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## **Abstracts for oral presentations**

*Some presentations are not introduced in the abstracts*

## **Green Growth in Fisheries and Aquaculture – Ensuring the Future of Ocean Food Resources**

*Carl-Christian Schmidt, Head of Fisheries Policies Division, OECD*

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The focus of this presentation is on how the Green Growth concept offers some new ways to address the increasing pressure on the marine environment. Against the backdrop of increasingly global fish markets and intensifying aquaculture production green growth can help ensure that capture fisheries and aquaculture are competitive now and in the future. Green growth means fostering economic growth and development while ensuring that the natural assets continue to provide the resources and environmental services on which our well-being relies. The OECD Green Growth Strategy (GGS) establishes a set of principles for policy makers along with a roadmap for how to achieve green growth in practice.

Achieving green growth in fisheries means using the resource as efficiently as possible to obtain the greatest possible benefits. This suggests a key role for the management system ensuring that stocks can deliver maximum value for the long term. Rebuilding and maintaining fish stocks at more productive levels of biomass and coupled with efficient and cost effective management systems are central to this. A green growth strategy for aquaculture would seek to continue the expansion of the sector while reducing its negative impacts and demand on natural resources. Negative impacts of aquaculture include discharges (nitrogen and phosphorous in particular), diseases, demand for wild feed stocks, escapees and local environmental degradation.

However, fishing and aquaculture are just two of a wide range of activities that take place in the marine environment. Coastal tourism, dredging, maritime transport, habitat for people, plants and animals, dumping and waste, agriculture run-off, oil and gas extraction and the extraction of marine ingredients for pharmaceuticals, biofuels etc. all take place in the same area or in close vicinity to fisheries. The co-existence of these activities adds pressure to the marine environment and may also add competition for space in ports (e.g. berthing space, space for fish processing activities). The co-existence of such activities pose a number of challenges related to conflicts over the use of the same inputs (e.g. fish, marine space and ports) and externalities between the activities (e.g. agriculture run-off, coastal encroachment/sewage and fish/aquaculture). To make informed decision about trade-offs between these diverse activities (who should have the right to use the marine space?) require information about the various activities' value added contribution, job creation and environmental externalities.

## **Saving the world from obesity with Norwegian fish proteins**

*Dr. Bomi Framroze, Chief Scientific Officer, Hofseth Biocare ASA*

Salmon offcuts from filleting constitute over 40% of the total mass after gutting. This valuable resource has up to now only been used for producing animal feed.

Hofseth Biocare has designed and built a novel plant in Midsund to process this material into isolated fractions of protein hydrolysate powder, salmon oil and calcium powder all specially formulated for human consumption.

Our talk today will focus only on the unique bio-efficacy observed with our salmon protein hydrolysate powder.

Our protein hydrolysate is a very palatable powder which has shown the fastest uptake of nitrogen in TIM-1 simulated human digestion trials and has further clinically shown a unique ability to increase haemoglobin levels in anaemic human subjects.

## **The Importance of Omega-3 for Brain Development and Function**

*Dr Alex Richardson, Senior Research Fellow, Centre for Evidence Based Intervention, University of Oxford, UK*

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The prevalence of disorders of mood, behaviour and/or cognition - including ADHD and related conditions, depression and dementia - has become an issue of major concern in most developed countries. Increasing evidence suggests that relative deficiencies of omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) are an important and modifiable risk factor for these and other disorders of mental health, wellbeing and performance.

Adequate supplies of omega-3 LC-PUFA (EPA and DHA) are critical for normal development and functioning of the brain and nervous system, as well as for general health. Marine oils (from fish, seafood or algae) are the main dietary source, but intakes in most developed countries are suboptimal. A direct dietary supply of DHA is needed to achieve optimal status, as synthesis of DHA from the short-chain omega-3 (alpha-linolenic acid, found in some vegetable oils) is unreliable in humans.

Epidemiological and biochemical studies show that higher intakes and/or tissue concentrations of EPA/DHA are associated with better behaviour, cognition and mood in both children and adults. Clinical trials also show that supplementation with omega-3 LC-PUFA can benefit both clinical and non-clinical populations, although further research in some areas is still needed. The importance of adequate intakes of omega-3 (particularly DHA) in early life is well established, but current evidence shows that an increased dietary intake can also improve mood, behaviour and cognition at almost any age. These findings have important implications for many domains of research, public policy and practice in addition to their obvious significance for producers and suppliers of foods and dietary supplements.

## **Impact of processing on nutritional quality of marine ingredients**

*Edel O. Elvevoll Dean, Professor, Faculty of Biosciences, Fisheries and Economics, UiT the Arctic University of Norway*

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As new insights in the interaction between genetic predisposition, specific health risks and nutritional needs, and the functioning of separate nutrients are provided there is growing awareness that the dietary source and form of food and thus ingredients may affect the overall health of the consumer.

Changes in consumer preferences toward functional foods, ready-meals and products with longer shelf-life have in general led to a higher degree of processing. Main objectives are to be able to offer a safe and stable product with even palatability, form and texture and these aims are reached with large-scale productions favouring the most economically viable processes and logistics. Potentially detrimental nutritive effects of processes or unit operations have been focussed to a lesser extent. During processing components with beneficial effects may be altered, destroyed or simply removed from the product.

The traditional extraction technique of oils from marine products involves heating or steam stripping of the raw material in order to release the lipids. In addition, marine oils are refined, a process in which improvement of sensory attributes or safety of the marine oil may destroy antioxidants and remove components with potential beneficial effects.

Processing and preparation may also cause huge losses of low molecular weight and water soluble compounds like trace elements and amino acids. Processing and unit operations may also alter the activity of molecules within an ingredient.

## **Dietary Protein and the metabolic syndrome: opportunities for marine ingredients?**

*Marco Mensink, Wageningen University, Division of Human nutrition, The Netherlands*

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The metabolic syndrome is a clustering of (clinical) features associated with an increased risk for type 2 diabetes and cardiovascular disease. Major components or consequences of the metabolic syndrome are insulin resistance and/or a disturbed glucose homeostasis, dyslipidemia, abdominal obesity, ectopic lipid accumulation, lipotoxicity and inflammation. Diet can play an important role in the pathogenesis, treatment and prevention of the metabolic syndrome, both, quantity and quality. Diets with an increased protein content, or a preferred protein source, like marine proteins, could be a mean to improve cardiovascular and metabolic risk factors. The presentation will consist of three parts

- An introduction to the metabolic syndrome and its key features insulin resistance, ectopic fat and lipotoxicity. In particular lipid accumulation in the liver ('fatty liver') will be discussed
- The association between dietary protein intake and several aspects of the metabolic syndrome will be reviewed, with the focus on hepatic lipid accumulation.
- The opportunities of marine ingredients to manipulate hepatic lipid content and other aspects of the metabolic syndrome will be discussed.

## **Fish proteins and peptides - beneficial effects on human health and possible industrial use**

*Oddrun Anita Gudbrandsen, PhD, Researcher, Department of Clinical Science and Department of Clinical Medicine, University of Bergen*

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The health benefit of eating fish has mainly been thought to be caused by the long chain omega-3 fatty acids EPA and DHA. Fish contains several other nutrients such as vitamin D and selenium, and in addition fish is an excellent protein source. Numerous trials using fish oil or purified EPA and DHA in has been conducted in both humans and animals, but very few scientific studies have been conducted to investigate the effects of fish filet or fish protein intake in humans. We have recently published the first results from a clinical trial using fish proteins, demonstrating that isolated cod protein significantly improved insulin sensitivity and cholesterol levels in overweight adults after 8 weeks intervention with no changes in physical activity and food intake in the participants.

The presentation will focus on the following:

- Presentation of reported effects of fish protein/peptide intake
- Discussion of the health implications of fish protein/peptide intake in humans
- Suggestions for possible use of fish protein/peptide for the food industry

## **The influence of dietary marine proteins, peptides and water soluble nitrogenous compounds on fish health and performance**

*Katerina Kousoulaki, Researcher, Nofima.*

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Fish nutrition studies largely focus on either major health and performance effects of suboptimal levels of single essential fatty acids, amino acids and vitamins or on effects of using different plant raw materials as alternative protein sources to fish meal. A smaller number of studies focus on effects of different qualities of fish meal or other raw materials in terms of freshness, oxidation of essential nutrients and development of toxic compounds. Whole marine raw materials are complex mixes of nutrients in dynamic balance influencing fish metabolism at different levels simultaneously. It is thus a challenging and rather bold task attempting to explain their functionality in living organisms. Recent results on i) Atlantic salmon fed very low fish meal diets, and ii) Ballan wrasse larvae reveal the significance of the water soluble fraction of fish meal and krill raw materials in the feed. Here, fish performance and health dietary effects of high quality marine raw materials, focusing on appetite regulation and lipid metabolism, are presented.

## **Marine Bioactive Peptides and Proteins including Betaines: potential industry applications and challenges**

*Dr Maria Hayes, Scientific Researcher, Teagasc Food Research Centre*

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The marine environment contains a vast array of plants and animals containing useful, biological compounds that may be released using biotechnological processes. The quaternary ammonium and tertiary sulfonium compounds glycine betaine and dimethylsulfoniopropionate are osmolytic, zwitterionic compounds that are found in seaweeds. In addition, bioactive peptides may be generated from protein rich seaweeds such as the red seaweed *Palmaria palmata* and *Porphyra* species. Bioactive peptides are peptides consisting of between 2-30 amino acids that may exert a health benefit to the consumer following consumption. These health benefits include antimicrobial, antioxidant, anti-inflammatory and heart health activities. The beneficial effects of Betaines relate to the maintenance of normal blood concentrations of homocysteine and in 2011, the Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Agency (EFSA) established a cause and effect relationship between consumption of Glycine Betaine and normal homocysteine metabolism and granted Glycine Betaine a health claim pursuant to Article 13 of regulation (EC) No 1924/2006.

The presentation will focus on three topics:

- Betaine content of Irish seaweed species; development of seaweed betaine extracts and characterization of these extracts using CE, NMR.
- Seaweed derived bioactive peptides with heart health benefits and potential delivery mechanisms of these benefits to the consumer through food product development
- Bioactive peptides derived from marine animals and potential uses

## **Improving value of raw materials by application of hydrolysis process – Pitfalls and Concrete applications in Aqua feed**

*Philippe Sourd, Fish Health Specialist, Aquativ*

Aquaculture is using more and more raw materials and struggles to find enough volumes to meet the high growth of farmed fish and shrimp production in a sustainable way. Feedstuffs of marine origin are still recognized as the best sources of macro- and micro-nutrients to meet fish and shrimp requirements. The numerous attempts to remove them totally from feeds have led to poor animal and feed performances where many metabolic functions, such as growth or immunity, were impaired. More than the well balanced amino acid profile of marine raw materials, their unique content in bioactive compounds essential for the animal metabolism is now well documented.

If marine hydrolysates are today considered for their benefits to prevent many metabolic disorders in humans, their use in aquaculture has long been restricted to their outstanding palatability and near perfect digestibility. However, the high concentration of bioactive peptide fractions in hydrolysates could find other interesting applications such as promoting aquatic animal health. This presentation will review the functional benefits that have been obtained from hydrolysates when used in aquafeeds. It will draw attention on the critical importance of the raw material quality, the hydrolysis process management and the analytical characterization needed to produce highly performing and standardized products. Finally, an insight of the actual use of hydrolysates in intensive aquaculture will show that the use of these high value ingredients is a market reality worldwide and contributes to enhancing the marine and aquaculture proteins life cycle while enhancing fish and feed performances in many species.



## **Firmenich Bjørge Biomarin in seafood – opportunities and challenges for a flavor-house in seafood**

*Oddvar Bjørge, Chairman of the Board, Firmenich Bjørge Biomarin AS, Norway*

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Firmenich is the third largest flavor house in the world with a total turnover of about NOK 18 billion, 6000 employees, and an extensive R&D record. In 2003 Firmenich acquired Bjørge Biomarin in Norway, a company specialized in Seafood. The short presentation will focus on three main points:

- Through our history as well as today we emphasize on superior flavor qualities, excellent delivery technologies, substantial R&D investments, close co-operation with a wide range of partners, and being close to our customers.
- Seafood fits into our range of tonalities, and to keep our position as the leading seafood flavor provider we need to be in the forefront of research and development as well as in delivery systems. The natural seafood products are today only a limited base. To be of value to the flavor industry more sophisticated products are needed for the savory market.
- As part of the Norwegian cluster of marine biotechnology companies, we emphasize on being relevant partners to these clusters. We build local knowledge through education and import of technologies, inviting key partners to the cluster, develop new technologies, and helping building stronger links to the markets. The main challenges for the Norwegian marine biotech industry, seen from a flavor house perspective, will be emphasized in the closing remarks of my speech.

## **Marine bioactives for dietary supplements and functional foods**

*Luce Sergent, Sustainable Development Manager, Copalis, France*

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The ocean has been providing food to human-being for centuries and extensive scientific research in the past few years has highlighted numerous health benefits from eating fish, especially fatty fish species, their omegas 3 fatty acid and vitamin content. However, marine products have a complex composition that can't be restricted to only one or two components.

With over 50 years' experience, COPALIS has developed a unique manufacturing expertise in the field of enzymatic processes for the fractionation and isolation of active fish bioactives for animal and human nutrition. The presentation will first give an overview of the enzymatic hydrolysis technique for the production of fish bioactives.

While most of the publications are about the anti-hypertensive and antioxidant activity of fish peptides, COPALIS research and development team has focused its research to design new peptides that meet consumer demand for healthy ageing products. The second part of the presentation will introduce two of these marine peptides:

- PROTIZEN, a fish protein hydrolysate with anxiolytic-like properties
- COLLECTIVE™, a fish protein hydrolysate with anti-wrinkle properties

## **Environmental pollutants in fish oil products and the importance of efficient removal**

*Carola Rosseland PhD, Research and Development Manager, Pronova BioPharma - now part of BASF*

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The health benefits of omega-3 are well established. They reduce risk of cardiovascular disease, and are used as a pharmaceutical for treatment of hypertriglyceridemia. A wide range of other positive effects are also well recognized. In addition to omega-3, the marine environment contains persistent organic pollutants (POPs) such as PCB and dioxin. Due to their chemical characteristics they are able to travel long distances by atmospheric transport and remain in the environment for a long period of time. Therefore, they are now ubiquitously present. High level toxicity of different POPs are well documented after accidental exposures, however, there is also an increasing concern that chronic exposure to low levels of POPs may contribute to the burden of a number of diseases. As POPs are lipid soluble, they accumulate in crude fish oil which is the starting material for many omega-3 products, and the question has been raised if the presence of pollutants may limit the health benefits from fish and fish oil products if they are not efficiently removed. As POPs accumulate, persist and bioaccumulate in the human body they might eventually achieve toxicologically relevant concentrations – even though exposure may appear limited.

Long chain omega-3 fatty acids are used as a pharmaceutical intended for patients with health problems. The presentation will focus on the biological relevance of low level exposure, especially in sensitive populations, and the importance of minimised concentrations of POPs to optimise the health benefits of omega-3.

## Marine Omega-3 - clinical nutrition applications

*Philip C. Calder, Professor of Nutritional Immunology, Faculty of Medicine, University of Southampton, UK*

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Marine omega -3

- What are they (very brief)
- Metabolism and incorporation in humans
- How do they act to influence cell function and human health

What is meant by “clinical nutrition”

Use of marine omega-3 in

- Prevention of cardiovascular disease
- Treatment of cardiovascular disease
- Treatment of chronic intervention (arthritis as an example)
- Preventing weight loss in cancer
- Improving cancer chemotherapy
- Patients requiring intravenous nutrition
  - Post surgery
  - Critically ill in intensive care

## Analyzing lipid oxidation – opportunities and challenges

*Stine Grimmer, Research Scientist, Nofima, Norway*

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The standard analytical methods for oil oxidation, peroxide and anisidine value, may not be good enough to describe marine oil quality. New quality standards for the determination of oil quality may be needed. This will require the development of new analysis parameters as well as documentation of realistic degree of oxidation in defined products. In addition, increased knowledge of the biological effects of lipid oxidation products is necessary. The presentation will focus on the following three topics:

- Novel methods to measure lipid oxidation
- Comparison of different methods for analyzing lipid oxidation on defined marine oils with increasing oxidation
- Effect of lipid oxidation

## **Production of high quality herring oil**

*Ana Carvajal, Research manager, SINTEF Fisheries and Aquaculture, Norway*

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Annually, the Norwegian herring industry generates almost 240 000 tons of rest raw materials that are mainly used for production of animal and fish feed. However, the rest raw material can be a valuable source for production of fish oil and protein products for human consumption.

The presentation will focus on the following topics:

- Utilization of herring rest raw materials for production of high quality oil for human consumption.
- Production of oil by using a mobile production plant (SINTEF Mobile SeaLab) and how the processing methods, thermal treatment and enzymatic hydrolysis, affected the quality of the oil.
- Effect of early addition of antioxidants to retain oxidation during oil production.

## The Omega3 industry – Process and technology development

*Iren Stoknes, R&D Manager, Epax Norway*

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The omega3 industry is a young industry even if it is based on long traditions in utilizing oil from cod livers. Since the 1980s fish body oils from South America have been the main raw material. The use of omega3 oils for human consumption is in rapid growth, and the main markets are within nutraceuticals (dietary supplements), pharmaceuticals and functional foods.

The industry is busy in developing and optimizing a series of unit operations needed for making high quality and concentrated omega3 products.

Main process and technologies for purification of oils:

- De-acidification
- Distillation
- Active carbon treatment
- Winterization
- Bleaching
- Steam deodorization

Main processes and technologies for concentrating omega3 fatty acids:

- Molecular distillation
- Urea precipitation
- Chromatography
- Supercritical fluid extraction/fractionation
- Enzymatic processing

Key factors for process and technology development:

- Be able to utilize marine oils with variable fatty acid content
- Secure high purity by combining and developing refining processes
- Secure high productivity and recovery of EPA and DHA by combining technologies.
- Develop more selective separation techniques at molecular level for marine fatty acids and fatty acid ethyl esters

## **Novel omega-3 ingredients from krill (*Euphausia Superba*)**

*Dr. Inge Bruheim, Research Director, Olympic Seafood AS, Norway*

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- A novel process, now running on board the krill fishing vessel Juvel, makes it possible to introduce omega-3 fatty acids from krill in a range of dry nutritional formulations.
- Conventional methods used in analysis of oxidative stability of omega-3 fish oils, such as peroxide and anisidine value determination, cannot be used in studying the oxidative status of krill lipids. Oxidation of omega-3 phospholipids results in formation of tertiary oxidation products such pyrroles and Strecker degradation products.
- It has been observed that gentle processing of krill into full fat meal results in minimal oxidative damage and preservation of the valuable nutritional components of krill.

## **Worldwide trends for fishmeal and fish oil –production, sustainability and market**

*Andrew Jackson, Technical Director, IFFO the Marine Ingredient Organisation, UK*

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The fishmeal and fish oil industry has seen enormous changes in recent years. These have included changes to the availability of raw materials as well as new markets for the finished products. All this at a time when there has been increasing concern over the sustainability of fishing. The presentation will cover the following topics:

- A review of recent production statistics for the industry
- Recent trends in raw material sourcing from both whole fish and by-products
- Growing trends for direct human consumption of fish and precautionary fisheries management reducing raw material availability
- Where are the raw materials for the future going to come from?
- Changing markets for fishmeal and fish oil and the trends for the future
- Growing importance of aquaculture and the omega-3 market
- The increasing importance of Asia
- Are the sources for marine ingredients sustainable and how can this be demonstrated?

## **Will fish oil be the limiting factor?**

*Petter Martin Johannessen, Supply Chain Director, EWOS, Norway*

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The global supply of marine ingredients is limited and unstable, and EWOS has contributed to a considerable more effective use of these ingredients through many years of continuously investing in research and development. The average content of marine ingredients in EWOS fish feed was 31 %, and 24 % of this was raw materials from byproducts from fish file production.

Research has always been an area of commitment for EWOS, and in 2012 we spent NOK 100 million on R&D. Our ambition is to maintain our strong reputation as the leader in our field and to be a trend setting innovator in the aquaculture industry. Already several of our innovations have changed the global fish feed industry, here a few examples:

- EWOS was the first fish feed producer that could supply feed in true bulk directly to the fish farm's feed silo, eliminating the need for bags.
- EWOS was first to introduce functional feed.
- EWOS introduced a new way of benchmarking growth performance
- EWOS has 'Sustainable Aquaculture' as its mission, and spends considerable resources on identifying alternative sources/ingredients in fish feed. Areas of commitment include:
  - Better utilization of fish waste and trimmings
  - Microalgae as source of marine Omega-3 in salmon feed

## **Marine ingredients from pelagic fish in Peru**

*Carlos Pinillos, CEO TASA, Peru*

Description of the speech:

- Sustainability of the Raw Material in Peru
- Evolution of the Fishery Industry in Peru
- Global Tendencies of Marine Ingredients

Omega-3 for human consumption – global trends

## Trends in Omega-3

*Dr. Albert Strube, Head of Product Management Omega-3, Human Nutrition, BASF SE*

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In the past years, Omega-3 ingredients have penetrated several market segments in nutrition and pharma applications, leading to a large diversity in Omega-3 uses. This trend still continues and in the meantime Omega-3 products have penetrated market segments beyond the traditional Pharma, Dietary supplement or Food segments, like e.g. Infant and Clinical Nutrition.

The market segment diversification requires a large number of different concentrations, in order to serve the demand and preferred Omega-3 concentration for each specific use, with a trend to higher EPA/DHA concentrates and customized EPA/DHA ratios.

Another trend is the tendency to alternative delivery forms, from neat oils to emulsions, syrups, gummies and powders, taking into account the preferences of specific consumer groups, e.g. for children, elderly or sportsmen.

## Omega 3 Fatty Acids from marine microalgae

*Dr Rob Winwood, Scientific Communications Manager of the Nutritional Lipids division of DSM Nutritional Products (EMEA).*

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Marine omega 3 fatty acids such as DHA (docosahexanoic acid) and EPA (eicosapentaenoic acid) are traditionally sourced from fish oils. These conditionally essential fatty acids are globally recognized as benefiting health in many areas. There are published EFSA health claims relating to DHA for maintenance of brain and eye health and for DHA and EPA, relating to heart health. The finite nature of our fish stocks lead to a search for alternative sources. The micro-algae eaten by fish and the source of EPA/DHA in their tissues were identified as an excellent alternative.

- Marine algae can be fermented in a contained environment to create biomass from which omega 3 fatty acid rich algal oils can be extracted.
- Algal sourced DHA, and more recently mixed DHA/EPA, have been commercially produced for use in supplements and foods
- Algal oils have excellent sustainability credentials and are produced in a highly controlled production environment. They also exhibit excellent taste/odour profiles.



## **Microalgae Production – From Lab to Pilot to Sustainable Industry – in Norway**

*Roald Audun Flo, CEO Biopharmia*

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The phytoplankton biomass in the oceans represents about 100 bill. tons, or 94,5 % of the total biomass. I.e. an unlimited and natural recourse. Microalgae are the very best enrichment source containing proteins, carbohydrates, lipids, vitamins, minerals, astaxanthin, antioxidants, all amino acids, needed for human, fish and animals.

Microalgae are the natural food for all life on Earth, containing no contamination, heavy metals, PCB, etc., in cultivated form.

Microalgae are the original source for omega-3, not fish and krill.

There are about 40.000 known microalgae species. Only a few thousands are in collections, and only a few hundreds have been researched for chemical content, and only a handful has been grown for industrial purposes.

The real number of microalgae are estimated to 5 – 10 millions. This means a huge potential for further research. There are microalgae everywhere in oceans, from Arctic to Antarctic. They can be cultivated everywhere – also in Norway. This is the sustainable and future oriented industry in Norway, using unlimited and renewable resources, using CO<sub>2</sub> in the production process, leaving no contaminating wasts, and produce high valuable products like nutraceuticals, pharmacy, medicine, functional food, cosmetics, food, beverage, feed, etc.

Biopharmia has developed and owns the worldwide patent rights to The Accordion Photobioreactor, an algae production technology designed for both photoautrophic and heterotrophic production. The photobioreactor is cost and space effective compared with other technologies, both regarding Capex and Opex, in addition to high productivity and effectiveness. Accordion represents a novel technology compared with other technologies.

Through laboratory tests, to pilot production, accepted and approved by world leading industries, Biopharmia is building industrial and sustainable production of microalgae in Norway, USA, Qatar, and other places, together with partners.

## Plenty more fish in the forest

*Margareth Øverland, Professor, Department of Animal and Aquacultural Sciences, the Norwegian University of Life Sciences (UMB), Norway*

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The aquaculture industry will be a major contributor to feeding the world's growing human population. The industry's output in Norway alone is projected to increase six-fold by 2050, but this will depend on several factors, including the supply of feed resources. Conventional sources like fish meal and fish oil are limited resources, so it is critical for future growth to develop alternative and sustainable high-quality feed resources using advanced technology. Today, plant ingredients are important alternatives, as these are available in large quantities. In the future, fish feed needs to be increasingly based on non-food raw materials that don't compete with human food. The presentation will focus on:

- Single-cell organisms like bacteria and yeast represent potentially sustainable ingredients due to their ability to convert natural gas or low-value biomass from forestry and the agricultural industry into high-quality feed ingredients.
- Bacterial meal produced by conversion of methane from natural gas by the bacteria *Methylococcus capsulatus* has shown to be a good feed ingredient for farmed fish. Bacterial meal contains 70% protein with a favorable amino acid composition and a wide range of components with functional properties that have shown to give beneficial health effects in salmonids.
- Another promising microbial ingredient is yeast. Use of new technology makes it possible to convert industrial by-products from the forest and agricultural industries into high-quality protein sources by yeast fermentation. Results from recent studies show promise for yeast products as protein sources in diets for salmonids.

New ingredients for fish feed based on these sources and new technologies can help secure a high-quality, sustainable food source for an ever-growing world population.

## **Cultivation of seaweed biomass for nutrients and energy**

*Jorunn Skjermo, Senior Scientist, SINTEF Fisheries and Aquaculture, Norway*

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In Norway, we have 175 brown, 205 red and 100 green macroalgal species out of which only 2 species are harvested and used commercially, either in the alginate industry or for production of extracts to be used in food, feed and fertilizers. As in the rest of Europe, the interest for seaweed cultivation is increasing in Norway and with one of Europe's longest coastlines and largest economic zones Norway has large areas suitable for seaweed cultivation. The presentation will focus on three topics:

- Cultivation of seaweed enables sustainable production of large biomass volumes for further processing into a long range of valuable products. Industrial scale technology will be a prerequisite for profitable biomass production in Norway.
- Despite much knowledge about the chemical composition of many macroalgae they so far represent an un-exploited raw material in Norway. Valuable components can be extracted and modulated for replacement of restricted feed resources, for use in fish and animal feed as well as in human food.
- Due to fast growth, high productivity and high carbohydrate content seaweed biomass is interesting for production of biofuels like ethanol and butanol. Because this production not requires human food crops, productive land area, fresh water, fertilizers or pesticides seaweed biomass represent a possibility for production of 3rd generation bioenergy.

## **Seaweed farming: Fertilized by effluents from fish aquaculture**

*Kjell Inge Reitan<sup>1</sup>, Xinxin Wang, Silje Forbord, Jorunn Skjermo and Aleksander Handå  
SINTEF Fisheries and Aquaculture AS and NTNU Department of Biology*

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The aquaculture sector in Norway is dominated of farming of salmonid fishes in marine waters, which constitutes 98% of the total aquaculture production volume. During the latest years the fish farms have increased in size and been moved to more exposed locations. The cage fish farming in open waters involves emission of nutrient to the surrounding waters, and these nutrients can be categorised into, particulate matters, dissolved organic matters and dissolved inorganic matters. The dissolved inorganic nitrogen and phosphorous from the fish farming activity can be a resource for cultivating of seaweed, and a question is if these resources can be utilised into large scale cultivation of seaweeds.

Cultivation of seaweed in close proximity to salmon aquaculture, will allow the seaweed to assimilate the waste from the fed aquaculture, and thereby recycle lost nutrients or energy similar as in natural based ecosystems. This concept is called Integrated MultiTropic Aquaculture (IMTA). A test of such system gave better length growth of the seaweed when cultivated close to the fish farm, compared to those seaweed cultivated at a reference station that was not influenced by the fish farm activity. Similar results have also been reported for experiments conducted in China, Chile, Scotland and Canada.

The nutrient emission from the fish farm activity can be regarded as an interesting resource for cultivation of seaweed. A calculation based on production statistics in Norway from 2009, with an assumption that seaweed farms can take up 10-30 % of the dissolved inorganic nitrogen originated from the salmon farming, shows that there is a potential of 577 000 - 1 730 000 ton fresh weight of seaweed production per year. This production will need an area of 80 - 240 km<sup>2</sup>.

## **Bioactive Peptides from Milk and Fish – Can They Cross the Barrier?**

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Marine species represent a unique source of novel molecules which could be of benefit in treatment of acute and chronic medical conditions. This lecture focuses on bioactive peptides which can be extracted from hydrolysed fish protein. These peptides have potent in vitro effects as mimetics of drugs which inhibit angiotensin converting enzyme, for example. The advantage of this approach is that it allows their recovery from fish proteins which would be wasted during processing. There is some evidence for clinical efficacy. The importance of the gastrointestinal barrier to their uptake will be discussed. In addition, the benefits of fish protein in restoring insulin sensitivity in animal models will be discussed.

The key theme of this talk is the importance of protein hydrolysis methods to recover and produce useful nutritional and pharmaceutical products from important fish species and those minor species which now require to be processed under EU regulations.

## **The Marine Ingredients Industry: Investment Opportunity or Future Dream**

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The marine ingredients industry has grown substantially the last 10 years. Today it represents an annual turnover in the range 8-10 billion NOK in Norway alone. There is an increasing interest in this industry from financial investors, Private Equity players and industrial companies.

We will highlight the different segments within the industry, with focus on growth opportunities and profitability. Give some examples on success stories and what investors are looking for.

### **A biorefinery model**

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Borregaard in Norway have been running a spruce based biorefinery for more than 50 years. The concept of this biorefinery will be presented as well as a new biorefinery concept based on the new BALI technology for co-production of lignin based performance chemicals and sugar based chemicals. A further development of such a biorefinery concept particularly adapted to Norwegian conditions will also be presented as an example of improved value creation for a biorefinery utilizing local or regional opportunities. This will integrate a wood based biorefinery with other industries like fisheries, aquaculture, off-shore and metal industry. There are many general lessons to be learned from one type of biorefinery based on one type of biomass to another type of biorefinery. Some of the main lessons will also be touched upon.

### **Omega-3, omega-6 and contaminants in Atlantic salmon**

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The composition of diets for farmed Atlantic salmon is in constant development, and the inclusion of fish oil and fish meal have gradually decreased since early 2000. Especially the content of the marine omega-3 fatty acids EPA and DHA, omega-6 fatty acids and contaminants change when marine ingredients are replaced with plant proteins and vegetable oils. Today, the content of EPA and DHA in farmed salmon is comparable with that of wild Atlantic salmon where 100 g fillet contribute with ca 1.5 g EPA and DHA. Hence, one portion of 150 g salmon fillet contributes with more than one week recommended intake for healthy people to protect against development of cardiovascular disease (0.25 g/day).

When the inclusion of fish oil decrease the inclusion of vegetable oils increase, which

contribute with alternative fatty acids such as monounsaturated fatty acids and omega-6 fatty acids. The balance of omega-6 to omega-3 is recommended to be 5 to 1 in a healthy diet. However, most western populations have too high intake of omega-6 fatty acids compared to total omega-3. Due to increased use of rapeseed oil in farmed Atlantic salmon diets, the content of omega-6 fatty acids have increased in Atlantic salmon since early 2000 and today's farmed Atlantic salmon is a source of omega-6. The balance between total omega-3 and omega-6 in farmed salmon is ca 1 to 1, thus a positive contribution for shifting the balance closer to 5 to 1. It is, however, important that the omega-6 content of farmed Atlantic salmon does not increase any further.

The content of the environmental contaminants dioxin and dioxin like PCB's in Atlantic salmon have received great attention over the past decade. When the diets change to contain less marine ingredients, the content of dioxins and dioxin like PCB's also decrease. This is reflected in Atlantic salmon fillet, thus based on today's composition farmed Atlantic salmon is safe food both relative to upper limits and tolerable weekly intake (TWI).

In conclusion, farmed Atlantic salmon is seafood in compositional evolution due to the replacement of marine ingredients with new alternatives. Farmed salmon and wild salmon is safe food and they are equally good sources of EPA and DHA. Farmed salmon is still a limited source of omega-6 contributing to an improved balance of omega-6 to total omega-3 in a healthy diet.

## **Abstracts for posters**

## 01: Cod and herring milt affects human immune cells

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Fish milt represents an essential part of byproducts obtained from cod and herring during seasoning fisheries. Fish milt has a high content of nucleic acids, protamine, essential vitamins, minerals and trace elements as well as omega-3 fatty acids. In this study, fish milt in the form of cod and herring milt powder and DNA isolated from cod and herring milt were evaluated as possible dietary immunostimulants. Milt powder, DNA from milt, RNA from baker's yeast, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the concentrations found in milt powder were evaluated in an inflammation model using lipopolysaccharide (LPS) stimulated peripheral blood mononuclear cells (PBMC) isolated from healthy human volunteers (n=6). LPS increased the secretion of interleukin 1 beta (IL-1 $\beta$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin 6 (IL-6) and granulocyte macrophage colony stimulating factor (GM-CSF) in all PBMC. There was a small to moderate increase in LPS induced IL-1 $\beta$  secretion when cod milt and cod DNA were included. Also LPS induced TNF- $\alpha$  and IL-6 secretion was increased adding cod and herring milt and DNA from cod and herring to the cultures. LPS alone did not increase interleukin 8 (IL-8) secretion compared to controls, but adding herring and cod milt and DNA from herring and cod together with LPS, IL-8 secretion increased substantially. RNA from baker's yeast affected granulocyte macrophage colony stimulating factor (GM-CSF) particularly, both regarding protein secretion and gene transcription. LPS induced IL-1 $\beta$  transcription was increased by adding cod or herring milt to the cultures. Cod and herring milt, DNA isolated from cod and herring and RNA from baker's yeast are able to modulate human PBMC cytokine secretion and affect gene transcription. Milt and DNA from cod seem to affect IL-1 $\beta$  transcription and secretion particularly, while milt and DNA from both cod and herring, together with a LPS as a costimulatory signal, were generally inducers of IL-8 secretion. TNF- $\alpha$  secretion was affected least of all. Milt and DNA from cod and herring, EPA and DHA, alone, could not induce any cytokine secretion or transcription.



## 02: High quality protein ingredients from marine macroalgae

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**Background:** Corn, rice and wheat provide approximately 60 % of the world's food energy intake and are considered as staple foods for around 4 billion people, mostly in developing countries. Both total protein and the level of some essential amino acids (lysine and threonine) are low in these cereals and finding additional protein sources would be of interest. Marine macroalgae are a diverse group of fast growing plants which occur in marine environments worldwide. Studies from Asia, where macroalgae are a part of the traditional diet, have shown that some species are rich in good quality protein. So far, the utilisation of macroalgae in the rest of the world has been limited, but considering both protein content and growth rate, at least some species could be potential protein sources. The aim of this study was to examine the protein content and amino acid composition of ten common marine macroalgae and compare them to corn, rice and wheat.

**Methods and results:** Amino acid composition of all samples was analysed chromatographically following acidic hydrolysis and protein contents were calculated from the sum of amino acid residues. Of the analysed species, *Enteromorpha intestinalis*, *Palmaria palmata* and *Vertebrata lanosa* ranged highest in protein and had a high relative content of essential amino acids (11-12 % protein and 38-43 % essential amino acids, respectively). Assessed against human requirements, the protein of these species contained sufficient amounts of most essential amino acids. Compared to meals of corn, rice and wheat, the algae meal contained equal or higher amounts of all essential amino acids. Levels of threonine were 3-4 times higher and levels of lysine were 3-9 times higher in algae meal compared to corn, rice and wheat meals. The results from this study show that several algae species could serve as valuable protein sources.

### **03: Oxidative stability of herring milt powder tested at accelerated storage conditions**

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Rest raw material from the pelagic fish sector is predominantly used for fish meal, protein concentrate and oil production. Herring roe is to some extent processed for human consumption, while the utilization of herring milt is still limited. Herring milt is a complex marine raw material with high content of protein, DNA, trimethylamine N-oxide, and polyunsaturated fatty acids. The goal of the present project was to further optimize a novel manufacturing process and evaluate the oxidative stability of a dried herring milt product. Herring milt was collected from a Norwegian herring fileting factory and stored frozen (-33 °C) until heat treated and processed into a dried powder in pilot scale at Nofima in Bergen. Three different antioxidants were added in a factorial design experiment and the oxidative stability tested at 35 °C with open access to air. A parallel kinetics study and sensory testing was used to determine the needed storage time before assessment of the samples based on sensory and headspace GC-MS analysis. Headspace analysis revealed some volatiles typical for oxidized marine lipids, but at levels below the perceived threshold for unpleasant taste and smell. In the presence of the best antioxidant the sensory properties of the milt powder were found to be still acceptable after 3 months storage at 35 °C.

#### **04: Characterization of seaweeds with the potential to replace sodium in food products**

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Health authorities worldwide have recommended reducing salt in processed foods in order to reduce the risk of high blood pressure. Seaweeds have the potential to act as salt replacers since they have naturally salty taste being abundant in minerals like potassium, magnesium besides sodium. This salty taste improves the flavor profile of foodstuffs. In addition, some seaweed contains a range of potential flavor components that can naturally enhance the flavor of the food. Mild processing can release potential flavor components like proteins, amino acids and reducing sugars. The aim of this work was to characterize three species of brown seaweed (*A. nodosum*, *F. vesiculosus*, *S. latissima*) which could potentially act as sodium replacers in food products through mild processing.

A seaweed flavor language was developed and the sensory properties of the seaweeds evaluated by different sensory panels using two sensory techniques, i.e. QDA (quality descriptive analysis) and FCP (free choice profiling). Chemical characterization was carried out, in addition to key aroma identification, in order to identify taste active compounds or their precursors. Sensory evaluation revealed differences between the unprocessed seaweeds. Besides high carbohydrate and mineral and low fat contents in all species, protein contents varied. The results indicated a greater taste-enhancing potential of *F. vesiculosus* compared to *A. nodosum* and *S. latissima* due to higher overall amino acids content and higher glutamate contents. Next steps in the project are to develop processing methods in order to release the components with flavor enhancing properties.

## 05: Seasonal variance in polyphenol and polysaccharide content in seaweed

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The bioactivity, anti-oxidative and potential utilization of chemicals extracted from seaweed has been extensively researched in the recent years. Polysaccharides, such as fucoidan and laminarin, have great bioactivity potential and can be used in functional food, nutraceuticals and cosmetics. The bioactivity includes anticoagulant and antithrombotic, antiviral, antitumor and immunomodulatory, anti-inflammatory, antioxidant properties and gastric protective effects.

We used seaweed samples collected on the east coast of Iceland in 2012 and studied seasonal variance in the polysaccharide content and bioactivity of extracts by comparing samples collected in March, June and October of that year. The species used were *Fucus vesiculosus* and *Ascophyllum nodosum*. Our focus was on the two polysaccharides that have garnered the most interest for their bioactive properties: fucoidan and laminarin. Polysaccharide extracts were subjected to standard bioactivity tests to determine variance between the two species as well as the time of harvest.

Research has focused on phlorotannins and polysaccharides from different species and shown variance in the amount between species. While it is common to state which species is worked with in research there is a lack of specifying which season the seaweed harvested. We will address this season variance and the effect on bioactivity. This factor is important as the different ratio of sugars and polyphenols can affect the bioactivity of seaweed extracts.

## **06: Consumer perception of seafood products enriched with marine based ingredients (e-poster)**

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Consumers increasingly search for food products with known bioactivity as means to improve their health or prevent diseases. The food industry is now moving from producing nutritious food into food promoting health and well-being, preventing illness and reducing the risk of chronic diseases. It is important to understand that consumers are not willing to sacrifice taste to realize these nutritional benefits. Therefore, development of enriched foods requires understanding of consumers' demands. The ingredients omega-3, seaweed extracts and fish proteins have various health beneficial effects and are of different familiarity to consumers. In home-use test (n=79) of seafood products carrying different information about enrichment of the products with these ingredients generally indicated negative liking of taste during consumption. Thereafter consumer attitudes towards enriched seafood concepts were studied using an on-line survey among consumers in Iceland (n=460) and Finland (n=432).

Consumers were questioned on perception of healthiness, naturalness, taste and buying intention of enriched product concepts with different information on ingredients and health effects. Although information on enrichment had rather negative effects on perceived taste, information about ingredients and health effects positively affected product perception and buying intention. Enrichment of convenience seafood products with marine based ingredients is a realistic option for the seafood producers however it is extremely important to consider labelling and information provided to the consumers

## **07: Isolation of marine phospholipids from the non-soluble fraction (sediments) after hydrolysis of salmon backbones**

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Marine phospholipids usually contain high content of long chain omega-3 fatty acids with well documented beneficial health effect. Therefore, the interest in marine phospholipids as carriers of n-3 fatty acids is increasing in supplement industry. Both new sources for marine phospholipids and technologies for separation of marine phospholipids are subjects of interests.

Marine biomass such as krill is already commercially utilised as a source of long chain omega-3 fatty acids in the form of phospholipids. The focus to use fisheries rest raw material for production of fish protein hydrolysates, fish oils for human consumption is increasing. Enzymatic hydrolysis for production of fish protein hydrolysates yields non-soluble fraction which still contains a high amount of lipids (up to 50%) including phospholipids (up to 60% of lipids). This non-soluble fraction could be used as a material for isolation and/ or concentration of marine phospholipids.

This work discusses the possibilities to use the non-soluble fractions: bones and sediments obtained after hydrolysis of salmon backbones for production of marine phospholipids.

The research leading to these results has received funding from the European Community's Seventh Framework Programme FP7/2007-2013 under grant agreement n° 289170 —APROPOS.

## 08: Methods to evaluate lipid oxidation: Strengths and weaknesses

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Oxidation is an important cause of quality deterioration in marine oils. Due to the high amount of unsaturated double bonds a large variety of different peroxides and secondary oxidation products can be formed during oxidation of marine oils. Measuring of all the oxidation products in the oil can therefore be time and resource consuming. Therefore, in industry per today the standard oxidative quality parameters are: the level of primary oxidation products measured by peroxide value and the level of secondary oxidation products – aldehydes - measured as anisidine value. Other methods that evaluate the amount of peroxides and aldehydes in the oils can also be employed. However, each method has its strengths and weaknesses.

This work discusses the principle of several methods that are used for evaluation of the oxidation status of oils. The methods that were tested were traditional iodometric titration methods for peroxide value test, and anisidine value for determination of the level of secondary oxidation products. The sources of possible misinterpretation of the results such as reaction time, presence of oxygen in the measuring system, the intensity of the mixing of the reactants were studied and discussed. Some of the fish oil products that are on the market contain flavors, color compounds and vitamins. Studying the effect of different additives on the measurement of the oxidative quality parameters showed that addition of some additives (like lemon taste) complicates the determination of the oxidation status of the product.

## **09: The potential use of Infrared and near-Infrared spectroscopy in marine oil processing**

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Omega-3 ingredients and concentrated omega-3 fatty acids of marine origin are gaining increasing interest within international food, ingredients and pharmaceutical industries. This is not least related to the potential health beneficial effects of intake of omega-3 rich products reported in recent years. Proper monitoring of the industrial processing of marine oils is a major prerequisite for cost-efficient production, for high product quality, for minimising waste, and thus for a sustainable use of marine resources. Important monitoring points relate for instance to the characterisation of raw material qualities, to product quality documentation according to product specifications, and to trouble shooting approaches in case of product quality deviations. Currently, chromatographic approaches are the main analytical instrumentations used for process monitoring in marine oil processing industries. The methods are accurate, but time-consuming and labour-intensive, and on-line monitoring approaches are almost non-existing at present. Laboratory-based at-line alternatives, however, based on infrared spectroscopy have been introduced to the market, but the general awareness of this methodology as well as its suitability for process monitoring is low. Recently, we have compared the performance of infrared and near-infrared sensors for the characterisation of fatty acid composition and glyceride composition (i.e. the content of esters, mono-, di-, and tri-glycerides). The latter sensor has on-line capabilities and could thus potentially be used on-line directly in a distillation process. In the presentation, the potential of the two sensors for use in marine oil processing will be demonstrated, and factors such as detection limits and prediction uncertainties will be discussed. The research results presented have been obtained in collaboration with Epax AS and GC Rieber Oils AS, and funding from “regionalt forskningsfond Midt-Norge” is greatly acknowledged.



## **10: Functional and bioactive properties of fishmeal from fresh byproducts of cod (*Gadus morhua*) and saithe (*Pollachius virens*)**

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Norway is globally one of the top producers of fishmeal but little effort has been put into development of new production technology or investigation of valuable ingredients for the commodity-, health and pharmaceutical markets. However, newly contracted vessels in the whitefish trawling-fleet have been equipped with on-board production facilities of fishmeal for direct human consumption. This will improve the utilization of totally fresh fish-processing by-products (FPCP) and should enforce products of higher quality as well as better conservation of interesting properties in the FPCP. Substantial scientific validation of the physiochemical and health promoting effects of bioactive components of this whitefish meal are however scarce. In order to explore new market opportunities, investigation of functional and bioactive properties of “at-sea test productions” of whitefish meal have been undertaken by Møreforsking Marin. Here we present data on biochemical, physiochemical and bioactivity properties of fishmeal produced from FPCP of cod and saithe. Results are discussed in relation to other fishmeals on the market.

## **11: Two stages processing of salmon by-products for the high quality product and more profitable and sustainable production**

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Two stages processing concept for utilization of salmon by-products were developed. This technology applied for fish-filleting by-products combine the gentle thermal treatment following by enzymatic hydrolysis. This thermo-enzymatic process yielded premium oil at the first stage of the process. The remaining raw material was further processed by enzymatic means. The flow of raw material into the enzymatic stage decreases due to separation of significant part of oil which leads to a decreased amount of enzymes needed. The energy consumption for unnecessary warming and heating of oil is also reduced. After hydrolysis and enzyme inactivation the second oil fraction as well as soluble fish protein hydrolysates (FPH) and sediments fraction obtained. The solid fraction containing insoluble proteins, lipids and bones can be used as material for extraction valuable ingredients like phospholipids or can be dried and used as feed. Remains of the processing might also be used for energy production and soil improvement. Sustainability of the process was evaluated by the method of life cycle assessment (LCA). Assessment includes chain of salmon by-products processing, transportation, feasible application and final degradation in the soil. In addition, the economic feasibility of proposed concept is evaluated. In order to handle the uncertainty of early stage concept design, Monte Carlo simulations are utilized to ensure more realistic results of key economic figures with probability distributions.

The research leading to these results has received funding from the European Community's Seventh Framework Programme FP7/2007-2013 under grant agreement n° 289170 —APROPOS.

## 12: Underutilized fish biomass for food, feed and bioenergy production

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Finland has underutilized fish resources, which is globally quite exceptional. The eutrophication of the Baltic Sea has resulted in growing abundance of low-valued cyprinids (Cyprinidae), which has caused multiple problems for fisheries. Therefore, temporary subsidies are targeted to fishing of cyprinids to remove fish-bound nutrients from the coastal waters and to find new markets for these fish species. Some 1 500 tons of cyprinids were caught along the Finnish coast in 2012. They were exported for food use or used as animal feed. More aggregated and larger volumes (some 7 000 tons) of low-value fish biomass arise from the fish processing industry. Depart from the previous these by-products are fatty and already partly utilized in processing.

The aim of this project is to study the feasibility of new technologies (for instance protein, fish oil and biogas production) to use underutilized fish and fish industry by-products in Finland. Raw material volumes were mapped and the economic feasibility of diverse commercial value chains was evaluated. Experiments producing dehydrated fish meal were carried out. Furthermore, the applicability of fish biomass to biogas production was investigated. Based on the results the businesses of the highest potential and the areas of insufficient scientific or technical knowledge will be revealed and outlined for further investigation.

Project (2011-2013) has been funded as a part of The Biorefine – New Biomass Products programme by Tekes – the Finnish Funding Agency for Technology and Innovation and two companies, i.e. Sybimar Oy ([http://www.sybimar.fi/eng\\_sybimar.php](http://www.sybimar.fi/eng_sybimar.php)) and Biovakka Suomi Oy (<http://www.biovakka.fi/?setLang=en>)

### 13: Enzymatic production of triacylglycerols: study of kinetics

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The beneficial effect of long chain omega-3 fatty acids (such as EPA and DHA) is well documented. Fatty fish or fish oil supplements are sources of those fatty acids. Using supplements of long chain polyunsaturated fatty acids in a concentrated form can reduce intake of lipids, but still ensure recommended daily intake of beneficial fatty acids. EPA/DHA in a triacylglycerol form is indicated to have better oxidative stability. Moreover, fatty acids in the triacylglycerol form are considered as a more natural from the consumer's point of view. Thus, up-concentration of EPA/DHA to triacylglycerol form from the ethyl esters is a process of interest in the supplement industry. In order, to have better control of the up-concentration process, understanding kinetics of the re-esterification process is important.

This work discusses re-esterification process between ethyl esters (high concentration in EPA and DHA) and glycerol using the enzyme Novozyme 435. The effect of temperature and pressure on the kinetics of re-esterification process is investigated. The re-esterification rate was followed by analyzing the changes in ethyl ester amount in the reaction mixture. Lipid class composition was followed by TLC-FID analysis. C-13 NMR study indicates: sn-1,3 positional selectivity of Novozyme 435 and even distribution of EPA and DHA in produced TAG molecules. Kinetics study shows that re-esterification reaction followed second order kinetics. The proposed mathematical equation could simulate the ethyl ester concentration changes up til 20mbar pressure 60-80°C. Increased pressure most probably led to increased ethanolysis reaction and the measured ethyl ester concentration values were higher than simulated ones.

## 14: Comparison of industrial endopeptidases for the hydrolysis of Atlantic salmon head and backbone rest raw material

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In Norway, the amount of rest raw materials from the fish fileting industry (pelagic and salmonoid fish) is around 800.000 tonnes. Most is still used for the production of feed grade fishmeal, protein concentrate and oil. However, there is a strong industrial interest for the development of higher value food products, including fish protein hydrolysates.

The main objective of the study was (1) to compare the efficiency of five food grade industrial endopeptidases for the hydrolysis of Atlantic salmon rest raw materials (head and backbone) and (2) to establish substrate specific figures for nitrogen factor ( $N_f$ ) and total number of peptide bonds ( $h_{tot}$ ) needed for the calculation of the degree of hydrolysis (DH).

The activity of the endopeptidases was determined at pH 6.5 and 50 °C by a universal protease activity assay with casein as substrate. Hydrolysis kinetic studies with activity levels of 5-80 U/g protein were performed at pH 6.5 and 50 °C, and the DH monitored by the pH-STAT and OPA methods.

The amino acid composition of the salmon substrate showed high levels of essential amino acids and taurine. The nitrogen factor was found to be 5.83 g protein/g N and the  $h_{tot}$  value 8.32 mequiv/g protein. A linear correlation between the OPA and pH-STAT methods was established ( $R^2 = 0.89$ ). No significant differences were observed for the individual enzyme regression coefficients. The effect of enzyme dosage decreased at levels above 20-40 U/g protein. A comparison of the cost efficiency of the individual enzymes for the specific substrate and conditions will be presented.

## **15: A novel and rapid approach for monitoring enzymatic protein hydrolysis using Infrared spectroscopy**

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Enzymatic protein hydrolysis of by-products of marine and animal origin is an emerging industry in Norway. In the marine sector, enzymatic protein hydrolysis has been a subject of focus for several years, and currently there are several commercial companies that produce protein hydrolysates from marine by-products. One of the main challenges regarding industrial enzymatic protein hydrolysis is to provide stable and optimal product quality based on raw materials of highly variable composition. Rapid and robust monitoring of raw material quality, the hydrolysis process and end-product quality is thus of great importance. There is, however, currently a lack of techniques to serve these purposes. A crucial point in the hydrolysis is to decide when to terminate the process and inactivate the enzymes. There are numerous techniques based on chemical reference methods to serve this purpose, but the techniques are generally time-consuming and not feasible for industrial use. Infrared spectra are known to be sensitive probes for protein structure, and feasibility studies using rapid infrared and near-infrared sensors for monitoring of hydrolysis reactions have shown promising results. The limiting factor for these analyses is, however, related to sensitivity issues related to low concentrations of the analytes in aqueous solutions, and interferences from water in the protein region of the infrared spectra. Thus, at Nofima we have recently developed an alternative approach for infrared analysis based on thin dried films of protein hydrolysates. Using this methodology we are now able to monitor the enzymatic hydrolysis of salmon tissue based on spectral changes in the protein regions of the infrared spectra. These results will be presented along with an overview of the quantitative aspects of the methodology. The approach is rapid, easy to use, could be automated, and can potentially be implemented in low-cost instrumentation.

## 16: Marine Ingredients: quantity, quality and use in Portugal (e-poster)

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**MARMED** project (2012-2014) aims the valorization of marine residues and sub-products from several companies from the marine sector, with demonstration of real cases (collaboration with industries as proof-of-concept) and on the other side to demonstrate the added-value and high-potential of applicability of those materials in biomedical applications, also with close industrial collaboration, that will evaluate the market potential of the applications to be developed and/or studied under the scope of this project.

Research was made on the quantities and types of marine ingredients (by-products) available in Portugal for valorization. A survey on marine by-products production was sent to marine industries: seafood, fish fleets, aquacultures, shellfish harvesters, transformation (canning, smoking, freezing, salting and drying). Contacts were established with by-products processing operators (fishmeal plants) and waste disposal operators to inquire about the quantities of by-products received annually.

Estimations of the major figures were drawn from the  $\approx 5\%$  of the companies that answered (till the present moment) to the survey or to our contact. Secondary sources like the National Institute of Statistics and the ICES working group were also consulted. The total discards - 3084.6 t (2011) - include: atlantic chub mackerel, bleu, whiting, boarfish, hake, sardine and sharks.

At the fish auctions, the total rejections are around 507 t/year. The withdrawals of anchovy, scad or horse mackerel, chub mackerel, european conger, pout, hake, ray, atlantic mackerel and sardine at the fish auctions are around 530 t/year. From the transformation industries the marine ingredients available are skin, bones, head, tails, fillets leftover and viscera of: cod fish, scabbardfish, black scabbardfish, salmon, sea bass, hake, sea bass, lusitanian shark, tuna, gill-head bream. Crustacea by-products are also available.



## 17: Oxidation of marine lipids in gastric juice – *in vitro* study

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The importance of long-chain polyunsaturated omega-3 fatty acids (LCPUFA) from marine sources in the diet, especially eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), has been well established. Lipid radicals and oxidized derivatives (e.g. core aldehydes, hydroperoxides, and epoxy compounds) and other products of oxidative decomposition of LCPUFA (e.g. low molecular weight aldehydes) are on the other hand believed to be cytotoxic and linked to development of cancer, atherosclerosis, and other diseases. The state of LCPUFA entering the blood stream is therefore crucial for the overall impact of marine lipids on health. Before the lipids are metabolized, they are retained in the stomach and blended with gastric fluid. It has been proposed that stomach environment acts as a bioreactor and can create environment for oxidation processes.

In the present study, oxidation of emulsified herring lipids was followed in an *in vitro* digestion model containing authentic human gastric juice, and a model lacking gastric juice components apart from hydrochloric acid. The aim was to evaluate whether marine lipids oxidize in the acidic gastric juice and whether gastric juice has the potential to act as a pro-oxidative medium. The effect of iron, hemoglobin and several antioxidants rich beverages (red wine, green tea, coffee, berry and orange juice) on oxidation of marine lipids under the stomach conditions has been investigated as well. The level of lipid oxidation was determined by measuring peroxide value (PV) and TBARS in the lipids, and the oxidation was also followed continuously by recording oxygen consumption (OUR) by fatty acids.

PV, TBARS and OUR increased in all models during incubation (2.5 h, pH 4, 37 °C). However, the markers showed no difference between oxidation in gastric juice and hydrochloric acid solution. Gastric juice reduced the prooxidant activity of iron ions measured as oxygen uptake rate, but did not reduce the activity of methemoglobin. Berry juice, green tea, red wine, and caffeic acid reduced oxygen uptake in the acidic environments while coffee, ascorbic acid and orange juice increased oxidation. Beverages accompanying foods containing marine lipids will therefore affect the course of post-prandial lipid oxidation.



## 18: Reduction of persistent organic pollutants in fishmeal and fish oil

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Persistent organic pollutants (POPs) are fat soluble chemical compounds that persist in the environment, bio-accumulate in the food chain and might cause adverse effects to human health and to the environment. POPs comprise pesticides, industrial chemicals and unwanted by-products. Fishmeal and fish oil has been identified as one of the most important contributors to the level of dioxins and PCBs in fish feed. Fish caught in some of the more polluted North-European fishing areas contain high POP levels resulting in fishmeal and oil with dioxin and PCB levels above the maximum permitted. To comply with present legislations and enable the use in feed applications such products have to be decontaminated.

Efficiency of alternative decontamination technologies to reduce the level of POPs in fishmeal (organic solvent extraction and oil leaching) and fish oil (activated carbon (AC) adsorption and short-path distillation (SPD)) have been studied and compared. Factorial design experiments, response surface methodology and quantitative structure property relationships have been utilized to model and optimize the alternative technologies.

The fishmeal oil leaching process has been found to give effects comparable to hexane and isopropanol extraction (> 75%). AC adsorption of PCDD/Fs is highly effective with obtainable TEQ-reduction of 99%. Adsorption of DL-PCBs is less effective and dependent on *ortho*-substitution. AC adsorption has no effect on PBDEs. Reduction by SPD of the individual congeners is linearly dependent on the number of chlorine or bromine substitutions and degree of *ortho*-substitution. High decontamination efficiency (> 90%) can be obtained by choice of favorable process conditions. Residual levels considerable below the maximum permitted levels are obtainable based on the studied technologies. However, some limitations exist and will be discussed.

## 19: Protective culture inhibiting *Listeria monocytogenes*

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It seems that most indigenous pathogenic bacteria in lightly preserved seafood products may adequately be controlled by strictly respecting production hurdles such as salt content, smoke and storage conditions but *Listeria monocytogenes* still remains a major microbial risk. The prevalence is usually low initially, but still, expiry date of e.g. smoked salmon is often influenced by the risk of *L. monocytogenes* to exceed the EU food safety criteria for ready-to-eat food with the tolerated limit of <100 CFU/g (EC 1441/2007).

Two French Institutes, Ifremer and Oniris, have successfully selected strains of *Carnobacterium* producing bacteriocins with promising anti-listerial properties, which full-fill the demands to a protective culture for seafood application. The strains have successfully been validated on a wide range of *L. monocytogenes*, they do not produce histamine, they do not exhibit any problematic antibiotic resistance, they do not modify the microbial biota except for inhibiting the growth of *L. monocytogenes*, and they do not have any negative sensory effects. Consequently, addition of these *Carnobacterium* should enhance the safety of seafood products. This concept is now being commercialised on license by the Italian starter culture manufacturer; Sacco S.r.l., as the protective culture Lyoflora FP-18.

## 20: An example of French strategy for the production of marine ingredients from salmon by-products: the Pesk&Co project

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Marine biological resources such as fish by-products are sources of valuable ingredients and bioactive molecules. Their exploitation using biotechnological tools is likely to lead to the development of new markets and industries, specifically in the areas of food, petfood, feed, cosmetics, and, in some cases, therapeutic agents.

For the first time in France, four companies located in Brittany on the West coast of France (Meralliance, Yslab, SPF and Glon, respectively) and the University of Bretagne Occidentale (Laboratory of Marine sciences - LEMAR UMR 6539) gathered their expertise to set up an integrative research and development project. The project Pesk&Co is financed with the support of *Région Bretagne, Conseil général du Finistère* and *Quimper Communauté*.

The Pesk&Co project aims to produce new marine ingredients such as structure proteins, functional peptides, polysaccharides for food, feed, and cosmetics applications.

The Pesk&Co strategy is based on :

- (1) The implementation of innovative processes such as clean technologies and biotechnological processes to embrace the total salmon by-products, from raw materials available to market.
- (2) The creation of a research and development center based on efficient multidisciplinary cooperation between specialists from the 5 partners of Pesk&Co project, in order to properly address the main objectives and stimulate synergies between companies, from salmon by-product processing to commercialization of active compounds.

The expected results will contribute to the added value obtained by a completely integrative project which regroups local skills and agents, to place Brittany Region on the global market of marine ingredients.

Partners :

<http://www-ium.univ-brest.fr/UMR6539/>

<http://www.meralliance.com/>

<http://fr.spf-diana.com/>

<http://www.yslab.fr/>

<http://www.groupe-glon.com/>

## **21: Herring roe phospholipids improved plasma lipids and glycemic control in healthy, young subjects**

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Herring roe is an underutilized source of n-3 polyunsaturated fatty acids (PUFAs) for human consumption with high phospholipid (PL) content. PL may improve bioavailability of n-3 PUFA; and provides choline. Arctic Nutrition's MOPL™30 is a herring roe PL:DHA-rich fish oil mixture, with DHA:EPA of 3:1. Healthy young students (21) received encapsulated MOPL30, 2.4 g/d EPA + DHA for 14 d. Blood parameters were measured before- and after supplementation. An oral glucose tolerance test (OGTT) was performed with 75 g glucose with samples at 0 (fasting), 10, 30, 60, and 120 min. Fasting TAG decreased and HDL-cholesterol increased post-supplementation ( $p < 0.05$ ). HDL/LDL increased and NEFA decreased ( $p < 0.10$ ). Fasting glucose was unchanged. In the OGTT, blood glucose reduced at 10- and 120 min with MOPL30 vs. pre-study values, improving glycemic profile. Plasma phosphatidylcholine (PC)- EPA, n3 DPA and DHA increased 159%, 13%, and 62% respectively. Likewise, EPA and DHA increased 134% and 48% in RBC PC, respectively. In RBC PC, the omega 3 index (EPA+DHA as % identifiable fatty acids) increased from 1.9% to 5.9%. In both pools, linolenic acid decreased 6-15% and various n6 and n9 PUFA were decreased. Serum insulin, free choline, and vitamins are being analyzed. Herring roe PL as MOPL30 improved lipid profile and glycemic control in young individuals with high habitual intake of n-3 PUFA and healthy eating habits, after only 2 weeks. A double-blinded randomized trial is underway to expand these findings.

## 22: Digestibility of herring roe egg proteins in a dynamic in vitro gastrointestinal model

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Fish proteins have attracted nutritional interest due to favorable effects on blood glucose and lipids, and anti-obesity and sports performance benefits. The goal herein was to determine the digestibility of herring roe proteins and constituent amino acids. Herring roe intact proteins, shells, and lumen (provided by Arctic Nutrition, Norway; whole extracts sold as Herring Caviar Protein Powder-HCP) were compared to ovalbumin (chicken egg white), using an in vitro dynamic, computer-controlled model of the stomach and small intestine (TNO Tiny-TIM). Total protein content of whole, shell, and interior was high at 87, 96, and 90% (dry wt. basis), respectively. Relative to ovalbumin, whole HCP was higher in Ile, Leu, Val, Lys, Thr, Tyr, and carnosine; and lower in Met, Cys, Trp, Phe, His, and Arg. Whole HCP was digested more rapidly and more completely (70% after 6 h) than ovalbumin (56%)- desirable for sports and clinical applications. Individual essential amino acids in HCP were digested more completely vs. ovalbumin for Ile, Leu, Val, Met, Trp, Phe, His, Cystine, and Arg. Digestible indispensable amino acid scores (DIAAS) for whole HCP (FAO reference protein; 6 mo-3 y) was “good” (>75% of the FAO reference protein). For the FAO reference protein for children and adults > 3 y, both whole HCP as well as the interior HCP scored a DIAAS >75%. HCP has good odor and stability for a fish protein, high protein content (87%), and contains bioavailable amino acids, and nucleotides. HCP can also be complemented with other proteins to make a perfect protein. The peptide profile of HCP and further investigations into protein stability are areas of future interest.

## 23: Sensory evaluation of omega-3 oils

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The bulk of the world's omega-3 production is located in the northwest of Norway and the industry focuses on marine oils with high nutritional value and quality.

There are currently no common sensory quality demands on the omega-3 oil, but the industry has a number of chemical documentation requirements for their products. Over the last years there has been an increased focus and commitment to document the sensory quality of marine oils. It is expected that sensory quality will be a marked demand in the future. At present most of the companies practice some sensory quality control. However, standardized methodology for sensory evaluation is lacking. Therefore, a group of six companies have joined forces to strengthen their expertise in the field.

The main objective of this study is to develop a sensory evaluation method for the omega-3 oils. The evaluation method will include the entire process and best practice of sensory testing with guidelines of how to choose sensory judges, prepare samples, training, testing and result evaluation. The marine oils include a wide range of oils produced from different species, using somewhat different production methods and with different choices of antioxidants. The project will develop a method that is robust and which will include most of these products.

Within plant oils, there are currently a number of requirements for both chemical and sensory evaluation. The olive oil industry has developed an aroma wheel with good description of both the positive flavors and the negative off-flavor of the product. The aroma wheel is commercially available in the same way as the aroma wheel customized for wine tasting and is a useful tool for the sensory panel during quality assessment. The present study is based on the experience from this work. Sensory and chemical properties for the marine oils are compared and a nomenclature and an aroma wheel are developed for the different marine oils available.

The results of this study project will make a basis for standardized sensory evaluation of omega-3 oils and is expected to be an important tool for marketing marine oils from the Norwegian omega-3 industry.

## 24: Microbial production of DHA

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Due to the growth of the marine aquaculture industry the demands for the essential omega-3 fatty acids EPA and DHA are rapidly increasing. The future needs can not be covered by fish oils, and new sources for these fatty acids are required.

Microbial production of EPA and DHA is a potential future, sustainable feedstock. Phototrophic and heterotrophic microalgae and some heterotrophic protists (thraustochytrids) can accumulate high levels of EPA and/or DHA as part of their lipids. Heterotrophic production provides considerably higher productivities than phototrophic production. DHA produced by heterotrophic marine microorganisms is already commercialized for applications as dietary supplements and functional foods, but the production costs currently prevent application as fish feed. However, with the increasing prices of fish oil, and improved production strains and processes for microbial production, microbial produced omega-3 fatty acids could also be a realistic alternative for feed applications.

A collection of approximately 100 thraustochytrids has been established by bioprospecting. Of these, 7 strains are commercially interesting DHA-producers. These strains accumulate more than 50 % lipids (mainly triacylglycerols) of the cell dry weight, and DHA constitute more than 30 % of the fatty acids. Other strains accumulate similar levels of lipids, and up to 10 % EPA or ARA (arachidonic acid), but no DHA. A high number of strains that do not accumulate lipids have very high levels of DHA (>70% of the membrane fatty acids). Many of the strains also produce carotenoids, which may contribute to an added product value.

One of the strains has been further characterized in fermentation studies. A productivity of 2.5 g/l-day and a volumetric concentration of 14 g/l DHA have been obtained. By this productivity, DHA can be produced for a selling price of 500 NOK/kg (~80 \$/kg), corresponding to 125 NOK/kg (~20 \$/kg) for an oil with 25 % DHA. Process optimization may bring the process down to 200 NOK/kg DHA (50 NOK/kg oil), and by use of other strains the prices may be even further reduced.

A new project with the aim to improve the productivity of DHA and carotenoids by physiological optimization and genetic engineering has recently been initiated.



## 25: Extraction of LC-PUFA fatty acids from marine biomass/microalgae using enzymatic hydrolysis and membrane separation technology.

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Long-chain n-3 polyunsaturated fatty acids, LC-PUFA, are essential fatty acids for humans and must be supplied through diet. Of these, docosahexaenoic acid, DHA (C22:6) has particular health benefits, and is used not only as a food supplement and special feed ingredients, but also as a pharmaceutical compound e.g. for treatment of coronary diseases.

In general, LC-PUFAs are obtained from fish, fish by-products and more recently from marine microalgae. Conventionally, marine and vegetable oils are isolated from the biomass by thermal-mechanical processes and/or solvent extraction, and further refined. Fatty acids or fatty acids ethyl esters are produced by hydrolysis or transesterification, respectively, to be further used for enrichment of fish oils and in the pharmaceutical industry. The fatty acids are subjected to extensive processing, and high temperature throughout these processes. This might be detrimental to the fatty acids, particularly the unstable polyunsaturated fatty acids. Thus, there is a need for new processes for better extraction of fatty acids, particularly LC-PUFA.

We have developed a new process for the hydrolysis of lipids and extraction of LC-PUFA, particularly DHA, from high density algae cultures [1]. The process comprises: (i) enzymatic hydrolysis of biomass lipid content, and simultaneous (ii) extraction of released fatty acids in a membrane contactor module.

The performance of several enzymes and polymeric membranes for the hydrolysis and separation of fatty acids, respectively, have been studied. Results from the optimization of the enzymatic hydrolysis reaction, membrane and process conditions will be presented, as well as applications of the technology.

### References

- [1] Miranda A.M.M., A.I.M., Torp E.G., Livingston A.G., "Process for extracting fatty acids from aqueous biomass in a membrane contactor module" US Patent No 8,455,669 (B2), 2013.