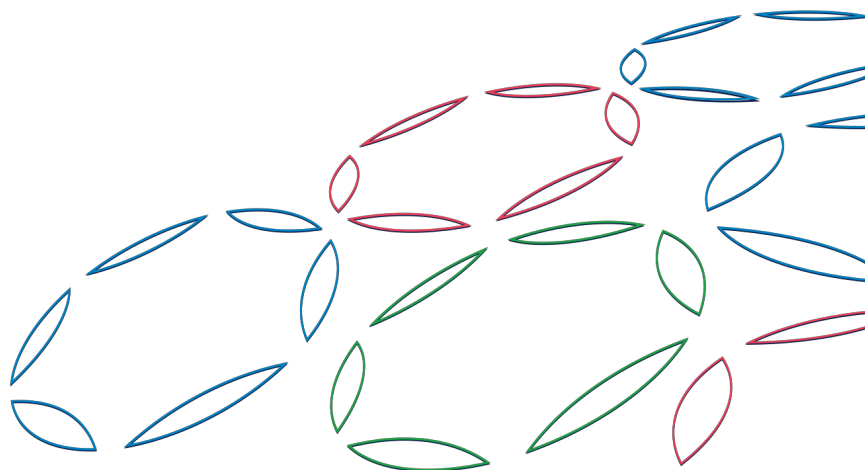# Nordic Centre of Excellence Network in Fishmeal and Fish oil

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Matís ohf

Icelandic Food and Biotech R&D

Report summary

Title	**Nordic Centre of Excellence Network in Fishmeal and Fish oil**
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Report no.	06-19 Date: Maí 2019
Project no.	62477
Funding:	AG-fund, EUfishmeal
Summary in English:	The main objective of this work was to summarise current knowledge on fishmeal and fish oil as well as identify the research needs and create a roadmap for future industry-driven research. The main conclusion was that the quality of raw material, fishmeal and - oil are not yet well defined. The real focus by the industry has mainly been limited to nutrients, such as proteins and fats and other components that makeup fishmeal. There has been less focus on the health benefits of dietary contents of fishmeal and –oil and the relationship between processing methods and the nutritional and technical properties of fishmeal. In addition, to proactively strengthen the market position and competitiveness, it is crucial for the industry to achieve a common understanding of the needs of their customers in line with a clear profile of the benefits of their products. A communication strategy as well as a research strategy is needed. Finally, the identity of the industry needs to be clear and transparent to promote a story about the industry to provide a clear and positive image of the industry to be communicated to the society. This means, that a communication strategy as well as a research strategy must be established, as there is a lack of communication along the value chain from the industry to the consumers. There is still a lack of understanding by the consumers of why fishmeal is produced, the reasons must be communicated in such a way that it reaches the average consumer. The industry members are interested in moving forward to sustain the future growth of the industry. Fishmeal and fish oil production has been prosperous for a very long time, but to remain so, cooperation among all stakeholders is crucial for continued progress.
English keywords:	Fishmeal, fish oil

Table of Contact

Introduction

Due to changes in markets and demands, there is an urgent need for improved knowledge about the nutritional value of fishmeal and oil and how to increase their value within the fish feed industry. To facilitate and strengthen the Nordic cooperation and bioeconomy, a Nordic Centre of Excellence in Fishmeal and Fish oil was established in 2018 with support from the Nordic Council of Ministers.

This report contains a detailed review of the current knowledge on raw material quality and seasonal variation, processing methods and the nutritional properties and characteristics of fishmeal and –oils. Processing methods, both traditional and new have been discussed along with applied analytical methods. The effect of fish species and seasonal variation on the nutritional and technical properties of fishmeal and –oils, have been explored. Preservation methods throughout the value chain have also been discussed as well as food grade production regulations and end use.

By identifying the current knowledge of fishmeal and oil, raw material and processing methods and use, the intention is to establish a platform for further research in this area. The objective of this work was to summarize the current knowledge and to identify unexplored areas. This will create a road map for future industry driven research in the field and to lead scientists and industry forward.

1 Background

Fishmeal and fish oil production plays an important role in the Nordic countries. However, production has been static the last decade, while the world´s protein and oil demand has increased, along with increasing public demand for improved sustainability and increased use of marine and terrestrial animal by-products. The quality of fishmeal and oils depends very much on the properties of the raw material used. The fish species, ocean conditions (temperature, pollution, feed, other species) where the fish was caught, seasonal effects and preservation methods are raw material parameters that influence the processing, yield and nutritional and technical quality of these products.

The Nordic countries have played a leading role in providing healthy and safe products for human consumption from marine resources and it is important for the Nordic bioeconomy that this status is maintained. The research and innovation proposed by this Network Centre of Excellence is based on a sustainable bioeconomical approach within and across industry sectors, promoting interdisciplinary cooperation to enhance the Nordic economy.

Changes in market demands for alternative protein and oil sources both for human and animal consumption, as well as increased competition from fishmeal and oil substitutes (e.g. plant, yeast, algae, and insect ingredients) call for improved knowledge and optimized processes and methods in the fishmeal industry to increase the nutritional and economic value of their products. This will benefit the entire value chain – fishery, industry, coastal regions, and consumers and increase the export revenues of the industry. The price of fishmeal decreased from approximately 2,400 USD per ton in June 2014 to 1,100 USD per ton in June 2017, while the use of soy meal for animal feed increased at the expense of fishmeal. The price of starter feed with the right chemical and nutritional composition is currently around 3,000-5,000 USD per ton, and prices for proteins for human consumption may reach 7,000 USD or even more. So, there are many good reasons to optimize the processing methods and product quality of the Nordic industry and adapt them to a changed market atmosphere.

1. Overview

What is fishmeal and fish oil production ?

Fishmeal is a powder obtained after cooking, pressing, drying and milling fresh raw fish and/ or fish trimmings. Fish oil is the liquid pressed from the cooked fish. From 100 kg of raw material a fishmeal and fish oil factory roughly produces 20 kilos of fishmeal and 5 kilos of fish oil.

The production process seen in Figure 1 can be split into seven main processes. 1) The raw material is boiled in its own juice by an indirect supply of steam. 2) Pressing of the raw materials results in a solid fraction – the presscake – and a liquid fraction – the presswater. 3) A decanter processes the liquid from which the solids are separated and returned to the presscake. 4) The fish oil is removed from the liquid fraction by centrifuging. 5) The remaining liquid called "stickwater" is evaporated. The resulting product is called solubles. 6) In the dryer the presscake and the solubles are dried by means of indirect steam or hot air to a fishmeal with a moisture content of 5-10%. 7) The fishmeal is added antioxidant, cooled and milled to form the product. After control and analysis the fishmeal is ready for packaging and delivery.
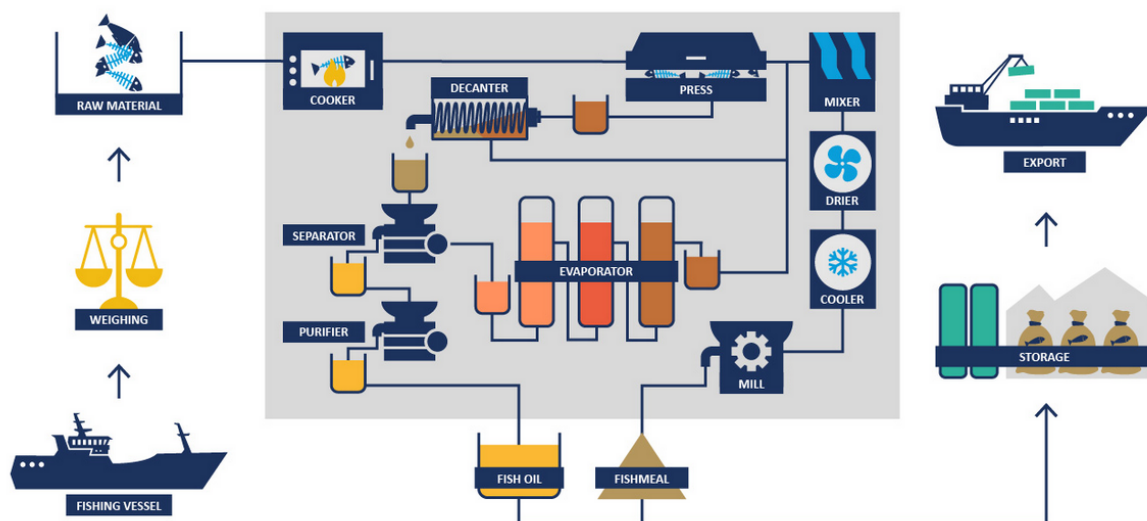

Figure 1 The production processes of making fishmeal and oil

Production is based on landings of small, oily, short-lived species such as blue whiting, capelin, sand eel, norway pout and sprat as well as by-products (trimmings) from the

consumption fish processing sector. All fish stocks used for the production of fishmeal and fish oil in European countries are subject to strict catch limitations based on biological advice from advisory bodies such as ICES and STECF.

A growing amount of raw material comes from recycled trimmings. The fillet yield for most fish species varies between 30% and 65% of the mass of the fish and the cut offs constitute a valuable resource for the fishmeal and fish oil producers. Full use of the valuable marine resources is thus obtained. Another source of supply are fish that do not meet the requirements for direct human consumption such as undersized or damaged fish. Trimmings and other rest raw material currently constitute around 30 % (globally) of the raw material for fishmeal production (Jackson and Newton, 2016).

The value in fishmeal and oil

Fishmeal and fish oil are important marine ingredients. When used for aquaculture, human consumption and animal feeds, the products bring the important omega-3 fatty acids EPA and DHA in to the human food chain via farmed fish and fish oil supplements. EPA and DHA are central components in all cell membranes and their health benefits are well documented. EPA and DHA contribute to the function of the heart and cardiovascular system as well as to the immune system and are particularly important for the development of the brain and vision early in life.

Fishmeal and fish oil are considered the most nutritious and most digestible ingredients for farmed fish feeds and feed for many land-farmed animals.

All marine fish rely on marine omega-3 fatty acids to varying degrees and the composition of micronutrients in fishmeal, including amino acids, vitamins and minerals, support growth and optimal physiological function of animals and farmed fish. This makes fishmeal and fish oil indispensable feed ingredients.

- High protein content of 62 to >70 %

- Rich in long chain omega-3 fatty acids EPA and DHA

- Contains minerals; calcium, phosphorus, magnesium, potassium and selenium

- Contains vitamins; B1, B2, B6 and B12

- High digestibility

Inclusion rates in compound feeds for aquaculture have shown a downward trend as they are used more selectively, Figure 2 (Ytrestøyl et al. 2015; FAO, 2018). This is due to a combination of growing global aquaculture, a stagnating supply of fishmeal and fish oil and an increased recognition of the benefits of the valuable ingredients in fishmeal and fish oil in various stages of animal growth.
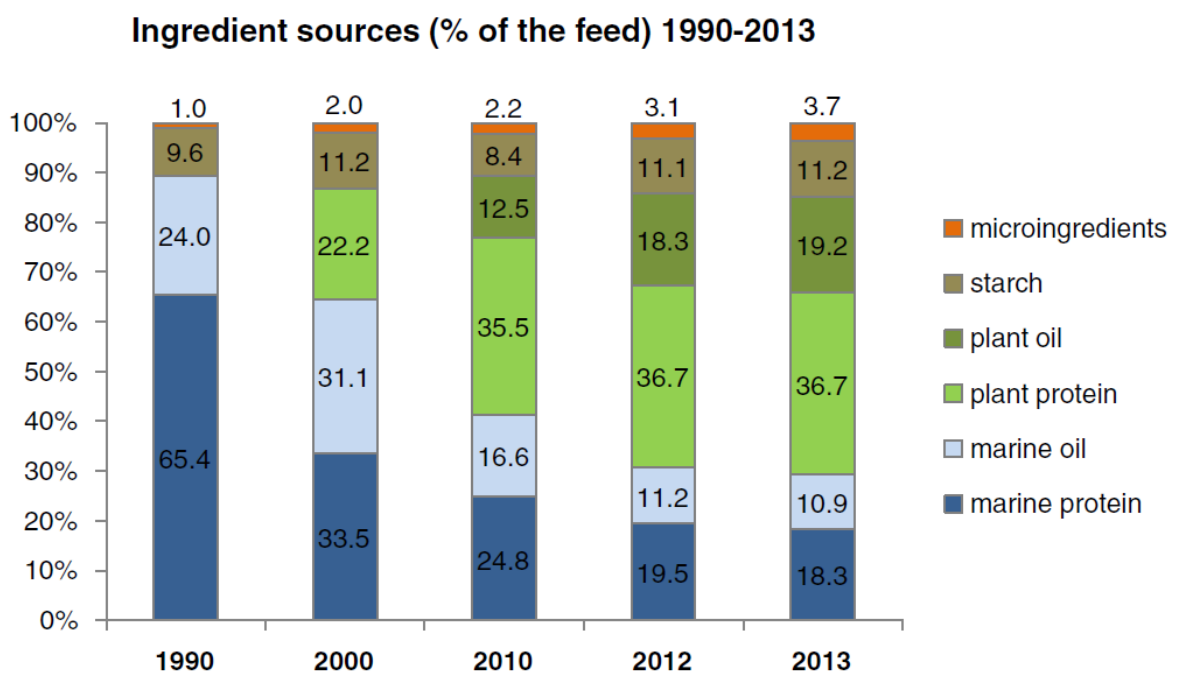
Ingredient sources (% of the feed) 1990-2013

	1990	2000	2010	2012	2013
(top value)	1.0	2.0	2.2	3.1	3.7
starch	9.6	11.2	8.4	11.1	11.2
plant oil			12.5	18.3	19.2
plant protein		22.2	35.5	36.7	36.7
marine oil	24.0	31.1	16.6	11.2	10.9
marine protein	65.4	33.5	24.8	19.5	18.3

Legend:
- microingredients
- starch
- plant oil
- plant protein
- marine oil
- marine protein

Figure 2 Nutrient sources in Norwegian salmon farming as percentage of total diet (Ytrestøyl et al. 2015).

2. Nordic Centre of Excellence Network

The Nordic Centre of Excellence in Fishmeal and Fish oil was established in 2018 in order to facilitate and strengthen the Nordic cooperation in this particular research field. The Nordic Centre of Excellence Network in Fishmeal and Fish oil is supported by the Nordic Council of Ministers and EUfishmeal. Project partners were MATIS (coordinator), Nofima, DTU Food, DTU Aqua and EUfishmeal.

The research and innovation proposed by the Network Centre of Excellence is based on a sustainable bio-economical approach within and across industry sectors, promoting interdisciplinary cooperation to enhance the Nordic economy. Its establishment is also in line with the Fishmeal and Fish Oil Symposium organized in Hirtshals, Denmark from 29th – 30th August 2016, see below. Around 100 participants took part in the Symposium on "Perspectives for Fishmeal and Fish Oil in light of the management of forage fisheries, alternative uses of fishmeal and the development of new feeds and technologies". The aim was to bring stakeholders together to discuss trends, challenges, and opportunities for the fishmeal and fish oil value chain. Among the results from the Fishmeal and Fish Oil Symposium in Hirtshals 2016 was a wish for the Nordic regions to collaborate more with a view to reducing the overall burden of research costs. There is a need to advance the research agenda throughout the value chain i.e. from forage fishing at one end of the value chain to better understand the nutritional importance of the fishmeal and fish oil inclusion rates in aquaculture diets and fully understand the unique nutritional qualities of fishmeal and fish oil. The Symposium participants highlighted the usefulness of having started a process and the importance of a follow-up. In this regard, it was suggested that a platform could be developed providing a tool to communicate the latest research and general news about the fishmeal and fish oil value chain. The overall objective of the *Network of Excellence* is to continue the valuable cooperation launched by the Symposium.

Workshop by Nordic centre of Excellence, held in November 2018

The "Workshop in Fishmeal and Fish oil" was held in Copenhagen, Denmark on November 14th-15th. The workshop was organized by EUfishmeal as part of The Nordic Centre of Excellence Network in Fishmeal and Fish oil. Other partners in organizing the event were DTU Aqua, DTU Food, Matis and Nofima,

To ensure that viewpoints of a wider range of audience would be considered when identifying the main focal points of the workshop and to suggest the most relevant presenters, a steering group was formed around the preparation of the workshop. The steering group consisted of the following persons.

Alfred Jokumsen - DTU Aqua, Denmark

Anne Mette Bæk - EUfishmeal

Charlotte Jacobsen – DTU Food, Denmark

Jóhannes Pálsson – FF Skagen, Denmark

Marvin Ingi Einarsson – Matís, Iceland

Odd Eliasen – Havsbrún, Faroe Islands

Ola Flesland – TripleNine Group A/S, Norway

Søren Anker Pedersen – EUfishmeal

Tor Andreas Samuelsen – NOFIMA, Norway

The workshop was successful with 75 participants from several European countries. Researchers, producers, salesmen and customers presented current knowledge in five sessions on topics as raw material, production of fishmeal and oil, analytical methods, preservation methods throughout the value chain and final products – key/desirable properties of various final products. After each session research needs were presented to the audience and discussed. The last part of the workshop included group discussions and a final panel discussion chaired by Matis CEO Sveinn Margeirsson, to define and establish the road map for the industry and science community.

The main topics discussed during the panel discussion where:

1) Communicating with customers

2) Importance of organizing the value chain and important future research areas

3) Determining the health benefits of fishmeal and oil

The panel discussion was summarised by Sveinn Margeirsson in the end of the session.

Members of Nordic centre of Excellence Network

Organization: The EUfishmeal Secretariat, Søren Anker Pedersen

EUfishmeal represents European fishmeal and fish oil producers. On a global level, Europe is producing 16% of the world´s fishmeal and 23% of the world's fish oil.

The member countries are Denmark, Faroe Islands, Germany, Iceland, Ireland, Norway and United Kingdom and they account for an average yearly production of more than 500,000 tonnes of fishmeal and 160,000 tonnes of fish oil with a total production value of approximately 1 billion €/year and. Products are exported world-wide.

Research institutions: Matís Research Institute (Matís) (IS), Marvin Ingi Einarsson and Stefán Þór Eysteinsson; Technical University of Denmark, Institute for Aquatic Resources (DTU-AQUA) (DK), Alfred Jokumsen; Technical University of Denmark, The National Food Institute (DTU-FOOD) (DK), Goncalo Marinho and Charlotte Jacobsen; Nofima Food Research Institute (Nofima) (NO), Tor Andreas Samuelsen

> **Matís** is an Icelandic government owned, non-profit, independent research company, founded in 2007 following the merger of three former public research institutes. Matís pursues research and development aligned to the food and biotechnology industries as well as providing Iceland's leading analytical testing service for public and private authorities. Matís'vision is to increase the value of food processing and food production, through research, development, dissemination of knowledge and consultancy, as well as to ensure the safety and quality of food and feed products. Matís has about 100 employees composed of food scientists, chemists, biochemists, molecular biologists, engineers and fisheries scientists with expertise in Food Science, Aquaculture, Genetics and Environmental Sciences.

> **Technical University of Denmark**, DTU, is a public university conducting research, education, research-based consulting services to the public authorities in Denmark. DTU comprises 18 institutes. One is the National Institute of Aquatic Resources, DTU Aqua, where the Section for Aquaculture in Hirtshals conducts applied aquaculture research and advice services, including research in Recirculation Aquaculture Systems (RAS), fish feed and nutrition, fish physiology, and environmental impacts of fish farming, organic aquaculture and certification, fish welfare. DTU Aqua has several full scale rearing facilities and advanced laboratory facilities.

> Another institute at DTU is the National Food Institute, which researches and communicates sustainable and value-adding solutions in the areas of food and health

for the benefit of society and industry. The institute's tasks are carried out in a unique interdisciplinary cooperation between the disciplines of nutrition, chemistry, toxicology, microbiology, epidemiology and technology. The vision is that the National Food Institute creates welfare for the future through research into food and health. The institute makes a difference by producing knowledge and technical solutions which prevent disease and promote health, make it possible to feed the growing population and develop a sustainable food production.

Nofima, the Norwegian Institute of Food, Fisheries and Aquaculture Research, was established by a merger January 1st, 2008, and is owned by the following stock holders:

The Norwegian Ministry of Trade, Industry and Fisheries	56,8 %
The Agriculture Nutrient Research Foundation	33,2 %
Akvainvest Møre and Romsdal AS	10,0 %

Nofima is one of Europe's largest institutes for applied research within the fields of fisheries, aquaculture and food. We carry out internationally recognized research and develop solutions that provide a competitive edge throughout the value chain.

The main office is located in Tromsø, and the research divisions are located in Bergen, Stavanger, Sunndalsøra, Tromsø and Ås. The institute has around 350 employees and an annual turnover in 2017 of NOK 595 million.

Industrial partners: FF Skagen (DK), Johannes Palsson, Klaus H. Kristoffersen; Havsbrún (FO), Odd Eliasen; TripleNine Group A/S (NO), Ola Flesland.

2 Raw material

This chapter will focus on the effect of raw material quality and how seasonal changes can influence the properties of the final product. Sources of raw material intended for fishmeal and oil production will also be discussed including by-products and side streams. Along with that the quality criteria for raw material, fishmeal, oil intended for feed pet food, and human consumption will be covered.

2.1 Raw material quality

Maintaining optimal quality of the raw material is fundamental to ensure good quality fishmeal and fish oil, which will provide optimum nutritional value and obtain the highest prices. Fish raw materials are unstable and very perishable. Spoilage, development of off-flavor and odors must be prevented by controlling protein degradation and lipid oxidation (Thorkelsson et al., 2009). Usually, refrigerated seawater is used to chill the fish and keep them fresh, avoiding damage (IFFO, 2018). Moreover, the catch must be harvested and processed within the shortest possible period of time.

The requirement for raw material quality/freshness

The freshness of the fish raw material is the major factor affecting the quality of the produced fishmeal and fish oil. The bases ammonia, trimethylamine, dimethylamine, and others present in trace amounts can be formed during spoilage of fish. The total volatile basic nitrogen (TVB-N) expresses the combined total amount of nitrogen in these three compounds present in the fish and is a commonly used estimate of spoilage. TVB-N can be measured easily and quickly using a relatively simple apparatus and, for this reason, the TVB-N value is often used as a rejection limit in regulations and commercial specifications. The TVN content in the raw material of acceptable quality/freshness should not exceed 60 mg TVB-N/100 g of whole fish.

However, according to EFSA (2010), the total volatile basic nitrogen (TVB-N) is a spoilage parameter developed and defined for ice stored gutted fish and fish filets. It has not been investigated for the determination of the 'freshness' of whole fish as raw material intended to be used for the production of fish oil for human consumption. Moreover, the criterion of 60

mg TVB-N/100 g for whole fish is not based on scientific evidence (EFSA, 2010). In this context, the European Food Safety Authority recommends sensory assessment for the evaluation of the freshness of raw material for fish oil production for human consumption (EFSA, 2010).

Biogenic Amines

Biogenic amines present in fish are almost totally the result of the action of exogenous enzymes released by the various microorganisms associated with the seafood products (Frank et al., 1981). Biogenic amines are produced during enzymatic hydrolysis of amino acids, which starts right after death, and can be indicative of the extent of the enzymatic degradation of the protein in the raw material before processing. During spoilage of fish especially if the temperature rises to above 10°C, histidine may be converted to histamine. Other important biogenic amines include cadaverine, putrescine, and tyramine, resulting from the hydrolysis of lysine, arginine and tyrosine, respectively. While biogenic amines are naturally occurring compounds, which play a rule in many critical functions in the human body, the consumption of seafood with high concentrations of such compounds may constitute a safety issue for the consumer. Levels of histamine above 500–1000 mg/kg are considered potentially dangerous to human health (ten Brink et al., 1990).

Fish such as anchovies, herring, and menhaden have different amine patterns, and thus the concentrations of specific biogenic amines in the fishmeal will depend on the fish species used to produce the meal. Biogenic amines are heat-stable and, thereby, do not volatilize or evaporate during drying. However, they are also water-soluble, and thus they can be separated from the press cake during drying and concentrated in the soluble fraction (stickwater). This means that when the condensed soluble is added back to the press cake during the drying process, the final meal will contain relatively higher levels of biogenic amines than meals that contain only the press cake.

Since the presence of histamine results from bacterial activity, the development of histamine could according to some researchers be an indicator of fish quality deterioration (Taylor and Summer, 1986). Several studies have evaluated the usefulness of histamine and other amines as a quality indicator, with somewhat conflicting results. Some authors reported the existence

of a correlation between histamine development in fish and other quality indices such as volatile acids, hypoxanthine and TVB-N (Khayat, 1977; Putro and Saleth, 1985; Veciana-Nogués et al., 1990). On the other hand, high concentrations of histamine have also been found in fish samples with low TVB-N and low bacterial count levels, thereby considered fresh (Mendes, 2009). Therefore, the use of efficient and rapid methods of histamine detection is highly important because there is not a direct relationship between changes in fish freshness and the histamine level (Arnold and Brown, 1978; Mendes, 2009). It is important to highlight that the presence of biogenic amines does not necessarily result in any perceptible changes to the sensory analysis; thereby, spoilage will not necessarily protect the consumers from the action of hazardous biogenic amines.

Although, the detection of histamine in fish muscle is a clear sign that decomposition has taken place, its occurrence is highly variable since its production is a result of several factors such as: (1) the species of fish and the individual fish, (2) the part of the body of the fish sampled, (3) time and temperatures of storage and (4) types and numbers of bacteria present in the fish (Rawles et al., 1996; Mendes, 2009).

Many countries established maximal limits or guidelines on levels of histamine from fishery products. The European Union demands that nine samples must be analyzed from each batch of fishery products from fish species associated with a high amount of histamine, particularly fish species of the following families: Scombridae, Clupeidae, Engraulidae, Coryphaenidae, Pomatomidae, and Scombresosidae (Official Journal of the European Union, 2005). The histamine content in the samples must fulfill the following requirements to make samples acceptable for human consumption:

- The mean content must not exceed 10 mg/100 g

- Two samples may have a value of more than 10 mg/100 g but less than 20 mg/100 g

- No sample may show a value exceeding 20 mg/100 g.

Dioxins and dioxin-like PCBs

Dioxins are environmental pollutants derived both from natural, but mostly, anthropogenic activities. Although the formation of dioxins is local, they have a global environmental

distribution, and accumulate in the food chain, mainly in the fatty tissue of animals. *Table 1* gives an overview of dioxin levels found in fishmeal and oil from different origin/stocks. These results show a large variation in the concentration of dioxins in fishmeals and oils derived from different stocks. Moreover, their concentrations seem to be generally higher in fishmeals and oils derived from European stocks compared to those from South Pacific stocks. However, it is important to highlight that currently dioxins are largely removed during oil refining processes. Fishmeal is also analyzed for dioxins and if required can also be purified for dioxins and dioxin-like PCBs using extraction processes (Oterhals and Nygård, 2008; Oterhals and Kvamme, 2013; TripleNine Fish Protein, 2018). This ensures the production of fishmeal and oil with levels of dioxins and dioxin-like PCBs that comply with EU regulations. Kawashima et al. (2006) proposed combining supercritical extraction with CO_2 and adsorption on activated carbon to remove PCBs and other contaminants from fish oil, which could reduce the level of contaminants such as dioxin and PCBs by more than 95%.

Table 1 Levels of dioxins found in fishmeal and fish oil originating from South America and Europe.

Commodity	Level	Origin	
		South Pacific area (Chile, Peru)	*European area*
Fish Oil	*Low*	0.16 ng/kg fat	0.7 ng/kg fat
	Mean	0.61 ng/kg fat	4.8 ng/kg fat
	High	2.6 ng/kg fat	20 ng/kg fat
Fishmeal	*Low*	0.02 ng/kg dry matter	0.04 ng/kg dry matter
	Mean	0.14 ng/kg dry matter	1.2 ng/kg dry matter
	High	0.25 ng/kg dry matter	5.6 ng/kg dry matter

Source: FAO 2002

Threshold values for dioxins, furans, and dioxin-like PCBs found in foodstuffs have been established by the European Commission (Table 2). In this context, raw materials containing such contaminants in concentrations exceeding the recommended maximum limits and action limits must be avoided (Recommendation 2004/705/EC).

Table 2 Combined maximum dioxin and dioxin-like PCB levels and action limits in seafood for human consumption (including fish oil).

Maximum levels[1]	Sum	Dioxins & Furans	Dioxin-like PCBs
Fish	8 pg/g	4 pg/g	4 pg/g
Marine oil, incl. fish body oil and liver oil	10 pg/g	2 pg/g	8 pg/g
Eel	12 pg/g	4 pg/g	8 pg/g
Action limits[2]			
Fish and fish products	6 pg/g	3 pg/g	3 pg/g
Marine oil incl. fish oil	7.5 pg/g	1.5 pg/g	6 pg/g
Eel	12 pg/g	4 pg/g	8 pg/g

[1] EC Regulation 199/2006; [2] EC Recommendation 2006/88/EC

Raw material freshness and product quality

The freshness of the fish raw material is the major factor affecting the quality of the produced fishmeal and fish oil, which will impact the product nutritional value and the price obtained. Comparison of the concentration of TVB-N, most relevant biogenic amines, and protein and oil content of fishmeal produced from herring and anchovy of different freshness can be seen in *Table 3*. As expected, there is a clear correlation between the raw material freshness and the concentration of TVB-N and biogenic amines. Moreover, there is a considerable reduction in the protein content, and especially, in the oil content of the fishmeal produced from moderately fresh and stale raw material compared to the one produced from fresh raw material. Enzymatic and bacteriologic activity in the fish can rapidly decrease the content and quality of the protein and oil. Protein decomposes into amines and ammonia, and both reduce the protein value and recovery of protein (Keller 1990) and hence could reflect the lower protein content of the fishmeal. Lipid oxidation resulting from non-enzymatic processes such autoxidation and photosensitized oxidation, as well as catalyzed by enzymes such as lipoxygenase lead to the formation of oxidation products, which have a detrimental effect on the nutrition value of lipids and sensory properties of fish (Decker et al., 1988; Jacobsen et al., 2009).

Table 3 Analytical values for fishmeal made from raw materials of different freshness.

Analyses	Fresh		Moderately Fresh		Stale	
	Herring	Anchovy	Herring	Anchovy	Herring	Anchovy
TVN (mg /100g)	22	14	62	30	143	50
Protein (%)	73.5	69.6	73.1	67.5	69.4	65.8
Oil (%)	18.7	7.7	8.1	7.4	10.9	9.4
Cadaverine (ppm)	330	28	1000	1850	1600	4701
Putrescine (ppm)	30	51	230	503	630	1599
Histamine (ppm)	<30	35	440	446	830	916
Tyramine (ppm)	<30	-	400	285	800	657

Source: Summarized from Pike and Hardy (1997)

The presence of biogenic amines is one of the main factors that affect the quality of fishmeal. According to Pike and Hardy (1997), the recommended quantity of biogenic amines for high-quality fishmeal should be less than 1000 ppm for histamine, and the total sum of all four of the main biogenic amines (cadaverine, putrescine, tyramine, and histamine) should be less than 2000 ppm. *Table 3* shows that only the fishmeal produced from fresh raw material fulfills the recommended quality criteria.

Production of feed, pet food and food for human consumption

Production of fish oil intended for human consumption must comply with the Food Hygiene Regulations set by the European Commission (EC). Relevant regulations: EC 852/2004, EC 854/2004, EC 853/2004 as amended by EC 1020/2008, and EC 2074/2005 as amended by EC 1022/2008.

General requirements for raw material and production of fish oil for human consumption

Such production and products must meet the relevant requirements for fishery products found in the Hygiene Regulations. In general, this means that the raw materials used and the fish oil must:

- Come from establishments, including vessels, registered or approved pursuant to the Hygiene Regulations.

- Derive from fishery products which are fit for human consumption and are handled throughout the food chain as such. Animal by-products and fishery products not fit for human consumption cannot be used as raw material for fish oil for human consumption.

According to regulations, the raw material must be chilled as soon as possible after the catch. When chilling is not possible on board the vessel the raw material must undergo chilling as soon as possible after landing and be stored at a temperature approaching that of melting ice.

However, by way of derogation, the food business operator may refrain from chilling the fishery products when whole fishery products are used directly in the preparation of fish oil for human consumption, and the raw material is processed within 36 hours after the catch, provided that the freshness criterion laid down are met.

The freshness criterion is based on the total volatile basic nitrogen (TVB-N), which shall not exceed 60 mg of nitrogen/100 g of whole fishery products used directly for the preparation of fish oil for human consumption. However, where the raw material is still fit for human consumption the competent authority may set limits at a higher level for certain species.

The production process for fish oil must ensure that all raw material intended to produce crude fish oil is subject to a treatment including, where relevant, heating, pressing, separation, centrifugation, processing, refining and purification steps before being placed on the market for the final consumer.

Provided that the raw materials and the production process comply with the requirements applying to fish oil intended for human consumption a food business operator may produce and store both fish oil for human consumption and fish oil and fish meal not intended for human consumption in the same establishment.

Refined fish oils are very different from other fishery products such as fish fillets or shellfish because they undergo a manufacturing process that kills microbes and eliminates moisture in which microbes can survive. These products also undergo manufacturing steps that remove

heavy metals, pesticides and other toxins that are commonly found in seafood and are already covered by other EU legislation.

Specific requirements for fish oil for human consumption

The Commission has the possibility to propose hygienic requirements for fish oil for human consumption, including rancidity requirements. Point 4 of Chapter VI.B of Section VIII of Annex III to Regulation (EC) No 853/2004 includes a clause stating that, pending the establishment of specific Community legislation, Member States must ensure compliance with national rules for fish oil placed on the market for the final consumer.

2.2 Sources of raw material for fishmeal and oil production

According to FAO (1986) the fish that are processed to produce fishmeal and fish oil may be divided into three categories: (a) fish caught for the sole purpose of fishmeal production (e.g. Peru, Norway, Denmark, South Africa, and the U.S.A.); (b) by-catches; and (c) fish offcuts and offal/trimmings from the consumption industry incl. aquaculture.

A wide variety of fish species is used to produce fishmeal and oil in different countries. *Table 4* gives an overview of the top producing countries and the main species used for fishmeal and fish oil production. In Europe, the Nordic countries are the main producers, where the annual landings of small pelagic fish by country ranges from 365,000 to 950,000 tons, and the production of fishmeal and fish oil ranges from 52,000–188,000 tons and 8,000–52,000 tons, respectively (*Table 5)*. Fishmeal and fish oil are produced by processing mainly small, bony, oily fish, such as sand eel, anchovy, herring, capelin, and menhaden, for which there is little demand for human consumption. A smaller percentage is manufactured from fish offal, trimmings or cuttings, and other side streams mainly from the filleting and canning of edible fish such as tuna, cod, haddock, hake, and pollock (IFFO, 2018). The composition and quality of the raw material are predominant factors in determining the properties and yield of the products (FAO, 1986). *Table 6* shows the proximate composition of fish stocks commonly used for fishmeal and oil production. The main constituents of the fish vary very little with regard to protein (14–18%) and inorganic matter (1–4%). On the other hand, the fat (oil; 2–31%) and water contents (60–80%), which make up to 72–78% of the whole fish are highly

variable (Ruiter 1995; Ariyawansa 2000). The separation of fatty substances (lipids) from the other constituents of the whole fish is one of the major operations in the manufacture of fishmeal and oil (FAO, 1986).

Table 4 Main fish species used for fishmeal and fish oil production by the top producing countries.

Countries	Species
Chile	anchovy, horse mackerel
China	various species
Denmark	pout, sand eel, sprat
European Union	pout, capelin, sand eel, and mackerel
Iceland and Norway	capelin, herring, blue whiting
Japan	sardine, pilchard, mackerel
Peru	anchovy
South Africa	pilchard
Thailand	various species
USA	menhaden, pollock

Source: Miles and Chapman (2006)

Seasonal changes

Seasonal changes in fish composition are related to variation in factors such as diet (e.g. zooplankton production cycle), fish size, and reproductive cycle. Dubrow et al. (1976) evaluated the seasonal variations in chemical composition and protein quality of Atlantic Menhaden (*Brevoortia tyrannus*) harvested during the course of four different fishing seasons. No seasonal trend in protein quality was detected, but the lipid content increased considerably towards the end of the fishing season (October/November). The proportion of polyunsaturated fatty acids was found to be higher at the beginning of the fishing season (June/July), while there was a trend toward a lower proportion of polyunsaturated fatty acids (PUFA) during the course of the season; the opposite trend was observed for saturated fatty acids (SFA).

Hardy and Mackie (1969) studied the seasonal changes in the lipid components of Sprat (*Sprattus sprattus*). The lipid content fell during the fishing season from 18% in October to

11.5 % in March, and the degree of unsaturation also decreased. This latter diminution was mainly due to the reduction in the levels of PUFA of the triglyceride fraction of the lipids.

Aidos et al. (2002) studied the seasonal changes in crude oil and lipid composition of herring (*Clupea harengus*) fillets, by-products, and respective oils produced from the by-products. The lowest levels of polyunsaturated fatty acids (PUFAs) in the oil were found from January to March (14-16%), coinciding with the post-spawning and starvation period. In contrast, the highest levels of PUFA were found from June to August (23-24%).

Studies evaluating the effect of seasonal variation in the composition of the fish raw material on the properties of the produced fishmeal and oil are scarce. Since during fishmeal processing, the water and lipid phases are largely removed it is generally expected that a meal of similar composition can be obtained regardless of the fish species used (Burt et al., 1992; Ariyawansa, 2000). However, Bragadóttir et al. (2004) evaluated the composition and stability of fishmeal produced from Capelin harvested at different seasons. The protein content of the produced fishmeals was remarkably stable regardless of the season of harvest (69.6-71.7%). On the other hand, the lipid content was significantly lower in spring fishmeal (8.4%) compared to all other seasons (10.9-11.9%), with an inverse relationship with water content. Rancidity was highest in autumn, but free fatty acids were highest during spring and summer. These seasonal variations in the FFA content of the meal were most likely the result of variations in the fresh capelin from which the meal was produced. High autolytic activity was observed in the capelin from the heavy feeding period in the summer (Bragadóttir et al., 2002).

Fish meat is mainly composed of myofibrillar and sarcoplasmic protein and connective tissue. The mutual distribution between the protein fractions varies depending on the fish species (Suzuki 1981). Collagen is the main component of the connective tissue. Upon heating under moist conditions, collagen transforms to water-soluble gelatin (Suzuki 1981). The partitioning between the non-soluble press-cake protein and the water-soluble stickwater protein phases during fishmeal production depend on the natural variation of endogenous protease activity in the raw material and freshness of the fish (Samuelsen et al., 2014). High enzyme activity and/ or spoilage give increased level of water-soluble and a high content of suspended solids in the soluble phase (Høstmark, 1987). These variations are dependent on the fish species, levels of

feed (zooplankton) in the fish stomach and gut, seasonal variations in fat content, level of roe and milt and raw material freshness, all impacting the technical quality of the fishmeal (McBride *et al.*, 1959; Suzuki, 1981; Aksnes, 1988; Schmidtsdorff 1995; Flesland *et al.*, 2000; Bragadottir *et al.*, 2002; 2004; Samuelsen *et al.*, 2013; 2014).

Table 5 Landings of small pelagic fish, and fishmeal and fish oil production (in tons, average 2014-2017) by the Nordic countries

Countries	Species	Landings[1]	Fishmeal[2]	Fish oil[2]
Norway	Capelin	66,000	127,000	35,000
	Norway pout	29,000		
	Blue whiting	399,000		
	Sand eel	86,000		
	European sprat	13,000		
	Atlantic herring	357,000		
	Total	**950,000**		
Iceland	Capelin	190,000	111,000	36,000
	Blue whiting	196,000		
	Atlantic herring	100,000		
	Total	**486,000**		
Denmark	Atlantic herring	135,000	188,000	52,000
	Norway pout	17,000		
	Blue whiting	46,000		
	Sand eel	180,000		
	European sprat	202,000		
	Total	**580,000**		
Faroe Islands	Capelin	15,000	52,000	8,000
	Norway pout	1,000		
	Blue whiting	287,000		
	Sand eel	0		
	Atlantic herring	62,000		
	Total	**365,000**		

Source: 1Natioal Fisheries Statistics 2018, 2EUfishmeal 2018

Table 6 Proximate composition of whole fish. North Sea herring (NS herring); Norwegian Spring Spawning herring (NVG herring).

Fish	Protein %	Fat %	Ash %	Water %
Capelin	14	6-15	2	73-78
Blue whiting	15	2-5	2	79
Sand eel	18	7	2	73
Norway pout	16	2-3	4	73-77
Sprat	15	18	3	70-73
NS herring	17	10-17	2	60-80
NVG herring	17	4-10	2	60-80
Mackerel	18	16-31	1-2	60-74

Source: FAO 2018, Nifes 2018; PELAGIA 2018

Both the condition of the wild stocks and the seasonality of the catches are important factors with considerable impact in the fishmeal and fish oil processing industry. In Iceland, pelagic fish accounts for 70% of the catch. A decline in the catch of capelin has been observed, while blue whiting and herring catches have shown an upward trend. There is a marked seasonal variation in the catch; the capelin fishing season takes place mostly in the first months of the year, blue whiting in early spring and summer, and herring in summer and autumn (Thorkelsson et al., 2009). Moreover, as the composition of the fish may vary widely during the year, systematic sampling and analysis of seasonal variations provide important information when considering the establishment of a fishmeal industry (FAO, 1986).

By-products

The processing of fish for human consumption e.g. filleting and canning generates large amounts of side streams such as heads, viscera, frames, skins, tails, fins, scales, mince, blood, etc. This side streams material may constitute up to 70% of whole fish and shellfish, whereas fish fillet yield after processing is species-dependent and is often in the range of 30–50% of the fish (Olsen et al., 2014). The side stream material or by-products derived from the processing of edible fish constitute a valuable raw material which can be processed into fishmeal and fish oil. However, such by-products are still underutilized by the fishmeal and

fish oil industry, and therefore, there is still great potential to increase fishmeal and oil production from this raw material. The by-products can come from wild caught fish or aquaculture processing and the main source is from finfish such white fish trimmings (pollock, cod, hake, haddock, and others) as well as salmon (both wild and farmed), tuna, herring, mackerel. It should be noted that by-products from one species generally cannot be used to produce fish meal to be used in fish feed for the same species. Other raw material sources include cephalopods such as squid and crustaceans such as shrimp (both wild and farmed). Some modern fishing vessels have the equipment to preserve or process by-product on board into fishmeal and oil or alternatively, established coastal facilities have prompt collection methods to ensure that processors have a reliable outlet for their by-product. Larger aquaculture operators may also have access to advanced facilities that preserve and process by-product raw material into fishmeal and oil and this is particularly the case for farmed Atlantic salmon (IFFO, 2018).

It is a positive development that the fish processing by-product is increasingly used as raw material to produce fishmeal and fish oil. Utilizing the by-product reduces side streams and produces high-value products that contribute to the improved health of humans and animals. It must be kept in mind though that some countries have restrictions on using animal by-products in animal feed. For instance, EU regulation, EC 1069/2009, stipulates that animal by-product may not be used to feed animals or farmed fish of the same species.

It is estimated that around 33% of the total global fishmeal production currently comes from by-products (Jackson and Newton, 2016). Around 10% originates from the aquaculture by-products, 19% from capture fisheries by-products, and 71% from whole capture fisheries (**Figure 3**).
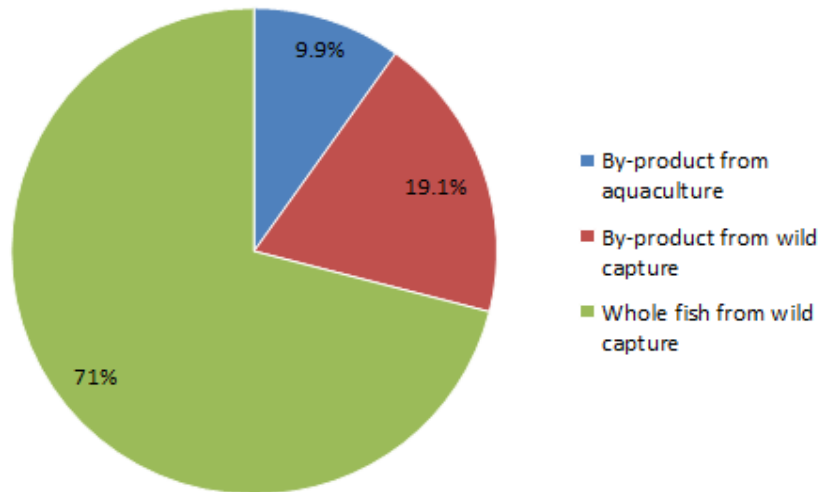
Figure 3 . Share of different sources of raw material used for the production of fishmeal (Jackson and Newton, 2016)

The share of different fish species and by-products originated from different fish species used to produce the fishmeal utilized by three of the largest fish feed manufacturers is provided in Table 7. Blue whiting, anchoveta, sardine and capelin stocks contributed most to the production of the fishmeal utilized. Concerning the utilization of by-product, fish trimmings accounted for 12–32.2% of the total fishmeal utilized by the three feed manufacturers in 2015. Trimmings were used to produce 32.2% of total fishmeal and fish oil used by EWOS.

Table 7 Source of raw material for the production of the fishmeal and fish oil (EWOS) utilized in fish feed production by three key feed manufacturers – BioMar, Skretting, and EWOS, 2015.

	Biomar		Skretting	EWOS (FM and FO)
Species	**Volume MT**	**Share %**	**Share %**	**Share %**
Whole forage fish				
Blue whiting	35,149	22	33	12.8
Anchoveta	33,625	21	14	28.2
Sardine	24,862	16		7.2
Capelin	18,005	11	Icelandic 8 Barents Sea 3	6.8
Krill	10,114	6		
Lesser sand eel	8,380	5	7	
Sprat	2,661	2	European 11 Baltic 3	4.4
Herring – Icelandic summer spawning		2		
Herring – Norwegian spring spawning		1		
Menhaden	1,996	1		3.3
Other	1,939	1		5.0
Norway pout	1,824	1	2	
Jack mackerel	1,731	1		
Other marines krill				0.08
Total marine	**159,459**	**87%**	**83%**	**67.7%**
Trimmings				
Herring - Norwegian spring spawning			7	16.3
Herring - Icelandic summer spawning			1	
Capelin			Barents Sea 1	1.1
Unidentified/various			8	3.5
Whitefish offal				7.8
Hake				1.0
Atlantic mackerel				2.5
Total Trimmings	**19,174**	**12%**	**17%**	**32.2%**
Certification				
IFFO RS approved fisheries		**92%**	**96%**	**93%**

Source: SEAFISH 2016

The global production of fish oil from by-products accounts for 26% of the total fish oil production (**Figure 4**; Jackson and Newton, 2016). The lower value compared to fishmeal is mainly because Asia processes a large volume of farmed shrimp side stream which does not yield any oil.
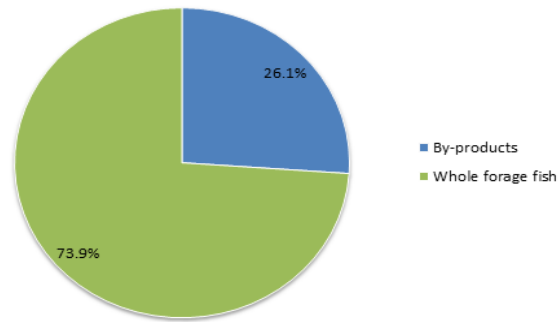

Figure 4 Source of raw material used for the production of fish oil in percentage (Jackson and Newton, 2016)

The growth in the utilization by-products is encouraging although there is still a large proportion of the by-product of fish for human consumption that is discarded instead of being turned into high-value products that contribute to the supply of nutrient-rich food. It is estimated that globally there are an additional 11.7 million tons of by-product produced in processing plants that are not collected for the production of marine ingredients (Jackson and Newton, 2016). According to Jackson and Newton (2016), Asia (excluding China), at 4.6 million tons, is the area with the largest potential for the utilization of by-product and even in Europe, it is estimated that there are an additional 0.6 million tons that could be used.

According to OECD/FAO (2014), the world fishery production is expected to be 17% higher by 2023 mainly due to the growth in aquaculture fish production. The aquaculture growth rate is expected to slow down slightly compared to the previous decade, but it is still expected to grow by 2.5% per annum. The increased production of aquaculture will ensure a growing potential supply of raw material for the production of fishmeal and fish oil. Fishmeal production is in fact estimated to grow by 25-30% over the next 10 years as a result of increased by-product availability whereas fish oil production is estimated to increase by only around 5-10% over the same period (Jackson and Newton, 2016). Developing and optimizing the collection and processing of this valuable source of raw material should be encouraged as much as possible.

Responsible sourcing

In Europe, more than 90 % of all fishmeal and fish oil produced is sourced from fisheries certified as sustainable fisheries (IFFO RS, MSC and others).

In order to support sustainable market growth, decrease the impact on the environment and provide stakeholders with a tool to demonstrate responsible practise, the IFFO Governance Board (IFFO RS GB) put together a multi-stakeholder Technical Advisory Committee (TAC) to develop a business-to-business Global Standard for the Responsible Supply (IFFO RS) of marine ingredients.

IFFO RS demands that a factory must demonstrate to an independent auditor that it has:

- Responsible Sourcing: of fishery material (non IUU) from fisheries that comply with the key principles of the FAO Code of Conduct for Responsible Fisheries.

- Responsible Traceability: of fishmeal and fish oil back to fisheries that are compliant with this Standard

- Responsible Production: of safe fishmeal and fish oil

The European Feed Manufacturers' Federation (FEFAC) has performed research in responsible sourcing of ingredients for aqua feed. They found that the main raw materials used in the production of fish feed were vegetable raw materials, marine raw materials, land animal by-products and additives. Concerning the use of vegetable raw materials in fish feed, ingredients such as different types of soy, beans, wheat, peas and rapeseed oil are used. For this type of feed to be sustainable, the production of the land based raw materials must also be sustainable. FEFAC has developed the FEFAC "Soy Sourcing Guidelines" to contribute to a mainstream transition towards responsible soy (FEFAC, 2016).

The Aquaculture Stewardship Council (ASC) is developing a Feed Standard. The ASC Feed Standard will define requirements for both responsible factory practices, and requirements that define parameters for responsible ingredients for the three main ingredient groups used in aqua feed: marine ingredients, terrestrial plant ingredients and terrestrial animal ingredients.

3 Production of fishmeal and oil

3.1 Traditional processing methods

The fishmeal process is fairly standardized worldwide, it consists of heat coagulation combined with mechanical fat separation and thermal dewatering steps (Schmidtsdorff, 1995). This section goes through every step in the fishmeal process and describes the most common components and the main production principles. The focus of this section is on the traditional fishmeal setup seen in Figure 5.
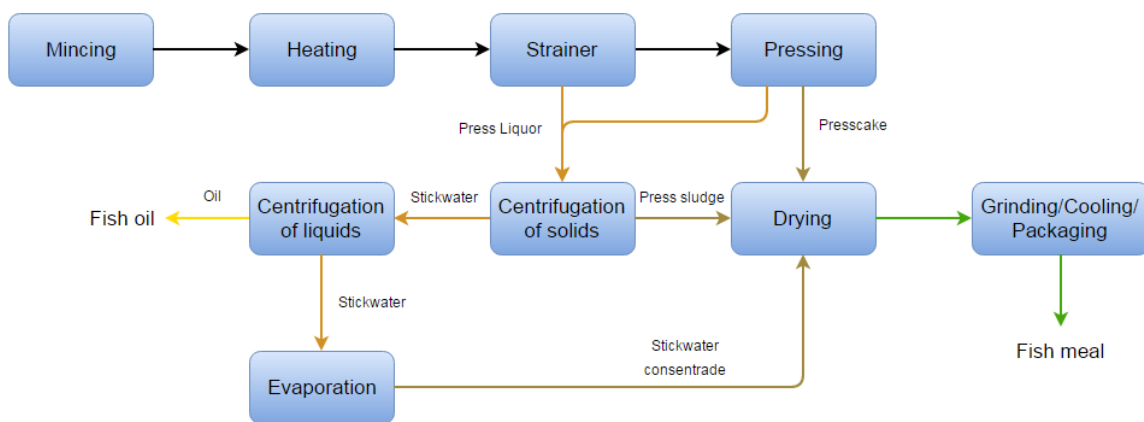

Figure 5 Main processes of onshore production of fishmeal and oil.

Mincing

The first step is mincing of the raw material. When fish or other rest raw materials enter the processing plant, they are usually gathered in the large hopper with a feeding screw at the bottom which leads to a mincer. The mincer evenly tears the material into the right particle size. This is however dependent on the raw material as whole fish requires mincing while other materials such as offals should preferably not be minced (FAO, 1986).

One type of mincers commonly used is the knife hasher. It consists of a rotor with staggered knives and a frame with a row of stationary knives. There is a wide variety of mincers available, but the most important thing is the particle size received (FAO, 1986).

Heating

The heating process is carried out to extract the oils and moisture from the material for better sieving as well as to inactivate bacteria, viruses, and parasites that can ruin the material. This is called the coagulation process. The temperature in this process has gained increased discussion in the past decade as research has shown that the fat cells are broken down before the temperature reaches 50 °C (Ruiter, 1995). Another research has shown that coagulation of proteins occurs around 75 °C quite rapidly (FAO, 1986). Inactivation of viruses, bacteria, and parasites is another factor that needs consideration in relation to temperature and residence time. According to Nygaard (2010), the minimum duration time required in cookers is at least 20 minutes at temperatures above 70 °C for wild minced fish. This ensure that viruses, bacterias, and parasites will be successfully inactivated after that time. This indicates that the heating procedure should be conducted around 75 °C for 20 minutes for optimal results. And theoretically, there should be no reason to heat the material further which has been done for centuries, and where 100 °C has been the target (FAO, 1986). It should although be mentioned that the temperature is also dependent upon the components within the plant as some separators require more heating of the material. Higher temperatures can also be used to ensure rapid temperature increase. Heating above 75 °C can, therefore, be accepted in some cases. A residence time of 25 min may be reduced as deactivation also can be conducted as the material travels in the dryer, evaporators or heat exchangers. Moreover, higher temperatures are commonly used to accelerate the heating.

Most common cookers used in the fishmeal industry are steam or water heated. The steam can be retrieved from electric or fuel steam kettles, excess condensated steam or material vapor from dryers or evaporators.

Pre-cookers are commonly used to lower energy cost in fishmeal plants. They are often powered by excess steam or condensate from the evaporators or other equipment to contribute to better energy efficiency. It moreover reduces the steam needed in the main cooker, as the material is often around 40-50 °C after the preheating (Arason, 2001). The risk with the preheaters, however, is increased enzymatic activity in the material and more viscosity in the press cake and stickwater. The reason for this is that the excess heat source does not hold enough energy to contribute to rapid heating (Arason, 2001). Pre-cookers should therefore

rather be used to heat up blood water and blend it into the screw cooker to promote faster heating of the raw material. This also reduces the time the material is at this critical temperature between 40-50°C, which is ideal for enzymes to start degrading the raw material.

Conventional screw cookers are the most common cookers used in the fishmeal industry (Figure 6). The cooker is equipped with a steam heated jacket and heated screw in the middle to feed the material and to increase the heat transfer.
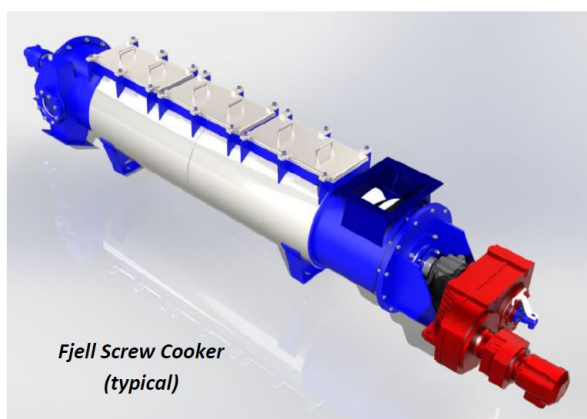

Figure 6 Conventional screw cooker from Fjell (AMOF Fjell, n.d.).

Figure 7 Compact coagulator in cross-sectional view (Process Cooling, 2001).

Compact coagulator is a type of pipe heat exchanger. They are commonly used as pre-heaters but can also serve as main cookers either with multiple units connected in series or in a larger version. They are constructed as normal pipe heat exchangers with a steam heated jacket that ensures heat transfer to the material. In addition, they are constructed with rotating blades inside that evenly scrape the contact area to prevent the formation of burnt material (Figure 7). It moreover ensures effective heat transfer as the material is continuously in motion. Common practice is to recirculate stickwater that has already been separated from the mass to accelerate the heat transfer. Compact coagulators have gained increased popularity in the fishmeal industry; they are compact, require less space and are designed to be able to receive excess heat sources such as a vapor. Their rapid heating results in shorter holding time which

reduces viscosity and enzymatic activity (Earle & Earle, 2004). They are moreover simple to operate as well as being easy to clean and maintain (FAO, 1986).

Straining

After heating, oil and most of the water is released and can be removed to a large extent by strainers. The goal is to make the material more porous to increase the processability in the press. The material is separated down into two streams; press liquor and wet press cake. The press liquor consists of oil, water-soluble nitrogen compounds (protein, peptides, amino acids, putrefaction products etc.), vitamins and minerals, and suspended fine particles.

Pressing

After the straining process, the wet press cake is fed into a press which squeezes the rest of the liquids out of the material. The press cake is then ready for drying while the press liquor goes into further processing.

Screw presses are a standard component in the fishmeal industry. The most common is the twin-screw press seen in Figure 8 but presses with one screw are also available. The compression ratio of these presses is dependent on the material and the processing methods. The press liquor contains, by estimate, 70% stickwater and 30% press cake in (FAO, 1986).
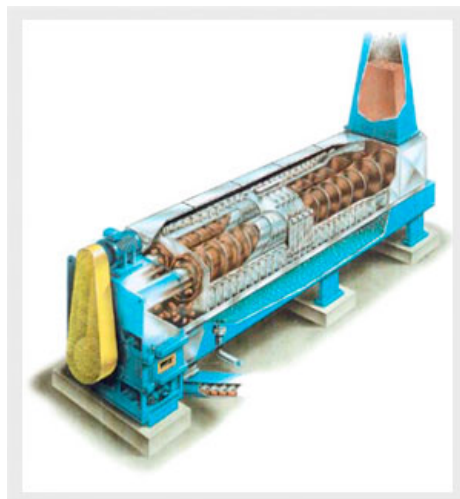

Figure 8 Twin screw press from Stord, The inlet is at the right side and outlet on the left (ATI Group, n.d.)

Decanter centrifugation of solids

Decanter centrifuges are used to remove solid particles from the press liquor. They have gained increased attention as a replacement for the main screw press in smaller fishmeal plants (Gíslason et al., 2014). This presents both advantages and disadvantages for the whole production. Firstly, using decanter centrifuges instead of the traditional screw press or mesh strainer will simplify the process, they are more compact, with more controllable separation than presses and strainers, they separate faster, the heat load on the material is reduced, they promote better hygiene and easier cleaning of the components. Most important though is their ability to process soft and very fluid material, which is where the press would fail. However, they have their disadvantages as well, the press cake can have higher moisture content and more soluble dry matter can be found in the stickwater which calls for more intensive separation of oils. Higher moisture content will moreover lead to higher energy consumption in the dryer (FAO, 1986).

According to European food regulations, screw presses may not be used when producing fish oil for human consumption. The reason for that is that screw presses are not so easily cleaned compared to decanters which are generally more hygienic and easier to clean.

In practice, there are two types of decanter centrifuges. First, the two-phase centrifuge which separates liquid and solid phase. Second, the three-phase centrifuge which separates liquid, solids, and oil phase. Even though this section is called decanter centrifugation, other separation methods could be well suited for this section, as vibration screen or other strainers. Centrifuges or so-called horizontal decanters are however considered more effective (FAO, 1986).

Two-phase horizontal decanter centrifuges use rotational forces that can be over 3000 times greater than the force of gravity (Alfa Laval, 2013). They are constructed as a horizontal cylinder with a bowl in one end where the solid phase is collected and discharged and with a rotational screw/scroll conveyor in the middle to feed the solid phase further into the bowl. The sludge is brought in at the opposite end through piping and enters the cylinder in the middle (Figure 9).
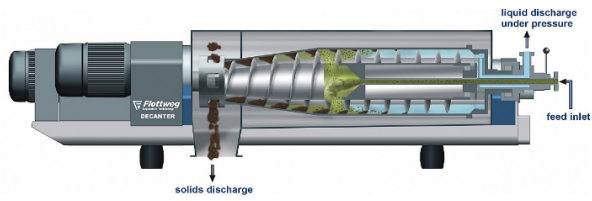
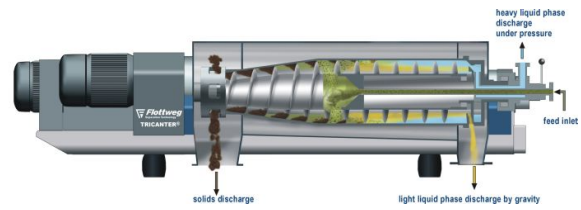Figure 9 Horizontal decanter centrifuge (Flottweg, n.d.)

Figure 10 Horizontal tricanter centrifuge (Flottweg, n.d.)

Three-phase horizontal tricanter centrifuges (Figure 10) have similar structure and function as decanters. Their alternative is that they can separate the material down to three material streams. The one with the highest density, the press cake, the water in the middle and oil with the lowest density.

Centrifugation of liquids

Centrifugation of liquids includes the process of separating the stickwater and oil. This is most often conducted in vertical centrifuges, either a nozzle type which operates continuously or a self-cleaning one (FAO, 1986). The liquor sludge is then fed into the evaporator system while the oil is led to further polishing. The oil can go through several stages of centrifugation before it enters the oil polishing. How far the producer wants to go, it's a matter of the degree of refining. Oil polishers, other centrifuges, and decanters require buffer tanks and stickwater to be heated to 95-90 °C for increased separation and purity of the end product.

Evaporation

After separation of solids in decanters or other centrifuges, a large portion of the oil and solids has already been removed from the press liquor. The next step is to process the excess stickwater, which undergoes evaporation for thickening to retrieve extra yield. Even though stickwater contains a low portion of solids (6-9%), it is estimated that it can hold up to 20% of the final weight of the fishmeal. The stickwater contains compounds such as dissolved and suspended proteins, minerals, residual oils, vitamins and amines/ammonia (Hall, 2011). Evaporation of stickwater is highly energy demanding and as an effort to reduce the energy cost, evaporators can be constructed in series, called multiple effect evaporators. The main principle is that steam is used on the first stage to evaporate and the vapor that comes out is used as a source of heat for the next evaporator. Thus, they can reuse the heat source at

different stages with different evaporation temperature. The excess vapor that comes out of the last stage can moreover be used for other purposes in the plant such as preheating of the material.

Four stage multi-effect evaporators are common in onshore plants. Having more stages is not preferred as it will require too high temperatures in the first stages (Arason, 2001).

The factor that greatly affects the degree of evaporation is the viscosity. It determines how much water that can be removed before running into pumping failure or before unwanted material formation on the heating surface occur. Stickwater is therefore usually concentrated to 30-50% dry matter to prevent this (Hall, 2011). However, viscosity decrease with higher temperatures and the last stage of evaporation is often conducted in the first evaporator stage with the highest temperature (Schenz and Morr, 1996) (FAO, 1986). Special care must be taken to prevent overheating the material since too high temperatures can damage heat sensitive components such as vitamins, proteins, amino acids, in particularly cystine, lysine, and tryptophan. Thus, it's not adviced to heat the material up to 130 °C (FAO, 1986).

There are many types of evaporators. Stickwater is heat sensitive and can be highly viscous on the last stages so evaporators should be chosen with that in mind. The falling film evaporators are most commonly used. The liquid stickwater is fed in at the top and fall down the pipes while evaporation occurs. The liquids are partly drained out at the bottom of the evaporator while the vapor is ejected through a pipe into a separation unit where unwanted liquid drops, within the vapor, are condensed. Falling film evaporators (Figure 11) are widely used for heat-sensitive liquids that require short residence time (5 to 10 s or more) and high heat transfer coefficient (Geankoplis, 1993).
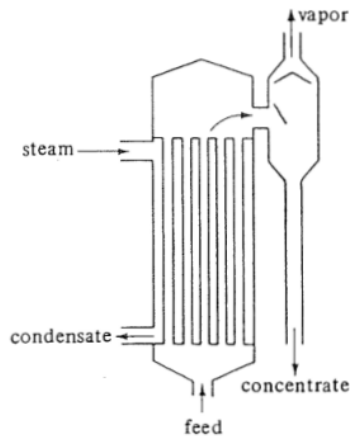
Figure 11 Falling film evaporator (Geankoplis, 1993

Other types such as rising film evaporators, mechanical vapor recompression evaporators (MVR) and plate evaporators can also be used and are seen on the following figures 12 and 13.
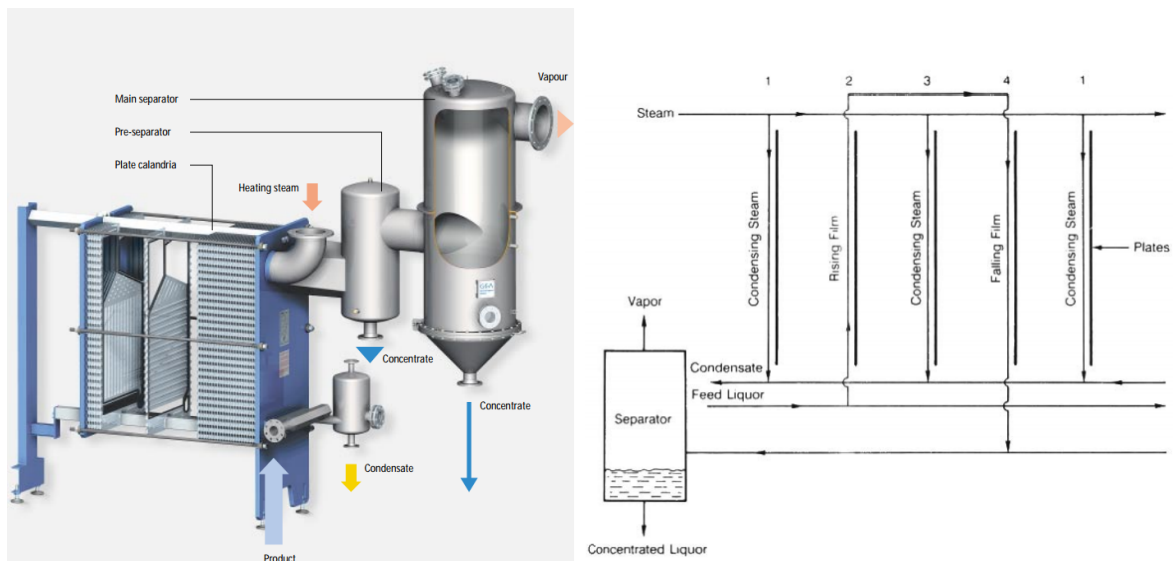

Figure 12 (Right), the outlook of GEA plate evaporator with a steam trap (GEA, n.d.). (Left), the function of plate evaporator and different production streams (Whalley, 1991)
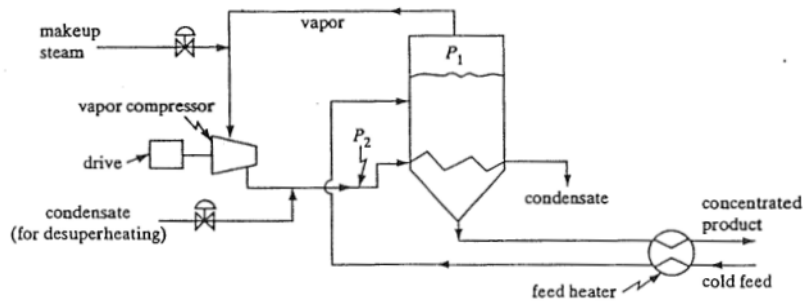
Figure 13 A simplified diagram of the MVR evaporation process. The MVR consists of a compressor, heat exchanger, falling film tower and drive units (Geankoplis, 1993).

Drying

In a conventional fishmeal plant, with processing of stickwater, the dryer receives press cake from the screw press with a moisture content of around 50%. This press cake consists of sludge from the centrifugation steps, concentrated stickwater from evaporators (around 60% moisture) and press cake from the press. These material streams need to be blended and, in some cases, a hammer mill is used to reduce the particle size to facilitate the evaporation of moisture from the material. Reducing the particle size increases the total surface area, which will contribute to more effective drying as well as a more stable and uniform end product. Target moisture content out of the dryer is below 12%. The dryers used in the fishmeal industry can be of two types; indirect steam dryers or direct air rotary dryers. The selection is dependent on several factors including their material chemical properties, fuel supply, available space and the production capacity (FAO, 1986).

The main difference between the two dryer types are the convection used. The indirect steam dryer uses hot steam to heat up the contact surface., This can be heated tubes, discs, coils, etc. The material is heated in contact with the heated surface, leading to evaporation of the moisture within, while air is blown through the dryer in opposite direction with the material to capture the vapor. The steam dryer is constructed with heated screw in the middle, which serves as conveyor, heat source and ensures blending of the material (FAO, 1986). The direct air dryer uses hot air for the convection, which can be extracted through heat exchanger or directly from fire. The convection through air contributes to more efficient mass transfer, which results in shorter holding time. The disadvantage is that when heat is directly extracted from fire, hazardous chemicals from the fuel can contaminate the material (FAO, 1986).

Understanding the difference between direct and indirect drying and its effect on material properties is crucial for successful drying. When drying of the material, whether it is a direct or indirect, the material undergoes two processes:

Process no. 1- the removal of moisture from the outer surface of the material. The removal is dependent on factors such as air humidity, airflow, pressure and the surface area of the material (Mujumdar, 1987).

Process no. 2- the transport of moisture within the material to the outer surface. This transfer is dependent upon the material properties, temperature, time and the moisture content (Mujumdar, 1987).

At the first stages of drying when the press cake is relatively wet, the removal of moisture occurs at a higher speed than at the last stages when the moisture content has decreased. That is mainly due to process no. 2 since the transfer of moisture within the material becomes a limiting factor. In direct-air rotary dryers, too high temperatures can lead to shell formation on the outer surface of the material, thus limiting the transfer of moisture (process no. 2). If too high temperatures are conducted in an indirect steam heated rotary dryer it can lead to burning of the material on the last stages and the material can form a shell on the heating surface. To take advantage of this, secondary dryers can be installed. They run at lower temperatures to prevent burning and shell formation. This can be conducted for both direct and indirect dryers (Arason, 2001). Figure 14 describes how the drying curve changes in relation to the moisture content. At first, the drying rate is stable with constant removal of moisture. When the moisture content lowers, other factors become more limiting and the drying rate decreases. According to Arason, the drying rate of minced fish starts to decrease when the moisture content reaches 30 to 40% (Arason, 2013). Another reason for using two-stage drying configuration is to not damage the digestibility of proteins. This is very important for the feed manufacturers as reduction of biological protein digestibility will reduce growth and feed efficiency in fish. The digestibility of the proteins is not temperature dependent when the water content is over 40% but when the water content drops below 40% the temperature of the meal and drying time determine the digestibility (Arason, 2013). If drying is only performed in one stage steam dryer, it may affect the digestibility since too high temperatures

are present during the last stage of drying. As an example during production of Norse-LT 94 indirect steam dryers could only be used as pre-dryers (Flesland et al 2000).
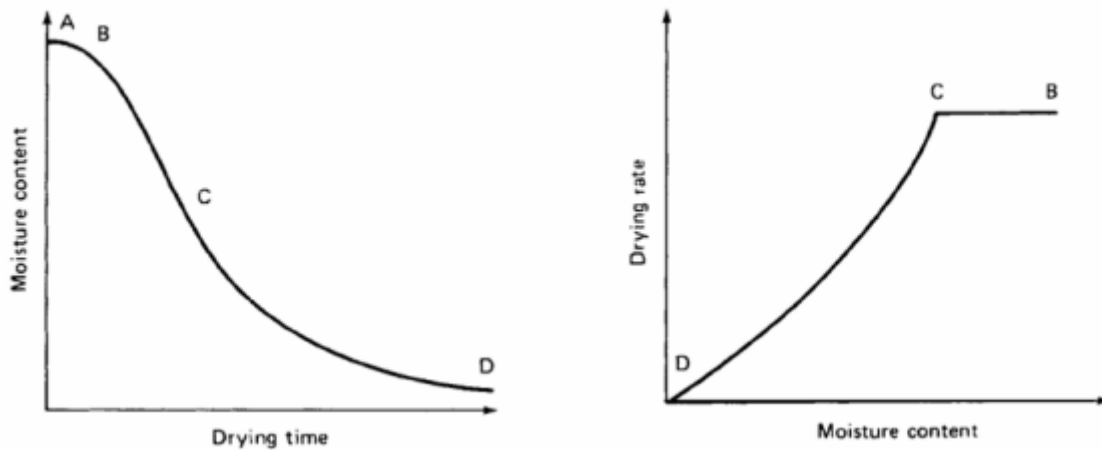
The advantages of indirect steam dryers are their ability to process large quantities with relatively simple equipment and lower installation cost. They do not require extensive air cleaning, and the fishmeal becomes less dusty than in the direct ones, which makes them more acceptable for mink feeding. Indirect steam dryers also allow for renewal of the steam and vapor, which can be used for preheating or other purposes in the plant. They do not necessarily require blending of the stickwater concentrate and the press cake, due to the constant circulation of the material in the dryer (FAO, 1986).

The advantages of direct air rotary dryers are short holding time (between 10 to 20 min compared to 30 min in indirect dryer) and flexibility when it comes to choosing fuel supply as they can be powered with conventional fuels to fish oils. They also require less maintenance and their processes are more controllable (Arason, 2001) and they enables production of fishmeal with high biological protein digestibility (Flesland et al. 2000).

Cooling

When the meal leaves the dryer, it has a temperature at approximate 80 °C. The air within holds a lot of moisture, which has to be removed as quick as possible, otherwise, the material can absorb that moisture. This will result in higher water activity that can have negative effect

on e material quality. Cooling the material before milling procedure is also beneficial. Cooling will also reduce the moisture content by 1-2%, giving a fishmeal with within the advisable moisture range of 9-11 % (Arason, 2001). The target for the cooling step is to reduce the material temperature from around 80 °C to room temperature. (FAO, 1986).

The most common way to cool fishmeal is to use air blowers. Various versions are available. In some coolers the air is blown in the same direction as the material flow and in others the opposite direction (Arason, 2001).

Milling

After the cooling procedure, the dried material undergoes milling to retrieve homogeneous particle size. The physical properties of the material can, however, be a concern as a large part of the material is already in right particle size or even smaller than wanted. Thus, it is often required to set up vibrating sieves to separate the smaller particles from the larger ones that need to be milled (FAO, 1986). This moreover ensures the removal of unwanted particles such as pieces of wood, cloth, fish hooks and nails that could have entered into the process. But more importantly, it ensures the right particle size for the customer. Most purchasers require the meal to pass through a 10-mesh screen which is a widespread scale for the particle size (FAO, 1986). The risk that follows continuous milling of all the material is the creation of dust. The material becomes dusty which can cause contamination or loss, as the dust has access through the packing. Too small particles can moreover cause oxidation as oxygen seems to have better access with more surface area (Gíslason et al., 2014).

3.2 Novel processing methods

The fishmeal production process is quite standardized as previously mentioned and the industry has not changed much in the last decades and has remained relatively conservative. The focus has been much more on energy efficiency rather than actual material-, protein- or oil qualities. Alternatives to fishmeal and oil is to produce fish protein concentrate (sometimes called fish silage) or to produce fish protein hydrolysate where enzymes are used. These products can be used to make variety of products from food, feed and fertilizers. Instead of

heat coagulating the raw material as done with fishmeal it is treated either by acids, enzymes or other chemicals, which breaks down the proteins and separates the oil.

Fish protein concentrate

FPC is produced from fish offcuts and offal from filleting, gutting and other fish processing operations. The raw material is minced and thereafter added formic acid to reduce the pH to lower than 4. This gives optimal conditions for protein hydrolysis by the fish pepsin and are stabilizing microbiological activity. The hydrolysed crude fish silage is heated and processed over a 3-phase decanter centrifuge to remove oil and particulate matter before concentrated to an FPC with a typical dry matter of 40-50% (Sikorski et al., 1995).

According to the FAO, there are three major types of fish protein concentrates (FPC):

- Type A: a virtually odourless and tasteless powder having a maximum total fat content of 0.75%.

- Type B: a powder having no specific limits as to odour or flavour, but definitely having a fishy flavour and a maximum fat content of 3%.

- Type C: normal fishmeal produced under satisfactorily hygienic conditions.

The fat content is specified when defining types of FPC because fat, when oxidised, can produce a strong, often rancid, taste in the product. The protein content of FPC depends on the raw material used and the extent to which water has been removed, but the products normally contain at least 65 percent protein and, in type A, up to 80 percent (Windsor, 2001).

Other types of fish protein concentrate such as those produced via hydrolysis of fish proteins using enzymes or other chemical processes (fish protein hydrolysates) following concentration of the product into a paste or extract are not included in the previously mentioned definition.

FPC can be prepared from any type of fish or fishery waste (e.g. fish offal, trimmings or cuttings, and other side streams), provided such production and products meet the relevant requirements for fishery products established by the EC Hygiene Regulations (EC

1020/2008). FPC is prepared from fish by extracting out the water and the oil, which combined account for up to 80% of the whole fish, screening or settling out the bones and drying, so that the resultant FPC is higher in protein (85% to 95 %) and lower in ash content than fishmeal. Several approaches for preparing FPC have been investigated including chemical, biological, and physical methods (Dubrow and Stillings, 1971). Nevertheless, the most widely investigated methods are based on the use of chemical solvents to remove the water, fat and fishy-tasting components from whole fish (Dubrow and Stillings 1971; Windsor, 2001; **Error! Reference source not found.**). Although, technically FPC can also be prepared from fishmeal (Windsor, 2001).

Excellent quality FPC can be prepared from a variety of raw materials (Power, 1964; Dubrow and Stillings 1971; Iwaya and Yamaguchi 1979). High-quality protein concentrate has been obtained from cod fillets, whole cod, headed, eviscerated cod, and cod trimmings using solvent extraction (Power, 1964). The fish protein concentrate thus produced had a high protein efficiency ratio, satisfactory color, odour, taste, and texture. Following another approach, cooking and pressing whole fish before the solvent extraction step was tested by Dubrow and Stillings (1971) in order to reduce the amount of solvent used. Heating the fish at 109°C for as long as 80 min had no significant negative effect on the chemical and nutritional properties of the produced FPC.
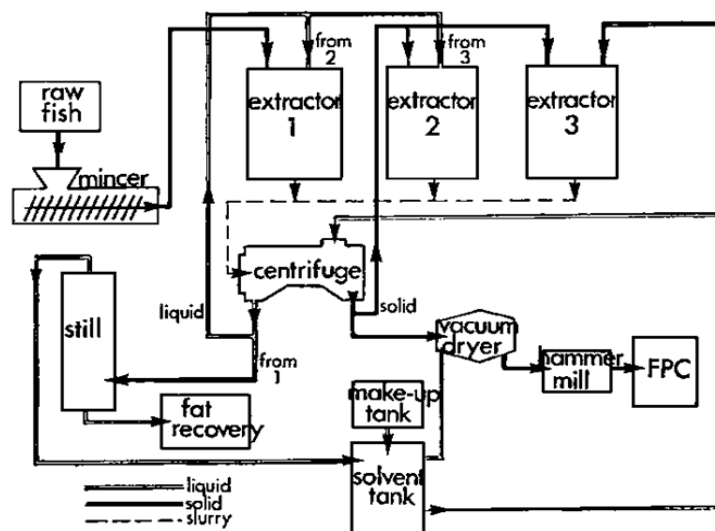

Figure 15 Flow diagram for the production of FPC (solvent extraction;Windsor, 2001).

Fish protein concentrates are a source of high-quality proteins, with a balanced amino acid profile and high digestibility (Iwaya and Yamaguchi, 1979). Therefore, FPC can potentially be used to supplement diets that contain inadequate amounts of high-quality protein and/or fortify food for those with special requirements (e.g. sports nutrition).

At the beginning of the '60s when there was a rapid and widespread growth of interest in FPC for human food, the possibilities of upgrading fishmeal were naturally a prominent item in the deliberations of various working parties. However, different organization including the United Nations Protein Advisory Group have gradually hardened their position that in order to be suitable for consumption by the protein-starved people of the world a food must also be acceptable by the standards of the well-fed; and in this regard, any suggestion of an upgraded animal feed supplement should be avoided.

Despite subsidies from national or international agencies, all attempts made in the following decade to introduce FPC into staple cereal foods have failed to get beyond the trial stage, e.g. in South Africa, Morocco and Chile (Lovern, 1969). In a review of the problems in the development of fish, protein concentrates during the 60's Lovern (1969) highlights some of the challenges:

- Even the FPC type A (virtually fat-free, odorless, tasteless product) acquired a slight fishmeal-like taste and odor after storage (reversion flavors).

- Solvent-extracted FPC has a gritty texture that is detectable in the mouth even after very fine grinding when it feels chalky.

- Addition of FPC in cereal products at inclusion levels of 5% and above has a detrimental effect on the product color, taste, volume, and structure.

- A substantial increase in production cost for FPC in the degree of sophistication now envisaged overcoming these technological challenges.

Fish protein hydrolysate (FPH)

FPH are produced by protein hydrolysis and this is achieved by the cleavage of fish proteins into peptides. Hydrolysis can be completed by either enzymatic and/or chemical reactions.

Hydrolysis directly affects the physicochemical and functional properties of proteins (Kristinsson and Rasco, 2000). Protein hydrolysis produces peptides with different or enhanced functional properties and bioactivities compared to native proteins (Kitts and Weiler, 2003). Biological processes using added enzymes are more frequently employed compared to chemically based methods because they require milder conditions and are easier to control. Enzymatic hydrolysis is a very promising technique for the future because it produces FPH with various functionalities, health-promoting bioactivities, and high nutritional value (Kristinsson and Rasco, 2000). The modification of structure, chain length, and amino acid sequence depends on various factors such as treatment, hydrolytic conditions, type of enzyme, temperature, and protein concentration (Foh et al., 2011). Additionally, FPH are determined by the raw material used. Different proteinaceous substances yield hydrolysates with different nutritional value as well as functional properties. Generally, FPH can be produced from fish flesh and by-products such as skin, bone, etc. as well as minces containing indigenous proteases. A scheme for FPH production is described in Figure 15. Production of FPH utilizing enzymes is advantageous in that they are lower in start-up cost and they can be scaled up or down pending supply and demand of materials (Table 8).
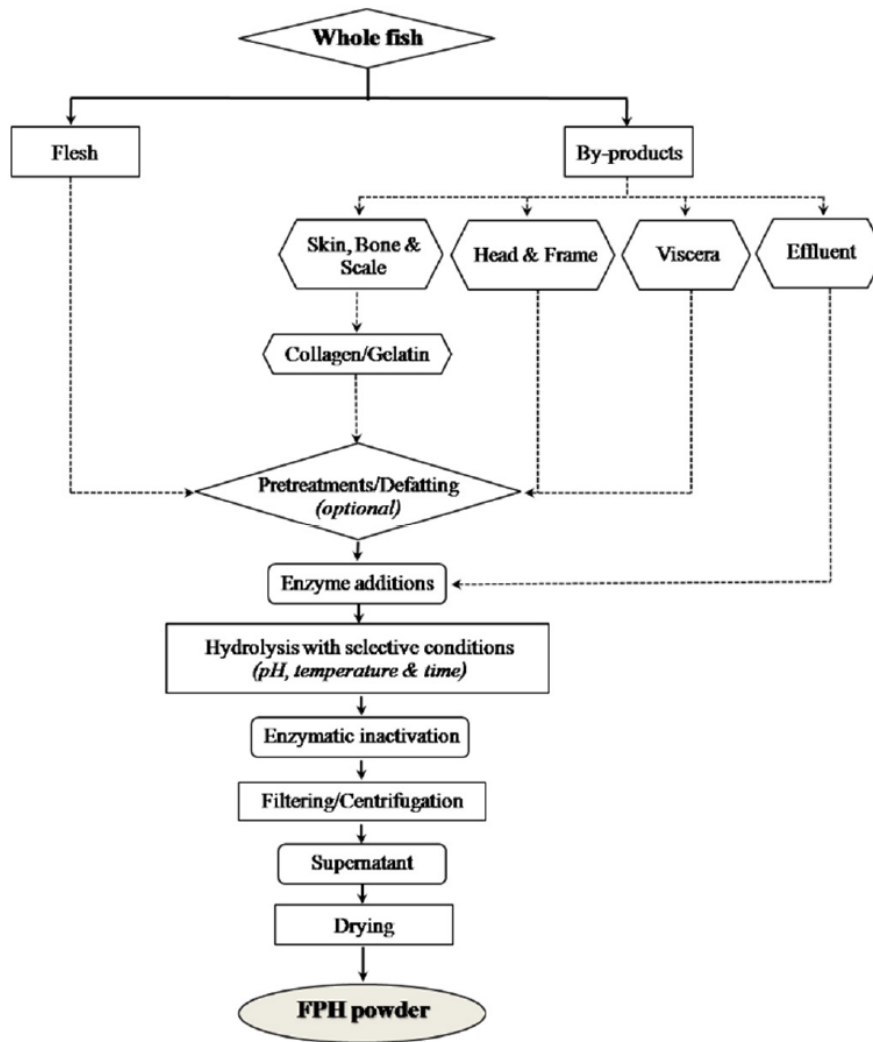
Figure 16 Schematic description of FPH production process from whole fish, flesh, and byproducts.

Table 8 Comparison of the chemical process and enzymatic process to produce fish protein hydrolysates from fish byproducts (Kristinsson and Rasco, 2000; Sanmartin et al., 2009).

Processing method	Advantages	Disadvantages
Chemical process (acid and alkali)	High protein recovery Short processing time Low processing cost	Absence of homogeneous hydrolysates Bitterness Poor functionalities High salt content Metal corrosion of equipment Reaction is hard to control Form toxic substances like lysino-alanine Form D-amino acids, which are not absorbed by humans
Enzymatic process	Less bitterness of final hydrolysates Maintain functions and nutritive value of final hydrolysates Low salt content in final products Produce homogeneous hydrolysates	High processing cost Long processing time

Major challenges in commercializing bioactive fish protein ingredients for human consumption is lack of high, consistent quality and oxidative instability. In the past, FPH hydrolysates were utilized for fertilizer or animal feed due to poor organoleptic and functional properties. Many seafood species are rich in polyunsaturated fatty acids that are extremely susceptible to oxidation in addition to being rich in oxidation accelerating agents (pro-oxidants) such as hemoglobin and iron. This is especially the case when using low-value raw materials for the hydrolysis process. During a conventional enzymatic hydrolysis process, the reaction conditions can be quite severe due to temperature and pH extremes (Kristinsson and Rasco, 2000). Under these conditions, fatty acids and pro-oxidants interact and oxidation develops rapidly, having a negative effect on the quality of the final peptide product. It not only can have a negative effect on nutritional value and organoleptic quality but also on the bioactive properties as identified by Halldorsdottir and coworkers (2014). A way to increase the quality of FPH substantially is by adding natural antioxidants to the protein substrate prior to hydrolysis. Studies indicate that by using natural antioxidants during hydrolysis the quality of the resultant FPH are considerably higher than FPH not produced with antioxidants. The natural antioxidants inhibit oxidation during hydrolysis, contributing to an increase in the

bioactivity and decrease the unpleasant taste and smell such as a bitter taste of the final FPH product. However, there are a lot of different antioxidants that all have different functions and work through different mechanisms. Therefore, it is necessary to investigate each process thoroughly to predict, which antioxidant is best for each material and conditions. According to extensive research that Matís has conducted on the subject, antioxidants derived from seaweed show the most promising results. According to sensory analysis, FPH produced with seaweed extract (Seaweed-FPH) has a lower intensity of all attributes tested as compared to FPH not produced with seaweed (control) (Figure 16). Matís holds a pending patent on the subject entitled "Use of natural antioxidants during enzymatic hydrolysis of aquatic protein to obtain high-quality aquatic protein hydrolysates" (Halldorsdottir et al., 2012).
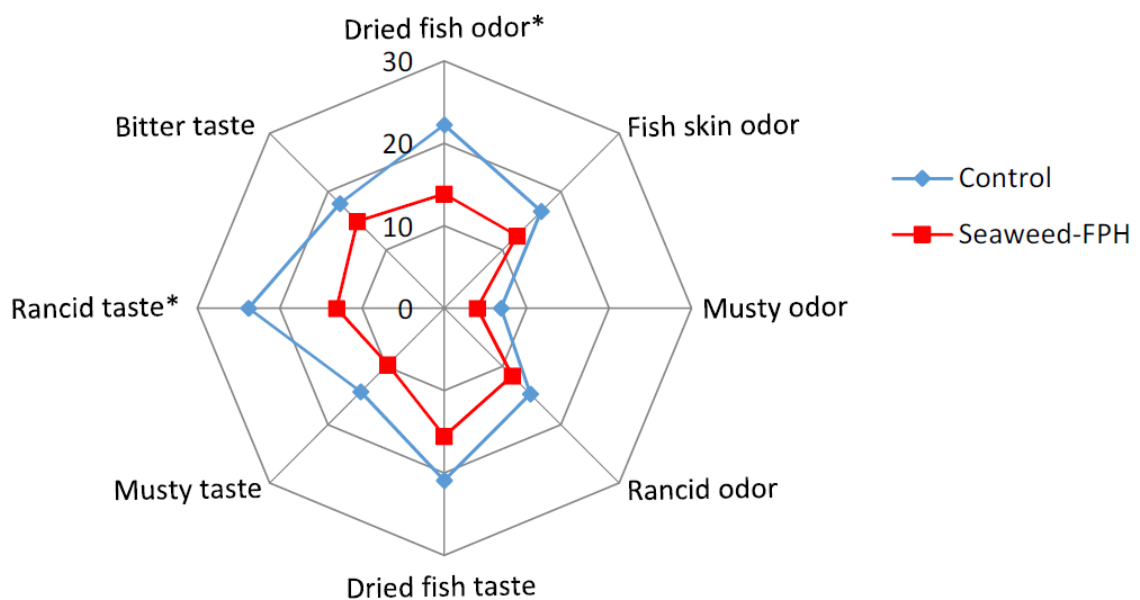

Figure 17 Sensory analysis results on FPH produced with seaweed extract (Seaweed-FPH) as antioxidants as compared to FPH produced without seaweed extract (Control).

Isolation of peptide fractions has been conducted using minced muscle from de-headed and gutted blue whiting. This type of processing scheme could be used to isolate hydrolysates of specific molecular weights and bioactivities (Vandanjon, L., Johannsson, R., et al., 2007).

4 Analytical methods

Introduced in this chapter are traditional analytical methods that are currently applied to raw material, fishmeal, and oil as well as newer methods such as NIR and GC-MS. Methods for analysis of the technical quality of fish meal are also covered.

4.1 Methods traditionally applied on raw material, fishmeal, and oil

The two main fishmeal specifications used today in Norwegian aquaculture feed production are NorSeaMink and the high-quality Norse-LT 94 (Table 9). On the world commodity market similar specifications are used (Schmidtsdorff 1995). The specifications are based on a set of chemical and biological analysis.

Table 9 Specification for NorSeaMink and Norse-LT 94

	NorSeaMink	Norse-LT 94
Crude protein (%)	Min. -, Typical 71	Min. 68, Typical 71
Water-soluble protein (% of crude protein)	-	Max. 32, min. 18
Moisture (%)	Max.10, min. 5	Max.10, min. 6
Fat (Soxhlet) (%)	Max. 13	Max. 13
Ash, without salt (%)	Max. 14	Max. 14
Salt (sodium chloride) (%)	Max. 4	Max. 4
Total volatile nitrogen (%)	Max. 0.20	Max. 0.18
Cadaverine (g kg^{-1})	Max. 1.8	Max. 1.0
Histamine (g kg^{-1})	Max. 0.7	Max. 0.5

Source: (Norsildmel 2018)

Proteins

Protein content in fish and fish meal is determined by analyzing the total content of nitrogen in the sample, which is then multiplied by a conversion factor (f= 6.25) to convert this value to the protein content. Total protein content is usually analysed either by the Dumas method or by the Kjeldahl method. The Kjeldahl method is still the standard method, which most other methods are being compared against, but since the Dumas is faster to perform its use in industry is increasing at the expense of the Kjeldahl method. Another advantage of the Dumas method is that it does not require the use of toxic catalysts or concentrated sulphuric acid as is the case for the Kjeldahl method.

Water-soluble protein

Water soluble proteins can be measured using either Kjeldahl distillation or Dumas combustion and are an estimation of how much protein from the fish meal is soluble in water. Most methods today depend on shaking a known amount of fish meal in water and then measuring how much of the proteins were solubilized in the water.

Moisture

The moisture content is evaluated by identifying the percentage of dry matter in a sample. A sample is placed in a ceramic bowl and into an oven for 4 hours at 104°C and into a desiccator for 30 min. The sample is weighed again, and amount of weight lost used to calculate amount of water in the original sample (ISO 6496. 1999, 2007).

Free fatty acids

Free fatty acids (FFA) are formed due to hydrolysis of triglycerides (lipolysis). FFA content in the crude oil will depend on factors such as fish species and harvest season. FFA may constitute up to 12 % of the total lipids, but typical values are lower (Bimbo, 1998). FFA are more susceptible to lipid oxidation than triglycerides and they will also give rise to off-flavors in the final product. For this reason, FFA must be removed during refining of fish oil. FFA are usually quantified by titration with NaOH with phenolphthalein as an indicator (AOCS, 1998). This method is valid for raw material and fish oil (EFSA 2010).

Ash

The amount of inorganic material in fish meal is determined using the ISO 5984-2002 (E) method in which the sample is carbonized before being incinerated at 550°C. The amount found of inorganic material in fish meal differs depending on species.

Salt

Salt content is determined by the Volhard method. The solution is acidified with nitric acid and the chlorides precipitated in the form of silver chloride by means of silver nitrate. The excess silver nitrate is titrated with a solution of ammonium thiocyanate.

Oxidation of the oil, in oily fish like herring, gives rise to rancid odours and flavours. These can limit the storage life of such species more quickly than the protein changes that govern the extractable protein value. An important stage in the oxidation is the addition of oxygen to the fatty acid molecules to form hydroperoxides; the amount of these can be used as a measure of the extent of oxidation in the early stages. The correct term lipid hydroperoxide value is frequently shortened to peroxide value. To measure peroxide value in fish raw material the oil must first be extracted from the fish by a method that does not itself encourage further oxidation. The oil containing peroxides is treated with potassium iodide, following that the iodine is liberated and measurement of the amount of iodine enables the peroxide value to be calculated. Other methods to determine peroxide values also exist (see below), but the method based on the reaction between peroxides and iodide is the standard method (AOCS, 1998). Increase in the peroxide value is most useful as an index of the earlier stages of oxidation; as oxidation proceeds the peroxide value can start to fall. Therefore, a single measurement of PV can only be used as an index of current oxidation status if the peroxides formed are stable enough so that they do not decompose after formation. This is not true for most lipids. This method is valid for raw material and fish oil. The PV in crude oils will depend on the quality of fish used for oil extraction, the process of oil extraction and the storage conditions of the crude oil. As previously described elevated temperatures and high levels of Hb-Fe will catalyze oxidation. Hence, if fish have been squeezed and bruised onboard the fishing vessel and/or if the fish has been stored several days on board the vessel maybe even at a too high temperature before being processed, it may be severely oxidized when it reaches the processing plant. This will lead to crude oils with high PV, e.g. above 10 meq/kg. As mentioned above a wide range of methods have been developed for measuring PV, including both wet chemical and instrumental methods. The analytical principle of many methods is the ability of the lipid hydroperoxides to oxidize either iron or iodide ions (Nielsen et al., 2003). The main disadvantage of the AOCS standard method (AOCS, 1998) is that it requires large sample amounts. Nevertheless, this method is recommended as a standard method for PV determination in crude oils in the industry (EFSA, 2010).

Most of the methods mentioned above are empirical and unspecific. Thus, other components present in the food may also react with the reagents used in the different methods. For example, iodide may under certain circumstances react directly with unsaturated double bonds

in the case of the titration and micro methods. Likewise, the iron salts may react with e.g. metal chelators that have been added to the food/fat to prevent lipid oxidation. Due to the lacking specificity of the various methods, different PVs may be obtained when the same samples are analyzed by different methods (EFSA, 2010). For further information on non-conventional methods employed for PV determination see section 4.2 *Non-conventional methods*.

Thiobarbituric acid value (TBA value)

The hydroperoxides, mentioned above, can react further to give a wide range of compounds, some of which are responsible for the rancid odours and flavours in oily fish and for cold storage odors and flavors in white fish. One such compound called malonaldehyde, and a number of related compounds, can be separated from the fish either by distillation or by preparing a protein-free extract. The reaction of these compounds with 2-thiobarbituric acid gives rise to coloured products, the amount of which, the TBA value, is measured using a spectrophotometer. The increase in the TBA value is a measure of the extent of oxidative deterioration in oily fish, but, as in the case of peroxide value, the TBA value can fall again at a later stage of spoilage. This method is only used for raw material (EFSA, 2010).

Anisidine value

The anisidine test is a method commonly used in the oil industry as a measure of the level of secondary oxidation products (carbonyl compounds). The principle of the anisidine test is that the before mentioned compounds react with p-anisidine to form a coloured complex that absorbs at 350 nm. The anisidine value is defined as the absorbance of a solution resulting from the reaction of 1 g fat in 100 mL of isooctane solvent and 0.25 % anisidine in glacial acetic acid (AOCS, 1994). The anisidine test has previously been attributed to the reaction between p-anisidine and 2-alkenals, but other carbonyl compounds than the 2-alkenals are able to react with p-anisidine as previously mentioned. The anisidine method is, therefore, an unspecific method and furthermore, it is not very sensitive. The anisidine test measures the carbonyl compounds that may contribute to off-flavour formation as a result of oxidation. Therefore, results obtained from the anisidine test could be expected to correlate well with sensory data, but this is not always the case, especially not in food emulsions (EFSA, 2010).

Quantitative determination of anisidine value by FTIR spectroscopy also has been shown to be feasible (Sedman et al., 1997), while NIR seems to be less suitable for a spectroscopic determination of AV (Yildiz et al., 2001). The method is used for raw material and for fish oil (EFSA 2010).

Total volatile bases (TVB)

Ammonia and trimethylamine are examples of bases; another base, dimethylamine (DMA), can also be formed during spoilage of fish, together with traces of others. These bases, other than ammonia, are known chemically also as amines. The combined total amount of ammonia, dimethylamine, and trimethylamine is called the total volatile base content of the fish (usually expressed as mg-N/l00 g minced fish) and is a commonly used estimate of spoilage. The increase in the amount of TVB parallels the increase in TMA but the analysis is easier to carry out than that for TMA. Alternative terms used are total volatile basic nitrogen (TVB-N) and total volatile nitrogen (TVN) since the results of the analysis are always given in terms of the nitrogen content of the bases. Corresponding French and German names are azote basique volatile total (ABVT) and flűchtiger Basenstickstoff. TVB can be measured easily and quickly using a relatively simple apparatus and, for this reason, a TVB value is often used as a rejection limit in regulations and commercial specifications. This method is only used for raw material (EFSA 2010).

Analytical methods characterizing the technical quality

The following analytical methods have been established to better characterize the technical properties of fishmeal in relation to the feed extrusion process and physical feed quality:

- *pH*: Measured in the water phase obtained by suspending fishmeal (10% DM basis) in distilled water at room temperature (Samuelsen et al., 2013).

- *Peptide size distribution of the water-soluble protein fraction*: Water-soluble protein can be extracted with hot-water and measured by use of HPLC size exclusion chromatography and detection based on UV adsorption (Oterhals et al. 2015). The amount of, and the molecular weight distribution is very important for the technical quality of the fishmeal which impacts the feed production process and physical feed quality.

- *Flow-figure*: Measured by gently sifting FM through a US sieve no. 8 on top of a cylinder with a diameter of 50 mm. The procedure is continued until a stable cone height is achieved and height measured in cm (Høstmark 1985).

- *Loose bulk density***:** Measured based on the weight of 100 ml fishmeal in a measuring cylinder after tapping (ISO 5311 1992).

- *Particle size distribution***:** Can be measured using AOAC (2005) method 965.22 combined with an air jet sieve (U.S. Sieve No.120, 180, 230, 325). The cumulative mean particle size can be estimated based on the measured size class distribution. A dust fraction can be defined as the percent passing through the 325-mesh sieve (<44 μm). Particle size and amount of dust are also important parameters which impact the particle moisture uptake in the feed process and consequently physical feed quality.

Both flow-figure and bulk density reflect the structure of the fishmeal particles. Fishmeal with a fibrous particle structure (high flow-figure and low bulk density) gives high internal friction (Høstmark 1985). Flow-figure and loose bulk density show a significant negative correlation for herring meal and the main impact on these properties were related to the type and combination of dryers (Figure 18; Samuelsen 2015). Fishmeal with lowest friction forces (low value of flow-figure and high value of loose bulk density) were produced on a Hetland indirect hot air dryer and the fishmeal with highest friction forces was produced on a Jäckering Ultra-rotor mill dryer (flash dryer). In between were combinations of steam pre-dryer and final air/vacuum dryers with the increasing friction forces in the order Hetland indirect hot air dryer < Dyno-Jet indirect hot air dryer < indirect vacuum dryer (Flesland *et al.* 2000; Høstmark *et al.* 2001; Samuelsen *et al.* 2013). Poor powder flowability was found for herring meal with flow-figure ≥ 5.2 cm due to high fibrous particle structure. Flow-figure and loose bulk density show a significant correlation for both herring meal and fishmeal from sand eel but with a lower y-axis intercept for herring compared to sand eel (Figure 19; Samuelsen *et al.* 2014). The shift indicates differences in particle structure and properties between the studied groups. Comparing fishmeal from different species, both flow-figure and loose bulk density may, therefore, give an inaccurate measure of particle structure and particle friction forces.
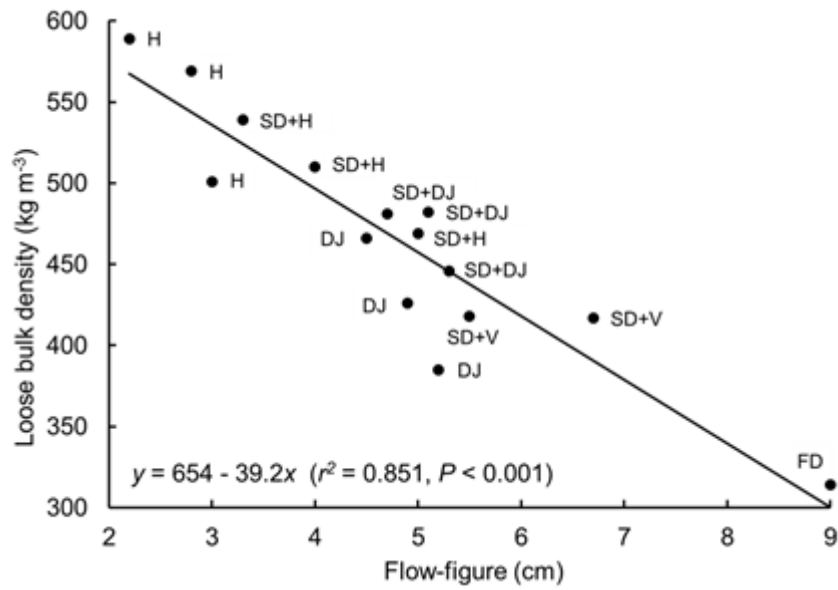
Figure 18 Relationship between flow-figure and loose bulk density for herring meal dried at different types or combination of dryers (Samuelsen 2015). Abbreviation: DJ, Dyno-Jet indirect air dryer (Stord International A/S, Bergen, Norway); FD, flash dryer (Ultra-rotor mill dryer, Altenburger Maschinen Jäckering GmbH, Hamm, Germany); H, Hetland indirect air dryer (Kværner Hetland A/S, Bryne, Norway); RSF, refrigeration by fresh water; RSW, refrigeration by seawater; SD+, indirect steam dryer used as pre-dryer; V, indirect vacuum dryer (Stord International A/S, Bergen, Norway).
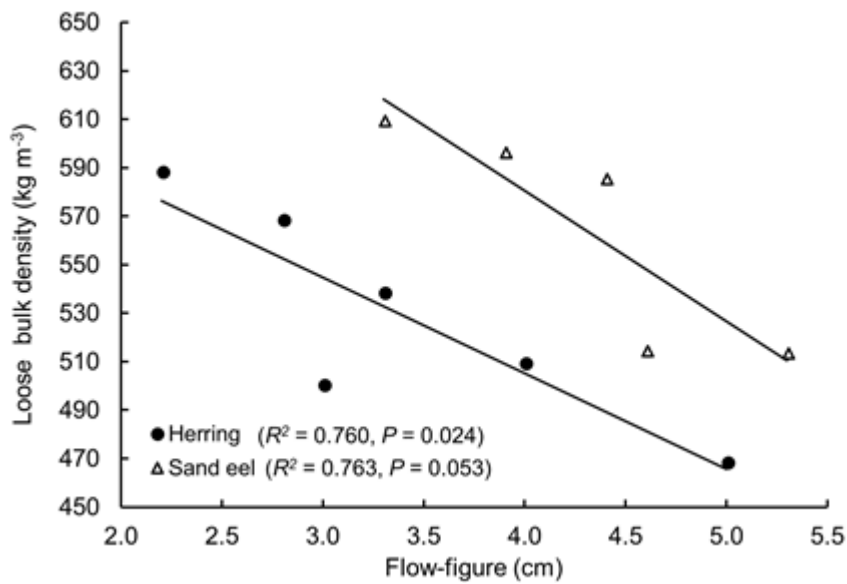

Figure 19 Relationship between flow-figure and loose bulk density for herring and sand eel meals respectively (Samuelsen et al. 2014).

4.2 Non-conventional methods

Peroxide value (PV)

During the past decades, several new instrumental methods have been developed for measuring PV including methods such as GC-MS (Frankel et al., 1979), HPLC (Hartvigsen et al., 2000b, Müllertz et al., 1990), IR and NMR spectroscopic techniques (Khatoon and Krishina, 1998) NIR (Yildiz et al., 2001; 2002; 2003) and FTIR (Ma et al., 1997; Sedman et al., 1997) and Differential Scanning Calorimetry (Tan and Man, 2002). Promising instrumental methods for routine control analysis of peroxide values seem to be NIR, FTIR and DSC techniques. NIR spectroscopy offers several advantages over conventional methods including high speed, non-destruction of the sample, a possibility of automation, on-line measurements and no reagents required/minimal sample preparation. Yildiz et al. (2001; 2002) have developed a very simple method to measure peroxide values in soybean and corn oil by NIR spectroscopy. The only sample preparation is the transfer of a small oil sample (which has been tempered at 25°C) to a 2 mm quartz cuvette, which subsequently is placed in the cuvette holder of the NIR instrument. The PV in a set of test samples predicted by NIR were compared to PV measured by the AOCS titration method and the correlation between the two methods was good (r > 0.99). The NIR method and the other spectroscopic methods can be used as long as they are calibrated against the standard method (EFSA 2010).

Near-infrared spectrograph (NIR)

NIR is a promising spectroscopy method that can be used for rapid analysis while requiring minimal sample preparation and is most often non-destructive for the sample. There is no chemical consumption required thus giving it an advantage over conventional chemical methods. The spectrum obtained from the analyzed product can give information on both chemical and physical properties of the product.

The spectral region of NIR extends from 730 nm to 2500nm and can be recorded in Spectra can be recorded in reflection, transmission, and transflection modes. NIR spectroscopy is dependent on the vibrational behavior of molecular bonds and the peaks observed in NIR

spectra are caused by the vibration of hydrogen groups such as C-H, O-H and N-H (Barnes et al, 1989).

NIR can provide real-time information from processing lines on the composition of the product and has been for both quality control and product optimization in this capacity within the food industry for several years, specifically for meat, milk and fish (Uddin et al 2006; Sivakesava et al, 2002; Alomar et al, 2006).

NIR has successfully been integrated into the seafood industry both for the prediction of quality as well as for the prediction of chemical composition for an array of different products (Uddin et al 2005; Uddin et al 2006; Masoum et al, 2012). Prediction of the chemical composition of fishmeal and surimi has been successful as well as the prediction of lipid content in several fatty fish species (Uddin et al 2006; Rasco et al, 1991; Lee et al, 1992; Cozzolino et al, 2002; Nielsen et al, 2005). Furthermore, NIR has been used to assess the digestibility of feeds and could potentially be used to predict the digestibility of fishmeal (Bastianelli, 2013).

Gas Chromatographic methods for determination of volatile oxidation products

In contrast to the anisidine and TBA test, gas chromatographic (GC) methods to determine secondary oxidation products offer the sensitivity and specificity that the former methods are lacking. GC methods are thus capable of determining volatile oxidation products that either contribute to or serve as markers for the off-flavour formation in oxidized lipid-containing foods. Depending on the method to collect and trap the volatiles a large number of different volatiles can be determined by GC methods including aldehydes, hydrocarbons, ketones, and alcohols. The most common methods to collect the volatiles are the static headspace, the solid phase microextraction method, and the dynamic headspace method. The static headspace method is based on the principle that at a given temperature and in a sealed container volatile will diffuse from the liquid/solid phase and vaporize into the headspace above the sample and after a certain length of time, an equilibrium will be established. At equilibrium, the concentration in the headspace will be proportional to the concentration of the volatiles in the sample.

The static headspace method is suited to highly volatile compounds and for routine consecutive analyses of many samples. It is often used as a quick method to determine hexanal originating from n-6 fatty acids and propanal originating from omega-3 fatty acids. These aldehydes are often used as markers of oxidation in vegetable oils and fish oils, respectively. The static headspace method is usually not sensitive towards some of the higher molecular volatile compounds such as the 2,4-decadienal and the 2,4-heptadienal, which have low flavour thresholds, and which may be important to flavour deterioration.

The solid phase microextraction (SPME) technique is very simple: The sample is weighed into a vial, which subsequently is sealed with a cap provided with a needle-pierceable septum. After equilibration at the desired temperature, a SPME fiber is inserted into the vial. Subsequently, extraction of the volatiles onto the fibre is usually conducted under stirring for a period of e.g. 10-30 minutes. Then, the fibre is conditioned in the GC injector for a certain time period before analysis. This method is usually less sensitive than the dynamic headspace method. Calibration curves for selected compounds can be made as described for the static headspace method. A drawback of this method is that there is competition between volatile compounds for space on the SPME fiber. In case, certain compounds with high affinity for the SPME fiber are present in high amounts they may take up all the space on the fiber and thereby other compounds may not be detected.

The principle of the dynamic headspace sampling method is that the volatiles are "forced" to be released from the food matrix by purging or sweeping a liquid sample with nitrogen or helium in a tube or vessel heated in e.g. a water bath. Subsequently, the volatiles are trapped on a tube containing a porous polymer (e.g. Tenax™, Chromosorb™, Carbosieve™). The volatiles are then desorbed from the absorbent tube by heating, and depending on the instrument, the volatiles may be further concentrated by trapping on a cold trap at low temperatures (e.g. minus 30°C). Subsequently, the cold trap is heated and the volatiles transferred by a carrier gas onto the capillary inlet of the GC, where they are separated on the GC column. After sampling of the volatiles, they are analyzed by either flame ionization detection (FID) or mass spectrometry (MS). This is also the case after static headspace and SPME sampling. Different methods are available for further identification of the volatiles (spiking with authentic standards, kovats index and mass spectra library). The advantages of the dynamic headspace method is that it is sensitive and that a more complete picture of the

volatiles present in the sample is obtained compared with the static headspace and SPME methods. The disadvantage is that the sampling procedure is laborious and time-consuming, but automated systems are available for the later part of the sampling procedure, i.e. the transfer of the volatiles from the absorbent tube to the GC. Another disadvantage is that a large range of volatiles usually will be detected and therefore it can be difficult to ascribe importance to any single species. However, by the use of multivariate data analytical statistics, it is possible to look at many variables at the same time and to make mathematical models that describe the relation between sensory and GC data (EFSA 2010).

Analytical methods characterizing rheological properties

Glass transition (T_g) and flow-starting temperatures (T_f) are very important parameters defining the state and flowability of fishmeal or a feed mass in the extrusion process and friability of the feed. The parameters can also be used to document the plasticizing effect of small molecular weight components like amino acids and other N-compounds as these compounds lowers both the T_g and T_f of fishmeal, feed mass or feed. The T_g of an amorphous solid is a temperature range where the solid transits from a brittle glassy to a soft rubbery state (Abiad et al. 2009). The rubbery polymer reaches a state where it can be considered as a highly viscous melt when heated above T_g. The flow-starting temperature (T_f) can be defined as the temperature where a melt starts to flow through a capillary die at constant pressure (Fujio et al. 1991). Closed-chamber capillary rheometry enables the measure of both T_g and T_f at elevated moisture levels and high pressure and temperatures (Fujio *et al.* 1991; Igura *et al.* 1997). The Phase Transition Analyzer (PTA; Strahm *et al.* 2000) is a closed-chamber capillary rheometer developed for this purpose. In the PTA compaction and flow relative to an initial sample height at constant pressure and at increasing temperature can be measured and T_g and T_f defined.

Viscosity characterization is important to define the effect of fishmeal, starch or feed mass viscosity on extruder heat dissipation and cooking efficiency. There are two measuring principles that can be used; 1) Viscosity measured with excess water. The most common instrument used in the feed industry is the Rapid Visco Analyser, (RVA; Newport Scientific, Warriewood, NSW, Australia; Whalen et al. 1997). In the instrument, a suspension is cooked under a defined temperature and shear protocol, and its viscosity continually recorded. RVA

parameters as cold viscosity (maximum viscosity at 25 °C); peak viscosity (maximum viscosity at 95 °C); hold viscosity (minimum viscosity at 95 °C) and final viscosity (maximum viscosity after cool-down to 25 °C) can be determined (Samuelsen 2018). 2) Viscosity measured in low water, high-temperature environment with the use of a capillary rheometer. A capillary rheometer consists of a piston and a capillary die where the barrel wall temperature is controlled by a heating cabinet. By controlling the flow rate (setting the piston speed) and measuring the pressure drop across the capillary it is possible to measure and characterize the viscous behavior of high viscous non-Newtonian fluids (Bengoechea et al. 2007).

5 Preservation methods throughout the value chain

Preservation through the value chain is essential to maintain the quality of products. Whether it is raw material on its way to processing or fishmeal and oil through transportation. Raw fish is very sensitive and starts to degrade fast if it is not chilled. Fishmeal and oil are also very reactive and will oxidize if antioxidants are not used.

5.1 Raw Material

Maintaining the raw material quality is fundamental to ensure good quality fishmeal and fish oil (IFFO, 2017). The shelf life of fish can be extended by several preservation methods.

Cooling

Fish is a highly perishable product and spoilage begins as soon as the fish dies. Fish should, therefore, be processed as soon as possible after death (FAO, 1992). Initially, quality loss is due to biochemical reactions caused by endogenous enzymes. This "autolysis" liberates amino acids, fatty acids and other low-MW compounds, which in turn promotes microbial growth. Microorganisms cause spoilage by decomposing such compounds while liberating volatile and/or poisonous compounds. Total volatile nitrogen (TVN), consisting mainly of trimethylamine (TMA) and ammonia (NH3), is used as a quality criterion for fishmeal raw material. The changes that occur during storage of fish depends on fish species, feeding status, storage conditions, etc.

The easiest way to preserve fish and reduce the risk of spoilage is to properly chill the fish down to temperatures around zero degrees or lower to slow down the enzymatic activity. This method is often called, super chilling, and is becoming a more prominent cooling method onboard fishing vessels fishing for human consumption. In principle, the term means to chill below zero degrees. When fish is super chilled, a proportion of the water inside the fish is frozen, more specifically the water in the fish viscera as the viscera usually have higher water content than the fish muscle, especially in fatty fish species such as mackerel and this water will freeze first. It is important to freeze water in the viscera because most of the enzymes bind the water phase in the viscera and by freezing the water the enzymatic activity can be reduced dramatically. On the other hand, going too far down can have negative effects on the fillet qualities, especially in the case when fishing for human consumption. Large ice crystals will then start to form in the fillets causing muscle gaping as well as drip and poorer texture. The chilling temperature should be decided with the raw material in mind and the moisture content if not to reduce the quality of the fillets.

The desired temperature is chosen with the help of the following Figure 20. It shows the proportion of frozen water in fish with specific water content at a specific temperature. For example, mackerel, where the fillet is 54% water, and viscera is 73% water. At -1.5°C, 30% of the water in the viscera is frozen while inside the muscle it is only 0-2% of the water that is frozen.
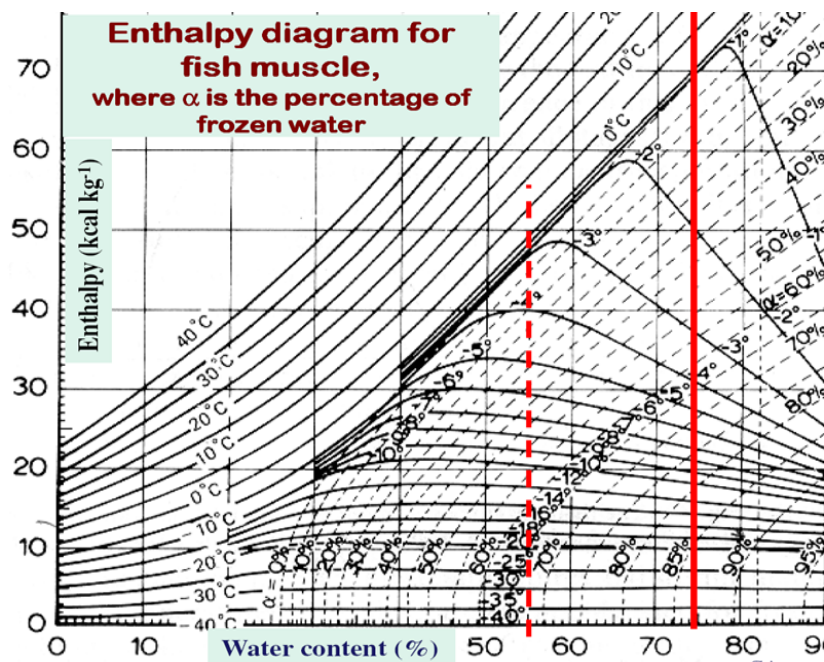
Figure 20 Enthalpy diagram for fish muscle

In principle, there are two types of chilling system that are being used on board fishing vessels. It's the chilled seawater systems (CSW) where seawater is mixed with slurry ice or ice cubes and the refrigerated seawater systems (RSW) where the seawater is circulated through a refrigeration system. These systems often use a mixture of seawater and freshwater as an effort to reduce salt uptake, especially when fishing for more sensitive lean fish species.

Unpublished results from experiments that were conducted in cooperation between Matís and Síldarvinnslan in Iceland indicated the importance of adequate chilling. They compared different storage temperatures and analyzed the TVN development over 9 days in storage (Figure 21). The results concluded that the shelf life of blue whiting could be extended to almost five days by chilling down to 0°C compared to 5°C, considering the maximum level of 60 TVN in the raw material. Furthermore, trials were also done on blue whiting chilled down to 0°C with different concentration of seawater vs freshwater and the salt uptake in the fish was measured. The results showed that the salt uptake almost doubled when the fish was stored in pure seawater compared to a mixture of seawater and freshwater (Figure 22). The blue whiting had a salt content of 1,5% after 6 days in seawater at 0°C which would translate to blue whiting fishmeal with approximately 8,5% salt, which is on the higher end and the meal would not fit into the high-quality specifications.
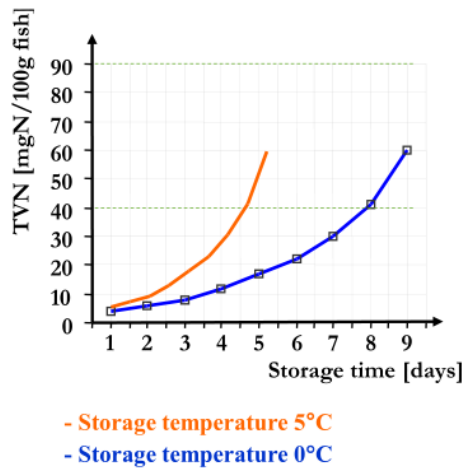
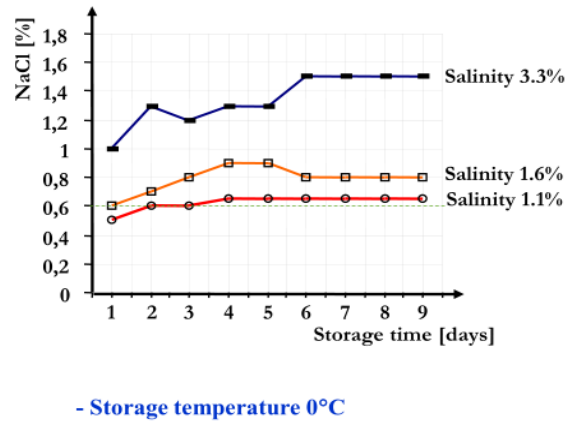Figure 21 TVN development in blue whiting stored at 5°C and 0°C

Figure 22 Development of salinity in blue whiting stored in water with 3,3%, 1,6%, and 1,1% salinity

Chemical preservation

Chemical preservation can be used in combination with cooling. Previously, agents like formaldehyde and nitrite were widely used (FAO/SIFAR, 2001). Today, only organic acids are used to any extent. They are weak acids that do not dissociate completely in water. The dissociated and undissociated state coexist in a pH-dependent equilibrium (Figure 23). The mode of action of organic acids is that the undissociated (neutral) state of the acid can penetrate the bacterial cell membrane and disrupt vital functions inside the cell (Brul and Coote, 1999).

In Norway, low concentrations of acetic acid (approximately 0.2 %) in combination with RFW provide valuable shelf life extension for species like blue whiting, which is captured far from land. Acetic acid cannot be combined with RSW because some marine bacteria are able to convert seawater sulfate to toxic H_2S gas in the presence of acetate (Nygaard, unpublished data).

For short-term preservation of fishmeal raw material, acetic acid is preferred to the stronger acids (e.g. formic acid) because it is more undissociated at any given pH (Figure 24). A low pH should also be avoided because digestive enzymes with low pH-optimum in the fish stomach/gut will degrade fish tissues and because the stronger acids are more corrosive to the equipment onboard.
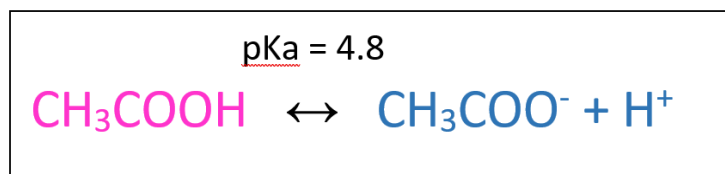
61

$$pKa = 4.8$$

$$CH_3COOH \leftrightarrow CH_3COO^- + H^+$$
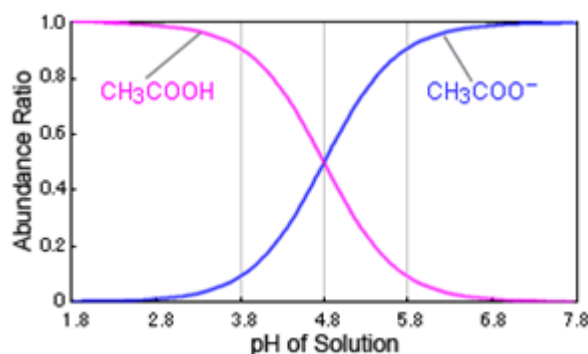
Figure 23 Dissociation of acetic acid

Figure 24 Fraction of undissociated and dissociated acetic acid vs pH of the solution. The pKa of acetic acid is 4.8, at this pH 50 % of the acid is undissociated with antibacterial activity. For comparison, the pKa of formic acid is 3.8 which implies that pH must be lowered by 1 unit to obtain the same fraction of undissociated acid.

Fishing practice

The fishing practices, the type of fishing gear, the towing time, the amount of fish in each haul, the amount of fish in the storage tanks and the type of landing and storing equipment can all greatly affect the quality of the raw material. Essentially, fish is very sensitive species and is easily damaged by stress and load. Most of the microorganisms and enzymes are found in the fish viscera and in the blood. When fish is under pressure and friction, bruises and wounds can easily form on the fish creating access for enzymes and microorganisms to areas where they are not found in the same numbers, such as in fillets. If the fishing practices do not take into consideration the pressure and stress the fish undergoes, these enzymes may have better access for breaking down the raw material which will eventually promote microbial growth.

5.2 Fishmeal and oil

Through transportation and storage in supply chains, fishmeal and oil can get exposed to many external obstacles. Humid air, sunlight and other factors are an example of these obstacles, which can dramatically affect the quality of the product. Fishmeal is very sensitive to oxidation of fat and humid air and fish oil is also very sensitive to oxidation from sunlight

and oxygen present in the water phase in the oil. Antioxidant chemicals as ethoxyquin have been used in the past to stabilize fishmeal and oil and to prevent oxidation.

Water activity and oxidation

When fishmeal is dried, the water evaporates, and the moisture content decreases parallel to the water activity. The water activity is defined as the partial vapor pressure of water in a substance divided by the standard state partial vapor pressure of water. Indicating when the relative humidity reaches equilibrium with pure water. The water activity is also dependent upon dissolved chemicals within and can be used to lower the water activity (salt, sugar, et al.). Figure 25 describes the reaction rates for foods in relation to water activity.
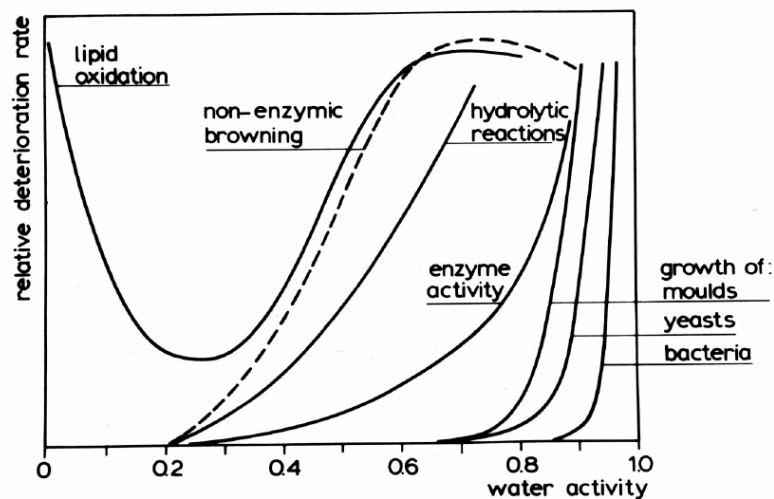

Figure 25 Reaction rates in food as a function of water activity (Rockland and Stewart, 1981)

The aim of fishmeal production is to lower the water activity to approximately 0.2 to reduce the risk of degradation, see Figure 26 (Ariyawansa, 2000). This creates worse conations for enzymes to break down the raw material and reduces the yeast- and bacterial activity as well as the risk of mould growth. However, when fishmeal is overdried it can, on the other hand, lead to lowering of the water content below 0.2 causing oxidation of lipids. This can moreover be a safety concern since too dry fishmeal is flammable due to oxidation (Thomas Miller, 2016). There are more than one instances where fishmeal has caught on fire during transportation on sea due to heating of the material. Antioxidants do, however, not affect the water activity but are used to stabilize the material by reducing the risk of oxidation. The amount used is dependent upon many factors, but it is most commonly standardized for each

particular species. According to FAO, adding 700 ppm of BHT-antioxidant or 200 ppm of ethoxyquin is enough to stabilize herring meal. The chemicals can be injected into the material after the drying procedure which requires blending, or it can be added with the stickwater which blends the press cake before entering the dryer. Both methods are practiced today (FAO, 1986).

The use of antioxidants has raised concerns among regulators as it may have an adverse effect on animals consuming it. The Food and Drug Administration of The United States (FDA) has, for example, decreased the maximum amount of ethoxyquin in pet food from 150 ppm (0.015%) to 75 ppm due to health concerns (FDA, 2016). More research is needed to prove these concerns as the FDA nevertheless stated themselves along with the European Food safety authorities (Aquilina, Bampidis, Bastos, Bories, Chesson, Cocconcelli & Wester, 2015).

Fish oil is very rich in omega-3 polyunsaturated fatty acids that are considered healthier for human consumption than the saturated ones (Ackman, 1988). However, it is much more difficult to store the unsaturated fatty acids as they are more sensitive to oxidation. The oxidation often occurs due to so-called autoxidation of fat when exposed to oxygen (Labuza, 1971). One alternative to reduce the risk of oxidation is to add antioxidants to preserve the oil (FAO, 1986). Nitrogen injection can also help with reducing oxidization. Matís ltd. conducted a study in cooperation with the fishmeal plant producer Héðinn ltd. in 2014, which concluded that injection of nitrogen in the heating process resulted in reduced oxidation. This limits oxygen uptake which leads to a more stable end product (Gíslason et al., 2014). Fish oils can also be protected against oxidation by addition of antioxidants. Traditionally, synthetic antioxidants such as BHT, propyl gallate and citric acid have been used. Also, for fish oil there is an increasing focus on replacing synthetic antioxidants with natural antioxidants such as tocopherol and rosemary extracts. In a recent study, Thomsen et al. (2017) demonstrated that high doses of tocopherol (500 ppm) and rosemary extract (5000 ppm) almost completely inhibited formation of volatile oxidation products in fish oil.

Packaging and storage

The main principle behind the packing of fishmeal is that the fishmeal has to be able to ventilate. Heat and moisture must have access out of the packaging to prevent condensation inside. Strict and stable temperature and humidity control are required during storage to prevent the material from absorbing moisture from the environment.

The most common practice is to package fishmeal into bags. They can be of various sizes and of different material. The most commonly used bags are the hessian bags, multilayer paper bags (without and with plastic lining), sheet bags or woven plastic bags (low-density polyethylene). These types have their own advantages and disadvantages, but the most important principle is that they can let moisture and heat out without being too porous so that oxygen has unlimited access (FAO, 1986).

When it comes to stacking the bags, the same principles should be kept in mind; ventilation of moisture and heat, and prevention of access to oxygen. Therefore, it's not required to stack bags up to more than 5 m in width surface area (FAO, 1986). Too tight stacking can lead to undue heating in the material which causes moisture migration, condensation, mould growth and lumping.

Bulk storage is another alternative. Fishmeal is often stored in silos or even sheds. Bulk storage reduces the work needed by employees and makes transportation and handling of the material easier. In general, bulk storage can be distinguished by two principles; the open type or the sealed type. In the open type, air has access through the bulk density by doors or other valves and openings. In the sealed type, space is sealed off from the surroundings, but this requires aeration of the material and especially on the first stages when the material is most reactive. Silos can be used for this kind of storage, it involves constant conveying of the material from the bottom which ensures cooling of the material and promotes uniformity of the material (protein, fat, and water), particle size and color (FAO, 1986).

The most preferable method of storing fish oils is in mild steel tanks. (FAO, 1986). This prevents oxidation due to sunlight and conduction of oxygen through the constructed material. The tanks should be constructed with a cone in the bottom to have the option to drain out existing water. This is especially important in the first days and it is recommended to drain off

existing water daily. It is also preferred not to store the oil in too cold condition as it can cause transformation of oils into solids. It is recommended to have some kind of heating of the tanks to prevent solids formation and to ease the transportation. Finally, it is also recommended to eliminate air in the tanks to avoid oxygen or to use nitrogen to remove the existing oxygen in the tank (Arason, 2001).

6 Key properties of various final products

This chapter describes and discusses fishmeal and fish oil use in the feed and aquaculture industry as well as the use for human consumption.

6.1 Feed production process

Extrusion processing is a technology that enables production of high-quality feed pellets and is, therefore, the preferred technology for large-scale manufacturing of fish feed. The extrusion process involves the use of water and high temperature achieved by steam injection and mechanical heat dissipation in an extruder barrel to transform the feed mix into a plasticized and flowable material that can be shaped through a die, cut into pellets, dried and added oil in a coating operation. The main unit operations are as follows:

Major ingredients are weighed and dosed to a grinder. In the grinder, usually a hammer mill, the particle size is reduced to <1 mm with use of a screen aperture at 1.5 to 1.0 mm. Grinding is advantageous. Uniform and small particles prevent segregation during mixing with micro-ingredients, and small particles are easier to hydrate and will heat up more quickly than coarser particles in the preconditioner.

Due to the low residence time in the extruder (<1 min) the feed mix is conditioned prior to extrusion in a preconditioner by use of steam and water, and with a typical residence time of 1.5 to 4 min. In the preconditioner, the feed particles are hydrated and heated to a typical moisture content of 18 to 30% and a temperature of 77 to 95 °C (Rokey 1994; Strahm 2000; Riaz & Rokey 2012). As the preconditioner also have high mixing capabilities fish protein concentrates (silage) and oil can be added in this stage.

Extrusion is the key process as the physical product quality as well as pellet expansion and oil adsorption capacity are defined in this unit. The most commonly used extruders for fish feed

processing are single screw or co-rotating, fully intermeshing twin-screw extruders. In the extruder screw, the feed mix is cooked by mechanical energy dissipated into heat (internal energy) and the addition of water and/or steam. During this treatment, the mix is transformed into a plasticized and flowable material (melt) that can be shaped through a die and cut into pellets. The temperature upstream the extruder die during fish feed production is typically in the range of 120 to 145 °C (Sørensen *et al.* 2009; Sørensen *et al.* 2010; Samuelsen & Oterhals 2016). The extrusion process is mainly operated by adjusting the water and steam level, feed rate and screw speed.

The extrudate has to be dried to prevent mould and bacteria growth and to fix the final porous structure and physical quality. The extrudate has water content of approximately 18 to 30% (Rokey 1994; Sørensen 2012) and is dried to around 8% (Sørensen 2012). The most commonly used dryers in fish feed processing are conveyor dryers (single pass, multi-pass, two stage or multi-stage) where the air flows transversely through the product bed in separate zones with the lowest air temperature in the outlet zone.

Prior to vacuum coating, the feed may be pre-cooled and sifted to prevent evaporation and dust accumulation in the coater. Most of the oil (lipids) is added in the vacuum coater. In this unit, the air is withdrawn from the dry pellets before adding oil into the coater. After a predetermined mixing time, the air is slowly released back in order to let the oil be drawn into the porous pellet structure (Strauch 2005). After vacuum coating, the finished feed is cooled and sifted to prevent evaporation and dust during packaging.

The final feeds are delivered to the farms in bulk or big bags and are conveyed pneumatically to the sea cages. The treatments expose pellets to stress that may give product loss due to cracks and dust formation. Consistent and high physical quality is therefore important to reduce feed loss and discharge of nutrients to the aqueous environment. The feed also has to be expanded to adsorb the desired amount of oil in the vacuum coating process but have an optimal microstructure to minimize oil leakage during transport and feeding. Target bulk density specifications also need to be met to prevent floating feed that may escape the sea cages.

Various types of equipment are available for analysis of physical feed quality parameters.

The durability of feed pellets

Simulates the stress to which pellets are exposed when conveyed pneumatically through tubes.

Holmen durability: A sieved pellet sample is conveyed around in a closed circuit by a high-velocity air stream (Samuelsen et al. 2013). Pneumatic durability is calculated as the percent of pellets remaining on predetermined screen size.

Doris durability: A sample is transported in a screw conveyor to a rotating fan. Impact with the fan and the walls downstream the fan generates cracks and dust, which are measured using different screen sizes (Aas et al., 2011). The following Doris parameters are determined; unbroken, fractures and dust.

Pellet hardness

Pellet hardness can be determined using a texture analyzer. The pellets are broken individually between a flat-ended cylindrical probe and the bottom plate. The major break of the pellet (the peak force) is measured (Samuelsen & Oterhals 2016)

Fat adsorption (lipid coating) and leakage

Fat adsorption capacity can be investigated using a lab-scale vacuum coater (Samuelsen & Oterhals 2016). Pre-heated oil is sprayed in excess amount into the coater and mixed with the dried uncoated pellet under vacuum. The air slowly released back. Surplus oil is removed by gently compressing the pellets between oil-absorptive linings before the recording of the sample weight. Fat leakage can be measured on the previous samples by incubating the feed in a heating cabinet. Blotting paper and the feed are put into a plastic box and the box weighed. After incubation pellets and dust are removed from the box, and the new weight of the box and blotting paper is registered (Samuelsen et al. 2018).

Bulk density

Bulk density can be measured by loose pouring the feed to a 1000 ml measuring cylinder and sample weight recorded.

Sinking velocity/floating

Sinking velocity can be measured in a transparent vertical tube filled with salt water at a predetermined temperature. A stopwatch is used to measure the pellet sinking time in a predetermined depth interval.

6.2 Impact of fishmeal technical quality on the feed production process and physical pellet quality

Fishmeal has unique technical properties which differ extensively from plant-based protein ingredients (Draganovic et al., 2011). As an example, the replacement of fishmeal with plant proteins in commercial fish feed formulations demands use of a higher moisture level (around 30%) and thermomechanical energy in the extrusion process to achieve target pellet durability and density specifications (Draganovic et al., 2011, 2014). However, fishmeal is also documented to be a complex and variable ingredient that impact the extrusion process and physical feed quality. Nofima has performed a series of studies to identify the underlying mechanisms of this variation and to discuss possible physical, chemical and rheological explanations (Samuelsen et al., 2013; 2014; Oterhals & Samuelsen 2015; Samuelsen 2015; Samuelsen & Oterhals 2016). The studies were based on high-quality fishmeal from the three fish species; herring, sand eel, and blue whiting. The investigations were designed to cover the variability in fishmeal physicochemical and technical properties possible to obtain in commercial fishmeal processing and both seasonal and process variation were included. Due to the complexity of the fishmeal ingredient, a combination of several factors explaining the variation in fishmeal technical properties was identified. However, some clear properties were documented which had a high impact on the extrusion process and physical feed quality:

In a study performed on herring (Samuelsen et al., 2013), a different combination of dryers gave a wide range in fishmeal particle structure (flow-number, range 2.2-9.0 cm; confer chapter 4). In addition, the fishmeal batches were milled with screen aperture ranging from 2 to 8 mm (hammer mill; Jesma-Matodor AS, Vejle, Denmark) giving a cumulative mean particle size in the range of 75-266 μm. In pilot extrusion trails performed on a TX-52 co-

rotating, fully intermeshing, twin-screw extruder (Wenger Manufacturing Inc., Sabetha, KS) and with the replacement of fishmeal in salmon feed diets a reduced cumulative mean particle size combined with increased flow-figure affected extruder screw specific mechanical energy and viscous heat dissipation positively. The measured flow-figure contains information about the structure of the fishmeal particles and a high value reflects the content of fibrous and/or fine particles in the sample. Increasing the level of small and fibrous particles in the feed mixture can be expected to increase the particle to particle contact area within the extruder screws. This will give higher viscous heat dissipation and improved extruder cooking efficiency and consequently higher physical pellet quality. In the same study, an increase in salt level combined with reduced pH value improved extruded pellet hardness. Proteins are charged molecules. In the low water content environment within the extruder, electrostatic interactions will be important and also influenced by soluble salts and pH.

The most prominent effect and the main result from the studies was the positive effect of increased level of water-soluble protein on extruder specific mechanical energy and pellet hardness and durability. In the studies, the effect of a large range of water-soluble protein levels was investigated (9-42% of total protein). The normal level of water-soluble protein (on a total protein basis) in high-quality fishmeal is in the range 18-32% (Schmidtsdorff, 1995), but both higher and lower levels might be found in commercial parcels. Fish caught during periods with extensive feeding on zooplankton contain high proteolytic activity. This gives increased enzymatic degradation during transportation, storage, and processing (Aksnes, 1988; Oterhals, et al., 2001) and a high level of water-soluble protein in the fishmeal comparable to the highest level. At some older production plants and in case of by-product processing onboard fishing vessels, it is still the industrial practice to discard the stickwater; the produced fishmeal being comparable in composition to the low level in these studies.

The observed main effects of the water-soluble protein on extruder specific mechanical energy and pellet hardness and durability can be explained by two different mechanisms:

1) Cross-linking of large water-soluble polypeptides. A larger portion of high molecular weight water-soluble proteins (>20 kDa) was found for herring and blue whiting meal compared to sand eel (Samuelsen et al., 2013; 2014; Oterhals & Samuelsen 2015). These fractions correspond to fish gelatine (Mohtar et al., 2010). Powdered gelatine readily swells

and dissolves in the presence of water and heat, forming a viscous melt. The gelatine molecules reform into triple helices upon cooling. Groups of triple helices might align themselves in parallel arrays, forming micro-crystallites that act as cross-links and enhance texture formation and physical feed quality (Arêas, 1992).

2) The plasticizing effect of the low molecular weight peptides and amino acids (<6 kDa) Higher values of these fractions are found for sand eel compared to herring and blue whiting meal. A plasticizer is generally added in the form of water in the process to improve ingredient processability, cooking efficiency within the extruder screws and final product properties. A plasticizer diffuses into the biopolymers and increases molecular mobility and lowers both the glass transition (T_g) and flow-starting (T_f) temperatures, which can be measured by use of the Phase Transition Analyzer (PTA; Fujio et al. 1991; Igura et al. 1997; di Gioia & Guilbert 1999; Strahm et al. 2000; confer 4). The studies documented that the water-soluble protein fraction in fishmeal was conserved through the extrusion process without loss and had a plasticizing effect similar to moisture in fish feed formulations (**Fig. 25**). In general, the documented plasticizing effect could be attributed to low molecular water-soluble N-compounds including protein and non-protein amino acids, peptides and N-containing putrefaction products (Oterhals & Samuelsen 2015; Samuelsen & Oterhals 2016). These compounds may also contribute to intermolecular binding network in the dried pellet through hydrogen and ionic bond interactions. The plasticizing and binding effects can be extended to FPC or protein hydrolysates with a high degree of hydrolyzation. These may be cost-effective processing aids serving multiple purposes as nutrients, plasticizers, and binders in extruded fish feed.
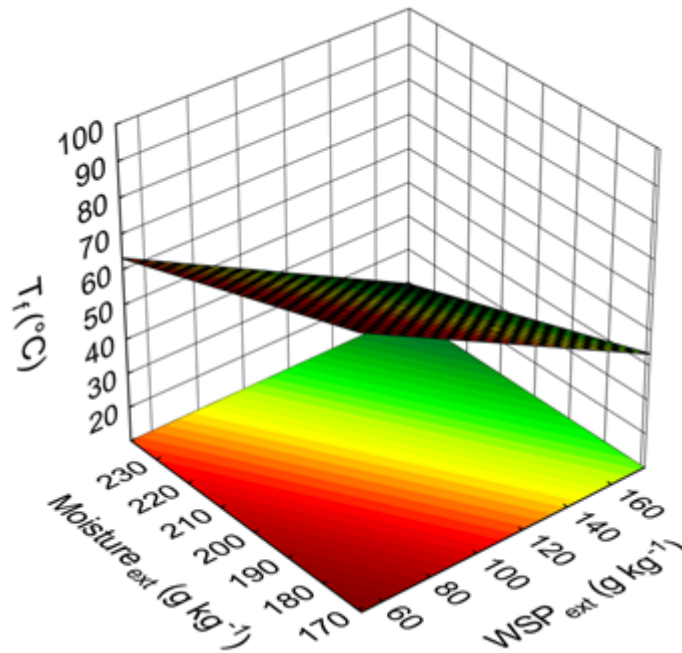
Figure 26 Flow-starting temperature (Tf) response surface plots based on moisture and water-soluble protein (WSP) in the extrudates (Samuelsen & Oterhals 2016)

Compared to herring meal, sand eel requires more thermomechanical treatment in the extruder screw to produce durable feed pellets. This may be to different chain length or fragmentation pattern of the myofibrillar protein (Suzuki 1981) influencing the thermal properties of the non-soluble protein fraction of the fishmeal.

6.3 Fishmeal and Fish oil in Aquaculture Feed

Aquaculture production is significantly increasing and provides an increasing share of the supply of fish for human consumption, while the wild catch fishery has been stabile around 90 million tonnes since 1985. About 70 million tons of wild-caught fish were utilized for direct human consumption, while about 20 million tons of the remaining capture fish were used as raw material for the production of fishmeal and fish oil (FAO, 2018).

However, around 80 million tons of fish were produced in aquaculture in 2016 (FAO, 2018), which means that aquaculture provides more than 50 % of the global fish production for human consumption, cf. Figure 27.

World capture fisheries and aquaculture production

● Capture production ● Aquaculture production

NOTE: Excludes aquatic mammals, crocodiles,
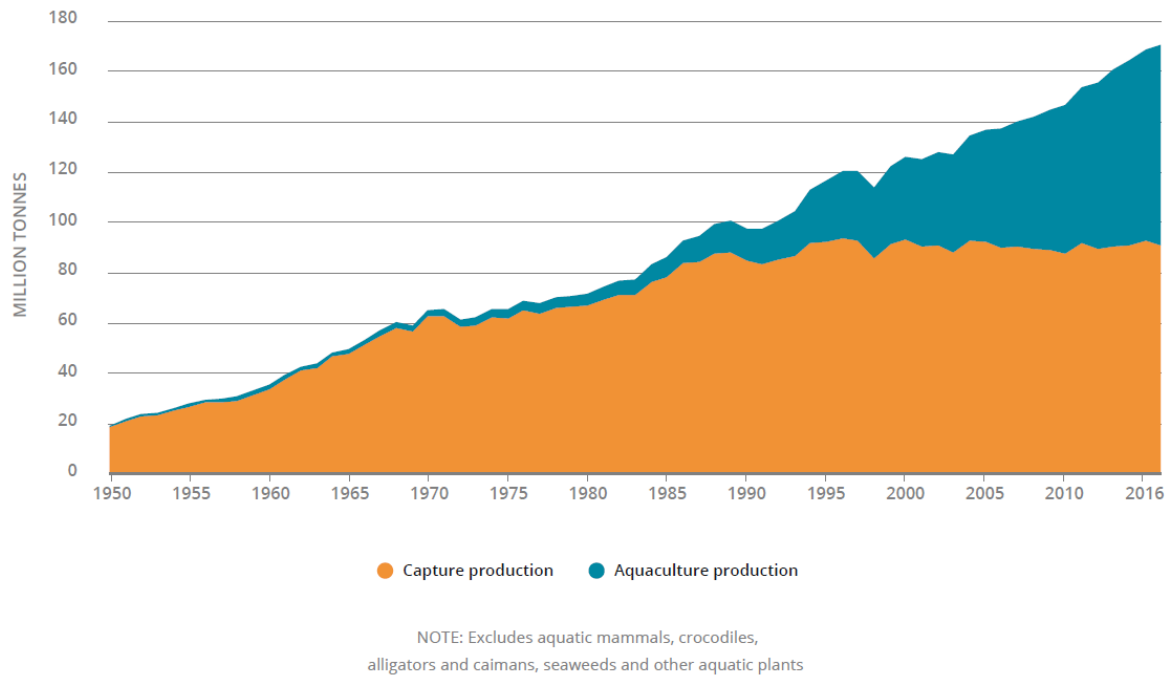alligators and caimans, seaweeds and other aquatic plants

Figure 27 World wild catch fisheries and aquaculture production (FAO, 2018).

In addition to the mentioned 20 million tons of wild-caught fish aimed for production of fishmeal and fish oil, fish by-products/trimmings from the processing industry is contributing with increasing shares (currently around 30 %) of the raw material for manufacturing of fishmeal and fish oil (Jackson and Newton, 2016). However, the composition of fishmeal made from trimmings is by various extent different from fishmeal manufactured from whole fish, i.e. the ash content in the by-product fishmeal is higher due to bone fractions in trimmings, a resultant lower protein content and unbalanced amino acid profile compared to the unique amino acid profile of fishmeal based on whole fish. Further, fishmeal produced from trimmings is not allowed in feed for farmed fish of the same species (EU regulation, EC 1069/2009).

Fishmeal and fish oil is no longer the overall main ingredients in aquaculture feed due to increased demand from aquaculture, public concerns about decreasing wild stocks and responsible and sustainable sourcing of raw materials, increases in prices and increasing availability of alternative competing feed ingredients, Figure 28. Hence, most aquaculture feeds will continue to have reducing inclusion levels of marine ingredients in the diets.

Fishmeal and fish oil will increasingly become strategic ingredients used at critical stages of the life cycle and specific productions to secure optimum performance, e.g. organic fish feed, where an addition of artificial amino acids is not permitted. The human health importance of EPA and DHA also calls for strong demand for fish oil, either for direct human consumption or via farmed fish.
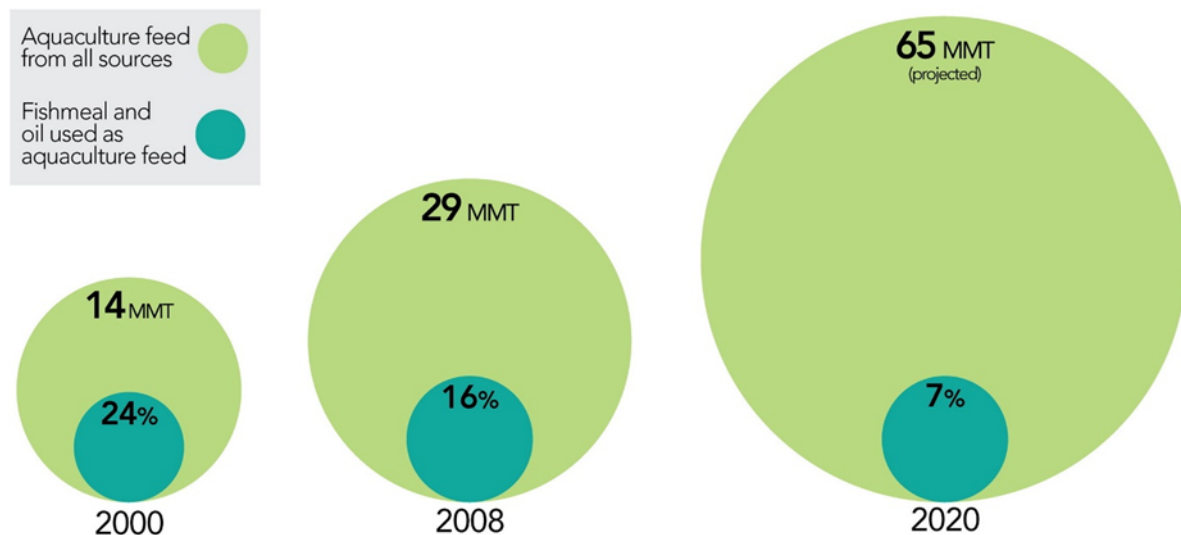

Figure 28 Growth of commercial aquaculture feed use. As the production of commercial aquaculture feed increases, shown here as actual and projected production from 2000 to 2020, overall use of FM and FO remains the same but the proportion of feed made from fish has and will continue to decline (Tacon and Metian, 2015, Tacon et al., 2011)..Source: Fry, J.P. et al., 2016. Environmental health impacts of feeding crops to farmed fish. Environment International, 91, pp.201–214. Available at: http://dx.doi.org/10.1016/j.envint.2016.02.022

Feed costs account for more than half of the production costs in aquaculture. In conventional aquaculture feed ingredients include a broad range of marine (fishmeal and fish oil), vegetable protein- and oil sources and other alternatives and possibly specific artificial amino acids to fulfill the nutritional requirements of the specific species. However, options of sourcing ingredients for organic aquaculture is very limited. Hence, feed for carnivorous organic aquaculture animals shall be sourced with the following priorities according to the EU Reg. 889/2008/EU / Reg. 710/2009 art. 25k: (1) Organic feed products of aquaculture origin; (2) Fishmeal and fish oil from organic aquaculture trimmings; (3) Fishmeal and fish oil and ingredients of fish origin derived from trimmings of fish already caught for human consumption in sustainable fisheries; (4) Organic feed materials of plant or animal origin and especially plant material shall not exceed 60% of total ingredients; and (5), amended in 2014, by EU Reg. 1358/2014, - Fishmeal and fish oil and ingredients of fish origin derived from whole fish, crustaceans or molluscs caught in fisheries certified as sustainable under a scheme

recognized by the competent authority in line with the Council Regulation (EC) 889/2008 and not used for human consumption. Furthermore, the organic regulation does not allow balancing the dietary amino acid profile by supplementing with synthetic free amino acids to fulfill the dietary requirements of the specific species. This is in contradiction to feed for conventional aquaculture, which can be balanced with synthetic free amino acids.

6.4 Fishmeal for aquaculture feed

It is a fact that fishmeal of high quality provides a balanced amount of all essential amino acids, minerals, phospholipids and fatty acids reflected in the normal diet of fish (Hardy, 2010; Lund et al., 2012), and hence secure high utilization by the fish and minimum discharge of nutrients to the environment. The performance of a certain protein, e.g. fishmeal, can be expressed by its biological value, which is a measure of the proportion of the protein/amino acids, which are incorporated into fish muscles. Hence, the biological value of a feed protein depends on its amino acid configuration, which means, that optimum fish performance (feed intake, weight gain) is achieved when the protein fed contains an ideal amount and proportion of all essential amino acids. This feed protein is called an "ideal protein". Fishmeal protein is closer to an ideal protein than most available alternative vegetable protein sources. But adding a small amount of a certain deficient essential amino acid to the vegetable protein diet improves the biological value of its protein measured by fish performance. Raising the concentration of this amino acid in the diet can further improve performance but only up to a certain level. Then adding more of this amino acid cannot induce further improvement. The "Liebig barrel" (Figure 29) illustrates this limitation of protein synthesis due to the lack of an essential amino acid. The shortest stave of the barrel represents the first limiting amino acid (here Met).

Figure 29 The "Liebig barrel" illustrates the limitation of protein synthesis due to the lack of an essential amino acid. The shortest stave of the barrel represents the first limiting amino acid (here Met)
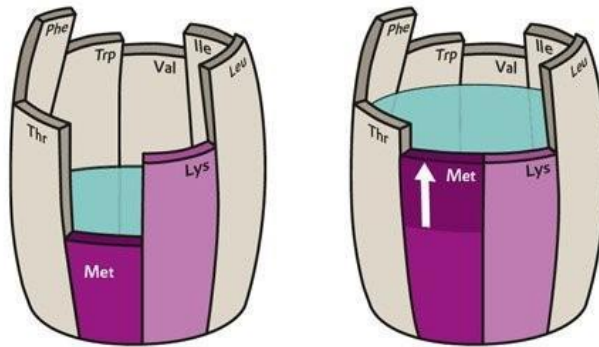
It is necessary to fill this gap in order to compose an ideal feed protein. Of course, if a first limiting amino acid exists optimum supply of a second (Lys) and third limiting amino acid (Thr) will also contribute to the formation of an ideal protein and "help completely fill the barrel". This is the so-called concept of the first limiting amino acid, i.e. if the rate of protein synthesis is lowered due to an inadequate supply of a limiting amino acid then increasing the limiting amino acid should increase protein synthesis, and thereby improve fish performance.

Lysine and methionine are often the most limiting amino acids when fishmeal is replaced by plant protein sources (Mai et al., 2006). Farmed fish need a balanced dietary amino acid profile and especially the essential amino acids have to be provided in the diet in specific proportions. If this is not the case the surplus amino acids will be burned off and the result is compromised growth, fish welfare and environmental impact (nitrogen discharge) - instead of being converted to fish meat. Therefore, a carefully balanced amino acid profile is important for the performance of the fish, as well as the minimization of nitrogen discharge.

Fish do not have absolute protein requirements but require the amino acids that compose the proteins. Atlantic salmon has documented requirements for the amino acids Arg, His, Ile, Leu, Lys, Met, Cys, Phe, Tyr, Thr, Try and Val, while Tau is not regarded as required (NRC, 2011). Recent publications indicate higher requirements of lysine and threonine at the smolt stage than the requirements reported as mean values by NRC. This may also be the case for other amino acids.

The essential amino acid (EAA) requirements for optimal growth of Mediterranean fish species, such as sea bass, are Lys, Arg, Met, Cys, Try and Thr. This EAA profile correlates tightly to the whole-body EAA composition of gilthead seabream.

Dietary amino acid disproportions may be regarded as the primary cause of changes in feed consumption and there is some evidence that voluntary feed intake in sea bass may be partially conditioned by limiting or excessive levels of certain dietary EAA. Diets with lower proportions of tryptophan resulted in the loss of appetite to juvenile seabass, while diets lacking in methionine induced a reduction in feed intake. Diets lacking in tryptophan could also be responsible for spinal deformities in sea bass fingerlings, as well as crystalline lens opacity and increased levels of Ca^{++} and Mg^{++} in the liver.

Histidine (His) is an essential amino acid, which cannot be synthesized by fish or any other aquatic animals and therefore must be supplied in the diet.

His is a very important precursor for the synthesis of proteins, vitamins (e.g. Vit C) and enzymes and it plays a vital role in the structure and binding functions of hemoglobin. Further, His has been shown to prevent cataracts in salmonids, i.e. permanent lens opacity of both eyes.

Synthetic amino acids available as supplements for conventional aquaculture diets are produced via chemical or fermentative production processes. When applied correctly to feed these amino acids have a digestibility of 100 percent. However, supplementation with synthetic amino acids is not permitted in feed for organic aquaculture (EC 834/2007 Article. 15 1d. (IV)), which seriously is challenging production of well-balanced feeds for organic aquaculture due to the limited options of only combining limited available aquatic and plant protein sources to balance the dietary AA profile.

Fish oil for aquaculture feed

Fish oil is a major natural source of the long chain omega-3 HUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can be synthesized by salmonids and by other carnivorous marine species only at a limited rate, and thus are required in the diet. Omega-3 HUFAs are produced by marine phyto- and zooplankton, which is consumed by the wild

marine fish larvae (Baron et al., 2013). Hence, in addition to fishmeal, fish oil is a "strategic ingredient" to be used at critical stages of the life-cycle, when optimum performance is required, e.g. farmed larvae and broodstock need the "strategic ingredients" to secure optimum development, growth, and reproduction.

Atlantic salmon and rainbow trout can convert ALA to EPA and DHA, but the conversion is not very efficient (Ruyter et al., 1999, 2000a, b, c, Tocher et al. 2000; Bell et al. 2001; Bell and Dick, 2004). Therefore, their essential fatty acid (FA) requirements must be provided by dietary EPA and DHA to obtain good growth and health (Ruyter et al. 2000a, b, c). Studies on sea bream showed that a replacement up to 66% of fish oil can be operated by a vegetable source with comparable results. However, nutrient absorption in the fish intestine was impaired by total substitution of fish oil by vegetable oil.

Diets based on vegetable oils have shown generally good growth results in salmon, but with major challenges in the body lipid composition (Thomassen & Røsjø, 1989; Sargent et al., 2002; Grisdale-Helland et al., 2002; Torstensen et al., 2005). However, Omega-3 fatty acids have important biological functions in the fish (Montero et al., 2010; Torstensen et al., 2013), and a change in dietary fatty acid composition is expected to affect fish performance and health. The omega-3 fatty acids serve as the building blocks of cell membranes, regulate gene expression, and are precursors of a range of bioactive substances that regulate inflammation, physiology, and satiation. By optimizing dietary fatty acid composition, the retention of EPA+DHA can be optimized and thereby improving fish health as well as securing the farmed salmon as a good source of EPA+DHA for human consumption. The balance between n-6 and n-3 FAs in the diet seems to be important, as it may have an impact on several aspects of fish health (Lands, 1992). However, the optimal n6/n3 ratio for conversion of 18:3 n-3 to EPA and DHA in salmonids is not known. The feed oils for the future may consist of a mix of different oils that provide an optimal dietary ratio between groups of FAs (C18 n-6 / C18 n-3 / C20 n-3 / C22 n-3) and utilize the innate ability of the fish to produce EPA and DHA. The optimal oil mix (FA combination) may vary for different life stages and in different environments.

Fishmeal replacement in aquaculture feed

Replacing fishmeal in diets for aquaculture, and in particular for organic farming, is not straightforward due to the unique content of protein, excellent amino acid profile, high nutrient digestibility, high palatability, adequate amounts of micronutrients, as well as general lack of anti-nutrients in fishmeal (Gatlin et al., 2007; Kaushik and Seiliez, 2010; Krogdahl et al., 2010; Lund et al., 2012).

Many studies have investigated the effects of replacing fishmeal with various plant protein ingredients (Borquez et al., 2011; Glencross et al., 2011; Pratoomyot et al., 2010; Torstensen et al., 2008; Yang et al., 2011). Complete replacement by plant proteins has usually not been successful due to problems related to the anti-nutrient factors, altered patterns of amino acid uptake when replacing fishmeal with plant-based protein ingredients, and impairment of immunocompetence (Bendiksen et al., 2011; Borquez et al., 2011; Geay et al., 2011; Larsen et. al., 2012; Lund et al., 2011).

Replacement with alternative sources requires to ensure that the dietary amino acid profile is optimized according to requests by the species, for example by adding free amino acids (conventional feeds), and/or by combining several plant protein sources with different amino acid composition (e.g. organic feeds). E.g. recent studies at BioMar have shown, that it is possible to include only 5 % fishmeal in addition to various vegetable protein concentrates supplemented with free amino acids for feed for conventional salmon and trout without negative effects on performance.

The replacement of fishmeal by vegetable proteins is further complicated in finfish species because not only the overall dietary amino acid profile is important for efficient utilization of amino acids, but also the timing by which amino acids from different protein sources appear in the bloodstream after a meal.

Larsen et al. (2012) investigated differences in amino acid up-take, i.e. plasma free amino acid concentration patterns in juvenile rainbow trout (Oncorhynchus mykiss) fed either a fishmeal-based diet (FM) or a diet (VEG), where 59 % of fishmeal protein (corresponding to 46% of total dietary protein) was replaced by a mixture of plant proteins from wheat, peas, field beans, sunflower, and soybean. Results showed that the appearance of most amino acids

(essential and non-essential) in the plasma was delayed in fish fed the VEG diet compared to those fed the FM diet. Essential and non-essential amino acids furthermore appeared more or less synchronously in the plasma in fish fed the FM diet, while the appearance was less synchronized in fish fed the VEG diet. The apparent protein digestibility coefficient was higher in the VEG diet than in the FM diet, supporting that protease inhibitors from plant protein ingredients were not the cause of the delay. The apparent digestibility coefficient of carbohydrates (calculated as nitrogen-free extract (NFE)) was much lower in the VEG than in the FM diet (51 versus 76%). Combined with a higher NFE content in the VEG diet, there was 2.7 times more indigestible NFE in the VEG than in the FM diet, which may suggest that the uptake of amino acids (AA) was affected by dietary carbohydrates.

Total ammonia-nitrogen (TAN) excretion was slightly, but non-significantly, higher in VEG fed fish than in FM fed fish. In conclusion, the study showed that amino acid uptake patterns were affected when replacing fishmeal with plant-based protein ingredients.

Replacement of fishmeal with alternative plant protein sources requires that anti-nutrients (such as trypsin inhibitors, tannins, lectines or glucosinolates) (Chebbaki et al., 2010) are efficiently removed from these sources to meet the high protein requirement of fish. Extrusion processing may also reduce the level of anti-nutritional factors in addition to increasing the nutritional value of protein-containing ingredients. Furthermore, procedures for the removal of anti-nutrients have to follow specific rules for ingredients used in organic aquaculture feed. Further, there is less availability of relevant organic plant sources to balance and optimize the amino acid profile of organic feed compared to conventional plant sources (Lund et al., 2011; Rembiałkowska, 2007).

A specific issue in replacing fishmeal by plant protein ingredients is that they contain significantly less phosphorus than fishmeal and the phosphorus is largely present as phytate-phosphorus. Phosphorus is an essential mineral required to support normal growth and development in fish including bone mineralization. Phosphorus is primarily obtained from the diet; however, not all phosphorus in a diet can be fully utilized by the fish. While the digestibility of P in fishmeal ranges between 40 and 60 percent, phytate-phosphorus in plant ingredients is not bio-available to the fish as they lack the enzyme (phytase) necessary for releasing the phosphorus. Bio-available phosphorus refers to that fraction of phosphorus in

the diet that the fish can naturally digest (physical break down of the feed) and absorb across the intestine. Non-bioavailable and other phosphorus compounds that are not digested and absorbed are egested in the feces and are principally lost to the fish.

Supplementation of microbial phytase, an enzyme that breaks down phytate and releases phytate-bound phosphorus, has been effective in improving P bioavailability of plant feed ingredients provided the activity of this enzyme is maintained and water temperature is optimum for feed utilization (FAO, 2018). E.g. addition of phytase to the feed was found to increase phosphorus, as well as nitrogen bioavailability and utilization in plant-based diets used in sea bream aquaculture (Morales et al., 2013). However, phytase is not allowed in organic aquaculture, which is challenging to use plant ingredients in organic feed.

The following study illustrates very well the difference between total phosphorus and bio-available phosphorus (Dalsgaard et al., 2009). The study examined the effects of adding phytase to diets with a high share of plant-based proteins (Figure 30). Diet A, B, and C in the study contained a similar amount of phytate phosphorous (i.e., non-available phosphorous; 0.29 % wet weight) but increasing concentrations of total phosphorus (0.89, 0.97 and 1.12 % wet weight, respectively). Diets with suffix "1" were supplemented with phytase, while diets with suffix "0" were not supplemented with the enzyme.
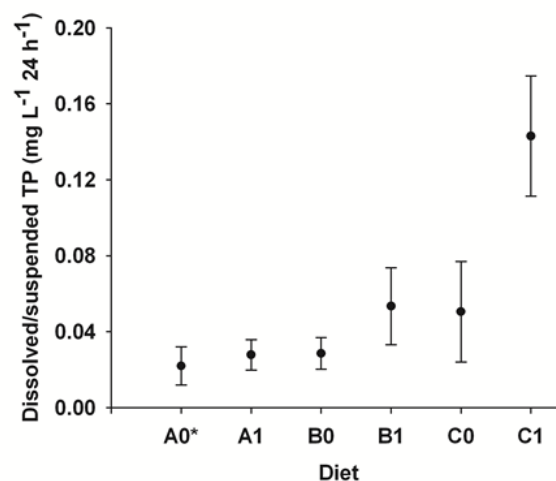
Figure 30 Excreted total phosphorus (mg L-1) measured in tanks with rainbow trout (mean ± SD, n = 4* or 6) fed three dietary total phosphorus levels (A = 0.89 %, B = 0.97 %, C = 1.12 %), and without (= 0) or with (= 1) phytase supplementation. (Reproduced from Dalsgaard et al., 2009).

Fish excrete any bio-available phosphorus in excess of their requirements to the water, and this was what happened for fish fed diet B when supplemented with phytase (i.e., diet B1 vs.

B0). Hence, the enzyme-aided to release phosphorus from dietary phytate, increasing the overall bio-availability of phosphorus in diet B1 to a level that exceeded requirements. For fish fed diet C the bioavailability of phosphorus already exceeded requirements prior to adding the enzyme (i.e., increased P excretion in fish fed diet C0). Adding phytase (C1) was therefore of no advantage of the fish, which simply excreted the additional phosphorus that became available.

According to EC 889/2008 fishmeal and fish oil from trimmings are prioritized as an ingredient for feed for aquaculture animals, whether it is from organic aquaculture trimmings or ingredients of fish origin derived from trimmings of fish already caught for human consumption in sustainable fisheries. However, using fishmeal from trimmings in fish feed imply as well as potential nutritional as environmental concerns. Fishmeal derived from trimmings might conflict with national environmental legislation due to too high P-concentrations. Fishmeal from trimmings is lower in protein and higher in phosphorus content compared with high-quality fishmeal (FF, Skagen, 2018). The presence of carcass remnants (head, skin, bones) in trimmings also increases the phosphorus content of the fishmeal. Using this meal for feeding fish puts limitations on the inclusion level to comply with environmental legislation. F. ex. Danish environmental legislation only allows the phosphorus content of the fish feed to be max. 0.9% (max. 1% on dry weight basis) (Miljø- and Fødevareministeriet, 2016).

The phosphorus content of traditional fishmeal has been measured to 2.1 - 2.2%, while the phosphorus content of trimmings was 2.5% (FF, Skagen, 2018). Based on these findings, using 41% of traditional fishmeal in the diet will theoretically result in 0.9% of phosphorus in the diet, not taking into account other potential phosphorus sources. Under the same conditions, using the same amount of trimming-meal would result in phosphorus content of 1 % in the feed for organic aquaculture. Thus, to comply with the environmental legislation, the diet could contain 36 % trimming meal, while, conventional fish feeds contain less fishmeal, and for conventional feeds, in contradiction to organic feeds, a long list of alternatives exists, with the diets balanced by supplementing free amino acids.

Carnivorous fish requires relative high dietary protein content, i.e. 38-48% of the diet, depending on fish size, with the highest requirement and quality for fry and brood-stock. This

means that, to produce an adequate feed, the inclusion rate of fishmeal from trimmings should be high, which conflicts with the limitations of max. 0.9% dietary phosphorus content. Furthermore, the available organic plant sources are limited and their amino acid profiles are not adequately balanced to make an optimum fish feed. As discussed previously, the breakdown of surplus amino acids is likely to result in increased environmental impact and reduced growth, health, and welfare of the fish.

The manufacturing process to obtain fishmeal and oil from trimmings is similar to that of wild-caught industrial fish (Sand eel, blue whiting etc.). However, due to the carcass remnants and the little remaining meat, the protein content of the meal from trimmings is 65–68 % (FF, Skagen, 2018). Further, the digestibility is supposed to be less than 90%, whilst it should be at least 90% in high quality fishmeal (FF, Skagen, 2018).

Fish oil replacement in aquaculture feed

Linseed oil, as well as less commonly used oils like camelina oil and chia oil are candidates that are rich in C18 n-3 (Ruyter et al., 2000a, 2000b). Camelina oil has been tested in diets for Atlantic salmon (Hixon et al., 2014), and 100 % exchange of fish oil with camelina oil caused a small but not significant drop in growth rate. Salmon lipid composition reflected the dietary fatty acid profile, with a higher content of 18:3 n-3 in fish fed the camelina oil diet. Echium (Echium plantagineum) oil is another promising oil, with high content of C18:4 n-3, one step further to a long chain n-3 FA compared to 18:3 n-3. Echium oil has been tested in diets for Atlantic salmon (Codabaccus et al., 2010), and higher biosynthesis of eicosatetraenoic (20:4 n-3) and 20:5 n-3 was indicated in the Echium oil group compared to fish oil and canola oil groups.

Several studies have shown that there are species differences in the capacities for conversion of essential FAs. It is well known that rainbow trout can synthesize DHA from ALA and this process is also taking place when fish are fed diets high in 22:6n-3. The studies indicate that rainbow trout has better ability than Atlantic salmon to convert the shorter vegetable n-3 FAs to the important long-chain EPA and DHA.

For the replacement of fish oil, marine fish species, such as sea bass and sea bream, have a lower tolerance to vegetable oil compared to freshwater or anadromous fish species such as

salmonids. This lower adaptation of marine fish species to vegetable oil can be linked to their lower efficiency in synthesizing LC-PUFA from n-3 and n-6 precursors present in plants. A high or total substitution of fish oil by plant oils induced decreases in growth rate of gilthead sea bream and European sea bass.

Indeed, LC-PUFA, used as structural components of cell membranes, are also the principal precursors of eicosanoids, that are involved in many physiological processes such as osmoregulation, immune responses, blood coagulation, and reproduction.

The lower nutritional value in the flesh of marine fish fed vegetable diet is generally due to the low content in EPA end DHA.

Isochrysis sp., partially substituted in sea bream diets, showed to be a good source of polyunsaturated fatty acids and in particular of docosahaesenoic acid (DHA).

Progressive substitutions of fish oil with Cottonseed Oil (CSO) did not affect fish growth, feed conversion ratio, and protein utilization but hepatosomatic and visceral fat indexes increased with increasing dietary CSO (a rich source of n-6 PUFA).

Microalgae and especially marine species are promising alternative fatty acid sources for interest in aquaculture feed. It was shown that microalgae oils from Isochrysis, Nannochloropsis, Phaeodactylum, Pavlova, and Thalassiosira contain sufficient omega-3 LC-PUFA to serve as an alternative for fish oil.

Lipids are also essential components of the diets for shrimps and are mainly used for direct energy production and cell membrane building. Typically, crustaceans have limited ability to ex-novo synthesize HUFA, as observed in marine fish (Mourente, 2005), at least at the beginning of maturation. Similarly, there are difficulties for the ex-novo synthesis of cholesterol, useful to synthetize steroid hormones (Kontara et al., 1997). Some lipids are more important than others because they cannot be synthesized de novo or not in sufficient amounts by shrimps. Phospholipids (e.g. lecithin) and cholesterol are the two main categories of essential lipids for shrimps. They are also used as emulsifiers for lipid digestion. Without phospholipids in their diet, shrimps are unable to digest lipids properly.

The requirements for lecithin and cholesterol are often linked together. Cholesterol is part of cell membranes and is a precursor of ecdysone, a steroid prohormone needed in the moulting process and as well involved in the biosynthesis of steroid hormones, bile acids, and vitamin D.

Natural cholesterol is produced from only one source so far, i.e. it is extracted from sheep wool grease, but the yields are very limited. Other potential sources of cholesterol are egg yolk, animal by-products, shellfish.

Cholesterol is extracted from lanolin, which comes from sheep wool grease, in a multistep extraction procedure. The lanolin can be refined to produce pure cholesterol (91%), which is added to the feed for shrimp.

Other alternative feed ingredients to fishmeal and fish oil

In addition to the plant proteins, there are several other potential feed ingredients, such as microbial organisms (bacteria, fungi, microalgae), terrestrial animal by-products (PAP, blood meal) wild-harvested and/or cultured annelid worms, insect larvae/pupae, gastropods (e.g. golden apple snail) which also may be candidates to replace fishmeal in aquaculture feed (Bergleiter et al. 2009; Sørensen et al., 2011; Van Huis and Oonincx, 2017).

Microbial ingredients, i.e. products from bacteria, yeast and microalgae are expected to have important potential in future salmonid and other carnivorous fish species (such as sea bass and sea bream) feed. A special aspect of some of these products is that they can be produced with different kinds of waste as raw material, and thus contribute to circular economy by recycling of valuable nutrients. A large number of products, produced from various single-cell organisms grown on different materials, have been investigated (e.g. Anupama and Ravindra, 2000; Mathews et al., 2011; Rajoka et al., 2006). Depending on the type of organism, the proximate composition, and amino acid profile can be much similar to that of fishmeal (Øverland et al., 2010). A number of products have been tested as protein sources in fish feeds, and the suitability varies among the different products, inclusion levels and fish species tested (Oliva-Teles and Gonçalves, 2001; Li and Gatlin, 2003; Berge et al., 2005; Aas et al., 2006; Palmegiano et al., 2009; Romarheim et al, 2011; Øverland et al., 2013).

Microalgae as a raw matter or as a feed ingredient for fish have also gained interest, as they are the natural start of the food chain in the oceans. Microalgae are fed on by zooplankton, which again is fed on by fish. The idea is to harvest from the first trophic level or cultivate in closed systems and provide feed ingredients for farmed fish by culturing microalgae. Living microalgae are used in aquaculture for fish feeding during the early stages and the benefit of the addition of marine Isochrysis sp. to cultivated zooplankton for European sea bass was demonstrated on the immune and digestive system, Cahu et al., 1998. Modern process and algae cultivation in photobioreactors or fermentation systems can provide algae under a flour form, which can be used with the same form as fishmeal to produce formulated pellets. The chemical composition of microalgae varies depending on species, cultivation parameters and the potential as a feed ingredient varies accordingly (Skrede et al., 2011). Diets containing the microalga T-Iso (Isochrysis spp.) resulted in improved growth of gilthead sea bream juveniles, and the chemical composition of sea bream fillets also met the needs of consumers, although the level of proteins was lower compared to a conventional diet and the level of fats was higher. T-Iso resulted in high digestibility and supported the best performance of fish fed on a diet based on 70% of microalgae, probably due to its high protein efficiency (Palmegiano et al., 2009).

The evaluation of microalgae Isochrysis sp. in partial substitution of fishmeal in gilthead Sea bream (Sparus aurata) pellets showed better performances than control diets. The best performances of fish fed on 70% algae diet were probably due to the protein composition and the amino acid profile in comparison to other diets. Other algae species as Tetraselmis suecica was able to replace up to 20% of European sea bass protein without hampering growth performance and major quality traits fish.

Processed animal protein (PAP) is an important ingredient in feeds and provides a valuable source of animal by-product utilization. Nutritional quality of rendered animal protein ingredients is affected by composition, the freshness of raw materials, and processing conditions. PAP has a high nutritional value making it an excellent alternative to imported proteins such as soya. It has a significantly higher protein value (45-90% on a fed basis) than plant feed ingredients. PAP contains 10 % phosphorus, which is low in relation to the content of amino acids. Blood meal is also a feed ingredient with high protein content (80% in full

blood) and excellent protein digestibility (Bureau et al., 1999). It has a high content of lysine and histidine, while the content of isoleucine is low (El-Haroun and Bureau, 2007; Breck et al., 2003). While there may be consumer and producer concerns about the feeding of PAP to fish, due to the potential transmission of prions, the scientific panel opinion published by the European Food Safety Authority (EFSA) in 2011 concluded that processed animal protein in feed for food producing non-ruminants, respecting the proposed ban on intra-species recycling, presents a negligible risk to human health (EFSA, 2011).

The use of insects as a source of protein in fish diets is also being explored. The chemical composition of prepupae larvae varies with species, age, a method of processing and the substrate the maggot is produced on (St-Hilaire et al. 2007a,b; Aniebo and Owen, 2010). The nutritional value of insects as feeds for fish, poultry, and pigs has been recognized for some time in China, where studies have demonstrated that insect-based diets are cheaper alternatives to those based on fishmeal. The insects used are the pupae of silkworms (Bombyx mori), the larvae and pupae of house flies (Musca domestica) and the larvae of the mealworm beetle, Tenebrio molitor. Silkworm pupae are an important component of cultured carp diets in Japan and China. Dried ground soldier fly larvae have been fed to chickens and pigs with no detrimental effects (Newton et al., 1977; Hale, 1973). In recent years there has been some interest in the use of housefly maggot meal as a substitute for fishmeal in tilapia and African catfish diets (Adesulu and Mustapha, 2000; Fasakin et al., 2003; Ajani et al., 2004; Ogunji et al., 2006). Bondari and Shepherd (1987) observed that channel catfish and blue tilapia fed on soldier fly larvae for 10 weeks were acceptable as food by consumers. Growth and organoleptic quality were not affected when common carp were fed on non-defatted silkworm pupae, a major by-product of the sericulture industry in India (Nandeesha et al., 2000). Ng et al. 2001 demonstrated that T. molitor larvae meal was highly palatable to the African catfish (Clarias gariepinus) and could replace up to 40% of the fishmeal component without reducing growth performance.

St-Hilaire et al. (2007a) described a study in which they determined if black soldier fly (Hermetia illucens) pre-pupae and housefly pupae could be used as a partial replacement for fishmeal and fish oil in rainbow trout (Oncorhynchus mykiss) diets. Their data suggested that a rainbow trout diet in which black soldier fly pre-pupae or housefly pupae constituted 15%

of the total protein had no adverse effect on feed conversion efficiency over a 9-week feeding period. However, rainbow trout fed on black soldier fly diets low in fish oil had reduced levels of omega-3-fatty acids in the muscle. According to the researchers, modifying the diet of the fly larvae could improve digestibility and fatty acid content of the pre-pupae, which in turn could enhance the fatty acid profile of the fish fed on the fly pre-pupae. The use of the black soldier fly in manure management, yields abundant numbers of fly pre-pupae, and fly pre-pupae may be an economical and sustainable feed ingredient for carnivorous fish diets. Yellow mealworm meal could partially (35%) replace fishmeal in the diet of European sea bass (Dicentrarchus labrax) without affecting mortality or growth but replacing 70% of the fishmeal did depress growth (Gasco et al. 2016).

6.5 Products for human consumption

Fish oil

If fish oil is to be used for human consumption, it has to live up to a number of different quality criteria. According to Jacobsen et al. (2009) some of the most important quality criteria for fish oil include:

> 1) The content of long-chain omega-3 polyunsaturated fatty acids

> 2) The content of different lipid classes

> 3) The presence of environmental contaminants such as dioxin, PCB, etc.

> 4) Lipid oxidation

Fish oil quality and properties will depend on the quality of the raw material and the production process. During the refining process, crude fish oil goes through a number of physicochemical processes in order to remove undesirable compounds, such as contaminants (e.g. dioxins and PCBs), and other components that can contribute to the development of unpleasant flavour and colour of the oil (e.g. residual proteins, water, pigments, free fatty acids, phospholipids, and lipid oxidation products). At the same time, the desirable properties including the nutritional value of the oil must be kept. The properties of the obtained refined fish oil will determine both its application and price. Quality guidelines for some important

parameter for refined fish oils are provided in Table 10 as proposed by Hamm (2009). While some other proposed guidelines have referred a peroxided value of 5-10 meq O_2/kg, such values would result in some smell that would make the oil unacceptable for inclusion in food products. On the other hand, if the oil is incorporated into oil capsules and sold as dietary supplements then such peroxided values could actually be acceptable.

Table 10 Some quality guidelines for refined fish oils.

Parameter	Quality guidelines
Colour	<3.0 Red, 30 Yellow
Odour and taste	Bland
Matter volatile at 105°C	<0.2 %
Insoluble impurities	<0.05 %
Soap content	<0.005 %
Iron	<0.12 mg/kg
Free fatty acids	<0.10 % (as oleic acid)
Copper	<0.05 mg/kg
Peroxide value	<0.1 meq O2/kg
Nickel	<0.20 mg/kg

Source: Hamm 2009

The refining process is a requirement for the subsequent successful and high-yield production of omega-3 concentrates (Rubio-Rodríguez et al., 2010). The development of omega-3 concentrates comprises the following steps: de-acidification (optional), transesterification, concentration, deodorisation/earth treatment, and antioxidant addition and fill off (for detailed description see EFSA, 2010).

The obtained omega-3 concentrates must fulfil certain quality requirements in order to be acceptable for inclusion in food products. In the USA, the FDA has established a set of quality guidelines for omega-3 concentrates with the food-grade specification, which must be fulfilled in order to receive a generally recognized as safe (GRAS) status (Table 10).

The form of the omega-3 FAs i.e. the natural triacylglycerol form as opposite to the methyl or ethyl esters (via transesterification) has also been a matter of discussion. Omega-3 FAs in the form of acylglycerides have been reported to present higher stability against oxidation (Boyd

et al., 1992) apart from being more efficiently absorbed by humans compared to those in the form of esters (Lawson and Hughes, 1988). Furthermore, stability is also higher when omega-3 FAs are bound to sn-2 position of the glycerol structure than to the sn-1,3 position (Wijesundera et al., 2008). In this context, the development of cost-effective technological solutions for the production of acylglycerides concentrates with omega-3 in sn-2 position is highly desirable (Rubio-Rodríguez et al., 2010).

The concentration of free fatty acids must be minimal as hydrolysis and acidity have a major detrimental effect on the quality of omega-3 concentrates.

Oxidative stability is another major quality criterion for the omega-3 concentrates. Therefore, exposure to factors that accelerate lipid oxidation (i.e. heat, intense light, metal ions, and oxygen) must be minimized throughout processing. In this regard, the levels of primary oxidation products (peroxides) must be kept at a minimum during the entire process. For this reason, antioxidants are generally added to the omega-3 concentrates after cooling down following the deodorization step.

Additionally, the presence of impurities and off-flavours in the omega-3 concentrates must be imperceptible to human sensory analysis.

Finally, the levels of dioxins and dioxin-like PCBs must be below the threshold values set by EU (Table 3 in chapter 2.1 *Raw material quality*).

Table 11 Some quality guidelines for omega-3 concentrates (FDA GRAS Notification 2002; 2006).

Parameter	Quality guidelines
Appearance	Light yellow to yellow at room temperature
Triglycerides	>50 %
Odour and taste	At worst, a slightly fishy odour and taste
Acid value	<1.0 %
Peroxide value	<2.5 meq O_2/kg
p-Anisidine value	<20
Totox value	<25
Density	0.85-1.00 g/ml
Tocopherols	>2.0 mg/g
Moisture	<0.1 %
Unsaponifiables	<2.35 %

Source: EFSA 2010

Products such as virgin fish oils or extra low oxidised fish oils have been introduced in the market (EFSA, 2010). The production of 'extra low oxidized oils' differs from traditional production methods for fish oils and fish meals. 'Extra low oxidised oils' are produced from materials from food operations. This can for example be material after filleting of high quality (i.e. very fresh) salmon or herring. The raw material is processed very shortly after catching. The process involves heating to below 100°C, for example to a temperature around 90-95°C just for the time needed for the material to pass through an indirectly heated tubular scraped surface heat exchanger. The heated suspension is then separated in a suitable decanter in order to isolate the oil. The semisolid protein phase that is obtained from the same process can be valuable starting material, for example for production of marine protein hydrolysates. Because of the gentle processing conditions and selection of raw materials these oils are generally suitable for direct use as ingredients in food and beverages. EFSA highlights though that scientific literature references are lacking regarding the production process of virgin fish oil.

7 Results from the workshop in Fishmeal and Fish oil - 2018

As mentioned in chapter 1.2 the workshop was divided into five main categories following the chapters in this report.

- Raw material

- Production of fishmeal and oil

- Analytical methods

- Preservation throughout the value chain

- Final products – Key/desirable properties of various final products

The presenters of each presentation identified research needs or the research gaps for their topics which they presented and discussed with the workshop participants after each talk. These research needs are listed below along with a summary of the discussion that followed.

In the end of the workshop, a penal discussion was held to further discuss the results of the workshop and how the industry science community can react. The panel discussion was led by Sveinn Margeirsson former CEO of Matís.

7.1 Identified research needs

Raw material

Establish the relationship between freshness of raw fish and the properties (including the level of lipid oxidation) and yield of the obtained refined fish oil.

Determine whether TVB-N is a suitable spoilage parameter for whole/un-gutted fish used as raw material for production of fish oil intended for human consumption and if it relates to lipid oxidation. If this is the case, scientific research should be carried out to establish the foundation of setting TVB-N limits for whole fish.

Develop sensory methods such as Quality Index Method (QIM) schemes for fish species intended as raw material for fish oil production (EFSA 2010).

Better understanding of effect of raw material freshness/quality on animal growth performance. How to further increase high-quality fishmeal and oil production from by-products.

How to optimize collection of trimmings (by-products) from smaller processing plants.

By-products from aquaculture yield poorer quality oil (lower content of omega-3 PUFA).

How important is the salt content in fishmeal in terms of quality, fish growth and feed intake?

How much salt do lean fish species take up when super-chilled in seawater at -2°C?

Is it economical to land super-chilled raw material for fishmeal?

How can we further improve the quality of lean fish species?

How do we measure protein quality throughout the fishmeal processing.

Is enzymatic hydrolysis a reasonable addition both in regards to quality and cost?

Are there other methods than heating and adding enzymes to break down the raw material more efficiently?

Are there other methods than heating and adding enzymes/acid to break down the raw material during pre-treatment?

Discussion

Questions were raised about the freshness indicator for fish oil intended for human consumption. Currently, it is based on TVN, 60 mg/kg, in the raw material and as pointed out TVN is an inefficient indicator for fish oil quality. It is suggested that the quality of the finished product should be a better indicator.

Research is needed into which production method is the best for each raw material. Different messages were communicated by different machine producers. Fishmeal machinery producers emphasized that the production method determines the overall quality of fishmeal, whereas other machinery producers emphasized that raw material

quality, from sea to shore, is the key to quality fishmeal production. The answer most probably is that both are right, but the actual effects must be identified.

Collaboration between the industry and researchers is the key for any progression within a traditionally static industry.

Production of fishmeal and oil

Several physical and chemical analysis were used to characterize the technical properties of fishmeal. Can these analyses be used as quality control analysis on a fishmeal factory or should other rapid methods be developed?

Fishmeal is a complex and variable ingredient creating challenges for the fish feed producers. Are there ways to "narrow" the observed differences in technical properties to enable delivery of a more predictable product?

According to EFSA (2010) some of the knowledge gaps and research needs concerning the production and use of fish oils for human consumption include: - Investigate the threshold level of oxidation of refined fish oil (as measured by peroxide and anisidine values) that may have detrimental effect on health (e.g. oxidative stress). - Thorough evaluation of the impact of individual oxidation products originating from refined fish oil on human health. - Establishment of quantitative relationship between peroxide and anisidine values and the specific volatile oxidation products. - Establish a clear relationship between crude oil PV and the PV in the final oil.

Develop alternative extraction methodologies for Fish Protein Concentrates; specially to increase flavour stability (avoid reversion flavours) and avoid "gritty texture" and "chalky mouthfeel".

Develop/test new/alternative food systems where FPC can be incorporated at meaningful levels without impairing the properties of the product.

Discussion:

NIR (Near-infrared technology) has made its way into the fishmeal and oil industry during the last decade and will continue to do so until production methods have been fully optimized. NIR represents a very rapid method for data mining quality during production, both when used online during production and offline during final product quality assessment.

Questions about the real technical properties of the fishmeal were raised and should be investigated further. Mechanical properties of the fishmeal, such as viscosity, water adsorption, binding properties etc. are studied by the feed producers, whereas fishmeal producers have yet to fully investigate these properties.

The real focus of the industry has not really been towards much else than nutrients, such as proteins and fats and other components that makeup fishmeal. A relationship between the quality of the fishmeal and the technical properties must be established for fishmeal producers to further understand their own product.

Within the industry, there is a gap of knowledge on crude fish oil. Fish oil produced by EU fishmeal members is almost solely sold as crude fish oil. There is a will within the industry to bridge this gap so that crude fish oil can be refined by industry members and marketed directly for human consumption.

Analytical methods

Standard method for protein determination in FM using Dumas principle?

An alternative to the AV method is needed for fish oil (for human consumption)

 For headspace GC-MS there is no standard method and labs are doing the analysis in many different ways

As the differences in fishmeal rheological properties impact the feed extrusion process and physical feed quality, may then rheological measurements be alternative tools better describing fishmeal technical properties?

Is it possible to establish a set of analysis enabling to predict and control the fishmeal behaviour in the feed extrusion process?

Discussion:

There is a need for standardization for protein determination within the fishmeal industry as both the Dumas method and the Kjeldahl method seem to be used by laboratories intermittently. The Dumas method has gained popularity in recent years and is adequate for the time being if the same method is carried out by all laboratories.

Both the Dumas and Kjeldahl methods determine the total nitrogen content within a sample and then estimate the protein content using a pre-determined factor, which for fishmeal is 6.25. This factor should be investigated and further validated so that producer can be assured that they are getting accurate protein analysis.

Amino acid profiling can potentially be used to standardize a correction factor within the industry, but it is unknown if a method to estimate true protein content can be produced.

GC-MS headspace method for the measurement of volatiles in fish oil is most likely the way forward when compared to the older methods as it has the potential of being much more reliable. However, much work is still needed as currently there is a lack of standardization in sample handling, sample measurement etc. Also, more research is needed into the correlation between different volatiles and how they affect the quality of different fish oils.

For the determination of technical properties, there is a need to decide on the best, easiest methods to understand the meal behaviour in the extrusion process and there is also a need for a quick analysis for controlling the extrusion process.

Preservation methods throughout the value chain

Develop new methods for shelf life extension of bulk-stored whole fish

Develop new methods for shelf life extension of bulk-stored rest raw material from the consumer industry

As most studies reported in the literature are carried out with stripped or refined oils more research should be carried out with unrefined fish oil with different fatty acid compositions and levels of natural antioxidants, e.g. - How can BHT (or ethoxyquin if still used) be replaced by natural antioxidants in unrefined oil? - Are there differences in antioxidant efficacy in refined vs unrefined oils? How is antioxidant efficacy influenced by fatty acid composition and presence of endogenous antioxidants or pro-oxidants in unrefined fish oils?

Discussion:

To preserve raw material quality there is a need to schedule landing of the fish to ensure efficiency. "Just-in-time" processing can be key in persevering raw material quality and ensuring final product quality as it shortens the time between catch and processing. Temperature control on board remains crucial for any sort of quality production. The physical aspect of storage must be investigated in more detail and communicated better to the fishermen as research has shown that catching less fish makes it easier to ensure the quality of the raw material. Questions on the suitability of the silo type systems were raised and it questioned if they are truly the best way to store the fish or if there are better, more efficient ways to store fish after catching.

Trimmings must be treated in the same way as fresh fish with regard to the "just-in-time" processing theme. Getting the trimmings as quickly as possible from the consumer plant to the fishmeal plant is a key factor in determining the eventual quality of the final product. The possibility of using additives to conserve the quality of the raw material must be investigated and the importance of excluding light during storage and controlling the temperature must be communicated to trimming producers.

Unrefined fish oil contains antioxidants, any optimizing or refining of crude fish oil must take that into account as some added oxidants can act as prooxidants whereas on

the flip side efficacy of what is added to the oil can be improved to synergize with the already present antioxidants.

Key properties of various final products

Impact of processing methods on nutritional value of fishmeal and fish oil for aquaculture feed?

Nutritional value of solubles? – Macro-/micro nutrients – Feed/Food?

Impact of dietary fishmeal on fish health/stress tolerance and immune defence?

Identification of unique nutrients in FM for humans (peptides, hormones, vit. etc.)?

Strategic use of FM and FO (e.g. fry feed)

Low molecular weight water-soluble proteins, fish silage and protein hydrolysates improve transformation and binding of fishmeal and other ingredients in the feed extrusion process. These results documents the possibility to develop a new processing aid for the fish feed industry serving multiple purposes as nutrient, plasticizer and binder in extruded fish feed. Can this information be used to develop a higher value fishmeal product?

There is an urgent need of a communication strategy/clear messages between the FM and FO industry and the stakeholders at all levels (fishermen, current and potential new customers/ markets/retailers of FM and FO, consumers, authorities, NGO etc. Lack of common understanding throughout the chain that industrial fishery is sustainable also in terms of keep sustaining wild stocks – including utilization of trimmings from processing of consumption fish and contribute to providing value added products throughout the value chain.

More focus on innovation incl. a research and development strategy.

> **Discussion:**
>
> To increase the value of fishmeal and oil products in the short term the focus should be on the most adequate ways to add value. Currently an option may be the new knowledge on the fatty acid cetoleic acid (C22n1:11). It is now known that cetoleic

acid increases the retention of EPA and DHA by 2% in model systems. Scandinavian fish oil has traditionally lacked EPA and DHA compared to fish oil produced in South-America. Typical Scandinavian fish oil contains about 18% of EPA and DHA, the value of each % of EPA and DHA is about 30$. With 150 thousand tons of Scandinavian oil sold each year, this new knowledge on cetoleic acid potentially brings 9 million USD added value. However, extensive knowledge on cetoleic acid and other unique nutrients calls for further research.

Increased requests of fishmeal and -oil for aquaculture feeds call for more strategic use, e.g. in fry feeds with focus on essential macro- and micro nutrients.

Identifying the most relevant parameters to describe the technical/nutritional value of the feed is important to better serve our customers as well as to increase the fishmeal industry's power of deciding a price point.

7.2 Summary from the panel discussion

Future strategy

Innovation has been lacking within the fishmeal industry and it has been described by many as a stagnant industry. Innovation, however, is the key to keeping up with other industries providing protein and oil worldwide. There is a need to create a goal for the industry as a whole, a vision that can be followed.

Perceptions about the fishmeal and fish oil value chain are often skewed and the industry needs to develop a communication strategy that can address this. Creating a story for the consumers and customers isn't sufficient as there is a need to be able to communicate the story and get it across. But it is also of importance to analyze the market and know who the fishmeal and oil consumers are so that the correct message is communicated.

A plan must be made by the industry and it must be ready within 2 years' time so that the fishmeal industry does not fall too far behind.

Communicating with customers

There is a lack of communication between the industry and its consumers, messages from the industry have been relayed by IFFO down the supply chain but the messages do not seem to make it beyond the retailers. There is still a lack of understanding by the consumers of why fishmeal is produced, the reasons must be communicated in such a way that it reaches the average consumer. The fishmeal industry in Europe is sustainable but there is no real awareness of this among the average consumers. Consumers also do not seem aware that the industry is increasing the value of raw material that is otherwise thrown away. The industry must come together and form a clear message stating its goals and why the production of fishmeal and oil is important. The European fishmeal industry is very small globally and for it to succeed it must be a united industry when it comes to communicating a clear message to consumers.

Importance of organizing the value chain and important future research areas

The consensus was that there is room for improvement when it comes to scheduling landings but that practical realities hinder this to a certain degree. Maintaining the quality throughout the whole production process should be prioritized as it affects the nutritional benefits of the fishmeal and the health benefits as well. Continued sustainability is an important research avenue, not only the sustainability of fishing but also the energy efficacy of the ships, production factories etc.

Creating a clear strategy for the industry is of the upmost importance. First, a communication strategy must be formed then followed by a research strategy. Raw material quality needs to be correlated with the quality of fishmeal for the feed producer. It must be established where the value for the feed producers lies. Full understanding of how the processing equipment impacts the quality of the final product.

Determining the health benefits of fishmeal and oil

The health benefit focus has mostly been on fish oil due to the extensive research into EPA and DHA. More focus needed on fishmeal to increase knowledge. Producers see the benefits but there is a need for documenting and researching it. Many micronutrients are present in fishmeal which are good for many different feed users. Cooperation with the next step in the value chain is important to increase the value of fishmeal and oil.

Final remarks by Sveinn Margeirsson CEO of Matís

Communication is key for any sort of growth within the industry, but communication today can be very hard. The European fishmeal community is very strong and must remain so for the industry to truly grow. The industry members are obviously interested in moving forward but there is a lack in human capital within the industry so there is a need to recruit more people to sustain the future growth of the industry. Fishmeal production has been prosperous for a very long time, but changes are coming, and cooperation is essential for continued survival.

The identity of the industry is not sufficiently clear, there needs to be a story behind why companies are in the industry and that identity must then be communicated to the consumers and possibly future employees.

8 Discussion and conclusion

The main objective of this work was to summarise current knowledge on fishmeal and fish oil as well as identify the research needs and create a roadmap for future industry-driven research. The main conclusion was that the quality of raw material, fishmeal and - oil are not yet well defined. The real focus by the industry has been mainly limited only towards nutrients, such as proteins and fats and other components that makeup fishmeal. There has been less focus on the health benefits of dietary contents of fishmeal and -oil. Or the relationship between processing and effects on nutritional properties of fishmeal. In addition, to proactively strengthen the market position and competitiveness, it is crucial for the industry to achieve a common understanding of the needs of their customers in line with a clear profile of the benefits of their products. A communication strategy as well as a research strategy is needed.

Fishmeal and fish oil production plays an important role in the Nordic countries. However, production has been static in the last decade, while the world´s protein and oil demand has increased, along with increasing public demand for improved sustainability. The inclusion of fishmeal and oil in fish feed has decreased dramatically in the last decade and is expected to continue to decrease with growing aquaculture production. However, the demand for protein and other nutrients will increase and the industry needs to keep up with the development in the feed industry.

One of the conclusions of this work was, that raw material, fishmeal and fish oil qualities need to be better defined as well as the relationship between them. Research is needed to further prove the relationships between TVN and e.g. fish oil qualities (lipid oxidation etc.), but other methods may be more effective of measuring freshness in general, e.g. the quality index method (QIM) or biogenic amines. Regarding fishmeal, the real focus by the industry has been mainly limited only towards nutrients, such as proteins and fats and other components that makeup fishmeal. Further, there is a need for standardization of protein determination within the fishmeal industry as both the Dumas and the Kjeldahl methods do not always give the same results.

Production of fishmeal is a fairly standardized process which has not changed much the last decade. There is a need for a better understanding of how processes and the methods used affect the material and nutritional properties at different stages during processing. It is essential to preserve the freshness of raw material with adequate cooling and make sure the cooling chain is maintained during landing. Moreover, it's important to preserve both fishmeal and oil since they are very sensitive towards external condition during transport and storageThe relationship between the quality of the fishmeal and the technical properties must be established for fishmeal producers to further understand their own products, as these properties are important for feed producers, farmers and other customers. The industry should emphasize the amino acid composition of fishmeal to further understand the properties of their products and which amino acids are lacking in plant-based ingredients, e.g. methionine and lysine.

Production of water-soluble proteins from fish silage or fish hydrolases may improve technical properties in mixtures with other ingredients during feed manufacturing and which has proven to be effective. Fish solubles from high quality raw fish could also be used to make products for human consumption, or extracting other nutrients such as peptides, hormones, vitamins etc. for humans. The unique X-factor of fishmeal remains to be defined as well as knowledge on the influence on the immune defense, health and stress tolerance of the fish is lacking.

Innovation has been lacking within the fishmeal industry and it has been described by many as a stagnant industry. To tackle the obstacles ahead a clear strategy for the industry is of crucial importance. A communication strategy as well as a research strategy is called upon. Cooperation with the next step in the value chain is important to increase the value of fishmeal and oil. There is a lack of communication along the value chain from the industry to the consumers. There is still a lack of understanding by the consumers of why fishmeal is produced, the reasons must be communicated in such a way that it reaches the average consumer. The identity of the industry needs to be clear and transparent to promote a story about the industry to provide a clear and positive image of the industry to be communicated to the society.

The industry members are interested in moving forward to sustain the future growth of the industry. Fishmeal and fish oil production has been prosperous for a very long time, but to remain so, cooperation among all stakeholders is crucial for continued progress.

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