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Executive Summary
According to the Food and Agriculture Organization of the United Nations (FAO), a 60% increase in global food production is necessary if the global population continues to increase as it has. The mesopelagic species may be a new source of food or feed for consumption in the future. This report focuses on different aspects of the conversion of mesopelagic species from a largely untapped resource to a more commercially viable one. We've identified that the commonly applied processes of creating fishmeal and fish oil, fish protein hydrolysates or silage are probably the most commercially viable. Additionally, we've taken in the production of bioactive peptides and the use of fish bones as other potential pathways. The report also includes a techno-economic feasibility study of the different processes, legal considerations, market possibilities and environmental impacts of the industrial processes and the products mentioned above.

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1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), a 60% increase in global food production is necessary if the global population continues to increase as it has. In 2021, the number of people affected by hunger globally was 828 million, which is 9.8% of the world population. This added up to an increase of 49 million in one year. The COVID-19 pandemic has had severe effect on hunger and food security, and the economic impact of the pandemic is apparent with 11.7% of the global population living with severe food insecurity (2021). Malnourishment is an increasing problem. In 2022, 149 million children under the age of five were suffering from impaired growth and development caused by chronic lack of essential nutrients, and 39 million people were overweight (WFP & UNICEF, 2022). According to the 2030 Agenda for Sustainable Development, we aim to end hunger, achieve food security, improve nutrition and promote sustainable agriculture by the year 2030, but we are currently moving away from this target (Sachs et al., 2022). We need new sources of food and feed to sustain the needs of our growing global population. Seafood has great potential to contribute to food security, either directly for human consumption, or as feed ingredients for aquaculture or other food producing organisms. The mesopelagic species represent an unexploited resource to expand the current realm of economically and biologically viable fisheries for food consumption. Exploitation of any previously unexploited biomass could potentially have significant impact on the ecosystem, so thorough assessments of stock size and population dynamics is necessary prior to full scale fisheries (Costello et al., 2020; Paoletti et al., 2021). Biomass estimations have been elaborated on and current best estimates are presented elsewhere (Irigoien et al., 2014; Pauly et al., 2021).

In a previous report from WP3 of the Meeso project; D3.4, different processing procedures for the mesopelagic biomass were tested and discussed. Nutritional analyses, covering among others oxidation and proximate analysis, contaminant analysis, and nutrient composition was presented. Bioactivity of processed biomass and a sensory evaluation of filtered hydrolysates was also described. In the current report, the focus lies on potential product categories from mesopelagic biomass: Their applications, production process, product characteristics, economic factors, potential legal specifications, and environmental impact.

LIST OF ACRONYMS

ASCVD: Atherosclerotic Cardiovascular Disease
BOB: Bay of Biscay
CAGR: COMPOUND ANNUAL GROWTH RATE
CV: CARDIOVASCULAR
DH: DEGREE OF HYDROLYSIS
EC: EUROPEAN COMMISSION
EFSA: EUROPEAN FOOD SAFETY AUTHORITY
EU: EUROPEAN UNION
FAO: THE FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
FDA: FOOD AND DRUG ADMINISTRATION

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FFA: FREE FATTY ACID
FOSHU: FOODS FOR SPECIFIED HEALTH USE
FPH: FISH PROTEIN HYDROLYSATE
IMR: INSTITUTE OF MARINE RESEARCH
MHLW: MINISTRY OF HEALTH, LABOUR, AND WELFARE
NDA: THE SCIENTIFIC PANEL ON DIETETIC PRODUCTS, NUTRITION AND ALLERGIES
NLEA: NATIONAL LABELLING AND EDUCATION ACT
PUFAS: POLYUNSATURATED FATTY ACIDS
RDA: RECOMMENDED DAILY ALLOWANCE
REDUCE-IT: REDUCTION OF CARDIOVASCULAR EVENTS WITH ICOSAPENT ETHYL-INTERVENTION TRIAL
TVB-N: TOTAL VOLATILE BASIC NITROGEN
BMI: BODY MASS INDEX
CCK: CHOLECYSTOKININ
GLP-1: GLUCAGON-LIKE PEPTIDE 1
ACE: ACETHYLCHOLINE ESTERASE

1.1. Mesopelagic species

Estimates of global mesopelagic biomass vary greatly and has increased over the last decades, from 1 billion tons in 1980 (Gjøsæter & Kawaguchi, 1980) to 11-15 billion tons in 2014 (Irigoien et al., 2014), leaving a great potential for commercial harvesting. The catch composition of mesopelagic hauls varies depending on geographical location and season. In a study from 2020, conducted as part of the MEESO project, the six most abundant species of the mesopelagic biomass in deep Norwegian fjords were studied (Alvheim et al., 2020). The most abundant fishes were *Maurolicus muelleri (M. muelleri*) and *Benthosema glaciale* (*B. glaciale*). In addition to this, they found the decapod *Eusergestes arcticus (E. arcticus)*, the decapod genus *Pasiphaea*, the euphausiid Northern krill (*Meganyctiphanes norvegica*) and the scyphozoan helmet jellyfish *Periphylla periphylla* (*P. periphylla*) to be highly abundant. Figure 1 shows all mentioned species. Even though these six species made up the majority of the biomass, their composition in the different Norwegian regions varied, as can be seen in Figure 2.

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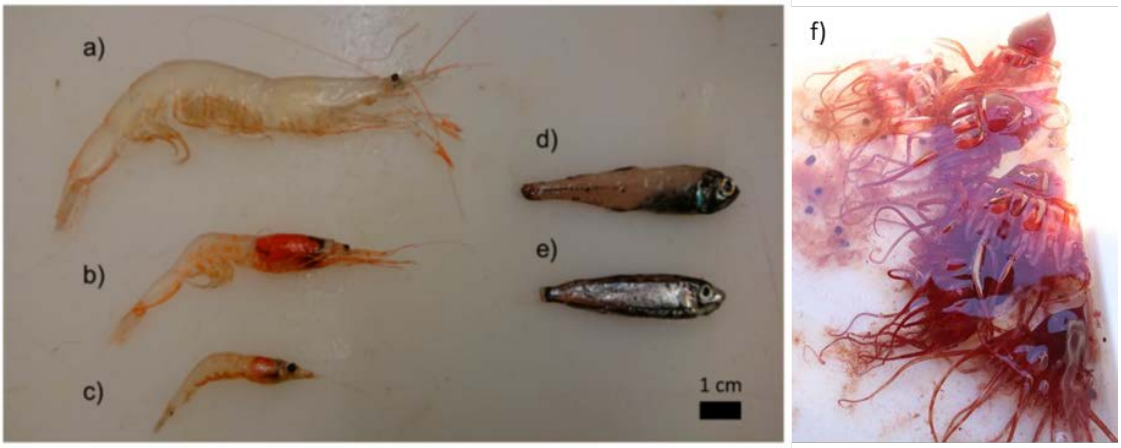

Figure 1. Mesopelagic species in Norwegian fjords. (a) Pasiphaea sp., (b) E. arcticus, (c) M. norvegica, (d) B. glaciale, (e) M. muelleri and (f) P. periphylla (a-e from (Alvheim et al., 2020), f from https://www.nrk.no/nordland/denne-maneten-invaderer-norske-fjorder-1.12354953 (photo: Øystein Nygård/NRK).

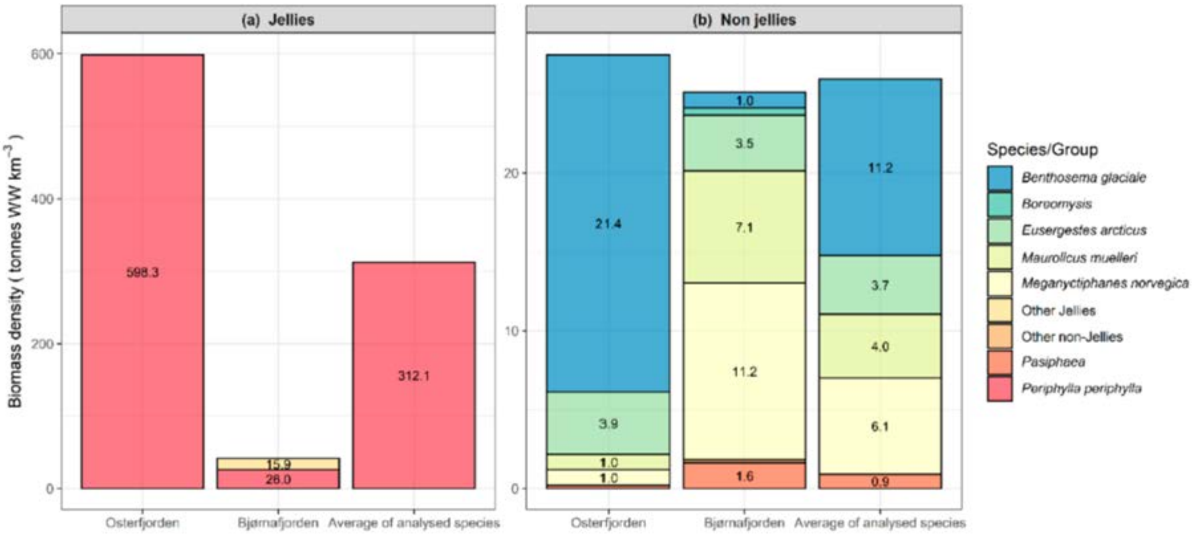

Figure 2. Biomass density of mesopelagic species in Norwegian fjords (Alvheim et al., 2020).

1.2. Quality of mesopelagic biomass

In WP3, mesopelagic biomass from two geographical regions has been studied for nutritional composition and levels of contaminants/undesirables: Biomass from the Bay of Biscay (BoB) and biomass from Norwegian fjords. The details surrounding these studies were largely covered in the report D3.4 and will only be briefly described here.

Mesopelagic samples obtained in the BoB were pure samples composed exclusively of specimens of *M. muelleri*. The mesopelagic samples from Norwegian fjords consisted of species of mesopelagic fish, the glacier lanternfish (*B. glaciale*) and the silvery lightfish (*M. muelleri*), the decapod *E. arcticus*, the decapod genus *Pasiphaea* (comprising *P. multidentata*, *P. sivado* and *P. tarda*), the euphausiid Northern krill (*M. norvegica*) and the scyphozoan helmet jellyfish (*P. periphylla*). The results from the study conducted in the Norwegian fjords was published in the MDPI journal Foods in 2020 (Alvheim et al., 2020). The proximal compositions of all species have been analysed and is presented in Table 1. The proximate composition of *M. muelleri* from BoB and Norwegian Fjords showed some differences. The

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protein and fat content were slightly higher from the BoB, compared to the same species collected from Norwegian fjords. In comparison, the protein and fat contents of another pelagic fish, blue whiting, was 16.1 (g/100g) and 3.9 (g/100g), respectively. Blue whiting was included as a control, as it is commonly used to produce fish meal and fish oil for the aquaculture industry. The protein content of all the species from the Norwegian fjords, except the Helmet jellyfish, was slightly lower, but quite close to blue whiting (ranging from 12.3 – 15.5 g/100g). The fat content varied between the different species but was generally highest in the fish species. All mesopelagic species, except the jellyfish, had higher fat contents than blue whiting, typically regarded as a very lean fish. Calculated by difference, the total ash content in the mesopelagic species were similar and ranged from 2.6 to 3.4 g/100g. The authors concluded that the mesopelagic organisms studied were high in micronutrients and valuable marine compounds and can be a great contribution to global food and feed supply. Briefly, the results showed that the mesopelagic species were nutrient dense, and contained high levels of vitamin A1, calcium, selenium, iodine, eicopentaenoic acid (EPA), docosahexaenoic acid (DHA) and cetoleic acid. For more detailed description of the contents of the mesopelagic species collected in Norwegian fjords, see Alvheim et al. (2020).

Table 1. Observed proximate composition of mesopelagic species collected in the BoB and Norwegian fjords. Blue whiting included for comparison. Data from Norwegian fjords from (Alvheim et al., 2020).

Species (common name)	Collection region	Protein g/100g (min-max)	Total fat g/100g (min-max)	Dry matter % (min-max)
Benthosema glaciale (Glacier lantern fish)	Norwegian fjords	14.0 ± 0.5 (13.5–14.6)	13.7 ± 3.7 (6.1–16.0)	30.8 ± 3.9 (22.0–33.7)
Maurolicus muelleri (Silvery lightfish)	Norwegian fjords	12.3 ± 0.4 (11.9–12.7)	17.8 ± 8.1 (7.1–24.7)	33.3 ± 8.1 (23.0–41.2)
Maurolicus muelleri (Silvery lightfish)	Bay of Biscay	16.32 ± 1.41 (11.94–18.33)	5.75 ± 2.36 (2.17–10.10)	24.97 ± 2.63 (18.76–29.3)
Meganyctiphanes norvegica (Northern krill)	Norwegian fjords	15.5 ± 0.9 (14.8–16.8)	5.5 ± 0.6 (4.9–5.9)	24.0 ± 1.9 (21.3–25.3)
Pasiphaea sp.	Norwegian fjords	14.1 ± 4.6 (42–50)	5.4 ± 2.7 (3.3–8.4)	21.7 ± 5.1 (15.9–24.1)
Eusergestes arcticus	Norwegian fjords	15.5 ± 0.5 (14.9–15.9)	9.4 ± 3.1 (4.9–12.1)	27.5 ± 3.6 (22.3–30.7)
Periphylla periphylla (Helmet jellyfish)	Norwegian fjords	0.95 (0.90–1.00)	0.45 (0.34–0.56)	4.82 (4.76–4.87)
*Micromesistius poutassou** (Blue whiting)	Data from: https://sjomatdata.hi.no/#search/	16.1 (15.5–17.1)	3.9 (2.9–5.8)	20.8 (18.4–22.9)

The fatty acid composition and amino acid composition of the *M. muelleri* biomass from BoB was studied. The fat content varied greatly between samples, from 2.17 % - 10.10 %, and the fatty acid composition also displayed great variation. In addition, the samples of *M. muelleri* of the BoB presented an erucic acid content between 0.0 and a maximum of 0.79% in the sample of East 2017 and the samples from Norwegian fjords were between 0.03 and 0.20%. The maximum intake of erucic acid recommended by the European Food Safety Authority (EFSA)

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is 7 mg/kg bw/day, which would mean a maximum fish consumption of 100g/ 70kg person/day for the most contaminated fish. Depending on the final commercial destination of mesopelagic biomass, an estimate of the expected consumption per person per day should be made to conclude whether it may exceed the recommended limits.

There were no major differences in the content of the different amino acids between the different samples and campaigns. The potential of these raw materials in some specific food applications, such as glutamate for obtaining fish aroma, can be further evaluated. Oxidation and degradation of the biomass was studied, indicating that the biomass is highly sensitive to oxidation and enzymatic degradation. For *M. muelleri* harvested in the BoB, the proximal composition was evaluated for samples collected at different locations and different years. Briefly, there were no major differences in protein, fat and ash content from different geographical areas collected in the same campaign, but some smaller differences could be observed for biomass collected in the same area in different years. The observed differences can be attributed to the size of the fish or to natural seasonal variation. Seasonal variations in fat content and fatty acid profiles need to be further explored. Some authors have commented that larger specimens (older) tend to have a higher content of total lipids and a higher ash/protein ratio (Toppe, et al 2007). In this evaluation, no major differences were seen when comparing samples collected from the same area, but in different season (spring and autumn).

The mesopelagic biomass mentioned above, captured in the BoB and in Norwegian fjords, has been analysed for the presence of contaminants. The implications that these contents have for the use of mesopelagic biomass as food and feed is also discussed. Heavy metal contents of *M. muelleri* from the BoB was analysed. The results indicate that cadmium content might exceed the maximum tolerable levels established by the European regulations for fish meat, established at 0.05 mg/kg (EC, 2006). Cadmium generally accumulates in the viscera. Due to the small size of the mesopelagic fishes, it is expected that they will be processed or consumed whole. This may lead to elevated concentrations in the products, exceeding maximum levels or intake recommendations, and therefore needs close monitoring. Levels of lead and mercury were under the maximum tolerated levels. There is no maximum authorised level for arsenic in European legislation for fish or fish oils, although it is worth mentioning that the levels detected are significantly lower than those published in previous studies in the same species. In addition, only a small fraction of the total arsenic was present in the inorganic form, being the most toxic one (Wiech et al., 2020). A recent study (Tibon et al., 2022) looking into the speciation of arsenic in the same samples of mesopelagic species as analysed in Berntssen et al. (2021); Wiech et al. (2020), found large proportions of potentially toxic arseno-lipids. However, as the toxicity of arseno-lipids again depends on the present form, future work is needed for a complete analytical characterization of individual compounds before it can be concluded on the toxicity.

To utilise mesopelagic biomass as a new source for food and feed production, safety studies need to be performed to evaluate the suitability of such new biomasses. In WP3, two articles have been published on this topic (Berntssen et al., 2021; Wiech et al., 2020). In Wiech et al. (2020), trace elements, organic pollutants, and potentially problematic lipid compounds from

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six of the most abundant mesopelagic species in Norwegian fjords (mentioned in the section above), were analysed and compared to food and feed maximum tolerable levels and food intake recommendations. Some potential safety issues concerning the mesopelagic biomass were identified: high levels of fluoride in Northern krill, wax esters in glacier lanternfish, and long-chain mono-unsaturated fatty acids in silvery light fish. The authors also estimated contaminant loads in processed mesopelagic biomass, indicating potentially high levels of contaminants (trace elements) in the protein fraction. For fish meal, this did not appear to be problematic, while for the oil fraction, amounts of dioxins and furans were above maximum levels for food and feed ingredients. For detailed results, please see the referenced article.

In Berntssen et al. (2021), a theoretical whole-chain feed and food safety assessment of ingredients from mesopelagic biomass and the resulting farmed fish fed these ingredients was performed. The study was based on analysis of processed mesopelagic biomass. The measurements indicated that fluoride contents will exceed legal feed safety limits, which was not unexpected due to the high levels of fluoride in crustaceans. Apart from this, levels of studied undesirables, such as dioxins, metals, and metalloids, were low when compared to aquafeed ingredients from pelagic fish. The authors estimated that using mesopelagic processed aquafeed ingredients could reduce levels of dioxins and polychlorinated biphenyls (PCBs) by approximately 30 % in farmed Atlantic salmon. With respect to trace elements, mesopelagic meal displayed similar levels as compared to pelagic fish meal in the Norwegian market, except for iron levels which were lower in the mesopelagic biomass. The fluoride levels from the processed mesopelagic biomass were high, with reference to the legal feed safety limits. The study showed that the fluoride levels varied greatly, and the amount of krill in the catches in a fishery targeting *M. muelleri* will determine if the concentration of fluoride in mixed mesopelagic meals are below or above the ML in feed materials. For a more detailed overview, please see the referenced article.

1.3. Raw material handling

The autolytic activity is high in mesopelagic fish, causing rapid decomposition. Because of this, some sort of conservation of the biomass must be performed quickly after harvesting. In addition, the raw material composition can vary greatly (Grimaldo et al., 2022; Olsen et al., 2020). This makes good practice in raw material handling essential if mesopelagic biomass is to be harvested commercially. The catch composition varies between hauls depending on geographical location and season, which means that the mesopelagic biomass can be highly diverse. As an example, if the biomass is dominated by krill, a lower amount of lipids can be expected compared to a biomass consisting mainly of fish (Olsen et al., 2020). Also, the levels of unwanted substances will vary depending on the species composition of the biomass (Olsen et al., 2020). The composition of the biomass must be considered, as it can greatly affect any potential end products.

Small fish with thin skin dies very quickly in the trawl and starts picking up salt from the seawater. Cooling with fresh water and limited towing time made the final product lower in salt and therefore higher in protein. The easiest way to preserve fish and reduce the risk of spoilage

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is to quickly chill the fish down to temperatures around zero degrees or lower to slow down the enzymatic activity. This method is referred to as super-chilling and is becoming a more prominent cooling method onboard fishing vessels targeting food market and human consumption (Einarsson et al., 2019). Gutting also normally improves quality and shelf life of many fish species. Ideally, however, a consistent supply of fresh raw materials with low variations is preferred (van ’t Land et al., 2017).

The presence of biogenic amines is one of the main factors that affect the quality of fish meal. According to Pike and Hardy (1997), the recommended quantity of biogenic amines for high quality fish meal should be less than 1,000 ppm for histamine, and the total sum of all four of the main biogenic amines (cadaverine, putrescine, tyramine, and histamine) should be less than 2,000 ppm. The total volatile basic nitrogen (TVB-N) is a commonly used to estimate spoilage. The TVB-N content in the raw material of acceptable quality/freshness should not exceed 60 mg TVB-N/100 g of whole fish (Einarsson et al., 2019). Histamine levels are frequently used as a proxy for quality in which higher histamine levels indicate poorer quality. Chilling seafood to 2-5 ºC eliminates histamine formation by mesophilic bacteria, but psychrotolerant bacteria can produce toxic concentrations of histamine still. To control this production, storage conditions and product characteristics must be carefully selected.

If the intended use is for human consumption or for feed, the biomass should be well preserved and quickly processed, which means that the processing plant should not be far away from the generation site. For feed uses it is important to guarantee the traceability to the raw material source to avoid cannibalism when employed as aquaculture feed ingredient. The specific regulatory demands for human consumption and feed production will be elaborated on in section 5. Legal considerations.

1.4. Previously assayed processes

In the previous report, D3.4, most of the tested processes have been described. In the current report, these will be mentioned, but not thoroughly presented. Several processes have been identified as suitable for processing of the mesopelagic biomass. Most processes are feasible both on-board and on-land, as new vessels are increasingly equipped with capabilities to both hydrolyse and create silage or meal. The different processes assayed were silage, hydrolysate, and meal production with compositional analyses, yield and assessment for suitability as feed in aquaculture. Mesopelagic biomass has been processed both consisting of one species (homogenous) and consisting of a mixture of species (heterogenous). The processed biomass was also assayed for bioactivity. More detailed information of processing and bioactivity studies can be found in report D3.4.

1.5. Input from stakeholders

To drive the development of mesopelagic fisheries forward, it is essential to gather different stakeholders together, to discuss possibilities and limitations in the industry. Several workshops and meetings have been performed as part of WP3 in the MEESO project to facilitate interaction and discussions between different stakeholders that are relevant with respect to possible mesopelagic fisheries. A summary will be given below:

An industry workshop was held on the 29th of March 2021. The workshop was held by researchers from Wageningen University on behalf of the MEESO project, hosted using Microsoft Teams, lasting 3.5 hours. The workshop successfully brought together different stakeholders from six European countries, and approximately 45 people participated from Norway, Iceland, Ireland, Denmark, the Netherlands, and Spain. The workshop consisted of presentations, breakout sessions targeting relevant questions/problems, and a plenary discussion. The objectives of the workshop were as follows:

- To bring stakeholders from the fishing industry, processing companies, gear manufacturing companies, and research together to share insights and develop contacts
- To understand country-specific factors and interests regarding fishing in the mesopelagic zone
- To gather questions that stakeholders have about the mesopelagic zone and offer preliminary answers
- To understand which questions are of greatest priority for the various stakeholders.

A joint meeting was held in November 2021 between AZTI's researchers from both MEESO and SUMMER projects, and stakeholders of the Cantabrian Sea case study: a fishmeal processor partner (Barna) in SUMMER and a fishing company (Velaspex). The most important logistical aspects on land and at sea that can affect the viability of using this resource as a raw material for fish meal and fish oils were discussed. The primary takeaways from the discussion were:

- Due to the rapid degradation of the biomass, it is not clear if commercial fishing can be profitable without a processing plant on board.
- Existing Basque fleets might not be suitable for implementing an onboard processing plant, and the investment cost for that is high. The low price of the mesopelagic resource calls into question the recovery of such investment.
- The main problem from the side of processing cost is the high-water content (expensive drying technology), and how to deal with ice in case of storage of fish with ice on board.
- It is important to clarify whether catches of mesopelagic fish can be kept in good condition in commercial fisheries, where catches cannot be stored immediately. If under laboratory conditions TVB-N levels do not meet legal limits, there are doubts that these conditions can be improved in a commercial fishing activity. An experiment is currently underway that will look into the degradation processes in Mueller's pearlside (*M. muelleri)* and provide important inputs to quality and processing yield under variable storage conditions.

The 25th of March 2022, an industry-focused seminar was organized in Limerick City, with the title "Building our knowledge on the mesopelagic zone". The seminar was co-organized by MEESO and the Irish Sea Fisheries Board. The aim was to communicate our view of the current situation surrounding sustainable development of mesopelagic resources and commercial

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possibilities. The seminar consisted of a total of ten twenty minutes presentations, plus time for questions and discussions. A complete summary of the talks and topics covered can be seen in the previously submitted report from WP3, D3.4. The seminar was ended with an open-panel discussion. There were more than 30 attendees (in-person and virtual participation).

2. Relevant processes

2.1. Fish meal and fish oil

Fish meal is typically a dark brown powder, but there are numerous variations based on fish species, particle size, lipid- and moisture-content etc. Fatty acids constitute the liquid component of fish oil. Fish oils are typically rich in PUFAs, which are required to maintain fluidity in membranes in cold environments. Most of the fish meal and oil are produced by the wet pressing method with these steps: cooking, which coagulates protein and releases bound water and oil, pressing, decanting, and centrifuging. The pressing step compresses unbound particles (primarily protein and bones), the decanting removes sludge from the liquid phase (press liquor) and centrifugal separation separate oil and stickwater. After the stickwater has been concentrated, it is combined with the press cake and dried, resulting in two distinct products: fish oil and meal (Einarsson et al., 2019). 100 kg of raw material produce approximately 20 kg of fish meal and between 3 and 6 kg fish oil (Einarsson et al., 2019). Typical fish meal contains 64 to 67 % crude protein, up to 12 % fat, and 10 to 20 % ash. In addition to a higher protein content of 68 to 72 %, specialty products typically have a lower biogenic amine content, which reflects the freshness of the product.

The two main fish meal formulations used today in Norwegian aquaculture feed production are NorSeaMink and the higher quality Norse-LT 94, both representative for the world commodity market (Einarsson et al., 2019). Typically, the sales values of fish oil are determined for oils containing 2-2.5 % free fatty acid, 3.5 % unsaponifiable material, and 0.3 % impurities (Iñarra et al., 2020). When these thresholds are exceeded, the price is negatively affected. Other factors that may have a negative effect on price include odour and dark colour (Kousoulaki et al., 2009). Initial experiments on the creation of fish meal with mesopelagic biomass has been performed as part of the MEESO project. Additionally, we have collected rudimentary data from other partners with previous knowledge on the subject. The biochemical parameters collected on Mueller’s pearlside is presented in Table 2. For clarity of reading, the water content has been adjusted mathematically to 10 % regardless of actual content.

Table 2 Biochemical parameters collected on Mueller’s pearlside

	Protein (wt %)	water-soluble protein (wt %)	fat (wt %)	ash (wt %)	Adjusted water content (10 %)
Northern Sea (Norway)	62.8	29.7	15.7	13.3	10
Northern Sea (Iceland)	63.8	28.8	11.3	17.8	10
Fish meal BoB	63.7	-	13.6	13.2	10

- Indicates no available data

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2.2. Fish protein hydrolysates

Fish protein hydrolysate (FPH) is produced from the protein fraction of whole fish or fish sidestreams. The hydrolysed fractions are composed mainly of proteins, peptides, and amino acids. In its concentrated form it is a stable and long-lasting product. The hydrolysate may be presented either as concentrate or as powder with 5-10 % humidity. The colour is normally creamy, and it smells and tastes like fish. Typical parameters are ~80-90 % protein, less than 5 % humidity, negligible amounts of fat and less than 5% ash. Depending on the degree of hydrolysis, protein hydrolysates may have advantageous functional properties such as solubilization, gelling, and antifoaming agents in contrast to protein concentrates. Additionally, FPHs may have bioactivities or other promising properties relevant to certain areas: Moderate antioxidant activity, high nutritional and functional value, less coagulation in reaction to heat, good contents of high value amino acids, good properties as a nitrogen source for microbial culture media, immunostimulant activity for aquaculture applications, reduce cooking loss, improve water holding capacity, reduce drip loss, and more (Gao et al., 2021; He et al., 2013).

The hydrolysates can be obtained by either enzymatic hydrolysis, which is most common, or by acid hydrolysis. The parameters that are commonly adjusted include time, temperature and the enzyme used. Additionally, pH may be adjusted, but pilot-scale observations suggest that this is not a scalable solution. Briefly, the steps of an enzymatic hydrolysis involve homogenization, addition of water and enzyme, and after incubation follows centrifugal separation and concentration. This will normally result in an aqueous protein rich phase, oil and sediment phase. The enzymatic hydrolysis can be performed by one protease or by a mixture of proteases. Antioxidants can be added to prevent lipid oxidation as fishy taste and odour can reduce applications range. An excessive hydrolysis degree can cause bitterness and loss of functional properties (Zhang et al., 2021). Different drying processes can be selected. Additional steps that may be employed is the separation of the water phase with membrane filtration or steps to purify the oil phase, but this is considered beyond the scope of this report. The difference with the silage technology (see 2.3.) is that the process is more controlled and rapid.

Enzymatic hydrolysis trials with *M. muelleri*

To obtain hydrolysates, 6 different enzyme-combinations of commercial enzymes and test conditions have been used on samples collected in the BoB, and 4 combinations on samples collected in the northern sea. Both locations included experiments with endogenous enzymes. The composition of the hydrolysate with the highest amount of protein obtained in the BoB was: Protein: 7.74 ± 0.01 %, dry matter: 9.82 ± 0.06 %, ash: 0.95 ± 0.05 %, fat 1.1 %, Degree of Hydrolysis (DH): 63 %. Four fractions resulted from the hydrolysis trials and the yield in each fraction was: 9.09 % of a lipid-water-protein emulsion, 67.38 % of liquid hydrolysate, 13.68 % of nondigested pellet and 9.65 % of bones. The yield of the total protein from the raw material obtained in each fraction was: 13 % in the emulsion, 58 % in the liquid hydrolysate, 19 % in the undigested pellet (FPC) and 10 % in the separated bones. Most of the oil was contained in the emulsion phase (45 %) and the rest was in the undigested fraction (34 %) and in the liquid hydrolysate (20 %).

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The hydrolysis trial carried out with the biomass of the Norwegian Fjords gave a hydrolysate with a 6.5 % of protein, 8.6 % dry matter, 1.4 % ash and 0.2 % fat. The protein yield in the liquid hydrolysate was 59 %, and 31 % in the undigested pellet.

2.3. Silage

Fish silage is a liquid product made from the entire fish or a portion of it, including the viscera, to which acids, enzymes, or lactic acid-producing bacteria have been added. Fish silage is a low-investment, low-cost, and simple-to-produce alternative to fish meal. Liquefaction is caused by the action of enzymes naturally present in the fish and is accelerated by the acid, which creates the ideal environment for the enzymes to break down the tissues and inhibits the growth of spoilage bacteria. The quality of the silage is contingent on the freshness of the ingredients (Olsen & Toppe, 2017).

First, the raw material (fish) is minced; then, a hammer mill is used to obtain suitable particles. A grinder with a screen containing holes measuring 110 mm in diameter can also be used. Immediate addition of 3.5 % by weight of 85 % formic acid (35 kg acid per one tonne of fish). It is essential to mix thoroughly so that all the fish is in contact with acid, as pockets of untreated material will putrefy. To avoid bacterial activity, the mixture's acidity must be at or below pH 4. After the first mixing, the silage process begins naturally, nevertheless, periodic stirring is necessary to maintain consistency. The production tank can be any size but must be circular. The warmer the mixture, the quicker the process. Silage prepared from fresh white fish offal liquefies in around two days at 20 °C, 5-10 days at 10 °C, and considerably longer at lower temperatures. Extended hydrolysis leads to overall deamination, also reflected by the decrease in essential amino acids (EAA) and increase in total volatile basic nitrogen. An increase in acid addition could improve the stability of the pH and minimize NH_3 production, improving the quality of the silage. However, there is a consensus that acid-sensitive amino acids decrease in fish silage, in particular tryptophan. But the main decrease seems to occur within the first month, indicating that the amino acid degradation stabilizes after a certain period of storage (van ’t Land et al., 2017).

Once the silage is prepared it can be handled like any other liquid and transported in containers suitable for the further purpose of the product. If the oil must be removed and used for other purposes, it can be separated by heating and centrifuging. Fish silage can be concentrated to reduce its bulk, but more experimental work needs to be done to assess the commercial advantage of such process. One drawback with fish silage is the high-water content which makes it difficult to use directly in dry or moist feed. The silage may however be used locally after drum-drying or co-drying with other feed ingredients like soybean-, feather- or poultry by-products meals or cereal brans (Olsen & Toppe, 2017).

The composition of fish silage can vary, with the following ranges: moisture content 56-78 percent, protein content 13-18 percent, oil content 5-25 percent, and ash content 1-4 percent. The chemical composition of fish silage determines the characteristics of the product. The

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viscosity of liquid fish silage is based on its chemical content. The viscosity of high-fat silage is significantly greater than that of low-fat silage. Additionally, the decrease of viscosity in silage is closely related to the increase in the DH. One of the quality factors of fish silage is the freshness of the raw material. Measurements of TVB-N and ammoniac are used as an indication of freshness of the raw material for high-quality fish meat. As for fish meal, raw material with a value higher than 50mg TVB-N/100g should not be accepted for high-quality fish silage. If the silage contains high level of fat, oxidation will occur. Peroxide value is a measure of the early stage of oxidation of fat.

Experiments

Two silage trials were performed with the biomass of the BoB and two trials on the biomass obtained in the North Sea, both were *M. muelleri.* The BoB biomass was treated for 30 days and 90 days whereas the North Sea biomass, aimed for bioactivity measurements, was treated for 2 days. The maximum liquefaction of the BoB biomass was reached day 12 when the DH was 56 %. However, this continued increasing until day 90 reaching 93% DH in the liquid fraction, as the enzymes continued digesting the protein. In day 12, the proportion of each fraction was as follows: Oil 1.28 %, interphase 9.84 %, liquid hydrolysate 69.88 % and solid digestate 19.69 %. In day 12, the yield of protein in the liquid phase was 61 %, 32 % in the solids and 7 % in the emulsion phase. The oil yield was an 18 % in the oil phase, a 21 % in the liquid hydrolysate, a 20% in the solids and the remaining 41 % in the emulsion phase. This proportion was maintained stable until day 90 when the degree of hydrolysis was the highest. The composition of the hydrolysate was: Protein 14.28 ± 0.28 %, dry matter 20.02 ± 0.14 %, and ash 3.27 ± 0.21 %. The North Sea biomass silaged for 2 days, had a protein yield of 55.2 % in the solid phase and 40.7 % in the aqueous phase, whereas the oil yield was 76.2 % in the oil phase and 14.5 % in the solid phase.

2.4. Bioactive peptides

Proteins may contain amino acid sequences with biological activity that is apparent only after cleavage of the parent protein. Bioactive peptides may be released during gastrointestinal hydrolysis, enzymatic hydrolysis with commercial enzymes or during food processing such as cooking, fermentation, or ripening. To identify these peptides, both *in silico* and *in vitro* processes are common (Daliri et al., 2017). Several bioactivities have been identified in different hydrolysates, e.g., antidiabetic, cholesterol-lowering, antihypertensive, anticancer, antimicrobial, multifunctional, anti-inflammatory and antioxidant (Daliri et al., 2017; Whitaker et al., 2021). In the MEESO project, samples (silage, aqueous extracts, hydrolysates) have been assayed for different bioactivities. Assayed indications included heart health, type 2 diabetes, inflammation, pain, gut health, mental health and sarcopenia from a range of different bioassays (Naik et al., 2021). The assayed biomass was Mueller’s pearlside, Antarctic krill, or mixed catches of the two, and blue whiting. Discovered bioactivities involved COX-1 and COX-2 inhibition (targets for inflammation and pain), MAGL inhibition (target for antinociceptive, anti-inflammatory and anticancer), antioxidant activity (anti-aging, anti-inflammatory), renin inhibition (heart health). The bioactivity observed from the project will be further elaborated in another report (D3.7).

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2.5. Fish bones

The bones should be converted into an edible form by softening its structure. This can be achieved by utilizing different methods including hot water treatment, cooking of fish bone in superheated steam (120-130 °C under pressure). Or addition of hot acetic acid solutions (hydrochloric acid, lactic acid, acetic acid). The treated bones need to be subjected to saponification, degreasing and degumming. The cleaning of the bones may be done by chemical or enzymatic treatment (with proteases). Process yield depends on the dry matter content of the bones, which is different in fresh and in cooked bones (side streams from the canning industries) and depends also on the degree of elimination of organic matter during the process. Some authors report yields of 4 to 5 % in obtaining a calcium preparation from cod and salmon backbones respectively (Bubel et al., 2015). For backbones coming from the canning process, which dry matter content is much higher (65-70 % face to a 22-34 % of fresh bones) the observed yield in previous work done in AZTI is of around 30 %.

Besides the solubility, the flavour is a factor to consider when choosing a calcium supplement to add in the formulation of foods fortified or functional. The production of tricalcium phosphate and collagen supplement involves high water consumption in the cleaning steps or enzyme treatment steps, and energy for drying. The resulting fish bones as a co-product of the hydrolysis of *M. muelleri* in the BoB trials were analysed for proximal composition and heavy metals. The enzymatic hydrolysis yielded 110-130 g of bones per kg of fish. The content of protein, ash and total fat falls within the expected range compared to previous published analyses (Toppe et al. 2007) considering that the spines come from the process of enzymatic hydrolysis and have followed a subsequent washing that increases their mineral content (Table 3). The levels of cadmium found in bone are within the tolerated limit for dietary supplements (1.0 mg/kg) but do not exceed the limit of 2 mg/kg (with a moisture content of 12%) established for animal feed.

Table 3. Centesimal composition and heavy metal content of M. muelleri spines resulting from enzymatic hydrolysis.

Protein	%	25.86 ± 0.37
Collagen	%	8.00 ± 0.38
Ashes	%	60.50 ± 0.02
Grease	%	1.95 ± 0.07
Humidity	%	4.74 ± 0.13
Calcium	%	18.79 ± 1.03
Phosphorus	%	10.44 ± 0.55
Ca:P		1.80 ± 0.004
Zinc	**(mg/kg)**	532.42 ± 23.67
Lead	**(mg/kg)**	<0.2
Cadmium	**(mg/kg)**	0.95 ± 0.04
Arsenic	**(mg/kg)**	< 0.2
Mercury	**(mg/kg)**	< 0.05

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Efficient methods for recycling and reuse of minerals in fish bones from other marine fish (such as herring, blue whiting and cod), as ingredients for aquaculture have previously been developed and their impacts in Atlantic salmon documented (Albrektsen, Lock, et al., 2018; Albrektsen, Østbye, et al., 2018; Ytteborg et al., 2016). More than 80 % of phosphorus in the bones was recycled by demineralisation of the fish bones. Methods for hydrolysis of collagen proteins in the fish bones after demineralisation has also been developed (Albrektsen, 2017), with the aim to ensure total utilization of available resources.

3. Applications

Applications of the different product categories is spread between human nutrition as food, pharmaceutical or nutraceutical, feed and bioenergy or fertilizer. The expected price ranges and willingness to pay will often be associated with area of application with pharmaceutical and nutraceutical products at the top, food and feed in the middle and fertilizers or bioenergy at the bottom (Whitaker et al., 2021). The amount of necessary product development is also commonly tied strictly to the expected price.

3.1. Human nutrition

The most dominant product categories in human nutrition are the fish oil, FPHs, the bioactive peptides, and the fish bones. FPHs are exceptionally diverse in applications and potential applications when one looks exclusively on the protein content. However, it is known that the fish flavour, which is often associated with the marine hydrolysates, is not desirable for many consumers. In food products, the hydrolysates have been used as tasting and flavouring agents for bakery goods, ice creams, sweets, soups, mayonnaise etc. They are also used as texturizing, jellying, binders or emulsifying agents, salt and monosodium glutamate replacers, and in fish sauce. Additionally, FPHs have been employed as essential amino acids, bioavailable and highly digestible proteins.

10 % of the produced fish oil is consumed in human nutrition both as nutraceuticals and pharmaceuticals (EUMOFA, 2021). In Europe, fish oils are widely used in the manufacturing of edible oils and fats, for example margarine. Other uses include the paint and varnish industry. In addition, there are several other specialized uses for small quantities of fish oils. Fish oils usually have to be low in free fatty acids, less than 2 %, to obtain the best price. Production of high-quality fish oils depends on the use of fresh raw material, proper purification and good storage (FAO, 1971). Bioactive peptides are also relevant products aiming at human nutrition. Marine derived proteins and peptides have potential uses as novel products in food, beverage, nutraceutical, pharmaceutical and cosmetic industries. However, the claims often associated with bioactive peptides are subject to stringent regulations.

The use of fish oil supplements for cardiovascular (CV) protection has been a controversial topic for years. Recently, Vascepa produced by Amarin, a prescription fish oil comprising high dose icosapent ethyl, received an expanded indication from the US Food and Drug Administration (FDA) for CV risk reduction in some patients with elevated triglyceride levels. Icosapent ethyl is the only prescription omega-3 approved for CV reduction. Icosapent ethyl

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can be used as an adjunct to statin therapy to reduce cardiovascular events in patients with elevated triglyceride levels (≥150 mg/dL) and established atherosclerotic cardiovascular disease (ASCVD) or diabetes and at least two other CV risk factors. These recommendations are based on outcomes from the Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT), which demonstrated a 25 % reduced risk of major CV events in patients receiving icosapent ethyl, and a 35 % reduced risk reduction in participants with a history of ASCVD.

The biotechnology company Hofseth Biocare announced the launch of ProGo bioactive peptides in the U.S., which helps maintain iron-rich blood, promotes energy utilization, supports red blood cell production, supports gastrointestinal and immune system health, and assists in iron absorption from daily diet. Additionally, NeoMatrix Therapeutics, Inc., a clinical-stage company, announced positive topline data from its Phase 1 clinical trial of NMT-cP12, a bioactive peptide for intravenous treatment of burns within 2 to 4 hours of injury. BWPH – a fish protein hydrolysate from *Micromesistius poutassou* induced CCK and GLP-1 secretion in STC-1 cells, was subsequently demonstrated to increase plasma concentrations of CCK and GLP-1, improve body composition and reduce body weight upon oral administration (1.4 g) to 120 overweight (25 kg/m2 ≤ body mass index (BMI) < 30 kg/m2) adults over 90 days. BWPH is now commercialised and marketed as Slimpro® (Nobile et al., 2016). In addition, peptides purified from dried bonito (katsuobushi) via thermolysin digestion exhibiting ACE-inhibitory activities *in vitro* were also shown to exhibit anti-hypertensive effects in spontaneously hypertensive rats and borderline (high normal) and mildly hypertensive adults (1.4 g/ day orally administrated over 5 weeks) (Fujita et al., 2001; Yokoyama et al., 1992). Katsuobushi oligopeptide received official approval as Foods for Specific Health Use (FoSHU) in 1999 by the Ministry of Health and Welfare in Japan.

Fish bones can make for products high in elemental calcium, phosphate, and collagen. It may also contain magnesium and trace elements (e.g., iodine, iron, zinc, and selenium). The composition of fish bones is diverse among species, but generally contain 45-60 % tricalcium phosphate and 35-55 % collagen on a dry matter basis. The fish bones have potential both as products in human nutrition and the nutraceutical market. Phosphates are employed in food applications due to their multifunctional properties (as pH regulators, binding agents, electric charge stabilizers). They may have a synergistic effect on preservatives as well as acting as texturizers due to their water binding capacity of proteins.

3.2. Feed, bioenergy, or fertilizer

The feed category is dominated by fish meal and silage as the largest contributors. Fish meal is predominantly used for animal feed, with aquaculture accounting for the majority at approximately 70 % and increasing to 78 % in recent years, swine accounting for 25 % and decreasing to 14 % in recent years, and poultry somewhat stable at 5 %. Additionally, nearly 70 % of the produced fish oil is used in aquaculture (EUMOFA, 2021). Silage is used in several areas of feed (animals, mink, fox, farm animals and aquaculture) and pet food in addition to energy and fertilizer. FPH are also relevant as feed as they are highly digestible and bioavailable

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as protein source. The use-cases as feed are plenty although aiming for human consumption is more common. Fish bone meal can also be used in fertiliser formulations.

4. Techno-economic feasibility of the proposed processing options

The processing options analysed in this report are fish meal and oil, silage (acid autolysis) and fish protein hydrolysates (including bioactive peptides).

When analysing the techno-economic feasibility, one of the main critical factors is the raw material quantity to be processed and its price. Whether a processing option is feasible or not, depends on it providing enough added value to the final product, making the whole process economically viable. If more value can be obtained from the products, the probability of obtaining profitability for all the actors of the value chain increases. Both aspects are being considered in the MEESO project. Regarding the capture possibilities of mesopelagics, in a fishing simulation made in MEESO project in BoB, 230-470 tonnes/day were captured per vessel. The maximum capacity per vessel is 1,400 tonnes. There are two boats in the Basque Country that could land each one 1,100- 1,400 t each three days. 75 days along 6 months of campaign when the boats do not fish cod (October-Mars). That means a total volume per week of 2,200- 2,800 tonnes and a total volume per campaign of 34,000- 70,000 tonnes in the Basque Country, with two more boats available in the Spanish fleet.

Regarding the raw material price, a previous study on the economic feasibility of the processing of salmon side-streams showed that all these options were profitable, but the price considered for the raw material was 115 €/tonne (€/t) (Venslauskas et al., 2021). This is a price usually paid per tonne of fish processing by-products, otherwise destined to landfill, while in the case of species that are captured expressly for the purposes hereby described the prices should be much higher. Whether a higher price can be paid will depend on the possibility of obtaining valuable co-products from this new biomass. In the same study, the sensitivity analysis showed that the prices of the fish meal and oil, as well as the price of a high-quality FPH, were the most critical aspects that determine the economic viability. In a similar study on the processing options for the discards of the Basque fleet, an acquisition price of raw material of 200 €/t has been used as average reference price (Iñarra et al., 2020). This is a value well below the average price of fish for fresh human consumption. The economic analysis showed that in order to attain a discount rate of 5 %, a maximum of 193 €/t and 89.2 €/t could be paid for the raw material for the production of FPH, and for fishmeal and oil respectively. The reference price for FPH was 12,000 €/t, market price for certain flavour concentrates. The reference price for fish meal used was 800 €/t and 900 €/t for the fish oil. However, this price is expected to increase in the coming years due the increasing demand (see 6.2.).

4.1. Fish meal and fish oil

The techno-economic feasibility of the production of fish meal and oil is based on the following assumptions: As there is no limitation from the market to the production due to the increasing demand from the aquaculture feed sector, the quantity to be processed depends on the recommended quota of the biomass. This is also investigated in the MEESO project. In a

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previous study conducted by the Institute of Marine Research (IMR) in the Icelandic fishery from 2009 to 2013, it was recommended a precautionary exploitation of the stock not exceeding 30,000 tonnes in that area. However, catches were very unreliable, from 46,000 tonnes in 2009 to none in 2013.

Due to the estimated levels of organic contaminants found in fish oil in the samples from the Norwegian Fjords (Wiech et al., 2020), it might not be suitable for food or feed unless refining and cleaning methods are employed. However, maximum levels for animal feed were not exceeded. The levels of organic contaminants found after processing of biomass from the North Atlantic were below the maximum levels in feed and lower than in analysed commercial fish oil samples (Berntssen et al.2021). The concentrations in fish meal were also below the maximum levels. Applying a feed-to-fillet transfer model for Atlantic salmon, it was estimated that processed mesopelagic aquafeed ingredients would reduce the level of dioxins and PCBs by ~30%.

Because of rapid degradation of these small sized fishes, on board processing may be advisable, and a suitable preserving method should be implemented on board. Maximum time to preserve the fish before landing must be established. Ensilaging of the fish with formic acid on board the vessel is a potential method for preserving. Existing Basque fleets might not be suitable for implementing an onboard processing plant, and the investment for that is high. The low price of the mesopelagic resource, estimated in 0.2 - 0.7 €/kg calls into question the recovery of such an investment. Therefore, the study on the economic feasibility must be done in two scenarios: (1) Assuming that the preservation of fish on board is technically feasible, followed by transportation and processing in an existing fish meal plant on land. (2) Processing the fish on board in an existing factory vessel.

We assume that the existing plants have enough capacity for processing this new raw material. Thus, the main associated costs will be processing costs, basically transportation and energy costs. The major sources of energy for the plants themselves are fossil fuels mostly used for heating (cooking of raw material, drying of fishmeal, evaporation plant (Fréon et al., 2017). Energy consumption translated into MJ would be around 1760 MJ for a 100–200 t/h plant with evaporation plant and waste heat recovery, assuming 40 MJ per kg of heavy fuel (FAO, 1986). There are some constraints associated with the high water-content of the mesopelagic biomass. The drying step of the production is considered the most energy demanding, which might result in very high energy costs. Moreover, due to the small size of the fish, preservation on board with chilled water or ice could increase the water content and make its processing unfeasible from the energetic point of view.

The study on the biomass nutritional composition gives an idea of the expected fishmeal and oil yield and hence the value of the raw material. Given an average triglyceride content of 52.94 % for the *M. muelleri* (Grimaldo et al., 2020) the expected oil yield ranges from 0.8 to 3.7 % for the biomass of the BoB and 2.6 to 9.2 % for the biomass of the Norwegian fjords, referred to the weight of fresh fish. The water to evaporate to produce a fishmeal with a 95 % dry matter ranges from 54 to 73 kg/100 kg raw fish. In the fish meal production, the cost of raw material

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constitutes in many cases more than 50 % of the total production costs of the products (FAO, 1986). However, the rising energy costs can skew the prices. The yearly first sale price of sprat varied between 0.19 €/kg and 0.28 €/kg from 2007 to 2020, and herring prices varied between 0.38 €/kg to 0.73 €/kg. The price fluctuations of the raw material for the reduction industry are closely linked to volumes landed in Denmark and to the price level of fishmeal and fish oil (EUMOFA, 2021).

4.2. Fish protein hydrolysates

Investment costs

Figure 3 presents the flow chart of a typical process for obtaining FPH. A facility for around 10,000 tonnes/year would need an investment around 5 million € (source: GEA).

The facility would include the following equipment:

- Entrance mill
- Metal detector
- Hydrolysis reactors
- Three-phase decanter
- Centrifuge
- Membrane filtration units (UF+NF/RO)
- Vacuum evaporator
- Spray dryer

Considering installation, auxiliary equipment, terrain and construction, the total investment reaches approximately between 9.2 and 11.5 million €. In a preliminary estimation, considering this investment and the obtained yields, for a final price for the FPH of 6.1 €/kg and additional revenues of 0.5 €/kg for the fish bones as fertilisers, 0.9 €/kg of fish oil recovered from the emulsion phase and 1.2 €/kg for the solids as fishmeal, the activity would be profitable for a raw material price of 0.2 €/kg, which is the price usually paid for fish processing co-products/side streams, with a payback period of 5 years. The cost of the raw material would in this case be 39 % of total production costs that are 4.61 €/kg. Meanwhile, a raw material cost of 0.7 € would mean 76 % of total production cost that would be 8.3 €/kg. Therefore, a lower price for the main product, the FPH, would make the plant economically not feasible.

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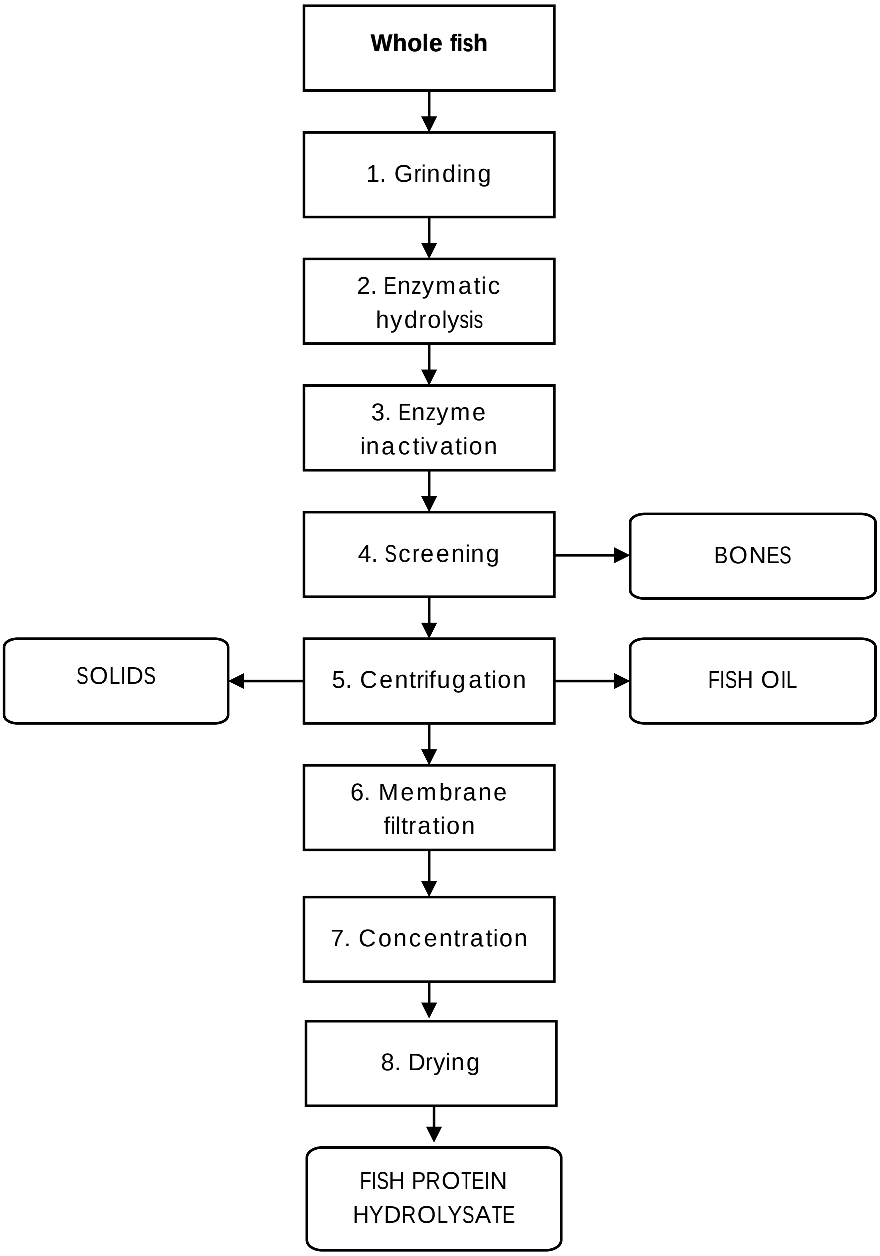

Figure 3. Scheme of FPH production (AZTI).

Energy requirements

Hydrolysate production involves heating raw material and hydrolysis in the first stage, maintenance at hydrolysis temperatures in the second stage, and final heating to end hydrolysis by enzymes in the 3rd stage. Followed by centrifugation and spray drying, which cost is estimated in 1 € /kg final product. The upper bounds of energy consumption for such a process respectively would be 583 kJ of energy for the 1st stage, 3.8 kJ for the 2nd stage, 252 kJ for the 3rd stage and 0.746 kWh for centrifugation and then 4880 kJ for spray drying energy consumption. Costs depend on energy prices in the location of production, but this also clearly emphasizes the enormous contribution spray drying has on energy consumption.

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Raw materials

There is a sustainable supply of mesopelagic fish species currently from harvesting. Seasonal storage may be required like pelagic processors also have the cost of storage though concerning pelagic species/off-cuts. If you make a comparison with the dairy industry, recently a dairy processor in Ireland invested 35€ million for a new I.M.F standards compliant spray dryer (included commissioning and validation). If FPH hydrolysate production includes spray drying a greenfield site near the coast would be preferable. The cost of raw material (20-100 €/t) has a limited influence on the cost of the final product; but the cost of the final product is highly dependent of the amount of raw material available.

In terms of capital investment in a plant to process mesopelagic species into hydrolysates and OPEX, companies like Landes Marine Ingredients announced in 2020 that they would invest $30 million US dollars in two plants to convert salmonid by-products and silage into feed ingredients. Proposed capital investment costs are in the region of 15 million €. A biorefinery approach can be adopted during production of FPH so that three products can be produced - fish oil, peptides, and liquid protein. Spray drying costs are the most expensive part of capital investment. The cost of the raw material for production of mesopelagic FPH is advantageous compared to production of FPH from salmon or sardines. Products that would be competing with mesopelagic FPH from cheaper fish raw materials are produced currently by companies including BII Ltd. and Copalis Sea Solutions and these companies sell to the same markets in the functional foods, cosmetics, pet care space. To offset costs, any company investing in mesopelagic FPH could target the Pharma market with the fish oil product. However, this would also require investment for Pharma production, compliance with Pharmacopeia in the relevant countries where they plan to sell the product and pharma grade manufacturing facilities. Mesopelagic fish oil would need to be purified significantly to be able to compete in the Pharma market with the likes of Amarine. However, oil extracted from Muellers' pearlside is commonly of low quality and additional purification would be needed for it to compete in the market. Omega-3 pharma grade oils can command $300 dollars per month for patient treatment and it is a large market in the USA. In a previous comparative study of the economic feasibility of different processing options for the salmon by-products it was concluded that the two-stage processing of the fish side-stream utilizing valuable marine biomass by producing premium fish oil and high-quality fish protein hydrolysate was a most economically feasible concept. The annual revenues of this process were significantly higher than the processing for obtaining fish meal and oil or the fish silage process (Venslauskas et al., 2021).

The possibility of paying a higher price for the raw material will depend on the price obtained from the FPH. While FPH can reach 8 €/kg for fish flavour production, it will be paid only 3-4 €/kg as protein ingredient for aquaculture feeding. FPH prices depend on the protein content, being highest for a >90 % protein and lower ash content. FPH can be then used as ingredient in high value formulations like nutraceuticals but if the market demand could absorb the expected volume to produce must be carefully considered.

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4.3. Silage

At bigger industrial fish processing units, the by-products are often processed into fish meal and fish oil. However, at small scale processing units, investing in a fish meal plant is not economically viable unless several tonnes of raw material are available on daily basis. When this is not the case, preservation of the raw material by acid silage could be a simple and inexpensive alternative. It could also be used as a rapid stabilization pre-processing method to preserve the fish on-board until it reaches the fish meal plant. Some larger fishing vessels use acid preservation (acid silage) of side streams and by-catch. In Norway, fish silage is commonly used to process side streams from salmon processing plants. Silage is quite stable and can be stored for months, however, some loss of specific amino acids may occur. As compared with a fish meal plant, the cost of silage equipment is lower. For a 10,000 tonnes/year capacity plant, the investment has been estimated in 960,000 € while for a fish meal plant of the same capacity 7.4 million € (Venslauskas et al., 2021). The equipment for a silage plant usually includes, entrance mill, agitation tank, acid tank and automatic dose system, storage tank with agitation, heater, three -phase decanter and storage tanks for oil and the protein concentrate.

Silage can be used directly in liquid form for piglet feeding. However, when dried and when the quality of the silage is good enough, it can be used as fish meal and reach similar prices, but then, the investment must include drying equipment with the consequent increase in the production costs. As a reference, the price of silage in 2013 was 230 €/t while the price of fish meal was near 1,290 €/t (Venslauskas et al., 2021).

4.4. Fish bones

FPH production generates purified fish bones as co-products of the process. The yield obtained per kg of fish processed was around 10 %. This can constitute an additional source of income for the FPH plant. Depending on the capacity of the plant, the costs for producing 1 kg of bulk calcium powder supplement varies between 1,5 and 1 €/kg produced. The process can be combined in a multipurpose plant whole biorefinery concept with the production of other fish by-product-derived products like the production of gelatine from fish bones and skins.

5. Legal considerations

The countries in the European Union (EU) follow the same regulations for food and animal by-products and side streams/co-products (see table 4 for an overview of the main relevant European legislation). The intended use of the biomass defines the legal considerations that must be taken and the regulations to be followed. Briefly, we can divide the use of mesopelagic biomass as “intended for human consumption” or “not intended for human consumption”. If the biomass is to be used for human consumption, it falls directly under food hygiene legislation, meaning that the processes must follow the same regulations that are used in “regular” food production (e.g., fish fillet production). In 2002, the General Food Law Regulation (Regulation (EC) No 178/2002) was implemented, which included the establishment of EFSA, an independent agency responsible for scientific advice and support. Food-related regulations include regulations of microbiological criteria (Regulation (EC) No

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2073/2005), food additives (Regulation (EC) No 1333/2008), maximum levels for certain contaminants (Regulation (EC) No 1881/2006), and others. Also, the Novel Food regulation (Regulation (EC) 2015/2283) might impact some of the potential products arising from mesopelagic biomass. The regulation states that if the food has not been "significantly consumed" in the EU before 15th of May 1997, an authorization is necessary:

> *"Novel Food can be newly developed, innovative food, food produced using new technologies and production processes, as well as food which is or has been traditionally eaten outside of the EU."*

The novel food regulatory framework incapsulates both processes and the consumed biomass. If such a novelty is to be approved, an application for approval must be sent the EC, in compliance with the EC implementing rules and the EFSA guidance on safety assessment before market introduction. An article focusing on the use of marine bioactive peptides for supplements and functional foods has been published as part of the work in WP3 of the MEESO-project. The article covers, among other topics, regulatory aspects and approval of marine biomass for human consumption (Whitaker et al., 2021). In relation to the bioactive peptide's commercial use, there may also be claims connected to their bioactivities. In the EU these are regulated under the EC Regulation on nutrition and health claims (Regulation (EC) No 1924/2006). Regulations related to health claims are further elaborated on in the MEESO report D3.7.

If the use of the biomass is not intended for human consumption, it falls under the EU regulations for animal by-products (materials of animal origin which are not consumed by humans). The Animal by-products Regulation (Regulation (EC) No 1069/2009) has been implemented to ensure that such products impose no risk to human/public or animal health. Briefly, the animal by-products are categorized into three categories based on the risk of transmitting diseases: Category 1 to Category 3. Category 3 animal by-products are considered to have low risk of transmitting disease and can therefore be used to make feed for food producing animals. Categories 1 and 2 are considered to have high and intermediate risk of spreading diseases, respectively, and are therefore not used for food or feed purposes. Category 2 can be used as fertilizers or soil improvers, while Category 1 can be used for production of renewable fuels.

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Table 4. Main European Legislation relevant for food or medicinal products in EU

Claim type	Regulation
Relevant food and functional food regulations/claims	
Food additive	Regulation EC No 1333/2008
Origin, composition, acceptance of additives	Regulation EU No 231/2012
Organic products	Regulation (EU) 2018/848
Food (organizations of the market in fisheries and aquaculture)	Regulation (EU) No 1379/2013
Nutrition and health claims for foods including food supplements	EU Regulation EC/1924/2006
RDA for vitamins and minerals	Annex III Nutrition Information Regulation (EU) 1169/2011
Novel Food Regulation	(EU) 2015/2283
Medicinal claims (cannot be made on food but can be made on pharma type products – i.e. Omega-3s)	Human Medicinal Products Directive 2001/82/EC
Relevant Feed and Feed additive claims	
Commission regulation 68/2013	
Toxic contaminants	Directive 2002/32/EC
Regulations regarding cosmetics	
Cosmetic products	Regulation 1223/2009/EC
Justification of claims used in cosmetic marketing	Commission regulation 655/2013 fo 10 July 2013
Regulations regarding fertilizers	
Heavy metal levels	Regulation EU2019/1009
Bio-stimulants	Subject of CEN

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6. Markets

6.1. Fish meal and fish oil

The countries with major industrial fisheries are Peru, Iceland, Denmark, Chile, Norway, and South Africa. Annual production of fish meal and fish oil in the EU fluctuates between 400,000 and 600,000 tonnes, and 120,000 and 200,000 tonnes respectively. Denmark is the largest producer accounting for 40-50 % of the total industrial fish volume (EUMOFA, 2021). During the period 2000-2010, the annual average production of fish meal in the EU was over 540,000 tonnes, while it was approximately 474,000 tonnes during the period 2010-2019. The declining trend is associated with an increase in human consumption of small pelagic species (herring, mackerel) and a general decrease in fishing for feed production. In 2019, Norway and Denmark produced a combined total of 390,000 tonnes of fish meal and 120,000 tonnes of fish oil. Due to its salmon and trout aquaculture production, Norway is one of the world's largest consumers of fish meal and fish oil. In Europe, the average annual production of fish oil between 2010 and 2019 was 155,000 tonnes (EUMOFA, 2021).

From January 2009 to January 2021, the price of fish meal in Europe increased by 37 %, reaching 1,164 €/t. The price level fluctuated during that period in accordance with global price trends. The price of fish oil increased by 85 % to 1,419 €/t during the period in question (EUMOFA, 2021). Fish meal and fish oil prices are expected to increase by 30 % and 13 %, respectively, in nominal terms by 2030, because of the strong global demand. The production of fish meal and fish oil is projected to grow moderately over the coming years, due to better utilization of side streams from the fish processing industry (FAO, 2020). Increased use of other low-trophic species such as krill, can be expected.

6.2. Fish protein hydrolysates

The FPH-market is lucrative, but there is stiff competition in this area in Europe, Asia, South and North America. Hydrolysates of marine origin also face competition from other hydrolysate markets. This includes the whey protein hydrolysate market, which currently dominates the health and wellness space with companies like Kerry Foods, Arla Food Ingredients, and other dairy co-operatives globally, with a large presence in the EU and USA market – the largest market for functional foods currently. The high value Pharma sector for fish oils is dominated by BASF, Amarin and a few other players. Regulation of pharma grade products in the USA and European markets is stringent. All companies selling existing FPH products in the EU for health benefits are currently sold as supplements. No company has achieved a health claim from the European Food Safety Authority (EFSA). The company Senmi Ekisu Co. Ltd (partners of Hofseth) have a novel food claim in the EU for their antihypertensive sardine powder Valtyron®. The CalGo® product also targets the supplements market. The health targets for FPH generated from mesopelagic species are similar to other fish protein hydrolysate manufacturers (based in the USA and Chile using Sardine and other pelagic species as well as BII based in Ireland but with Norwegian investors) in terms of weight management, BMI control, blood pressure and cholesterol, satiety and weight loss. Treatment of anaemia is a novel

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target market. Care should be taken when carrying out clinical trials and research to select the correct human population to complete the studies, so that a clinical/pharma claim, or even functional food health claim, can be obtained in the EU. EU – EFSA regulations are considered the “gold-standard”, and if products/companies are compliant with EU regulations with regards to use of ingredients as functional foods with health claims in Europe, they will likely be compliant and secure health claim status elsewhere (US, Japan, Canada, and Australia).

The FPH market can be segmented according to: (1) Fish species, (2) Technology used to produce the hydrolysate, (3) Product form (powder, liquid, paste), and (4) application (pet/companion feeds, animal nutrition, aquaculture, human nutrition, functional foods/nutraceuticals, and Pharma and agriculture applications). According to The Insight Partners (2021), the market is estimated to be valued at US$588.86 million dollars (€ 497.91 million) by 2027 and will have a compound annual growth rate (CAGR) of 4.8 % from the present time (2021).

6.3. Silage

The production of silage is derived from natural sources, which includes all fish species. Before making a substantial investment, the production of silage may serve as a preparatory step towards the production of fish meal by demonstrating the availability of a sufficient raw material supply. Depending on quality and cost, silage can join the same market as fish meal.

In 2021, the available co-products in Norway were 1089,000 tonnes (27 % of the total biomass). Approximately forty % of the available co-products were processed into silage before being used for other purposes, such as animal feed or biogas/energy (this category is primarily from aquaculture when the biomass is categorised as by-products and unsuitable for human consumption). The processing of fish meal and fish oil accounted for 17 % of the available co-product biomass. The majority of fish processing co-products (67 %) are utilised for animal feed, followed by biogas (20 %) and human use (13 %) (Myhre et al., 2022).

6.4. Bioactive peptides

In 2020 the bioactive peptide market was valued at US$ 48.62 billion, projected to reach US$ 95.71 billion by 2028, growing at a CAGR of 8.86 % (Research, 2022). The nature of bioactive peptides would indicate that most market potential is in the pharmaceutical, nutraceutical, or feed markets since they are more likely to demand qualities beyond standard nutrition. The food supplements sales are rapidly increasing in several countries which makes it likely that marine bioactive peptides (including from mesopelagic sources) would become more attractive to consumers. Table 5 shows some examples of marketed bioactive peptides, the producer, price point and their health claims. Several commercial facilities for scaling up the process exist; a comprehensive database can be found at https://biopilots4u.eu/. Additionally, Nofima AS in Norway owns and operates a pilot scale production facility with necessary approvals for creating food, called Biotep (Biotep - Nofima).

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Table 5. Examples of marketed bioactive peptides.

Product name	Claim	Producer	Price point (B2C)
PeptACE™	Antihypertensive	Natural factors Nutritional products Ltd., Canada	£34.94 (for 90 capsules - supplement)
Vasotensin®	Antihypertensive	Metagenics, USA	$US80.00 (for 120 tablets)
Levenorm®	Antihypertensive	Ocean Nutrition Canada Ltd., Canada	N/D
Peptide ACE 3000	Antihypertensive	Nippon supplements Inc., Japan	N/D
Precardix®	Antihypertensive	Marealis Health Inc., Norway	$49.96 (for 60 tablets)
Valytron®	Antihypertensive		
Stabilium® 200	Stress relief	Yalacta, France	€38.53 (for 60 tablets/capsules)
AntiStress 24	Stress relief	Forte Pharma Laboratories, France	€11.99 (for 60 capsules)
Protizen®	Stress relief	Copalis Sea Solutions, France	€38.53 (capsules)
PeptiBal™	Immune modulatory	InnoVactiv Inc., Canada	€68.40 (30 capsules)
Seacure®	Gastrointestinal health	Proper Nutrition, USA	€89.00 (30 capsules)
Marine collagen	Joint and skin health	Seagarden Norway	€52.81 (1 jar - 300 g)
ProGo®	Weight management	Hofseth Biocare Norway	€369.99/5 kg (price of similar product)
SlimPro®	Weight management		$49.90 (60 capsules)

Key companies in this space include Archer Daniels Midland Company, Seagarden AS, Phermpep Co. Ltd., Arlak Biotech Pvt. Ltd., Naturade, Royal DSM, MYOS RENS Technology Inc., Natural Factors Inc., Valio Oy, GenScript, HELIX BIOMEDIX, Oryn Therapeutics, Selecta Biosciences, NIBEC, vivitide, PEPTIDE INSTITUTE, INC, BCN Peptides, Setlance srl., APEPTICO Forschung und Entwicklung GmbH, and BIONANOPLUS. Restraints in this area include the cost to develop a product and to document evidence of safety, lack of complete knowledge regarding the mechanism and functions of bioactive peptides in terms of health benefits.

6.5. Fish bones

The recently revised report on the mineral supplements industry hints at an incremental opportunity of US$ 15 billion by the end of 2031. According to this analysis by Persistence Market Research, the mineral supplements market amounts to a total revenue worth of US$ 15 billion in 2021, which is anticipated to double by the end of the decade. Analysts have also predicted a demand for mineral supplements to expand at a robust CAGR of 7.4 % over the next ten years. The UK is expected to hold 18 % of the market share in the European region (http://www.persistencemarketresearch.com/market-research/minerals-supplements-market.asp).

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Figure 4 Some examples or mineral supplements from fish bone in the market

PHOSCALIM ® 7-9 €/kg

Mineral extract obtained by enzymatic hydrolysis of specially selected lean **fish (cod, haddock)**. It is an excellent source of calcium, phosphorus, collagen, trace elements, hydrolyzed proteins and glycosaminoglycans.

OSSOMER 253 €/kg

Each 750mg capsule contains: 750mg MCHC. 195mg elemental calcium, 98mg elemental phosphorous, 33mg elemental magnesium, 90mg marine collagen, 100IU cholecalciferol (vitamin D3)

CALCEO Forte 440 €/kg

Extract enzyme of spines of fish, hesperidin, extract marrow of bamboo, vitamin D

Dr. Earth 3-6 €/kg

Fishbone meal fertiliser. Slow-release product.

Kalsytech equine 247 €/kg

MCHC, with complex carbohydrates

Table 6 Some commercialised calcium supplements (prices are given in USD per kg active principle):

Supplement (nutraceutical form, in capsules)	***Price (USD/kg)***
Calcium carbonate – generic	107
Calburst (Nature Made)	136
Caltrate+D (Lederle)	179
Os-Cal+D (SK Beecham)	130
Tums 500 (SK Beecham)	160
Viactiv (Mead Johnson)	250
Calcium (Natures Bounty)	64
Calcium citrate – Citracal+D (Bayer)	225
Calcium Citrate+D (Nature Made)	90
Calcium complex (carbonate, lactate, gluconate) Calcet (Mission)	500
Bone Support – Calcium Supplement with Calcium Citrate & Hydroxyapatite 1000mg + Magnesium, K2, Vitamin D3 (Pure Micronutrients)	700
Calcium Citrate with Vitamin D3 (Solgar)	250
Calcium Citrate with Vitamin D-3 (Best Naturals)	250
Calcium phosphate – Posture-D (Selfcare)	307
Bulk Supplements Pure Dicalcium Phosphate (DCP) Powder	30

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Among the more employed calcium supplements are calcium carbonate, calcium phosphate, calcium lactate, calcium gluconate, calcium citrate, calcium glycerophosphate, calcium oxide, calcium pantothenate, calcium pyrophosphate and calcium sulfate. There is a new range of supplements with high bioavailability consistent in chelates of minerals with amino acids (e.g. glycinate and lysinate of calcium). The advantage of marine calcium from fish bones is that is combined in natural form with phosphorus, which benefits its absortion and bioavailability. Calcium and phosphorus occur as a 2 to 1 ratio predominantly in crystalline structures called hydroxyapatite. Fish bone is regarded as an important natural source of biological apatite compound since it contains a high source of minerals especially calcium and phosphorous. Processed fish bones improve the availability of the minerals present and can be used as a supplement material for health products (table 6) or as an ingredient in feed (figure 4). Fish bone meal can also be used directly in lower value applications like in fertiliser formulations (figure 4).

7. Environmental impact

The analysis of environmental impact only refers to that related to the processing of mesopelagic fish, mainly *M. muelleri* with different technologies evaluated:

- Production of fishmeal and fish oil (render)
- Production of fish protein hydrolysates
- Production of silage

For each of these options the process is evaluated with the Gate-to-Gate approach, not considering the fishing and transport to the processing plant, which is common to all of them. The functional unit will be one tonne of mesopelagic fishes processed.

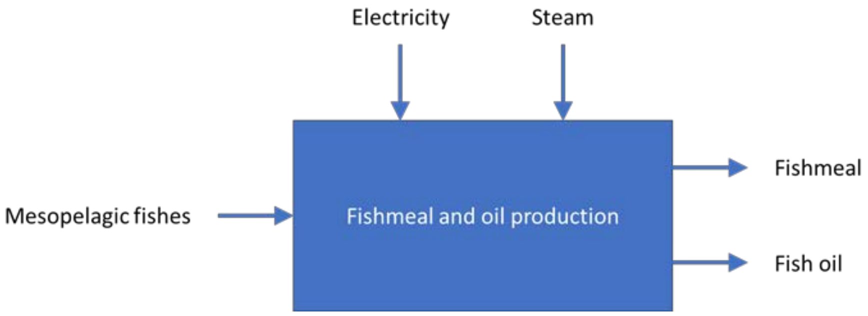

Figure 5. Input/output scheme of a fish rendering plant for the fishmeal and fish oil production

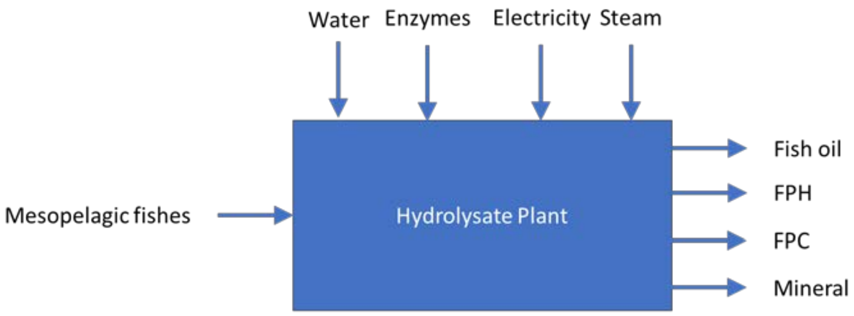

Figure 6. Input/output scheme for fish hydrolysate production

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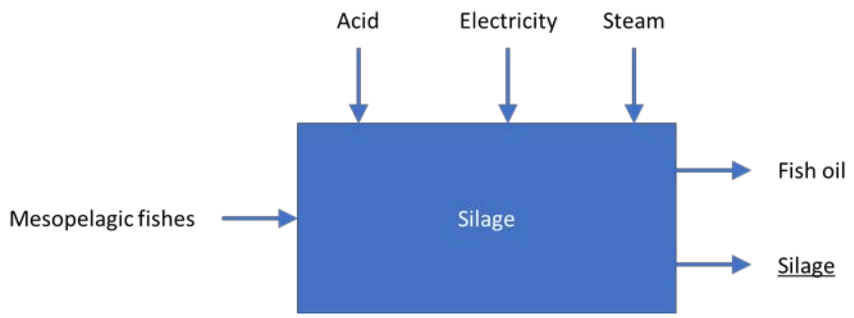

Figure 7. Input/output scheme for the silage production

The data used for the calculations of process yields are based on the average composition of the biomass of *M. muelleri* (table 7) and the results of the trials done (see section 2). For the energy consumption bibliographic references were used (Venslauskas et al., 2021).

Table 7. Average raw material composition used for the calculations of process yields.

	Raw material (%)
Dry matter	28
Protein	14
Fat	11
Ash	3

Table 8. Material and energy balance of the fish processing plant.

		Unit	Fishmeal and fish oil plant	Hydrolysate plant	Silage plant
Inputs	Raw material (Mesopelagic fishes)	kg	1000	1000	1000
	Acid	kg			40
	Enzyme	kg		2	
	Electricity	kWh	40	60	30
	Steam	kg	0.7	1.3	0.5
	Water	kg		500	
Outputs					
	Oil	kg	30	40	50
	Fishmeal*	kg	200		
	FPC*	kg		30	
	FPH*	kg		75	
	Fish bones	kg		100	
	Silage	kg			950

* Product dried with 5 % moisture.

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Environmental impact of fish processing has been evaluated using SIMAPRO 9.3.0.3 with Ecoinvent 3.0 Databases. In table 9 results of impact categories for processing one tonne of *M. muelleri* are shown. Regarding the carbon footprint (Climate change impact category), fish rendering is the most favourable process with 16 kg CO_2 equivalents (eq.) per tonne of raw material, followed by the hydrolysis with 45 kg CO_2 eq. per tonne of raw material. The production of silage would be the worst process and accounts for 195 kg CO_2 eq. per tonne of raw material.

Table 9. Environmental impact of processing 1 tonne of M. muelleri *to produce silage, fishmeal and oil, and fish hydrolysate.*

Impact category	**Unit**	**Fish Silage**	**Fish rendering**	**Fish Hydrolysis**
Climate change	kg CO2 eq	194.6E+0	15.6E+0	45.5E+0
Ozone depletion	kg CFC11 eq	18.0E-6	859.1E-9	3.6E-6
Ionising radiation	kBq U-235 eq	15.8E+0	9.1E+0	17.0E+0
Photochemical ozone formation	kg NMVOC eq	385.1E-3	35.1E-3	140.0E-3
Particulate matter	disease inc.	8.2E-6	262.8E-9	2.6E-6
Human toxicity, non-cancer	CTUh	1.6E-6	137.5E-9	1.2E-6
Human toxicity, cancer	CTUh	46.3E-9	4.2E-9	30.1E-9
Acidification	mol H+ eq	740.7E-3	84.3E-3	416.7E-3
Eutrophication, freshwater	kg P eq	44.6E-3	15.9E-3	36.8E-3
Eutrophication, marine	kg N eq	119.8E-3	15.0E-3	158.9E-3
Eutrophication, terrestrial	mol N eq	1.2E+0	128.3E-3	1.3E+0
Ecotoxicity, freshwater	CTUe	3.1E+3	209.5E+0	1.6E+3
Land use	Pt	435.8E+0	54.4E+0	913.1E+0
Water use	m3 depriv.	237.5E+0	4.9E+0	96.3E+0
Resource use, fossils	MJ	2.3E+3	336.9E+0	784.3E+0
Resource use, minerals and metals	kg Sb eq	815.3E-6	16.4E-6	240.0E-6
Climate change - Fossil	kg CO2 eq	194.3E+0	15.5E+0	44.3E+0
Climate change - Biogenic	kg CO2 eq	173.0E-3	35.3E-3	936.7E-3
Climate change - Land use and LU change	kg CO2 eq	115.4E-3	42.0E-3	257.6E-3
Human toxicity, non-cancer - organics	CTUh	67.6E-9	1.8E-9	54.2E-9
Human toxicity, non-cancer - inorganics	CTUh	426.8E-9	12.6E-9	107.4E-9
Human toxicity, non-cancer - metals	CTUh	1.1E-6	124.2E-9	994.8E-9
Human toxicity, cancer - organics	CTUh	13.9E-9	1.2E-9	7.1E-9
Human toxicity, cancer - inorganics	CTUh	707.9E-18	34.1E-18	158.7E-18
Human toxicity, cancer - metals	CTUh	32.4E-9	2.9E-9	23.0E-9
Ecotoxicity, freshwater - organics	CTUe	69.6E+0	1.3E+0	376.3E+0
Ecotoxicity, freshwater - inorganics	CTUe	292.3E+0	10.4E+0	192.4E+0
Ecotoxicity, freshwater - metals	CTUe	2.7E+3	197.8E+0	1.0E+3

In Figure 8, “EF 3.0 Method (adapted) V1.02 / EF 3.0 normalization and weighting set” has been used to calculate single score environmental impact to ease the comparison among categories. Fish silage results again in the higher global environmental impact with 16.3 mPt, followed by the fish hydrolysis with 6.5 mPt and fish rendering with 1,6 mPt. In all the

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processes, “Climate Change” and “Resource use, fossils” are the main category impacts as it is also shown in figure 9.

The next impacts contribution varies in each case, being “Water use” and “Ecotoxicity, freshwater” for silage process, “Eutrophication, freshwater” for fish rendering and “Ecotoxicity, freshwater”, “Water use” and “Eutrophication, freshwater” for hydrolysis.

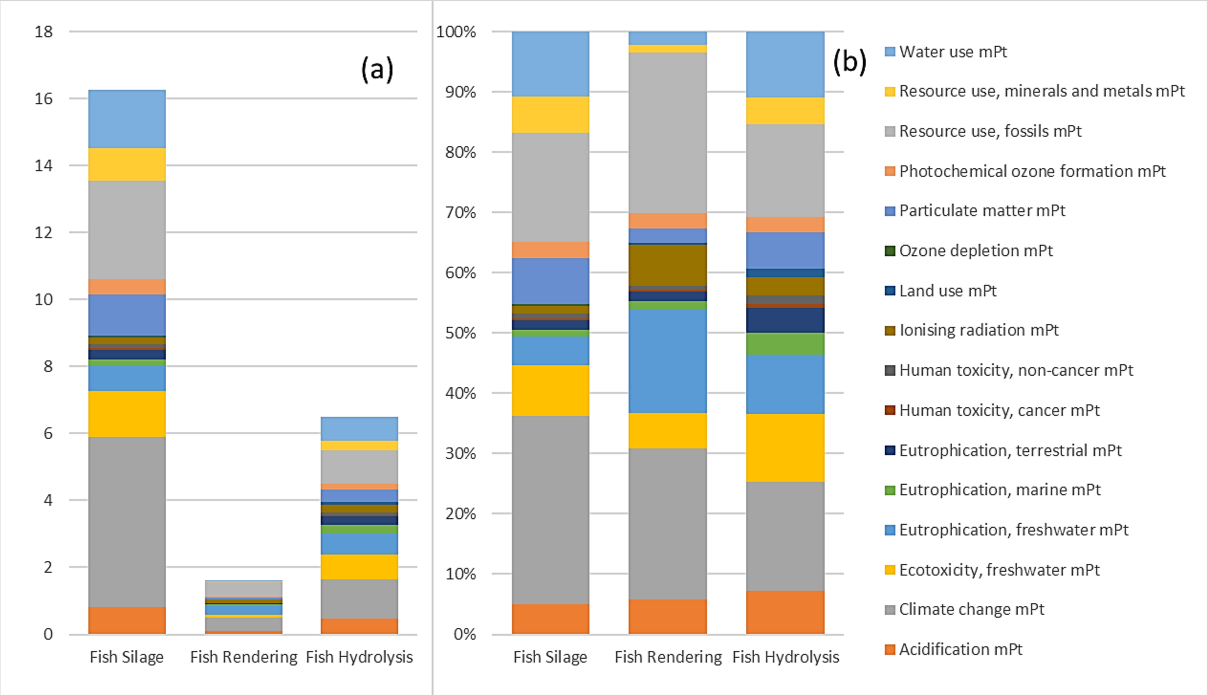

Figure 8. Single score impact for each process in absolute (a) and relative (b) contribution of each impact category.

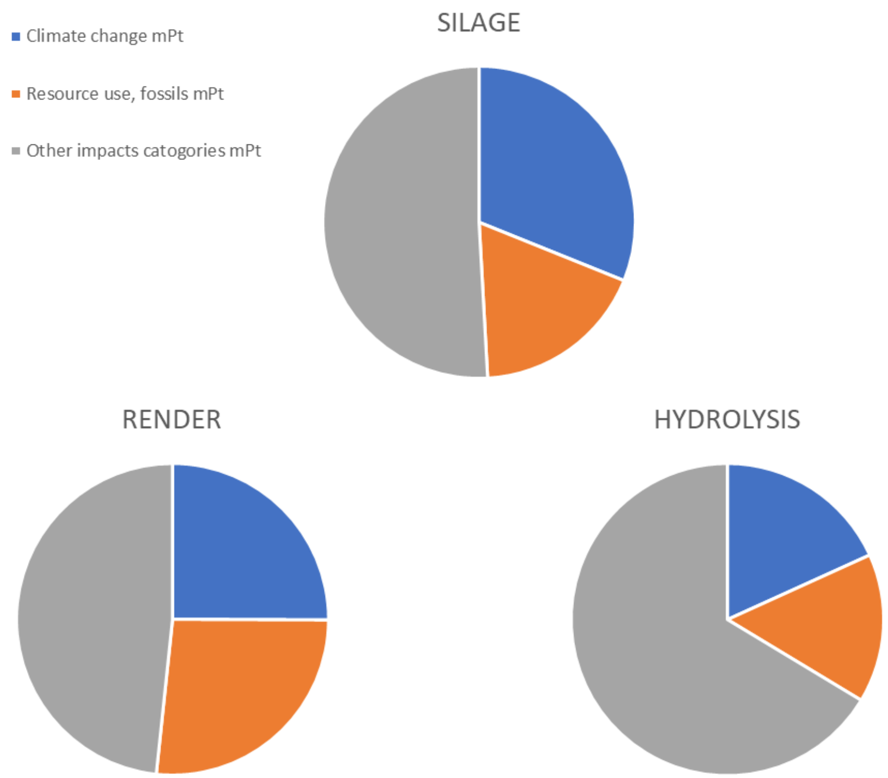

Figure 9. Impact share using EF 3.0 Method (adapted) V1.02 / EF 3.0 normalization and weighting set single score.

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If we evaluate the contribution of each input to the total environmental impact, different aspects could be highlighted. To produce fish silage (figure 10) the main impact is related to the use of formic acid, therefore the optimization of the process, and minimization of acid use would greatly improve the process sustainability.

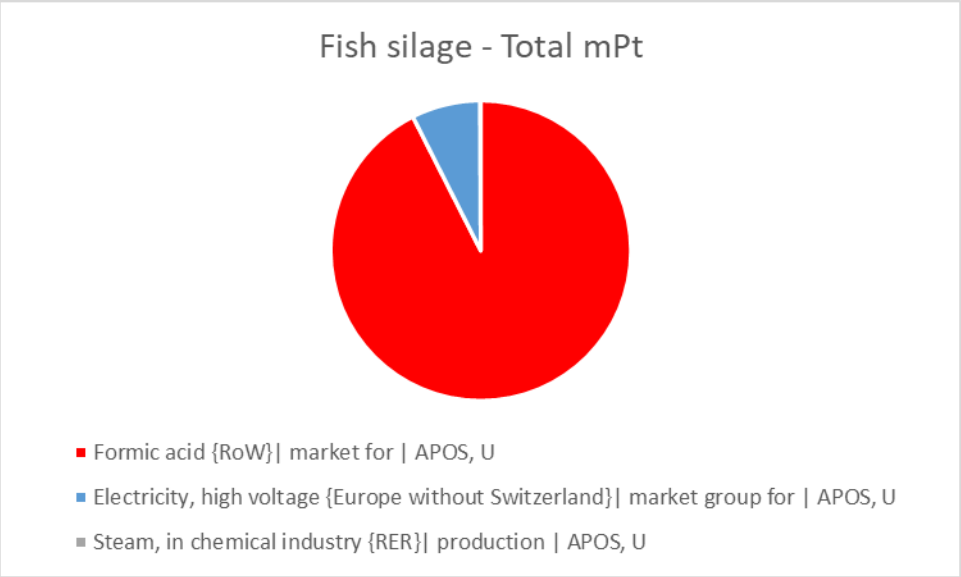

Figure 10. Total single score impact share among inputs for the fish silage process

As an energy intensive process, the main impact in fish rendering is the use of electricity (figure 11). In such processes, the increase in plant size and the energetic integration of processes might benefit to up to a 10 % energy consumption and the subsequent total environmental impact.

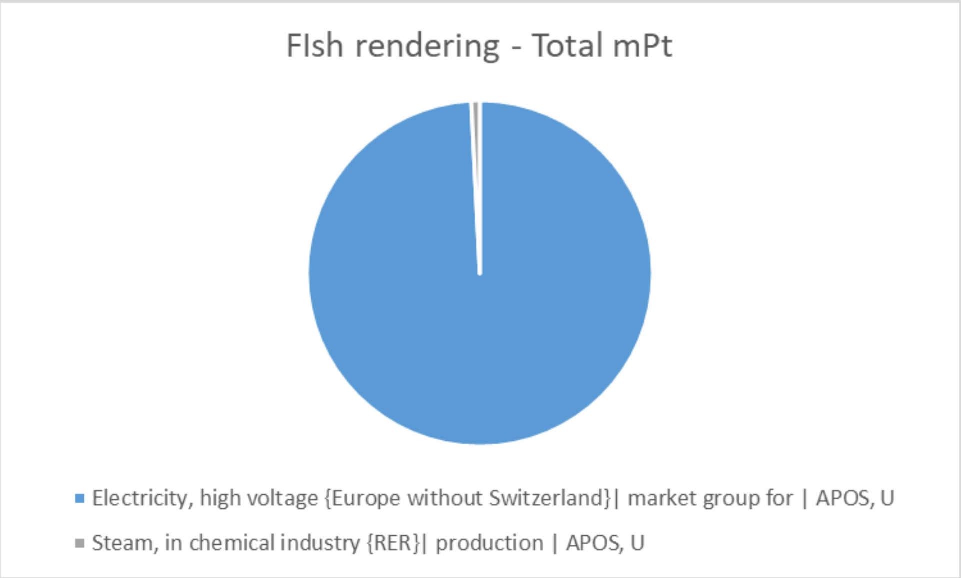

Figure 11. Total single score impact share among inputs for the fish rendering process

For the hydrolysis process, figure 12, the use of enzyme accounts for more than 50 % on the impact. As in the silage, the optimisation of process, with the minimisation of enzyme consumption would have an important environmental impact reduction. Furthermore, this would also improve the profitability of the process, being the cost of enzymes an important part of the OPEX.

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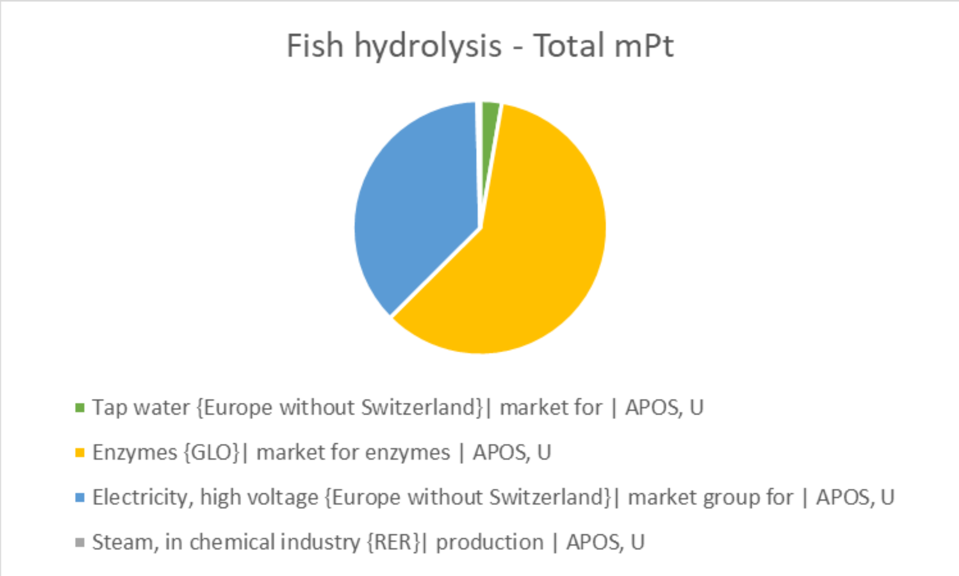

Figure 12. Total single score impact share among inputs for the fish hydrolysis process

The enormous differences in process yields, mainly since fish silage is a wet product, makes the comparison of product impact difficult. Also, allocation of impacts among products may result in important changes. Table 10 displays the results of the main environmental impact categories and total single score for the main product of each process allocating 100 % of the impact to this product. Fishmeal will result in lower carbon footprint per kilogram of product, while the fish hydrolysate presents the highest value. Trends are the same if we compare the resource use or the total single score. Therefore, even if the processing of a tonne of *M. muelleri* in the silage process has the biggest environmental impact (16.3 mPt/ tonne), due to the high mass yield the resulting main product, the silage, would perform better than the fish protein hydrolysate (17.1E-3 vs 86.7E-3 mPt/kg respectively).

Table 10. Products Carbon footprint and total single score per kilogram of product with a 100 % impact allocation to the main product.

		Fish Silage	*Fishmeal*	*Fish hydrolysate*
Climate change	kg CO2 eq / kg product	204,9E-3	78,1E-3	607,0E-3
Resource use, fossils	MJ / kg product	2,4E+0	1,7E+0	10,5E+0
Single score	mPt / kg prod	17,1E-3	8,1E-3	86,7E-3

Another common approach is to distribute the environmental impacts among the products or the outputs in order to accurately reflect their individual contributions to the environmental impact of the system under study and according to the produced amount: mass allocation, or to its value: economic allocation. In table 11, mass yield and economic assumptions are shown with the resulting impact with both types of allocation.

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Table 11. Products Carbon footprint and total single score per kilogram of products with mass and economic allocation.

		Silage plant	*Fishmeal and fish oil plant*	*Hydrolysate plant*
MASS YIELD				
Oil	%	5%	13%	16%
Fishmeal	%		87%	
FPC	%			12%
FPH	%			31%
Fish bones	%			41%
Silage	%	95%		
PRODUCTS VALUE				
Oil	€/kg	0.80	1.20	1.50
Fishmeal	€/kg		1.00	
FPC	€/kg			1.20
FPH	€/kg			4.00
Fish bones	€/kg			1.00
Silage	€/kg	0.15		
MASS ALLOCATION				
All products	mPt / kg Prod	16.3E-3	7.0E-3	26.5E-3
ECONOMIC ALLOCATION				
Oil	mPt / kg Prod	71.3E-3	8.2E-3	19.7E-3
Fishmeal	mPt / kg Prod		**6.9E-3**	
FPC	mPt / kg Prod			15.7E-3
FPH	mPt / kg Prod			**52.4E-3**
Fish bones	mPt / kg Prod			13.1E-3
Silage	mPt / kg Prod	**13.4E-3**		

With these different allocation strategies, general trends are similar; the fishmeal is the preferred and most environmental favourable option, followed by silage. These can be better observed in figure 13. High differences could be observed mainly in the hydrolysate when using economic allocation due to the important differences in product prices.

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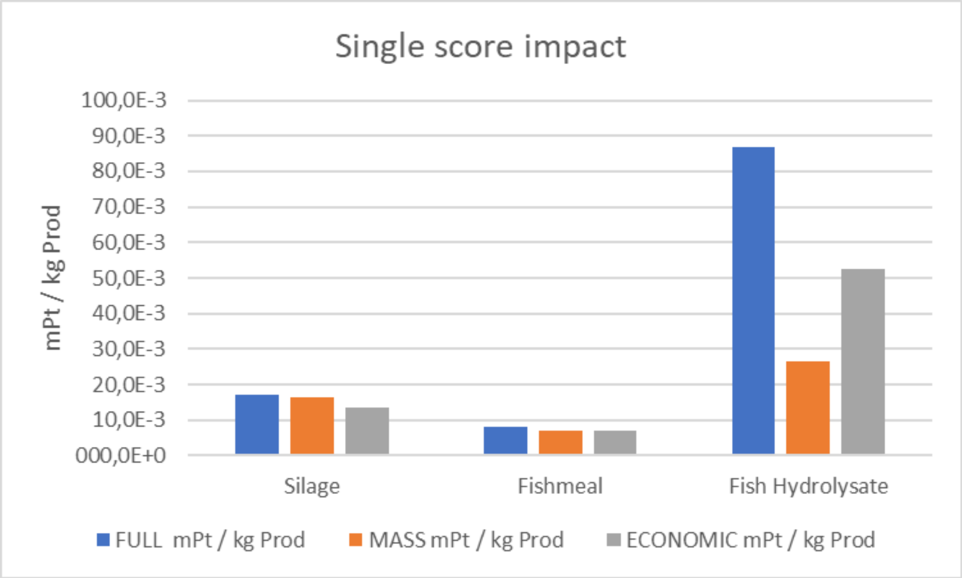

Figure 13. Comparison of single score impact or main resulting product with different allocation assumptions (FULL: 100 % allocation to main product; MASS: mass allocation; ECONOMIC: economic allocation)

Finally, the proposed products can be used directly, and some are being formulated as ingredients and further processed into high value products, like nutraceuticals, feed or food supplements. When further processed, the environmental impact of the whole value chain should be considered.

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8. References

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