



# 46<sup>th</sup> WEFTA CONFERENCE

12-14 October 2016. Split, Croatia

## BOOK OF ABSTRACTS

From  
Local Fish  
to Global  
DISH

Diverse  
Innovative  
Sustainable  
Healthy

# BOOK OF ABSTRACTS

## 46<sup>th</sup> WEFTA CONFERENCE

„From Local Fish to Global DISH“

12-14 October 2016, Split, Croatia

### ORGANIZERS

West European Fish Technologists' Association – WEFTA



Faculty of Food Technology and Biotechnology, University of Zagreb



### UNDER THE AUSPICE OF

European Hygienic Engineering & Design Group (EHEDG)



Ministry of Health of the Republic of Croatia



Tourist Board of Split



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- Ana Gavrilović (Department of Aquaculture, University of Dubrovnik)
- Ivan Župan (Department of Ecology, Agronomy and Aquaculture, University of Zadar)

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# PROGRAM

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## Tuesday, 11<sup>th</sup> October 2016 / Pre-Conference day

13:30	WEFTA WG Analytical Methods Meeting
16:00	WEFTA Representatives Meeting
18:00	Registration and Welcome Reception

## Wednesday, 12<sup>th</sup> October 2016 / Day 1

8:00	Registration	
8:30	Opening ceremony: <b>Josip Marković</b> , Croatian Ministry of Agriculture and Fisheries <b>Damir Ježek</b> , Dean of Faculty of Food & Technology and Biotechnology, Uni Zagreb, Croatia <b>Sanja Vidaček</b> , Conference Chair	
9:00	<b>WEFTA AWARD WINNER 2015:</b> <b>Heidi Nilsen</b> Norway	<b>Superhuman “fishy” vision – using light spectroscopy in seafood quality assessment</b>
9:30	<b>T. Altintzoglou</b> Norway	Interdisciplinary Research: Good on paper, but how good do we need to be in practice?

### SESSION I: Innovation

Innovative technologies and new perspectives for improving and assuring the quality of products

- Novel processing technologies: HEP, HHP, ultrasound, cold plasma, ohmic heating, microwave heating, super chilling, new packaging solutions etc.
- Emerging quality assurance and authentication methods
- Clarifying underlying mechanisms for improving the quality

### Chairpersons: Narcisa Bandarra and Guillaume Duflos

09:35	<b>M. Careche</b> Spain	LF NMR relaxometry can be used to verify that fish have been subjected to freezing in order to comply with EU regulation about prevention of parasite infection
09:55	<b>E. Giese</b> Germany	Determination of fish oil quality by <sup>1</sup> H NMR, FT-IR and FT-NIR spectroscopy in combination with artificial neural networks
10:15	<b>S. Pedros Garrido</b> Ireland	Effect of high-power ultrasound on the decontamination of salmon ( <i>S. salar</i> ) and mackerel ( <i>S. scombrus</i> ) natural flora
10:20	<b>T. Jančić</b> Croatia	Determination of histamine in fish by Surface Enhanced Raman Spectroscopy – comparison with reference HPLC method
10:25	<b>B. Martínez López</b> Denmark	Development of salt concentration local measuring techniques on fish muscle

### 10:30-11:00 Coffee break and posters

#### POSTERS (Session: Innovation)

- F. Jessen: Protein changes in shell and epidermis of shrimp (*Pandalus borealis*) after maturation on ice or in salt brine
- J. Lercke Skytte: Non-invasive volume estimation of fish fillets/cutlets using structured light
- C. Altinelataman: Electrical conductivity of refrigerated sea bass meat in semi-solid form and aqueous solution to determine quality
- M. Durmus: The effects of nanoemulsion on biogenic amine formation of vacuumed packed sea bass
- M. Durmus: The effects of nanoemulsions on fatty acid profiles of vacuumed packed sea bass at 2±2°C
- N. Toy: Antimicrobial effects of *Lactococcus* isolated from commercial cold-smoked fish
- N. Toy: Natural Food Preservative: Nisin
- Y. Ozogul: The impact of nanoemulsions based on sunflower and olive oils on the shelf life of sea bass fillets stored in ice
- E. T. Aksun: The effects of packaging material on quality changes of fish pate produced from skipjack tuna (*Katsuwonus pelamis*) during the refrigerated storage
- F. Özogul: The impact of two levels of clinoptilolite (1 and 5%) on the formation of biogenic amines and ammonia by food-borne pathogens in tyrosine decarboxylase broth
- F. Özogul: Seafood toxins and their health concern

T. Bongiorno: The effect of the pre-slaughter feeding/fasting schedule on post mortem quality traits of rainbow trout  
A. Gavrilović: Development and evaluation of marinade on the basis of bitter orange with *Lactobacillus Plantarum* O1 for bio-preservation and flavoring of sea food  
C. M. Messina: Research activities aimed to support the value-chain of canning tuna industry - from traceability to shelf-life prediction  
N. Abel: Influence of modified atmosphere, storage temperature, and fatty acid composition on the carbon dioxide solubility in fish mince  
T. Skåra: Effect of salt content and temperature on the cook loss of fresh cod (*Gadus morhua*)  
J. Lerfall: MAP technology to increase the shelf life of refrigerated saithe (*Pollachius virens*)  
S. K. Stormo: The impact of freezing and thawing on a high quality fish product  
J. Szarek: Comparison of the pathomorphological evaluation of the liver and posterior kidney in rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) reared by two different technologies  
S. P. Aubourg: Quality enhancement of chilled megrim (*Lepidorhombus whiffiagonis*) by the employment of alga *Undaria pinnatifida* ethanol extracts  
M. G. Karlsdóttir: Effect of brining and frozen storage on physicochemical properties of well-fed Atlantic mackerel (*Scomber scombrus*) intended for hot smoking and canning  
T. Yoshioka: Quality Preservation of Fish by Slurry Ice  
V. Šimat: Changes in oxidation stability of fish oil during thermic treatment and storage under poor conditions  
B. Tokur: Processing effects of newly designed toroidal cans on texture and color quality of canned tuna  
F. Leroi: Genetic and phenotypic characterization of *Lactococcus piscium* from diverse origins  
V. Verrez-Bagnis: Preliminary study for the development of a probe-based fluorescence melting analysis for quick testing of the presence of more than one scombridae fish species in food products  
M. H. Ekgreen: Biological variation of the raw material and processing conditions affect the yield and quality of fast-marinated herring  
T. Vukušić: Quality of fish (*Sparus aurata*) treated by cold plasma  
N. Gokoglu: Effects of Organic Acids on Physicochemical Characteristics of Octopus Muscle  
S. Zavadlav: Assessment of storage time of frozen squids (*Cephalopod: Loliginidae, Loligo gahi*) by impedance analysis  
M. G. Karlsdóttir: Effect of temperature fluctuation during transportation on quality and stability of frozen mackerel products  
C. Smyth: Assessing the quality of raw cod (*Gadus Morhua*) using microbiological, sensory and chemical indicators  
C. Fogarty: Shelf-life extension of fresh salmon (*Salmo salar*) using organic acids and phenolic compounds present in essential oils

11:00	<b>L. Méndez</b> Spain	A novel strategy for improving quality and shelf life of farmed rainbow trout during frozen storage: dietary intervention with essential oils
11:20	<b>M. G. Karlsdottir</b> Iceland	Estimation of lipid degradation of frozen saithe ( <i>Pollachius virens</i> ) and hoki ( <i>Macruronus novaezelandiae</i> ) muscles by colour analysis
11:40	<b>K. Konno</b> Japan	Superchilling storage of fish meat for keeping its freshness
12:00	<b>J. Robbens</b> Belgium	Effect of storage and transport in tubs on shelf life and quality of plaice ( <i>Pleuronectes platessa</i> )
12:20	<b>S. P. Aubourg</b> Spain	Shelf-life extension of chilled mackerel ( <i>Scomber scombrus</i> ) by including a jumbo squid ( <i>Dosidicus gigas</i> ) skin extract in the icing medium
12:25	<b>E. T. Aksun</b> Turkey	Effect of different plant extract coatings on physical quality parameters of frigate mackerel ( <i>Auxis thazard</i> ) during iced storage
12:30	<b>J. Calanche</b> Spain	Impact of freshness on sensory quality of thawed seabream ( <i>Sparus aurata</i> )
12:40	<b>N. Gokoglu</b> Turkey	Tenderization Effect of Freezing and Frozen Storage on Squid Muscle

**13:00 -14:15 Lunch****Chairpersons: Françoise Leroi and John Fagan**

14:15	<b>K. Heia</b> Norway	Direct filleting of Atlantic Salmon – Blood issues?
14:35	<b>N. Wiernasz</b> France	Lactic acid bacteria selection for biopreservation application on fishery products
14:55	<b>F. Özogul</b> Turkey	Probiotics function of cell-free extract of several lactic acid bacteria strains on histamine and other biogenic amines formation by common food-borne-pathogens
15:15	<b>E.K. Boga</b> Turkey	The effects of cell free extract of <i>Lactobacillus plantarum</i> and <i>Pediococcus acidophilus</i> alone or in combination with thyme and laurel extracts on microbiological and chemical quality of fermented sardine
15:20	<b>M. Durmus</b> Turkey	Antimicrobial and antioxidant effects of nanoemulsions based on herb oils (rosemary, laurel, thyme and sage) on the quality of rainbow trout
15:25	<b>F. Özogul</b> Turkey	Organic acids (lactic, acetic, succinic, propionic, formic and butyric) production by lactic acid bacteria in various fish infusion broth
15:30	<b>H. Yazgan</b> Turkey	Inhibition impacts of organic acids (lactic, fumaric, succinic and ortho-phosphoric) on biogenic amines formation by spoilage bacteria and common food-borne pathogens
15:35	<b>Y. Ucar</b> Turkey	The effects of organic acids on biogenic amine production by fish spoilage and food borne pathogens in tyrosine decarboxylase broth

**15:45-16:15 Coffee break and posters**

16:15	<b>A. Dehaut</b> France	Extraction and characterization of microplastics in seafood: identification of a suitable protocol and application to field study
16:35	<b>T.T. Dang</b> Denmark	High pressure and enzyme facilitating the shell loosening of cold-water shrimps ( <i>Pandalus borealis</i> )
16:55	<b>D. Cando</b> Spain	Gelation of mechanically extracted raw blue crab meat
17:00	<b>S.P. Aubourg</b> Spain	Effect of high-pressure treatment on biochemical changes in sardine ( <i>Sardine pilchardus</i> ) during a subsequent 9-month frozen storage
17:05	<b>I. Ucak</b> Turkey	Effect of high pressure processing in reducing <i>Photobacterium phosphoreum</i> and histamine formation in marinated herring

**Thursday, 13<sup>th</sup> October 2016 / Day 2****Chairpersons: Heidi Nilsen and Johan Robbens**

9:00	<b>S. Merkle</b> Germany	Content of minor components in fish oils of different fish species and processing technologies
9:20	<b>G. Lorentzen</b> Norway	Shelf life of salt-cured cod ( <i>Gadus morhua</i> L.) stored at elevated temperatures
9:40	<b>U. Çelik</b> Turkey	Investigation of Flesh Quality and Shelf Life at different storage conditions of European Sea bass, <i>Dicentrarchus labrax</i> , fed with diets included natural and synthetic additives
9:45	<b>L. Balakireva</b> France	Novel microplate-based spectrophotometric assay allowing quantitative measurement of dietary nucleotides IMP, inosine and hypoxanthine in aquafeed samples
9:50	<b>F. Lian</b> Ireland	Low-field Nuclear Magnetic Resonance (LF-NMR) Relaxometry to Monitor Quality Changes in Ready-to-eat Crab Meat ( <i>Cancer pagurus</i> ) as Affected by Cooking Method and Storage
9:55	<b>T. Ageeva</b> Norway	Effects of food deprivation on quality aspects of fillet products from live stored cod ( <i>Gadus morhua</i> L.)

10:00	<b>B. Tokur</b> Turkey	A new industrial design for the production of canned tuna: Effects on lipid quality after processing
10:05	<b>H. A. Yatmaz</b> Turkey	Effect of transglutaminase coating on the formation of biogenic amines in mackerel fillets

### 10:15-10:45 Coffee break and posters

#### POSTERS (Session: Diversification)

- M. G. Karlsdóttir: Stability of lightly salted cod (*Gadus morhua*) fillets during frozen storage
- T. Bongiorno: Processing fresh mussels (*M. galloprovincialis*) by sous vide technology: effect on the microbiological characteristics
- G. Parisi: Fishburger from aquacultured European species: nutritional quality and future prospective
- G. Parisi: Valorisation of horse mackerel, a discard fish
- B. T. Rotabakk: Consumer test for smoked salmon from diploid and triploid Atlantic salmon (*Salmo salar*)
- M. C. Gómez-Guillén: Melanosis development in differently sulphite-treated shrimp (*L. vannamei*)
- J. Borderias: Effect of high hydrostatic pressure on physicochemical gelling properties of crabmeat
- S. Kristoffersen: Shelf life of Skrei (*Gadus morhua* L.) fillets stored at refrigerated conditions
- F. Korkmaz: Sensory and chemical changes in carp (*Cyprinus carpio* L., 1758) fillets marinated with sunflower oil, sunflower oil plus pomegranate sauce and sunflower oil plus plum sauce during storage a +4oC for 1 month
- J. Pinedo: Control of lipid oxidation in fresh fish through the inclusion of barley on rainbow trout (*Oncorhynchus mykiss*) diet
- Z. Linhartová: Proximate and fatty acid composition of thirteen important freshwater fish species in the Czech Republic
- A. R. Köşker: Seasonal Changes in the Chemical Composition of the Beadlet Anemones (*Actinia equina* Linnaeus, 1758) from Mersin Bay in the Northeastern Mediterranean Sea

#### SESSION II: Diversification

Diversification of products for the rising global demand for food

- Novel products from rest raw materials and underutilized species
- Quality aspects of new species from aquaculture
- Reformulation of traditional products for modern consumer / global trade

Highlighted topics:

Products from freshwater species

Algae as a food source: cultivation, processing, quality and healthy aspects

#### Chairpersons: Magnea Karlsdóttir and Themistoklis Altintzoglou

10:45	<b>INVITED LECTURE</b> <b>E. Tribilustova</b> Denmark	Marketing strategies for freshwater fish and imported species on the EU market
11:10	<b>M. Daugbjerg</b> Iceland	Bioactive properties of Fish Protein Hydrolysates produced with Seaweed Extract related to skin health
11:30	<b>D. Skipnes</b> Norway	A proposed toolbox for food quality analysis of heat processed macroalgae: colour, texture, and microbiology
11:50	<b>M. Kjerstad</b> Norway	Preliminary study on the utilisation of Norwegian red sea cucumber, <i>Parastichopus tremulus</i> (Gunnerus, 1767) ( <i>Holothuroidea</i> , <i>Echinodermata</i> ), from Norwegian waters: resource, biology and market
12:10	<b>J. van Houcke</b> Netherlands	Attitude and purchase intent of Dutch consumers for refined and non-refined Pacific cupped oysters ( <i>Crassostrea gigas</i> )
12:30	<b>A. H. Hellevik</b> Norway	The future convenience product from the Norwegian salt cured and clip fish industry
12:50	<b>I. Peral</b> Spain	Introduction of new fish products derived from mackerel ( <i>Scomber spp.</i> ) in children's school menus through a social, sustainable and economic approach
12:55	<b>H. Harrysson</b> Sweden	Isolation of proteins from Swedish seaweeds, <i>Porphyra umbilicalis</i> , <i>Ulva lactuca</i> and <i>Saccharina latissima</i> , by the pH-shift method
13:00	<b>J. Pinedo</b> Spain	Barley vs. Red beet: two carbohydrates sources on rainbow trout diets. Effect on growth performance and quality parameters of fish fillets



13:05	<b>H. M. Lourenço</b> Portugal	Natural elimination of contaminant metals in oyster <i>Crassostrea angulata</i> from Sado estuary (Portugal)
13:10	<b>C. M. Messina</b> Italy	Valorization of low value fisheries species in Sicily (Italy), by realization of functional food: a case study of high technological readiness level

### 13:15 - 14:00 Lunch

#### SESSION III: Health

Healthy meal for happy consumer

- Emerging food safety issues- status and control
- Functional foods and nutritional value
- New bioactive compounds with health benefits
- Communication to and with consumers
- Adulteration, misbranding and traceability

#### Chairpersons: Mercedes Careche and Sigrún Halldórsdóttir

14:00	<b>INVITED LECTURE</b> <b>M. Tamplin</b> Australia	Integrated Predictive Models and Sensors to Manage Seafood Safety and Quality in Supply Chains
14:25	<b>U. Schröder</b> Germany	The importance of effective traceability tools by the example of monkfish ( <i>Lophius spp.</i> )
14:45	<b>M. Zunabović-Pichler</b> Austria	Prevalence and genotypic characterisation of <i>Listeria monocytogenes</i> in cold smoked fishery products sold in Austrian markets
15:05	<b>I. Sánchez-Alonso</b> Spain	In vitro infectivity and allergenicity of <i>Anisakis simplex</i> L3 after freezing fish muscle
15:25	<b>M. Tejada</b> Spain	The pepsin digestion method may underestimate the content of <i>Anisakis</i> in fish products and this is dependent on the status of the larvae
15:45	<b>H. M. Moreno</b> Spain	Surimi processing effects on the levels and allergenicity of $\beta$ -parvalbumin
15:50	<b>S. Cakli</b> Turkey	Influence of lysozyme process on sea bream ( <i>Sparus aurata</i> ) quality

### 16:00-16:30 Coffee break and posters

#### POSTERS (Session: Health)

- I. Bušelić: Population and genetic structure of *Anisakis spp.* from fish landed at local market of Island of Korčula, Adriatic Sea
- I. Mladineo: Quick insight into anti-*Anisakis* seroprevalence in professionals employed in fish-ery industry of Split-Dalmatian County, Croatia
- O. K. Topuz: Development of algal oil supplement: Effect of fluidized bed drying of algal biomass on the lipid fraction and fatty acid composition of *Nannochloropsis oculata* oil
- Y. Özogul: Effects of natural extracts on fatty acid profile of vacuumed packed fish ball made from mackerel (*Scomber scomber*) during chilled storage (4±2oC)
- H. A. Yatmaz: Chromatographic analysis of biogenic amines in seafood
- I. Ucak: *Morganella psychrotolerans*, a histamine-producing bacterium in seafood
- S. P. Aubourg: Optimisation of the production of EPA- and DHA-high-content butter from salmon refined oil and by employment of CO2 supercritical conditions
- L. Méndez: Rapid detection of anisakids in fish products by parallel reaction monitoring (prm) mass spectrometry
- C. Prentice: Effect of different levels of Common carp by-products protein hydrolysates in Zebra fish (*Danio rerio*) diets to evaluate the lipid peroxidation in brain and muscle
- P. Montero: Encapsulation of active compounds into phosphatidylcholine liposomes for the design of functional surimi gels
- M. Krpan: Assessment of added water in seafood products on Croatian market
- V. Šimat: Biological potential of three Adriatic brown seaweeds
- S. Vidaček: Prevalence of the flagellate *Giardia duodenalis* in mussels (*Mytilus galloprovincialis*) and oysters (*Ostrea edulis*) in Croatia

A. Mcdermott: Establishing thermal resistance profiles of *Listeria monocytogenes* isolated from Irish seafood products

L. Dediu: Preference for fish and fish products consumption depending on different market channels in Romania

A. R. Kosker: The Health Risk of Tetrodotoxin in Mediterranean Sea

F. Özogul: Tasting trout fish soup by consumers in the fish restaurant and taking their opinions

16:30	<b>N. Bandarra</b> Portugal	Dietary Oil Sources Have an Influence on Senegalese sole ( <i>Solea senegalensis</i> ) Fatty Acid Composition and its Bioaccessibility for Human Consumption
16:50	<b>C. Tullberg</b> Sweden	Formation of lipid oxidation products (MDA, HHE, and HNE) during static and dynamic in vitro digestion of cod liver oil
17:10	<b>O. K. Topuz</b> Turkey	Development of novel food supplement from rainbow trout egg: Effect of fluidized bed drying on the polyunsaturated fatty acid (PUFA) content of lipid fractions
17:15	<b>L. Méndez</b> Spain	Liver metabolism as target to understand the functional effect of EPA and DHA: regulation of protein pathways involved
17:20	<b>C. Prentice</b> Spain	Dipeptidyl Peptidase IV and Prolyl Endopeptidase Inhibitory Properties of Argentine croaker ( <i>Umbrina canosa</i> ) Hydrolysates
17:25	<b>C. Altinelataman</b> Turkey	European seabass ( <i>Dicentrarchus labrax</i> ) and gilthead seabream ( <i>Sparus aurata</i> ) muscle hydrolysates as a perspective source of biopeptides

## Friday, 14<sup>th</sup> October 2016 / Day 3

**Chairpersons: Vida Šimat and Flemming Jessen**

9:00	<b>B. Alfaro</b> Spain	Seafood offered by school foodservice: health and hedonic perception in children
9:20	<b>M. Geirsdóttir</b> Iceland	Bioactive properties of collagen hydrolysates from cod skin
9:40	<b>C. M. Messina</b> Italy	Valorization of shrimps by-products for pharmaceutical applications: effects of astaxantin and protein hydrolysates in vitro
10:00	<b>K. Kappel</b> Germany	What do chefs de cuisines purchase in order to sell as common sole?
10:05	<b>A. Cadun</b> Turkey	Development of Fish Cracker by Addition of Flaxseed ( <i>Linum usitatissimum</i> ) as an Alternative of Healthy Snacks
10:10	<b>S. Condon Abanto</b> Ireland	Effect of storage temperature on shelf-life and microbiological profiles of ready-to-eat whole brown crab

**10:15-10:45 Coffee break and posters**

### **POSTERS (Session: Sustainability)**

N. Gringer: Peelability and quality changes during ice maturation of shrimp (*Pandalus borealis*)

M. G. Karlsdottir: Packing of fresh fish products in boxes and tubs intended for sea transport

M. G. Karlsdottir: Quality and safety of Atlantic cod (*Gadus morhua*) fillets during cold storage, as affected by different thawing methods of pre-rigor frozen headed and gutted (H/G) fish

S. Cakli: Trypsins from fish processing waste: characteristics and biotechnological applications

S. Cakli: Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications

V. Iaconis: Tenebrio molitor larvae meal as alternative ingredient in diets for farmed blackspot seabream (*Pagellus bogaraveo*): effect on physical and chemical fillet traits

K. Grigoryan: Assessment of the efficiency of the biocides used for treatment of cold storage rooms for chilled fish

M. E. López-Caballero: Antioxidant and antimicrobial activity of Maillard reaction products obtained by reaction of shrimp waste hydrolysates with glucosamine

M. Van 't Land: Protein changes in fish silage produced using different combinations of undersized quota species

A. Cadun: Recovery Of Lipids From Marine Sources and Seafood Processing Waste: Methods, Current And Potential Uses

## SESSION IV: Sustainability

Sustainable processing - cleaner production strategies

- Reduction of energy / water during processing, storage, in retail and transport
- Reduction of waste / wastewater generation during processing
- Novel green extraction and refining methods
- Trends in sanitation

### Chairpersons: Helena Moreno and Ingrid Undeland

10:45	<b>INVITED LECTURE</b> <b>E. Bugge</b> Norway	What does the fish industry need? Emerging sustainable solutions"
11:10	<b>E. Misimi</b> Norway	Automated Classification of Atlantic Cod's Roe, Liver and Milt using Hyper-spectral Imaging in Visible Spectral Range
11:30	<b>J. Hinchcliffe</b> Sweden	Sustainable feed formulation for Swedish aquaculture from herring by-products using the ph shift method
11:50	<b>B. Forghani</b> Sweden	Flocculation as a mean to aid the recovery of protein from shrimp process water
12:10	<b>M. Abdollahi</b> Sweden	Tuning the pH-shift protein-isolation method for maximum hemoglobin-removal using blood rich marine resources
12:30	<b>S. Cakli</b> Turkey	Trypsin from viscera of cultured rainbow trout ( <i>Oncorhynchus mykiss</i> ): Characteristics and the effects of seasonal variation
12:35	<b>A. Roncarati</b> Italy	Strategies to improve the management of flat oyster ( <i>Ostrea edulis L.</i> ) from aquaculture using the short storage and package in closed-circuit system
12:40	<b>M. Abdollahi</b> Sweden	Comparing the efficacy of the pH-shift protein isolation process with the conventional washing method in development of mixed surimi
12:45	<b>D. Kanski</b> Croatia	Fish Forward project – sustainable seafood for the environment, people and developing countries
12:50	<b>M. Van 't Land</b> Belgium	Belgian bycatch fish silage as fish meal substitute in shrimp feed: fish silage characterization and variation
12:55	<b>John Fagan</b> Ireland	- Announcement: 47 <sup>th</sup> WEFTA CONFERENCE
13:10	<b>Sanja Vidaček</b>	- Closing ceremony

**13:30 - 14:15 Lunch / Snack**

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# ORAL PRESENTATION ABSTRACTS

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## SUPERHUMAN “FISHY” VISION – USING LIGHT SPECTROSCOPY IN SEAFOOD QUALITY ASSESSMENT

**Heidi A. Nilsen\***, Martin H. Skjelvareid, Svein K. Stormo, Karsten Heia

**\*2015 WEFTA AWARD WINNER**

*Nofima, Muninbakken 9-13, N-9291 Tromsø, Norway*

Assessment of fish and fish product quality is a challenging task, especially when performed on an industrial scale and on a variety of fish species. The perceived quality is dependent on a large number of attributes, such as nutritional content, color and shape, odor, taste, presence of defects (nematodes, bones etc.), handling, and storage conditions. Human evaluation of these parameters is time-consuming and requires a high level of expertise, and hence there is a need for fast, automatic and objective methods for quality assessment.

Light spectroscopy has been explored and developed as a tool for seafood quality assessment since the 1980s. The use of this technology is motivated by the possibility to conduct high-speed, non-invasive analysis by-line or on-line, meeting the demands of the industry regarding speed and accuracy.

This presentation gives an overview of the possibilities and application of light spectroscopy, looking into the use of visible and near-infrared spectroscopy, and the development of hyperspectral imaging for fish quality control. Methods and tools include the evaluation of sample constituents, assessment of freshness and quality defects, as well as hyperspectral imaging for whole-sample quality evaluation.

## INTERDISCIPLINARY RESEARCH: GOOD ON PAPER, BUT HOW GOOD DO WE NEED TO BE IN PRACTICE?

**Themistoklis Altintzoglou, Geir Sogn-Grundvåg, Gøril Voldnes**

*Nofima - Norwegian Institute for Food, Fisheries and Aquaculture Research, Tromsø, Norway*

EU and national research programs/projects are increasingly larger, seeking solutions to research problems that cover value chains and multiple disciplines. To acquire such projects, applicants are asked to build interdisciplinary teams. But what does interdisciplinarity really mean? Is it always useful? If not, how interdisciplinary do we need to be and under what circumstances? We elaborate on these important questions by providing a theory based discussion of the concept of interdisciplinarity and its usefulness.

We also draw on experiences from a large research project, CATCH which is led by Nofima. The project has a complex research aim, thus, we need to combine disciplines to find the best ways to improve the whole value chain for capture-based aquaculture of cod. Various disciplines and industrial partners work together to solve this wide task. This introduces a number of challenges related to interdisciplinarity. A work-package is devoted to facilitate and study interdisciplinary collaboration and learning. Based on recommendations from the literature

on interdisciplinarity, we arrange frequent meetings, ask the right questions to increase knowledge transfer, create awareness of interdisciplinarity and facilitate rich interaction between partners. Does it work? This presentation will describe our experiences and reveal the most useful tools to facilitate interdisciplinarity.

WEFTA is an interdisciplinary platform for sharing of research results. But how good are we in communicating results to other disciplines? Do researchers from other disciplines than our own understand the results we present and are they useful across disciplines? How much do we need to do to change that? This presentation will close with links between theories and practice from research projects and what these can teach us when communicating in interdisciplinary platforms such as WEFTA.

## LF NMR RELAXOMETRY CAN BE USED TO VERIFY THAT FISH HAVE BEEN SUBJECTED TO FREEZING IN ORDER TO COMPLY WITH EU REGULATION ABOUT PREVENTION OF PARASITE INFECTION

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Freezing and frozen storage of fish is nowadays the most adequate technological treatment to control the risk associated to consumption of raw or undercooked parasitized fish. EC Regulation (EU 1276/2011) which obliges reaching at least -20 °C in all parts of the product for not less than 24 h, or -35 °C for not less than 15 h covers a wide range of conditions which can have a dramatic effect on the muscle characteristics, affecting the eating quality of fish muscle.

In this work we first studied the changes occurring in hake muscle as affected by freezing conditions previously found sufficient to inactivate *Anisakis* parasites at the highest final temperature set in the Regulation (-20 °C), and secondly we used this information to find a tool to discriminate between fresh and frozen fish at different freezing rates that may be found both in industry and home freezers.

The freezing rate and storage time affected the sensory quality of the hake so that it is suggested that total freezing times of 70 min or less at a maximum temperature of -20 °C and thawing after 24 h could meet both safety in terms of *Anisakis* mortality and eating quality.

Significant changes in physical chemical characteristics between fresh vs frozen and thawed hake, and also among samples subjected to different freezing rates have been observed. Among them, discriminant analysis has shown that LF NMR relaxometry can be used as a tool to verify if a sample has been frozen and, to some extent, if it has been subjected to fast or slow freezing rates. This opens a possibility for authentication, which could aid in the verification of implementation of the EC regulation.

## DETERMINATION OF FISH OIL QUALITY BY <sup>1</sup>H NMR, FT-IR AND FT-NIR SPECTROSCOPY IN COMBINATION WITH ARTIFICIAL NEURAL NETWORKS

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Fish oil based dietary supplements have become increasingly popular, which is mainly due to their high content of the health promoting omega-3 fatty acids. However, these polyunsaturated fatty acids are extremely prone to oxidation. Traditional methods for determining fat quality parameters such as peroxide value (PV), anisidine value (AnV), and acid value (AV) is time-consuming, labor- and solvent-intensive and require high amounts of sample. Therefore, the aim of this study was to assess the applicability of <sup>1</sup>H NMR, FT-IR and FT-NIR spectroscopy to the prediction of PV, AnV and AV in fish oil using artificial neural networks (ANN). For this purpose, 84 different fish oils were analyzed by the traditional wet chemistry methods as well as by the aforementioned spectroscopy techniques. Some of the fish oils were subjected to storage trials with varying tempera-

ture and light exposure in order to increase the range of calibration. Before the prediction models were built using ANN, various methods of spectral preprocessing, variable selection and data dimensionality reduction were applied.

<sup>1</sup>H NMR, FT-IR and FT-NIR spectroscopy provides the advantages of being non-destructive, fast and less labor-intensive than traditional wet chemistry methods. Moreover, further quality parameters can be obtained from the same spectra.



## EFFECT OF HIGH-POWER ULTRASOUND ON THE DECONTAMINATION OF SALMON (*S. Salar*) AND MACKEREL (*S. Scombrus*) NATURAL FLORA

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High-power ultrasound (US) is a novel processing technology based on the transmission of low frequency sound waves (20-100 kHz) in a liquid medium creating regions of alternating compression and expansion. These pressure changes cause cavitation and gas bubble formation which are the basis for the application of ultrasound for cleaning and microbial inactivation or decontamination.

The aim of the current study was to evaluate the effect of a novel decontamination system using US on the natural flora of salmon (*Salmo salar*) and mackerel (*Scomber scombrus*). Fish was cut in small fillets of similar size without skin for salmon and with skin for mackerel, in order to maintain fillet integrity. Samples were treated in a low frequency ultrasonication bath (40 kHz) with a continuous flow of potable water maintaining a constant volume and temperature below 15°C. Fish samples were also immersed in the bath without applying ultrasound (IM). Selected treatment times (min) were: 0-5-15-25-35-

45, before concentrations of Mesophilic and Psychrophilic viable counts (MVC – PSC), *Enterobacteriaceae* (ENT) and *Pseudomonas* spp. (PSE) were determined. Both treatments showed significant reductions ( $p \leq 0.05$ ) of all microbial groups when compared to untreated controls (0 min). Reductions of up to 1.5 Log CFU/g for PSC were achieved in salmon when US was applied while reductions ranging from 0.8 to 1.3 Log CFU/g were observed for the other microbial groups. In mackerel maximum reductions of 1.2 Log CFU/g and 0.8 Log CFU/g were obtained for MVC and ENT respectively when US was applied. For IM treatments, reductions of between 0.5-0.9 Log CFU/g were achieved in salmon and mackerel for all bacterial groups studied. In general significant differences were observed between IM and US treatments irrespective of microbial group for both fish species ( $p \leq 0.05$ ). These findings suggest that high-power ultrasound may be a suitable technology to reduce microbial contamination on salmon and mackerel.

## DETERMINATION OF HISTAMINE IN FISH BY SURFACE ENHANCED RAMAN SPECTROSCOPY – COMPARISON WITH REFERENCE HPLC METHOD

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Histamine is a biogenic amine responsible for majority of health problems associated with seafood consumption occurring worldwide. Histamine formation in fish is result of bacterial, enzymatic decarboxylation of free histidine due to time – temperature mishandling of fish. Legislative limit regarding histamine content of fishery products in EU countries is set at 100 mg kg<sup>-1</sup> for fishery products from fish species associated with high histidine content and 200 mg kg<sup>-1</sup> for enzyme matured products. Conventional methods for histamine analysis often require time consuming protocols for extraction and derivatization of histamine accompanied by skilled personnel and high-cost equipment (HPLC) or use of enzymes and test kits (ELISA) with considerable costs.

A rapid and sensitive method of histamine determination in fish based on Surface Enhanced Raman Spectroscopy (SERS) using simple and widely

available silver colloid SERS substrate was developed. Principal components analysis effectively distinguished SERS spectra of fish samples spiked with different concentrations of histamine (0 – 400 mg kg<sup>-1</sup>). Partial least square regression models confirmed reliability of this approach ( $R^2_{\text{pred}}=0.962$ , RPD=7.250) in legislative significant histamine concentration range (0 – 200 mg kg<sup>-1</sup>).

Performance of SERS based method has been tested on real samples in comparison to reference HPLC method and results show SERS has a potential to be used as rapid screening tool for histamine determination in fish.

## DEVELOPMENT OF SALT CONCENTRATION LOCAL MEASURING TECHNIQUES ON FISH MUSCLE

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Accurate prediction of salting times in fish muscle relies on robust estimations of the diffusion coefficient, the solubility limit of the salt in the muscle, and in a lesser extent, the mass transfer coefficient. Because of the analogy with heat transfer that is commonly used for the modelling of a mass transfer process such as salting, the maximum amount of salt admissible by the muscle is assumed to be the concentration of the salting

environment instead of the solubility limit of the salt in the muscle, thus disregarding the discontinuity in the concentration profile occurring in the muscle-salting environment boundary. By adjusting the concentration of the salting environment to a value that ensures that the solubility limit is maintained at the boundary can lead to a better control of the process and subsequently to an optimization of the producing costs.

## A NOVEL STRATEGY FOR IMPROVING QUALITY AND SHELF LIFE OF FARMED RAINBOW TROUT DURING FROZEN STORAGE: DIETARY INTERVENTION WITH ESSENTIAL OILS

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During frozen fish storage, changes in chemical constituents may lead to marked quality losses as a result of texture, flavour, and colour deterioration and the breakdown of nutritional components. Several strategies have been described to inhibit or at least slow down these changes, allowing sensory and nutritional retention of food. Among them, dietary interventions with different compounds might be a good choice to keep fish quality in aquaculture species, minimizing the need for processing technologies post-mortem. Specifically, essential oil has been lately proposed as components of fish feeding for preserving quality and shelf life, although they have not yet been sufficiently studied especially during frozen storage.

For this reason, the aim of the present work is to evaluate the protective use of an essential oil containing eucalyptol, carvacrole and thymol includ-

ed in the basal diet of rainbow trout (*Oncorhynchus mykiss*) on the shelf life and organoleptic quality of frozen fish fillets during 6 months of storage at -10°C. The study was carried out by combining the evaluation of organoleptic properties with tools to assay the chemical changes produced in lipids and proteins, particularly by using a novel redox proteomics approach based on labelling protein carbonyls by fluorescein-5-thiosemicarbazide (FTSC), protein separation on gel electrophoresis and protein identification by mass spectrometry.

Results revealed the effectiveness of essential oil in maintaining colour and water holding capacity of frozen fillets as well as in inhibiting lipid and protein damages. As consequence, essential oils managed to delay the onset of lipid oxidation and rancidity of trout fillets by decreasing the generation of lipid oxidation by-products. Moreover,

this antioxidant effect on lipid was accompanied by the inhibition of carbonylation of specific protein targets in both sarcoplasmic and myofibrillar fractions of trout fillets, which was closely correlated to the minor loss of protein solubility and water holding capacity of flesh.

These findings highlight the usefulness of essential oils as novel, safe, inexpensive and simple strategy to enhance fish quality and to extend its shelf life during frozen storage, resulting in high quality products, which require less processing post-mortem.

## ESTIMATION OF LIPID DEGRADATION OF FROZEN SAITHE (*Pollachius virens*) AND HOKI (*Macruronus novaezelandiae*) MUSCLES BY COLOUR ANALYSIS

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Hygiene of the processing environment is a significant factor in the production of microbiologically safe and good-quality products in the fish industry. Pathogens and spoiling microorganisms which adhere to interior equipment, chilling cameras and working surfaces may be transferred to fish products during their processing. In the presented work assessment of efficiency of two biocides 0.1% nanosilver solution and 1% peracetic acid for treatment of the walls of fresh fish chilling rooms has been conducted. Treatment of cold storage room surfaces has been done after prewashing. Microbial swab tests were carried out before, directly and in 24 hours after disinfection and after sanitation. Swabs with neutralizing agent (HiMedia) have been used. Simultaneously, swabbing of trout skin after washing and from chillers has been performed. Swabs were analyzed for the following groups of bacteria: total aerobic

count, coliform bacteria, *Staphylococci*, yeasts, fungi, gram negative and oxidase positive bacteria. In case of 0.1% solution of nanosilver strong durable effect against gram negative oxidase positive bacteria (*Aeromonas*, *Pseudomonas*, *Schewanella*, *Vibrio*, *Alcaligenes*) has been occurred comparing to 1% solution of peracetic acid. Mentioned bacteria had high frequency of occurrence on trout cold storage room walls. Tested bacteria possessed low adaptability against 0.1% solution of nanosilver during three years of continuous usage.

## SUPERCHILLING STORAGE OF FISH MEAT FOR KEEPING ITS FRESHNESS

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The simplest technique to keep the freshness of fish is its storage at low-ered temperature. As frozen storage of fish sometimes damages its quality, storage of fish without freezing is required. Recently, the technology to store fish below 0°C, superchilling, has been introduced by using slurry ice. In this paper, change in freshness of fish meat when stored under superchilling conditions was studied by measuring K-value.

Flounder, which is one of the most important fish species as Sashimi, fillet wrapped with plastic film was stored at three temperatures; at 5°C (refrigeration), 0°C (storage on ice), and -2°C (superchilling). The freshness of fish meat was assessed by analyzing the serial decomposition of ATP. K-value was defined as a relative amount of Inosine + Hypoxanthine to total amount of compounds from ATP in %. ATP and its related compounds were extracted from 1 g of fish meat with 10 ml of 5% perchloric acid. The composition was analyzed on HPLC (Shodex Asahipak GS-320HQ, 0.1 M Phosphate buffer, pH 2.8).

IMP (tasty nucleotide) was the major compound in the extract after hydrolysis of ATP at any temperatures. Lowering storage temperature expanded the period to show high IMP content. When stored at 5°C and 0°C, sharp decline of IMP content in a latter phase was characteristic; in 7 days at 5°C and 14 days at 0°C. This gave a biphasic progress of K-value increase. However, storage at -2°C did not show such quick increase of K-value up to 21 days.

A quick increase of K-value in the latter phase disappeared when the meat was stored in the presence of chloramphenicol (antibiotics, 150 ppm). Thus, involvement of deterioration bacteria in the quick increase of K-value was proposed. Moreover, K-value increase at -2°C was slower than that at 0°C in the presence of antibiotics. It was concluded that super chilling keeps freshness of fish by suppressing bacterial growth and suppressing IMP decomposition.

## EFFECT OF STORAGE AND TRANSPORT IN TUBS ON SHELF LIFE AND QUALITY OF PLAICE (*Pleuronectes platessa*)

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In the Flemish Fish Auctions there is an increasing demand by fish processors to deliver plaice in tubs instead of in traditional boxes. The larger volume of the tubs (630 l) simplifies the logistical procedure in the processing factories. In classical fish boxes, fish is stored in ice, where the melt water is drained through holes in the boxes. In tubs, plaice is stored in chilled water with a layer of ice on top. The goal of this trial was to investigate the effect of storage and transport in tubs on the fish quality. Plaice iced and stored in traditional boxes was compared to plaice stored in tubs filled with chilled water and an ice layer on top. Water content, quality index method, chemical and various microbiological analysis were performed on fish stored up to 15 days. The temperature of the fish was logged throughout the whole experiment. During the shelf life experiment, a temperature difference within the same tub of up to 2.8 °C was recorded. The threshold level of 4°C for the fish temperature was never exceeded despite some fluctuations

of the ambient temperature. Although the fish in tubs reached slightly higher temperatures than the fish in the boxes, this had no adverse effect on the quality of the fish. The sensory and chemical analysis showed that plaice in tubs had a longer shelf life than plaice in boxes. In accordance, the microbiological parameters were also better in the tubs than in the boxes, although these differences were not significant. Transport did not affect any parameter. The plaice in tubs did have a significant higher water content in the fish meat, already after two days of storage. The overall results show that the 630 l tub is well suited for storage of plaice in chilled water. This way of chilling gave better quality of the fish after storage compared to fish stored in traditional trawl boxes.



## SHELF-LIFE EXTENSION OF CHILLED MACKEREL (*Scomber scombrus*) BY INCLUDING A JUMBO SQUID (*Dosidicus gigas*) SKIN EXTRACT IN THE ICING MEDIUM

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By-products of aquatic species are body parts that are removed before they reach the final consumer to improve their preservation qualities, reduce the shipping weight or increase the value of the main product. By-products can comprise blood, viscera, heads, bellies, bones, skin, trimmings and fins. Jumbo flying squid (*Dosidicus gigas*) is one of the largest known cephalopods and has shown an increasing economic interest in a wide number of countries. During its processing, large amounts (up to 60% of whole weight) of by-products that may contain high concentrations of biological active compounds related to antimicrobial and antioxidant properties are generated. The present work focuses on Atlantic mackerel (*Scomber scombrus*) traded as a chilled product. This small pelagic fish species can constitute food products of great economic importance in many European countries, although it remains underutilised because of its limited

chilled shelf life. In order to extend its shelf life, jumbo squid skin (JSS) was employed in this work as a source of pigmented preservative compounds. For it, aqueous solutions including acetic acid-ethanol (0.5%, v/v) extracts of JSS were tested at three different concentrations as icing media, being the effects studied throughout a 13-day chilled storage. A significant inhibition ( $p < 0.05$ ) of microbial activity (aerobes, psychrotrophs, Enterobacteriaceae, lipolytic bacteria; trimethylamine formation) was observed in mackerel corresponding to the batch with the highest JSS extract presence. Additionally, fish corresponding to batches including any of the JSS concentrations tested showed lower ( $p < 0.05$ ) counts of proteolytic bacteria and a better maintenance of pH value than their control counterpart. Sensory analysis revealed a marked increase of shelf life time in chilled mackerel corresponding to ice including the highest JSS content. Such fish

was found to be still acceptable at the end of the storage period, while all other batches were considered rejectable. The marked microbial activity inhibition observed could be explained

on the basis of the presence in ice of ommochrome compounds obtained by acetic acid-ethanol extraction (i.e., lipophilic compounds extraction) of the JSS.

## EFFECT OF DIFFERENT PLANT EXTRACT COATINGS ON PHYSICAL QUALITY PARAMETERS OF FRIGATE MACKEREL (*Auxis thazard*) DURING ICED STORAGE

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Plant extracts, particularly essential oils that contain antimicrobial and/or antioxidant activities have been comprehensively studied as an alternative to synthetic preservatives in packing films and as coating material for seafood industry.

Physical quality parameters (texture and colour) of frigate mackerel (*Auxis thazard*) coated with thyme and olive leaf extracts were investigated during 18 days of iced storage. Frigate mackerel fillets were divided into 3 groups; the control group without any coating (C), thyme extract coatings (TEC) and olive leaf extract coatings (OLEC). Fillets were dipped in solutions with %1 thyme or olive leaf extracts for 10 min. After that, coated and non-coated fillets were vacuum packed and stored in ice at 3±1°C. White and dark muscle of frigate mackerel were used separately for textural and colour analysis.

Results of texture measurement of all groups showed that textural deterioration increased with storage time.

While hardness and chewiness values were significantly higher ( $P<0.05$ ) in white muscle, the values of adhesiveness, resilience and springiness were higher in dark muscle in all samples. Among coated samples, thyme extract was more effective for all parameters of texture of both white and dark muscle of iced frigate mackerel than olive leaf extract coating.

During iced storage, colour deterioration according to instrumental colour analysis for lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) of frigate mackerel was also observed for all groups. The  $L^*$  and  $b^*$  values increased while  $a^*$  value decreased rapidly for all parts of all groups. The most significantly colour change for all groups occurred in  $L^*$  value during storage period. Mainly, thyme extracts prevented lightness loss compared with other groups. Initial  $L^*$  value was found 91.45 in frigate mackerel and this value decreased to 87.75 in the control group and the final  $L^*$  value was 89.01 and 89.03 in TEC and OLEC groups, respectively.

Consequently, thyme and olive leaf extract coatings improved texture and colour parameters of frigate mackerel, especially thyme extract coating.

*Keywords: frigate mackerel, plant extract coatings, quality parameters, colour loss, texture*

## IMPACT OF FRESHNESS ON SENSORY QUALITY OF THAWED SEABREAM (*Sparus aurata*)

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The aim of this study was to evaluate the impact of freshness on the quality of seabream submitted to a frozen storage in different presentations. The fish were slaughtered and divided in 3 treatments (Whole, Gutted and Fillets). All of them were stored at refrigeration (0 +/-1°C) for different times (5, 9, 11 and 18 days). Electrical conductivity (TM) was assessed using a torrymeter® for establish the grade of freshness before 1 month frozen storage (-30 °C) in whole and gutted fish. After this time, physico-chemical (TVB-N, TBARS, pH), microbiological (Psychrotrophic Viable Count) and sensory parameters (Quality Index -QI- and Descriptive Analyses -intensity scales- for cooked fish) were studied. Significant differences were found between treatments over time for TM, TVB-N and TBARS where fillets were the worst evaluated. QI was satisfactory 5 days after slaughter and after 1 month frozen storage for all the treatments and significant differences were noted. Fillets had the lowest assessment for flavour meanwhile two distinct groups for whole and gutted fish were appreciated,

a very successful initial assessment at 9 days and then a questionable assessment. Related to sensory descriptors, seafood smell (optimal quality) was not perceived in any treatment. Bitter flavor showed very high significant differences over the storage time for gutted fish and texture (juiciness and flaky) was not distinct. Sensory profile was different and specific for each sampling time. Whole fish was related to seafood smell at day 5 while gutted had umami flavour and fillets presented an unfavourable assessment (flaky texture). At day 9, butyric smell appeared in whole while gutted had a bitter flavour and fillets continued to be the worst. After 11 days, fillets were very spoiled (flaky, butyric and bitter). Gutted and whole fish showed a bitter residual flavour. At the end of the experience (18 days), all treatments indicated a marked spoilage. The initial freshness is the key for the quality of thawed fish; freezing itself was unable to maintain the initial quality of the raw material. In conclusion, fillets were the worst evaluated followed by gutted and whole were the best preserved.

## THE DETERMINATION OF SHELF LIFE OF SOUS-VIDE APPLIED *Luciobarbus esocinus* (Heckel, 1843)

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In this study, *Luciobarbus esocinus* (Heckel, 1843) obtained from the Keban Dam Lake in Turkey were prepared according to sous-vide method and stored at +2 C°. Moisture, ash, fat, protein, salt, pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARs), mesophilic aerobic bacteria, total psychrophilic bacteria, and yeast-mold numbers were determined in raw material. The sensorial, chemical and microbiological changes of products obtained under the cold storage were analyzed in 3 days interval with 3 replicates.

The data obtained from chemical analysis was evaluated statistically; the differences amongst different cooking temperature applied groups in term of moisture, protein, fat and ash were found insignificant ( $p>0.05$ ). But pH, TVB-N values in groups during storage period was found significant ( $p<0.05$ ). The total aerobic psychrophile, total aerobic mesophile and yeast-mold numbers at groups treated different cooking temperature was found insignificant ( $p>0.05$ ). It was observed that products prepared ac-

ording to the sous-vide method are liked considerably.

In conclusion, it was determined that application of sous-vide technology had a positive effect upon shelf life of fish products.

## TENDERIZATION EFFECT OF FREEZING AND FROZEN STORAGE ON SQUID MUSCLE

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Squid is the most important member of cephalopods. It has commercial importance and high quality proteins. Texture of meat affects considerably acceptability by the consumers. Squid mantle texture is known to be related to its particular structure. Texture can be improved by various technological processes. Freezing is a physical process involving the transformation of water molecules from an amorphous state to highly structured ice crystals. The phase change can lead to protein denaturation caused by alterations in the chemical and physical environment of protein. The aim of this study was to investigate tenderization effects of freezing and frozen storage on squid (*Loligo vulgaris*) muscle by investigation of changes in proteins and texture. Before treatments head, skin, viscera and tentacles of squid were removed. Squid samples were cut into pieces of 4x4 cm. These pieces were packed in polyethylene bags. The bags were frozen in a blast freezer at -45°C until the thermal centre reached -18°C. Frozen samples were stored in a deep freezer at -18°C for 30 days. After freezing and during frozen stor-

age total soluble protein and water holding capacity decreased, total free amino acid and cooking loss increased in squid muscle. According to instrumental texture analysis results, freezing and frozen storage affected textural characteristics of squid. Sensory texture parameters of squid were affected by freezing and frozen storage. The Scientific and Technological Research Council of Turkey (TÜBİTAK) supported this study (Project number 213O163)

## DIRECT FILLETING OF ATLANTIC SALMON – BLOOD ISSUES?

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Industrial slaughtering of Atlantic salmon is a process that involves stunning, gill cutting and bleeding (30 minutes in bleeding tanks) prior to further processing. Some of the fish are filleted and some fish is packed as whole on ice and shipped to the market.

Direct filleting of Atlantic salmon is an alternative, and is currently under development and testing at Nofima. Here the fish is stunned, decapitated and directly filleted, and the traditional bleeding tanks are replaced with gravity induced bleeding for 2-5 minutes. Using this approach, blood can efficiently be collected and together with the rest of the trimmings serve as high quality rest raw material. However, there have been some concerns whether the level of bleeding through such a process is sufficient?

Hyperspectral Imaging is a method that has been developed for measuring the amount of residual blood in fish fillets. Since oxidized blood is easier to identify than fresh blood, the method was applied on smoked salmon fillets to measure the blood levels in the main veins after bleeding.

The sensitivity of the analysis is good, and in combination with human interpretation, the bleeding efficiency was evaluated.

Our results show that Hyperspectral Imaging is a valuable tool for blood analysis of salmon fillets, and that direct filleting removes blood from the fillets as efficiently as or better compared to traditionally bled fish. The efficiency of the direct filleting method depends to a large extent on the orientation of the fillets, after filleting, and length of time of the gravity induced bleeding before filleting.



## LACTIC ACID BACTERIA SELECTION FOR BIOPRESERVATION APPLICATION ON FISHERY PRODUCTS

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As fragile food commodities, fishery and seafood products microbial and organoleptic qualities can quickly deteriorate. In this context, microbial quality and security improvement during the whole food processing chain, using hurdle technology, a combination of mild preserving technologies such as biopreservation, modified atmosphere packaging, chitosan coating, and superchilling, are of great interest. In this work, 36 lactic acid bacteria (LAB), from 5 species (*Carnobacterium maltaromaticum*, *Lactococcus piscium*, *Leuconostoc gelidum*, *Vagococcus penaei*, *Vagococcus fluvialis*) isolated from previous projects and known to possess antimicrobial activity, had been screened further, for their potential application as bioprotective cultures in fishery products. LAB strains were selected firstly on their inhibitory activity against 6 spoiling bacteria (*Shewanella baltica*, *Photobacterium phosphoreum*, *Brochothrix thermosphacta*, *Lactobacillus sakei*,

*Hafnia alvei*, *Serratia proteamaculans*) and 1 pathogenic bacteria (*Listeria monocytogenes*), commonly isolated from salmon and cod based products. Experiment were carried out in fish juice model, both in salmon and cod juice (1.5% NaCl, 1% D-glucose, 0.01% L-methionine, L-cysteine, TMA, buffered at pH 6.7 in 0.1 M phosphate buffer), miniaturized in 96-well plate assays. Inhibitory tests are based on a 96h at 15°C co-culture between LAB and targeted bacteria, respectively inoculated at 10<sup>6</sup> and 10<sup>2</sup> CFU/ml. Enumeration of targeted bacteria was performed with a micro-enumeration method by plating 5 µl of each 10-fold dilution on selective medium. Inhibition is quantified by comparison with control in pure culture. LAB spoilage potential was also investigated, both in cod and salmon juice, by performing a sensory analysis. 12-trained panellists have been asked to score (0 to 10) each LAB strain characteristic odor intensity. These two first screen-

ing key steps allowed highlighting LAB sensory signatures and inhibition profiles existence that seem to be species correlated. LAB selection was refined on 4 next criteria: I) chitosan resistance at 0.02% final concentration in salmon juice solution at pH 6.0 and pH 6.6; II) freezing resistance (- 80°C,

3h); III) biogenic amines production profiles (tyramine, histamine production); and IV) antibiotics resistance. According to this screening approach, 6 LAB strains were selected for further inhibitory assays in cod and salmon, alone or in combination with other hurdle technologies.

## PROBIOTICS FUNCTION OF CELL-FREE EXTRACT OF SEVERAL LACTIC ACID BACTERIA STRAINS ON HISTAMINE AND OTHER BIOGENIC AMINES FORMATION BY COMMON FOOD-BORNE-PATHOGENS\_

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Probiotics are metabolic products of non-pathogenic microorganisms including *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Lactococcus*, *Bacillus* and yeasts that have favorable impacts on human health contributing to the maintenance of intestinal microbial balance and enhance the host immune system. In order to monitor unfavorable compounds such as biogenic amines in fermented food, some lactic acid bacteria (LAB) strains that have probiotic function may play important roles as starter cultures, co-cultures, or bioprotective cultures to enhance food quality and safety. Biopreservation functions of LAB are often used in food industry because of their wide range of antimicrobial metabolites including organic acids, diacetyl, acetoin, hydrogen peroxide, antifungal peptides and bacteriocins without safety implications.

The impact of cell free extract (CFE) of several lactic acid bacteria strains

(*Leuconostoc mesenteroides* subsp. *cremoris*, *Pediococcus acidophilus*, *Lactococcus lactis* subsp. *lactis*, and *Streptococcus thermophilus*) on histamine and other biogenic amines production by *Salmonella Paratyphi A*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* was studied in histidine decarboxylase broth (HDB). The percentage of CFE concentrations obtained from LAB were 50 and 25 and the control was without CFE.

Histamine formation by *E. coli*, *S. Paratyphi A* and *S. aureus* was found to be 11163.5 mg/L, 10387.5 mg/L and 7330.6 mg/L, respectively. All of the CFE of LAB strains significantly ( $P < 0.05$ ) lowered histamine (HIS), ammonia and other biogenic amines production by common four foodborne pathogens since the metabolites produced by the fermentation process result in the reduction of food spoilage bacteria and their toxins production. Although, some of the CFEs obtained

by LAB strains showed stimulation impact on dopamine and tyramine, most of the CFEs revealed considerable inhibition effects on putrescine, cadaverine, spermine, trimethylamine and agmatine formation. Consequently, the results of this current study indicated that the functions of CFEs var-

ied depending on not only LAB strains and their growth conditions, but also on the CFE concentrations used and the pathogens interaction with LAB.

*Keywords: Lactic acid bacteria (LAB), probiotics, cell-free extract, food-borne pathogen, histamine, biogenic amines*

## THE EFFECTS OF CELL FREE EXTRACT OF *Lactobacillus plantarum* AND *Pediococcus acidolactici* ALONE OR IN COMBINATION WITH THYME AND LAUREL EXTRACTS ON MICROBIOLOGICAL AND CHEMICAL QUALITY OF FERMENTED SARDINE

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Effects of cell free extract of *Lactobacillus plantarum* and *Pediococcus acidolactici* alone or in combination with thyme and laurel extracts on microbiological and chemical quality fermented and vacuum packaged sardine were investigated during 8 week of storage. Sardine fillets were divided into 7 groups which are control group without any cell free extract and thyme/laurel addition, LP and PA group with cell free extract of *Lb. plantarum* (LP) and *Pd. acidolactici* (PA) at doses of 8%, THPA and THLP group with thyme at doses of 0.5% and cell free extract of *Lb. plantarum* and *Pd. acidolactici* at doses of 8%, and LAPA and LALP group with 0.5% laurel and cell free extract of *Lb. plantarum* and *Pd. acidolactici* at doses of 8%. According to sensory assessment, shelf life of fermented fish was found as 5 week for control, 6 week for LP, PA, LALP and LAPA, and 7 week for THPA and THLP. THPA and THLP had the lowest TBA values than other groups. Initial TVB-N value was 17.48 mg/100 g and

remained below 35 mg/100 g until 7<sup>th</sup> weeks for all groups. The lowest bacterial growth was observed in THPA and THLP group during storage periods. At the point of sensory rejection, total psychrophilic counts of control, LP, PA, LALP, LAPA, THLP and THPA were 5.92, 6.48, 6.42, 6.06, 6.23, 6.10 and 6.0 log cfu/g, respectively. At the beginning of storage, the highest lactic acid bacteria count was observed for control group, although LP and PA group had the highest lactic acid bacteria count towards the end of storage. *Enterobacteriaceae* slightly increased with storage time and the highest bacterial growth was found for control (3.68 log cfu/g), LP (3.60 log cfu/g), PA (3.51 log cfu/g) and LALP (3.51 log cfu/g) group. The study result revealed that the use of cell free extract of *Lb. plantarum* and *Ped. acidolactici* in combination with thyme extract was the most effective way to improve shelf life of fermented fish.

## ANTIMICROBIAL AND ANTIOXIDANT EFFECTS OF NANOEMULSIONS BASED ON HERB OILS (ROSEMARY, LAUREL, THYME AND SAGE) ON THE QUALITY OF RAINBOW TROUT

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Nanotechnology can be applied to all areas of food science such as food processing, packaging, safety, nutrition and nutraceuticals. Nanoemulsions are gaining popularity and also known as ideal carrier for the delivery of lipophilic substances because of ease of preparation, small particle size, their enhanced bioavailability, bioefficacy and kinetic stability. Nanoemulsions are also regarded as self-preserving antimicrobials due to bound water in their structure and thus no available water to microorganisms.

Essential oils (EOs) are plants secondary metabolites, which have been used in medicine, cosmetic, and food industry. The use of EO as aroma and flavor ingredients has increased recently because of the growing consumer demand for natural products and the replacement of synthetic additives in the food industry. As per our knowledge, there is no report on the preservation of fish with nanoemulsion based on herb EOs. Hence, the proposed research evaluates the effects of nanoemulsions based on EOs

(rosemary, laurel, thyme and sage) on the quality of cultured rainbow trout as fish preservatives. This project was funded by the Scientific and Technological Research Council of Turkey (TÜBİTAK, TOVAG-2130283).

Nanoemulsions were prepared according to Joe et al. (1999) with minor modification. Physical properties of nanoemulsions were analyzed in terms of viscosity, particle size of droplets, thermodynamic stability, refractive index, and surface tension. Fish fillets were immersed for 3 min in the nanoemulsions prepared with different oils. During 24 days of storage, sensory (raw and cooked), chemical (TBA (thiobarbituric acid), pH, TVB-N (total volatile basic nitrogen), PV (peroxide value), FFA (free fatty acids)) and microbiological quality (total mesophilic aerobic and psychrophilic aerobic bacteria, *Enterobacteriaceae* count) of rainbow trout fillets in ice at  $2\pm 2$  °C were carried out. Nanoemulsions of essential oils enhanced organoleptic quality of rainbow trout. Shelf life of rainbow trout was found as 14 days

for the control, 17 days for the treatment groups. The use of nanoemulsions based on all essential oils reduced the values of the biochemical parameters, especially in rosemary and thyme groups. The lowest bac-

terial numbers were obtained from rosemary followed by thyme group. Among the essential oils, it can be concluded that rosemary and thyme can be recommended for nanoemulsion as a preservative for fish fillets.

## ORGANIC ACIDS (LACTIC, ACETIC, SUCCINIC, PROPIONIC, FORMIC AND BUTYRIC) PRODUCTION BY LACTIC ACID BACTERIA IN VARIOUS FISH INFUSION BROTH

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Lactic acid bacteria (LAB) is the most important group of microorganisms used in fermented foods. They have vital importance because of their physiological features including substrate utilization, metabolic capabilities and probiotic properties. Discovery of organic acids formation by LAB has great significance to the food industry since the most suitable starter cultures have ability to improve food quality and safety. LAB are selected according to their technological characteristics such as their effectiveness in lactic acid production and pH reduction ability, as well as antimicrobial activity against food-borne pathogens.

A very limited number of research have been performed on organic acid production by LAB strains. Thus, production of lactic, acetic, succinic, propionic, formic and butyric acid by LAB was investigated in various fish infusion (anchovy, sea bass, tilapia and trout) and MRS broth (control) by using HPLC method in order to find out

the ability of organic acid formation by LAB strains.

Significant differences were found in organic acid levels ( $P < 0.005$ ) among bacterial strains. Succinic acid formation was the highest by LAB although acetic acid was produced at the lowest levels. The highest lactic acid production was observed with *Lb. lactis* subsp. *lactis* and *Pc. acidilactici* in anchovy infusion broth, with values of 2403 and 2345 mg/L, respectively. Acetic acid production was 822 and 803 mg/L by *Lb. acidophilus* and *Lb. delbrueckii* subsp. *lactis* in anchovy infusion broth, respectively while succinic acid production ranged from 142 mg/L to 9231 mg/L. Propionic acid formation by *Pc. acidilactici* was 3747 mg/L in sea bass infusion broth whereas *Lb. lactis* subsp. *cremoris* produced less than that in MRS broth (2344 mg/L) and butyric acid production was above 1000 mg/L in MRS broth by *Lb. acidophilus*.

The present study result indicated that organic acid production varied



significantly depending on LAB strains and growth medium. This specified that fish infusion broth play crucial role in organic acid production. In fact, LAB strains tested were very promising sources for novel products and

applications to provide food safety due to their high ability to form organic acids.

*Keywords: Lactic acid bacteria, organic acids, HPLC, fish infusion broths, MRS broth*

## INHIBITION IMPACTS OF ORGANIC ACIDS (LACTIC, FUMARIC, SUCCINIC AND ORTO-PHOSPHORIC) ON BIOGENIC AMINES FORMATION BY SPOILAGE BACTERIA AND COMMON FOOD-BORNE PATHOGENS

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The effects of lactic, fumaric, succinic and orto-phosphoric acids at level of 0.5% on ammonia (AMN) and biogenic amines (BAs) production by seven reference strains (*Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603, *Campylobacter jejuni* ATCC 33560, *Yersinia enterocolitica* NCTC 11175, *Salmonella Paratyphi A* NCTC13 and five bacteria isolated from spoiled fish (*Enterobacter cloacae*, *Proteus mirabilis*, *Vibrio vulnificus*, *Photobacterium damsela* and *Pseudomonas luteola*) were investigated using HPLC method in histidine decarboxylase broth.

Significant differences were observed among the reference strains and fish spoilage bacteria in terms of ammonia (AMN) and BAs production ( $p < 0.05$ ). All strains tested showed amino acid decarboxylase activity. AMN produc-

tion by bacterial strains ranged from 7.13 mg/mL to 78.29 mg/mL (*P. luteola*). The highest histamine production was observed by *C. jejuni* (8.62 mg/L) and *P. damsela* (6.13 mg/L). Presence of lactic acid in histidine decarboxylase broth significantly increased histamine production by *C. jejuni*, although significant inhibition in histamine production was observed with fumaric acid addition. *P. luteola* did not have ability to produce histamine in the presence of orto-phosphoric acid. Putrescine and cadaverine production by *P. damsela* was the highest (17.80 vs. 13.58 mg/L) compared to other bacteria. Tyramine production by bacteria lower than 9 mg/L. Succinic acid generally induced lower tyramine accumulation in histidine decarboxylase broth. *Y. enterocolitica* and *P. damsela* produced 98.90 and 24.64 mg/L of serotonin, respectively.

Organic acids considerably reduced serotonin production by these bacteria. TMA was one of the main amine produced bacteria, with ranging value of 1.89 mg/L and 160 mg/L. TMA accumulation was the lowest by *E. cloacea* and *K. pneumoniae* in the presence of succinic acid and by *P. luteola* in the presence of lactic acid.

The result of the study showed that the effect of organic acids on AMN and BAs production by references strains and fish spoilage bacteria in histidine decarboxylase broth varied depending on organic acid used, specific amine and bacterial strains.

## THE EFFECTS OF ORGANIC ACIDS ON BIOGENIC AMINE PRODUCTION BY FISH SPOILAGE AND FOOD BORNE PATHOGENS IN TYROSINE DECARBOXYLASE BROTH

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The impact of lactic, fumaric, succinic and ortho-phosphoric acids at level of 0.5% on ammonia (AMN) and biogenic amines (BAs) formation by seven reference strains (*Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603, *Campylobacter jejuni* ATCC 33560, *Yersinia enterocolitica* NCTC 11175, *Salmonella Paratyphi A* NCTC13 and five bacteria isolated from spoiled fish (*Enterobacter cloacae*, *Proteus mirabilis*, *Vibrio vulnificus*, *Photobacterium damsela* and *Pseudomonas luteola*) were studied using in tyrosine decarboxylase broth (TDB).

All tested bacterial strains were capable of decarboxylating more than one amino acid. Significant differences ( $P < 0.005$ ) were observed in AMN and BA production among all strains in TDB. All bacteria tested produced more than 3.97 mg/L of AMN. The highest AMN production was observed by *Phot. damsela* (32.30 mg/L) in the presence of fumaric acid. The most produced amines by bacteria were tyramine, dopamine, agmatine and serotonin. Histamine production by bacteria was generally below 10 mg/L, although *Pro. mirabilis* produced histamine at the highest concentration (28.89 mg/L) in

the presence of fumaric acid. Tyramine production by *Kleb. pneumoniae*, *Camp. jejuni* and *Vib. vulnificus* was significantly inhibited by addition of organic acids. However, significant increases in tyramine production were observed by *S. Paratyphi A* and *Yer. enterocolitica* in the presence of lactic acid and ortho-phosphoric acid and by *Phot. damsela* and *Ent. cloacea* in the presence of succinic acid. Putrescine and cadaverine production by fish spoilage bacteria were below 43 and 32 mg/L, respectively. The highest concentration of dopamine (DOP) was produced by *Pseu. luteola* which was 159.80 mg/L while agmatine (AGM) was 82.64 mg/L for *S. Paratyphi A*. Organic acids significantly suppressed dopamine production by *Pseu. luteola*, *Prot. mirabilis*, *S. Paratyphi A*, whereas ortho-phosphoric acid and lactic acid was generally reduced agmatine production by bacteria.

The result of the study showed that the effect of organic acids on AMN and BAs production by reference strains and fish spoilage bacteria in TDB varied depending organic acids used, on specific amine and bacterial strains.

## EXTRACTION AND CHARACTERIZATION OF MICROPLASTICS IN SEAFOOD: IDENTIFICATION OF A SUITABLE PROTOCOL AND APPLICATION TO FIELD STUDY

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Marine litter consists in 60 to 80% of plastics including microplastics, usually defined as particles with a size below 5 mm. Microplastics now constitute a major worldwide concern with physical and chemical threats relative to adsorbed persistent organic pollutants or plastic additives such as polybrominated diphenyl ethers (PBDE), bisphenol A or phthalates.

To date, international bodies recommend using standardized, effective and cost efficient analytical methods since microplastics studies implies sampling a large number of individuals. Nonetheless, protocols currently dedicated to microplastics isolation

are still facing limits such as particle sizes, polymer degradation or huge dwell times. The purpose of this work was to benchmark six existing protocols using acidic, alkaline, oxidizing or enzymatic solutions. Studies were focused on the impact of protocols on polymers and their efficiency digesting organic matter. A first step allowed selecting and slightly modifying the three most promising protocols after tests on six of the most produced plastics in the world. These three protocols were then applied to 15 different polymers and their impacts were assessed based on weighing, microscopic observations, identification by

pyrolysis coupled with GC/MS (Pyr-GC/MS) and Raman microspectrometry. Selection of suitable protocols, *i.e.* with no incidence on polymers, was performed by comparison of the results obtained before and after application of digestion methods. Finally, a single protocol using 10% KOH (60° C, 24h) was retained after tests performed on seafood tissues, consisting in evaluation of digestion efficiencies and spiking tests. Based on these re-

sults, a large scale study was launched on six species of fish and shellfish primarily consumed in France, to assess the prevalence of microplastics in their tissues. This study confirms the possibility of routinely use this method in the laboratory.

The selected protocol could now be proposed as an efficient and cost effective isolation process of microplastics from seafood tissues.

## HIGH PRESSURE AND ENZYME FACILITATING THE SHELL LOOSENING OF COLD-WATER SHRIMPS (*Pandalus borealis*)

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Shell-loosening before peeling is a particularly important step in the shrimp processing. The process can affect the yield, organoleptic quality and nutritious values of the finished products. From a processing viewpoint, the currently used brine and ice methods are concerning of quality-degrading and time-consuming issues since it may take up to 4-5 days to have the ready-to-peel shrimps. The emerging high pressure processing and the enzymatic hydrolysis are two alternative technologies used in this study in order to improve the shell-loosening process while still maintaining the quality of shrimps (*Pandalus borealis*). This study aimed at firstly investigating the protein changes in the epidermis and the shell of control and treated shrimps, secondly accelerating the shell-loosening process (improving the peelability) by the two above-mentioned technologies, and

lastly investigating the microstructural changes of shrimps subjected to high pressure and enzyme treatment. The protein changes were examined using 2D gel electrophoresis. The peelability was measured using a newly developed method based on the tensile-force test with particular fixtures and parameters. With light microscopy, fluorescence microscopy and transmission electron microscopy, the microstructural changes in the muscle-shell connection under the two shell-loosening technologies were compared with those of fresh shrimps. The findings obtained from this study provide new insights for developing the alternative and effective shell-loosening technologies.

## GELATION OF MECHANICALLY EXTRACTED RAW BLUE CRAB MEAT

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In North America, blue crab (*Callinectes sapidus*) meat is commercially available only in cooked, hand-picked form. Mechanical extraction of raw crab meat will increase meat yield and reduce production costs, but the lump texture is destroyed and thus less appealing. A process is thus needed to restructure the meat, without inducing yield loss, into a lump form suitable for typical blue crab application in foods. Microbial transglutaminase (MTGase), an enzyme typically used for protein-protein covalent cross-linking, was combined with high pressure processing (HPP; 300 MPa/10min/room temperature), to induce meat gelation (restructuring). Variables studied included additive levels (sodium chloride, tetrasodium pyrophosphate, egg white, sucrose, MTGase), low temperature pre-incubation ('setting': 40°C/40min), and heating (90 °C/30min). Breaking force (BF) and breaking deformation (BD) were determined as the most representative parameters of a gel quality.

It was observed that, for raw gels, either HHP or setting alone enhanced BF&BD as compared to gels without any treatment; but these two treatments used in combination resulted in gels with significantly higher breaking force (strength) and deformability. For cooked gels, there was a similar trend in BF as for the raw gels, though BD was lower than in raw gels and did not exhibit significant differences among cooked samples.

The results clearly demonstrated that, by using optimized combinations of these treatments, mechanically extracted raw blue crab meat can be successfully restructured into the more commercially acceptable product form of either raw or cooked lumps.



## EFFECT OF HIGH-PRESSURE TREATMENT ON BIOCHEMICAL CHANGES IN SARDINE (*Sardine pilchardus*) DURING A SUBSEQUENT 9-MONTH FROZEN STORAGE

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High-hydrostatic pressure technology has shown to retain the sensory and nutritional properties of food while inactivating microbial populations and endogenous enzyme activity. However, the employment of relatively strong high pressure (HP) conditions has been reported to produce lipid oxidation and protein breakdown when marine species are encountered. Sardine (*Sardina pilchardus*) is a small pelagic fish species captured and consumed in large amounts in South-European and North-African countries. One of the factors limiting its commercial use is the difficulty of its preservation under frozen conditions. The present research focuses on the quality retention of sardine under frozen conditions (-18 °C for 9 months). In it, the effect of a previous HP treatment (pressure levels: 125, 150, 175 and 200 MPa; pressure holding time: 0 min) on different biochem-

ical constituents was tested. Inhibition ( $p < 0.05$ ) of lipid hydrolysis development (lower free fatty acid formation and lipase activity) could be observed in frozen fish subjected to previous HP treatment, this effect being stronger with increasing pressure applied. According to the different lipid oxidation indices (peroxides, thiobarbituric acid, fluorescence and polyenes), previous HP conditions applied did not show a definite effect on the lipid oxidation development ( $p > 0.05$ ). Additionally, HP treatment did not lead ( $p > 0.05$ ) to a substantial modification of the activity of acid phosphatase and cathepsins B and D, as well as on the electrophoretic patterns of sarcoplasmic and myofibrillar protein fractions. However, HP processing led to a decrease in myofibrillar proteins content in frozen fish, this effect being higher in samples corresponding to the 175- and 200-MPa treatments.

This research showed that employment of HP conditions in the 125-200 MPa range results in minor biochemical modifications throughout a further 9-month frozen storage. Results of the present study indicate the need

of HP conditions optimisation, particularly when combined with freezing, frozen storage and thawing to ensure a higher quality retention and thus the commercial acceptability of the frozen product.

## EFFECT OF HIGH PRESSURE PROCESSING IN REDUCING *PHOTOBACTERIUM PHOSPHOREUM* AND HISTAMINE FORMATION IN MARINATED HERRING

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Histamine can be formed in seafood during various steps between catch and consumption. This is important as products containing 50–100 mg/kg or more are considered toxic and likely to cause histamine fish poisoning (HFP). HFP is a mild disease with allergy-like symptoms including rash, nausea, headache and sometimes diarrhea. Histamine is produced by the decarboxylation of free histidine, and results in the activation of either endogenous or bacterial histidine decarboxylase. *Photobacterium phosphoreum* is known to be a psychrotrophic and halophilic histamine producing bacteria, which is may be commonly found in the marine environment. Histamine formation by *P. phosphoreum* cannot be prevented by chilling of seafood to 0–5°C alone. With the increasing demand for minimally processed foods, non-thermal processing has become an alternative to conventional methods. High pressure processing (HPP) is one of the non-thermal technologies used in the preservation of many food products since it has the capacity to inactivate product-spoiling microorganisms at low temperatures. The objective of this

study was to investigate the inhibitory effects of HPP (100, 300 and 500 MPa for 5 min) on the growth of *P. phosphoreum* and histamine formation in marinated herring during refrigerated storage for 3 months. At the beginning of the storage, viable cell counts were 2.64 and 2.56 log CFU/g in control and 100 MPa pressure treated group, respectively, whereas bacteria population exceeded 7 log CFU/g in these groups at the end of the storage. No bacteria growth was observed in 300 MPa and 500 MPa pressure treated groups until 45<sup>th</sup> and 60<sup>th</sup> day of storage, respectively. Besides viable cell counts did not reach limit value during the storage period in both 300 MPa and 500 MPa treated groups. HPP treatment (300 MPa and 500 MPa) significantly reduced the histamine formation. While histamine level reached 116.41 mg/kg at 60<sup>th</sup> day of storage in control, this level remained under 50 mg/kg in both 300 MPa and 500 MPa treated groups throughout the storage. According the results, growth of *P. phosphoreum* can be inactivated and histamine formation can be reduced at pressure 300 MPa and above in marinated herring.

## CONTENT OF MINOR COMPONENTS IN FISH OILS OF DIFFERENT FISH SPECIES AND PROCESSING TECHNOLOGIES

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N-3 polyunsaturated fatty acids (n-3 PUFA), in particular EPA and DHA, are mainly responsible for the health benefits resulting from fish consumption. Encapsulated fish oil or n-3 PUFA concentrates produced from fish oils are convenient alternatives for a desirable increased intake of these essential minor components. For producing fish oil intended for human consumption, essential processing steps including oil extraction, refining, purification, and enrichment are usually applied. Refining of crude fish oil is often necessary to remove environmental contaminants such as dibenzo-p-dioxins, dibenzofurans (PCDD/Fs), dioxin-like polychlorinated biphenyls (dl-PCBs) and non-dioxin-like PCBs (ndl-PCBs) in order to comply with legal limits and to enhance shelf-life. However, during the deodorization of fish oil, process contaminants such as 2-monochloropropane-1,3-diol (2-MCPD) esters, 3-monochloropropane-1,2-diol (3-MCPD) esters and glycidyl esters may be formed.

The aim of the present study was to provide a comprehensive overview on the content of environmental and process contaminants in fish oils derived from different fish species treated with different processing technologies. Furthermore, concentrations of EPA, DHA and n-3 PUFA have been analyzed in different fish oil products.

The contents of PCDD/Fs, dl-PCBs and ndl-PCBs are well below the legal limits for food grade fish oil in analyzed crude fish oils from farmed fish, but not in fish oils originating from captured fish. A reduction in the amount of PCDD/F and dl-PCBs could be achieved by filtration with active carbon. 2- and 3-MCPD ester and glycidyl ester contents are considerably higher in the investigated refined fish oils compared to the crude fish oils. Additionally, the analyzed and labelled concentrations of EPA, DHA and n-3 PUFA values showed satisfying compliance, not confirming frequent deviations from labelled concentrations as stated in earlier reports.

## SHELF LIFE OF SALT-CURED COD (*Gadus morhua* L.) STORED AT ELEVATED TEMPERATURES

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Dried salt-cured cod is a commercially important product for Norway and it is exported to markets in Latin America, Southern Europe and Africa. From Norway, the fish is usually exported as split. Once arriving at destination market, the fish is normally cut followed by package in polystyrene boxes and finally wrapped with a plastic film. These products are more consumer friendly, which fit better with the logistics of retail and of the modern family life. Some markets of this product do have not adequate refrigeration facilities which may result in red discoloration due to growth of extreme halophiles. According to Codex Alimentarius, salt-cured fish with red discoloration is considered as defective and it cannot be sold.

In our work, we have studied the shelf life of packaged dried salt-cured cod, i.e. the number of days until the product obtain a red discoloration, during storage at 25, 30 and 35 °C. At all temperatures, the product have been stored at 60 and 80% relative humidity (RH). In addition, products with different water content were included.

Temperature and humidity have been selected based on relevant storage conditions for this product.

The results show that shelf life varies by temperature, RH, water content and the initial level of extreme halophiles. In general, products stored at 60% RH had a longer shelf life compared to corresponding products stored at 80% RH, and storage at 25 °C revealed a longer shelf life than storage at 30 and 35 °C.

A majority of dried salt-cured products are sold and consumed seasonally, implying a generally short time span from buying and selling. In Brazil, 60-70% of the annual sale occur during Easter, Christmas and New Year. Products not sold in these peak seasons may need to be stored for a prolonged period of time, and thereby risking a red discoloration. Having a label showing the exact shelf life of the product will be informative to the consumers and thereby reduce the risk of complains due to red discoloration.

## INVESTIGATION OF FLESH QUALITY AND SHELF LIFE AT DIFFERENT STORAGE CONDITIONS OF EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX*, FED WITH DIETS INCLUDED NATURAL AND SYNTHETIC ADDITIVES

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This study was carried out to examine the shelf life and fillet quality of sea bass fed with diets which are included grape seed extract (GSE) and Vitamin E. In the study, fish were fed with 4 experimental diets (Control, GSE, Vitamin E and mixture of GSE - Vitamin E) for 18 weeks. There were no significant differences observed between the growth parameters of fish fed with experimental diets ( $P>0.05$ ). In the feeding trial in order to evaluate the relationship between the experimental diets and the growth performance of fish economically, economic conversion and economic profitability ratios were calculated due to reveal the most cost-effective and proper diet used in this trial. After the feeding trial, fish were harvested and stored at 4°C in ice. Samples were taken at day 0, 7, 14 and 21. In the storage trial, biochemical (crude protein, crude lipid, humidity and ash), chemical (TVB-N, TBA, pH, fatty acid profile) colour change and microbiological analyses were performed. Results from

analyses indicated that fish were fed with GSE included diet the exceeded the upper limit for microbiological counts later than the others. The lowest TVB-N value was detected from GSE extract when the upper limit for microbiological count exceeded (14<sup>th</sup> day of storage). The consumable levels for TBA never exceeded throughout the storage period. However, there were observed increases for TBA when the upper limit for microbiological count exceeded (14<sup>th</sup> day of storage). For the colour change,  $L^*$  value of the groups showed increases entire storage periods. Overall, GSE extract was overcoasting for culture conditions than the other diets, in the storage trial better results were obtained from this diet group than the others.

## NOVEL MICROPLATE-BASED SPECTROPHOTOMETRIC ASSAY ALLOWING QUANTITATIVE MEASUREMENT OF DIETARY NUCLEOTIDES IMP, INOSINE AND HYPOXANTHINE IN AQUAFEED SAMPLES

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Nucleotides participate in nearly all biochemical processes important for animal growth providing the cells with the energy (ATP), metabolic enzyme cofactors (NAD<sup>+</sup>, FAD, and CoA) and building blocks for nucleic acids synthesis. The supplementation of feed with dietary nucleotides was shown to accelerate intestinal recovery and to enhance immunity and resistance to bacterial infections thus diminishing the needs in antibiotics

Fish muscle is naturally rich in ATP that is a second abundant muscle metabolite after amino acids. Muscle-derived products, fishmeal and protein hydrolysates, are excellent natural sources of dietary nucleotides inosine 5'-monophosphate (IMP), inosine and hypoxanthine, formed post-mortem through ATP catabolism. Inosine and IMP are also known as feeding stimulants useful to increase conversion of diet into live fish and to minimize the waste.

The content of IMP, inosine and hypoxanthine in muscle-derived products depends on the freshness of raw

material. In particular, hypoxanthine, last of three ATP catabolites adsorbed by enterocytes, hepatocytes and immune cells, can be rapidly destroyed by autolytic and microbial xanthine oxidase to uric acid, metabolic waste.

The enzymatic reaction leading to NADH formation from IMP, inosine and hypoxanthine was investigated for the first time and suggested as a novel tool for nucleotide quantification. This reaction was employed for the development of a novel microplate-based spectrophotometric assay for nucleotide determination fish muscles, fishmeal and protein hydrolysates. The proposed assay was carried out in a 96-microwell plates and the absorbance was measured at 340 nm by microwell-plate absorbance reader. The optimum conditions of nucleotides extraction and enzymatic reactions were established. Under these conditions, linear relationship with good correlation coefficient was found between the absorbance and the concentration of IMP ( $R^2 = 0.9976$ ), inosine ( $R^2 = 0.997$ ) and hy-

poanthine ( $R^2= 0.9989$ ) in the range of 0.25-50mg per mL. No interference was observed from other compounds present in water extract of fish muscle, fishmeal, protein hydrolysates or formulated aquafeeds. Accuracy of the assay assessed by measuring the mean analytical recovery was found to be 97.4 %, 91.6% and 96.8% (IMP,

inosine and hypoxanthine, respectively). Since the assay does not require organic solvents nor toxic compounds and can be performed with cuvette spectrophotometer, it has, therefore, great practical value not only for quality control laboratories but also for on-site quality control.



## LOW-FIELD NUCLEAR MAGNETIC RESONANCE (LF-NMR) RELAXOMETRY TO MONITOR QUALITY CHANGES IN READY-TO-EAT CRAB MEAT (*Cancer pagurus*) AS AFFECTED BY COOKING METHOD AND STORAGE

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The manufacturing process of ready-to-eat (RTE) muscle meat from European edible crab (*Cancer pagurus*) includes cooking, picking and an eventual in-pack pasteurisation before refrigerated storage. Current cooking practices at industrial level generally involve a severe heat treatment where crabs or crab claws are immersed in boiling salted water for a variable period of time. The application of milder cooking temperatures in conjunction with novel technologies (e.g. ultrasound-assisted cooking) may help lessen the risk of overcooking thus resulting in a higher quality product. In muscle meat, quality changes due to different processing are often related to variations in myofibrillar water distribution which can be determined by LF-NMR relaxometry, a quick and highly sensitive method applicable to the intact muscle. The technique uses proton transverse relaxation time ( $T_2$ ) decay to measure myowater mobility

which, in turn, can be linked to the texture and visual quality of the muscle. The aim of the present study was to examine the use of LF-NMR relaxometry to characterise water distributions in RTE crab muscle meat and determine how these are affected by different cooking process and storage conditions. Crab claws, from male exemplars, were cooked at (i) 97°C for 15 min, (ii) 75°C for 18 min, and (iii) 75°C for 15 min in an ultrasound-assisted process (ratio power/crab weight 180 W·kg<sup>-1</sup>). LF-NMR  $T_2$  relaxation measurements were conducted on the white meat after processing and at defined intervals during refrigerated storage in vacuum pouches. Continuous distribution of relaxation times showed the presence of four water populations in cooked crab meat centred at 2-15 ms ( $T_{2b}$ ), 35-47 ms ( $T_{21}$ ), 115-270 ms ( $T_{22}$ ), and 300-750 ms ( $T_{22b}$ ). The  $T_{21}$  proton population, generally associated with intra-myofibrillar water, accounted for

the majority of the total signal and its amplitude was significantly ( $p < 0.05$ ) lower in samples cooked at 97°C for 15 min compared to their mildly processed counterparts. A shift of the  $T_{22}$  component towards slower relaxation times was observed during

storage, suggesting increasing mobility of extra-myofibrillar water. The results indicate the potential of LF-NMR measurements to monitor quality of white crab claw meat cooked using both conventional and novel assisted methods.

## EFFECTS OF FOOD DEPRIVATION ON QUALITY ASPECTS OF FILLET PRODUCTS FROM LIVE STORED COD (*Gadus morhua* L.)

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The migration pattern of northeast Atlantic cod (*Gadus morhua* L.) makes the Norwegian coastal cod fisheries highly seasonal with the main supplies of fresh cod from January to April. The concept of live storage of wild cod is therefore becoming constantly more evident in Norway. The cod can be kept alive in sea cages up to 12 weeks after capture, whereas the first four weeks the fish can be legally stored without feeding. The aim of the present study was to investigate whether a long-term food deprivation period affects the pattern of *rigor mortis*, contraction and weight loss of fillets, loins and tails.

The fish were caught in Andenes (Norway) in March 2015 using Danish seine and stored alive without feeding for 82 days. The fish were sampled 2, 26, 54 and 82 days after capture. At each sampling, body weight and length as well as weight of liver and gonads were recorded. Development of *rigor mortis*, measured as muscle hardness, was registered during the first 120 hours after slaughter. Addi-

tionally, the fish were filleted from 4 to 48 hours after slaughtering, and quality assessments of fillet products were performed after 7 days ice storage.

As starvation proceeded, the duration of the pre rigor phase was reduced; 24 hrs after slaughter, the muscle hardness of non-starved fish (73.6 g/cm<sup>2</sup>) was significantly ( $p < 0.05$ ) lower compared to fish exposed to starvation for 82 days (248.2 g/cm<sup>2</sup>). The contraction of the fillets decreased with prolonged starvation period, while the weight loss was relatively stable. All fillets produced *pre rigor* were shorter and had slightly higher drip loss in contrast to their counterparts produced in *rigor*. The drip loss in tails was significantly higher compared to fillets and loins independent of nutritional status and filleting time.

## A NEW INDUSTRIAL DESIGN FOR THE PRODUCTION OF CANNED TUNA: EFFECTS ON LIPID QUALITY AFTER PROCESSING

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Thermal processing during canning is based on applying thermal treatment at required temperature and time to destruct pathogenic microorganisms and enzymes. For microbial safety, extended application of high temperatures causes certain changes in physical, chemical and sensory properties of canned products resulting in increase the production costs. While the extended heat treatment in canned fish destructs the unsaturated fatty acids of (EPA and DHA), amount of free fatty acids (FFA) and PV (peroxide value) increase with other possible quality degradation. Therefore, the objective of this study was to increase the quality of canned tuna by applying an innovative approach during the canning process by modifying the can geometry.

For this purpose, 160 and 1705 g standard can geometries were modified into a toroidal shape, and sterilization times were determined to reduce the number of *C. botulinum* spores from  $10^{12}$  to  $10^0$  (for sterilization val-

ue of  $F_0=12 \times 0.21=2.52 \text{ min}$ ) by using mathematical approaches. The results were validated with experimental temperature data, and lipid quality control tests (fatty acid composition, free fatty acids and TBA) were carried out after processing to compare results with standard can geometries.

Besides the reduction in the process time (more than 30% compared to the industrially applied process time) without compromising the process safety, notable increases in lipid quality were obtained. Lower sterilization time prevented the further increase of free fatty acids (FFA) and PV (peroxide value) compared to the standard can sizes.

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## EFFECT OF TRANSGLUTAMINASE COATING ON THE FORMATION OF BIOGENIC AMINES IN MACKEREL FILLETS

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Biogenic amines are produced by decarboxylation of specific amino acids. Seafood products contain high protein and the amount of biogenic amine are generally increase during storage with enzymatic and microbiological activity. The presence of biogenic amine can create a health risk in the members of Scombridae family such as mackerel, bonito, and tuna fish due to containing high levels of free amino acids. Microbial transglutaminase (MTGase) is an enzyme utilized in the food industry in many areas. The amino acids are cross-linked to the proteins in the presence of MTGase, instead of decarboxylation. Since MTGase promotes the polymerization of proteins via amino acids, and the formation of biogenic amines can be suppressed. In this study, the effect of microbial transglutaminase (MTGase) at various levels (1, 4, 7 and 10 units) on the formation of biogenic amines of mackerel (*Scomber scombrus*, Linnaeus, 1758) fillets were investigated during refrigerated storage ( $4\pm 2^{\circ}\text{C}$ ) for 8 days. Control and 4 unit MTGase treated samples had the highest putres-

cine levels as  $42.14\pm 1.51$  mg/kg and  $38.09\pm 0.01$  mg/kg, respectively. Comparing of cadaverine levels 1 unit MTGase treated samples had the lowest cadaverine content. Group of control and 4 unit MTGase treated samples had the highest histamine contents and the values were  $3.60\pm 0.18$  mg/kg and  $2.82\pm 0.14$  mg/kg, respectively. Tyramine was not detected during the storage. Spermine and spermidine contents of groups decreased during the storage. The content of biogenic amines increased sharply in control samples. It can be considered that 7 unit MTGase treated samples were more effective in suppressing the formation of biogenic amines in mackerel fillets comparing to other MTGase treatments and control samples. As a result of the study, a new usage of MTGase will reveal in the food industry.

## FISH TRADE AND CONSUMER TRENDS IN EUROPE

**Ekaterina Tribilustova**

### INVITED SPEAKER

*Eurofish International Organisation*

With over 510 million inhabitants, the EU represents the largest market for fisheries and aquaculture products in the world with an average annual consumption of 25 kg per capita. During the past years, consumption of fisheries and aquaculture products in volume terms been decreased since 2008, while household expenditures on those products kept the upward trend. In 2015, the total trade flow of fisheries and aquaculture products in the EU reached EUR 49.4 billion and 13.8 million tonnes. In 2005-2015, the value of the EU-28 trade flow increased 61%, whereas it grew 11% in volume terms. At present, consumer trends for fisheries and aquaculture products represent an interesting mix of established and new trends which sometimes contradict and transform into the challenge for consumer purchasing decisions. Over the past years, EU consumers bought less fishers and aquaculture products, but they had to spend more for it due to increased prices, which consequently reflected in the change of consumption patterns.

Health-conscious attitude in choosing products became the foremost trend in

the past year since millions more consumers were encouraged to eat healthier. The concept of “natural”, “GMO and additives-free”, and “ecologic products” does not only indicate production, but also preparation and supply. In particular, the supply system supporting the “Km zero” concept” became adopted by some of the leading retail groups which preferred to buy fisheries and aquaculture products locally in the different fish markets, instead of having centralized suppliers.

An increasing number of European consumers use technology to save time and buy fishery and aquaculture products, although traditional sales channels will continue to be the principle places for purchasing fish and seafood. However, almost 80% of the EU population were estimated as internet users in the end of 2015, and several studies showed that more consumers were willing to use technology to improve their diet with smartphone apps as well as proliferating order-and-pay apps to make purchases and “dining in” easier.

## BIOACTIVE PROPERTIES OF FISH PROTEIN HYDROLYSATES PRODUCED WITH SEAWEED EXTRACT RELATED TO SKIN HEALTH

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Studies indicate that Fish Protein Hydrolysates (FPH) possess interesting bioactive properties and that quality problems due to lipid oxidation can be tackled by using antioxidants during processing. Seaweed Extract (SE) has shown to both inhibit oxidation during processing and contribute to the bioactivity of the final product. However, the shelf life of FPH produced with antioxidants has not been extensively investigated. For past years, focus has been on investigating bioactive properties of FPH by ingestion, whereas their biological effect on skin has not been widely explored although bioactive peptides are one of the most interesting components in skin care products. In this study FPH were produced on industrial scale by Iceprotein with the following antioxidants; L-ascorbic acid,  $\alpha$ -tocopherol, and with or without Seaweed Extract

(SE) for comparison. To evaluate the shelf life of the FPH, they were stored in -80°C, 4°C and 22°C for 12 months and quality assessment was done by measuring the Thiobarbituric acid (TBA) formation during storage and with sensory analysis. The following bioactive properties of FPH were evaluated; antioxidant activity, effect on collagen maintenance (elastase and collagenase inhibition) and anti-inflammatory properties. According to results of quality assessment (TBA and sensory analysis) there was no significant difference between samples. The TBA was slightly higher for samples stored at 22°C for 12 months than other samples but no rancidity was detected in sensory analysis in any sample after 12 months of storage. Results indicate that both versions of FPH possess antioxidant activity. FPH with SE had significantly stron-

ger anti-inflammatory properties than FPH without SE. FPH with SE showed ability to maintain collagen (elastase and collagenase inhibition) whereas FPH without SE did not. Results indicate that SE boosts the properties of the FPH which is potentially due to a synergistic effect between these two

bioactives. A skin care product was prepared with and without FPH with SE for consumer testing. Generally, consumers considered the product including FPH with SE to increase the softness and glowing of their skin as compared to the product excluding FPH with SE.



## A PROPOSED TOOLBOX FOR FOOD QUALITY ANALYSIS OF HEAT PROCESSED MACROALGAE: COLOUR, TEXTURE AND MICROBIOLOGY

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Macroalgae are getting increasingly popular as a food component in Europe. The bright green colour and desired taste (umami) that is typical for e.g. *Laminaria digitata*, makes it suitable for salads, soups, pestos and sushi. Macroalgae sensory parameters (colour, texture and taste) are highly influenced by heating.

Here, we propose practical and rapid methods to analyse these parameters, as well as microbial quality, as an effect of thermal treatment. The methods were developed using commercially grown brown algae; winged kelp (*Alaria esculenta*) and sweet kelp (*Saccharina latissima*), that were vacuum packaged at harvesting and stored frozen. A standardized protocol for Digieye-based colour analysis is proposed and was used to study colour changes at 88 combinations of time and temperature up to 60 minutes at temperatures up to 100 °C. Based on this, a colour chart for use in the field and by the industry was also proposed for rapid testing. The elasticity modulus (E-modulus) and the notch

sensitivity were used as parameters for texture measurements analogous to a method frequently used for salad. Enthalpy changes observed by differential scanning calorimetry were discussed in relation to the quality changes observed.

Traditional microbiological methods based on culturing and plate counting were customized for macroalgae. Several culturing media and incubation conditions were tested. We propose that total aerobe colony count be performed using marine agar incubated at 25 °C for 7 days. Aerobe and anaerobe spore formers can be enumerated by spread plating on tryptic soy agar with 0.6% yeast extract (TSAYE) followed by incubation (aerobic and anaerobic) at 30 °C for 8 days. Cold tolerant bacteria may be analysed using Plate Count Agar amended with 1% NaCl incubated at 8 °C for 7 days.

The underlying mechanisms of the product quality changes were investigated and microbial activity was assessed in order to document sensory parameters and food safety of mac-

roalga food. The practical toolbox developed for the food quality analysis of macroalgae may contribute to stan-

dardization of protocols and facilitate industrial thermal processing design for the macroalgae food industry.

## PRELIMINARY STUDY ON THE UTILISATION OF NORWEGIAN RED SEA CUCUMBER, *Parastichopus tremulus* (Gunnerus, 1767) (Holothuroidea, Echinodermata), FROM NORWEGIAN WATERS: RESOURCE, BIOLOGY AND MARKET

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The demand for imported sea cucumbers on the Asian market has increased during the last 15 years. Along the Norwegian coast both *Cucumaria frondosa* (Gunnerus, 1767) and *Parastichopus tremulus* (Gunnerus, 1767) (Norwegian red sea cucumber) could be of interest for the Asian market. In this study we focus on *P. tremulus* and present information on the value-chain (fishery, processing, and markets). Three test fisheries (pilot studies) on fishing and exporting *P. tremulus* to China were undertaken in 2000, 2003 and 2004. The results showed a highly varied and patchy distribution of this resource, and were followed up by subsequent research fisheries (scientific cruises). *P. tremulus* was recorded from Finnmark to Møre & Romsdal County, as well as from the Norwegian Deep and Skagerrak.

Trawling was conducted between depths of 50 to 500 m, with largest catches taken from depths of ~100-300 m. Production and marketing tests for *P. tremulus* showed that it has significant potential as a commercial product on the Chinese market. Chinese customers liked the properties of *P. tremulus*, such as its size, meat content, taste and nutritional content. Production procedures are good, but can be optimised. *P. tremulus* has a special colour and an unknown appearance. Therefore, long-term marketing efforts to launch the product in the high end market are required. There is a growing demand for both local and imported sea cucumber in China and the price level for sea cucumber is generally increasing. DNA barcoding on *P. tremulus* was also conducted.

## ATTITUDE AND PURCHASE INTENT OF DUTCH CONSUMERS FOR REFINED AND NON-REFINED PACIFIC CUPPED OYSTERS (*Crassostrea gigas*)

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Oyster refinement by using land-based pond systems is a new activity in the Dutch oyster sector and aims to promote growth and the sensorial properties of the oysters. In this study a multidisciplinary approach was chosen in order to evaluate the attitude and purchase intent of Dutch consumers for refined and non-refined Pacific cupped oysters (*Crassostrea gigas*).

Using questionnaires the most important parameters for the quality perception (e.g. meat content, shell shape, odour, taste, country of origin, cultivation method) by Dutch consumers (n=85) were evaluated. Next the importance of the production process (refinement or no refinement) amongst other drivers for consumer purchase intent on the willingness to buy and willingness to pay of Dutch consumers (n=56) were evaluated. Finally, the actual products, refined and non-refined oysters, were evaluated by Dutch consumers (n=72) using Qualitative Descriptive Analysis (QDA). The effect

of information about the production process was studied.

Results indicate that taste, texture and odour are the most important sensory quality characteristics of oysters for consumers. Country of origin and flavour profile showed significant effects on the willingness to buy and willingness to pay by Dutch consumers. Native oysters are preferred over non-native oysters by Dutch consumers. Including the attribute sweet in the flavour profile led to a higher preference by the consumers. Information about the production process had no effect on both the willingness to buy and willingness to pay. QDA results showed significant differences between refined and non-refined oysters for the attributes sweetness, saltiness and firmness. Information on the production process lowered the consumer perception of overall odour and sweetness of the refined oysters. Likewise overall appreciation of refined oysters increased when consumers were aware on the production process.

## THE FUTURE CONVENIENCE PRODUCT FROM THE NORWEGIAN SALT CURED AND CLIP FISH INDUSTRY

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Exporting fresh (not frozen) rehydrated products from salt cured and dried fish (clip fish) to the Portuguese and Brazilian markets is a challenge. The short shelf life of these products makes it almost impossible; still the Norwegian clip fish industry is going for it.

Clip fish is one of Norway's most recognizable export products, especially in "home markets" like Portugal and Brazil where clip fish has been enjoyed for centuries. However, times are changing. The younger generation is less willing to invest the time required to prepare traditional clip fish. The Norwegian clip fish industry has lost large market shares during the last five years to rehydrated and desalted clip fish products from low-wage countries such as China and Portugal. The industry is facing restructuring to meet this challenge. The Norwegian industry wishes to develop processes for producing rehydrated and desalted cod (CoPro) at an industrial scale using Norwegian raw materials, in Norway. Successful product development requires new competence along

the entire value chain. This effort has resulted in a new project: "The future convenience product from the Norwegian salt cured and clip fish industry".

The project mobilizes a multidisciplinary consortium of industry actors and research partners with the goal of producing sustainable, profitable CoPro that is competitive in current and future markets. There will be a focus on processes such as industrial desalting and innovative packaging technologies that protect the characteristic sensory parameters, while extending shelf life. Pilot scale testing will be performed in collaboration with technology suppliers tasked with developing innovative and scalable processes for automated production. The successful launch of a competitive CoPro product is the ultimate goal; however, the project will also generate new knowledge in seafood processing technology, with a focus on extending shelf life without compromising quality.

## INTRODUCTION OF NEW FISH PRODUCTS DERIVED FROM MACKEREL (*Scomber spp.*) IN CHILDREN'S SCHOOL MENUS THROUGH A SOCIAL, SUSTAINABLE AND ECONOMIC APPROACH

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Governments recognized school meals as an essential tool for the development and growth of children, communities, and society as a whole. Food served in schools and canteens must meet school food standards so that children have healthy and balanced diets, and this include providing high-quality oily fish. In the Basque Country (North of Spain), more than 92,000 menus are delivered each day at these centers and more than 17 million menus each year, thus representing a matter of concern for consumers (families), food industry, institutional caterers and Education Authorities.

Therefore the aim of this project was the introduction of new fish products derived from mackerel (*Scomber spp.*) in children's school menus, through a social, sustainable and economic approach, adding value to all levels of the food chain. The work done was focused on three main fields of interest:

I) Social dimension, through the promotion of consumption of healthy oily fish among children, increasing

the variety of locally sourced fish and introducing new product references (non fried-non coated) without bones and easy swallowing in school diets, following the recommendations on the improvement of school menus marked by the Department of Education of the Basque Government.

II) Sustainability of food chain, creating new food business opportunities for the fishing primary sector trough the incorporation of processing activities to the traditional ones and adding value to mackerel captures.

(III) Industrialization viability: Designing industrial processes from the fish flesh obtaining to mackerel precooked portion development at pilot and industrial scale, characterization of sensory and nutritional profile, analyzing their technical and economic feasibility and taking into account all partners (extractive sector, food processing industry, caterers) specificities and requirements (product price, supply, cooking method, portion size, etc.).

As a result, a complete commercialization chain has been developed for the introduction of a new safe and nutritious mackerel fish product, in the

school menus of the Basque Country contributing to the alignment of all industrial, government and consumer expectations and needs.

## ISOLATION OF PROTEINS FROM SWEDISH SEAWEEDS, *Porphyra umbilicalis*, *Ulva lactuca* AND *Saccharina latissima*, BY THE pH-SHIFT METHOD

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There is an increased demand for vegetable proteins all over the world. Some seaweed species here constitutes interesting raw materials and studies have shown that seaweed proteins contain all for humans essential amino acids. However the tough polysaccharide-rich cell wall and the abundance of polyphenols reduce the digestibility of the proteins. Further, total protein levels in most seaweed species are too low to directly use them as protein ingredients in feed and food. Therefore food grade, scalable and environmentally friendly techniques for isolating the proteins are desirable. Previous studies reporting isolation of proteins from seaweed have used solubilization in water or alkali and subsequent precipitation with ammonium sulfate followed by a time consuming dialysis step, which will not be feasible in a large scale. Isolation of proteins by the pH-shift process, i.e., acid or alkaline protein solubilization followed by isoelectric precipitation has successfully been applied to various fish muscle sources in larger scale and hence this could be a suitable method also for seaweed. Proteins in seaweeds however have different solubility optima and pre-

cipitation optima than fish muscle, and besides, are bound to cell wall constituents; hence the pH-shift technique must be optimized for this biomass.

The red, green and brown seaweed *Porphyra umbilicalis*, *Ulva lactuca* and *Saccharina latissima* are all found in wild along the Swedish west coast. However, currently cultivations of these three species are being developed to create the basis for a Swedish seaweed industry. The red and green seaweed are during this development subjected to different breeding techniques, with the goal to achieve larger cultivations and more nutrient-rich breeds.

The aim of this study, which is part of the *Seafarm* and *Sweaweed* projects, is to adapt a pH-shift process protocol for all these three seaweed species; which varies a lot in physiology and composition. The pH-dependent protein solubility is examined along with isolation of the proteins at the optimal precipitation pH. A pre-treatment step with distilled water, i.e. osmotic shocking, is also considered to make the proteins more accessible for isolation. The results from these trials will be presented and discussed.



## BARLEY VS. RED BEET: TWO CARBOHYDRATES SOURCES ON RAINBOW TROUT DIETS. EFFECT ON GROWTH PERFORMANCE AND QUALITY PARAMETERS OF FISH FILLETS

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Rainbow trout is a carnivorous fish which in the natural environment thrives on food practically devoid of carbohydrates (CHO) as also occurs in other carnivorous fish such as sea bass, eel, yellow tail and plaice. Although carbohydrates are the cheapest energy source, most teleost do not tolerate high dietary CHO level, and maximum dietary inclusion level is fish specie dependant. Under aquaculture conditions, it is important to provide appropriate levels of CHO in the diets to ensure efficient utilization of the other nutrients. It is generally considered that for marine and cold water species incorporation of digestible CHO should not exceed 20% of the diet. For rainbow trout data on the CHO sources, their utilization and effect on the quality of final products is still limited.

The aim of the present study is to compare the effect of two CHO sour-

es (barley and red beet) on rainbow trout diets on productive and quality parameters.

During 12 weeks rainbow trout were fed in a monitorised and controlled room using recirculation system at a temperature of 14-17°C and dissolved oxygen levels of 7-9mg/L. Fish were randomly allocated in 15 tanks with 60 fish per tank. Rainbow trout were fed 5 isoproteic and isolipidic diets (45 %CP and 18 %CF). Barley diets contained graded levels of barley (0-31,8 %). Red beet diets contained graded levels of red beet (0-28 %). During the experiment, productive and quality parameters were studied. Among the productive parameters, growth (Relative biomass increment (RBI), Specific growth rate (SGR), feed conversion ration (FCR) and feed intake (FI)), protein and fat retention efficiency and protein, fat and CHO digestibilities were determined. Quality parameters indi-

cators of freshness and organoleptic properties were monitored (colour, texture and sensory analysis).

Comparing the effect of barley and red beet on the productive parameters, no significant differences were observed on the growth performance, protein and fat retention efficiency and protein and fat digestibilities when barley was used. The use of red beet decreased significantly RBI, SGR and increased significantly FCR, while no significant differences were observed on the FI. A significant decrease on protein and fat retention efficiency was observed with the inclusion of red beet and no significant differences appeared on digestibilities of the different nutrients. When quality parameters were analysed, the colour of fish fillets were not significantly affected with the inclusion of barley, while the inclusion of red beet increased significantly luminosity ( $L^*$ )

and redness ( $a^*$ ) of fish fillets as it was seen in QDM analysis. The use of barley in rainbow trout diets increased significantly the gumminess of fish fillets till a concentration of 8 %, while increasing levels of red beet significantly increased elasticity of fish fillets. Freshness of fish fillets were analysed using QIM, the inclusion of 4 % of barley and increasing levels of red beet makes gills colour get redder. QDM analysis showed that the inclusion of barley increased the lightness of redness of fish fillets, in contrast of what has been seen with the colorimeter.

Barley could be used as an alternative ingredient in rainbow trout diets as no alter their growth parameters and red beet makes them a bit worse. On the other hand, both ingredients in different ways enhance quality parameters of fish fillets, increased redness and luminosity and different parameters of texture.

## NATURAL ELIMINATION OF CONTAMINANT METALS IN OYSTER *Crassostrea angulata* FROM SADO ESTUARY (PORTUGAL)

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Shellfish are recognized as a high quality food, greatly appreciated in Portugal. However, as a result of release to the environment, various substances from Human activity, these organisms can accumulate metals with potential impact on human health. Portuguese production areas of bivalve molluscs, in particular some estuaries, as Sado estuary, have shown over the years a high heterogeneity of chemical contamination and therefore it is highly relevant to obtain more detailed information in order to contribute to a better use and recovery of these marine resources. Thus, in order to assess the temporal evolution of metal contamination in oyster populations from Sado estuary and to investigate the possible elimination of these metals, samples were collected monthly in a natural bank, and, were transferred to an aquaculture production located in a different area of the same estuary. Cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg) and zinc (Zn) metals were analysed, since they are the main contaminant metals in oysters from the Sado estuary.

Total Hg and Pb levels in aquaculture or natural bank samples, were always lower than the limits set by the EU. All samples of *Crassostrea angulata* from the natural bank revealed Cd levels higher than the limit allowed by the EU (1.0 mg / kg, ww) which confirms its interdiction in the Sado estuary. After two months, Cd concentrations in aquaculture samples were lower than those from natural bank, being below EU limit after 8 months. Levels of Cu and Zn in *Crassostrea angulata* samples of the natural bank were between 150 and 280 mg / kg and 580 and 2000 mg / kg, respectively. As for Cd, it was also found that after some months, contents of Cu and Zn in oysters from aquaculture were much lower than those observed for the oysters from natural bank. This study suggests that the oyster transposition to an aquaculture seems to be an effective process to eliminate these metals, improving their quality for human consumption.

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## VALORIZATION OF LOW VALUE FISHERIES SPECIES IN SICILY (ITALY), BY REALIZATION OF FUNCTIONAL FOOD: A CASE STUDY OF HIGH TECHNOLOGICAL READINESS LEVEL

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The growing demand for fish products of safe and local origin and the need to reduce fishing on target species, requires continuous diversification in the seafood industry, to meet the needs of consumers. One solution is represented by the use of surplus species of local fisheries, of little commercial value and often ignored by the consumer, which, if subjected to new preservation and processing methodologies, allow creating new product categories, which open up new market opportunities. In our study, we set-up and applied, on some local surplus fisheries species from western Sicily, some processing technologies, aimed to valorize and extend the shelf life of fish products: the cold smoking with addition of natural antioxidants and the salting-drying, with low salt content. The cold smoking process was

applied on fillets of the species *Coryphaena hippurus*, a surplus seasonal species. The process demonstrated to be effective in extend the shelf-life and ameliorate some sensorial aspects of quality. The salting-drying process was applied on the species *Spicara smaris*, after the setup of a protocol aimed to reduce the sodium content of the processed product. For this purpose, the sodium chloride was partially replaced with potassium chloride, during the salting phase. The partial replacement seems to be the best way to reduce the sodium content; both salts, in fact, have similar properties, the potassium, in addition, does not seem to be correlated with the onset of hypertension and cardiovascular diseases like the sodium. This allows obtaining a product that can be considered a functional food, intended also for

consumers that suffer of hypertension. Both processes are in advanced phase of technological readiness level (TRL8) since have been tested also in a real environment through involvement of producers and consumers in the research project.

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## INTEGRATED PREDICTIVE MODELS AND SENSORS TO MANAGE SEAFOOD SAFETY AND QUALITY IN SUPPLY CHAINS

**Mark L. Tamplin**

### INVITED SPEAKER

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Seafood supply chains must optimise product safety and quality in order to maintain and increase domestic and international market access. This is important for extended supply chains that are complex, especially considering seafood is highly perishable. Supply chain design and monitoring can be improved using predictive models, especially when coupled with environmental sensors. Microbial kinetic models were produced to estimate spoilage and pathogenic bacterial growth and inactivation, as well as product sensory attributes. Specifically, models were produced for *Vibrio parahaemolyticus* and Total Viable Counts (TVC) in live Pacific oysters (*Crassostrea gigas*), and for *Listeria monocytogenes*, TVC, and Quality Index Metrics (QIM) in whole head-on gutted salmon (*Salmo salar*). From this information, secondary models were

developed to estimate safety, spoilage and quality values, as a function of product storage temperature. Models for Atlantic salmon demonstrated acceptable performance for TVC, *L. monocytogenes* and QIM, over a range of 0-15°C. In addition, a strong relationship was observed between TVC and QIM, allowing simultaneous estimations. Two models were produced for *V. parahaemolyticus* in Pacific oysters—one for growth above 15°C, and another for inactivation below 15°C. In addition, a risk assessment of Tasmanian oyster supply chains demonstrated that post-harvest cooling was the most sensitive node for quality and safety. When coupled with real (or near)-time sensors and software interfaces, food safety and quality can be proactively managed.

## THE IMPORTANCE OF EFFECTIVE TRACEABILITY TOOLS BY THE EXAMPLE OF MONKFISH (*Lophius spp.*)

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Traceability along the supply chain of fishery products has become established in a greater extent in Europe in the last years. Nevertheless, there are still some issues that need to be brought to mind. Particularly correct labelling along the full supply chain is a prerequisite for offering high-quality fish products. As a result of growing global trade in the last decade, traditional fish species are more and more competing with imported new fish species from all over the world. While a few years ago, exclusively monk fish *Lophius piscatorius* and *L. budegassa*, originating from the Northeast Atlantic and Mediterranean Sea were traded on the European market, nowadays also “new monkfish” species as the Chinese monkfish *L. litulon* from the Pacific or *L. vomerinus* from South Africa are for sale. The new monkfish species are available as low-priced frozen fillets on the German market. This may offer opportunities for fish substitution as well as fraudulence. Especially one and the same German commercial designation “See-teufel” for all seven monkfish species of the genus *Lophius spp.* could hamper

the informed choice. Although legislation requires the scientific name of fish species for unprocessed products, most of the consumers are not aware of the different monkfish species. Therefore, they might often prefer the lower priced fillets, believing to purchase the well-established Atlantic monkfish.

As a consequence, European fishermen and fish industry may suffer from this new product range. Our comprehensive investigations on monkfish aimed to clarify the following questions: i) Are there quality discrepancies among the traditional and new introduced species? and ii) Can we differentiate these fish species/products by means of available protein- and DNA-based methods in order to use them as reliable traceability tools? Results should provide transparency in the fish supply chain and retail stores to prevent a negative development in the fishery economy by fraudulent competition as well as to protect consumers against fraud or enable them making the right choice.

## PREVALENCE AND GENOTYPIC CHARACTERISATION OF *Listeria monocytogenes* IN COLD SMOKED FISHERY PRODUCTS SOLD IN AUSTRIAN MARKETS

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As most of the popular fresh fish and fishery products in the Vienna market are imported, the production conditions in the countries of origin are mainly unknown to the customers. Hence, the safety and hygiene of those commodities should be understood in order to minimize the risk of food threats for the fish trade in Austria and finally the consumer. Current scientific publications indicate an increased incidence of *L. monocytogenes* in certain fish species, such as halibut, which is also traded in Vienna. According to the zoonosis report the prevalence of *L. monocytogenes* is highest in RTE fishery products. However, low listeriosis incidents are identified related to this product group. In this study 250 cold smoked fish products were taken from retail stores and market halls in a one-year period including possible seasonal fluctuations. The detection and enumeration of *L. monocytogenes* is carried out according the reference method (ISO 11290) including an alternative method. Imo0733 gene positive isolates were serotyped by two multi-

plex PCR systems. Next to the microbial spoilage profiling, physico-chemical characteristics such as aw-value, pH, salt concentration, phenolic substances are examined to elaborate potential relationships for the incidence and contamination behavior of this pathogen. Overall 22 % of the examined smoked fish samples were contaminated with *L. monocytogenes*. The legal threshold was albeit exceeded by only two products and is therefore relatively slight. Serotyping revealed that 84 % of the positive isolates belong to the group 1/2a, 3a, typically connected to food, whereas serotype 4b is generally associated with cases of illness. Subtyping of more than 200 *Listeria* isolates through GTG5 PCR revealed country specific transmission routes. Further, next to the seasonal prevalence variations heavy spreaders of *Listeria* exist in the fish supply chain. Physico-chemical parameters even vary within fish batches resulting in an underestimation of the growth potential in cold smoked products.



## **IN VITRO INFECTIVITY AND ALLERGENICITY OF *Anisakis simplex* L3 AFTER FREEZING FISH MUSCLE**

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The importance of *Anisakis* infection of fish is well recognized by the fisheries sector and food safety authorities since humans can get infected by *Anisakis* larvae in the third stage (L3) through consumption of fish parasitized with live L3, whenever the fish is being consumed raw or when the technological processes, cooking, or storing practices the fish product has been subjected to, are insufficient to kill the larvae. Freezing fish muscle and their subsequent frozen storage is the most adequate technology treatment to control the risk of infection of consuming raw or undercooked infected fish. Most of the studies found in the literature describing the survival of *Anisakis* L3 along freezing do not take into account the possible infective characteristics of those who have survived technological treatments. It is thought

that the larval penetrability may play an important role in the pathogenicity for the invasion of the gastrointestinal mucosa of patients. In this study the ability of the larvae to enter into an agar layer and the rate of mortality in an artificial gastric fluid were checked in larvae who had survived a freeze thaw treatment. Results showed that from those larvae surviving a freeze thaw cycle, only 10 % could penetrate an agar gel, as compared to 50 % in controls. 80 % of treated larvae died within 24 h under acid conditions simulating those of the gastric fluid, suggesting that they have an impaired infectivity as compared to the untreated ones. However, the release of allergens suggests that the pathogenic potential of these moribund larvae cannot be discarded.

## THE PEPSIN DIGESTION METHOD MAY UNDERESTIMATE THE CONTENT OF *Anisakis* IN FISH PRODUCTS AND THIS IS DEPENDENT ON THE STATUS OF THE LARVAE

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Pepsin solution has been applied as a tool to quantify parasitic infections and to estimate the number of parasites in the fish musculature. The objective of this piece of work was to study the effect of pepsin digestion on the recovery of dead and live *Anisakis* larvae.

In a first trial, experimentally infected mince fish, were frozen at different final freezing temperatures (-10 °C, -15 °C, -20 °C, and -30 °C). After freezing and thawing, larvae were visually recovered from the mince and analysed for mobility. They were then placed again in the same mince and performed a pepsin digestion. Results showed that none of the larvae that had survived a freeze/thaw cycle were alive after the digestion with pepsin. They also showed that the range of recovery of intact larvae was between 46-60%.

To confirm this result, a new set of experiments was performed in 5 separate

days to give an insight of the day to day differences in the viability and recovery of larvae after pepsin digestion. When this digestion was performed with untreated larvae, there was, also in this case some percentage of *Anisakis* that could not survive pepsin and were recovered dead (0-27%), and the total recovery was quite variable (84-100 %). However, when larvae had been previously treated by a freeze thawing cycle the total recovery could be as low as 60%. Also in this case, the percentage of larvae that resisted the digestion with pepsin and could be found alive was much lower than with untreated larvae (25-63 %).

These results point out at an analytical problem with the pepsin digestion method when analyzing treated (i.e. frozen) fish, since the actual starting infective larvae in a given frozen product may be significantly higher than the standard pepsin method is showing.

## SURIMI PROCESSING EFFECTS ON THE LEVELS AND ALLERGENICITY OF B-PARVALBUMIN

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Fish and seafood products consumption has been increased in the last years because of their nutritional value. This fact has made that adverse reaction to fish such as those caused by allergens, parasites and toxins are becoming common. Particularly, fish allergy occurrence might vary with the geographical eating habits, fish processing and fish species. In 95 % of the reported fish allergies, the  $\beta$ -parvalbumin (PV) has been identified as the major allergen displaying a high cross-reactivity among fish species with commercial value. PVs are a family of proteins ranged between 10-15kDa and their detection in fish is further complicated by the impact of fish processing. By cooking and canning PV structure can be degraded or alter which makes it difficult they detection by antibodies. On the other hand in the elaboration of surimi, during fish meat washing, PV can be mostly removed. Surimi, which is mechanically deboned fish flesh that has been washed and mixed with cryoprotectants, is widely used as raw material

in the elaboration of seafood products, so the determination of the PV levels and allergenicity in this raw material and in the seafood products made of them, is of public health relevance.

To determine the levels and state of PV in fish, surimi and in the prototype surimi product all from *Alaska Pollock*, we focused on developing native extraction procedures of extended use. We found that TBS buffer together with several sonication steps allow the quantitative extraction of soluble PV as judged by SDS-PAGE and Western-blotting. At the present stage, results indicate that PV level decreases along with processing, decreasing from *Alaska Pollock* muscle followed by surimi as raw material and lastly in the prototypes of surimi products. These results take part to a preliminary work and more analyses related to allergenicity of PV after gastric and intestinal digestions are going to be performed.

## INFLUENCE EXAMINATION OF LYSOZYME PROCESS WHICH IS STORED IN ICE ON SEA BREAM (*Sparus aurata*) QUALITY

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Increment of attention in food industry especially on bio protection methods in which natural origin antimicrobial components are used enhances the potential usage of natural enzymes on food. Lysozyme goes in for GRAS as nisin in U.S.A. and Canada. It is also acknowledged as natural and safe biological antiseptic and widely used in food and drug engineering. Sea bream that is used in the study is separated into two groups and half of their inner parts are cleaned. Groups that are formed according to fish that is cleaned and not cleaned (whole fish) are stored in 0-4°C after being treated with lysozyme.

Lysozyme enzyme that is used as antimicrobial is bought commercially. Microbiologic analyses on groups that are stored in 0-4°C are done through *Salmonella spp.*, *Listeria monocytogenes*, *E. coli*, *Staphylococcus aureus* existence and total counting of aerobic mesophilic bacteria causing deterioration, psychrophilic bacteria counting, yeast-mold counting, *Enterobacteria-*

*ceae* counting and lactic acid bacteria counting. Chemical analyses are done through; TVB-N, TBARS and sensory analyses storing period.

With this study, it is shown that lysozyme which is a natural antimicrobial enzyme has effects of quality and shelf life on sea bream. Also, lysozyme's effect on food pathogen and deterioration bacteria's number and activation of described types is found. When lysozyme's price and utility in industry is considered, its availability in industry increases because it has an advantage of effective improvement on quality and when it is used in low concentration, it enables inhibition for pathogen and microorganisms which cause deterioration.

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## DIETARY OIL SOURCES HAVE AN INFLUENCE ON SENEGALESE SOLE (*Solea senegalensis*) FATTY ACID COMPOSITION AND ITS BIOACCESSIBILITY FOR HUMAN CONSUMPTION

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*Solea senegalensis* were fed three iso-nitrogenous diets with increasing substitution of fish oil (FO) by a blend of vegetable oils (50 % soybean oil, 25 % rapeseed oil and 25 % linseed oil): control with fish oil - CTL; 50 % replacement of FO by vegetable oils - D50; 100 % total replacement of FO by vegetable oils - D100; during 10 months. At the end of the experiment, the effect of the different diets on the fatty acid profile of sole as well as on the bioaccessibility of each  $\omega$ 3-PUFA determined by an *in vitro* digestion model taking into account the digestive action upon different lipid classes, such as triacylglycerols (TAG) was analysed.

The fatty acid composition of the diets varied significantly. The total  $\omega$ 3-PUFA varied from 22.6 % in CTL to 15.9 % in D100 diet and the total  $\omega$ 6-PUFA increased from 12.3 % in CTL to 36.2 % in D100 diet. Oleic acid (18:1) and linoleic acid (18:2  $\omega$ 6) augmented from 12.4 % and 11.3 % in CTL to 30.0 and 36.0 % in D100, respectively; docosahexaenoic acid (DHA, 22:6  $\omega$ 3) declined from 7.8 % in CTL to just 1.1 % in D100. The fatty acid composition of the fish mirrored these dietary changes, since EPA and DHA contents were reduced from 2.6 and 17.8 % in CTL fish to 0.2 and 4.1 % in D100 fish, respectively.

Bioaccessibility of  $\omega$ 3-PUFA varied

widely between 36 and 74 %, depending on the particular fatty acid and the diet fed to the farmed fish. In fact, the bioaccessibility of linolenic acid (18:3  $\omega$ 3) was higher (70-74 %) than that of DHA (56-69 %). The bioaccessibility of eicosapentaenoic acid (EPA, 20:5  $\omega$ 3) and other  $\omega$ 3-PUFA was lower for fish fed D100. Given the fact that other fatty acids' bioaccessibility

was not similarly affected, it is possible that these variations are related to the positional and lipid class distribution of  $\omega$ 3-PUFAs, which would be different in each group. Representing a smaller share of the total fatty acids, these  $\omega$ 3-PUFAs would be more concentrated on the sn-2 position of TAG, which is less hydrolysable.

## FORMATION OF LIPID OXIDATION PRODUCTS (MDA, HHE, AND HNE) DURING STATIC AND DYNAMIC *IN VITRO* DIGESTION OF COD LIVER OIL

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Marine long-chain n-3 polyunsaturated fatty acids (omega-3) have attracted interest due to the positive health effects they have been associated with. However, omega-3-rich oils are highly sensitive to lipid oxidation, something that could interfere with its beneficial effects. A few recent *in vitro* studies have shown that fish lipids not only oxidize during storage, but also under gastrointestinal tract (GIT) conditions. Lipid oxidation is known to generate certain highly reactive oxidation products, like malondialdehyde (MDA), 4-hydroxy-trans-2-hexenal (HHE), and 4-hydroxy-trans-2-nonenal (HNE); all three with documented carcinogenic and genotoxic properties. To date, little is known about formation of these specific compounds in presence of human GIT juices, and also, what levels that can be reached in the GIT. In two separate *in vitro* studies, MDA, HHE, and HNE-formation in fish oils have therefore been investigated under GIT conditions.

In the first study, two static GIT *in vitro* models were set up to follow oxidation in cod liver oil during digestion. In one model, digestive enzymes and bile of porcine origin were used, and in the other model, human digestive juices were used. The digests were analysed for MDA, HHE, and HNE by LC-MS, and for free fatty acids by GC-MS. Results showed that the aldehydes increased over time during intestinal conditions in both models. However, rate and maximum aldehyde levels reached were significantly higher with human digestive juices.

In the second study, the formation of MDA, HHE, and HNE in cod liver oil subjected to (i) pre-oxidation, (ii) emulsification (20% o/w), and (iii) enrichment with EDTA and fish hemoglobin (Hb) were evaluated in a dynamic *in vitro* digestion model. The highest levels of aldehydes recorded were obtained in the presence of Hb. Pre-oxidized oil generated higher aldehyde

levels during digestion than fresh oil, and emulsification was slightly protective in the gastric tract. In general, higher aldehyde levels were attained in intestinal lumen than in the initial meal, demonstrating that GI digestion promotes oxidation also in a dynamic digestion model.

These results emphasize the importance of keeping high quality of marine omega-3 products and to find ways to inhibit the Hb pro-oxidative effect.



## DEVELOPMENT OF NOVEL FOOD SUPPLEMENT FROM RAINBOW TROUT EGG: EFFECT OF FLUIDIZED BED DRYING ON THE POLYUNSATURATED FATTY ACID (PUFA) CONTENT OF LIPID FRACTIONS

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Rainbow trout fish (*Oncorhynchus mykiss*) is one of the main commercial fish species widely farmed in the Europe. Rainbow trout egg, also named as roe has a high content of nutritive lipids, such as phospholipids and omega-3 polyunsaturated fatty acids ( $\omega$ -3 PUFA), particularly eicosapentaenoic (EPA) and docosahexaenoic (DHA). Since rainbow trout egg has been known as source of biological valuable protein and  $\omega$ -3 PUFAs, processing of eggs with novel food processing methods and serving of those valuable components to human nutrition as a functional dietary supplement can not only increase its value but also contribute to public health. Drying process inhibits activity of microorganism and enzymes; improves food preserving and reduces the bulk of food for cheaper transport and storage. But during drying, some thermolabile compounds including PUFAs could be oxidized. The purpose of this research was to investigate influence of drying treatment on the PUFA profile of lipid fractions in egg oil. Rainbow trout eggs were dried in fluidized bed dryer. Lipid fraction of trout egg was determined in

fresh and dried eggs. Effect of drying process on the PUFA content of lipid fractions was determined by the lipid fraction and fatty acid profile analyses.

Result showed that crude lipid of trout egg consisted of 59.5% phospholipids (PL), 38.1% neutral lipids (NL) and 2.4% glycolipids (GL) fractions. Crude lipids of trout roe and its fractions had a high content of PUFAs, followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). DHA (C22:6 *n*-3), an important *n*-3 fatty acid, was the dominant PUFAs in all lipid fractions of crude lipid of trout egg, where as the main SFA and MUFA were palmitic acid (C16:0) and oleic acid (C18:1 *n*-9). The high content of PUFAs is contributed to nutritional requirement for embryonic and early larval development stages of fish species. PUFA and omega-3 fatty acids contents of lipid fraction of trout egg were not significantly affected by drying process. Thus, fluidized bed drying can be served as an efficient drying method without any nutritional losses for the production of food supplement from trout egg.

## LIVER METABOLISM AS TARGET TO UNDERSTAND THE FUNCTIONAL EFFECT OF EPA AND DHA: REGULATION OF PROTEIN PATHWAYS INVOLVED

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Fish oils are considered an important source of bioactive compounds to formulate functional foods and supplements. During the last years, a growing interest has been focused on the benefits of these fish oils with high contents in EPA and DHA in prevention and palliation of diet-induced disorders such as dyslipidemia, type 2 diabetes and obesity, all of them linked to a high risk of cardiovascular diseases (CVD). However, the recent clinical trials have failed to replicate protective effects of EPA+DHA on CVD lacking of definitive conclusions. It is currently recognized that the right design of functional foods requires the study of their *in vivo* mechanisms at molecular level in order to draw the right intake in terms of concentrations and formulations.

Our previous findings showed that supplementation with fish oils, especially those oils having balanced pro-

portions of EPA and DHA were able to improve parameters related with oxidative stress, insulin resistance and inflammation in animal models. On this basis, we are investigating the mechanism of action of fish oils considering the metabolic pathways modulated by EPA and DHA, through the understanding of liver protein regulation using animal models. Since high-fat high-sucrose (HFHS) diets are well-known model diets to intentionally promote inflammation processes and metabolic disorders, we have studied the protein targets modulated by the consumption of fish oils in Wistar rats subjected to such dietary unhealthy background. A Functional Proteomics approach based on a shotgun quantitative analysis using iTRAQ (isobaric tags for relative and absolute quantitation) complemented with 2D-DIGE (two-dimensional fluorescence difference gel electrophoresis) was em-

ployed to illustrate the capacity of fish oils in regulating liver proteins depending on the dietary context.

Our findings reveal a role of the consumption of fish oils, especially visible under a background of HFHS diets, in regulating specific liver proteins involved in key metabolic pathways. Moreover, our findings highlight the

importance of dietary context in order to assay the functional properties of seafood. Therefore, this research lays the groundwork for discovering new functional uses of seafood rich in EPA and DHA as well as for the personalized design of nutraceuticals and functional foods based on them.

## DIPEPTIDYL PEPTIDASE IV AND PROLYL ENDOPEPTIDASE INHIBITORY PROPERTIES OF ARGENTINE CROAKER (*Umbrina canosai*) HYDROLYSATES

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The Argentine croaker (*U. canosai*) is a fish species caught in large amounts in the Southern Brazil coast. However, this species is underutilized and has low commercial value. Many studies used these species to obtain minced fish, which can be utilized for development of higher commercial value as protein hydrolysate. Actually, studies have been published of different biological activities, as antidiabetic and anti-Alzheimer's through of inhibition of dipeptidyl peptidase IV (DPP-IV) and prolyl endopeptidase, by protein hydrolysates obtained from aquatic species. For this reason, the potential *in vitro* of Argentine Croaker hydrolysate to act as a source of dipeptidyl peptidase IV (DPP-IV) and prolyl endopeptidase inhibitory peptides was studied. First, Argentine croaker myofibrillar proteins (CMP) were obtained and characterized. From this raw material two hydrolysates with different degrees of hydrolysis - DH (10 and 20%) were prepared using enzyme Protamex. They were then

characterized for their molecular weight profile (MW) and *in vitro* bioactive properties such as inhibitory enzyme dipeptidyl peptidase IV (DPP-IV) and prolyl endopeptidase (PEP). CMP showed a protein content of 88.7%, lipids 3.9%, ash 3.3% and moisture 2.0%. It was found that the increase in the DH favored the peptides with lower MW. The greatest 50% inhibitory concentration (IC<sub>50</sub>) value of DPP-IV, was observed in the presence of sample hydrolyzed of CMP with higher DH (20%) and lower MW (1845 Da), was  $0.50 \pm 0.02$  mg/mL. However, the greatest IC<sub>50</sub> value of PEP ( $0.72 \pm 0.01$ ), was observed with the hydrolyzed with lower DH (10%) and higher MW. These results suggested that the protein hydrolysates from Argentine croaker myofibrillar proteins are a good source of natural inhibitors of dipeptidyl peptidase IV and prolyl endopeptidase and/or as alternative to synthetic inhibitors currently used. *Keywords* - Fish, protein, hydrolysate, prolyl endopeptidase, dipeptidyl peptidase IV

## EUROPEAN SEABASS (*Dicentrarchus labrax*) AND GILTHEAD SEABREAM (*Sparus aurata*) MUSCLE HYDROLYSATES AS A PERSPECTIVE SOURCE OF BIOPEPTIDES

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European seabass (L) and Gilthead seabream (C) muscles were subjected to a designed proteolysis by Alcalase (Lalc, Calc) and Chymotrypsin (Lch, Cch) to compare their peptides profiles. A total of 765, 794, 132 and 232 peptides respectively have been identified. The antioxidant capacity in vitro was higher for Alcalase hydrolysates, probably due to higher content of short peptides (<5 amino acids). A higher content of antioxidant peptides was predicted for Lalc and Lch; however, the observed antioxidant capacity in vitro was slightly higher for Seabream hydrolysates, reaching the maximum value of  $848.11 \pm 60.78$  mmol TE/g protein for Calc (TEAC assay). All the hydrolysates studied inhibited oxidative stress in MDCK1 cell lines by 5-12% (the maximum activity  $12.8\% \pm 4.5\%$  registered for Lalc), proliferative activity in MTT assay in

MDCK1 cell culture (the highest proliferative capacity  $147.0\% \pm 3.1\%$  observed for Calc), and dose-depending chemopreventive activity in human colon adenocarcinoma cell line HT-29 (Lch showed 40-60% decrease of HT-29 cells viability at concentrations 0.05 – 0.5 mg/mL). Surprisingly, water extracts of the investigated species' muscles without enzymatic treatment also exhibited a comparable but slightly reduced proliferative, antioxidant, and chemopreventive action in cell lines, probably due to biological activity of short peptides and/or native components of fish muscles.

*Keywords: Seabass hydrolysate; Seabream hydrolysate; enzymatic hydrolysis; proliferative; antioxidant; chemopreventive.*

## SEAFOOD OFFERED BY SCHOOL FOODSERVICE: HEALTH AND HEDONIC PERCEPTION IN CHILDREN

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The choice of consuming fish as a part of a main meal is generally accepted as health promoting. A good understanding of the early development of food choice partners and the reasons behind eating healthy or unhealthy food is important as nutritional influences on health can be established in childhood. The aim of this work was to study the nutritional understanding (healthy and unhealthy food) and hedonic perception of school meals, various dishes from primary school canteen, in particular fish dishes, by children with different age.

Three groups of children of 6-7 years (n=39), 8-9 years (n=35) and 10-12 years (n=26) were interviewed in a primary school. The study was targeted to 16 dishes, included four fish dishes: baked hake with lettuce, breaded hake with lettuce, tuna with peppers and mackerel burger. The test session involved two tasks, a "structured sorting task", where they had to sort 16 colour photographs of dishes offered in the school canteen in 4 pre-determined groups (healthy/unhealthy and hedon-

ic perception of food). In the second task, the same pictures were rated for the overall liking with the use of 5-point hedonic smiley-scales and classify in healthy or unhealthy.

Regarding the sorting task, the results of the three age groups were very similar. All the groups showed a good knowledge of the nutritional value of the tested dishes offered in the school canteen. All the fish dishes were categorized as healthy by the majority of the participants. The ANOVA showed significant differences in liking between products. Based on the results, baked and breaded hake with lettuce were highly appreciated for all ages. Tuna with peppers was less liked in all the children groups. The 6-7 year group scored higher preference to mackerel burger than other groups.

In conclusion, these results contribute to our understanding of children's nutritional perception and its relation to fish dishes preferences of school meals, what is of particular importance to quality improvement and strategic planning in school foodservice.

## BIOACTIVE PROPERTIES OF COLLAGEN HYDROLYSATES FROM COD SKIN

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Collagen Hydrolysate (CH) is becoming increasingly important to the expanding nutraceutical market because of its sensory profile, appealing functional properties and strong links to positive health effects. Mainly sourced from pig- and calfskin, CH from wild-caught fish skin has very similar characteristics and serves as an excellent alternative since it is not connected to factory farming, diseases or religious restrictions.

The aim of this project was to evaluate the effect of different enzymes to hydrolyse collagen from cod skin on the hydrolysates properties. Collagen was extracted from fresh cod skin by first washing with 0.1 M NaOH solution to remove non-collagenous proteins, lipids and colour, acidification in 0.05 M acetic acid solution followed by extraction in hot water over night. After extraction the samples were freeze dried and kept frozen until analysed. The dried collagen (5% solution) was hydrolysed with four different proteases from Novozymes (Protamex,

Neutrase, Flavourzyme and Alcalase) and collagenase from Sigma for two hours at 45°C, the enzymes were inactivated in a 90°C water-bath followed by cooling, freezing and freeze drying. The degree of hydrolysis (DH) was measured by with ophthaldialdehyde (OPA) method. The hydrolysates composition was evaluated by protein analysis and SDS page. Bioactive properties of the hydrolysates were evaluated using chemical methods: Oxygen Radical Absorbance Capacity (ORAC), Metal Chelating (MC), Reducing Power (RP), inhibition of alpha amylase and alpha glucosidase, inhibition of Angiotensin Converting Enzyme (ACE) as well as elastase and collagenase inhibition and cell assay to evaluate chondroprotective effect.

The hydrolysate collagens did not show high bioactive properties measured and no MC, alpha amylase, alpha glucosidase, Elastase, Collagenase or ACE inhibition was detected. Low ORAC and RP activity was detected but clear increase was found

in the ORAC values as DH increased while the opposite trend was seen for RP, lower reducing power with higher degree of hydrolysis. The hydrolysate with highest DH was fractionated to

different molecular sizes (> 30 kDa, 10-30 kDa, < 10 kDa 5-10 kDa, < 5 kDa) and the bioactivity of the different fractions are now further evaluated.



## VALORIZATION OF SHRIMPS BY-PRODUCTS FOR PHARMACEUTICAL APPLICATIONS: EFFECTS OF ASTAXANTIN AND PROTEIN HYDROLISATES IN VITRO

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Marine natural products have already shown their value in pharmaceuticals, but despite this proven value, the majority of marine by-products are currently used in the production of fish oil, fishmeal, fertilizer, pet food and fish silage, which hold low market value considering their high protein and potentially high valued contents. Furthermore, a considerable amount of marine products are considered to be unsaleable and so are discarded. The declining supply of seafood from the wild, as well as stricter environmental controls of dumping discards, provides further incentive to utilize all products that may come from fish. Shrimp waste contains high biological value components such as protein, chitin and astaxanthin. The hydrolyzed protein (HP) may be supplemented in diets formulated for aquaculture, and represent also a sources of biologically active peptides with

considerable potential applications in pharmacology. Astaxanthin, the main carotenoid in crustaceans, is used as a food additive, in diet for aquaculture and in the production of drugs and nutraceuticals.

Shrimp waste (exoskeleton and cephalothorax) were obtained from industries of fish processing in Sicily. More than ten sampling were done in one year. After collection, samples were thawed and minced. The homogenate was utilized for production of protein hydrolyzates (PH) and astaxanthin. The enzymatic hydrolysis reaction was carried on employing three different commercial proteases, Protamex, Flavourzyme and Alcalase. Astaxanthin was extracted from the dried matrix with a mixture of hexane/isopropanol 3:2 (v/v). A protocol of extraction by Supercritical Fluid Extraction (SFE) was defined. The use of Protamex and

Flavourzyme resulted in more efficient hydrolysis. SFE technique allows obtaining extracts free of any hazard caused by the use of traditional toxic solvents, hence, it results qualitatively suitable for use in nutraceutical, pharmaceutical industry. The bioactivities of PH and astaxantin were assessed in human cell culture. In normal cells, the PH showed a significant antioxi-

dant power, preventing the damage induced by free radicals. In cancer cells, astaxantin resulted effective in reduce proliferation and in induce apoptosis, attested by a set of specifics biomolecular markers.

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## WHAT DO CHEFS DE CUISINES PURCHASE IN ORDER TO SELL AS COMMON SOLE?

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Common sole (*Solea solea*) is a highly appreciated food fish, which is served as a special delicacy in restaurants. But unfortunately, consumers often don't get true common sole but less valuable fish species (like striped catfish or tropical soles), especially when they have ordered dishes with common sole fillets. In a former German survey of restaurant sole samples, even species were detected, which could not be identified due to lacking DNA reference sequences in the public databases. The question is now: what kind of products do restaurant chefs purchase when they intend to adulterate sole fillet dishes with less expensive species? In order to solve

this question, several products with fish fillets resembling sole fillets were bought from German traders and were analysed with DNA barcoding and phylogenetic methods. The results indicate that sole dishes are often substituted with cheap *Cynoglossus senegalensis/spp.* products from the West African coast, which contain a smorgasbord of flatfish fillets from different *Cynoglossidae* as well as *Soleidae* species. The results also emphasize the urgent need to increase DNA sequence coverage of West African flatfish species in public databases as a prerequisite for an effective control of fish product labelling.

## DEVELOPMENT OF FISH CRACKER BY ADDITION OF FLAXSEED (*LINUM USITATISSIMUM*) AS AN ALTERNATIVE OF HEALTHY SNACKS

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Flaxseed (*Linum usitatissimum*) is nowadays emerging as one of the nutritive and functional ingredient in food products due to the high beneficial compound such as n-3 alfa linolenic acid (ALA). Incorporating flaxseed oil into crackers made of whiting fish (*Merlangius merlangus*) flour can improve its nutritional properties. The objective of this research is to gain the best formulation of fish crackers as an alternative of healthy snacks rich of both protein and fatty acids. The research was divided into two stages; preliminary and main research. The preliminary research was conducted by production of fish flour and analysis of its physical and chemical properties. The main research was conducted by production of crackers

through formulation based on fish flour (10, 12.5 and 15% of the total flour used) and flaxseed oil (0, 50 and 100% of the total vegetable oil used) followed by analysis of the chosen formula product (proximate composition (fat, protein, moisture, carbohydrate, ash) microbiology (total aerobic plate count), sensorial, chemical (TBA) and physical (colour and texture profile analysis, statistical analysis). The production of fish flour from whiting fish yielded up to 15% of fish flour (moisture content 5.06%, dry based). The best crackers product was obtained according to sensory and fatty acid composition analysis.

*Keywords: crackers, fish flour, flaxseed, functional food*

## EFFECT OF STORAGE TEMPERATURE ON SHELF-LIFE AND MICROBIOLOGICAL PROFILES OF READY-TO-EAT WHOLE BROWN CRAB

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In the past, the brown crab (*Cancer pagurus*) market largely consisted of live product. However, the opening of new markets (e.g. China and USA) has created a demand for ready-to eat products. This in turn creates an opportunity to add value but also affords manufacturers the chance to improve existing commercial processes (e.g. milder heat treatments resulting in higher quality product). In addition, the export of such products in a ready-to-eat format could eliminate the problem of losses during transport. An important consideration in all of this however, is product shelf life following these new processes and for this reason, the aim of the present study was to assess the effect of the storage temperature on the shelf-life and microbiological profiles of ready to eat whole crab cooked under mild temperatures and packed under vacuum. The shelf-life of the product was calculated by fitting the modified

Gumpert equation for mesophilic bacterial growth at different storage temperatures, with the end of shelf life being viewed as the point where the mesophilic bacterial load reached 6 Log<sub>10</sub>. A shelf-life of 16, 12, 9 and 7 days was found for white meat at 2, 5, 7 and 10 °C respectively, while for brown meat a shelf-life of 20, 14, 11 and 7 days, was found at 2, 5, 7 and 10 °C respectively. In addition, a linear relationship was found between the storage temperature and shelf-life of both kinds of meats. The microbiological profiles of both meat types at the end of the shelf-life (4 and 10 °C) were studied. In all cases the main microbial species detected were *Bacillus*, *Pseudomonas* and *Staphylococcus* spp. At 4 °C, microbial counts were similar regardless of the type of meat, though at 10 °C higher counts were found for all bacterial groups in the brown meat. This study revealed that, although the storage temperature has a bigger ef-

fect on the shelf-life of brown meat, at temperatures below 10 °C the shelf life will be determined by the white meat. In addition the work also found

that, *Bacillus*, *Pseudomonas* and *Staphylococcus* spp. were the main spoilage microorganisms.

## WHAT DOES THE FISH INDUSTRY NEED? EMERGING SUSTAINABLE SOLUTIONS

**Elvin Bugge**

### INVITED SPEAKER

*Aquatic Concept Group, Norway*

Maintaining a high quality of a product from “alive fish to a dish” is essential for the fish industry. Since 2010, there has been an increased focus of the salmon Industry into the processes that influence the quality aspects.

To maintain a high quality and to assure the safety of a product, the processing should be fast, performed at low temperature and in a way that the product cross-contamination is eliminated. Important source of the product contamination is processing equipment. The cleanability of equipment is closely linked to the formation of bacterial biofilm. The main reason for this build-up of biofilm is the complexity and poor design of the machinery. EHEDG (*European Hygienic Engineering & Design Group*) has made guidelines for the hygienic processing and the hygienic design of the equipment that have helped to improve the standards. The main objective of the hygienic design is to reduce/eliminate the risk of the equipment becoming a source of product contamination.

The key environmental issues associated with fish processing operations are the high consumption of water, consumption of energy and the discharge of wastewater. Since cleanable equipment will need less water, energy and chemical to reach the required hygienic level, hygienic design often leads to economic and environmental benefits for the Fish Industry.

The presentation will give an overview of the latest innovations in salmon processing, both regarding new hygienic solutions and the industries' maintenance of optimal product quality as well as sustainable solutions in the processing.

## AUTOMATED CLASSIFICATION OF ATLANTIC COD'S ROE, LIVER AND MILT USING HYPERSPECTRAL IMAGING IN VISIBLE SPECTRAL RANGE

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In Norway, the whitefish industry generates 0.34 million metric tons (44% of the total catch) of rest raw material. The most frequent rest raw materials are heads, tongues, roe, liver and milt. Only 30% (113,800 tons) of these rest raw materials are utilized while the remaining 226,000 tons are waste. Therefore, the potential for increased utilization of rest raw material is large and would lead to a more sustainable and profitable whitefish industry. The main reason for failing to achieve higher utilization rates for rest raw material from white fish is the lack of technological solutions that can enable automated classification and sorting on-board fishing vessels. Currently, separation of fractions of whitefish roe, milt and liver is done manually, which is a laborious and costly process.

Automated classification of roe, milt and liver, due to the similarities in the appearance of these materials as manifested in colour and texture, is a very challenging research task. To en-

able automated classification, we performed a complete characterization of roe, liver and milt in the visible/near infrared (vis/NIR) (420 – 990 nm) and short-wavelength infrared (SWIR) (960 – 2500 nm) wavelength ranges. We generated prediction models based on a single wavelength (Model I) and as a combination of two optimal wavelengths (Model II). Classification performed using reflectance measured at 444 nm (Model I) achieved 74%, 71% and 65% correct classification rates for roe, liver and milt, respectively. Classification rates for Model II were 96%, 97% and 100% for a combination of reflectance measured at 415 nm and 990 nm, and these are regarded to be sufficient for practical industrial applications. In practice, the combination of two different wavelengths can be solved by triggering two lasers alternately every second frame of the camera to generate almost simultaneously two images that can be used for analysis and image classification.



## SUSTAINABLE FEED FORMULATION FOR SWEDISH AQUACULTURE FROM HERRING BY-PRODUCTS USING THE pH SHIFT METHOD

**James Hinchcliffe, Nils Gunnar Carlsson, Elisabeth Jönsson, Susanne Erikson, Kristina Sundell, Ingrid Undeland**

*Chalmers University, Gothenburg*

Aquaculture is expected to be the fastest growing food sector this century. In Sweden, work is being done to establish a knowledge base for the development of sustainable marine aquaculture, focusing on two species: Spotted wolffish, *Anarhichas minor* and European lobster, *Hommarus gammarus*. One of the aims is to develop new feeds for the two species during larval and juvenile on-growth stages. Currently, formulated feeds are highly dependent on raw materials from wild fisheries and are costly to produce. The development of diets composed from sustainable materials with suitable texture that increases survival and quality is an important challenge. In this study, pH-shift processing was used to isolate protein concentrates from herring by-products, in order to investigate the potential incorporation of these concentrates as an alternative protein source in aqua feeds.

Herring by-products were sorted into back bones (B), heads (H) and guts (G) which thereafter, in different combinations (B+H+G, B+H or G), were subjected to pH-shift processing. For each raw material combination, the acidic version of the pH-shift process was compared

with the alkaline process version; accompanied by different separation forces (8000×g vs. 800×g), to identify protein yield as well as potential functional and chemical differences in protein isolates, and how these could affect the potential of isolates as a feed ingredient. Among studied factors were protein yield as well as mineral content (e.g. calcium), total lipid content, omega-3 content and protein content of the concentrates.

Results showed that alkaline pH-shift processing generally gave higher protein yields (54-66%) than the acid version (45-54%) while there were no significant differences between protein yields obtained from the different raw material combinations or from high vs. low separation forces. Among detected chemical differences between concentrates, the acid process version generated significantly higher calcium levels than the alkaline version, something which is particularly important in lobster feeds. Further, gut-concentrates obtained the highest lipid content and, as expected, concentrates produced with low g-force separations contained higher moisture than those made at 8000×g.

## FLOCCULATION AS A MEAN TO AID THE RECOVERY OF PROTEIN FROM SHRIMP PROCESS WATER

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Throughout seafood processing, large volumes of water are used, which dissolves significant amounts of high-quality protein, currently being turned into waste. Besides the loss of a large amount of marine biomass, in which there is a potentially high market value, an elevated cost is associated with the discharging of process water high in BOD/COD. Currently, industrial pre-treatments to reduce BOD/COD are normally done with non-food grade flocculants, something which does not allow further upgrading of the sludge beyond biogas.

The present study is part of the Nordic project "Extracting Novel Values from Aqueous Seafood Side Streams –NoVAqua", and aimed at evaluating the feasibility of using food-grade protein flocculation in combination with mild separation to treat shrimp process water in order to efficiently recover proteins. The effect of pH and the addition of natural and synthetic polymers on the flocculation of protein in the process water were assessed. Protein flocculation using

anionic polymers was maximized at pH 4 where nearly 73 % of the initially dissolved protein was recovered by simple sedimentation whereas the maximum protein recovery using cationic polymers was around 50 % at pH 6. Also the synthetic cationic polymers tested exhibited around 50 % recovery at the native pH of process water (pH 8.7).

Initial separation tests showed larger success with aggregates formed in presence of the anionic polymers compared to the untreated dissolved protein of the crude water. Separation trials with the other flocculants are ongoing. Overall the results so far showed the efficiency of adding polymers and adjusting the pH (to 4 and 6 in case of anionic and cationic natural polymers, respectively) in order to recover the protein load from shrimp process water. It is envisioned that the recovered shrimp protein using natural polymers could be a potential ingredient in both food and animal feed.

## TUNING THE pH-SHIFT PROTEIN-ISOLATION METHOD FOR MAXIMUM HEMOGLOBIN-REMOVAL USING BLOOD RICH MARINE RESOURCES

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During the last decade, the pH-shift method has been recognised as a promising technique for protein recovery from unconventional complex raw materials (e.g., whole dark muscle fatty fish and by-products). However, one of the main challenges that may prevent an optimal use of the protein isolates is the difficulty to remove enough heme-pigments (hemoglobin, Hb, myoglobin, Mb) to make the color of the isolates appealing and prevent their pro-oxidative effects. The present study was aimed to map the distribution of Hb in the different fractions formed during alkaline and acid pH-shift processing of blood rich fish raw materials. Strategies are also tested to facilitate Hb-removal in the different steps of the process considering protein recovery, moisture content of protein isolate and color changes.

To allow exact mass balance calculations, a model system consisting of minced cod white muscle fortified with known amounts of trout Hb was used as raw material. The basic pH-shift protein isolation protocol applied

involved X volumes of water, solubilisation at pH 2.5 or 11.5, and precipitation at pH 5.5. Hb was analysed with the Hornsey method, protein with the Lowry method and colour ( $a^*$ ,  $b^*$ ,  $L^*$ ) with a Minolta colorimeter.

Results showed that the alkaline process version could remove considerably higher amount of Hb (77.2 %) compared to the acidic version (37.2 %) when pH 5.5 was used for subsequent precipitation of the protein. Adjustment of precipitation pH to 6.5 improved total Hb removal up to 91% and 74.0% for the alkaline and acid process version, respectively. When phytic acid (PA) was added to the first supernatant of the alkaline process version, it could improve Hb removal up to 93.1 % without negative effect on protein yield. A combination of 1 cycle prewashing with PA, pH adjustment to 5.5 and then pH-shift process could increase Hb removal up to 96 % and 92 % in the alkaline and acid isolates, respectively.

## TRYPsin FROM VISCERA OF CULTURED RAINBOW TROUT (*Oncorhynchus mykiss*): CHARACTERISTICS AND THE EFFECTS OF SEASONAL VARIATION

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Fish have been consumed worldwide with increasing demand. The total production of fishery products in Turkey is 537.345 tons with 302.212 tons of capture and 235.133 tons of aquaculture (TUIK, 2015). Aquaculture is one of the fastest growing food-producing sectors in Turkey and the rainbow trout (*Oncorhynchus mykiss*) is the most important aquacultured species with 113.593 tons produced annually. During processing a huge amount of nonedible portion generated as by-products is conventionally, processed into low-value products such as fish meals, fish oil, and natural fertilizers or discarded. Viscera are one of leftovers, which can be a promising source of enzymes, particularly trypsin. However, trypsin activity can be varied with seasons. The study aimed to characterize trypsin from viscera of rainbow trout cultured in summer and fall. Trypsin was extracted and partially purified using ammonium sulfate 80% saturati-

on and acetone precipitation at -20°C. The activity was assayed using BAPNA as a substrate. The yield of 62.5% and 37% was obtained in summer and fall, respectively. The optimum temperature and pH of partially purified trypsin of rainbow trout obtained from both seasons were 50 °C and 8. Trypsin was stable to heat treatment at temperature range of 20-50°C for both seasons. For pH stability, trypsin was stable in the pH range of 5-10 and 4-11 for summer and fall samples, respectively. Trypsin activity in rainbow trout cultured in summer and fall was; 0.05 u/ml and 0.024 u/ml, respectively.

Thus, viscera of rainbow trout can be an alternative source of protease, especially trypsin, for industrial applications. The recovery of trypsin also helps in minimizing ecological problems.

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## STRATEGIES TO IMPROVE THE MANAGEMENT OF FLAT OYSTER (*Ostrea edulis* L.) FROM AQUACULTURE USING THE SHORT STORAGE AND PACKAGE IN CLOSED-CIRCUIT SYSTEM

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In the Adriatic Sea, shellfish fishermen are exploring different strategies to improve their economic situation, such as management of natural stocks in marine protected areas and development of shellfish aquaculture systems.

The main purpose of this trial was to evaluate the effects of a short storage in closed-circuit system of flat oyster (*Ostrea edulis*) on their quality, especially in terms of nutritional traits and yield of flesh. Flat oysters ( $68.8 \pm 17.3$  g) were divided into three groups of 270 oysters each, submitted to different storage and package: Group A (oysters packaged after harvest on the vessel, subdivided into 9 packs of 30 animals each, and transferred into refrigerated cell at 4 °C); Group B (oysters placed in the tank for a 48 hours short-storage, then they were subdivided into 9 packs of 30 animals each, and transferred into refrigerated cell at 4 °C); Group C (oysters placed in the tank prepared for a 48 hours short-storage, then subdivided into 9 packs of 30 animals each and placed back into tank for another

24 hours before being transferred into refrigerated cell). Packages were analysed at different times at cell transfer (t0) and 12 days (t12). As Control, 30 oysters were left in storage tank and other 30 oysters were maintained in long-line shellfish farm.

Results showed reduction of quality traits among the groups. Significant differences were shown at the end of the trial (t12). However, use of package, in which the oysters were placed horizontally with the lower valve positioned at the bottom and closed by clips, allowed to maintain constant temperature and humidity. In particular, B and C groups, application of a short storage in closed-circuit system allowed reduction of incidence of sand and mud inside the valves. No mortality was recorded in all the groups. Findings indicate that the availability of a land-based facilities for storage and final processing can be a solution to differentiate farmed product from the low price of unpackaged/loose harvested product.

## COMPARING THE EFFICACY OF THE PH-SHIFT PROTEIN ISOLATION PROCESS WITH THE CONVENTIONAL WASHING METHOD IN DEVELOPMENT OF MIXED SURIMI

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Focusing more on the aquaculture and underutilized resources as well as newer and more efficient technologies in recycling and reducing wastes are two solutions to answer the increasing need for seafood products. The present study was aimed to enable efficient use of valuable resources through the recycling of common tilapia and silver carp protein by pH-shift method and improve the quality of the surimi gel by manipulating factors such as optimizing blending condition. In this regard, the protein isolate was initially recovered from gutted tilapia and silver carp at acidic and alkaline pH using the pH-shift method. Then, their blending condition in the terms of pH-history (alkaline and acid), the time (before after protein precipitation) was investigated and its efficiency was compared with the conventional washing method. The results showed that blending proteins recovered from two species using both washing and the pH-shift method can

be a tool to improve the functional properties of tilapia surimi. However, the pH-shift method showed higher efficacy in production of blend surimi with desired functional properties at the same blending ratio compared to the conventional washing method. The pH-history and blending step significantly affected the characteristics of the blend surimi. Blending the protein isolates obtained from alkaline process version and before precipitation (at high pH value) further improved the viscoelastic and functional properties of the blend isolate, due to favourable conformational change and better mixing of the protein isolates.

## FISH FORWARD – SUSTAINABLE SEAFOOD FOR THE ENVIRONMENT, PEOPLE AND DEVELOPING COUNTRIES

### Danijel Kanski

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Fish Forward is a project launched during the European Year for Development, co-financed by the EU, for more environmental, social and economic sustainability in fish and seafood consumption.

In 2015, World Wide Fund For Nature (WWF) started a project in 11 member states of the European Union. A project goal is to increase consumer awareness of the global ecological and social consequences their fish consumption has. At 23% the EU is the main importer of fish and seafood in the world. Half of this comes from developing countries. Fishing and fish farming have direct effects on poverty alleviation and food security for more than 800 million people. An awareness campaign will show how our fish consumption can change the world: how each consumer can contribute to a more responsible global economy for humans and nature by independently and consciously buying sustainable products.

Vision of the project is to increase awareness of the relationship between fish consumption and its global

effects on the environment and people – especially in developing countries. This will be made through multiple steps, namely:

- To secure and improve the livelihoods of fishing communities in Europe and developing countries through responsible and sustainable seafood production and fish consumption.
- Informed and responsible citizens make informed buying decisions.
- Increased responsible buying decisions by European consumers for sustainable fish and seafood products – also from developing countries.
- Responsible and sustainable product ranges traded in fish and seafood.
- The implementation of legal conditions for the production of sustainable fish and seafood.
- Promotion of responsible and sustainable fishing and fish farming methods.

- Support efforts to end illegal, unreported and unregulated (IUU) fishing.

Project pillars:

- Improve understanding among consumers of the necessity to buy sustainably
- Presentation of living conditions of fishing communities in developing countries in multiple case studies
- Motivation and advice for the retail sector to source sustainable and responsible seafood products in their product range
- Promotion of laws and guidelines supporting legal, sustainable and responsible production of seafood
- Preparation of scientific studies



## BELGIAN BYCATCH FISH SILAGE AS FISH MEAL SUBSTITUTE IN SHRIMP FEED: FISH SILAGE CHARACTERIZATION AND VARIATION

**Mike van 't Land**

*Institute for Agricultural and Fisheries Research (ILVO) & Gent University, Belgium*

As of 2015, the EU is gradually implementing a landing obligation for all European fisheries, banning the practice of discarding undersized fish species. Fisheries will be prohibited from selling the undersized fish for human consumption. Therefore, together with the underutilized fish processing byproducts, a market needs to be found to valorize these raw materials. In Belgium, fishery byproducts currently end up as waste or biogas, resulting in the demise of valuable proteinaceous resources. Therefore, a scope determination was conducted to explore the prospects of utilizing byproducts from Belgian fisheries. During this project the production of fish silage for the aquafeed industry will be investigated in the form of a case study – locally produced fish silage as fish meal substitute in shrimp feed. A characterization was made of the quality and stability of fish silage produced from undersized Belgian bycatch. Also, the effect of species variation on fish silage characteristics was determined. Four types of fish silage were produced in triplicate using different combinations of commonly discarded species: plaice, sole, flounder and whiting. Species inclusion levels

were based on estimated discard ratios and variations thereof. Fish silage was produced using formic acid and stored for 3 months with periodic sampling. The low pH (4.1-4.5) created an antimicrobial environment and initiated enzymatic hydrolysis by endogenous enzymes. Initial results suggested that the produced fish silage was comparable to good quality fish meal, consisting of 68.2% ( $\pm 2.5$ ) protein (%DM). However, loss of organic nitrogen due to the hydrolysis process indicates a slight decrease in nutritional value over time, dropping to 60.7% ( $\pm 2.5$ ) protein (%DM). This is also reflected in the increase of total volatile nitrogen (37.4-128.4 mg/100g). Thus, subsequent experiments will focus on processing optimization to increase the stability and quality of the fish silage, followed by substituting fish meal with fish silage at several inclusion levels in *Litopenaeus vannamei* diets. This research is part of the GeNeSys project which incorporates system innovations along the complete value chain. The project strongly encourages transdisciplinary research and close stakeholder participation, allowing for a faster and realistic idea-to-market strategy.

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# POSTER ABSTRACTS

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## PROTEIN CHANGES IN SHELL AND EPIDERMIS OF SHRIMP (*Pandalus borealis*) AFTER MATURATION ON ICE OR IN SALT BRINE

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Mechanical peeling of boiled cold-water shrimps requires a preceding maturation period, a procedure that at the same time leads to reduced quality. It is hypothesized that during maturation the protein profile of the epidermis layer and shell of the shrimp is changed, mainly due to proteolytic activity and altered protein solubility, and that some of these changes facilitate the shell loosening that is required for mechanical peeling. The aim of this study was to obtain knowledge about the changes in the protein profile that occur during maturation of the shrimp.

Non-matured, frozen shrimps (n=20) and shrimps that were matured using two different procedures: ice maturation for 4 days (n=20) or maturation in 2% salt brine for 40-44 h (n=20) were compared. Proteins from shell and epidermis were profiled by 2D-gel based proteome analysis and proteins of interest were identified by tandem mass spectrometry.

Data analysis (ANOVA) of the 2D-gels revealed 136 protein spots in the epider-

mis and 120 protein spots in the shell differing significantly ( $p < 0.01$ ) between the three experimental groups. Some protein spots decreased (42 in epidermis and 50 in shell) and other increased (49 in epidermis and 58 in shell) in amount during maturation in either ice or salt brine or in both. Besides, 45 protein spots in epidermis and 12 in shell differed significantly between the two maturation procedures. The found protein changes were very substantial with 89 of the epidermal proteins and 87 of the shell proteins changing between 2 and 8 fold in amount during the maturation process. Identification of the interesting proteins is in progress and the results will be presented and discussed.

The following work will use this knowledge and correlate the changing protein profiles to peelability in order to select proteins that can be used as markers for optimizing the maturation process resulting in a higher peeling yield and higher final product quality.

## NON-INVASIVE VOLUME ESTIMATION OF FISH FILLETS/CUTLETS USING STRUCTURED LIGHT

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Measuring the volume of food products is a difficult task. It is laborious and requires interaction with the sample, which increases the risk of damaging or contaminating the sample. Especially, for processes where the sample continuously undergo volumetric changes (e.g. cooking), non-invasive methods are critical.

For this purpose, the computer vision technique known as *structured light* can be utilized. It is a relatively cheap setup that in its most basic form consists of a projector and a camera. By projecting structured patterns to a sample, spatial correspondences between the camera and projector can be established. For a calibrated camera-projector pair, simple triangulation can hereafter be used to estimate a depth map. If the investigated sample has a smooth continuous surface, which is a reasonable assumption for many fillet/cutlet products, the depth map can be used to estimate the volume of the sample.

The ability to non-invasively measure sample geometry allows for studies

where the geometry is continuously changing, e.g. quantifying shrinkage during heating or salting. Also, computer-guided filleting systems typically rely on traditional 2D images, and here additional depth information can be used for better decision making in order to minimize spoilage.

In the presented study, we seek to validate the precision of the technique on simple geometrical shapes of various sizes. Furthermore, we apply the technique on actual fish fillets/cutlets to estimate the volume and compare it to measures using the water displacement method. Finally, we present a proof-of-concept study regarding shrinkage estimation during heating of fish cutlets.

## ELECTRICAL CONDUCTIVITY OF REFRIGERATED SEA BASS MEAT IN SEMI-SOLID FORM AND AQUEOUS SOLUTION TO DETERMINE QUALITY

**Merve Taslak<sup>1</sup>, Can Altinelataman<sup>2</sup>, Yavuz Öztürk<sup>3</sup>, Kemal Demirağ<sup>4</sup>, Alper Erdem<sup>2</sup>, E. Burcu Şen Yılmaz<sup>2</sup>, Ufuk Çelik<sup>2</sup>**

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Quick, cheap, practical, and accurate methods are being developed on fish quality control. In this study, relationship between spoilage degree and electrical conductivity of refrigerated sea bass muscle was investigated. Fish material were supplied from fish farm, transferred to laboratory and refrigerated on same day. Electrical conductivity of samples was measured periodically by conductivity meter in three individual models and resistances of samples were measured by LCR meter. Models were set as;

1-Measuring conductivity of homogenized fish muscle (homogenization with plastic and steel knives in different concentrations).

2-Measuring conductivity of aqueous solution in which whole fish fillet was stored.

3-Measuring conductivity of aqueous solution where all drip loss of whole fillet was collected.

4-Determining resistance of homogenate with two different frequencies (20 and 100 kHz).

All results were compared to TVB-N (Total Volatile Basic Nitrogen) values and correlations were shown. Coefficients of determination ( $R^2$ ) between electrical conductivity and TVB-N, were found as; 0,964 for model 1 (with plastic knife), 0,798 for model 2, 0,933 for model 3 and 0,995 (at 100 kHz) for model 4. With this procedure, it was aimed to research applicability of practical and appropriate method for quality control of fish.

*Keywords: sea bass, electrical resistance, TVB-N, quality*

## THE EFFECTS OF NANOEMULSION ON BIOGENIC AMINE FORMATION OF VACUUMED PACKED SEA BASS

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Today shrimps are industrially peeled on automatic peeling machines after four to five days of maturation on ice. The TECHSHELL project is aiming at reducing the maturation time to one day, which will lead to an increased yield of 1%. Moreover, the CO<sub>2</sub>-emission will be reduced, as well as the expenses to ice and the shrimps will be of higher quality.

In order to be able to shorten the maturation period it is important to understand the quality changes that take place during the maturation. Thus, the changes in protease activity, in both the shell and in the epidermal layer between the shell and the muscle, during the ice were analyzed.

Moreover, video meter technology was applied to follow the color changes and a texture analyzer was used to measure the peelability during the maturation period. The peelability were measured both as the maximum force and the total work needed to separate the shell from the muscle.

It is shown that the general protease activity in the epidermal layer is rather

constant during the maturation period, whereas the activity in the shell decreases during the first three hours and then increases a bit again before dropping gradually during the fourth day. The video meter analysis revealed that spectrums for the non-matured shrimps and the four-day matured shrimps were significantly different, and in general the four-day matured shrimps had lost red color and appeared paler than non-matured shrimps. Texture analysis displayed less work and a lower maximum force needed on four-day matured shrimps compared to non-matured shrimps, indicating an increased peelability of the matured shrimps.

In conclusion, the industrial reasoning for ice maturation of the shrimps before automatic machine peeling is there. However, it also makes sense to shorten the maturation period in order to improve the color of the peeled shrimps. Furthermore, more knowledge about the protease changes during the maturation period is needed to understand the quality effects of these changes.

## THE EFFECTS OF NANOEMULSIONS ON FATTY ACID PROFILES OF VACUUMED PACKED SEA BASS AT 2±2°C

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The n-3 fatty acids (FAs) of particular interest for the prevention of cardiovascular disease (CVD) include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are found predominantly in fish and fish oils. There is also current interest from consumers and food industry in the application of natural ingredients in foods. The development of safe and effective natural ingredients to replace synthetic alternatives have been focused. Lipid oxidation process in fish meat may be retarded by the use of synthetic or natural antioxidants. Nanoemulsions have been attracting interest due to higher stability in terms of gravitational separation, flocculation, and coalescence of oil droplets and enhanced bioactivity of emulsified oils. This present study is focusing on the effects of commercial oils based nanoemulsion on the fatty acid profiles of vacuumed packed sea bass fillets during chill storage. This project was funded by the Scientific and Technological Research Council of Turkey (TÜBİTAK, TOVAG-1130379).

Nanoemulsions were prepared according to the method of Joe et al. (1999) with minor modification. Fish were

killed by use of hypothermia (dipping in ice-cold water). After death, the fish were transported to the laboratory in ice in one day. Fish were gutted, filleted and divided into seven lots. One lot (the control) was vacuumed packed and the other samples were treated with nanoemulsions. For each analysis day, 3 packages were randomly selected for each group and analyzed ten times (d 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18). The results showed that the oils contained mainly C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:0 (stearic acid), C18:1n9 (oleic acid), C18:2n-6 (linoleic acid), C20:5n-3 (EPA) and C22:6n-3 (DHA). There were significant differences in the fatty acid compositions of the control and treatment groups ( $P < 0.05$ ). Regardless of oil source used in this study, application of nanoemulsion in combination with vacuum packing maintained the PUFA content, especially EPA and DHA, of fish compared to the control. Hazelnut group gave the highest PUFA content, followed by canola and soybean at the end of storage period. These oils can be recommended for nanoemulsions as a preservative for fish.

## ANTIMICROBIAL EFFECTS OF *LACTOCOCCUS* ISOLATED FROM COMMERCIAL COLD-SMOKED FISH

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The lactic acid bacteria (LAB) is able to generate components including low-molecular-weight metabolites, weak organic acids, hydrogen peroxide, reuterin, diacetyl, bacteriocins that prevent the growth of pathogens in fermented food.

LAB strains were isolated from commercial cold-smoked fish. Total 235 colonies obtained from cold-smoked seafood products that resulted in the selection of 53 colonies presenting inhibitory activities. These isolates were identified as cocci and their fermentation ability by lowering pH. Antimicrobial activity of cell free solution of lactococcus isolates *in vitro* was investigated by disc diffusion assay.

Results showed that most of the selected strains were able to produce active compounds in cell free solution with antagonistic properties against indicator bacteria. Only 43 of lactococci isolates (81% of lactococcus isolates) revealed excellent inhibition zone diameters on the agar when *Staphylo-*

*coccus aureus* was used as indicator for preliminary detection of antagonistic activity (min 7.39, max. 14.41 mm). *Listeria monocytogenes* was used for secondary screening for antagonistic activity of these 53 strains. It was found that only 41 strains (75% of lactococcus strains) exhibited good inhibition zone diameters on the agar (min. 7.49, max. 13.40 mm), and 40 isolate (75% of lactococci strains) could inhibit *Klebsiella pneumoniae* (min 7.46, max 15.70 mm) as the third indicator following *Salmonella Paratyphi A* (42% of lactococcus isolates) (9.38-13.93 mm), *Pseudomonas aeruginosa* (49% of lactococcus isolates) (min. 7.76, max. 14.41 mm) and *Campylobacter jejuni* (39% of lactococcus isolates) (min. 8.40 max. 11.30 mm). The isolates exhibited widest zones of inhibition against *Escherichia coli* (25% of lactococcus isolates), *Pseudomonas aeruginosa* spp, *Staphylococcus aureus* (16% of isolate) and *Bacillus subtilis* (15% of lactococcus isolates). Tested pathogenic strains were inhibited by



selected LAB. The strain that showed best organic acid production and pH reduction ability has between 4:00 to 4:41.

The control of *L. monocytogenes* and other pathogens in cold smoked fish remain unclear, when compared with

free bacteriocin, organic acids from cell free solution elements and more studies are needed for clarifications.

*Key words: cold-smoked fish, cell free solution, lactococcus, antimicrobial activity, pathogen*

## NATURAL FOOD PRESERVATIVE: NISIN

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Lactic acid bacteria (LAB) have been recognized as a potential of biopreservatives due to the discovery of new food process and the desire by consumers for “natural” food products. Preservation of fermented food by LAB is due to sugars being converted to organic acids, (lactic, acetic) causing a reduction in pH and removal of carbohydrates as nutrient sources. In addition, LAB produce substances such as hydrogen peroxide, diacetyl, secondary reaction products, bacteriocins that have potential to inhibit harmful microorganisms. The bacteriocin nisin is a natural antimicrobial peptide, toxicologically safe, produced by some strains of *Lactococcus lactis* subsp. *lactis*. Although there are five nisin forms which are A, B, C, D and E, Nisin A is most active and appears to be the commercial compound. Nisin was certified by the Joint FAO/WHO Expert Committee on Food Additives for use as an antimicrobial in food. Nisin has a narrow spectrum of inhibitory activity on Gram-positive bacteria such as LAB, bacilli, and *Clostridia* and *Listeria monocytogenes* whereas it does

not generally inhibit the Gram-negative bacteria, yeast, or moulds.

Many natural antimicrobials such as nisin have a narrow spectrum of activity and are functioning only at very high concentrations. A feasible solution may be using mixtures of antimicrobials. The use of several antimicrobial compounds such as NaCl, proteolytic enzymes, EDTA, plant extracts, lactic acid which are combination with nisin may enhance the impact of the latter, which is more stable in acidic pH. Thus the activity of nisin against Gram-negative bacteria can increase. Potential use of nisin as bio-preservative has been studied in meat, dairy products or vegetable products, pasteurised soups, salad dressings, egg products, flour based products, alcoholic beverages, and seafood. The stability of nisin in a food system during storage is dependent on three factors that are incubation temperature, span of storage and pH.

Apparently, more studies on the effect of environmental alterations, interaction with food components and

potentiation by other food antimicrobials need to be approved with nisin and other bacteriocins for prevention of both foodborne pathogens and spoilage microorganisms.

*Keywords: Lactic acid bacteria, bacteriocin, nisin, bio-preservatives, food-borne pathogen*

## THE IMPACT OF NANOEMULSIONS BASED ON SUNFLOWER AND OLIVE OILS ON THE SHELF LIFE OF SEA BASS FILLETS STORED IN ICE

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Nanotechnology is a new technique used in food preservation. Nanotechnology focuses on the characterization, removal of the image, modelling, control, production, processing and modification of the structure of biological and non-biological less than 100 nm. Applications of nanotechnology in the food industry; the enrichment of nutrients in food and the development of new products can be categorized under the headings. One of the technological applications that will contribute to extend the storage life of foods is nanoemulsion. Nanoemulsions are known as antimicrobial preservatives since they connect the structure of water and thereby limit the access to water of microorganisms. Nanoemulsions have adverse effects on the structure and function on bacteria by destabilising the organism's lipid envelope.

The effects of nanoemulsion based on plant oils (sunflower and olive oil) on the sensory, chemical and microbiological quality parameters of sea bass (*Dicentrarchus labrax*) fillets stored in ice were investigated. According to

results of sensory analyses, the shelf life of fish fillets was found as 15 days for the control group and 18 days for nanoemulsions groups since nanoemulsion suppresses fishy odour. It was determined that the limit values of TBA did not exceed acceptable limits during the storage period. TVB-N values exceeded the limit values when product was not acceptable by sensory panel. TVB-N, TBA, PV, FFA, and pH values in the control were found to be higher than those of treated groups throughout storage period. According to the microbiological analyses, mesophilic aerobic bacteria counts were lower in the treated groups than those of the control group, indicating inhibitory effects of nanoemulsion on the growth of bacteria. Nanoemulsions based on sunflower and olive oils can be used to extend the shelf life of fresh fish.

## THE EFFECTS OF PACKAGING MATERIAL ON QUALITY CHANGES OF FISH PATÉ PRODUCED FROM SKIPJACK TUNA (*Katsuwonus pelamis*) DURING THE REFRIGERATED STORAGE

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Numbers of working women of the population have increased last decade thus the direct consumer's preference for ready-to-eat fish products has rose. Patties are a very popular and cheap cooked meat product manufactured worldwide. For this reason, impact of different packaging materials (white jar, dark jar and plastic can) on the sensory, chemical and physical quality of fish patties produced from skipjack tuna during refrigerated storage were investigated. Skipjack tuna divided into primarily 2 groups that were without any plant enriched group (C) and dill leaf-enriched group (DLE). After that each groups separated into 3 groups of packaging material as white jar (WJ), dark jar (DJ) and plastic can (PC).

All samples groups were stored in a refrigerator for 6 months at  $4\pm 1^{\circ}\text{C}$  after patties had been prepared from minced skipjack tuna meat. For quality changes, chemical (total volatile basic nitrogen (TVB-N), thiobarbituric acid

(TBARs), peroxide value (PV), free fatty acids (FFA), and pH as well as physical (texture and colour) analysis were determined.

The values of chemical analysis for all groups significantly increased during storage. Patties sample which were stored in glass jar have better values for TBARs, PV and FFA analysis than samples stored in plastic can. While PC groups rejected after 18 week of storage, WJ and DJ groups did not exceed the legal limits for consumption up to 24 week for mentioned analysis. Based on sensory evaluation, the shelf life of refrigerated fish patties was 20 weeks for control pate (C) group, while DLE enriched with dill leaf pate group resulted in a shelf life extension of 4 weeks. Comparing to packaging material, DJ group was mostly preferable group observed by panellist. Assessment of texture measurement, significant differences were found in plastic can and glass jar, especially hardness and juiciness parameters. Comparing

colour deterioration of patties samples, most important differences were found in DJ and the other groups. While dark colour of glass jar prevents lightness loss, values of redness and

yellowness increased rapidly for all groups. As a result, using glass jar improved the quality of fish patties compared to plastic can, especially dark jar.

## THE IMPACT OF TWO LEVELS OF CLINOPTILOLITE (1 AND 5%) ON THE FORMATION OF BIOGENIC AMINES AND AMMONIA BY FOOD-BORNE PATHOGENS IN TYROSINE DECARBOXYLASE BROTH

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Natural zeolites (clinoptilolite) are microporous and crystalline aluminosilicate minerals that retain and slowly release cations. Their low costs and nontoxic and heat stable properties make them attractive carriers for antimicrobial metal ions. Thus, zeolites' microbicide properties have a great interest not only in food pathogen application, but also in a wide range of applications against bacteria. The clinoptilolite can be used to decrease biogenic amines (BA) and ammonia (AMN) production by bacterial strains that are of health concern though dependent on bacterial strain and level used. Moreover, zeolite could be used as a natural additive to prevent the production of the most toxic BAs tyramine (TYR) and histamine (HIS), as well as cadaverine (CAD) and putrescine (PUT) produced by Gram negative food-borne pathogens

The influence of two levels of clinoptilolite (1 and 5%) on the production of BA and AMN by *Aeromonas hydrophila*,

*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella Paratyphi A*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Listeria monocytogenes* in tyrosine decarboxylase broth (TDB) was studied.

The impacts of zeolite on biogenic amines (BA) and ammonia (AMN) production differ among bacterial strains and the zeolite level used. Gram negative bacterial strains produced higher concentrations of AMN and BAs in TDB, and zeolite addition had more apparent effect, particularly for reducing AMN, putrescine (PUT), cadaverine (CAD), histamine (HIS) and tyramine (TYR) production. In Gram positive bacterial strains, the zeolite showed more stimulating effect on TYR production, but reduced CAD and PUT formation in three tested bacterial strains. *A. hydrophila* and *E. coli* produced the highest amounts of amines that was 1220 and 2630 mg/l, respectively. All strains were able to decarboxylate tyrosine to tyramine

(TYR) with *E. coli* being highest (>1600 mg/l). *A. hydrophila* formed >50 mg/l histamine (HIS) while the other strains produced none or very low concentrations (<4 mg/l). In addition, the strains were able to produce AMN (48.6-338 mg/l), spermine (SPM) (10.2-646 mg/l), agmatine (AGM) (3.86-167 mg/l), putrescine (PUT) (3.01-70.9 mg/l), cadav-

erine (CAD) (1.30-74.0 mg/l) and dopamine (DOP) (0.72-99.4 mg/l). Among Gram positive pathogens, *E. faecalis* was characterized as the main amine former (478 mg/l).

*Keywords:* Clinoptilolite, tyramine, histamine, biogenic amines, ammonia, food-borne pathogens



## SEAFOOD TOXINS AND THEIR HEALTH CONCERN

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Marine toxins have a great threat to human health, which are tetrodotoxin, palytoxin, yessotoxin, pfiesteria toxins, anatoxins, microcystins, nodularin, and domoic acid may be produced significant amounts by marine organism. Additionally, consumption of seafood contaminated by toxins results in different seafood poisoning syndromes including paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP), ciguatera fish poisoning (CFP) and azaspiracid shellfish poisoning (ASP). Tetrodotoxin (TTX) is a powerful neurotoxin found in pufferfish and other marine creatures as well as terrestrial animals. TTX binds to voltage gated sodium channels which blocks the flow of sodium ions through the channels producing respiratory and heart failure followed by death. Marine toxins are odorless, tasteless and cannot be eliminated by cooking or autoclaving. The poisoning after consumption of toxins-contaminated food is characterized by various clinical symptoms ranging from gastrointestinal discomfort (nausea, vomiting, diarrhea, and abdominal pain), through neurological disturbance (headaches, weak-

ness and confusion) to dermatologic effects (rash and itching) and cardiovascular distress (cardiac arrhythmia) and sometime in case of severe intoxication they can lead to death.

Marine toxins are formed in phytoplankton and bacteria, which transferred to higher trophic levels along with food chains. Diagnosis is based on clinical presentation (abdominal cramps, vomiting and gastro-intestinal disorders) and a history of eating seafood in the preceding 24 h. Thus, if the seafood is contaminated, intoxications cannot be removed using different processing techniques such as canning, marinating, and irradiation and salting. To prevent these disease, some measures have to be taken including extensive monitoring of toxins content in seafood and prohibition of the harvesting when toxic algal growth go beyond a certain level in marine environment. Moreover, more efforts should be provided concerning the toxins detection and monitoring in order to reduce the impact of seafood poisonings on human health.

*Keywords: seafood toxins, puffer fish, tetrodotoxin, shellfish poisoning, symptoms, treatments*

## THE EFFECT OF THE PRE-SLAUGHTER FEEDING/FASTING SCHEDULE ON *POST MORTEM* QUALITY TRAITS OF RAINBOW TROUT

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Few studies have investigated the influence of pre-slaughter feeding-fasting history of cultured fish on quality traits of raw fillets. Short pre-slaughter fasting periods (3-9 days) led to slight changes in Salmonid fillet quality, whereas longer starvation (35 days) resulted in improved pre-slaughter resistance to stress and delayed *rigor* (López-Luna *et al.*, 2014; Bermejo-Poza *et al.*, 2015; Mørkøre *et al.*, 2008). The aim of this study was to evaluate the effect of a 3-week pre-slaughter fasting or feed restriction period on the evolution of *post mortem* quality traits of fillet of large rainbow trout (*O. mykiss*). Fish groups, each consisting of 40 specimens (695 g) kept at 13.2 ± 0.1°C in 2.5 m<sup>3</sup> circular tanks, were fed a full ration (FR, 0.66% biomass day<sup>-1</sup>) or a restricted one (RR, 30% of FR) or were kept starved (R0) over three weeks before slaughtering. Fish were rapidly sacrificed by percussive and five fish per treatment were stored on ice for recording evolution of *rigor mortis*. Other fish were subjected to

biometry and dressing out yield measurements. Fillets were analyzed for proximate analysis and physicochemical traits over 72 h of cold storage (+4°C). Dorsal muscle samples were quickly excised and stored in liquid nitrogen until actin degradation analysis.

At slaughter, fish previously fed a full ration gained some weight (+14%) while a weight loss occurred in starved fish (-4.4%) and in those given a restricted ration (-1.6%). Fillet proximate composition was not affected ( $P > 0.05$ ) by the pre-slaughter treatment. A pre-slaughter restricted ration induced higher variability in fish size and certain quality traits such as WHC and texture. It also resulted in faster muscle actin degradation during the first *post mortem* hours ( $P < 0.05$ ).

Despite the weight loss and decreased muscle redness ( $P < 0.05$ ), pre-slaughter starved fish had higher slaughter yield, better muscle firmness after 24 h in cold, extended *pre-rigor* phase,

higher pH and improved WHC values after 7 h, than both full or restricted fed fish ( $P < 0.05$ ).

The present study suggest that an extended pre slaughter fasting period

could result in improved technological quality traits in rainbow trout.

## DEVELOPMENT AND EVALUATION OF MARINADE ON THE BASIS OF BITTER ORANGE WITH *Lactobacillus plantarum* O1 FOR BIO-PRESERVATION AND FLAVORING OF SEA FOOD

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Daily emergence of new aquaculture species and products on the world market is not motivated only by diversification of aquaculture products, but also by the demand for healthy products and safe food without the use of chemical preservation methods. Bio-preservation, as an innovative strategy, involves the use of natural preservatives which are safe for human consumption. Fish preservation by marinade is until now mainly used for the scombroid fish. A new product for flavoring and preservation of different types of fish and seafood with a specific organoleptic properties was developed during this study in the form of marinade on the basis of bitter orange (*Citrus aurantium* L.). The main preservative effect was achieved with *L. plantarum* O1 DSM32196 that was isolated from natural micro flora of sea bream digestive system. The preservation efficiency of different

concentrations of *L. plantarum* O1 DSM32196 in marinated sea bass fillets stored for two weeks at the temperature of +4°C to +8 °C was compared with the conservation efficiency of *L. plantarum* ATCC under the same storage conditions. Total count (cfu/g) of *Vibrio sp.*, *E. coli*, total coliforms, lactic acid bacteria and the concentration of histamine (mg/100 g) in marinated fillets were analyzed every day during the storage period for the efficiency evaluation. Furthermore, the pH of the meat and marinade was measured every day. The preservation efficiency of marinade that contained *L. plantarum* O1 DSM32196 was significantly higher in comparison to that which contained *L. plantarum* ATCC. The shelf life of this product was extended to two week period. According to the results of this study, the highest shelf-life can be achieved with the following formulation: 65-75% of 1.5-

2.5% brine solution, 20-25% bitter orange juice, 1.5-2% mixture of spices (dry leaf of laurel, *Laurus nobilis* L.; dry leaf of sage, *Salvia officinalis* L.; dry leaf of rosemary, *Rosmarinus officinalis* L.; black pepper, *Piper nigrum* L.; cayenne

pepper, *Capsicum annuum* L.; dry, row or extract of garlic, *Allium sativum* L.) and 1.5 – 2% suspension of *Lactobacillus plantarum* O1 DSM32196 in sterile physiological solution in concentration of  $1 \times 10^3$ - $1 \times 10^8$  cfu/ml.

## RESEARCH ACTIVITIES AIMED TO SUPPORT THE VALUE-CHAIN OF CANNING TUNA INDUSTRY: FROM TRACEABILITY TO SHELF-LIFE PREDICTION

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The requirements for compliance with current regulations, the demands of large-scale distribution, the opening of new markets and the increase in consumer demand, are the basis of the synergy between seafood industry and the research community, which is increasingly requesting support for the amelioration of the value-chain. As a part of collaboration with one Italian tuna processing industry, our laboratory worked in two areas of research applied to quality: the traceability of the product and the estimation of shelf-life of the canned finished product. For the action of traceability, a protocol for sampling and morphological relief on tuna rounds has been defined, which allows, applying a mathematical model, to track the original size of individuals of yellowfin tuna (*Thunnus albacares*) used for the production of semi-finished products. For the action of characterization of

the quality of the canned products, our research group has applied a specific sensory evaluation scheme for the product that, in conjunction with a series of multidisciplinary analyses, are allowing to make shelf life predictions, useful for commercialization.

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## INFLUENCE OF MODIFIED ATMOSPHERE, STORAGE TEMPERATURE, AND FATTY ACID COMPOSITION ON THE CARBON DIOXIDE SOLUBILITY IN FISH MINCE

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CO<sub>2</sub> has long been known for its inhibiting effect on many food spoilage bacteria. Thus, the use of CO<sub>2</sub> in modified atmosphere packaging (MAP) is a well-established method used to increase shelf life of foods. The influence of CO<sub>2</sub> on shelf life of foods is highly dependent on the amount of CO<sub>2</sub> dissolved into the product. Several factors has a significant influence on the solubility of CO<sub>2</sub>, including the initial CO<sub>2</sub> concentration of the packing gas, temperature, and the amount and type of fat present in the product. Despite being well studied separately, results on the interactions between these factors are limited.

To understand the influence of, and the interaction between, CO<sub>2</sub>, temperature, and fatty acid profile, an experiment using a model product based on silver smelt (*Argentina silus*) mince was designed, taking all of the above into consideration. The model product was added varying combinations steric-, oleic acid, and/or marine oil high in EPA/DHA. All samples were

prepared to an equal total fat content. The model product was packed using MAP with varying concentrations of CO<sub>2</sub> (balanced with N<sub>2</sub>) and stored at different temperatures for seven days.

The absorbed amount of CO<sub>2</sub> was evaluated by submerging the package under water and measuring the buoyancy force using a texture analyzer. The change in package volume could subsequently be related the amount of CO<sub>2</sub> absorbed. Furthermore, fatty acid profile analysis as well as differential scanning calorimetry was performed.

Both storage temperature and initial CO<sub>2</sub> concentration displayed a significant effect on the amount of absorbed CO<sub>2</sub>, having higher absorption with lower storage temperature or higher initial CO<sub>2</sub> concentration. This was seen for all groups. It was found that the different storage temperatures resulted in highly different development patterns during storage.

Samples containing the unsaturated marine oil reached the highest concentration of CO<sub>2</sub> through the entire storage period. These samples furthermore showed the smallest temperature dependency.

The analyses are still in progress and results will be presented at the conference.



## EFFECT OF SALT CONTENT AND TEMPERATURE ON THE COOK LOSS OF FRESH COD (*Gadus morhua*)

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It is common to prepare cod by simmering in salty water/brine. Consumers, however, prefer to minimize handling of fish and often start from packaged industrially prepared portions. This study describes the transfer from a recognized way of preparing cutlets in a salt brine, starting at 100 °C and leaving it off the heating plate (dynamic heating) for 10 min, to preparing it in a vacuum package at three different temperatures.

Sample cylinders (n=5 for each treatment) of fresh cod muscle (Ø =50 mm, H = 30 mm) were salted (10 % salt, 10 min) or not salted, vacuum packed (99%) in plastic bags and heated at 75, 85 and 95 °C at two different times at each temperature. Each time represented a specific pasteurization value = 2 min and = 861 min. In addition, a time series study was conducted at 95 °C, and cook-loss was measured at intervals from 2.5 to 60 min.

The core temperature was recorded both in the dynamic heating as well as in the constant temperature heating regimes, showing that the heating

process was very reproducible with respect to final core temperature.

The unsalted samples had a significantly higher cook-loss than the salted samples. For the unsalted samples there were no significant differences between the temperatures included in the experiment. The samples with = 2 showed significantly lower cook-loss than the samples with = 861.

A salt content of approximately 1.5% was achieved. Salted samples showed significantly lower cook loss than the unsalted samples, lower cook loss for = 2, and minimum cook loss at 95 °C.

The time series study showed an initial linear increase in cook loss from 2.5 to 15 min, with an approximate increase in cook-loss of about 1%/min and a leveling off at 20 % after 25 minutes.

These results can be used to optimize heating/preparation instruction for industrially prepared portions. The sample preparation and experimental setup can be of use to study effects of handling, storage and processing on the yield and final product quality.

## MAP TECHNOLOGY TO INCREASE THE SHELF LIFE OF REFRIGERATED SAI THE (*Pollachius virens*)

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Fresh fish are perishable and in order to prevent these negative changes, action to preserve the fish must be taken. Catabolic processes, in the form of autolytic reactions, will start at the point of death whereas specific spoilage organisms (SSO) will in most cases catabolize the fish spoilage. In addition, chemical decomposition is also significant. Modified atmosphere packaging (MAP) is an important way to increase the shelf life of food products, including fish. In MAP, a balanced concentration of CO<sub>2</sub>, N<sub>2</sub> and/or O<sub>2</sub> is normally used to reduce the growth of spoilage organisms (SO) and increase the shelf life of the product.

In the present study, analyses of microbiological growth, together with chemical and microbiological spoilage of fresh saithe (*Pollachius virens*) packaged in different atmospheres were conducted. The experiment was set up to investigate the effect of O<sub>2</sub> to increase the shelf life. High and low content of CO<sub>2</sub> was mixed with either high and low content of O<sub>2</sub> or N<sub>2</sub>, respectively. As a reference, vacuum packed fillet was used. The storage trial was conducted in a refrigerated room (4-5 °C) and continued

until three packages of each group were found not acceptable by a sensory panel judging fish odor. At each sampling day (3, 6, 8, 10 and 13 days post packaging) samples were analyzed for aerobic plate count (APC), psychrotrophic count (PC), hydrogen sulphid producing bacteria, lactic acid bacteria, Enterobacteriaceae, *Pseudomonas* spp. and *Brochothrix thermosphacta*. Moreover, samples were analyzed for degradation products of adenosine triphosphate (ATP) and contents of biogenic amines. In addition, pH and drip loss from the muscle tissue were measured throughout the shelf life period.

The results showed that a gas mixture containing O<sub>2</sub> were found to be positive for the shelf life of MA-packaged saithe. Discriminating factors were found to be; lower APC and PC, slower breakdown of ATP and lower contents of certain biogenic amines (putriscine, cadaverine and spermidine). Vacuum-packed saithe stand out negatively with highest drip loss and reduced shelf life and quality. We concluded that O<sub>2</sub> have a positive effect on the quality of MA-packaged saithe.

## THE IMPACT OF FREEZING AND THAWING ON A HIGH QUALITY FISH PRODUCT

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Freezing and frozen storage are efficient processing steps to ensure quality and to maintain long shelf life. The impact of freezing and frozen storage is well documented primarily for whole fish. In this work the effectiveness of freezing and thawing was studied and its impact on high quality vacuum-packed cod loin pieces.

Two different regimes (slow/fast) were used for both the freezing and thawing processes; (fast) blast freezing (-40 °C, circulating) vs (slow) conventional freezer (-20 °C, still air) and (fast) water-thawing (4 °C, circulating) vs (slow) air-thawing (4 °C, still air). In a full factorial design, we documented how freezing, thawing, double freezing and frozen storage impacts drip loss after thawing. The drip loss constitutes both the weight loss of the product and loss of possible valuable nutrients. The amount of drip loss is also an indication of how product quality is perceived by consumers in relation to meal preparation.

Our results show that drip loss after freezing and thawing are reduced

when the processes are effective (fast). If both processes are optimal the drip loss from a double-frozen loin-piece can be almost as low as a fresh (unfrozen) product. However, the quality gained through proper processing may be entirely lost in the last stage of processing (ineffective thawing) or frozen storage at elevated temperatures.

**COMPARISON OF THE PATHOMORPHOLOGICAL EVALUATION OF THE LIVER AND POSTERIOR KIDNEY IN RAINBOW TROUT (*Oncorhynchus mykiss*, Walbaum 1792) REARED BY TWO DIFFERENT TECHNOLOGIES**

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Introduction: Rearing the rainbow trout is usually done in a classical extensive technology with a fresh water flow (OS) and in intensive with water recirculation (RS). In both technologies the animal welfare is an important concern. Meanwhile the RS technology doesn't have a full scientific backing allowing for the acceptance by people concerned with bioethics and the healthy food. The morphological assessment of the liver and kidneys was to show which of the two organs and in which technology is more prone to structural changes.

Materials and Methods: The study was done during two years (in the spring and autumn) in 6 fish farms using the same fodder but different rearing technology – OS or RS. The studies were done on 960 trouts (n = 20) with a body mass of 350 - 500 g (A) and 501 - 850 g (B), with 480 trouts

from each rearing system. The liver and posterior kidney were fixed in 5% buffered formalin and the paraffin sections were stained with hematoxylin-eosin.

Results: The structure of the liver and the posterior kidney in majority of trouts was typical. The liver, especially in trouts B from RS technology, steatosis simplex, congestion and the presence of melanomacrophages were seen. Parenchymatous degeneration, necrosis of singular hepatocytes, lymphoid cell infiltration and extravasations were present much less often. In the posterior kidney most often infiltration of melanomacrophages and lymphoidal cells, congestion and parenchymatous degeneration were present. Other morphological changes were seen comparatively seldom. More lesions were seen in the liver than in the kidney. Morphological

changes were more often present in trouts B from RS technology in autumn.

Conclusions: The liver of the rainbow trout, both from OS and RS technology, showed more intense adaptive and regressive lesions than the posterior kidney. The intensity of changes was

higher in the intensive technology. It was especially well visible in autumn and in bigger trouts (501 - 850 g).

The participation was financed with funds from KNOW Consortium „Healthy Animal – Healthy Food” from the Ministry of Science and Higher Education – Grant 05-1/KNOW2/2015.

## QUALITY ENHANCEMENT OF CHILLED MEGRIM (*Lepidorhombus whiffiagonis*) BY THE EMPLOYMENT OF ALGA *Undaria pinnatifida* ETHANOL EXTRACTS

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Marine algae are known to be part of the diet in different Asiatic countries and constitute a source of beneficial nutrients, such as vitamins, trace minerals, lipids, amino acids and dietary fibres. Among them, *Undaria pinnatifida*, a brown seaweed reaching an overall length to about 60-100 cm, has deserved a great attention as being used for human nutrition as the “wakame” product. This alga, native to the Japan Sea and acclimated in a variety of localities all over the world has recently attracted a great attention for its antioxidant and antimicrobial properties. Flatfish (e.g., flounder, sole, turbot, plaice and halibut) species represent a very important seafood group. Among them, megrim (*Lepidorhombus whiffiagonis*) is abundant in the Northeast Atlantic waters. This species, which is considered one of the most fished species in the Grand Sole North Atlantic Fishing bank, has been exploited by a wide number of European countries. Due to the fast post-mortem deterioration of fish species, substantial efforts are needed for the optimization of the refrigeration systems employed on-board to meet

the increasing consumer demand for high quality and safe fresh products. The present research focuses the quality retention of megrim during chilled storage. In it, aqueous solutions including *U. pinnatifida* ethanol extracts were employed as icing media. The effects of the alga extracts on microbial activity inhibition and lipid oxidation stability were monitored in megrim muscle for up to 14 days of chilled storage. As a result, an inhibition of lipid hydrolysis (free fatty acid formation) and lipid oxidation (fluorescent compounds formation) could be obtained. Additionally, a partial inhibition on microbial activity development could be observed as a result of the *U. pinnatifida* presence in the ice system. Complementary analyses on this alga, showed a polyphenol content of 11±1 mg/g of lyophilised alga, antioxidant capacity of 27 % (DPPH assay) and antibacterial activity against *L. innocua* and *S. putrefasciens*. It is concluded that the icing medium proposed in this study may open the way to the development of a natural bio-preservation strategy for chilled seafood based on this popular alga.

## EFFECT OF BRINING AND FROZEN STORAGE ON PHYSICOCHEMICAL PROPERTIES OF WELL-FED ATLANTIC MACKEREL (*Scomber scombrus*) INTENDED FOR HOT SMOKING AND CANNING

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Atlantic Mackerel (*Scomber scombrus*) is a novel species in Iceland, caught during the heavy feeding period. The feeding has an impact on quality and stability of the products, which requires optimization of storage and processing conditions.

Physicochemical changes of brined and un-brined mackerel were analysed during frozen storage (6, 9, 12 months) at -18 °C vs. -25 °C with the aim of investigating the suitability of using well-fed frozen mackerel as raw material for canned and hot-smoked products. Heat treatments to a core temperature of 90 °C (representing canning) and 75 °C (representing hot-smoking) were applied.

Prolonged frozen storage showed negative effects on the raw material prior to heat processing due to an increased level of lipid oxidation, where

fish stored at -18 °C was of significantly poorer quality than fish stored at -25 °C. Moreover, the results indicated that heat treatment resulting in a core temperature of 75 °C showed higher water content, liquid holding capacity, heating yield as well as lower maximum shear force of texture compared to mackerel heated to a core temperature of 90 °C. Overall, analyses indicated that the fatty summer mackerel was well suitable for production of canned and hot-smoked products.

The present study demonstrated physical and chemical properties of mackerel as caught in Icelandic waters during the heavy feeding period and analysed its potential as raw material for high value canned and hot-smoked products as well as its stability during prolonged frozen storage.

## QUALITY PRESERVATION OF FISH BY SLURRY ICE

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It is well known that freshness of fish decreases rapidly after their catch. The quality decrease is irreversible and suppression of loss of freshness is always the most important subject for marine products. East Asian consumers prefer consuming fish as sushi and sashimi. In this case, freshness is the determinant quality factor. Slurry ice is approximately -2.5°C sorbet like ice made from seawater or salt water. Slurry ice consists of micron order small ice crystals made of pure water and weakly concentrated brine solution. We studied the effect of slurry ice to quality preservation of fish.

Slurry ice cooled pacific saury *Cololabis saira* rapidly to 0 °C, which was much faster than the case of traditional flake ice. Slurry ice is an efficient cooling medium for fish.

Live Atka mackerel *Pleurogrammus azonus* was immersed in slurry ice and energy related compounds of muscle were analyzed. Slurry ice treatment kept ATP content high and suppressed lactic acid production. It is reported excessive exercise accelerate ATP consumption and lactic acid accumulation in their muscles. Killing

fish by slurry ice have an effect to keep the freshness high.

For a global distribution of fresh fish, we conducted transportation test of pacific saury from Japan to Taiwan. Pacific saury caught off Hokkaido in Japan were chilled by slurry ice on the fishing vessel just after catching and sent to Taiwan by air in foamed polystyrene boxes filled with slurry ice. Fish were delivered to a Japanese restaurant in Taipei three days after catch. Temperature of fish was kept constant below 0 °C throughout the transportation. The freshness of the fish was sufficiently high for consuming as sushi. K-value of the samples was 7%, which was lower than commercially distributed pacific saury in Japan (17%).

These results indicated that strict freshness control from fishing vessels to markets by using slurry ice makes it possible to distribute high-value fresh fish in far area apart from fishing grounds.



## CHANGES IN OXIDATION STABILITY OF FISH OIL DURING THERMIC TREATMENT AND STORAGE UNDER POOR CONDITIONS

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Oxidation stability of cod liver oil during five hours cooking at 95 °C and poor storage conditions (direct sun exposure and high summer temperatures) over three months was determined. Deterioration of the oil and its oxidation stage were determined through analyses of peroxide value, *p*-anisidine value, thiobarbituric test, free fatty acids (FFA) and composition of fatty acids. Additionally, total oxidation value, nutritional lipid indices and uncertainty of listed analytical methods were determined. The results showed increasing oxidation of the cooked oils and high peroxide values (> 40 mmol O<sub>2</sub>/kg). This was not the case in oil stored in poor conditions; it showed an increase of *p*-anisidine value. Fatty acid composition did not vary much between the samples; however, dissolution of polyunsaturated fatty acids measured through polyene index was the highest (2.21) in oil sample cooked for five hours. The percentage of the most important omega-3 fatty acids, eicosapentaenoic acid (EPA)

and docosahexaenoic acid (DHA) was not significantly changed during thermic treatment nor poor storage conditions. The share of the total mono-unsaturated fatty acids in cooked oil samples decreased from the average 46% to 31% after 5 hours at 95 °C. Opposite to that, the total polyunsaturated fatty acid increased after 5 hours of cooking from an average of 32% to 46%. Relative standard deviation (RSD) of ten parallel analyses of oil samples was used to determine the uncertainty of methods. Analytical methods for peroxide value, *p*-anisidine value and thiobarbituric test showed more certainty at higher concentrations of analyte, while average RSD for FFA was 18.89%, which is relatively high thus >4 repetitions are needed to get accurate results.

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## PROCESSING EFFECTS OF NEWLY DESIGNED TOROIDAL CANS ON TEXTURE AND COLOR QUALITY OF CANNED TUNA

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A toroidal can design might be an innovation in canning to increase the heat transfer rate and to reduce production time for a better quality canned products including tuna. Toroidal cans enabled more than 30% reduction in the process time compared to the industrially applied process time.

Besides the possible advantage of reducing processing time for energy savings and decrease production costs, it was important to compare the quality control changes in the canned tuna. Since texture and colour quality are accepted to indicators of an extended thermal processing and possible heat damage to the quality of the canned foods, the objective of this study was to determine the effect of innovative toroidal cans on the textural and colour quality changes of canned tuna.

For this purpose, 160 and 1705 g standard industrial size cans were modified for toroidal geometrical shape, and computational – numerical studies were carried out with Ansys (Ansys Inc.,

Canonsburg, PA) to determine the required time to reduce the number of *C. botulinum* spores from  $10^{12}$  to  $10^0$  (for sterilization value of  $F_0=12 \times 0.21=2.52$  min) and the reduced process time for a still safe process. The numerical results were experimentally validated, and the textural and colour changes of the canned tuna were determined during storage of for months.

The results demonstrated that the instrumental texture parameters by TPA showed significant differences between the products processed in toroidal and standard cans. An Increase in lightness ( $L^*$ ), redness/greenness ( $a^*$ ) and yellowness/blueness ( $b^*$ ) was also observed in the toroid-canned product.

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## GENETIC AND PHENOTYPIC CHARACTERIZATION OF *Lactococcus piscium* FROM DIVERSE ORIGINS

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*Lactococcus piscium* is a psychrotrophic species belonging to the lactic acid bacteria group. Since the first description in 1990, more and more *L. piscium* have been isolated from food such as meat, seafood, vegetables, mostly packed under vacuum (VP) or modified atmosphere (MAP) and stored at chilled temperature. This species can have a bioprotective activity on seafood product and on the contrary be responsible of spoilage of meat and fresh salads. In this study, a phenotypic and genetic characterization of 96 strains of *L. piscium* from diverse origins was initiated in order to identify the determinants of positive or negative effect on food matrix. Growth kinetics were performed in controlled conditions of temperature, NaCl, carbon sources and oxygen and compared with our model strain CNC-MI-4031, isolated from salmon. Then, a MLST (Multi Locus Sequence Typing) approach was used to describe the phylogeny of the species. Five genes of the core genome were selected and sequenced. First results showed a remarkable phenotypic and genetic

diversity of this species and a genetic clustering of strains rather independent of strain origins.

## PRELIMINARY STUDY FOR THE DEVELOPMENT OF A PROBE-BASED FLUORESCENCE MELTING ANALYSIS FOR QUICK TESTING OF THE PRESENCE OF MORE THAN ONE SCOMBRIDAE FISH SPECIES IN FOOD PRODUCTS

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Scombridae fish are some of the species most consumed worldwide, mainly as tuna and bonito canned products. In Europe, the legislation required that tuna can must contain only one fish species that could be either *Thunnus* genus fish or skipjack tuna (*Katsuwonus pelamis*).

In the aim to quickly distinguish the presence of two or more tuna species in canned tuna, here we explored an assay using fluorescence melting curve analysis (FMCA) with a single locked nucleic acid (LNA)-TaqMan probe in its ability to simultaneously detect multiple DNA. Probe-based FMCA can detect DNA differences based on melting temperature (T<sub>m</sub>). Using LNA (modified RNA nucleotides with high specificity against native DNA targets) probes improves hybridization specificity between probe and template DNA, and increases T<sub>m</sub> significantly.

Two small regions of the mitochondrial cytochrome b (cytb) (112 bp) and cytochrome oxidase subunit 1 (COI) (82 bp) DNA were selected, and two LNA-TaqMan probes (corresponding to each gene fragment) were designed. Prior to FMCA it was necessary to amplify template DNA by PCR to acquire sufficient signal. Asymmetric PCR was performed to improve the hybridization of the probe on the 3' 5' fragments, using a *Taq* polymerase without exonuclease activity. Asymmetric PCR followed by FMCA analysis were done directly in a real-time PCR thermocycler.

The two LNA probes were 100% complementary to the target sequence of *Thunnus albacares* giving significantly higher T<sub>m</sub> value (respectively 76°C for cytb and 71°C for COI assays) than for other scombridae species tested (e.g. for skipjack tuna, T<sub>m</sub> value are 71°C for cytb and 46°C for COI). To differen-

tiate all the *Thunnus* species, skipjack tuna and the other scombrids, the two cytb- and COI-based assays were to be performed.

The results presented here are the first one acquired on DNA template

of reference scombridae species and they have to be validated on mixed reference tuna cans, and commercial cans.

## BIOLOGICAL VARIATION OF THE RAW MATERIAL AND PROCESSING CONDITIONS AFFECT THE YIELD AND QUALITY OF FAST-MARINATED HERRING

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One of the most important factors in a profitable production of fast-marinated herring (*Clupea harengus*) is the yield. In order to increase the yield and reduce waste, without compromising the quality, research and documentation of the marinating procedures in relation to raw material quality is needed. The existing processing conditions are mainly based on years of experience rather than documented investigations. Numerous studies concerning the marinating process of herrings have been conducted; however, most of these studies have only been executed at batch level, where it is assumed that the batches are more or less homogenous. It is well known that a great within-batch variation is found due to biological variation of the raw material. The biological parameters are important in relation to the total product loss, and are impossible in practice to uncover if the experiments are conducted in batches. The objective of this study is to investigate how the raw material and the different procedures (varying acid and salt concentration) affect the

product yield and quality of marinated herring. The herrings are tagged in order to track and investigate the effect of varying marinades, where the individual variation is taken into account. The herrings are subjected to different concentration of salt/acid brine and the weight change on single filet level is measured. One of the quality parameters is the amount of protein lost to the brine. Therefore, a fast method using NIR is developed to measure the total protein in the brine during the marinating procedure without destroying the sample. The underlying reasons behind the difference in product yield are investigated and the major conclusions are that the quality of the end product and the product yield both depend on the acid/salt concentration and the raw material characteristics. The study will ideally result in optimized marinating procedures, better utilization of the raw material and reduction in product waste leading to an increased profit for the herring industry.

## QUALITY OF FISH (*Sparus aurata*) TREATED BY COLD PLASMA

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Cold plasma processing is a new field of research in food processing. The main objective of this study was to determine microbial inactivation and possible quality changes caused by cold plasma treatment on the fish *Sparus aurata*. The variable input parameters were argon gas flow rate of (1, 1.5 and 2 L/min) and the treatment time (5, 10, 15 minutes). Experiments were designed using Central composite design (CCD). Quality changes were observed through analysis of lipid oxidation, water binding capacity, textural properties and color. Results showed reduction in the number of microorganisms (0.3 to 0.96 log CFU), with the largest reduction after the treatment of 5 minutes and the gas flow rate of 1.5 L/min. By increasing gas flow rate, color parameters L, a, b and  $\Delta C$  increased while, H value decreased. The analysis of lipid oxidation showed an increase in the TBA value after the treatment of 5 minutes at the flow rate of 1 L/min. The greatest drop in water binding capacity of 16% was measured after the treatment of 10 minutes and flow rate of 1.5 L/min.

Major changes in the textural properties of the treated fish, compared to the untreated sample, occurred after the treatment of 10 min and flow rate of 1 L/min, where was measured decrease of 46% in hardness, 13% of elasticity and 31% for the work.

## EFFECTS OF ORGANIC ACIDS ON PHYSICOCHEMICAL CHARACTERISTICS OF OCTOPUS MUSCLE

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Common octopus (*Octopus vulgaris*) is a cephalopod eaten mainly in Mediterranean, South American and Oriental countries. Marination in acidic solutions has been traditionally used as a way of meat softening and flavouring. The aim of this study is to investigate the effects of organic acid on physicochemical characteristics of octopus muscle. Before treatments head, skin, viscera and tentacles of the samples were removed and then cut into cubes of 4x4 cm. Octopus samples were immersed into the citric and lactic acid solvents (1 and 2%) and kept in a refrigerator (4°C) for 24 h. According to results organic acid treatments were effective on physicochemical properties of octopus muscle. Initial pH value of octopus muscle was 6.32. Lower pH values of octopus muscle treated with organics were found as compared to control samples. Water-holding capacity of octopus muscle significantly decreased after marination process. Muscle swells gradually as the pH decreases to below pH 4.5. Below pH 4.5, most of the thin filaments were extracted and the myofibrils fused together giving an amorphous, coag-

ulated appearance at pH 4.5. In this study pH of squid muscle marinated with organic acids did not reach critical values (below the isoelectric point). Decrease in water holding capacity is probably due to the denaturation of myofibrillar proteins, which play a role in water retention. Cooking loss in squid muscle significantly increased after marination with organic acids. The increase in cooking loss was observed in conjunction with a decrease in water holding capacity after marination. Lower total protein and free amino acid contents were found in octopus muscle treated with organic acids compared to untreated samples. In this study muscle pH in cephalopod muscles marinated with organic acids did not fall below 4.5 and probably expected protein degradation did not occur. So, total soluble protein and free amino acid contents of the samples did not increase. The Scientific and Technological Research Council of Turkey (TUBITAK) supported this study (Project number 213O163).



## ASSESSMENT OF STORAGE TIME OF FROZEN SQUIDS (Cephalopod: *Loliginidae*, *Loligo gahi*) BY IMPEDANCE ANALYSIS

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The aim of this study was to investigate the possibility of impedance analysis in the assessment of shelf life and quality control of frozen squids (*Loligo gahi*).

Frozen squid samples were kept in the freezer at -18°C during the period of 12 months and were examined every 60 days. The HP 4294A Precise LCR meter with needle-type multi electrode array was used to measure impedance magnitude ( $|Z|$ ) and phase ( $\varphi$ ) at 200 frequencies from 100 Hz to 100 MHz. Protein solubility, water holding capacity, color parameters and textural parameters, TBA, weight and sensors have also been determined.

The results showed that by the impedance measured at 10 MHz and 20 MHz the samples of frozen squid (*Loligo gahi*) can be grouped into 3 distinctive groups. However, impedance measurements, protein solubility, water holding capacity, texture and color parameters had no correlation with the duration of storage.

## EFFECT OF TEMPERATURE FLUCTUATION DURING TRANSPORTATION ON QUALITY AND STABILITY OF FROZEN MACKEREL PRODUCTS

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Atlantic mackerel (*Scomber scombrus*) is a valuable species in the marine industry. Mackerel is known for its wide-spread relocations and has become an important species in Iceland since 2006, when its catching rate increased. The majority of the catch was first used for fish meal and oil, and only a small portion of the catch was intended for human consumption. By finding ways to improve transportation and prolonged storage conditions will allow for the production of better and more valuable products, and lead to a more efficient use of mackerel catches.

Atlantic mackerel (*Scomber scombrus*) caught in Icelandic water was subjected to temperature abuse representing fluctuation (-15 °C for 1 month) followed by frozen storage at constant temperature (-25 °C) for 9 months. This interruption of the cold chain, products experience during transportation and storage from processor to customer. Furthermore, diversity in chemical composition of the raw material was

investigated, where mackerel caught during heavy feeding period (end of July vs. beginning of September) was analysed. Evaluation of physical quality attributes such as gaping and peritoneum deterioration was performed on fish fillets. Furthermore, lipid hydroperoxide (PV), thiobarbituric reactive substances (TBARS) and free fatty acids (FFA) level were measured for both constant and abused frozen storage temperature.

The temperature fluctuation resulted in quality loss, higher lipid oxidation and hydrolysis than fish stored at constant temperature during frozen storage. Moreover, results indicated seasonal variation in fatty acid composition of the mackerel, where fish caught in the end of July was characterized with higher amount of polyunsaturated fatty acids (PUFA) than fish caught in the beginning of September. According to the present findings, different storage temperature has a significant effect on the quality of frozen mackerel stored for the long term.

## ASSESSING THE QUALITY OF RAW COD (*Gadus Morhua*) USING MICROBIOLOGICAL, SENSORY AND CHEMICAL INDICATORS

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The objectives of this study were; (1) to develop a quality index (QI) for assessing the freshness of cod based on sensory attributes; (2) to examine the relationship between QI sensory score and the microbial status of the fish during storage and (3) to validate a chemical (ATP) based kit for assessing the freshness of cod (PRECICE® K-Freshness Assay Kit).

Fresh cod (*Gadus morhua*), obtained within 48 hours of capture, were stored on ice at 2°C, sampled at times t = 0, 2, 3, 6, 8 and 10 days and tested for total viable count (TVC) and total enterobacteriaceae count (TEC) using standard methods. In collaboration with a consumer taste panel (15 persons), a quality index was developed for both raw and cooked cod based on organoleptic attributes (appearance, odour, touch and/or taste). This panel scored raw and cooked cod against the attributes described in the QI at times t = 0, 2, 3, 6, 8 and 10 days. Finally, at each sampling time the 'freshness' of the stored cod was measured using the PRECICE® K-Freshness As-

say Kit, (which measures the products formed from ATP degradation including IMP, inosine and hypoxanthine) as per the manufacturer's instructions.

The initial TVC of 2.64-4.32 log<sub>10</sub> cfu cm<sup>-2</sup>, increased to between 6 and 7 log<sub>10</sub> cfu cm<sup>-2</sup>, after 10 days storage at 2°C. TECs remained at a low level throughout, reaching 1.5 log<sub>10</sub> cfu cm<sup>-2</sup> on Day 10.

## SHELF-LIFE EXTENSION OF FRESH SALMON (*Salmo salar*) USING ORGANIC ACIDS AND PHENOLIC COMPOUNDS PRESENT IN ESSENTIAL OILS

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Microbial spoilage is largely responsible for the short shelf-life (9-10 days) of fresh salmon (*Salmo salar*) resulting in limited access to export markets. Identifying suitable shelf-life extension technologies for fresh fish is an on-going industrial priority. Farmed salmon samples were treated by immersion in citric acid (1 & 5% w/v), lactic acid (1 & 5% v/v), citral (1% v/v), carvacrol (1% v/v), thymol (1% w/v) and eugenol (1% v/v) solutions for 30 seconds after which they were stored for 18 days at 2°C. At 3 day intervals, up to 18 days, samples were tested for total viable counts (TVCs), total enterobacteriaceae counts (TECs), lactic acid bacteria (LAB), Pseudomonads, H<sub>2</sub>S producing bacteria, *Brochothrix thermosphacta* and *Photobacterium phosphoreum*. The 5% lactic acid solution inhibited the growth of Pseudomonads and *Photobacterium phosphoreum*. Overall, using a microbial count cut off of 7 log<sub>10</sub> cfu/cm<sup>2</sup>, the 5% citric acid solution increased the shelf-life by

1-2 days whereas the 5% lactic acid solution increased the shelf-life by 2-4 days.

## STABILITY OF LIGHTLY SALTED COD (*Gadus morhua*) FILLETS DURING FROZEN STORAGE

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Lightly salted cod fillets entered the market about 20 years ago, as a substitute for dehydrated heavily salted cod. This product has been growing in popularity, especially in Spain. It is therefore important for the Icelandic producers to meet this increasing demand with high quality products. Lightly salting is a process that can be used to control utilization and nutritional status of whitefish fillets resulting in more uniform quality and increased storage stability. It can reduce fluctuations that are caused by seasonal changes in condition and characteristics of the fish muscle and therefore, have a positive effect on the processing of the fish. Lightly brining alone, is thought to have an effect on taste, texture, water holding capacity, color and shelf life of fish.

In present study, the storage stability of lightly salted cod fillets during frozen storage (-18°C and -25°C) was evaluated. The main aim was to explore the effect of different bleeding methods, size and age of the fillets on quality-related changes in the fish muscle during frozen storage. The

samples were received from processor A that uses a long line fishing technique and processor B that uses a bottom trawl fishing technique. Quality-related changes during frozen storage were measured using the following analysis: water holding capacity, glazing content, drip loss, color, cooking yield, proximate composition and enzymatic activity.

Fillets from bled fish had less drip loss and more whiteness compared with fillets from insufficient bled fish. The age of the fish before processing was also an important quality parameter. The results showed that large fillet size is more suitable for processing compared to small fillets, where the large fillets had higher lightness value and better cooking yield as well as total yield.

## PROCESSING FRESH MUSSELS (*M. galloprovincialis*) BY SOUS VIDE TECHNOLOGY: EFFECT ON THE MICROBIOLOGICAL CHARACTERISTICS

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*Sous-vide* cook-chilled (SVCC) is used to describe food that has been vacuum-packed and given mild heat treatment under controlled conditions of time and temperature and subsequently rapidly cooled and stored at chilled conditions until heated before serving (Rhodehamel, 1992; Hansen et al., 1995). Limited studies reported the application of this process in aquacultural products (Espinoza et al., 2016; Shakila et al., 2009), such as trout fillets (Gonzalez-Fandos et al., 2004), salmon (Garcia-Linares et al., 2004, Gonzalez-Fandos et al., 2005; Diaz et al., 2009) and carp (Can, 2011) and there are no reports on the opportunities to apply such process to molluscs. The aim of the present research was to test the effect of the application of the SVCC technique on fresh mussel microbiological characteristics. Commercial Mediterranean mussels (*M. galloprovincialis*) were individually inoculated with 100 µl suspension of *Pseudomonas* spp. (10<sup>7</sup> UFC/mL), packaged in a Oriented Polyamide/Polypropylene (OPA/PP, Orved S.p.A., Musile di Piave, VE, Italy) pouch and heat-sealed before being submitted to heat treatment in a steam oven (Lanoix Ali S.p.A., Treviso, Italy). After treatment, mussel pouches were im-

mmediately chilled at 3°C. Six different time temperature combinations (75/85/95 °C for 10 and 30 min), were tested in triplicate (3 specimens/group) and compared to raw and inoculated mussels. Total aerobic bacterial count (TBC), lactic acid bacteria (LAB), anaerobic sulphite-reducing clostridia, *Pseudomonas* spp. and pH were determined.

All time/temperature combinations resulted in a substantial reduction of the *Pseudomonas* spp. population (<1 log CFU/g) both in raw (4.9 log CFU/g) and experimentally inoculated (5.7 log CFU/g) mussels. The homogenate pH values (6.22) resulted not affected by the heat treatment. Such results were confirmed on natural bacterial load of mussel. A TBC reduction (2.4 log and 3,5 log, respectively) was registered after treatment at 85 °C both for 10 and 30 min, while the highest reduction (4.8 log) was observed at 95 °C for 10 min confirming the efficacy of *sous vide* cook treatments even for fresh mussels. The technological and sensorial traits, as well the stability of these products both under refrigeration and thermal abuse conditions during storage need to be evaluated.

## FISHBURGER FROM AQUACULTURED EUROPEAN SPECIES: NUTRITIONAL QUALITY AND FUTURE PROSPECTIVE

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European sea bass, gilthead sea bream, and rainbow trout are common species in the Mediterranean area, generally marketed as whole or fillets whilst few derived products are currently commercialized. After all, the recent economic crisis, the saturation of market, and the strong foreign competition are pushing seafood industry toward new marketing approaches such as product diversification. Recently, technologies such as mechanical separation process (MSM) have been demonstrated to be successfully applied to fish by-products for increasing processing yield. Nevertheless, scarce is the information on nutritional quality of MSM from species of interest for European aquaculture. The present study aimed to evaluate nutritional characteristics of fishburger obtained from mechanical separation process applied on damaged or out-size European sea bass, gilthead sea bream, and rainbow trout. Fatty acid profile, and mineral composition were determined. Besides, nutritional indexes (Atherogenicity Index, AI, Thrombogenicity Index, TI, and Hypocholesterolemic / Hypercholesterolemic Index, HH) were calculated. Despite the high percentage of fat contained in 100 g of fish muscle (more

than 8% in all the considered species), the characteristics of intramuscular fat are very interesting for human nutrition as a consequence of the predominance of PUFA $\omega$ 3, especially in seawater species. Present findings revealed that a portion (100 g) of MSM burger of sea bass and sea bream may provide 2150 and 1978 mg of EPA+DHA respectively (suggested intake: 250 mg per day) together with a  $\omega$ 3/ $\omega$ 6 ratio as 2.96 and 3.64. Trout instead would provide for around 700 mg EPA+DHA, but with a poor  $\omega$ 3/ $\omega$ 6 ratio (0.74). Very low AI and TI values were calculated for all the analyzed species. Burgers represented a good source of K and P, whereas the microelement selenium was found only in trace. Specifically, around 360 and 220 mg/100 g of K and P were quantified in seawater species, whereas trout contained approximately 440 and 217 mg/100g of K and P. In conclusion, mechanical separated meat from aquacultured species resulted in a high nutritional quality raw material for new products, such as fishburger. However, further investigations on consumers acceptance and preference are suggested together to the impact of different cooking methods on product characteristics.

## VALORISATION OF HORSE MACKEREL, A DISCARD FISH

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In 2012 global fish production (158 million tonnes) comes from fishery (58%) and aquaculture (42%). In Italy, only few species are commercialized and appreciated by consumers thus resulting in the production of high volumes of discards. Atlantic horse mackerel (*Trachurus trachurus*) despite its high nutritional value is one of the most underutilised species. Recently, mechanical separation process (MSM) has been demonstrated to be successfully applied in fish sector. Nevertheless, scarce information on its possible role in fish discard recovery are presented in literature. Thus, this study focused on the chemical changes, antioxidant content as well as oxidative stability of horse mackerel subjected to mechanical separation process and stored up to three months at -20 °C. Thirty-six horse mackerel were fished in the Tyrrhenian Sea. Fish were slaughtered by asphyxia in ice slurry. Immediately after death, twelve fish were minced by the MSM machine and utilised for obtaining 6 MSM burger (MSM samples), whereas the other were filleted. Twelve fillets (right) were stored as fillet (WF samples), twelve fillets (left) were grounded in order to obtain 6 fish-burger (FB samples). Samples for each

treatment were analysed at time 0 (T0), and after 90 days of frozen storage (-20 °C) (T90) for proximate composition, total lipids, fatty acid composition (FAs), primary (conjugated dienes) and secondary (thiobarbituric acid substances, TBARS) oxidation products, and antioxidant capacity (ABTS, FRAP, and DPPH). Some advantages and disadvantages emerged. Horse mackerel showed a low fat content (<1 g/100 g muscle), a high PUFAw3 content (around 57 g/100 g total fatty acids), especially EPA and DHA (around 52 g/100 g total FAs) regardless the treatment. However, polyunsaturated fraction increases its susceptibility to oxidation, as revealed by the high TBARS content at T0, calculated as 8 mg MDA-eq/100g muscle for WF, FB, and MSM. However horse mackerel had a high antioxidant power which may protect muscle against oxidative damages both during treatment and storage. Indeed no significant increase in TBARS content was found whilst at the same time antioxidant capacity significantly decreased. In conclusion, mechanical separation process seems can be applied without many detrimental effects on horse mackerel.



## CONSUMER TEST FOR SMOKED SALMON FROM DIPLOID AND TRIPLOID ATLANTIC SALMON (*Salmo salar*)

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The aim of the present study was to investigate if consumers could distinguish between cold smoked products produced from diploid and triploid Atlantic salmon (*Salmo salar*), respectively.

The interest of triploid Atlantic salmon in Norway have intermittently come up as a topic, most recently, as a consequence of the announcement of the 45 “green production concessions” in Norwegian aquaculture. Triploid salmon are in accordance with Norwegian law not considered as a genetically modified organism (GMO) based on the production method. Triploid salmon is produced by cell technology where pressure is used on fertilized eggs. During mitoses, pressurized fertilized eggs do not return to a diploid state and a triploid salmon is created. Consequently, triploid cells have one extra set of chromosomes.

The design for this test was a full factorial design with ploidity (diploid or triploid) and rearing temperature (5, 10 and 15 °C) as factors, giving six groups, and

the consumer test was performed as a triangle test. 10 fish of each group were used, giving a total of 60 fish (1.57±0.32 kg) and 120 fillets. All the fillets were covered with refined NaCl for 20 hours before they were rinsed in cold water to remove excess NaCl. Smoking was performed as 60 minutes of drying followed by 4 cycles of 50 min smoking and 10 drying. The fillets were vacuum packaged and stored in a chilled room for 28 days prior testing. A total of 144 consumers were divided into three groups (46, 48, and 48 consumers) testing salmon reared at 5, 10, and 15 °C, respectively. They were given two triangles, and asked to identify the odd sample in each triangle.

The consumers managed to distinguish between diploid and triploid cold smoked salmon reared at 10 °C (P=0.008). However, they did not distinguish between diploid and triploid salmon reared at 5 and 15 °C (P>0.263 and >0.344, respectively).

## MELANOSIS DEVELOPMENT IN DIFFERENTLY SULPHITE-TREATED SHRIMP (*Litopenaeus vannamei*)

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Melanosis in fresh crustacean rises faster than the microorganisms-related spoilage, being frequently the main rejection cause. Sulfite-based formulas are the most widely additive used to prevent melanosis. The search for an efficient dosage and mode of application of sulphites to fresh shrimp is a main goal to avoid exceeding the limits established by the legislative authorities. Pacific white shrimp *Litopenaeus vannamei* is one of the species with greater commercial interest. The objective of the present work was the assessment of melanosis development and residual content of sulphites in shrimp (*L. vannamei*) by comparing different treatments with metabisulphite and a commercial sulphite-based formulation (Nomelan). Pacific white shrimp undergoes a rapid process of melanosis in the absence of antimelanotics, 100% of individuals having melanosis in mild, noticeable or even severe degree in the third day of storage. Preventing the onset of melanosis in the carapace was shown to improve as the dosage of antimelanotic was increased. The average re-

sidual SO<sub>2</sub> content in muscle showed in general good agreement with the dosage level and melanosis prevention. At equal SO<sub>2</sub> concentration in the dipping solution, Nomelan Z500 produced fewer residues than metabisulphite. Dusting treatments tended to leave higher SO<sub>2</sub> residual levels in the muscle than dipping treatments, especially with the metabisulphite. When treatment was applied on live shrimp, melanosis progressed more slowly than if applied *post-mortem*, being the effect much more apparent the higher the concentration of SO<sub>2</sub> employed. *In vivo* treatment, however, produced higher sulphite residue in the muscle. Again, greater SO<sub>2</sub> retention with metabisulphite than with Nomelan was observed in *in vivo*-treated shrimp. Dipping treatment with Nomelan Z500 at 4% (w/v) for 20 min led to more than 95% of individuals free of melanosis on day 3 of storage with an average residual SO<sub>2</sub> content in muscle of 35 mg/kg.

## EFFECT OF HIGH HYDROSTATIC PRESSURE ON PHYSICOCHEMICAL GELLING PROPERTIES OF CRABMEAT

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The objectives of this work were to study the gelation of blue crab (*Callinectes sapidus*) meat as well as to increase its meat extraction yield. Two different processing methods were followed: A) extraction of meat after cooking and B) extraction of meat after hydrostatic pressure processing (HPP) at 100, 300 and 600 MPa during 5 min at 10 °C of raw whole crabs.

The crab meat was manually extracted after cooking or HHP and then passed through a strainer to remove the rest of shells. The meat was blended with 3% egg white, 0.5% NaCl and 0.5% MTGase and then it was gelled by applying two steps heating process: 40 °C/30 min+90 °C/30 min. Yielding, SDS-AGE electrophoresis, DSC and FTIR were performed on crabmeat before gelation and the quality of the gels was determined by the mechanical analyses.

Yield extraction increased from 18% in cooked meat to 21%, 25% and 27%

when 100MPa, 300Mpa and 600MPa respectively were applied. By SDS-PAGE it was observed myosin degradation in HPP samples in comparison with cooked one. Increasing HPP also resulted in a reduction of denaturation temperature and enthalpy of crabmeat myosin and an increase in the amount of random structures and  $\beta$ -turns as well as a reduction in  $\beta$ -sheet as compared to cooked crabmeat indicating protein unfolding. Crab meat pressurized at 100 MPa, with more preserved myosin structure, rendered gels with the highest values of hardness, springiness and cohesiveness.

All these results suggest that 100 MPa can be applied on blue crabs to meat extraction, as a commitment treatment between maximizing the meat extraction and maintaining the protein functionality to get gels with adequate texture.

## SHELF LIFE OF SKREI (*Gadus morhua* L.) FILLETS STORED AT REFRIGERATED CONDITIONS

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Skrei is a spawning Norwegian arctic cod (*Gadus morhua* L.) caught off the Norwegian coast in the period from January to April. The Norwegian Seafood Council aims to define a Norwegian standard characterizing the trademark for Skrei fillets. The objective of this trial has been to study the shelf life of Skrei fillets in response to different time and temperature conditions during processing and distribution. Parameters defining shelf life have been off-odor according to the method of fillet index and level of total volatile basic nitrogen (TVB-N).

Initially, surface- and core temperatures of fish were studied during landing, processing, distribution and display in supermarkets in Hamburg, Germany and Tromsø, Norway. The results show that a short period with temperatures of 5-6 °C in fillets during processing did not affect the odor or the level of TVB-N 4 days post *mortem*. During distribution, the level of ice melting correlated with level of exposure in the pallet. However, despite the registered variations under transport, the core temperatures in all fillets was near -1 °C. In contrast, the

measured core temperature in fillets purchased from retailers in Hamburg and Tromsø, varied from 3,9 and 6.8 °C.

Secondly, shelf life experiments were performed imitating temperature profiles observed in the initial phase of this study. In addition, shelf life at other relevant temperature profiles was studied. The results show that to obtain a shelf life of 12 days of Skrei fillets, storage temperature of 0 °C is required in all steps. In fact, a raise in temperature of fillets to 2 °C after an initial storage for 5 days in ice resulted in TVB-N levels way above the recommended level of 35mg N/100g after 12 days of storage.

In our observations, the greatest challenge to obtain a shelf life of 12 days for Skrei fillets is maintaining a continuous low temperature of 0°C when displayed for sale. Therefore, our recommendation is to focus on development of suitable solutions to maintain the initially high quality when presenting the fish for sale at the retailers.

## **SENSORY AND CHEMICAL CHANGES IN CARP (*Cyprinus carpio L., 1758*) FILLETS MARINATED WITH SUNFLOWER OIL, SUNFLOWER OIL PLUS POMEGRANATE SAUCE AND SUNFLOWER OIL PLUS PLUM SAUCE DURING STORAGE AT +4OC FOR 1 MONTH**

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Sensory and chemical changes in carp (*Cyprinus carpio L., 1758*) fillets marinated with sunflower oil, sunflower oil plus pomegranate sauce and sunflower oil plus plum sauce during storage at 4°C for 1 month were investigated. Appearance, odour and taste, and texture parameters in carp fillets was found as good quality during storage period. TVB-N showed fluctuation for all groups during storage and remained below 10 mg/100 g. Pomegranate sauce resulted in significant changes in TVB-N content in carp fillets ( $P<0.05$ ). Free fatty acids

(FFA) increased with storage time for all groups. Carp fillets treated with pomegranate sauce had the lowest FFA content at the end of storage period ( $P<0.05$ ). Increases in TBA values in carp fillets were low and remained below 0,6 mg MA/kg. Among groups, the highest peroxide value was found in carp fillets treated with sunflower oil plus plum sauce at 30 days (9.25 meq O<sub>2</sub>/kg).

*Keywords: marinade, quality, pomegranate sauce, plum sauce, storage, common carp*

## CONTROL OF LIPID OXIDATION IN FRESH FISH THROUGH THE INCLUSION OF BARLEY ON RAINBOW TROUT (*Oncorhynchus mykiss*) DIET

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The health benefits of fish consumption have exacerbated the demand and interest for fish during the last decade. Mainly fatty fish species, rainbow trout is an interesting example of source due to high levels of essential long chain n-3 fatty acids (FA): EPA (20:5 n3) and DHA (22:6 n-3). However these lipids are high susceptible to oxidation leading off-flavours, secondary metabolites and reducing the nutritional value of the fish quickly. Diet composition has a significant effect on fish quality. In this sense, a new ingredient on the diet could modify the quality of the fish and control the fatty oxidation which alters its value for human consumption and market appreciation. It seems that quality attributes of fish fillets are correlated with fat content and lipid class in tissue. Although a clear relationship normally exists between dietary composition and fish muscle fatty acid profile (FA),

lipid metabolism and accumulation in fish, it may significantly affect fish fillet quality such as sensory characteristics and lipid stability. Considering the inclusion of barley as new ingredient on rainbow trout diets, barley is a cereal with and added potential of improving fish health due to the high  $\beta$ -glucan content and antioxidants present on their composition. The objective of this study was to evaluate the effect of barley, used as feed ingredient, on the FA profile and on the control of oxidative parameters of fresh rainbow trout fillets.

During 12 weeks rainbow trout were fed in a monitored and controlled room using recirculation system at a temperature of  $16 \pm 0.7$  °C and  $6 \pm 0.34$  mg/L of oxygen. Fish were randomly allocated in 20 tanks with 25 fish per tank. Rainbow trout were fed 5 isoproteic and isolipidic diets (45 % CP and 18 % CF). Diets contained

increasing levels of barley (0-31,8 %). Each four weeks, samples were taken to study FA profile and  $\alpha$ -tocopherol content of fish fillets and different oxidative parameters: peroxide value (PV), diens, triens, TBARS. A sensory analysis was carried out to determine the effect of these oxidative parameters and correlate with objective measurements. Data were analyzed using SAS software by one and two-way ANOVA followed by a t-Student test and considering significant differences between values with a p-value < 0.05.

Compared to control diet, the FA composition of fish fillets fed with barley showed at the end of the trial a slight increase on SFA and MUFA. The inclusion of barley significantly decreased PUFA, n-3 and n-6, although significantly increased n-9. No significant differences were observed on  $\alpha$ -tocopherol content. Regarding oxidative parameters, no significant differences were found on diens and triens between experimental treatments. Trouts fed with higher concentrations of barley on the diet showed lower level of TBARS on fillet than those obtained from trouts fed with lower concentrations of barley. Panelists detected significant differences in the colour (luminosity and redness) associated with the concentration of barley, samples from fish fed higher barley con-

centrations showed lower rancidity perception in sensory analysis.

The results suggest that increasing concentration of barley in trout diet slightly modify the PUFA content but reduced the metabolites associated with oxidation such as PV and TBARS which improve fish fillet perishability.

## PROXIMATE AND FATTY ACID COMPOSITION OF THIRTEEN IMPORTANT FRESHWATER FISH SPECIES IN THE CZECH REPUBLIC

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We aimed to investigate proximate and fatty acid composition of important freshwater fish species in the Czech Republic. Sampled fish include seven species from intensive farming: African catfish, rainbow trout, wels catfish, Nile tilapia, brook trout, northern whitefish and pikeperch; eight species from semi-intensive culture systems: common carp, northern pike, pikeperch, grass carp, European perch, tench, silver carp and catfish; and three species from extensive culture systems: rainbow trout, tench and common carp.

The results of this work confirmed that the fat content and its fatty acid (FA) composition are highly influenced by the type of culture system especially by the diet. Intensively cultured fish reached the highest content of eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acid. The omnivorous common carp ( $7.62 \pm 2.92$  %) and herbivorous silver carp ( $6.87 \pm 1.37$  %) had the highest fat content from semi-intensively cultured fish. On the other hand, the carnivorous fish showed low fat contents usu-

ally around 1%. The fish from extensive farms were more balanced in their lipid content (around 3% for all species). Simultaneously we observed a significant dependence of FA composition on the fat content. The lean carnivorous fish contained lower amounts of EPA + DHA in comparison with the fatty northern whitefish containing 17.83% (intensively cultured). The content of saturated FA was below 34% in all analyzed fish. The highest content of monounsaturated FA was found in common carp ( $52.71 \pm 7.75$  %), and the lowest in European perch ( $17.83 \pm 1.12$  %). Northern pike, pikeperch and European perch contained with over 50% the highest proportion of polyunsaturated FA.

Nutritional quality was determined by: atherogenic and thrombogenic indexes which ranged from 0.27 to 0.63 and 0.20 to 0.61 and by ratios n-3/n-6 (0.54–3.45) and polyunsaturated/saturated fatty acids (0.67–2.01). Results demonstrated that the flesh of all studied species are of high nutritional quality.



## SEASONAL CHANGES IN THE CHEMICAL COMPOSITION OF THE BEADLET ANEMONES (*Actinia equina* Linneaus, 1758) FROM MERSIN BAY IN THE NORTHEASTERN MEDITERRANEAN SEA

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In this study, the effects of seasonal variation in nutritional compositions and fatty acid profile as a component of chemical compositions of Mediterranean Sea anemone species (*Actinia equina* Linneaus, 1758) living in Mersin Bay in the Northeastern Mediterranean Sea were investigated. Nutritional composition analysis of anemone samples showed that while the highest levels of protein and moisture were obtained in winter, the highest lipid and ash levels were obtained in autumn. These highest values for protein, lipid, moisture and ash were obtained as 13.07 %, 1.61 %, 81.67 % and 3.96 %, respectively. In terms of fatty acid analysis, during all four seasons the dominant saturated fatty acids (SFA) were palmitic (C16:0) and stearic acids (C18:0), the dominant monounsaturated fatty acids (MUFA) were oleic (C18:1n9) and vaccenic acids (C18:1n7) and the dominant polyunsaturated fatty acids (PUFA)

were linoleic acids (C18:2n6), linolenic acid (C18:3n3), gamma linolenic acid (C18:3n6), arachidonic acid (C20:4n6), eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, C22:6n3) for Mediterranean Sea anemones. While the highest values of palmitic acid, stearic acid, oleic acid, vaccenic acid and linoleic acid were obtained in spring as 9.27 %, 7.70 %, 2.64 %, 8.48 % and 2.61 %, respectively, the highest values of linolenic acid was obtained in both autumn and summer as 0.30% and the highest values of arachidonic acid was obtained in both spring and summer as 0.61%. In addition, the highest values of gamma linolenic acid, EPA and DHA levels were obtained in autumn as 0.44%, 14.83% and 14.10%, respectively.

## POPULATION AND GENETIC STRUCTURE OF *Anisakis* spp. FROM FISH LANDED AT LOCAL MARKET OF ISLAND OF KORČULA, ADRIATIC SEA

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A high anti-*Anisakis* IgE seroprevalence was detected previously in the healthy Croatian southern islands' population. In order to understand the potential source of antigen to inhabitants, we investigated a random sample of most frequent fish species landed at fish market in the Island of Korčula, area where the highest seroprevalence was observed. Additionally, we sampled damselfish and picarel, fish that are traditionally consumed freshly marinated only in this region. Statistical and genetic analyses were performed by grouping *Anisakis*-infected fish according to their feeding habits; large, smaller and benthopelagic pelagic predatory fish, and small bottom-feeding fish, respectively. No statistically significant difference was detected among each group pair in terms of prevalence, abundance or intensity of infection. The highest prevalence was recorded in dolphinfish and little tunny (100% (95% CI 36.85-100.00) and 100% (95% CI 47.29-100.00), respectively). Anisakidae community from this area consisted of *Anisakis pegreffii* (96.4%), *A. simplex* sensu stricto (3.17%), *A. typica*

(0.19%) and *A. zyphidarum* (0.19%) inferred by a single mitochondrial DNA locus cytochrome oxidase (cox2). In respect to the potential source of anisakid antigen for the high anti-*Anisakis* IgE seropositive population of Island of Korčula, the incidence of larvae in the European hake fillets (mean intensity 2.22 (bootstrap 95% CI 1.56-3.56)) is indicative as a factor in such epidemiology. This work was fully supported by Croatian Science Foundation (Hrvatska zaklada za znanost, HRZZ), grant number 5576, AnGEI: *Anisakis* spp.: genomic epidemiology.

## QUICK INSIGHT INTO ANTI-*Anisakis* SEROPREVALENCE IN PROFESSIONALS EMPLOYED IN FISHERY INDUSTRY OF SPLIT-DALMATIAN COUNTY, CROATIA

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Epidemiological study conducted on healthy population in Split-Dalmatian County in 2014 revealed that total anti-*Anisakis* seroprevalence amounted to 2%. The highest levels were observed in subjects from southern islands that answered positive for daily and weekly consumption of thermally unprocessed fish. In order to have an insight in the anti-*Anisakis* IgE status of professionals employed in mid Dalmatian fishery facilities, we distributed questionnaires and took blood samples for Western Blot (WB) analysis of sera. The study has been approved by the Ethical Committee of Institute of Public Health. In total 295 blood donors working at four canning factories responded to the questionnaire. Among these, a subsample of 30 sera was chosen based on responses collected from the questionnaire. Selected samples encompassed sera of a) individuals that showed allergic symptoms that increment after their working shift; b) individuals with allergies that were not exacerbated during work; c) healthy individuals without allergy or other clinical signs of disease (N=10), representing a control

population, of which 4 consumes fish regularly and the rest does not. WB was performed using a fresh homogenate of *A. pegreffii* infective third-stage larvae as antigen, while circulating anti-*Anisakis* IgE antibodies were detected by secondary monoclonal anti-human IgE antibody. Detection was visualised by reaction of NBT-BCIP (Nitro blue tetrazolium-5-bromo-4-chloro-3-indolil-phosphate) with alkaline phosphatase conjugated to the secondary antibody. In total 5 males (age 20-45 years) and 25 females (age 26-56 years) were tested, of which allergic individuals suffered from bronchitis (27%) and urticaria (20%). Results evidenced that 14/ 30 individuals had at least one of 14 known *Anisakis* allergens, while 7/ 14 showed antibodies for at least 1 of 4 major allergens (Ani s 1, Ani s 2, Ani s 7, Ani s 12). This study will be further evaluated using indirect sandwich ELISA, but *Anisakis* allergy needs to be seriously considered as a professional risk in fishery industry. This work has been fully supported by Croatian Science Foundation (HRZZ), grant number 5576, AnGEL: *Anisakis* spp.; genomic epidemiology.

## DEVELOPMENT OF ALGAL OIL SUPPLEMENT: EFFECT OF FLUIDIZED BED DRYING OF ALGAL BIOMASS ON THE LIPID FRACTION AND FATTY ACID COMPOSITION OF *Nannochloropsis oculata* OIL

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In recent years, oils rich in omega-3 polyunsaturated fatty acids ( $\omega$ -3 PUFA) have attracted much attention because of its recognized beneficial effect on human health. At present, fish oil is the major source of omega-3 fatty acids, but it may be produced by microalgae with additional benefits. Marine microorganisms contain large amounts of  $\omega$ -3 PUFA and are considered a potential source of these important fatty acids. Since *Nannochloropsis* sp. alga has been known as source of  $\omega$ -3 PUFAs, drying of wet algal biomass and extraction of  $\omega$ -3 PUFA rich oil processes represent the most critical steps in terms of biological value and energy efficiency. Drying processes evaporate the water from wet biomass. But during drying, some termolabile compounds such as  $\omega$ -3 PUFAs could be oxidized. The purpose of this research was to investigate influence of fluidized bed drying treatment on the PUFA profile of lipid fractions of algal oil. After the harvesting and concentration, wet biomass of *Nannochloropsis oculata* was dried in freeze and fluidized bed

dryer. Lipid fractions of algal oil were determined in wet and dried biomass. Effect of drying process on the  $\omega$ -3 PUFA content of lipid fractions were also determined by the lipid fraction and fatty acid profile analyses.

Results showed that crude lipid of algal oil consisted of 42.41 % neutral lipids (NL), 40.12% phospholipids (PL), and 17.47% glycolipids (GL) fractions. Fluidized bed drying of algal biomass didn't affect ratio of lipid fractions whereas fatty acid profile of lipid fractions was slightly affected fluidized bed drying process. Crude lipids of *N. oculata* and lipid fractions had a high content of PUFAs, followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). EPA (C20:5 *n*-3), an important *n*-3 fatty acid, was the dominant PUFAs in all lipid and its fractions of *N. oculata*, whereas the main SFA and MUFA were palmitic acid (C16:0) and palmitoleic acid (C16:1 *n*-7), respectively. Fluidized bed drying can be served as an efficient and economic drying method without any nutritional losses for the production of algal oil supplement.

## EFFECTS OF NATURAL EXTRACTS ON FATTY ACID PROFILE OF VACUUMED PACKED FISH BALL MADE FROM MACKEREL (*Scomber scomber*) DURING CHILLED STORAGE (4±2°C)

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Lipid oxidation shortens the shelf life of food and leads to its qualitative deterioration. Lipid oxidation is increased by various factors such as the presence of enzymes, pro-oxidants, high temperature, moisture, and trace metals. Addition of antioxidants can avoid oxidative deterioration of lipids and nutritional and organoleptic loss. Therefore, there is an increasing in consumer demands for the replacement of synthetic antioxidants by natural ones. Medicinal herbs for instance are well known for their antioxidants action due to the presence of various components including phenolic acids, flavonoids and carotenoids. The current study focuses on the effects of the addition of 0.05% of natural antioxidant extracts, obtained from thyme (*Thymbra spicata*), rosemary (*Rosmarinus officinalis*) and basil (*Ocimum basilicum* L.) on the fatty acid profiles of vacuumed packed mackerel balls. The results showed that the proportion of total saturated fatty acids (SFA) increased in all groups at the end of the storage, while the pro-

portion of polyunsaturated fatty acids (PUFA) decreased. Treatment of fish ball with natural extracts resulted in significantly ( $p < 0.05$ ) higher PUFA and monounsaturated fatty acids (MUFA) concentration during the storage period. Based on the results, natural extracts especially rosemary and thyme can be recommended to add in fish ball to prevent oxidation of PUFA (particularly EPA and DHA).

## CHROMATOGRAPHIC ANALYSIS OF BIOGENIC AMINES IN SEAFOOD

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Due to health risks and consumers demand for fishery products analysis of biogenic amines especially histamine, cadaverine, putrescine and tyramine became very important. These compounds are generally associated with the deterioration of seafood. As a result of decarboxylation of free amino acids, biogenic amines are formed. Biogenic amines have importance for many physiological functions but in high concentrations, they can have toxicological problems such as migraines, headaches, allergic responses and intestinal problems. Also these compounds can be used as a quality indicator. The European Union considered the maximum level of 100mg/kg histamine content for fish. Various methods can be used in extraction of biogenic amines. Due to complexity of seafood matrices extraction of biogenic amines is the most important step. In extraction procedure pH is very important for good recovery. After extraction step derivatization process or ion pairing methods can be applied. With different chemical reagents pre or post column derivatization process can be used (eg., dansyl

chloride, O-phthaldehyde) to determine biogenic amines. Also numerous instrumental methods are available for detection biogenic amines such as liquid (Fluorescence, UV and mass detectors), gas chromatography (flame ionization and mass detectors) and capillary electrophoresis.

## **MORGANELLA PSYCHROTOLERANS, A HISTAMINE-PRODUCING BACTERIUM IN SEAFOOD**

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Histamine production in seafood is important and responsible for scombrototoxic fish poisoning. Furthermore, histamine in food is of great interest not only for the potential risk to human health but also because it could have a role as chemical indicator of unwanted microbial contamination and processing conditions. Histamine is produced by the decarboxylation of free histidine, and results in the activation of either endogenous or bacterial histidine decarboxylase. Many different bacterial species are known to possess histidine decarboxylase and have the ability to produce histamine. *Staphylococcus spp.*, *Tetragenococcus muriaticus*, *Clostridium perfringens* and some strains of *Lactobacillus spp.* are among the best known gram-positive histamine producers. Furthermore, a wide range of gram-negative bacteria isolated from seafood is able to produce histamine. Members of the mesophilic Enterobacteriaceae are often identified as histamine producers. Today, it is clear that both mesophilic bacteria including *Morganella morganii*, *Hafnia alvei* and *Raoultella planctica* and psychrotolerant bacteria in-

cluding *Photobacterium phosphoreum* are important for histamine formation in seafood. Among many bacterial species in fish, *Morganella morganii* has been reported to be the predominant histamine-producing bacteria. However, recent studies of histamine fish poisoning (HFP) in Japan and Denmark showed the psychrotolerant histamine producing bacteria *Morganella psychrotolerans* to cause more incidents of HFP than the well known mesophile histamine producing bacteria. Psychrotolerant *Morganella psychrotolerans* has been isolated from modified atmosphere packed (MAP) fresh tuna stored at 20°C, cold-smoked tuna, frozen and thawed MAP garfish stored at 5°C and lumpfish roe stored at 5°C. The psychrotolerant isolates produce toxic concentrations of histamine at 0–5°C and appear to be important in histamine formation in chilled seafood. Nowadays, researchers study to develop mathematical models that allow growth and histamine formation by *M. psychrotolerans* to be predicted in relevant seafoods.

## OPTIMISATION OF THE PRODUCTION OF EPA- AND DHA-HIGH-CONTENT BUTTER FROM SALMON REFINED OIL AND BY EMPLOYMENT OF CO<sub>2</sub> SUPERCRITICAL CONDITIONS

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The objective of this research was to study the development of a butter product including a high content on n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA), i.e., eicosapentaenoic (C 20:5n-3, EPA) and docosahexaenoic (C 22:6n-3, DHA) acids. For it, n-3 LCPUFA concentrates were obtained by saponification reaction from salmon refined oil and subsequent inclusion with crystals of urea. Further, structured purified triacylglycerols (TGs) were prepared through the enzymatic acidolysis process between n-3 LCPUFA and caprylic acid under CO<sub>2</sub> supercritical conditions. Analysis of starting salmon oil, TGs and n-3 LCPUFA butter were conducted by differential scanning calorimetry (DSC), thin layer chromatography (TLC) and gas-liquid chromatography (GLC). The optimisation of the butter production process was carried out by assessment of the melting profiles and the solid fat content (SFC), being the results submitted to the response surface methodology (RSM) with a central rotational composite design composed of two fac-

tors (namely, speed and stirring times) with 10 experimental runs. As a result, the addition of purified TGs to butter formulations was achieved with a high content of EPA and DHA, thus achieving an average value of 243 mg of EPA+DHA per 7-g portion of butter, thus attaining the claim "EPA+DHA high-content product", according to the Chilean regulation. Melting thermograms of the butter formulations showed that the range of appearance of the peak of low melting point, -72,5 to -70,1°C, corresponded to the melting zone of the n-3 TG including both EPA and DHA. The combination of factors levels during the homogenisation process that showed to maintain at 20 °C the SFC similar to the commercial butter score (namely, 15.96 %) was 8,199 rpm and 4.4 min for speed and stirring times, respectively. This formulation presented a similar behaviour to the one provided by the commercial butter and was chosen as the experimental condition to be employed as optimum formulation in the present research.



## RAPID DETECTION OF ANISAKIDS IN FISH PRODUCTS BY PARALLEL REACTION MONITORING (PRM) MASS SPECTROMETRY

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Anisakids are fish-borne parasites that are responsible for a large number of human infections and allergic reactions around the world. World health organizations and food safety authorities aim to control and prevent this emerging health problem. In the present work, a new method for the fast monitoring of these parasites in fish products is described. The work builds on previous studies in the research group that resulted in the proposal of a series of peptides present in the proteome of *Anisakis spp.* and could be potentially used as biomarkers for the presence of parasites in fish samples. These peptides are part of a thermostable protein present in *Anisakis spp.*, Ani s 9. The thermostability of this protein allows an effective purification by heat treatment leading to an increase of the sensibility and specificity of the Proteomic method.

The overall objective of this work is the application of proteomic techniques for the identification of *Anisakis spp.* in fishery products using an approach based on Parallel Reaction Monitoring

(PRM) Mass Spectrometry.

To achieve this overall objective, we have developed the following partial objectives:

- Development of a fast Proteomic method aimed to identify a set of biomarker peptides.
- Confirmation and validation of the presence of the biomarkers peptides proposed for the detection of *Anisakis spp.* coming from different geographical origin.
- Identification and validation of the biomarkers peptides in different parasitized fish species.

The results presented in this work provide a useful and fast tool for the identification of *Anisakis spp.* occurring in fish in less than 2 h. The method has been validated in different fish species. The present strategy constitutes the fastest method for Anisakids detection, whose application in the food quality control area could provide to the authorities an effective and rapid method to guarantee the safety to the consumers.

## EFFECT OF DIFFERENT LEVELS OF COMMON CARP BY-PRODUCTS PROTEIN HYDROLYSATES IN ZEBRA FISH (*Danio rerio*) DIETS TO EVALUATE THE LIPID PEROXIDATION IN BRAIN AND MUSCLE

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Fish industry is one of the most important resources in many countries worldwide with approximately 140 million tons of fish production, including aquaculture, in which 110 million tons are for human consumption. Nevertheless, fish trade presents underutilized fish by-products which include head, skin, trimmings, fins, frames, viscera and roe that account for more than 60 % of total biomass. Discards of these fish by-products have a great ecological impact and also considerably affect the economic viability and sustainability of the fishing and aquaculture sector. Therefore, enzymatic hydrolysis is one of the techniques currently being developed nowadays in order to recover biomolecules from fish by-products. Fish by-products protein hydrolysates can be used as nutraceutical and food additives for functional foods for human consumption. On the other hand, Zebrafish (*Danio rerio*) is a common model organism for the study of vertebrate biology, physiology and human diseases. This work aimed to study the effect of Common carp by-products protein hydrolysates in Zebra fish di-

ets to evaluate the lipid peroxidation in brain and muscle. For the hydrolysis was used the enzyme Alcalase 2,4 L. Four experimental diets with different levels of Common carp by-products protein hydrolysates (0, 2.5, 5 and 10%) were formulated to evaluate the effect on the lipid peroxidation in brain and muscle. Brain lipid peroxidation of fish fed with diets 2.5, 5 and 10% was significantly lower ( $p < 0.05$ ) than those fed with control diet (0%). Fish fed with diet 5% showed the lowest ( $p < 0.05$ ) levels of muscle lipid peroxidation when compared to the other diets. Results of this study indicate that hydrolysates, even at low concentrations, could reduce the lipid peroxidation in Zebrafish brain and muscle. These are promissory results to the aquaculture, food and pharmaceutical fields since it could be a basis for a research line to develop new products as natural nutraceutical to reduce the risk and drawback of oxidative damage, including neurodegenerative diseases or as a food or feed to reduce muscle lipid peroxidation, favoring a higher shelf life.

## ENCAPSULATION OF ACTIVE COMPOUNDS INTO PHOSPHATIDYLCHOLINE LIPOSOMES FOR THE DESIGN OF FUNCTIONAL SURIMI GELS

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Liposomes are colloidal vesicular structures composed of one or more lipid bilayers that are used as a potential drug delivery system for pharmaceutical, cosmetic and biochemical purposes. The phospholipid-based liposomes have the ability to act as carriers for both lipophilic and hydrophilic compounds. In the field of food industry, they may offer a potential solution to enhance the efficacy and stability of bioactive substances to be included in the formulation of functional foods. They also have the additional advantage that do not dissolve when included in foods with high water activity. Liposomes can be stored effectively by freeze-drying. Moreover, addition of dried liposomes instead of liposomal suspensions greatly facilitates the processing and formulation of the final food product.

In the present work, different types of bioactive substances (collagen hydrolysate-*HC*, pomegranate peel extract-*PG* and shrimp lipid extract-*SL*) were encapsulated with high entrapment efficiency (>90% for *HC* and *SL*, and 63% for *PG*) into phosphatidylcholine vesicles and

subsequently freeze-dried. Squid surimi gels added with the dry liposomal preparations were subjected to simulated gastrointestinal digestion (SGID) and the residual antioxidant property (FRAP and ABTS values) was evaluated. Liposomal z-average particle size (75-81 nm) and zeta-potential (between -65 and -88mV) did not vary substantially depending on the bioactive used, however, they all showed a considerable increase in particle size after freeze-drying and rehydrating (199-283 nm). The incorporation of dried liposomes into the surimi gel decreased significantly the gel strength and also modified the colour parameters ( $L^*a^*b^*$ ). The bioactives showed very different antioxidant capacity, however, differences among the filled nanovesicles were considerably diminished. The antioxidant property of the water-insoluble shrimp lipid extract could be measured in aqueous medium when it was incorporated into the nanovesicle. The gels with *PG*-containing liposomes exhibited the highest FRAP values after SGID, however, no significant differences were found regarding the ABTS radical scavenging capacity.

## ASSESSMENT OF ADDED WATER IN SEAFOOD PRODUCTS ON CROATIAN MARKET

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Seafood is an important source of nutrients in the human diet. There is, however, growing concern regarding the correct composition and labeling of seafood. Chemical composition is commonly categorized in water, protein, lipid and ash. Water is the main component, both in volume and weight, in all seafood products. However, frozen products are often associated with excessively high water content. The addition of polyphosphates improves the water retention during processing and may lead to an unjustified water uptake and increase in weight.

The aim of present study was to determine the water, protein, ash, and polyphosphates content in fresh and frozen seafood samples with reference methods. Fresh and frozen salmon, shrimp, scallop, mackerel, and sea bream samples were purchased on Croatian market and prepared according to standard procedure prior to the analysis. In order to detect an excessive water addition, the water to

protein ratio was calculated, and nitrogen factor was compared with the literature.

Proximate composition of analyzed samples significantly differed among different seafood species and processing conditions. Water to protein ratio in fresh and frozen seafood samples ranged from 3.57 to 5.16, while polyphosphate content ranged from 3.67 to 6.56 g per kg. Calculated nitrogen factor for three fresh and two frozen seafood samples was below values published in the literature.

## BIOLOGICAL POTENTIAL OF THREE ADRIATIC BROWN SEAWEEDS

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Marine organisms are a rich natural resource of many biologically active compounds. Among them, seaweeds are well known as an excellent source of phytochemicals with good biological activities. Although exposed to high oxidative living environment, seaweeds suffer no oxidation damage, which suggest that their cells have antioxidant defense system. So far there has been no investigation in Adriatic Sea, thus this study presents first results on biological potential of three Adriatic brown seaweeds from the Phaeophyceae class. The research included Adriatic marine algae extracts of *Padina pavonica* (Linnaeus) Thivy, *Cystoseira amentacea* var. *stricta* Montagne and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin. The total phenolic content in seaweed extracts was determined by Folin-Ciocalteu method. As one antioxidant, method cannot fully describe the antioxidant activity of the samples; for the full evaluation of the antioxidant potential, multiple method approach was necessary. Therefore, we have measured the antioxidant activity by employing

few methods, based on the different reaction mechanisms (ferric reducing antioxidant power (FRAP), scavenging of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, Briggs-Rauscher (BR) oscillating reaction, etc.). Extract of *C. amentacea* was superior in comparison to other two extracts in both phenolics and related antioxidant efficiency. The total phenolic content in extract from this specie was 785 mg GAE/L, while other two extracts contained almost 8-fold (*P. pavonica*) and 10-fold (*C. compressa*) lower amounts. Similar results were obtained for antioxidant activities: reducing activity by FRAP method, as well as for free radical scavenging activity using DPPH radical, while in Briggs-Rauscher method extracts of *P. pavonica* and *C. compressa* did not show any activity. The obtained results emphasize *C. amentacea* seaweed as a potentially good source of biologically active compounds and open the numerous possibilities of its use in food, cosmetic and/or pharmaceutical industry.

## PREVALENCE OF THE FLAGELLATE *Giardia duodenalis* IN MUSSELS (*Mytilus galloprovincialis*) AND OYSTERS (*Ostrea edulis*) IN CROATIA

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*Giardia duodenalis* is ubiquitous intestinal protozoa which infects both humans and animals. It can be transmitted through food or water.

The aim of this study was investigation of the prevalence of *Giardia duodenalis* in shellfish farms from Croatia. Samples of mussels` and oysters` digestive gland, collected from thirteen different farming areas, were analysed by nested Polymerase Chain Reaction, followed by direct sequencing of triosephosphate isomerase gene.

All tested farming areas contained *Giardia* cysts, and 6.1 % of shellfish were positive. Prevalence varied depending on the type of shellfish and locations from which the samples were taken. Four assemblages of this parasite were identified: A, B, C and E. The most common was assemblage A, or more specifically subassemblage A

## ESTABLISHING THERMAL RESISTANCE PROFILES OF *Listeria monocytogenes* ISOLATED FROM IRISH SEAFOOD PRODUCTS

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Irish Brown crab (*Cancer pagurus*) is a valuable commodity to the fishing industry, with almost 9,000 tons caught annually by Irish vessels alone. Consumers regard crab highly and it is often eaten as a luxury. Unfortunately, this species is very susceptible to both quality deterioration and spoilage due to rapid bacterial growth. Current processing methods used may be detrimental to the quality of the product. Milder cooking processes could be applied if they were sufficient to destroy spoilage and pathogenic bacteria present in crabmeat. The present study investigated the thermal inactivation (55, 60 and 65 degrees C) of the pathogenic bacteria, *Listeria monocytogenes* in tryptic soy broth (TSB). D-values ranged from 2.6 (50 degrees C) to 0.7 min (60 degrees C) for crab isolates and from 2.5 (50 degrees C)

to 1.2 min (60 degrees C). The z-values for crab meat and seafood isolated were 5.38 and 6.26°C, respectively

## PREFERENCE FOR FISH AND FISH PRODUCTS CONSUMPTION DEPENDING ON DIFFERENT MARKET CHANNELS IN ROMANIA

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The pattern of fish consumption differs from country to country based on the availability of different types of products. The main objective of this study was to investigate the preference for different types of fish product depending on the socio-demographic factors, price and most frequently accessed market channels.

Cross-sectional data were collected in September 2015 from a sample of 743 individuals from different regions in Romania. Survey questions addressed consumption patterns, the market channels used for purchasing fish and fish products as well as the main factors influencing the purchase decision. In this paper the socio-demographic profiles of different consumer clusters based on the preference for one or more fish types or products will be presented and the implications for marketing and distribution through different channels will be discussed.

Fish is predominantly preferred as fresh or refrigerated (66, 2%), followed by live fish (54, 1%) while de the sea

food still remains the last choice of the consumers (31, 8%). Around two thirds of the surveyed consumers indicate the hypermarket (66,3%) or the fishery (67,5%) as the preferred market channels and price was mentioned as a decisive purchasing factor in 70,5% of the cases.

While these results are close to the traditional consumption patterns in the Romanian population, the subgroup analysis of socio-demographic characteristics reveal the emergence of new types of consumer preferences (e.g. sea food).

While the Romanians increase the range of fish and fish products they consume, the market channels they use for purchasing these products are decreasing in variability. When the geographical availability of fisheries is taken into account, the gradual increase in importance of the hypermarket becomes more obvious. In this context, the high dependence on the offer of hypermarkets raises the risk of an impoverished consumer choice in the near future.



## THE HEALTH RISK OF TETRODOTOXIN IN MEDITERRANEAN SEA

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Alien marine species migrate into the Mediterranean Sea via the Suez Canal. Some of the alien species have economic value, although some species are harmful on ecosystem, local species and human health especially in the Eastern Mediterranean Sea. Some members of the alien marine species contain marine toxins. Especially pufferfish species are remarkable in terms of marine toxin. Pufferfish is regarded as one of the “worst alien fishes” of the entire Mediterranean Sea. The increasing number of alien species in the Mediterranean has had ecological and economical effects especially on the north eastern Mediterranean in recent years.

Particularly, pufferfish, members of *Tetraodontidae* family, has many negative effects on local species, fishery industries and public health. Pufferfish is considered a delicate fish in the Far East, mainly in Japan where it is prepared by experts. However, according to European Commission directive No 853/2004, fishery products obtain from poisonous fish belonging to the *Tetraodontidae*, *Molidae*, *Diondontidae*, and *Canthigasteridae* families may not

be placed on the market. In particular, there is concern about the impact of pufferfish belonging to the *Tetraodontidae* family since they may contain tetrodotoxin (TTX). TTX is believed to be confined to regions of South East Asia, but recent studies have demonstrated that the toxin has spread to regions in the Pacific and the Mediterranean. TTX is a new phenomenon for most of the Mediterranean countries. Moreover, there is no sufficient information about TTX levels in Pufferfish species in Mediterranean Sea. The poisoning due to pufferfish consumption has reported in some Mediterranean countries including Turkey, Egypt, Israel, and Lebanon last decade.

TTX is most potent and oldest known marine toxin. TTX is a non-protein toxin that is weakly basic, odorless and colorless. There is no known antidote for TTX, which is a powerful sodium channel inhibitor. TTX is thermostable and also cannot be removed by washing. Therefore, the general properties and poisoning reports in Mediterranean countries of TTX has been given.

## TASTING TROUT FISH SOUP BY CONSUMERS IN THE FISH RESTAURANT AND TAKING THEIR OPINIONS

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In this study, trout soup was prepared and served to 100 customers who came into a fish restaurant for a traditionally cooked soup. Fish soup is a dish made by combining fish or seafood with vegetables and stock, juice, water, or another liquid. Trout soup was prepared with minced trout fillets, spices, onion, oil and water. Firstly, onion and minced trout fillets were cooked with oil and after that spices were added. Finally, after adding water the soup was brought to boil. When finished the soup was served to consumers in large soup plates. All the consumers remarked that it was the first time they tasted trout or any other fish soup and it was their first experience with trout soup. After tasting the trout soup, 85% of consumers said that they liked it and I would try it again, 5% of consumers said that the soup was good but they would not try it again, and 10% of the consumers decided they did not like the soup and they would not taste it again. During two months, although there was not a trout soup on the menu of the

restaurant, over 50 consumers ask for the trout soup insistently. The study showed that people preferred to have the fish soup instead of traditional soup since fish soup is healthier and rich in minerals, fatty acids, amino acid content and vitamins.

*Key words: trout, fish soup, tasting, consumer preference*

## PEELABILITY AND QUALITY CHANGES DURING ICE MATURATION OF SHRIMP (*Pandalus borealis*)

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Today shrimps are industrially peeled on automatic peeling machines after four to five days of maturation on ice. The TECHSHELL project is aiming at reducing the maturation time to one day, which will lead to an increased yield of 1%. Moreover, the CO<sub>2</sub>-emission will be reduced, as well as the expenses to ice and the shrimps will be of higher quality.

In order to be able to shorten the maturation period it is important to understand the quality changes that take place during the maturation. Thus, the changes in protease activity, in both the shell and in the epidermal layer between the shell and the muscle, during the ice were analyzed.

Moreover, video meter technology was applied to follow the color changes and a texture analyzer was used to measure the peelability during the maturation period. The peelability were measured both as the maximum force and

the total work needed to separate the shell from the muscle.

It is shown that the general protease activity in the epidermal layer is rather constant during the maturation period, whereas the activity in the shell decreases during the first three hours and then increases a bit again before dropping gradually during the fourth day. The video meter analysis revealed that spectrums for the non-matured shrimps and the four-day matured shrimps were significantly different, and in general the four-day matured shrimps had lost red color and appeared paler than non-matured shrimps. Texture analysis displayed less work and a lower maximum force needed on four-day matured shrimps compared to non-matured shrimps, indicating an increased peelability of the matured shrimps.

In conclusion, the industrial reasoning for ice maturation of the shrimps

before automatic machine peeling is there. However, it also makes sense to shorten the maturation period in order to improve the color of the peeled shrimps. Furthermore, more knowl-

edge about the protease changes during the maturation period is needed to understand the quality effects of these changes.

## PACKING OF FRESH FISH PRODUCTS IN BOXES AND TUBS INTENDED FOR SEA TRANSPORT

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The goal of the study was to find the best and most efficient method of packaging fresh fish for sea transport with the aim to maximize the storage life of the product, which is a key element in the marketing of fresh fish.

Experiments were made with the transport of fresh fish in tubs with slurry ice and compared with transport in expanded polystyrene boxes with regard to temperature control, product quality and shipping cost. Different product groups were compared, using different temperature conditions and packing methods to find the best outcome for fresh fish quality and storage life.

Present results clearly indicate that the cooling before packaging and low and stable storage temperature play a major role to maximize storage life of fresh fish products. Different packaging solutions were also a major factor, although the effect on extended

storage life was not as dramatic as the effects of temperature.

The results indicated also an increased likelihood of prolonged shelf life if fresh fish is packed in a tub with a slurry ice compared to traditional packaging in expanded polystyrene boxes with ice. In order to estimate the necessary amount of slurry ice to maintain acceptable temperature, a thermal model was developed. Economic analysis of different packaging and transport was also carried out and the results showed substantial savings with the use of tubs for the transport of fresh fish products in comparison with the expanded polystyrene boxes.

## QUALITY AND SAFETY OF ATLANTIC COD (*Gadus morhua*) FILLETS DURING COLD STORAGE, AS AFFECTED BY DIFFERENT THAWING METHODS OF PRE-RIGOR FROZEN HEADED AND GUTTED (H/G) FISH

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A major challenge in the whitefish industry is the seasonal variation in amount of landings throughout the year. While most of the whitefish is caught and processed during winter, the market demands an all-year deliveries of high quality products. This is especially the case regarding the large Norwegian fisheries of Atlantic cod (*Gadus morhua*), with a short four month commercial season. The large volumes of fish caught during a limited period leads to processing capacity challenges in the land-based industry. This challenge can be met by applying fish frozen at sea and, subsequently, thawed by procedures maintaining quality and safety comparable to fresh fish.

This study aimed to investigate the applicability of novel thawing strategies in order to provide an all-year supply of high-quality and safe cod products. Comparisons of thawing in

water, with and without air-circulation, and contact thawing was performed. Water-thawing with air-circulation provided faster thawing compared to water-thawing without air-circulation and contact thawing. However, differences between thawing methods with regard to fillet shelf-life were not significant until 14 days post-filleting. The hygiene conditions during the thawing processes were considered good and no implications of impaired food safety during any of the thawing strategies. The results indicates that fillets from thawed fish can be of similar quality as fresh fish.

## TRYPAINS FROM FISH PROCESSING WASTE: CHARACTERISTICS AND BIOTECHNOLOGICAL APPLICATIONS

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The digestive tract of fish in general is rich in hydrolytic enzymes, and internal organs are an important source of digestive enzymes such as proteases. These enzymes have a high potential in use of additives for different industrial products. The internal organs are considered as waste, this means an ecological and economical loss. Enzymes of internal organs of fish show differentiation in characterization, due to species of fish; this means a wide range of use for these enzymes. They have diverse applications in a wide variety of industries, such as the detergent, food, agrochemical and pharmaceutical industries. Proteases are derived from animal, plant and microbial sources. The most important digestive proteases of fish viscera are acid stomach enzymes and alkaline intestine enzymes. The main alkaline enzymes in fish viscera are trypsin, chymotrypsin and elastase, all belonging to the serine-protease family (EC. 3.4.21). An interesting alternative to convert the fish processing wastes into more marketable and acceptable products is to isolate and purify trypsins which can be used in

the food industry or in fish protein hydrolysate preparation. The purpose of this review is to provide an overview of trypsins from fish processing waste and to discuss their potential uses in biotechnological applications.

\*This study was supported by TUBITAK Project No: 214O569

## FISH PROTEIN HYDROLYSATES: PROXIMATE COMPOSITION, AMINO ACID COMPOSITION, ANTIOXIDANT ACTIVITIES AND APPLICATIONS

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The digestive tract of fish in general is rich of hydrolytic enzymes, and internal organs are an important source of digestive enzymes such as proteases. These enzymes, have a high potential as additives for different industrial products. The internal organs are considered as waste, this means an ecological and economical loss. Enzymes of internal organs of fish show differentiation in characterization, due to species of fish, this mean a wide range of use for these enzymes. They have diverse applications in a wide variety of industries, such as the detergent, food, agrochemical and pharmaceutical industries. Proteases are derived from animal, plant and microbial sources. The most important digestive proteases of fish viscera are acid stomach enzymes and alkaline intestine enzymes. The main alkaline enzymes in fish viscera are trypsin, chymotrypsin and elastase, all belonging to the serine-protease family (EC. 3.4.21). An interesting alternative to convert the fish process-

ing wastes into more marketable and acceptable products is to isolate and purify trypsin which can be used in the food industry or in fish protein hydrolysate preparation. The purpose of this review is to provide an overview of trypsin from fish processing waste and to discuss their potential uses in biotechnological applications.

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## ***Tenebrio molitor* LARVAE MEAL AS ALTERNATIVE INGREDIENT IN DIETS FOR FARMED BLACKSPOT SEABREAM (*Pagellus bogaraveo*): EFFECT ON PHYSICAL AND CHEMICAL FILLET TRAITS**

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Fishmeal (FM) is the main protein source for carnivorous freshwater and marine fish. Since the increase of FM request has dramatically caused the reduction of natural stocks, alternative protein sources have been tested in the last decades and, recently, insects have been identified as an alternative sustainable protein source for feeding fish. Its utilization seems to have numerous advantages, nevertheless more information are necessary for better understanding its possible role on fish quality. Aim of this study was to evaluate the partial replacement of FM with *Tenebrio molitor* larvae meal (TM) on fillet quality of farmed blackspot seabream (*Pagellus bogaraveo*), a promising finfish species for aquaculture. Three experimental isoenergetic diets were formulated: TM0, with FM as exclusive protein source, TM28 and TM50 replacing 28

and 50% of FM with TM, respectively. Fish were fed experimental diets (three replicate tanks per treatment; 14 fish/tank) for 131 days. At the end of the trial, eight fish per diet were randomly sampled for determination of physical (skin and fillets colour, pH, texture, Water Holding Capacity) and chemical (proximate composition: moisture, crude protein, ether extract, ash; fatty acids profile) fillet characteristics. Different diets did not affect the colour of skin; however, significant variations were observed in colour of fillets with higher yellowness when TM was added in the diets (2.51 and 2.67 vs 0.69 in TM25, TM50 and TM0, respectively); instead Hue\* resulted higher in fish fed FM diet (165.89 vs 134.45 and 132.91 in TM0, TM25 and TM50, respectively). No significant differences were detected for Water Holding Capacity, hardness, cohe-

siveness, resilience, gumminess and adhesiveness. pH was found lower in TM50 (5.97) than TM0 and TM28 (6.12 and 6.15) specimens. Fillets proximate composition was not affected by the diet, unlike PUFA<sub>n</sub>3 and PUFA<sub>n</sub>6%. Even though EPA and DHA significantly increased in TM0 fish (EPA: 5.81 vs

5.39 and 5.15%, DHA: 13.92 vs 12.83 and 12.28%, in TM0, TM28 and TM50, respectively), the differences were low in value. Since no detrimental effects on fillet quality were observed, the use of *Tenebrio molitor* meal as alternative protein source in blackspot seabream feeding seems to be encouraging.

## ASSESSMENT OF THE EFFICIENCY OF THE BIOCIDES USED FOR TREATMENT OF COLD STORAGE ROOMS FOR CHILLED FISH

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Hygiene of the processing environment is a significant factor in the production of microbiologically safe and good-quality products in the fish industry. Pathogens and spoiling microorganisms which adhere to interior equipment, chilling cameras and working surfaces may be transferred to fish products during their processing. In the presented work assessment of efficiency of two biocides 0.1% nanosilver solution and 1% peracetic acid for treatment of the walls of fresh fish chilling rooms has been conducted. Treatment of cold storage room surfaces has been done after prewashing. Microbial swab tests were carried out before, directly and in 24 hours after disinfection and after sanitation. Swabs with neutralizing agent (HiMedia) have been used. Simultaneously, swabbing of trout skin after washing and from chillers has been performed. Swabs were analyzed for the following groups of bacteria: total aerobic count, *coliform* bacteria, *Staphylococci*, yeasts, fungi, gram negative and oxidase positive bacteria. In case of 0.1% solution of nanosilver strong durable

effect against gram negative oxidase positive bacteria (*Aeromonas*, *Pseudomonas*, *Schewanella*, *Vibrio*, *Alcaligenes*) has been occurred comparing to 1% solution of peracetic acid. Mentioned bacteria had high frequency of occurrence on trout cold storage room walls. Tested bacteria possessed low adaptability against 0.1% solution of nanosilver during three years of continuous usage.

## ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF MAILLARD REACTION PRODUCTS OBTAINED BY REACTION OF SHRIMP WASTE HYDROLYSATES WITH GLUCOSAMINE

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The antimicrobial and antioxidant activities of Maillard reaction products made from glucosamine and protein hydrolysate from cooking Pacific white shrimp (*Penaeus vannamei*) water were investigated. The hydrolysate was obtained by using a crude enzymatic extract produced by *E. faecalis*. Both glucosamine and cooking shrimp water hydrolysate were heated at 100°C and aliquots collected at different times (0, 40, 60, 120 and 180 min) during reaction. The antioxidant activity of the protein hydrolysate increased significantly after reaction with glucosamine. Thus, the Fe<sup>2+</sup> chelating activity of the Maillard reaction products improved 4-fold after heating during 40 minutes and remained stable until the end of reaction. Meanwhile, the ferric ion reducing capacity (FRAP) steadily increased throughout

the heating reaction and achieved values 30-fold higher when compared to that of the shrimp hydrolysate. The ABTS radical scavenging capacity increased 15-fold after heating during 60 minutes and remained stable. Moreover, the Maillard reaction products obtained after 180 minutes of reaction conferred antimicrobial activity against *Staphylococcus aureus*. Thus, the reaction between glucosamine and cooking shrimp water hydrolysate at high temperature yielded Maillard reaction products with interest as potential natural food preservatives.

## PROTEIN CHANGES IN FISH SILAGE PRODUCED USING DIFFERENT COMBINATIONS OF UNDERSIZED QUOTA SPECIES

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Fish silage implies the liquefaction and stabilization of minced whole fish or fish offal by the addition of mineral and/or organic acids. It is considered a low-investment, low-cost and easy-to-produce fish meal substitute. Silage characteristics are influenced by the initial composition and quality of the raw materials. Therefore, according to the industry, a steady supply of low-variety raw materials is required to ensure product uniformity. However, many of the European demersal fisheries are mixed fisheries, thus supplying a large variation of raw materials.

During this research we investigated protein changes in four types of fish silage, each produced using a different combination of undersized Belgian quota species. The goal of this research is to determine the stability of the proteins in the silages over a 3-month time period and to ascertain the effect of raw material combination (RMC) on silage characteristics.

There was no difference in initial crude protein content (CP) between silages (74%) and in all silages CP decreased

over time. However, CP of less complex RMCs levelled off at approximately 64% after 3 weeks, whereas more complex RMCs exhibited a stronger decrease in CP over time and dropped to levels below 60% after 3 months. Accordingly, these complex RMCs also showed a slower but longer hydrolysis compared to less complex RMCs, 73.5% and >85% respectively. Longer hydrolysis leads to a loss of protein-N in the form of NH<sub>3</sub> and a decrease in nutritional value. The increase in total volatile nitrogen in this experiment can mainly be contributed to the release of NH<sub>3</sub>.

There seems to be an effect of RMC on fish silage characteristics in this experiment. Product pasteurization could limit hydrolysis, minimizing protein losses.

## RECOVERY OF LIPIDS FROM MARINE SOURCES AND SEAFOOD PROCESSING WASTE: METHODS, CURRENT AND POTENTIAL USES

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Total fisheries production in Turkey increase every passing year with growing aquaculture. According to FAO, (2014) World fisheries production reached 158 million tone. 91,3 million part of these production came from fishing and 66,6 million tone come from aquaculture. In Turkey, total fisheries production are 607.515,2 tone and 339.046,9 tone of these production came from fisheries and 268.468,3 tone came from aquaculture. While large parts of these products consume as fresh, rest of products process. When considered, fisheries products give rise to %40-60 wastes, a significant amount processing wastes occur.

These products are rich by the protein, lipid, mineral and vitamins and can be recovered with different methods. These recovered compounds are valuable than its raw material and can be referred as value added products. Beside, recovered compounds have various positive health effect and so it can be used in pharmaceuticals, medicine etc.

In this context, various researches are carried out. Differences in methods, species and nutritional compounds (protein, lipids etc.) are examined and evaluated in researches.

In this study, species and methods used in lipid recovery and information about its current and potential uses are reviewed.



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