AQUABIOPRO-*FIT* at a glance

PART I

Downstream processing methods of aquaculture and fisheries side stream biomass to produce targeted nutritional supplements

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PART I: Downstream processing methods of aquaculture and fisheries side stream biomass to produce targeted nutritional supplements

Summary:

This Part I encompasses 4 courses presenting information about the side stream biomass of marine origin downstream processing and transformation into valuable nutritional supplements.

Aquaculture and fisheries side stream biomass constitutes an important value-added resource in the EU fisheries and aquaculture industry; in Norwegian one as well, where most of it is utilised in animal feeds. Although in the EU the volumes of fish discards amount to over 5.2 million tons per year, there is a potential for increasing the utilization rate of this nutritious resource.

This Part presents information on the production of fisheries and aquaculture side stream biomasses in Europe and particularly Norway, their chemical characteristics, and exploitation opportunities. Special attention is paid to the sensory properties of the marine nutritional supplements and the methods for their sensory characterisation.

Information focused on the fish protein and hydrolysate commercial products for human consumption and fresh fish side stream products is presented. The market size, barriers for commercial availability, and opportunities for their higher-value production and commercialization are shown.

Special attention is given to fish collagen (incl. its partly hydrolyzed product gelatin) and fish oils, in particular to their potentially valuable applications in the medicine, food, and pharmaceutical industries. The production and applications of fish collagen and marine oil-based supplements, their main valuable characteristics, market positions, barriers, and prospects for their further exploitation are revealed in this Part. Information about the tunicate *Ciona intestinalis* and the possible compounds (cellulose, fatty acids, and proteins) that can be isolated and further valorized is also provided.

This Part presents as well comprehensive information for the currently applied and novel downstream processing methods used in the marine ingredient industry and research for the transformation of different fish biomass fractions into more stable, nutritious products of acceptable organoleptic properties. Special emphasis is given to thermal processing, protein hydrolysis, and the silage process. Details about the downstream processing of marine protein products in terms of technical decisions and equipment are given.

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Course 1.1: Volumes and qualities of fish side stream biomasses in Europe

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1.1.1 Norwegian aquaculture and fisheries

Side streams are materials that result from industrial processes, that do not constitute the main targeted processing products, often of lower value. Side streams can be further valorised by entering as raw materials in new processing applications for the creation of higher value products, in a circular economy manner. Side stream biomass of marine origin constitutes an important value-added resource in the Norwegian but also the EU fisheries and aquaculture industry, where most of them are used in a good way. Nevertheless, there is a potential for increasing the utilization rate, especially from the whitefish sector. Whitefish or white fish is a fisheries term for several species of demersal fish with fins, particularly Atlantic cod (*Gadus morhua*), whiting (*Merluccius bilinearis*), haddock (*Melanogrammus aeglefinus*), hake (*Urophycis*), pollock (*Pollachius*), and others. Whitefish (*Coregonidae*) is also the name of several species of Atlantic freshwater fish. Whitefish live on or near the seafloor, and can be contrasted with the oily or pelagic fish which live in the water column away from the seafloor.

In Norway, more than 950,000 tons of side stream raw materials was accessible from a base of 3.57 million tons of fish and shellfish in 2018 (Table 1.1.1).

Table 1.1.1 Norwegian fisheries and side stream volumes (2018).

	Whitefish	Pelagic*	Aquaculture	Shellfish	Total
Raw material (live weight in x1000 tons)	756	1296	1466	52.1	3570
Available side streams (in x1000 tons)	329	205	418	10.8	953.8
% side stream raw materials of live weight	42%	16%	29%	21%	27%

**Raw material species are herring, mackerel, blue whiting, capelin etc. (those who generate side streams). Source: Richardsen et al (2019)*

1.1.2 World-wide and EU fisheries side stream biomasses

The annual fish captures in the 28 EU member states including fisheries and aquaculture were 6.451 million tons in 2015 (EUMOFA (https://www.eumofa.eu/) 25/11/2016 from FAO and Eurostat (https://ec.europa.eu/eurostat/data/database) compared to 211,511 million tons world-wide. Approximately only 50% of the production is used for human consumption whereas the world-wide discards are estimated to be approximately 20,000,000 tons including processing wastes, non-target species and side stream raw materials. In EU the respective volumes of fish discards amount to approximately 5.2 million tons per year, including fish trimmings from slaughter houses, smokeries, fish canning factories, shellfish processing industries (AWARENET, 2004).

Below are listed the relative amounts of the different fish fractions deriving from round fish from fisheries and aquaculture in Norway and Europe (Figures 1.1.1 - 1.1.2; Tables 1.1.2 – 1.1.3).

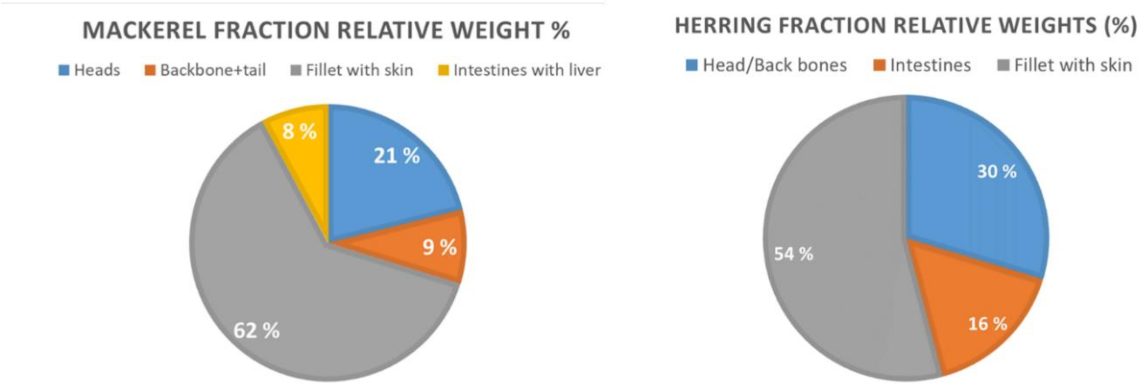

Figure 1.1.1 Relative proportions of fillet and side stream fractions in mackerel and herring from the Norwegian fisheries.

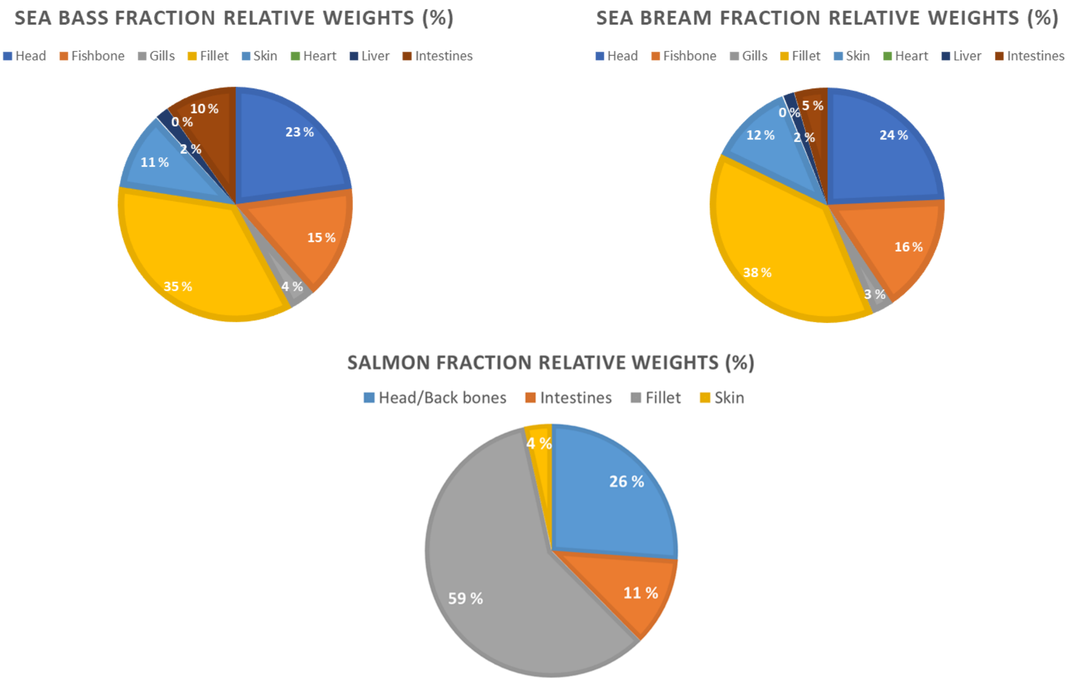

Figure 1.1.2 Relative proportions of fillet and side stream fractions in farmed European sea bass (sea bass), Gilthead sea bream (sea bream) and Atlantic salmon (salmon).

Mackerel has relatively higher proportion of heads as compared to herring and salmon, herring having the smallest heads in relative weight among the three species. Herring has however highest relative amount of backbones including the tails. Salmon and mackerel have similar proportions of fillet as compared to herring whereas visceral weight proportions are lowest in Mackerel and highest in herring (Table 1.1.2).

Table 1.1.2 Relative proportions (%) of fillet and side stream fractions in aquaculture and fisheries fish species.

<table>
<tr><th>Species</th><th>Heads</th><th>Gills</th><th>Backbone</th><th>Tail</th><th>Fillet</th><th>Skin</th><th>Liver</th><th>Intestines w/o liver</th></tr>
<tr><td>Salmon</td><td>9.61±1.14</td><td>2.24±0.43</td><td colspan="2">14.99±4.50</td><td>60.54±3.74</td><td>2.84±1.60</td><td>1.25±0.35</td><td>10.51±1.91</td></tr>
<tr><td>Herring</td><td>6.91±1.09</td><td>2.49±0.28</td><td colspan="2">20.25±4.72</td><td>54.11±5.26</td><td></td><td>1.35±0.49</td><td>14.89±3.71</td></tr>
<tr><td>Mackerel (2)</td><td colspan="2">21.13±0.85</td><td colspan="2">8.64±1.18</td><td colspan="2">62.37±1.28</td><td colspan="2">7.86±0.76</td></tr>
<tr><td>Mackerel (1)</td><td colspan="2">21.00±1.21</td><td>10.10±1.80</td><td>0.81±0.17</td><td colspan="2">60.54±1.46</td><td colspan="2">7.56±0.83</td></tr>
</table>

The biometric parameters and side stream fraction proportions of farmed European sea bass and Gilthead sea bream differ significant (P<0.05) for the two fish species. These differences were especially significant (P<0.001) for dressing percentage, which was higher in sea bream as compared with sea bass (95.30 vs. 93.76, respectively). Regarding filleting trimmings and side stream fractions, only gills, guts and fins showed significant (P<0.05) differences between the two species studied. The average relative proportions of heads (21.53 vs. 19.80), and fillet (34.12 vs. 30.57) usually commercialized with skin (10.25 vs. 9.28) were higher in sea bream, while there were higher proportions of the remaining fractions in sea bass.

Table 1.1.3 Relative weights and proportions of fillet and side stream fractions in farmed European sea bass and gilthead sea bream.

Fraction	**Sea bass**	**Sea bream**	**SEM**	**Sig.**
Carcass weight (g)	523.78	484.32	6.23	***
Length (cm)	35.40	27.21	1.01	***
Dressing percentage (%)	93.76	95.30	0.32	*
Cuts (% respect to carcass)				
Head	19.80	21.53	0.46	ns
Fishbone	13.50	14.57	0.51	ns
Gills	3.14	2.63	0.07	***
Fillet	30.57	34.12	1.03	ns
Skin	9.28	10.25	0.45	ns
Heart	0.19	0.23	0.02	ns
Liver	1.57	1.31	0.09	ns
Guts	8.47	5.04	0.47	***
Fins				
Dorsal	0.99	0.43	0.11	*
Pectoral	0.25	0.38	0.02	***
Anal	0.28	0.12	0.03	***
Caudal	0.93	0.58	0.06	***
Pelvic	0.35	0.24	0.03	ns
Others	4.45	3.89	0.33	ns

*SEM: Standard error of the mean; Sig.: Significance (ANOVA), ns: not significant; *: P<0.05; ***: P<0.001.*

1.1.3 Chemical analysis and characteristics of fish side stream raw materials and products

This section provides an overview of the chemical compositions of different pelagic and farmed fish species and their different side stream fractions as analysed in the scope of the AQUABIOPRO-FIT project. The chemical composition of fish tissues may vary according to fish size and season, and in the case of farmed fish also related to feed composition. The main chemical characteristics of major fish species fished or farmed in Norway (Herring, Blue whiting, mackerel and Salmon) and those farmed in the Mediterranean (Gilthead sea bream and European sea bass) are discussed in this section.

Crude protein in fish tissues is usually determined by the Kjeldahl method (ISO 5983-1997) which quantifies nitrogen (N) in the material which can then be multiplied by a factor to provide the protein amount in the analysed sample. The factor 6.25 is usually applied in the case of fish protein but may vary from tissue to tissue. Fish tissue moisture and ash can be determined gravimetrically after drying pre-weighed samples in porcelain cups at 105°C for 24 h and then incinerating the dried samples at 500°C for 12 h (ISO 6496-1999 and ISO 5984-2002, respectively). Total lipid in raw materials and body tissues can be quantified by several methods such as Bligh & Dyer extraction method (1959), Folch (1967) and others. For the analysis of total amino acid profile, samples can be hydrolysed in an acid solution (6 M HCl for 22 h at 110°C) and analysed by HPLC using a fluorescence technique for detection (Cohen and Michaud, 1993). Total phosphorous (P) can be determined by a spectrophotometric method (ISO 6491-1998). The water-soluble fraction of fish tissues can be extracted with boiling water and then the extract can be filtered using paper filter, and the crude protein content in the water phase determined by the Kjeldahl method. Analysis of fatty acid composition is commonly realised in Bligh & Dyer extracts by gas chromatography (GC).

1.1.3.1 Atlantic salmon, Herring, Blue whiting and Mackerel

The chemical composition of different fish species and fish fraction varies significantly. In Table 1.1.4 below is listed the analysed protein, fat, ash, and water content of different herring fractions as analysed in the scope of the AQUABIOPRO-FIT project.

Table 1.1.4 Chemical composition of herring fractions (in wet matter)

Herring	Fillet with skin	Back bone with tail	Guts with liver	Heads with gills	Skin	Fillet	Intestines	Liver	Gills	Heads w/o gills
Protein	16.6	17.3	20.0	12.3	6.1	18.0	20.4	17.1	12.0	12.4
Fat	19.9	14.8	13.7	13.6	25.3	15.5	10.1	7.0	9.7	15.4
Ash	1.02	2.34	1.28	5.40	0.49	1.41	1.96	2.06	5.72	5.29
Water	62.5	65.5	65.0	68.7	68.1	65.1	67.6	73.9	72.6	66.9

Often are the different fish fractions analyzed in dry form, which is easier to handle and preserve. In Figure 1.1.3, the ash (minerals) composition of different fish fraction of salmon (pink), herring (orange), blue whiting (gray) and mackerel (blue). The backbone, head and gill fractions are the richest ones in minerals, with higher levels present in Blue whiting among the considered species.

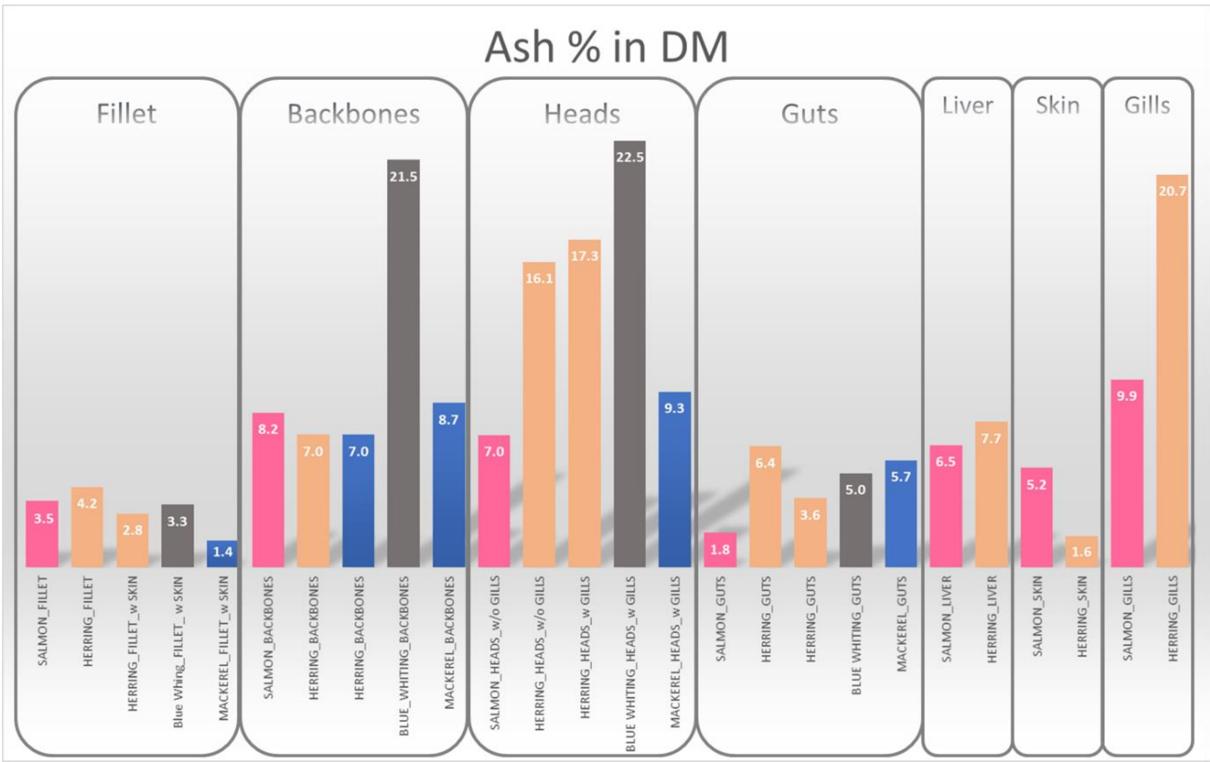

Figure 1.1.3 Ash content in different fraction of different fish species in % dry matter (DM).

In Figure 1.1.4, the analysed macrominerals (Calcium: Ca, Magnesium: Mg, Sodium: Na, Potassium: K and Phosphorous: P) content of different fish species and tissues (in dry matter DM). Blue whiting is the species that, as in the case of ash, contained the highest levels of calcium, magnesium and phosphorous in fractions that are rich in bone tissue, such as heads and backbones.

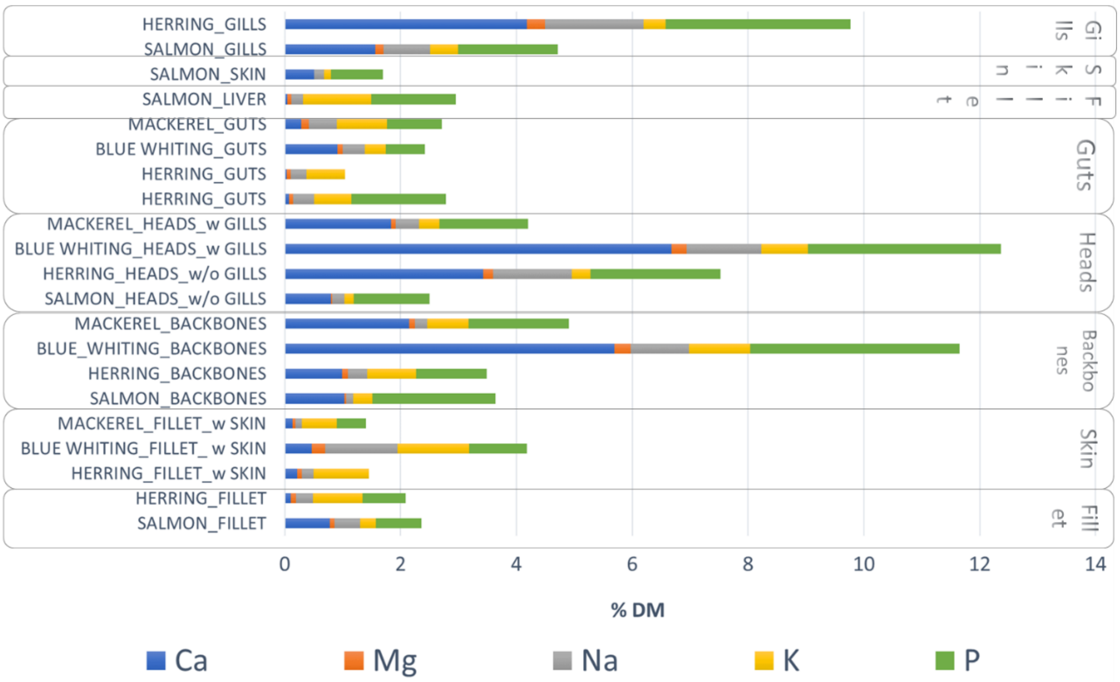

Figure 1.1.4 Macro-mineral content in different fraction of different fish species in % dry matter (DM).

The protein content of fish tissues is high in general varying according to the respective lipid and ash levels. The protein levels of different tissues in salmon, herring, blue whiting and mackerel (in DM) are presented in Figure 1.1.5 below.

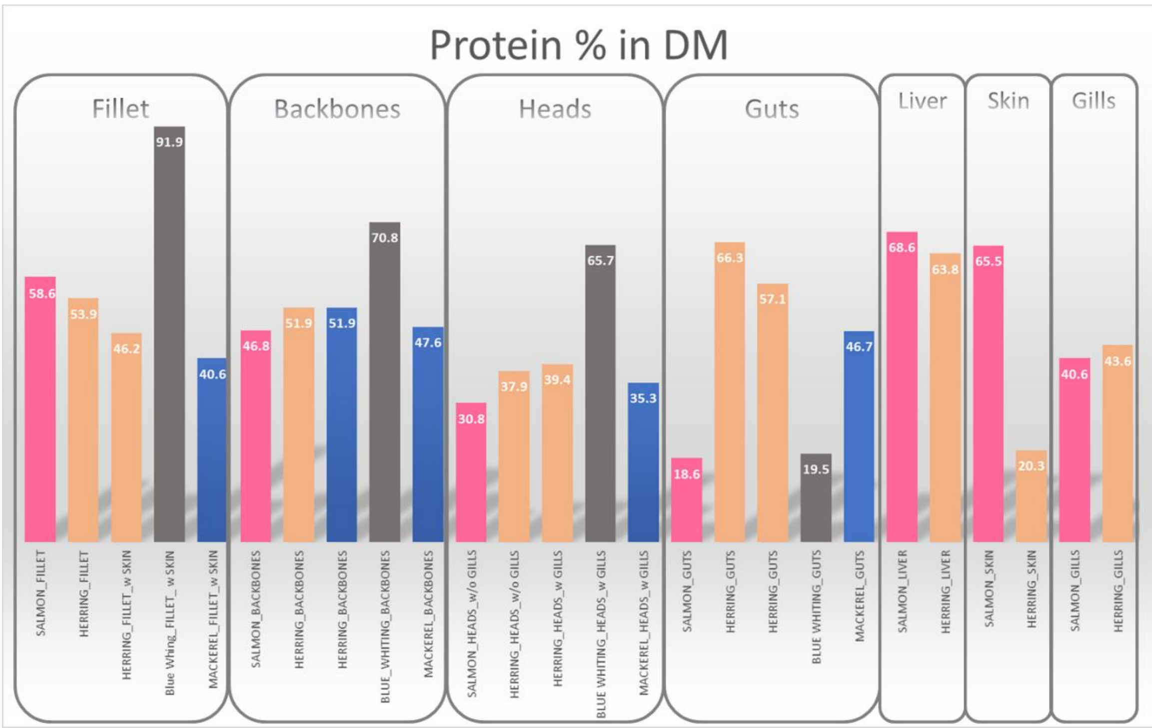

Figure 1.1.5 Protein content in different fraction of different fish species in % dry matter (DM).

The levels of essential amino acids such as histidine (His) and lysine (Lys) were found to be higher in filet and backbone fractions as compared to heads and intestines (in DM) (1.1.6). The same pattern was observed also in the case of non-essential amino acids glutamic acid (Glu) aspartic acid (Asp) and tyrosine (Tyr) (Figure 1.1.7) however the differences among the different tissues in this case were less prominent. Different pattern is observed in tissues that are richer in connective tissue (such as backbones and skins) where higher levels of amino acids hydroxyproline (Hyp) and glycine (Gly) were found, as those are the predominant amino acids in collagen (Figure 1.1.8).

Collagen is a general term to define a group of structural proteins of the extracellular matrix, organized in a fibrillar arrangement. Collagen is the main component of connective tissue (e.g. in cartilage, bones, tendons, ligaments, and skin). Collagens are the most abundant high molecular weight proteins in both invertebrate and vertebrate organisms, including mammals and fish. Collagen consists of amino acids bound together in a triple helix. Depending upon the degree of mineralization, collagen tissues may be rigid (bone), compliant (tendon), or have a gradient from rigid to compliant (cartilage). Bovine collagen is of Type I and Type III while fish collagen is composed of collage Type I.

Unlike protein and ash levels was the level of lipids lowest in fillet and backbone and head fractions in blue whiting as compared to the other analysed species. Lipid levels were in general higher in the main side stream fractions (heads, backbones, and guts) of salmon as compared to herring, mackerel, and blue whiting (Figure 1.1.9). Last, salmon fractions contained significantly higher levels of n-6 PUFA and lower levels total EPA+DHA as compared to herring, blue whiting and mackerel (Figure 1.1.10).

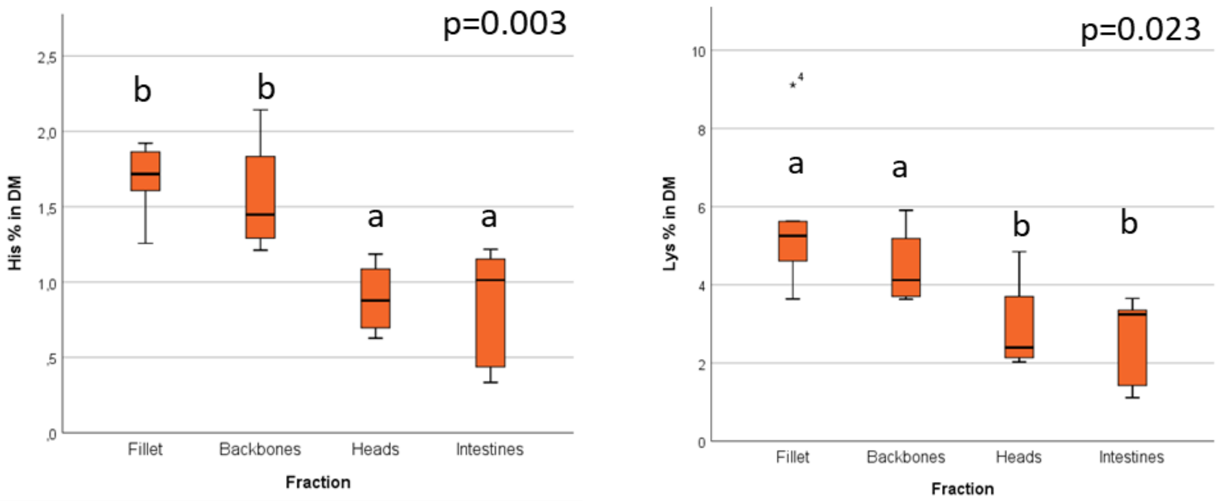

Figure 1.1.6 His and Lys content in different fraction of different fish species in % dry matter (DM).

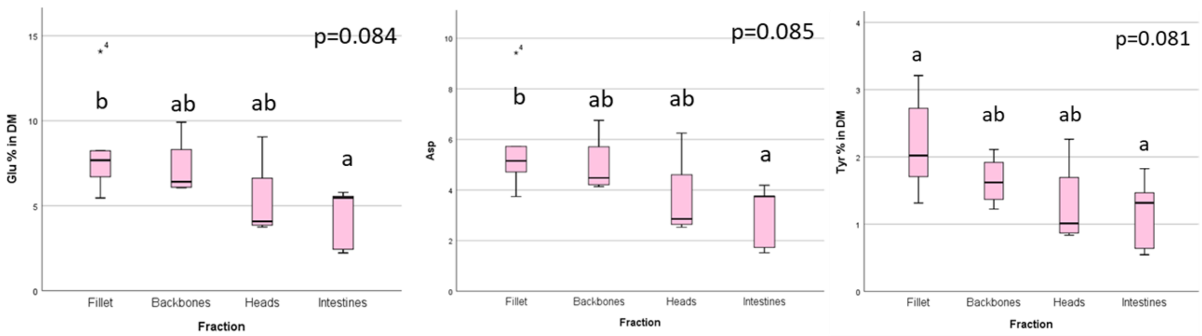

Figure 1.1.7 Glu, Asp and Tyr content in different fraction of different fish species in % dry matter (DM).

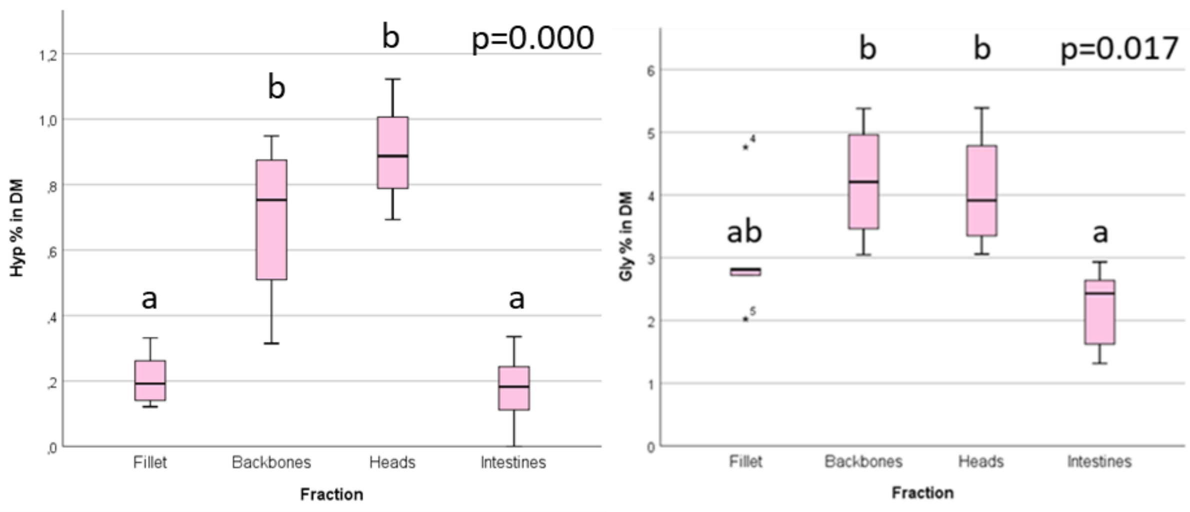

Figure 1.1.8 Hyp and Gly content in different fraction of different fish species in % dry matter (DM).

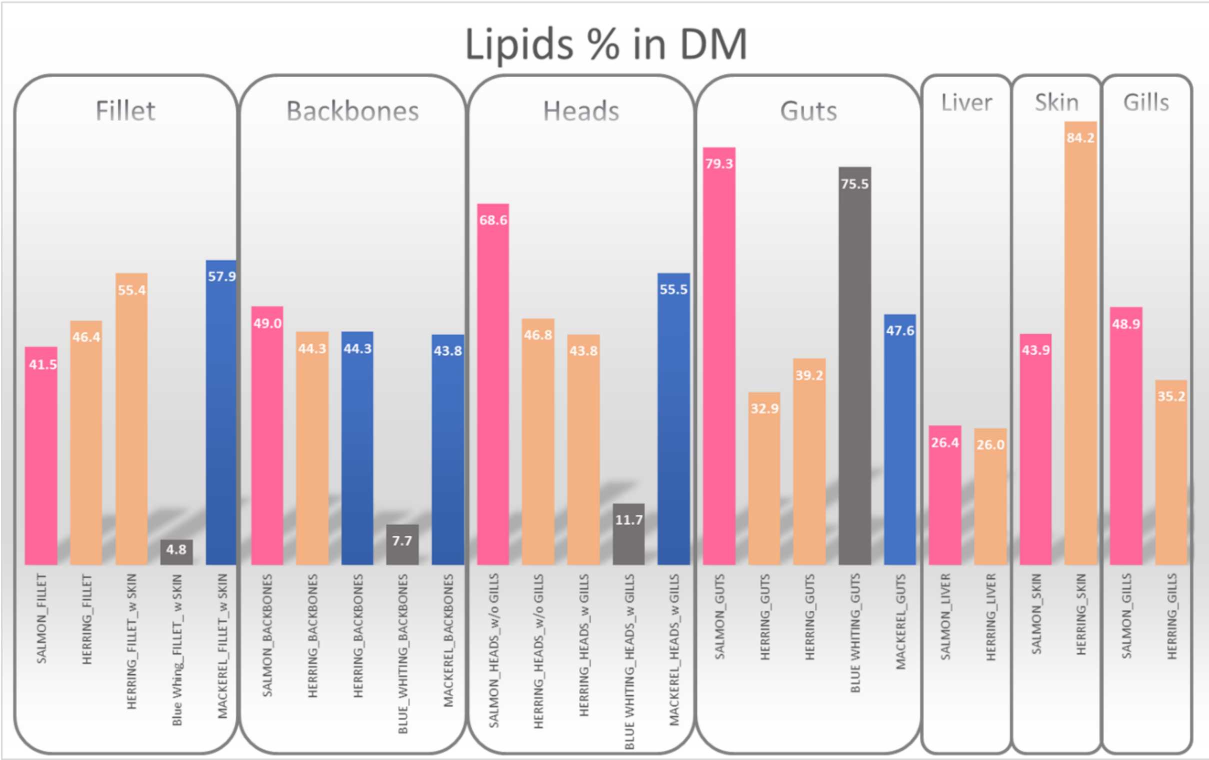

Figure 1.1.9 Lipid content in different fraction of different fish species in % dry matter (DM).

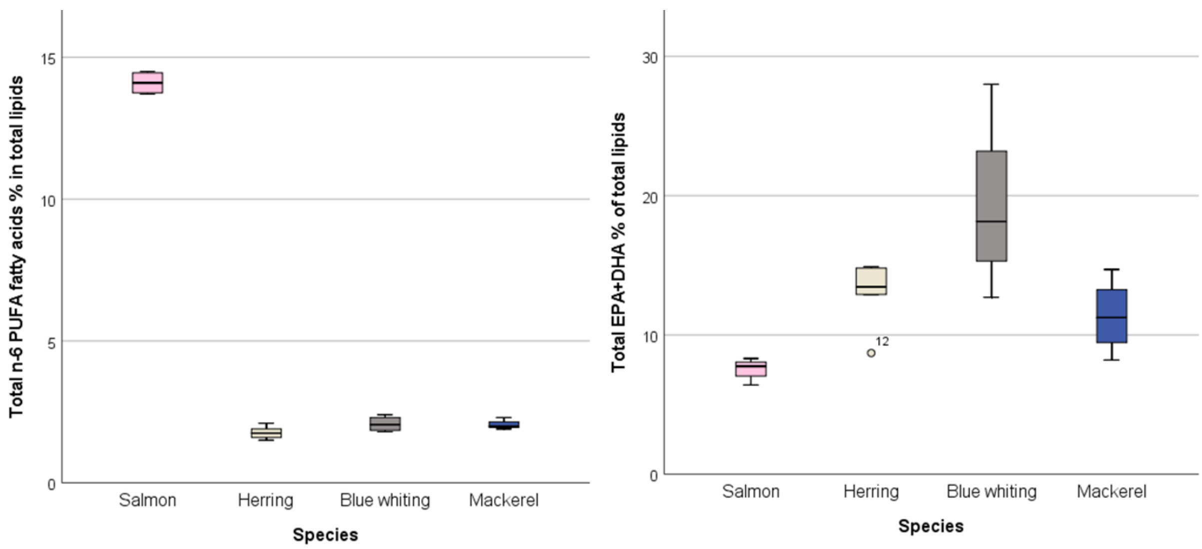

Figure 1.1.10 Total n-6 PUFA and EPA+DHA content in different fraction of different fish species in % dry matter (DM).

1.1.3.2 Sea bass and Sea bream

Moisture contents in sea bass and sea bream fractions (https://www.mdpi.com/2218-273X/10/2/232/htm) as analysed in the scope of the AQUABIOPRO-FIT project ranged between 37.61 and 72.71%, with fillet containing the highest amounts. Guts, head and skin are fish fractions with the highest fat content both in sea bass and sea bream, with values of 53.12, 35.27 and 17.10% in sea bass, and 34.11, 25.76 and 26.78% in sea bream, respectively. In terms of protein, the fillet and skin contained the highest amounts (higher than 20%), followed by gills, head, and fishbones. Differences among the two species studied and the different fractions were analysed also in terms of their mineral content (analysed as ash). Fishbone, gills, and head are the parts that contain higher ash amounts (greater than 5% of the total whole-body composition). Among the most abundant macro-minerals in fish are Ca and P, the content of which varies, however, depending on the analysed tissue. Higher Ca contents were observed in head and fishbone, while P is the predominant mineral in fillet and liver.

The content and composition of specific fatty acids are important for assessing the nutritional quality of fish. Lipid contents are highly variable both between and within fish species and fractions. Monounsaturated fatty acids (MUFA) are the predominant fatty acids in fish lipids (42--49 and 45-48 g/100 g of fatty acid methyl esters (FAMES) for sea bass and sea bream, respectively) followed by polyunsaturated fatty acids (PUFA) (27-32.0 and 31-34 g/100 g of FAMES for sea bass and sea bream, respectively) and saturated fatty acids (SFA) (25-26 and 20-218 g/100 g of FAMES for sea bass and sea bream, respectively).

The analysed concentrations of total polyunsaturated fatty acids (PUFA) (32 and 34 g/100 g of FAMES for sea bass and sea bream, respectively), n-3 PUFA (19 and 14 g/100 g of FAMES for sea bass and sea bream, respectively) and long chain n-3 PUFA (16 and 9 g/100 g of FAMES for sea bass and sea bream, respectively) were higher in the fillet. These differences were mainly due to the differences in C20:5n-3 (eicosapentaenoic acid or EPA) and C22:6n-3 (docosahexaenoic acid or DHA) contents, which are the main recognised health promoting fatty acids in fish oils.

The amino acid profile of sea bass and sea bream fillet and side stream fractions differed with the skin, guts, liver, and gills containing predominantly non-essential amino acids, whereas the fillet had higher contents of essential amino acids. The major amino acid in sea bass and sea bream tissues was glutamic acid, which represented around 10% of total amino acids. The ratio of essential/non-essential amino acids ranged between 0.64 and 1.08 and in the different fractions analysed, with fillet having the highest and gills the lowest value.

1.1.4 Utilization of side stream raw materials and products

The global population is expected to reach 9.5 billion by 2050, which will put a strain on our current food supply system, imposing the need to look at alternative and sustainable food sources, particularly proteins. Proteins and other bioactive ingredients from fisheries and aquaculture side streams and residues can contribute in filling the gap between the projected global demand for proteins and bioactive compounds in 2030 and the current production capacities.

Out of the total available fish side stream raw materials in Norway ca 80% (778 000 tons) are utilized towards ingredients (oils, proteins, supplements/premix) for animal feed and human consumption. However, rest raw materials mainly from whitefish about 170-180 000 have not been utilized as fish processing often occurs on board and the side streams are in these cases not brought to land.

From the raw materials available in all fishing sectors, whitefish has the largest amounts of unused residual raw material volumes. In 2018 it was estimated that 60% of whitefish fisheries processing side stream volumes were utilized, mostly landed from smaller coastal fleet vessels. Nevertheless, an increasing share is also taken care of by sea-going vessels.

There is almost 100% utilization of side stream raw materials in the pelagic sector, while in the salmonid aquaculture sector everything except the wastewater blood is used further in feed and food applications. Between 3.5 and 4.0% of the live weight of a salmon is blood, depending on method of slaughtering. With increasing slaughter volumes of salmon and trout, the amount of blood constitutes increasingly significant amounts that are moreover associated with fewer and larger processing locations. The wastewater blood amounts in 2018 were estimated at approx. 36,600 tons, but it is uncertain whether and when it will be economically possible to utilize this side stream biomass.

The side stream materials from the fishery and aquaculture industry are used in various applications. Some of these side streams are used directly for human consumption as fresh, frozen or dried seafood products (e.g. cod tongue, roe, dried heads, salmon bellies) in niche markets worldwide. Some of them are also used following further processing into higher value consumer products (protein extracts, marine oils such as cod liver oil used in nutritional supplement). The approximate figures of fisheries and aquaculture side stream utilization in Norway in 2017 are presented in Table 1.1.5.

Table 1.1.5 Fisheries and aquaculture side stream biomass applications in Norway (2017).

Processing	Demersal species	Pelagic	Aquaculture	Shellfish	Total	% of total RM
Fishmeal/Oil	18 250	136 624		900	155 774	21%
Silage	49 490	56 938	225 262		331 690	45%
Fur animal feed	27 078				27 087	4%
Hydrolysis of fresh guts			135 637		135 637	18%
Side streams for consumption (seafood)	61 313	1 196	14 338		76 847	10%
Edible oils and extracts	10 634				10 634	1.5%
Miscellaneous				2 500		0.5%
Total	166 756	194 758	375 237	3 400		

Source: Kontali AS

1.1.5 Nutritional supplements – Sensory properties

In many countries, research programs focused on discovering high-quality, sustainable protein sources are a top priority. The current main sources of protein are plants (57%), meat (18%), dairy products (10%), fish and shellfish (6%) and other animal products (9%). Side streams from fish and meat are such potential nutritious protein sources as they have a favourable amino acid composition, including essential amino acids. However, approximately 50% of the fish biomass in Europe is currently treated as waste, with protein rich side stream fractions, such as heads, bones, skins, and viscera, being presently underutilized. There are potentially more than 5 million tons of side stream biomasses available for higher-value applications. Today, these are mainly used within the feed sector as fishmeal and silage, in pet food or discarded. Thus, this is clearly an untapped opportunity to convert these fish side streams into valuable ingredients and products.

Aside from fish protein, protein hydrolysates have attracted attention from the food industry. Via an enzymatic process, the fish protein is converted into smaller peptides containing 2-20 amino acids. These amino acids and bioactive peptides act as antioxidants and antimicrobials as well as playing a role in controlling hypertension and immunomodulation. Potential other applications for fish protein hydrolysates are improving the nutritional value of formulations for the elderly or sports nutrition or improving the functional properties such as solubility, gelation, water holding capacity, emulsifier, or foaming.

Fish proteins can potentially be utilised in nutritional supplements as Fish Protein Concentrates (FPC), fish hydrolysates, bioactive fish peptides (gelatine and collagen) and other fractionated bioactive compounds found in fish (e.g. terpenoids, steroids, enzymes, alkaloids, fatty alcohol esters and glycolipids).

Fish oil is a major source of the omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which play an important role in regulating body functions and health. Lipids can be separated from fish side stream biomass and utilised in omega 3 nutritional supplements for humans that seek to improve their cardiovascular health.

1.1.5.1 Sensory properties of marine nutritional supplements

Protein hydrolysates and stick waters (discussed in Unit 1.4) are essentially aqueous phases composed of peptides and free amino acids, along with small water-soluble components present in the substrate. Small water-soluble components in the raw material will be present in the water phase and can influence the tastes and flavors of the final products. These components include NaCl and other mineral salts, nucleotides, non-protein amino acids, biogenic amines and possibly small amounts of lipid oxidation products. Moreover, marine fish tissues contain the nitrogenous compound trimethylamine oxide (TMAO). Fish contain relatively high amino acids and amines, including TMAO, in their blood which aids their increased osmoregulation needs resulting from living in hyperosmotic water environment (approx. 3% salt content). TMAO can be converted to trimethylamine (TMA) during bacterial spoilage, responsible for the unpleasant “fishy” smell of stale products.

The formation of bitter peptides generated in the hydrolysis process may pose a challenge in the production of commercial protein food products. Bitter taste is generally ascribed to small hydrophobic peptides, where both presence and position of hydrophobic amino acids are important for the bitter taste sensation. Moreover, it has been suggested that the formation of bitter taste is solely dependent on the chosen hydrolytic enzymes (proteases) and less influenced by the raw material. Thus, a proper choice of protease(s) and downstream processing conditions are important for the final taste properties of protein hydrolysates.

The most common tastes and flavors attributed to fish protein products are listed in Table 1.1.6. Even though bitter taste may be reduced by proper choice of processing conditions, the remaining tastes and flavors of a protein product are not dependent on enzyme and rather on raw material composition and quality, and ascribed to small molecules <200 Da. Thus, by introducing nanofiltration technology it may be possible to remove most of these molecules and consequently reduce the overall taste intensity.

Table 1.1.6 Sensory attributes commonly used to describe fish protein products

Attribute	Description of tastes related to the attributes
Flavor intensity	Strength of all flavors in the sample
Sweet taste	Basic sweet taste (sucrose)
Salt taste	Basic salt taste (sodium chloride)
Sour taste	Basic sour taste
Bitter taste	Basic bitter taste
Umami taste	Basic umami taste
Acidic flavor	Related to a fresh, balanced taste from organic acids
Metallic flavor	Related to taste of metal (ferrous sulphate)
Sea flavor	Related to taste of fresh, salty sea
Fish flavor	Taste of boiled white fish
Trimethylamine flavor	Taste of trimethylamine
Cloying flavor	Non-fresh, nauseating flavor
Rancid flavor	All rancid flavors (grass, hay, stearin, paint)
Fullness (mouthfeel)	Textural properties related to flow resistance
Astringent (mouthfeel)	Related to complex feeling of contractions and dryness of the mouth
Fatness (mouthfeel)	Surface textural property related to perception of fat in a product

1.1.5.2 Sensory analysis

Sensory evaluation is a science of measurement that applies principles of experimental design and statistical analysis to the use of human senses. A vast array of sensory tests is available, where both trained judges and untrained consumers can be used. The three types commonly used sensory testing are listed in Table 1.1.7.

Table 1.1.7 Test methods in sensory evaluation

Question of interest	Test	Panelists
Are the products different?	Discrimination	Screened for sensory acuity, oriented to test method. Trained/untrained judges.
How do the products differ in sensory characteristics?	Descriptive	Screened for sensory acuity. Usually highly trained judges.
How well are the products liked?	Affective	Screened for product acceptance. Untrained judges.

The description of the tastes and flavors present in different food products are often assessed by descriptive analysis by an expert sensory panel. Expert sensory panel is a specific number of trained sensory evaluators that perform simultaneously blind tests in the same product or series of products in order to establish often in a comprehensive and detailed way their sensory characteristics which can be related to respective sensory qualities of competitive products and consumer preferences. Descriptive sensory analyses allow the sensory scientist to obtain complete sensory descriptions of products and identify underlying ingredients, process variables and attributes that are important for the products. Descriptive sensory analyses require a trained panel with high a degree of commitment and motivation. The training phase begins with the development of a common language that comprehensively and accurately describes the product attributes.

There are several different methods of descriptive analyses, but most frequently generic descriptive analysis is used. This method combines different approaches from all methods of descriptive analyses. In a generic descriptive analysis, the samples are evaluated by a trained panel (8-12 judges) guided by a panel leader. The analysis is generally performed in the following stepwise procedure:

- ✓ **Attributes are chosen**: Attributes relevant for the products are chosen and discussed by the panelists.
- ✓ **Panelists are calibrated**: Samples with variance in the sensory attributes tested are used to calibrate the judges of the potential variance in attribute intensity.
- ✓ **The test is performed**: Samples are served in randomized order and the judges scale the chosen attributes on an unstructured line.
- ✓ **The results are analyzed**: Statistical evaluations of the results are performed. Analysis of variance (ANOVA) and multivariate techniques are most commonly used to evaluate sensory data. The panelists' performance, repeatability and reproducibility are validated through statistical software.

Projective mapping (or Napping) is a projective method which provides information about the overall similarities and dissimilarities among a set of products. Assessors are asked to try the samples and locate them on a sheet of paper according to their differences and similarities using their own words. The sheet of paper should have a rectangular shape (60x40 cm) and consequently, the X-axis become visually more important than the Y-axis. Thus, the assessors will prioritize the reasons why they perceive the stimuli differently. The idea of napping is to collect through the positioning of the samples, the first and second "reason" why they are perceived differently. Figure 1.1.11 gives an illustration of two potential napping- sheets from the same products, where the products are separated based on shape or color. The samples could also have been separated based on size.

Spectrum analysis and ranging tests are useful for evaluating single sensory attributes, such as bitter taste, where products are given scores (Spectrum) or ranked based on the desired attribute. Regardless, depending on research question, several methods for sensory evaluation of food stuffs are available.

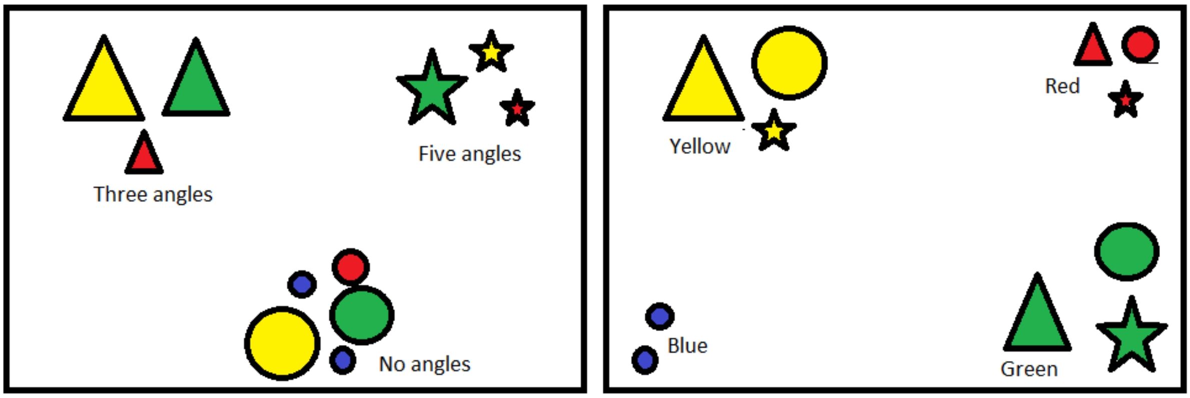

Figure 1.1.11 Examples of projective mapping where products are separated based on angles (left) and colour (right)

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Course 1.2: Fish proteins and protein hydrolysates - products and applications

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1.2.1 Fish protein products

Current fish protein products that can be found for human consumption are:

Fish Protein concentrates

Fish Protein concentrates (FPC) are defined as any stable fish preparation intended for human consumption where the protein is more concentrated than in the original fish source. The protein content in such products typically is in the range of 65 to 80%. The Food and Agriculture Organisation of the United Nations (FAO) defines FPC according to three classifications:

- ✓ Type A: A virtually odorless and tasteless powder having a maximum total fat content of 0.75 per cent.
- ✓ Type B: A powder having no specific limits as to odor or flavor, but definitely having a fishy flavor and a maximum fat content of 3 per cent.
- ✓ Type C: normal fish meal produced under satisfactorily hygienic conditions. Fish meal is a commercial product made from fish raw materials and species not usually not destined for human consumption, commonly used as farmed animal feed ingredient, mostly in aquaculture. Fishmeal is made from whole fish or side streams fractions, such as bones, skins, guts, and trimmings, from fisheries or aquaculture. Fish meal powder or cake are produced by cooking of the fish raw materials, separation of press cake and water/oil phases (stick water and fish oil), drying and dewatering of the different fractions and mixing back the dry fractions to a meal.

Type A products come in a powder form that can be incorporated in other foods such as bread, biscuits, soups, and stews at a level that does not affect their normal taste and texture. Good results have been obtained in macaroni products, milkshakes, dietetic fruits, and cereals. Examples of protein rich products from salmon filleting value chain can be found in here: http://www.fao.org/3/a1394e/a1394e.pdf .

Fish protein hydrolysates

Fish protein hydrolysates are proteins that are enzymatically broken into peptides of various sizes. The hydrolysis process makes the protein more digestible by increasing the solubility of peptides. However, the hydrolysis process releases bitter compounds, ascribed to small peptides containing hydrophobic amino acids, which makes their consumption difficult. Amino acids are grouped according to their side chains. Those with hydrophobic side chains are glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), methionine (Met), and tryptophan (Trp). The hydrophobic side chains are composed mostly of carbon and hydrogen and tend to be repelled from water, which has implications for the proteins' tertiary structure. Having said that, however, it has been shown that fish protein hydrolysates can be incorporated into food products and used as flavour enhancers.

1.2.1.3 Bioactive fish peptides

Bioactive fish peptides (https://core.ac.uk/download/pdf/91281072.pdf) are specific protein fragments that have a positive impact on body functions or conditions and may influence health. They are isolated from the protein hydrolysates and consist of between 3 and 20 amino acid residues, with their biological activities based on molecular weight. There are not many products containing bioactive peptides of fish, but those that exist are more related with anti-stress function. While there are products on the market, their health claims have not been approved by EFSA (European Food Safety Association).

Other bioactive compounds

Other bioactive compounds that are found in fish include terpenoids, steroids, enzymes, alkaloids, fatty alcohol esters and glycolipids.

Commercial products

As mentioned above, the number of fish protein products on the market has been limited in part due to their strong fishy smell and taste. The characteristic fishy odour of fish products derives mainly due to the presence of trimethylamine ($N(CH_3)_3$; TMA). The oxidised form of TMA trimethylamine oxide (TMAO) is practically odourless. However, there are some companies that sell various products, in capsule, powder and paste form, as shown in Table 1.2.1. It is worth noting that some companies refer to the fact that their products have a neutral smell and taste (Originates Biomega), while others highlight the fishiness and flavour of the products (Essentia and Biomega). Moreover, Firmenich, a major player in the flavour and fragrance industry, have recognised the importance of sustainable sources of protein, which includes marine ingredients, and the role they play in health and wellbeing. As a result, they have made a commitment towards the development and commercialisation of new ingredients from sustainable resources, such as proteins from fish side streams.

Table 1.2.1 Fish protein and hydrolysate products currently on the market

Company	Size	Country	Market distribution	Product name	Form	Description
Aroma Ltd	SME	New Zealand	Business to Business (BTB)	Protein-PLUS	Powder	From 100% fish meat, processed at low temperature freeze-drying tor to preserve key protein components. Dietary supplement.
Biomega	SME	Norway	BTB	Biomega®	Powder	Contains small proteins, peptides and amino acid form Salmon. Neutral taste and smell. Beneficial effects on blood pressure
Essentia	SME	Denmark	BTB	peptides	Paste	Flavour based on meat and fish proteins
Huzhou Zhenlu Biological Products	SME	China	BTB	Biomega®	Powder	Contains small proteins, peptides and amino acid form Salmon. Fishy taste and smell.
Copalis Copalis	SME SME	France France	BTB / BT Consumer BTC)	Salmigo	Powder	Marine bioactive peptide which helps to reduce fat deposition and to improve restitution.
				Nutripeptin®	Powder	
Firmenich	Large	Switzerland	BTB	Protizen		Marine bioactive peptide which helps to relief stress
Essentia Firmenich	SME Large	Denmark Switzerland	BTB BTB	Probase™		Flavour based on meat and fish proteins
				-	Powder	Hydrolysed protein powder
Hofseth Biocare	Large	Norway	BTB	Progo	Capsules	Hydrolysate salmon protein with 98 % of digestibility. For athletes to recover muscle mass after sport.
Huzhou Zhenlu Biological Products	SME	China	BTB	Fish protein powder		Fish protein powder. Suitable for human consumption, to achieve beneficial effect on skin.
Originates	SME	USA	BTB	Romega®		Herring caviar protein with neutral smell and odour. Contains EPA and DHA
Seagarden	SME	Norway	BTB/BTC	Fish Complex	Capsules/ Powder	Fish powder from 3 types of fish. Produced from whole fish.
Seagarden	SME	Norway	BTC/BTB	Cod Protein	Capsules/ Powder	Cod protein from wild-caught cod filets. Fishy taste and smell.

Market size of fish protein and hydrolysate products

Information regarding the market size of the fish protein and protein hydrolysates industry was difficult to find, in part due to the fact that there are not many products on the market, but also that the market reports often consider the protein and/or hydrolysates markets as a whole. The global **protein ingredients market size**, which includes protein from animals, fish and plants, was valued at **US$25.62 billion (approximately €22.1 billion) in 2016** and is expected to witness a Compound Annual Growth Rate (CAGR) of over 7% from 2016 to 2025. In 2015, the USA was the major producer and consumer of protein products and this market is expected to continue growing, favoured by a change in consumer behaviour, resulting in an increase in protein intake instead of fat. Consequently, this has been translated into new products, such a functional foods or sport beverages creation. Europe is the second largest market but is less developed for protein products than in the USA. This is most likely due to cultural reasons but also a lack of consumer knowledge about the function and benefits of protein.

The market for protein products is driven by various factors, such as obesity and sports. Proteins may be substituted for fat and sugar in food products, which thus can be marketed to obese people as a nutritional and healthy product that may also help in reducing weight. The demand for such products is expected to grow as the levels of obesity continue to rise. Furthermore, there is an increasing consumer interest in protein products due to the evidence that they support body building (see Figure 1.2.1) and keep muscles healthy and active as people age (https://www.grandviewresearch.com/industry-analysis/protein-ingredients-market).

Barriers and opportunities

As mentioned previously, the market for fish proteins and protein hydrolysates is currently quite limited. This is in part due to smell and flavour, which constitute a major barrier for these products. Moreover, other protein sources, such as whey, soy, and wheat, are well consolidated and thus, present an additional barrier for introducing new protein sources on the market. Finally, it is apparent that consumers do not fully understand the function and benefit of proteins, which has also meant that the market has mostly been limited those who are more conscious of health and fitness.

Despite these barriers, there are also several opportunities for fish proteins to have a stake in the protein market. The fact that there is a relatively untapped source of proteins from fish side streams means that there is the possibility to increase the current protein production without the need to extend agriculture or aquaculture practices. This not only offers a more sustainable source, but also contributes to a circular economy. Fish proteins are also a nutritious source of amino acids, ensuring that consumers can obtain all the essential ingredients needed for their body to function. Moreover, bioactive peptides have beneficial functionalities and can be used as antioxidants, antimicrobials and anti-stress ingredients. Enzymatic hydrolysis also helps to reduce allergenicity, thus creating hypoallergenic hydrolysates. This may be of particular interest for consumers who have allergies or intolerances to other protein sources, such as whey, soy and wheat.

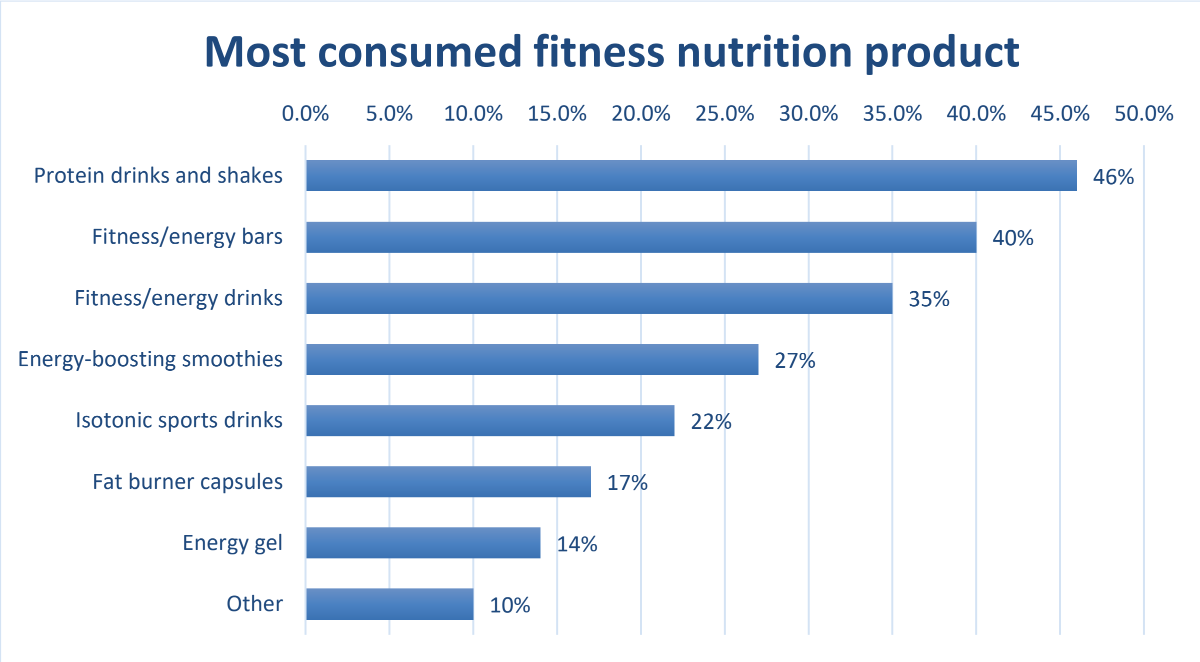

Figure 1.2.1 Most-consumed fitness nutrition products by consumers in 2016 (Statista Research Department 2016).

1.2.2 Fresh fish side stream products and applications

Fish mince

Minced fish is a comminute flesh produced by separation from skin and bones. Separation is a mechanical process (for producing minced fish) whereby the skin and bones are removed from the flesh. Bone separators working on different principles are available commercially, but the separator most widely used for fish is of comparatively simple design. The total yield of flesh of low bone content is higher than with filleting alone; up to twice as much can be recovered by separating flesh directly from headless gutted fish. When the fish is first filleted, an additional 8-12 % flesh can be separated from the filleting waste. Some people do not like fatty fish such as herring and mackerel partly because of the large numbers of small bones remaining in the fillets. Mince made from these fishes is almost free from bones and might therefore be more widely acceptable. Flesh from underexploited species, such as blue whiting, that are difficult to fillet efficiently (small size or awkward shape) can readily be removed in a bone separator. Mincing can increase the risk of oxidation due to membrane disruption, contact with metals and air. Mince spoils faster than fillets, mainly because the structure of the flesh is degraded during separation, and extra care must be taken to maintain good quality. Minced fish is also more easily denatured during freezing. Thus, fish used for making mince must be of very high initial quality, and processing has to be completed quickly, with emphasis on hygiene and low temperature.

Restructured fishery products

Restructured fishery products are products made from minced and/or chopped muscle and which are used, with or without ingredients, to make other products with a new appearance and texture (https://agris.fao.org/agris-search/search.do?recordID=US201400096810). For some time, there have been products in the form of fingers or other shapes intended basically for children's foods, which are covered in breadcrumbs or batter then frozen for use as fried products. Also, recent years have seen the development of a new generation of fishery products called analogues or substitutes, most of which mimic seafood or other high-value products. Such products are made essentially from surimi, which is ground, thoroughly washed and refined fish muscle. The reason for restructuring fish muscle is that the supply of high-quality fishery products is limited, and many are becoming exhausted because of severe overfishing. There are therefore not many options that do not entail the utilization of species that have not traditionally been commercialized either very much or at all. One of the chief advantages of restructured products is that the composition of the end-product can be modified by reformulation of the original product once this has been chopped or ground. In this sense, the process might be said to be one of eliminating some constituents or adding other new ingredients or additives. These ingredients or additives may be categorized as (a) favouring storage, (b) functional from a technological standpoint and (c) functional from a nutraceutical standpoint. There are several types of ingredients that perform more than one of these functions.

Fish burgers

A general protocol of preparing a given product once ingredients in the formulation are decided is as follows: Fish is headed, gutted, and viscera removed. Gutted fish is filleted mechanically or with knives. Fish fillets are first chilled at 2°C usually for 2–3 hours, and then frozen for up to four weeks depending on the constraints of the industrial process. One of the outcomes of this treatment is to harden the flesh, which improves the structural properties of fish meat in the final product. Following storage, fillets are minced using crushing and fine cutting with various choppers to yield homogenised mince. Preparations are made by blending appropriate amounts of fish meat with starch, non-starchy hydrocolloids, vegetables, dry fruit leathers, condiments, sucrose, etc. The homogenous mixture is stuffed in manually / automatically operated machines for pressing thus producing firm and free from air burgers of variable weight, height, and diameter. End products are stored under conditions of domestic freezing (about −20°C), preferably using bulk vacuum packaging, for quality control analysis (microbial growth, chemical composition, colour, etc.), and retailing. Fish burger, being a convenience food prepared out of comminute fish, spices, and starch, is an important delicacy in fast food trades and home preparations. Frying of fish burgers imparts an aromatic, savoury flavour to the traditional fish burgers and they are popular in mild heated lunch or dinner meals or simply eaten without heat treatment as a ready-to-eat (RTE) product. Different minced fish products from a variety of fish species are presented in Table 1.2.2.

Minced fish burgers of improved eating quality

In the development of processed fish products, it has often been used minced or filleted fish with additives, e.g., polyphosphates, which reduce drip loss especially in products made from thawed frozen fish. Spices can be added to improve taste according to market requirements. Fish muscle protein does not possess sufficient functionality to hold together a cohesive processed product, therefore are often corn flour or corn starch introduced to enhance the structure and cohesion of products made increasingly from a mixture of prime and inferior cuts of minced fish. Changing consumer expectations, with amplified focus over what people are eating is placing pressure on clean label products, using less additives in the products. People associate a diet with lifestyle choices and increasing personalization represent a continuation of this trend. High concentration of proteins is also requested as a healthy product for many consumer groups, e.g. for elderly people, patient groups and sports enthusiasts.

Table 1.2.2 Minced fish products (burgers) with different types of fish

Topic	Fish type	Reference
Freshwater Fish Burgers	Carp, Goldfish, Perch and Tench	(Branciari *et al.* 2017)
MA packaging of burgers	Sea bass (*Dicentrarchus labrax*)	(Danza *et al.* 2017)
Instrumental and sensory properties	Many types – fish burgers	(Kasapis 2009)
Transglutaminase on quality and gel properties	Silver carp	(Li *et al.* 2017)
Tuna Protein Isolates for Better Sensory Quality and Frozen Storage Stability	Silver carp	(Shaviklo *et al.* 2016)
Fish burger	Catla (*Catla catla*) Fresh water fish	(Vanitha *et al.* 2015)
Effects of Natural Extracts on the Quality Changes of Frozen Chub Burgers	Mackerel (*Scomber japonicus*)	(Ozogul & Ucar 2013)
Chemical, physical and sensory properties – various types of flour	Farmed Gilthead Sea Bream (*Sparus aurata*)	(Makri 2012)
The effect of mincing method on the quality of refrigerated whiting burgers	Whiting (*M. merlangus*, L. 1758)	(Kose *et al.* 2009)
Storage properties of refrigerated whiting mince after mincing by three different methods	Whiting (*M. merlangus euxinus*, N. 1840)	(Kose *et al.* 2006)

1.2.3 Market size, barriers, and opportunities

Commercialisation of fish based nutritional supplements face barriers mostly related to the smell and taste of such products. This topic is analysed in detail in another section of this training module (M1U1). Aside from the barriers, there are many opportunities. Firstly, the fact that fish industry side streams can be used for protein and oil extraction is a big opportunity as it is a cheap and sustainable source of raw materials. The health benefits of fish proteins are more and more appreciated by consumers and such products containing essential amino acids and bioactive peptides are attracting greater consumer trust. The same applies for fish oil, where an increased consumption of omega-3 fatty acids DHA and EPA is expected, as a means to mitigate heart disease and other illnesses. The fish collagen and gelatine market have also potential opportunities as consumer health awareness enhances the growth in the functional food and cosmetics (cosmeceuticals) markets. Increased evidence of the health benefits and the potential in disease prevention give also more opportunities for market development of trusted collagen sources.

The category of fish proteins and protein hydrolysates includes Fish Protein Concentrates (FPC), fish hydrolysates, bioactive fish peptides and other bioactive compounds found in fish. The global protein ingredients market size, which includes protein from animals, fish, and plants, was valued at US$25.62 billion (approximately €22.1 billion) in 2016 and is expected to increase 7% annually from 2016 to 2025. The market for protein products is driven by various factors, such as obesity and sports. Proteins may be substituted for fat and sugar in food products, which thus can be marketed to obese people as a nutritional and healthy product that may also help in reducing weight. The demand for such products is expected to grow as the levels of obesity continue to rise. Furthermore, there is an increasing consumer interest in protein products due to the evidence that they support body building and keep muscles healthy and active as people age.

Fish oil is a major source of the omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which play an important role in regulating body functions and health. It is reported that there is a growing demand for fish oil as a nutritional supplement, due to low consumption of omega-3 fatty acids in Western diets. Within this category, 30 products in the market have been identified. The global fish oil market is expected to increase from US$2.22 billion (€1.91 billion) in 2016 to reach US$3.69 billion (€3.18 billion) in 2025.

Collagen products that can be found in the market are divided in three categories: native collagen, gelatine, and collagen peptides. There are 33 competing products already launched in the market. In 2017, fish collagen dominated the market due to the presence of proteins, essential fatty acids, minerals, and vitamins. Marine collagen market is estimated at US$620.3 million for 2018 and is expected to increase at US$897.5 million by 2023.

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Course 1.3: Fish collagen and marine oil-based supplements - products and applications

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1.3.1 Fish collagen

Collagen is a general term to define a group of structural proteins of the extracellular matrix, organized in a fibrillar arrangement. Collagen is the main component of connective tissue (e.g. in cartilage, bones, tendons, ligaments, and skin). Collagens are the most abundant high molecular weight proteins in both invertebrate and vertebrate organisms, including mammals and fish. Collagen consists of amino acids bound together in a triple helix. Depending upon the degree of mineralization, collagen tissues may be rigid (bone), compliant (tendon), or have a gradient from rigid to compliant (cartilage). Bovine collagen is of Type I and Type III while fish collagen is composed of collage Type I.

Collagen is the most important protein produced by the human body, it is mainly formed by the amino acid glycine (33%), proline and hydroxyproline (22%) (primary structure) in a triplex helix which is formed by three α chains. Each alpha chain is composed of approx. 1014 amino acids with a molecular weight around 100 kDa. These chains are coiled into a left-handed helix with three amino acids per turn (secondary structure). The chains are twisted around each other into a triple helix to form a rigid structure (tertiary structure). The super helix represents the basic collagen structure (quaternary structure). This collagen structure is very stable because of the intramolecular hydrogen bonds between glycine in adjacent chains.

Nearly 28 types of collagen have been identified, but collagen type I is the most common in skin, bone, teeth, tendon, ligaments, vascular ligature, and organs. Collagen type II is present in the cartilages. For collagen type III, the skin, muscle, and blood vessels are the most common sources of this protein. Type IV has been reported in the epithelium-secreted layer of the basement membrane and the basal lamina. Collagen type V is one of the principal components of cell surfaces and placenta. Collagens are different according to their α-chain composition, depending on the repeat and length o he Gly–X–Y amino acid repetition, with and without interruptions, also the occupation of the X and Y positions by proline and its hydroxylated form, hydroxyproline, respectively.

Native collagen type I can be extracted from different fish tissue such as bones, skin, and scales or waste fish by-products, or other sources such as chicken, bovine, duck, and rabbit skin. Extraction can be carried out by an acid or alkaline treatment. Extraction under acid treatment is usually applied for extraction of collagen type I from tissues of porcine or fish skin origin. Acetic acid is the most common reagent for collagen extraction. The concentration of this acid will affect the final pH value changing the electrostatic interaction and structure. It also determines the solubility and extraction capacity from animal tissue. A combination of both acidic and enzymatic treatment produces a higher and more efficient collagen extraction process. Pre-treatment conditions, dialysis, and source of extraction are the main factors that determinantal collagen characteristics such as molecular weight, amino acid composition, and molecular structure.

Products and applications

Collagen is a high molecular weight structural protein that is found in the extracellular matrix of connective tissues including the tendons, ligaments, bones, skin, cartilage and teeth. Depending on the location in the body, these fibrous proteins have different mechanical requirements, sometimes imparting stiffness and support while in other cases storing mechanical energy through their elasticity. Gelatin is the partially hydrolysed form of collagen and consists of a mixture of peptides and proteins.

There are five most common types of collagen (https://en.wikipedia.org/wiki/Collagen)

- ✓ Type I: skin, tendon, vasculature, organs, bone (main component of the organic part of bone)
- ✓ Type II: cartilage (main collagenous component of cartilage)
- ✓ Type III: reticulate (main component of reticular fibres), commonly found alongside type I
- ✓ Type IV: forms basal lamina, the epithelium-secreted layer of the basement membrane
- ✓ Type V: cell surfaces, hair, and placenta

Collagen has mostly been sourced from porcine and bovine origin, but recombinant production and marine sources are also becoming more prevalent. In particular, the outbreak of bovine spongiform encephalopathy (BSE) in the 1980s and religious constraints have driven the demand for alternative sources. Moreover, marine collagen can be obtained from a variety of sources, such as fish side streams as well as marine sponges or jellyfish. The skin, scales and bone of fish are a source of Type I collagen, which is the most abundant form of collagen and is commonly used in healthcare and food products. However, Type II collagen can also be isolated from fish cartilage and is often used for medicinal purposes. With the increasing demand for personal care and health supplements, collagens are now widely used as ingredients in nutraceutical, cosmetics and pharmaceutical products.

Recent research into the effect of collagen on various body functions has shown that collagen has several benefits. These include the improvement in elasticity of skin by stimulating the production of collagen by the skin cells themselves, thus counteracting aging-related changes and potentially leading to a more youthful and vibrant skin, antioxidant properties that may prevent and even repair skin damage caused by environmental factors and positive effects on tendon flexibility, ligaments stability, muscle and bone integrity and bone metabolism.

The global demand for collagen and gelatine from the industry throughout the world is considerable and still rising. Marine collagen is often used in a purified form in cosmetic creams and gels. In sunscreen lotions, collagen can be used as a functional ingredient to protect the skin against UV damage and promote skin regeneration. Collagen can also be used as a supplement, to reduce the effects of ageing and maintain skin, hair, nails and body tissues by stimulating the synthesis of new collagen. Aside from the health benefits, collagen is also used to improve the rheological properties of sausages and as sausage casings, edible films and as a barrier to oxygen.

Gelatin is primarily used in the food and pharmaceutical industries. In food products, gelatin is used as an additive to enhance texture, water-holding capacity, stability and clarification. For bakery products, gelatin is used extensively as a setting agent, stabilizing substance or foam producing material in pies, breads, and cakes. Almost 10% of the edible gelatine is used in the pharmaceutical industry for capsules and emulsions. One of the most appreciated properties of gelatine is its gel-forming capacity. Fish gelatine, however, does not tend to form strong gels but may therefore have a market when non-gelling gelatine applications are required (i.e. shampoo with protein).

Competing products

On the market collagen products can be find in three different forms (https://www.peptan.com/):

- ✓ **Native collagen**, which is composed of a large triple helix chain of amino acid and strengthens the structure of the body.
- ✓ **Gelatin**, which is obtained by the partial hydrolysis of collagen. As gelatin dissolves in hot water and gels when it is cooled, it is frequently used in culinary applications, such as gummies, candies, jellies, sauce thickeners, etc. Gelatin also has a role in pharma applications, often used as excipient for making soft and hard capsules.
- ✓ **Collagen peptides**, small bioactive peptides obtained by enzymatic hydrolysis of collagen that are highly bioavailable. They act as building blocks, renewing body tissues, such as skin, bones and joints and are also recommended for improving skin moisture as well as hair and nail strength. Two examples of these products are shown in Figure 1.3.1.

Figure 1.3.1 Examples of hydrolysed collagen products that are currently on the market.

Collagen is also used as an active ingredient in functional foods, as shown in Figure 1.3.2.

Figure 1.3.2. Food products containing collagen that are currently on the market.

Given the various benefits and functionalities of collagen and gelatine, there are several companies selling these products. While the market for porcine and bovine sources is well-established, consumer health and religious preferences are also paving the way for marine-based products.

Information about the collagen and gelatine products that are currently on the market can be found in Table 1.3.1.

Table 1.3.1 Collagen and gelatine products that are currently on the market

Company	Size	Country	Market distribution	Product Name	Format	Description
Aroma Ltd.	SME	New Zealand	Business to Business (BTB)	Marine Collagen - Nz	Capsules/ Powder	-
Bega Bionutrients	SME	Australia	Private consumers	Collyss™	Powder	Marine collagen peptide from skins of sustainably caught fish.
				Cartidyss™	Powder	Marine Cartilage hydrolysate powder produced form sustainably
BioCare Finland	SME	Finland	Private Client	Calcium + Collagen	Capsules	Hydrolysed collagen and calcium from Salmon
Certified Nutraceuticals	SME	USA	-	Pure Marine Collagen	-	From fish skin collagen through an enzymatic hydrolysis process.
Connoils	SME	USA	BTB	Hydrolysed Collagen	Powder	From Bovine, porcine or fish
Copalis	SME	France	BTB	Collagen HM	Powder	Marine Hydrolysed collagen
Gelita	Big Company	Germany	BTB	Gelatin	Various	Produced from pig skin and bones
				Pargel®	Powder	Collagen for emulsifying and texturizing meat products
				Verisol	Powder	Collagen peptides for skin's collagen metabolism
				Fortigel	Powder	Collagen peptides to promote growth of cartilage tissue

				Fortibone	Powder	Collagen peptides that stimulates synthesis of bone collagen matrix
				Bodybalance	Powder	Collagen peptides to decrease fat mass and increase body mass in body composition
				Peptiplus	Powder	Collagen peptides to produce protein drinks and shakes
				Petagile	Powder	Collagen peptides for regeneration of joint cartilage of animals
Health aid	SME	United Kingdom	Private Client and Farmacy distribution	Radiance®	Tablets	Hydrolysed marine collagen, vitamin C and Zinc
Huzhou Zhenlu Biological Products	SME	China	BTB	Fish Collagen	Powder	Obtained from fish squama.
Kenney and Ross	SME	Canada	Private Client	Hydrolysed Collagen	Powder	Protein additive in nutraceutical, cosmetic, or food applications
				Fish Gelatin	Dried/Liquid	Similar characteristics to animal gelatin, does not gel at room temperature
Minervalabs	SME	United Kingdom	Private Client	Gold-Collagen	Liquid	Drink of hydrolysed collagen and some other ingredients
Niche4health	SME	Netherlands	Private Client/ BTB	Hydrolysed Collagen	Powder	Hydrolysed collagen with Peptan®

<u>**Nitta Gelatin**</u>	SME	Japan	BTB	Wellnex-Collagen	Powder	Collagen peptides from porcine, bovine and fish origin
<u>**Norland products**</u>	SME	USA	BTB	Hipure Liquid Gelatin	Liquid	Purified gelatin with similar properties to animal protein but easier handling characteristics
				Fish Gelatin	Powder	Manufactured by hydrolysis of collagen. Produced from the skin of deep water fish such as cod, haddock and pollock.
				Hydrolysed Collagen	Powder	Produced from the skin of deepwater ocean fish (Cod, Haddock and pollock). Hydrolysed with acid and then further hydrolyzed with a food grade enzyme to further break down the molecular weight.
				Fish Glue	Liquid	Organic fish glue with high initial tackiness, capacity of easy re-activation of adhesive by water.
				Seasource™	Capsules	Pure fish collagen made from wild-caught deep-sea fish
<u>**PLT Health Solutions**</u>	SME	USA	Private consumers	Collactive Ô	Capsules	Composed of collagen and elastin peptides. Is a sustainably produced ingredient provided by CTPP-Copalis
				Caviar (Caviar, Collagen, Hyaluronic Acid)	Tablets	Nutricosmetic with caviar, hydrolysed collagen from fish (Peptan ®), hyaluronic acid, Vitamin C and Vitamin A

Power Health Greece	SME	Greece	Private Client and Farmacy distribution	Marine Collagen	Tablets	Hydrolysed collagen form fish (Peptan®) Vitamin C and copper
Rousselot	Big Company	USA	BTB	Gelatin	Powder	Extracted from animal raw materials (Bovine and Porcine)
				Peptan®	Powder	Collagen peptides obtained from fish, porcine or bovine
Seagarden	SME	Norway	BTB/BTC	Nutricoll	Powder	Collagen peptides from wild-caught cod. Collagen is made of fish skin. Pure or with Vitamin C.
Waitaki Biosciences	SME	New Zealand	-	Marine Collagen	Powder	Manufactured from commercial sidestreams or non-endangered fish species that are sustainably harvested in New Zealand
Weishart	SME	Germany	BTB	Naticol	Powder	Naticol® is a type I fish collagen peptide, produced by enzymatic hydrolysis. It is a mixture of peptides with various molecular weights including some dipeptides and tripeptides. It is made from fish skin and scales.

Market size

In 2017, fish collagen dominated the market due to the presence of proteins, essential fatty acids, minerals and vitamins. As a result, nutraceuticals are one of the main applications for marine collagen. The main market for marine collagen was Asia-Pacific. The demand is high in Japan and China, with the latter being one of the major manufacturers and exporters for marine products. In the EU, the market is driven by an increased use of health supplements, with Germany and UK leading the way.

By the end of 2018, the marine collagen market is estimated to be valued at **US$620.3 million** (approximately €534 million) and is projected to reach **US$897.5 million** (approximately €772 million) by 2023 with a CAGR of 7.7%. The prices vary from €10 to €15/kg depending on the traceability, degree of hydrolysis, taste and purity. The market growth is triggered by the demand for more nutritious and enhanced sensory attributes in food products. Greater health and wellness awareness have also led to increased consumption of marine collagen for its health benefits and better bioavailability compared to other forms of collagen. Type I collagen is expected to have the highest growth over the coming 5 years, with the main sources of marine collagen arising from skin, scales and muscles of fish.

The competition in the global collagen market is intense, which is a reflection of the great number of competitors who have a strong platform as raw material suppliers. Gelita (DE) and Rousselot (FR) are two of the more prominent companies operating in the global collagen market (https://www.beautypackaging.com/contents/view_breaking-news/2018-04-19/marine-collagen-market-worth-8975-million-usd-by-2023/).

Barriers and opportunities

For collagen and gelatine, there are well-established markets for ingredients based on porcine and bovine sources, with large companies such as Gelita and Rousselot selling a wide range of products. Given this level of competition, it may be difficult for new products and new players to enter into the market. Moreover, high import duty and high processing costs are major challenges faced by the marine collagen market. While collagen is widely purported to be beneficial to one's health and wellbeing, there are no official health claims approved by EFSA to support this. Thus, it is difficult to market the products based on their health benefits. As a result, consumers are not fully aware of the advantages of collagen and collagen peptides.

Despite these potential barriers, there are also many opportunities for fish collagen. The BSE health scare along with religious and dietary factors have resulted in a demand for collagen from other sources. Moreover, the increase in consumer preferences for healthy foods is expected to result in the growth in the functional food and cosmetics (cosmeceuticals) market. As there is currently limited availability of marine collagen products, this makes the market more favourable for suppliers. The properties of marine collagen are also different to those from porcine and bovine sources, offering greater bioavailability and non-gelling behaviour at room temperatures and thus an alternative application range in cosmetics and food. Plus, as more evidence of the health benefits comes to hand, this will also create opportunities for collagen products to be used in the prevention of diseases, such as arthritis, osteoporosis and sarcopenia. The larger gelatine suppliers had previously expressed an interest to collaborate with Danish and Norwegian companies to source marine collagen, particularly for beauty products. Finally, the Marine Stewardship Council certified sustainable seafood produced in Europe is of interest for customers who are looking for trusted sources of collagen.

1.3.2 Marine oil-based supplements - products and applications

Fish oil is a major source of the omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which play an important role in regulating body functions and health. While there is strong evidence the regular consumption of omega-3 fatty acids can reduce the risks of heart disease, high blood pressure, arrhythmia, arthritis, and diabetes, they may also be beneficial in reducing the incidence of cancer, depression, psychosis and multiple sclerosis. Pregnant women are also encouraged to consume fish oil as EPA and DHA are important in foetal development, especially for the brain and retina. As a result of the scientific evidence regarding health benefits, EFSA has approved the health claims for EPA and DHA and recommends a daily intake of 250 mg to maintain normal cardiac function.

Oily fish, such as salmon, mackerel, tuna and herring, are major sources of omega-3 fatty acids. However, high levels can also be found in the oils from microalgae (such as *Schizochrytium* and *Nannochloropsis*), krill, seeds and nuts. DSM reports that they only use side streams from the fishmeal or edible canning industries as their source of fish oil, thus no fish are caught specifically for the production of oil. The main applications of fish oil are for aquaculture and human consumption. The fish oil for human consumption represents 18% of the market (Figure 1.3.3) and includes mostly supplements and functional foods. Fish oil products, which are usually sold as capsules to consumers, contain both DHA and EPA, although the amount of each and the ratio often vary. Products may also contain other ingredients, such as omega-6 fatty acids (mostly found in nuts and seed), coenzyme Q10 (ubiquinone, CoQ10), and vitamins (D_3 and E), to differentiate themselves on the market (see amazon.com for more details).

There is a growing demand for fish oil as a nutritional supplement, as more consumers become aware that the Western diets are deficient in important omega-3 fatty acids. However, most of the fish oil is used in aquaculture (75%, see Figure 1.3.3), as, together with fishmeal, they are the most nutritious and digestible ingredients for farmed fish. However, it has been reported that the decline in fishmeal and fish oil production, together with higher prices, has seen then need to look for alternative sources, such as fish side streams and krill. Moreover, these ingredients are being used more selectively and for specific stages in the production, namely as part of the hatchery, broodstock and finishing diets (https://www.businesswire.com/news/home/20180223005319/en/Global-Fish-Oil-Market-2018-Analysis-Forecasts; https://www.seafish.org/media/publications/SeafishFishmealandFishOilFactsandFigures_201612.pdf)

The other uses of fish oil (7%, see Figure 1.3.3) include animal nutrition and pet food and pharmaceuticals.

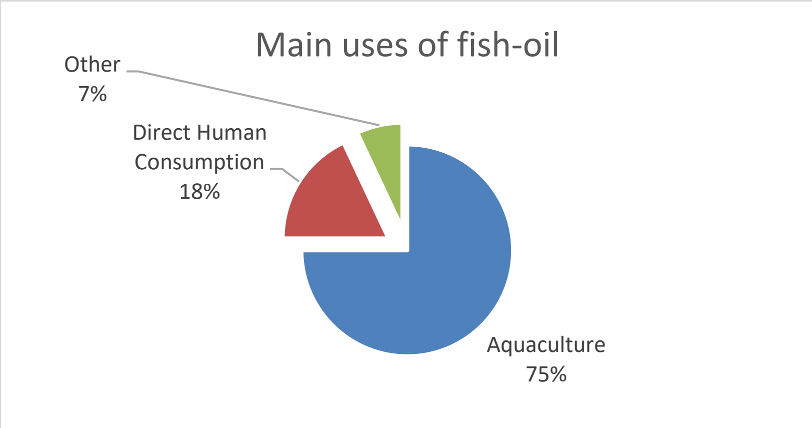

Figure 1.3.3 Use of fish oil by market in 2016 (Bachis 2017).

Competing products

The fish oil market is well established and involves big industrial players, including the AQUABIOPRO-FIT consortium partner PELAGIA, following the acquisition of a leading global brand of high-quality, marine-based omega-3 EPA/DHA fatty acid concentrates EPAX®, and others such as BASF, DSM and Croda. The products are mostly distributed in liquid or capsule form, although microencapsulation techniques have also been used to produce the fish oil in a powder form (DSM). Despite the presence of the larger companies, there are SMEs who have also entered the market, which shows that there are still opportunities for both large and small companies to have a share in this market (Table 1.3.2).

Table 1.3.2 Fish oil products currently on the market.

Company	Size	Country	Market distribution	Product Name	Format	Description
Alaskomega©	SME	USA	BTB	Alaskomega©	Capsules	Different concentrations of EPA and DHA could be chosen by consumer
Arista Technologies	SME	USA	BTB	Norwegian Cod Liver	-	Available fish-oils with different kind of concentrations of EPA and DHA, and also oils from more than 50 varieties of plants.
				Cod Liver Oil Concentrates	-	
				Fish Liver Oil	-	
				Krill Oil	-	
				Salmon Oil	-	
Aroma NZ	SME	New Zealand	BTB	Greenshell™ Mussel Oil	Capsules	Obtained from mussel, rich in EPA and DHA
Artic Nutrition	SME	Norway	Private consumer	Romega®	Capsules	Product Extracted from herring caviar oil.
BASF	Big Company	Germany	BTB	Omega Oil 1812 Tg Gold	Liquid	Fish oil available in different concentrations of EPA and DHA.
BioCare	SME	Finland	Private consumer	Omega-3 Lohiöljy	Capsules	Produced form Salmon sidestream productss
Biomega	SME	Norway	BTB	Biomega®Salmoil	Powder	Crude Salmon oil with astaxanthin.
Bioriginal	Big Company	Canada	BTB	Omegapure®	Liquid	Can be incorporated into food without change the taste or flavour.
				Omegaactiv®	Capsules	From silver herring
				Biopure DHA	Fish oils	Fish-oil containing five times more DHA than EPA.
BTSA	Big Company	Spain	BTB	Biomega Tech® Fish	Liquid, Powder	Fish oil refined and deodorized, developed by custom mixtures according consumer requirements.
Clover Corporation	SME	Australia	BTB	Nu-Mega Hidha® Tuna	Powder / Liquid	Crude oil product from high quality tuna oil.

				Oils		
				Ocean Gold	powder/ liquid	Product extracted from super refined tuna oil using cold pressure.
Croda.Inc	Big Company	United Kingdom	Private Consumer /Distribution	Incromega	Capsules	Omega 3 oil obtained using Puremax™ purification technology. Available in different concentrations of EPA and DHA. To support consumer health including cardiovascular health, pregnancy, rheumatoid arthritis, cholesterol and cancers.
DSM	Big Company	Netherlands	BTB	Meg-3®	Powder/ Liquid	Microencapsulated fish oil, available in different concentrations of EPA and DHA, and deodorized format.
Firmenich Bjorge Biomarin	Big Company	Switzerland	BTB	-	-	-
Golden Omega S.A	SME	Chile	BTB	Golden Omega	Concentrates	Available in bulk form, with different concentration of EPA+DHA
Hofseth Biocare	SME	Norway	BTB	Ome-Go®	Capsules	Produced from salmon sidestream products. They are doing clinical trials to see the effect of salmon omega-3 with the rest of normal omega-3 products
KD Pharma Group	Big Company	Germany	BTB	Kd-Products	Capsules	The oil is processed in Norway and Alaska. High Concentrates are manufactured in Germany using the oil form Norway and capsules from USA. Bulk Oils are available in different concentrations of EPA and DHA-
Norwegian fish oil ®	SME	Norway	BTB	Cod Liver Oil	Liquid	For kids
				Omega -3 Gummies	Gummies	Each gummy has a taste of multiple fruits that are well blended
				Omega-3	Capsules/ liquid	Concentrated format
				Omega-3 And Krill Oil	Capsules	Highly concentrated Omega-3 with 33% EPA

						and 22% DHA combined with a clean oil from krill.
				Salmon Oil	Capsules	Contain astaxanthin
				Shark Liver Oil	Capsules	Marine fatty acids, Omega -3 and vitamin A, Alkylglycerol AKG, squalamine, squalene (min 20 %) Used for immune system cells, inflammation, skin and eyes
Olvea Group	SME	France	BTB	Sardine Oil	-	From Morocco and Mauritania. Produced from canneries trimmings
				Cod Liver Oil	-	Caught in Iceland, available in a wide variety of cod liver oil ranging from crude to fully refined and ready to use products.
				Tuna Oil	-	Obtained from Tuna sidestream products coming from canneries timings
Originates	SME	USA	BTB	Essentiomega™	Liquid	Fish sources include anchovy, sardine and mackerel. Products produced by Naturmega in Colombia.
Pelagia	Big Company	Norway	BTB	TGN Epax EE Epax	Liquid	EPA/DHA ethyl esters and concentrates from fish sources
Stepan Lipid Nutrition	Big Company	USA	BTB	Marinol ®	Powder	Micro-encapsulated fish oil with a high content of concentrated natural fish oil

Market size

In 2016, the global fish oil market was evaluated at US$2.22 billion (€1.91 billion) and is expected to grow at a CAGR of 5.8% to reach US$3.69 billion (€3.18 billion) in 2025. The combined growth in the global aquaculture industry, as well as consumer awareness of the health benefits of fish oil, has driven the growth in the fish oil market. However, fishing quotas that limit the supply of fish have also impacted the growth of the fish oil industry. It is speculated that the utilisation of fishing side stream products may help alleviate these issues with supply and demand.

China is the largest consumer of fish oil, due to its proximity to aquaculture in the Asia-Pacific region. However, the global production is led by Peru, USA, Chile and China (Table 1.3.3).

Table 1.3.3 Fish oil production for the top 15 countries from 2008 to 2016 (SEAFISH 2016).

X 1000 tons	2008	2009	2010	2011	2012	2013	2014	2015	2016	Average 2012-2016
Peru	320	282	173	354	195	178	115	94	114	134
USA	86	76	61	79	63	104	80	83	101	85
Chile	171	152	105	139	140	103	144	107	81	112
China	32	38	44	47	45	50	45	40	65	48
Japan	62	64	60	55	55	54	62	60	62	58
Norway	39	42	40	45	41	42	58	51	61	50
Vietnam	26	20	49	28	30	40	53	48	49	43
Denmark	56	72	67	54	33	43	51	55	48	45
Iceland	72	62	43	49	56	46	32	48	28	41
India	4	5	12	17	23	21	37	20	27	25
Morocco	25	40	43	22	29	20	35	28	22	26
Ecuador	13	10	13	16	17	19	12	16	16	16
Mauritania				4	12	8	16	14	15	13
Germany	5	6	6	10	11	12	16	12	14	13
total	911	869	716	919	750	740	756	676	703	709

The total amount of fish oil produced strongly depends on the production in Peru, which is highly influenced by the climate phenomena El Niño and La Niña. El Niño, where the Pacific Ocean temperature can increase by 5°C, affects anchoveta production, which is one of the main fish used to produce fish oil. The combination of the last El Niño, which took place from 2014 to 2016, and reduced fishing quotas saw a decline in fish oil production during this period (Figure 1.3.4). However, in 2017, global production increased again due to the more favourable La Niña (lowering of ocean temperatures) off the Peruvian coast and increased landings of small pelagic fish in the Nordic countries.

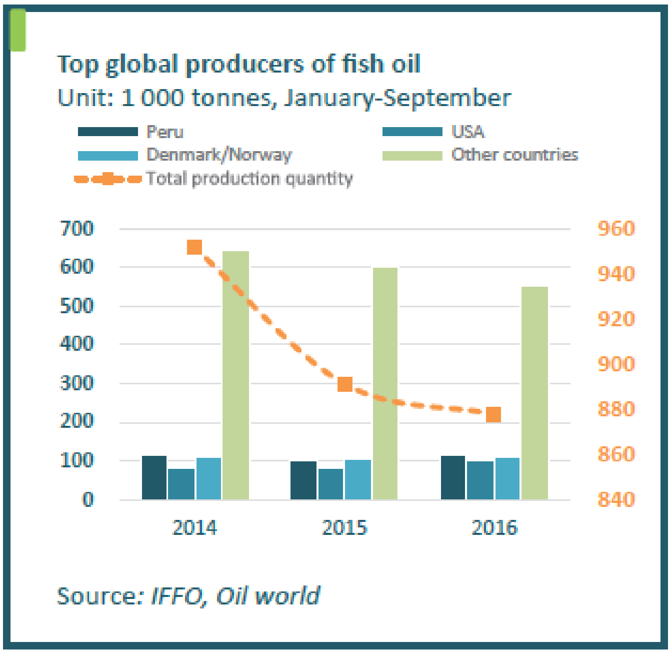

Figure 1.3.4 Top global producers of fish oil (FAO GLOBEFISH).

In Europe, approximately 190 000 tonnes of fish oil are produced per year, with Norway, Denmark and Iceland being the main producers. In 2017, 86 000 tonnes of fish oil were produced by these three countries, the highest production level since 2003. While the main source of oil is from pelagic fish, these recent figures also include oil from salmon trimmings. Once again, the production of fish oil is strongly dependent on the availability of the fish, which is related to the fishing quotas.

The EU also has an integral position with regards to the trading of fish oils. In 2016, the EU reached peak exports at 127 064 tonnes of fish oil valued at €237 million, an increase of 20% and 12% respectively compared to 2015. Denmark was the main exporter, showing a similar increase in export volume of 19% to 113 637 tonnes (€188 million), with Norway being the main non-EU market. On the other hand, in 2016, the EU imported 177 093 tonnes, corresponding to a 1% growth at an increase in value of €27 million. Norway is the main supplier of fish-oil to the EU (57 070 tonnes at €1115 /tonne) with the USA in the second position (39 929 tonnes at €1669/tonne) followed by Peru (21 996 tonnes). Of all the EU countries, Denmark is the largest fish oil importer, purchasing 85 755 tonnes of fish oil from non-EU countries.

Variations in production capacity and fishing quotas increase the volatility of the fish oil market and contribute to price pressure. In 2017, lower quotas for the second fishing season in Peru resulted in an increase in the fish oil price on a global level to around **US$1600/tonne** (approximately €1400/tonne). Price decreases were also seen in 2015, when the production capacity in Peru and China dropped (Figure 1.3.5). However, with the increased use as nutritional supplements and its importance in fish feed, demand for fish oil is expected to remain high. Thus, as long as the fish supplies are available, the price is likely to remain relatively stable in the near term. In the long-term, however, economic factors that affect the fishing industry, along with a project reduction in the use of fish oil in fish feed, is likely to result in lower fish oil prices by 2025.

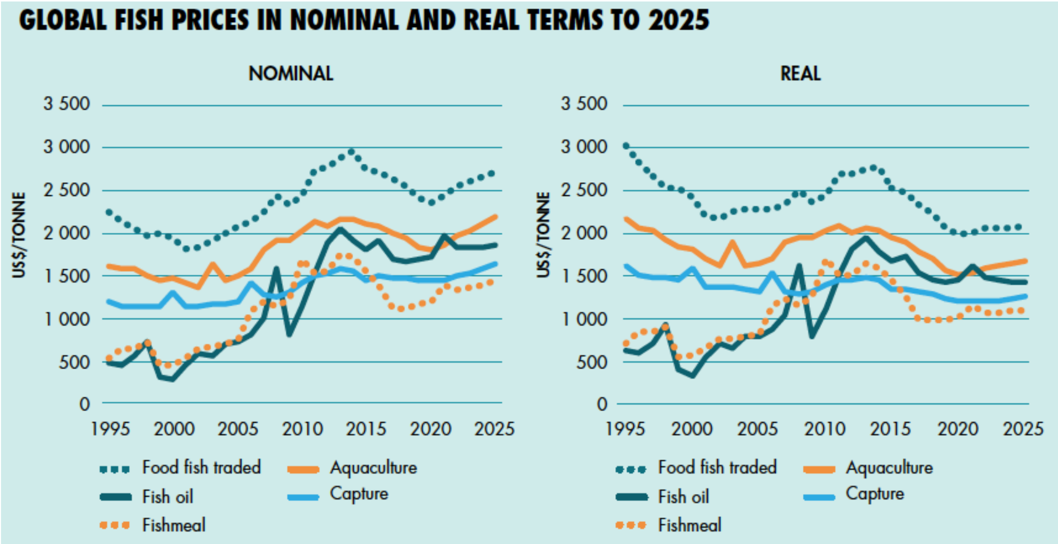

Figure 1.3.5. Global fish product prices in nominal and real terms to 2025 (FAO 2008).

Barriers and opportunities

As the fish oil market is already well established, trying to gain market share is one of the biggest barriers for other fish oil producers. Moreover, as the fish oil production is currently closely linked to the quotas and hauls from the fishing industry, the is volatility in the market and product pricing based on the availability of biomass resources. As a result of this, there is also increasing competition from other oil sources, such as microalgae, krill, seeds and nuts, which are also rich in omega-3 fatty acids. Moreover, the microalgae, seeds and nuts are suitable for vegans, which is not the case for the oils obtained from fish.

Perhaps the biggest opportunity for the AQUABIOPRO-FIT project is the use of sidestream products and side streams from the fishing industry as a source of fish oil. While some companies are already taking this approach (e.g. DSM), it is apparent that there are more could be done to make use of such raw materials. Furthermore, utilising these sidestream products may help to maintain certain volumes that may have otherwise dropped due to low fishing quotas. With the increased incidence of heart disease, consumers are becoming more aware of the health benefits of omega-3 fatty acids DHA and EPA. This is expected to see a shift in the market share towards health supplements, this is expected to increase demand particularly in Europe and other Western countries. With the fish oil price project to drop slightly over the next 10 years, this may also make fish oil supplements more affordable for a wider consumer market (https://www.businesswire.com/news/home/20180223005319/en/Global-Fish-Oil-Market-2018-Analysis-Forecasts).

1.3.3 *Ciona intestinalis* - products and applications

Tunicates are a group of sea invertebrates known as the only animals to produce cellulose. The name "tunicates" is derived from their outer covering, which is referred to as a tunic. Ascidians are the major group of tunicates, found mostly on hard surfaces, such as rocks, and include the filter feeding animal *Ciona intestinalis.*

Anatomically, ascidians are characterized by two main body parts; the outer tunic, which consists of cellulose, protein and ash and whose function is mainly supportive and protective, and the inner body that is covered by the tunic, which includes the vital organs (ovary, heart, stomach, intestine, etc.). These two components can be easily separated from one another, thus facilitating the possibility to valorise each part (grant agreement 790956 AQUABIOPRO-FIT BBI JTI 2017).

Ciona intestinalis is being used by the company Marin Biogas as a renewable source of biomass to produce biogas. The anaerobic fermentation process converts the carbon into biogas, while the residue is a nutritious material high in nitrogen and phosphorus that could be used as a fertilizer for plants. Moreover, the cultivation and harvesting of Ciona helps to remove excess nutrients from the sea, which translates to positive ecological impact by reducing eutrophication.

In recent years, the chemical composition and characteristics of *Ciona intestinalis* has been investigated in order to explore whether such creatures can be exploited as a bio-source for agriculture, human consumption and renewable energy. Aside from the biogas applications, there are very few examples of the valorisation of ascidians. However, some species are considered a delicious seafood for human consumption in some regions of Asia, Chile and the Mediterranean. Given that the exploitation potential for *Ciona intestinalis* is relatively unexplored, it is not possible to perform the same market analysis as was conducted for the fish proteins, oils and collagen. In this respect, the following is a description of the possible compounds that can be isolated from *Ciona intestinalis* for further valorisation.

- ✓ **Cellulose**

Cellulose is one of the most promising compounds that can be isolated from tunicates. In *Ciona intestinalis*, cellulose represents approximately 60% of the tunic and is chemically identical with plant cellulose. In the tunicates, cellulose is in the form of microfibrils and is associated with other compounds, such as proteins and lipids, that can be easily removed to obtain high-purity cellulose. Such cellulose is promising for high-performance films and packaging polymers, offering an alternative to synthetics polymers with the added advantage that it is biodegradable, nontoxic and suitable for edible packaging.

- ✓ **Fatty acids**

Fatty acids are also present in ascidians, with Zhao et al. (2015) having reported the quantity and profile of fatty acids from *Ciona intestinalis*. The lipids are contained in the inner body tissues of *Ciona intestinalis* are similar to other marine-based oils, with a high content of the polyunsaturated fatty acids, EPA and DHA. The lipid content typically represents between 1.22 and 6.16% of the inner body, with PUFA representing between 61.11 and 69.77 % of fatty acid content, where EPA and DHA are between 15.20 and 29.27%. While Zhao et al. (2015) mention that other studies have reported significantly lower fatty acid contents in *Ciona intestinalis,* there is no doubt that these animals are a potential source of such ingredients.

- ✓ **Proteins**

Proteins are present in both the tunic and inner body of tunicates in relatively high contents. The inner body is the major source of proteins, containing about

66-73%, in comparison with the tunic, where the protein is between 30 and 50% as a dry-weight ash-free content. The quality of protein is also promising, offering high nutritional and food values due to the content of essential amino acids, which is 66-73% in the case of the inner part and 32- 50% in the tunic.

Given the composition of *Ciona intestinalis*, possible applications for these compounds include fuel, animal and aquaculture feed, food, pharmaceuticals, biomedical applications, nanocomposites and packaging materials.

Opportunities and barriers

Perhaps the greatest barrier to bringing the products from *Ciona intestinalis* to the market is the fact that there is currently no market. In this respect, these products will have to compete with what is already on the market and may face reluctance from customers to use ingredients from such an untraditional biomass source. Should these ingredients be considered for food applications, another barrier may be the need for novel food approval from EFSA (https://www.ekocentrum.se/utstallare/marin-biogas-fornyelsebar-energi-fran-ett-renare-hav/ (in Swedish)).

On the other hand, the lack of a tunicate product market means that there is every opportunity to disrupt the current market. As mentioned above, tunicates may offer a novel, sustainable, alternative source for valuable ingredients, such as cellulose, proteins and oils, that show similar characteristics to other marine products. If the AQUABIOPRO-FIT projects demonstrate that these compounds can be easily isolated to produce a safe, nutritious product, there should be no reason why these ingredients cannot compete with other marine products.

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Course 1.4: Production technologies for fish protein-based products

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1.4.1 Thermal processing

By thermal processing, the fish based raw material is separated into a water-phase (stickwater) and solid phase (press cake), illustrated in Figure 1.4.1. Briefly, the raw material is heated to T > 80 °C to coagulate proteins, inactivate microorganisms, disrupt fat deposits and release oil and water. After the heat treatment, the material is mechanically pressed to squeeze out oil and water from the coagulated material, the press cake. The press cake is rich in non-water-soluble proteins, bones and minerals. The press cake is dried and ground to desired particle size to produce a protein rich powder. The liquid phase is separated into water and oil phases, where the water phase, the stickwater, contains water soluble proteins, peptides, amino acids, other water-soluble compounds present in the raw material. Stickwater is typically concentrated on an evaporator before being blended with press cake and dried to produce fishmeal. To read more about the fish meal process, see http://www.fao.org/3/x6899e/X6899E00.HTM .

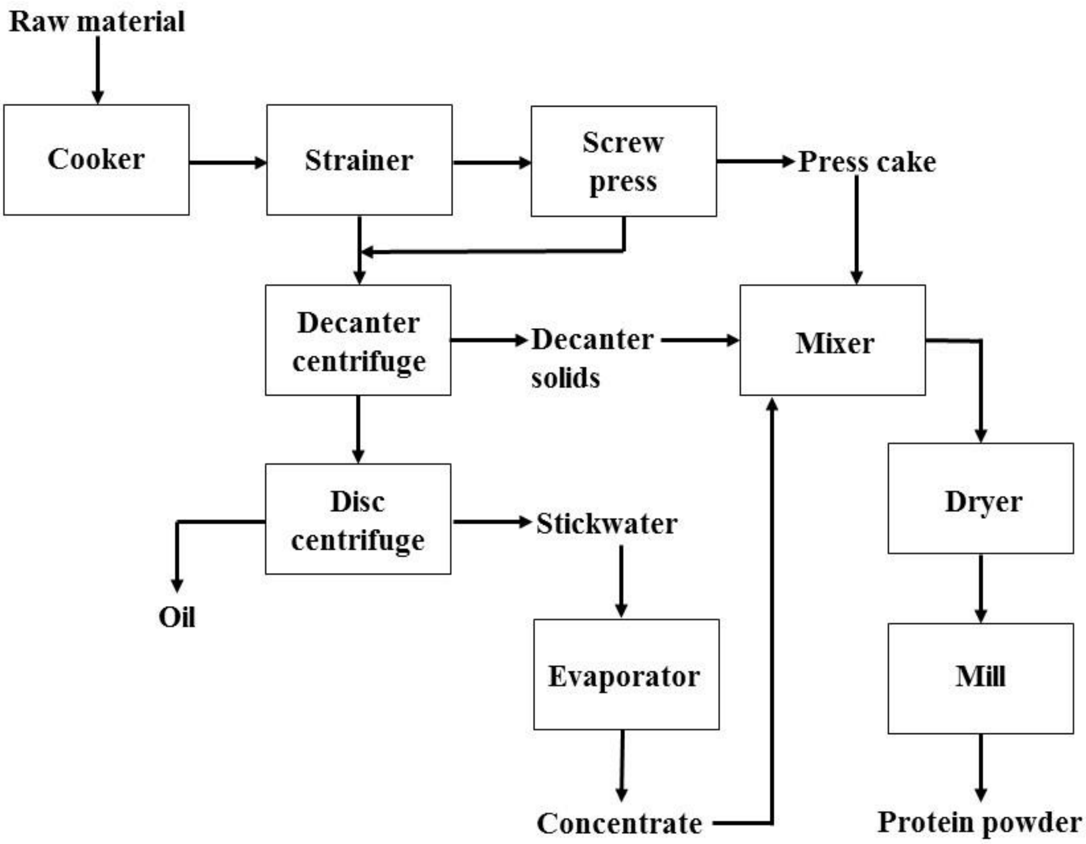

Figure 1.4.1 Flow diagram showing the main unit operations applied in thermal processing of fish raw materials (Aspevik et al., 2017)

1.4.2 Protein hydrolysis

Enzymatic protein hydrolysis is a method commonly used to extract proteins from fish side stream products. The main purpose of a hydrolysis process is to degrade proteins thus solubilizing them in water, increase protein recovery and the yield of valuable components. Protein hydrolysis can be achieved chemically or enzymatically, where the latter is preferred of products for human consumption. The hydrolysis process will yield a water-phase (the protein hydrolysate), a sludge phase and oil phase, if based on a fatty raw material. The fish protein hydrolysate (FPH) can be dried to a protein powder that is applicable within a variety of products.

The production of FPH includes the following steps:

- ✓ Preparation of raw material (mincing and dilution with water)
- ✓ Adjustment of reaction temperature
- ✓ Addition of enzyme
- ✓ Hydrolysis reaction for a predefined hydrolysis time
- ✓ Inactivation of enzyme
- ✓ Separation of the water, oil and semi-solid phases

Proper dilution with water, usually 1:1, can prevent product inhibition and maximize product yield, but added water is also a factor influencing processing costs. In industrial operations, final products are often dried and a compromise between desired product yield and water that needs to be removed is imperative. The hydrolysis process and enzyme performance depend on reaction temperature and pH. Several commercial proteases working well under the substrate natural pH are available, avoiding the need for pH-adjustment. At the end of the reaction, the enzyme activity is terminated by irreversible denaturation of the enzyme by heating the slurry to temperatures above 85 °C for at least 10 minutes. Finally, the slurry is separated into three phases: an oil phase, a water phase and a sludge phase.

Choice of enzyme

The enzymes responsible for cleaving peptide bonds belong to the family of proteases (also called peptidases or proteinases). Several types of proteases are known (Table 1.4.1) and can be classified based on critical amino acid required for the catalytic function, pH-optimum for their activity, their site of cleavage or the requirement of a free thiol group Proteases can be divided into endopeptidases and exopeptidase, based on their preference for cleavage. Endopeptidases catalyze the peptide bonds in the interior of the peptide chain, leaving two new, smaller peptides. The exopeptidases, on the other hand, require the presence of an unsubstituted N- or C-terminus, only releasing free amino acids or small di- and tripeptides. Enzymes are systematically classified by a European Commission (EC) number according to rules of nomenclature defined by the Nomenclature Committee of International Union of Biochemistry and Molecular Biology (NC-IUBMB 1992). Proteolytic enzymes are defined as number 3 (hydrolases) 4 (proteases) and 11-19 (exopeptidases) or 21-99 (endopeptidases). To read more about protease enzymes, see https://en.wikipedia.org/wiki/Protease .

Table 1.4.1 Classification of proteases (Brenda 2020)

Protease	EC Number	Peptidase type	Action
Exopeptidases	3.4.11	Aminopeptidase	Releases N-terminals
	3.4.13	Dipeptidase	Acts only on dipeptides
	3.4.14	Dipeptidyl peptidase Tripeptidyl peptidase	Releases N-terminal di-peptides and tripeptides
	3.4.15	Peptidyl dipeptidase	Releases C-terminal dipeptides
	3.4.16	Carboxypeptidase (serine)	Releases C-terminals (serine at active site)
	3.4.17	Carboxypeptidase (metallo)	Releases C-terminals (metal requiring protease)
	3.4.18	Carboxypeptidase (cysteine)	Releases C-terminals (cysteine at active site)
	3.4.19	Omega peptidase	Releases modified residues from N- or C- termini
Endopeptidases	3.4.21	Serine endopeptidase	Serine at active site
	3.4.22	Cysteine endopeptidase	Cysteine at active site
	3.4.23	Aspartic endopeptidase	Aspartate at active site
	3.4.24	Metallo endopeptidase	Metal requiring protease
	3.4.25	Proteasome endopeptidase	Very broad specificity
	3.4.99	Endopeptidase of unknown catalytic mechanism	

There are a multitude of distributors of commercial enzymes, where Novozymes (Denmark), Biocatalysts (UK) ABEnzymes (Germany), DuPont (US) and Enzybel (BE) are some examples of distributors of enzymes applicable for valorization of marine side stream materials. Proper choice of enzyme is important in the hydrolysis of marine side stream materials, as this will influence the processing costs and sensory and physicochemical properties of the final products.

1.4.3 Silage process

Fish silage is a liquid product made from whole fish or fish side streams that are hydrolyzed by endogenous enzymes in the presence of an added acid, usually formic acid. At acidic pH, the digestive enzymes break down the fish proteins into smaller peptides and free amino acids. The process is inexpensive and does not require high investment-costs and is regarded as especially useful when only small amounts of fishery side streams are available. The final liquid product can be concentrated to a fish protein concentrate. The process of producing silage entails several health and safety requirements and handling of acids should only be done while wearing personal protective equipment (goggles, rubber gloves etc.).

For preparation of fish silage, the raw material must be ground, and the mixture stirred to ensure good contact between the raw material and added acid. At pH <4, enzymes naturally present in fish viscera degrade and liquefy the fish tissue without risk of bacterial spoilage. Antioxidants should be added to prevent oxidation of fish oil. Production of fish silage is a relatively simple and low-cost technology but requires strict process control to avoid growth of spoilage bacteria. The rate of liquefaction depends on the type of raw material, its freshness, and the temperature of the process. Fatty fish liquefy more quickly than lean fish and the fish should be as fresh as possible. Moreover, the warmer the mixture, the faster the process. Fish silage of the correct acidity keeps at room temperature for at least two years without putrefaction. The final product is not suitable for human consumption, and silage technology should preferably be based on fish and residuals found unfit for food production. To readd more about silage, see more 1.4.3.

1.4.4. Downstream processing

Separation and Concentration

Whether the process is fishmeal, enzymatic hydrolysis, or silage, the resulting three phases (water, oil, and solid) must be separated before further processing.

- ✓ **Conventional separation of fish press cake, solubles and oil**

In the fishmeal process, a screw press performs the primary liquid-solid separation. The minced and cooked raw material is forced against a metal screen by a horizontally rotating screw conveyor. The liquid is pressed out through the screen and the solid phase (press cake) exits via the bottom of the machine at end of the screw (Figure 1.4.2.).

Figure 1.4.2. Screw press and conveyor screws

The press liquid is pumped into a (usually vertical) centrifuge (oil separator), separating the water (stickwater) and oil phases, also producing some sludge which may be blended back with the press cake. Most of the water in the protein-rich stickwater is removed on a falling-film evaporator before the concentrate is blended back into the presscake. The resulting fishmeal is then dried and milled. The oil phase, which is a valuable product, is further refined (purified) and may be used in aquaculture feed or human nutrition products (omega-3 oil or capsules).

Enzymatic hydrolysis, including the silage process, produces a greater amount of fine particulate solids. A screw press is unsuitable for this type of material, so a decanter centrifuge is typically used to separate the liquids from solids. A decanter centrifuge is a (usually horizontally) rotating cylinder with a conveyer screw inside that rotates at 5 to 60 rpm relative to the cylinder (which rotates at several thousand rpms, producing thousands of g-forces). The feed, containing liquids and particles, is pumped into the center of the centrifuge. The high g-forces cause the solids to settle to the inside surface of the cylinder. The solids are then moved by the screw, up a slope in the cone-shaped end of the cylinder, where the solids exit. The liquid builds up to a certain level and exits at the opposite end of the machine from the solids port. The water and oil are then separated in a vertical centrifuge (oil separator) as in the fishmeal process. Alternatively, 3-phase decanter centrifuges separate the solid, water, and oil phases in one process step (Figure 1.4.3).

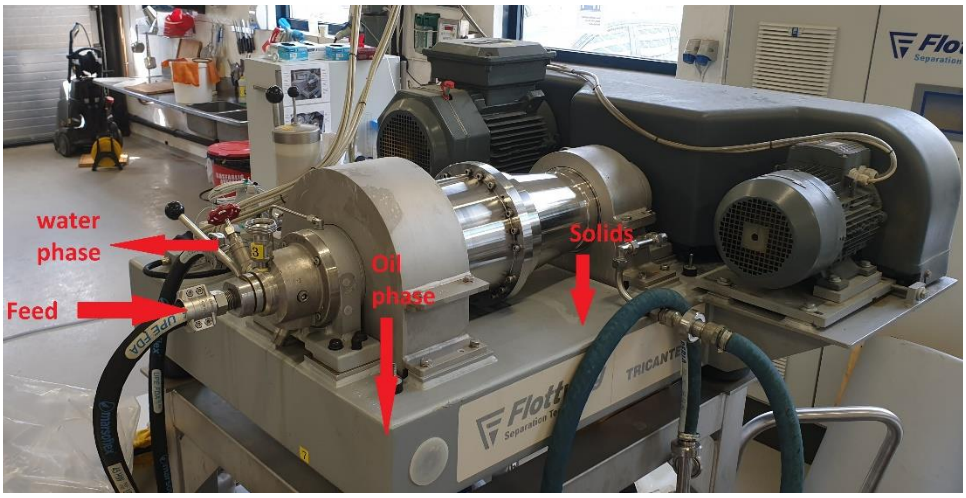

Figure 1.4.3 A phase decanter centrifuge used for separation of water-phase, oil phase and solids

Of the processes described previously, only enzymatic hydrolysis is normally used to produce concentrated protein products for human consumption. Instead of falling-film evaporators, crossflow nanofiltration (NF) is used to concentrate the protein-rich water phase. In addition to using less energy than evaporators, NF has the added benefits of removing monovalent salts and small molecules that contribute to off-flavors and odors.

- ✓ **Crossflow membrane filtration of solubles and hydrolysates**

Crossflow filtration differs from so-called dead-end filtration (Figure 1.4.4). In crossflow filtration, the feed liquid flows perpendicularly to the filtration membrane and can pass through the membrane or exit the system though another route, producing two streams—permeate (the liquid that has passed though the membrane) and retentate (the liquid retained by the membrane). In dead-end filtration, which is the process most people envision when they read the word "filtration," the feed liquid is forced through the filtration membrane (by gravity or pressure) and the filtrate is the only liquid stream produced.

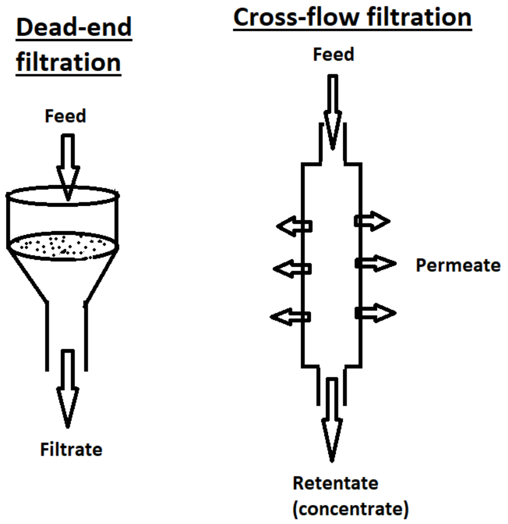

Figure 1.4.4. Crossflow versus dead-end filtration.

Crossflow filtration is often categorized into four types/levels: microfiltration (membrane pores in the micrometer range), ultrafiltration, nanofiltration (nanometer-scale pores), and reverse osmosis. Reverse osmosis membranes have pores so small that they only allow water molecules to pass while retaining sodium chloride and other larger molecules, producing permeate that is pure water. One step up in pore size from reverse osmosis, nanofiltration membranes allow passage of water, sodium chloride, and other (neutral or monovalent) small molecules. NF membranes are sometimes rated by the molecular weight cutoff (MWCO) - the approximate minimum molecular mass of molecules retained by a filtration membrane. In nanofiltration, the MWCO only applies to neutral or monovalent molecules, as divalent cations are more than 95% retained regardless of molecular mass. MWCOs for nanofiltration membranes are usually between 200 and 1000 Dalton. If the water phase is to be concentrated by nanofiltration, most of the suspended particles must be removed beforehand to avoid fouling (clogging) the NF membrane. This may be done by crossflow microfiltration.

The NF permeate, which contains salt and other undesired compounds, is discarded (or the water from the permeate may be recycled by reverse osmosis) while the retentate (concentrate) is taken on to further up-concentration by an evaporator, or spray-dried directly. To optimize removal of salt and other small molecules, water may be periodically added to the feed tank (while recycling the retentate to the feed tank), causing more salt and other molecules to be removed along with the added water. This technique is called diafiltration.

The water phase from an enzymatic hydrolysis is typically around 5% dry matter (%DM) and may be concentrated to 25 %DM or more by nanofiltration. An additional drying step is needed to produce a protein powder, spray drying being the most usual.

Drying

Different technologies can be used for drying marine protein products, involving variable amounts of energy and temperature levels, steam or air, with vacuum or not. In our project inexpensive methods such as tray (air) drying will be used when appropriate, or more energy demanding ones depending on the quality specification of the products and the physical properties of the material. Freeze drying, spray drying, vacuum, steam and superheated steam drying are among the methods that are discussed in this module.

- ✓ **Conventional dryers**

Selecting the right drying system is crucial for marine raw materials for many reasons, heat sensitivity is one of them. A poor choice of drying system and or drying conditions can have an adverse effect on physical and nutritional properties of the dried product and may give a reduction in biological digestible protein. The selection of a dryer for a particular raw material depends mainly on the type of end product, amount and type of moisture, drying kinetics, heat sensitivity, physical structure of the material to be dried, quality requirements of a dried food, and many other factors. Dryers are commonly classified based on the mode of heat transfer (e.g., conduction, convection, or radiation) and mode of operation (batch vs. continuous). For sheet forms or extruded food products, drying can be achieved on a belt conveyor or in a batch process by use of either conductive or radiative heat transfer along with convection and vacuum. However, pasty materials are conventionally dried using indirect rotary, paddle, or drum dryers and, recently, using beds of inert particles with some variant of the fluidized bed dryer as discussed previously for liquid suspensions.

- ✓ **Superheated steam drying**

In drying with superheated steam, the marine material is in direct contact with steam at temperatures above the condensation temperature. Superheated steam does not contain oxygen; hence, oxidative or combustion reactions are avoided. In addition, it eliminates the risk of fire and explosion hazard and the quality of the products tends to be better than that using a conventional hot air dryer. Superheated steam also allows pasteurization, sterilization, and deodorization of food products. This is particularly important for food and pharmaceutical products that require a high standard of hygienic processing. In addition, superheated steam drying provides higher drying rates in both constant and falling rate periods under certain conditions. Desirable organic compounds can also be contained using the superheated steam drying method. Mujumdar, 2007 has discussed the principles, advantages, and limitations as well as diverse applications of superheated steam drying technologies. Many foods and biomaterials are damaged at the saturation temperature of superheated steam. It may be necessary to include supplementary heat sources, for example, microwave radiation or conduction, to speed up drying rates at low steam pressures have provided a detailed discussion on superheated steam drying and dried various food materials using superheated steam.

- ✓ **Freeze drying**

Freeze-drying (also called lyophilization) comprises freezing a water solution or suspension of product then drying under vacuum. The water passes directly from the solid (ice) phase to gas phase before again freezing on the condenser coils or plates of the freeze drier. This process is called sublimation — a form of evaporation in which molecules pass directly from the solid phase to the gas phase, without going through a liquid phase (https://en.wikipedia.org/wiki/Sublimation_(phase_transition)). For water to sublime, the pressure must be below 6 mbar and the temperature must be below 0 °C. The freeze-drying process has two phases: primary drying which removes frozen water through sublimation and secondary drying which removes non-frozen bound water. As the ice crystals disappear, they leave behind a porous mass that is (usually) easily crushed to a powder. Freeze-drying typically leaves a product with less than 3% moisture. Freeze drying is a gentle drying technique, as the product is not exposed to high temperatures. However, it is more expensive method than many other drying methods because of the energy required for vacuum and cooling, and the cost of the machine. Therefore, commercial applications of this technique in the food sector are limited to high-value products.

- ✓ **Spray drying**

Spray drying is used when freeze drying of aqueous solutions is not economical or practical on a large scale. Perhaps the biggest advantage of spray drying is the quickness of the process. A solution or suspension of product can be dried to a powder in a matter of hours. It is also more amenable to large-scale production than freeze drying. A water solution or suspension is pumped into the spray dryer where it is sprayed into tiny droplets. At the same time, hot air is blown through the chamber causing the water in the droplets to evaporate. The resulting dry particles fall to the conical bottom of the spray dryer while moist warm air exits via another port. Spray-dried product also has little moisture, often less than 3%.

1.4.5 References

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