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Executive Summary

Several processing methods were evaluated in order to determine best potential on-board processing strategies. The studies were done in collaboration with land-based processing efforts to obtain synergies between on-board and on-land processing scenarios.

Methods evaluated included production of silage, hydrolysates and meal production. The yield for each process was determined. The compositional analysis concerning total amino acid and lipid profiles, oxidation status and content of contaminants was determined. Biomass was assessed for its suitability for feed use specifically for use in aquaculture and salmon farming.

Different hydrolysates were made using a variety of proteolytic enzymes and operating parameters. Nutritional composition was also investigated. The higher economic value of hydrolysates developed on land and their potential for use as value-add food products, functional feeds and nutraceuticals was explored.

The levels of undesirables and nutrients in common mesopelagic species from Norwegian fjords were investigated and a theoretical processing scenario was applied assuming that these species are being processed to fish meal or oil.

Results indicate that mesopelagic biomass collected from different trawls has potential for use in feed production either when the biomass is homogenous and consisting of one particular fish species or when the biomass is mixed and consisting of different mesopelagic species. The autolytic activity and impact on biomass are currently still under investigation and this will provide information that will help to improve stabilization of the biomass on-board.

In terms of use as a human food source or functional foods, the mesopelagic products (hydrolysates and aquatic fractions) show potential for use as functional foods for use in prevention of diseases associated with metabolic syndrome including high blood pressure or hypertension, type 2 diabetes (T2D) and inflammation. The Organoleptic properties, safety and efficacy of the developed hydrolysate products is still under assessment.

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Introduction

This report is meant to highlight on-board processing methods, their advantages and drawbacks, potential products and market possibilities and relevant aspects in relation to these topics.

The duration of the trawls conducted for mesopelagic fisheries can vary depending on the distance from shore to catch areas. It is known from on-board investigations that the mesopelagic biomass deteriorates quickly due to autolytic activity. Therefore, mesopelagic biomass needs to be stabilized quickly once caught. The most relevant different strategies for stabilization are immediate freezing, silage, meal production on-board, and on-board hydrolysate generation using enzymes. All these technologies exist already but have not, to date been applied to mesopelagic fish. Additionally, the most cost effective and relevant technology depends on several factors including (1) The duration of fishing trips and type of vessels used, (2), products produced from mesopelagic catch and market potential of the same and (3) suitability of process technology to the fishing vessels.

If the conducted fisheries are close to shore, freezing the biomass for further processing on-land is a possible solution. Freezing the entire catch is less applicable for longer haul fishing trips due to the requirement for large freezing spaces, which are an additional cost. On-board biomass utilization and preservation strategies include production of silage, fishmeal, and hydrolysates. In broad terms, one can say that the value of the products increases from silage to meal production and hydrolysate production. In addition, on-board production of feeds or foods allows for stabilization of the biomass and a lesser requirement for freezer capacity and in turn, reduced energy consumption costs. Processing steps may also include removal of water from the biomass, which will also help to reduce weight on board and fuel consumption costs.

In this draft report, we present results from initial evaluations of different product production methods and indicate future efforts that will be finalized so that the optimal production of commercial products from mesopelagic biomass can be realized.

Proximate and oxidation analyses of raw materials

Catches were attempted gathered at different stations along the west coast of Norway during the period 7-19th of November 2019 by Norway Pelagic AS. Catches from the three stations 4, 6 and 7 were transported to Nofima's facilities for further investigations (figure 1).

Figure 1 Sample stations on the west coast of Norway. Stations 4, 6 and 7 proved successful in obtaining mesopelagic species.

Table 1 gives an overview of the different analyses performed and results obtained. Oxidation recorded at station 4 and standard error is apparently double that of station 6. This is due to one of two parallels having very high oxidation levels in this sample. The proximate analyses fluctuate less between the two stations. Both anisidine and peroxide levels are markedly lower in the sample containing both Krill and Mueller's pearlside. This may be due to the known antioxidant astaxanthin present in Krill-oil. The amount of fat present in station 7 is lower than what was recorded for biomass collected at stations 4 and 6, indicating that the amount of fat present in Mueller's pearlside is greater than in Krill.

Table 1 Oxidation and proximate analyses from the three stations 4, 6 and 7. MP: Mueller's pearlside, Mix: 43% Krill, 57% Mueller's pearlside

Station	**4**	**6**	**7**
Composition	100% Mp	100% Mp	Mix
Anisidin	36 ± 15	14 ± 1	12.5 ± 0.5
Peroxide	85.5 ± 30.5	33.5 ± 2.5	18.7 ± 11.3
Protein Kjeldahl (N*6.25)	15.1 ± 0.4	12.55 ± 0.25	11.5 ± 0.1
Total solids	25.1 ± 0.1	23.45 ± 0.55	20.1 ± 0.2
Ash	3.25 ± 0.05	2.8 ± 0	3.15 ± 0.05
Fat Bligh & Dyer	7.2 ± 0	8.8 ± 0	5 ± 0.1

Sampling of mesopelagics in the Bay of Biscay

The mesopelagic samples from the Bay of Biscay were analysed by AZTI and other partners and are shown in the results sections. The analysed samples were pure samples, composed exclusively of Mueller's pearlside species and came from the two JUVENA oceanographic campaigns conducted in September 2019 and 2020 carried out by AZTI and the Spanish Institute of Oceanography (IEO) for the estimation of the stock of the anchovy's fisheries in the Cantabrian Sea. The design of the sampling campaign was as shown in the map in figure 2.

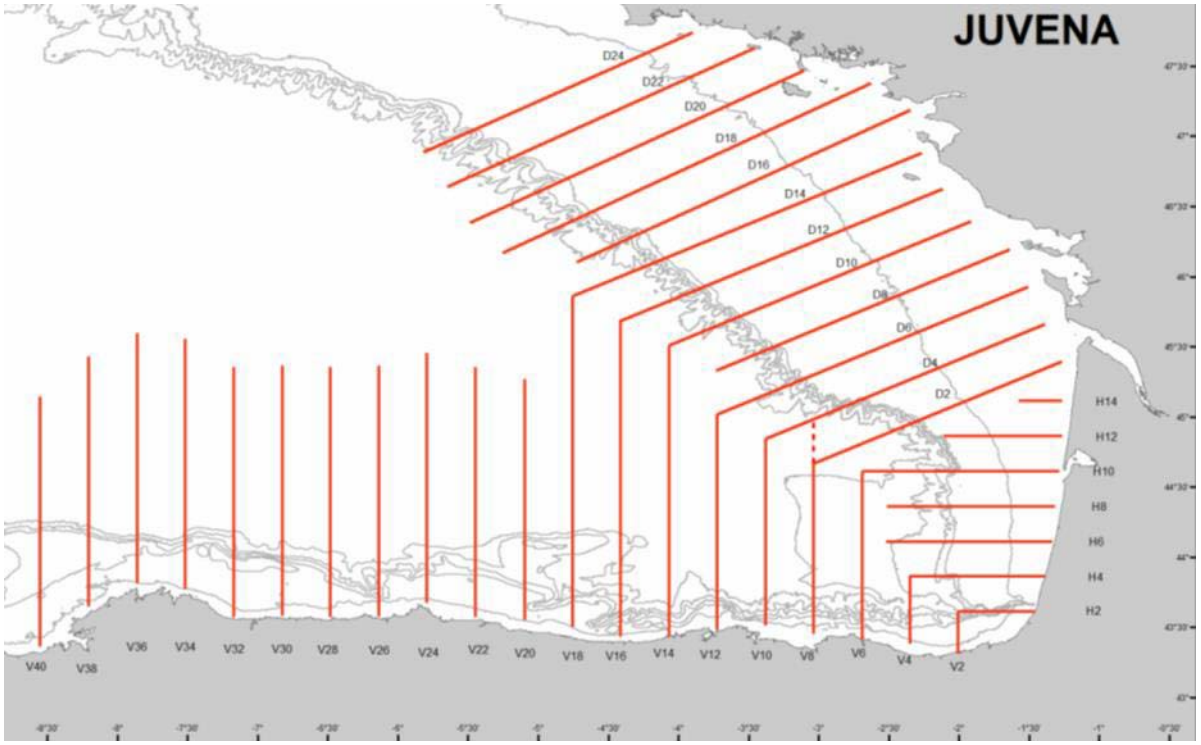

Figure 2 Sampling design of Juvena Campaign in the Gulf of Biscay.

Samples from at least two distant geographic locations within the Bay of Biscay and two consecutive years (figure 3) were chosen for comparative analysis of nutritional composition and contaminants as described. A sample that was kept frozen from a previous 2017 campaign was also analysed. For the comparative analysis of each parameter, replicates of the same sampling point were those corresponding to each geographical area classified as follows:

- 2017: Arcachon
- 2019: Gijón (9007, 9009, 9014), La Rochelle (9028, 9124)
- Arcachon (9011). 2020: Northeast Cantabrian (Quimper 9037 and 9039), West Cantabrian (Gijón, Ribadeo and Llanes: 9002, 9010 y 9020 respectively).

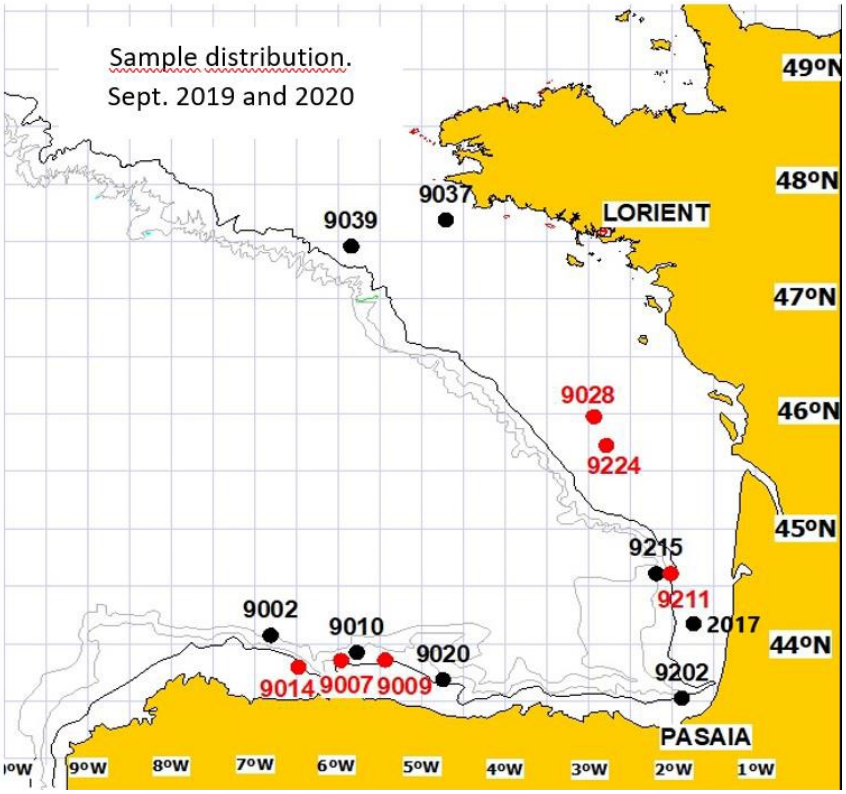

Figure 3 Geographical distribution of samples analysed. In red those of the Juvena 2019 campaign and in black the 2020s. In addition, a 2017 sample whose origin is indicated on the map was analysed.

All samples were frozen and glazed on board (figure 4) and were kept in separate bags from the outset different amounts of biological material for proximate analysis, contaminant analysis and hydrolysis trials. All were kept at -18ºC until analysis.

Figure 4 Frozen sample of Mueller's pearlside individuals as they are collected on the ship during campaigns.

Analysis of nutritional composition of mesopelagics

All samples were analysed for proximate composition (protein, total fat, water and ash), amino acid profile, total and free amino acids, collagen, calcium, phosphorus, iron, zinc, fatty acid profile, indicators of oxidation (peroxide index and TBA) and contaminants (heavy metals and biogenic amines).

Analysis of contaminants

Five samples obtained from sufficiently distant geographical locations were collected and sent to the Institute of Marine Research for contaminant analysis. These are the geographic sampling points and the year of the campaign:

- 9007 Vigo (2019)
- 9010 Vigo (2020)
- 9037 North East Cantabrian (2020)
- 9028 La Rochelle (2019)
- 9211 Arcachon (2019)

The results of the analyses are presented later (Analyses of undesirable products).

Biorefinery process. Hydrolysis trials.

For the biorefinery process, four different commercial enzymes were used: Alcalase 2.4 LFG and Protamex® provided by Novozymes, and papain and bromelain (Sigma Aldrich, USA). An additional hydrolysis was done with the endogenous enzymes of the fish samples.

Yields and composition of each fraction obtained were determined. All fractions were analysed for protein, dry matter and ash. The soluble fractions were analysed for degree of hydrolysis and molecular size profile of proteins obtained.

As a simple and low-cost method, alternative to enzymatic hydrolysis with commercial enzymes, and thus, compare the effectiveness in the separation of the different fractions, an acidic (silage) autolysis test was performed for 21 days (figures 5 and 6). Samples were taken daily for analysis of pH, protein solubilised, degree of hydrolysis and characterization of the molecular weight profile of the hydrolysed protein.

Figure 5 Images of the evolution of fish silage in laboratory (left to right: first day, intermediate time, after 21 days of silage).

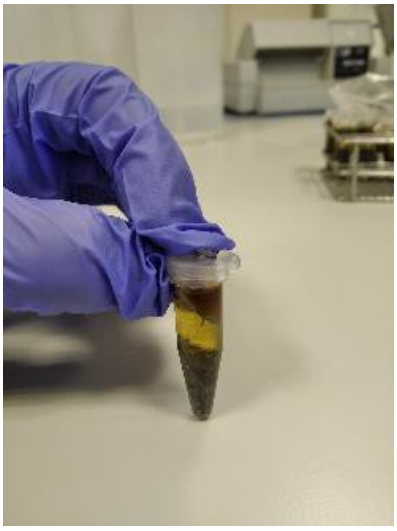

Figure 6 Phase separation after centrifugation of a sample.

Comparison of silage, meal production and hydrolysis

In order to evaluate on-board possibilities for processing, silage, meal production and hydrolysis were compared with respect to contents in the aqueous, lipid and solid phases. Four different batches of biomass were investigated using three different processes resulting in 36 different products. The biomasses are illustrated in Figure 7.

Figure 7 Mueller's pearlside received at Nofima from different catches. Left to right: Mueller's pearlside sept. 2019, Station 4, station 6, station 7 (also containing Krill).

Mesopelagic biomass (consisting of Mueller's pearlside or a mixture of Mueller's pearlside and krill) underwent three different processes at laboratory scale – fishmeal, silage, and enzymatic hydrolysis. Each process yielded three products: oil, an aqueous phase, and a solid phase which were analysed as such, without further concentration or modification (in industrial production, the aqueous phase would be concentrated, then recombined with the solid phase and finally dried, to produce fishmeal). The raw material amino acid and fatty acid composition is suitable for use as aquaculture feed ingredients, and the material presented no difficulties in processing. The typical quality analyses (free fatty acids, volatile nitrogen, peroxide and anisidine values) reflect the freshness of the raw material and have little bearing on the general suitability of the biomass for feed production.

Experimental procedures

Silage process: To 1000 g of ground raw material were added 2.5 g of Grindox 1032 (tocopherol blend) and 25 g of 99% formic acid. The mixture was stirred at 22 °C for 47 hours and then heated to 90 °C in a microwave oven and kept at 90 °C for 10 minutes. After centrifugation at 20,000 × g for 30 minutes, the liquid phase was decanted into a funnel, and the oil and water phases were separated.

Fishmeal process: To 1000 g of ground raw material were added 2.5 g of Grindox 1032 and 500 g of water. The mixture was heated with stirring to 85 °C for 56 minutes, kept at 85 °C for 10 minutes, and then pressed in a tincture press. The liquid phase was then centrifuged and poured into a funnel where the oil phase and the water phases were separated. The sediment after centrifugation was mixed with the press cake and homogenized.

Hydrolysis: To 1000 g of ground raw material were added 2.5 g of Grindox 1032 and 500 g of water. The mixture was heated with stirring to 55 °C for 20 min. 1.1 g of Promod 439L enzyme solution was added, and the mixture was stirred at 55 °C for 60 minutes. It was then heated to 90 °C for 20 minutes and kept at that temperature for 10 minutes. The mixture was then centrifuged at 20,000 × g, 40 °C for 20 minutes. The liquid phase was decanted into a funnel, and the oil and water phases were separated.

Pilot scale hydrolysis: 65 kg raw material and 68 kg water were combined and heated to 55 °C with stirring. FoodPro enzyme solution (65 g) was added. Stirring was continued at 55 °C for 60 min. The temperature was raised to 90 °C for 15 min and held at 90 ° C for 10 min. The mixture

was then pumped directly into a 3-phase decanter centrifuge. The aqueous phase was then filtered on a 20 µM cloth then a 0.1 µM ceramic membrane (crossflow filtration). The MF permeate was nanofiltered on a 200 MWCO spiral membrane and diafiltered with 6 x 20 L of water yielding 16 kg retentate, 9.0 Brix. 5.6 kg of the retentate was lyophilized to give 363 g of beige powder (91% protein).

The oil and protein recovery were calculated for each process and as seen in Figure 8 varied between the processes, with most protein in the water phase for the enzymatic hydrolysis process, as well as for the oil recovery. The use of the different methods was evaluated together with manufacturing possibilities as well as the quality of the protein isolated and the quality of the oil isolated. The fish meal was suitable for feed inclusion, however neither the meal nor the silage are suitable for human consumption.

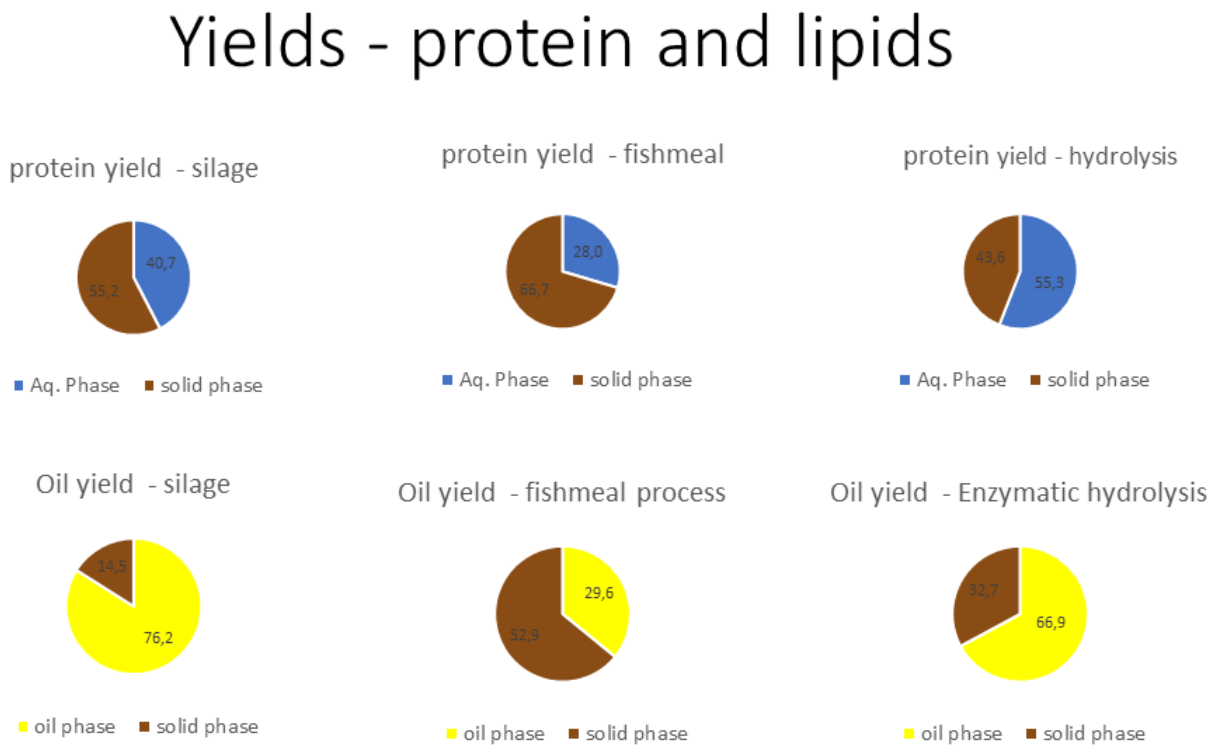

Figure 8 Oil and protein recovery as a result of process. Yields are percentages of the amounts of protein and lipids in the raw material and do not sum to 100% because of losses in the processing

Investigation of hydrolysed biomass

To evaluate the suitability of mesopelagic catch for applications in human and animal nutrition, several different hydrolysates were generated using different enzymatic treatments and conditions.

Enzymatic hydrolysis

Enzymatic hydrolysis was performed using the three commercial enzymes; Alkalase 2.4L (Al2.4), Endocut 01-L (01-L), FoodPro PNL (PNL) at optimum temperatures ranging from 50-55 °C. Additionally, endogenous enzymes (i.e., no additional enzymes included at the reaction start, abbreviation Endo) were used at 30 °C. All enzyme-reactions had identical setups: 1:1 biomass mixed with water (~500 g of each), 1 h hydrolysis time and 15 min deactivation at 90 °C. After deactivation, the hydrolysates were separated by centrifugation (30 min, 7,000 × g) into oil-phases,

hydrolysates, and sediment fractions. Weight measurements were performed on all the separate fractions and are presented in table 2. This was performed on Krill (manually separated from station 7), Mueller's pearlside and mixed raw materials from station 7. In table 2, the previously mentioned oil-level differences are easier to spot, as Krill consistently display lower levels of oil than Mueller's pearlside.

The efficiency in terms of the yield of resulting hydrolysate generated can be used as a proxy for protein yields generated from the biomass using selected enzymes and conditions. The most efficient enzymes for use in hydrolysis of mesopelagic fish in order are Alcalase 2.4 L>FoodPro PNL>Endocut 01-L>Endogenous enzymes.

Table 2 relative weight measurements on fractions obtained from enzymatic hydrolysis on Krill, Mp and mix, ww: wet weight, dw: dry weight.

Enzyme	Species	Oil	Sediment ww	Sediment dw	Hydrolysate ww	Hydrolysate dw
Al2.4	**Krill**	0.8 %	20.5 %	46.6 %	59.1 %	7.1 %
	Mp	2.1 %	15.3 %	39.6 %	75.9 %	6.0 %
	Mix	1.0 %	22.1 %	37.5 %	68.5 %	6.9 %
01-L	**Krill**	0.1 %	26.8 %	43.1 %	62.2 %	6.5 %
	Mp	2.3 %	19.4 %	38.4 %	66.7 %	5.7 %
	Mix	1.6 %	21.2 %	38.5 %	60.6 %	6.3 %
PNL	**Krill**	0.4 %	23.3 %	47.3 %	68.3 %	6.6 %
	Mp	2.3 %	15.3 %	40.5 %	72.4 %	6.0 %
	Mix	1.5 %	19.9 %	39.4 %	54.1 %	6.2 %
Endo	**Krill**	0.2 %	23.3 %	47.1 %	73.7 %	6.0 %
	Mp	2.2 %	19.5 %	44.7 %	72.7 %	5.2 %
	Mix	1.2 %	18.8 %	45.1 %	76.0 %	5.7 %

Figure 9 depicts the mw distribution of all the hydrolysed samples as obtained from size exclusion chromatography. The figure gives an overview how much of each category the samples contain; 800+ Da, 390-800 Da, 250-390 Da. Additionally, the orange trace indicates the average mw of the sample. Two general trends can be drawn from this figure. The average mw is higher in the endogenous samples. This is very much expected as endogenous enzymes rarely display efficacy in the same range as commercial enzymes, rarely have known optimum temperatures and presumably exist in lower concentrations than the 0.1 % of commercial enzyme which is added.

The other visible trend is that Mueller's pearlside alone or krill alone generally have higher average mw regardless of enzyme used compared to the mix of both species.

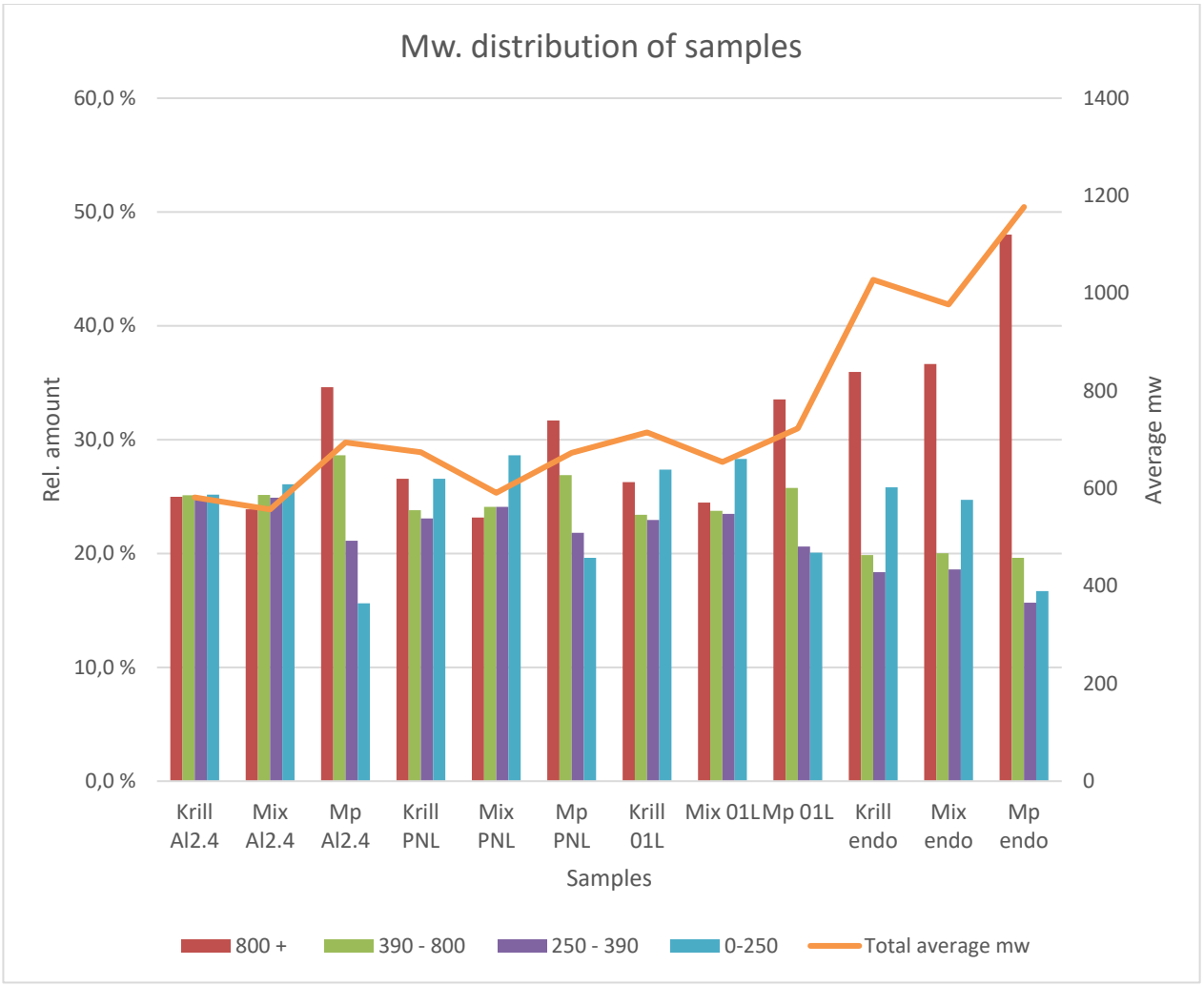

Figure 9 mw distribution of hydrolysates (water phases) described in table 2.

Endogenous enzyme activity assay on Mueller's pearlside

To investigate the endogenous enzyme activity on Mueller's pearlside, an experiment was conducted in which parameters were the same as for enzymatic hydrolysis, except the protocol being four h and samples were taken for SEC every 30 min.

Figure 10 displays the evolution of the hydrolysates taking place. The first 60 min seem to primarily involve increasing of average mw (orange trace going from 2525-3208 Da) and is perhaps a consequence of the enzymes liberating proteins from the material. After the initial 60 min, the average mw drops to a minimum of 1550 Da at 210 min. The final measurement, at 240 min seem to be another increase in average mw. This is not expected and no good explanation for it can be given presently. The increase is probably a measurement error as proteins are not known to spontaneously form during this reaction.

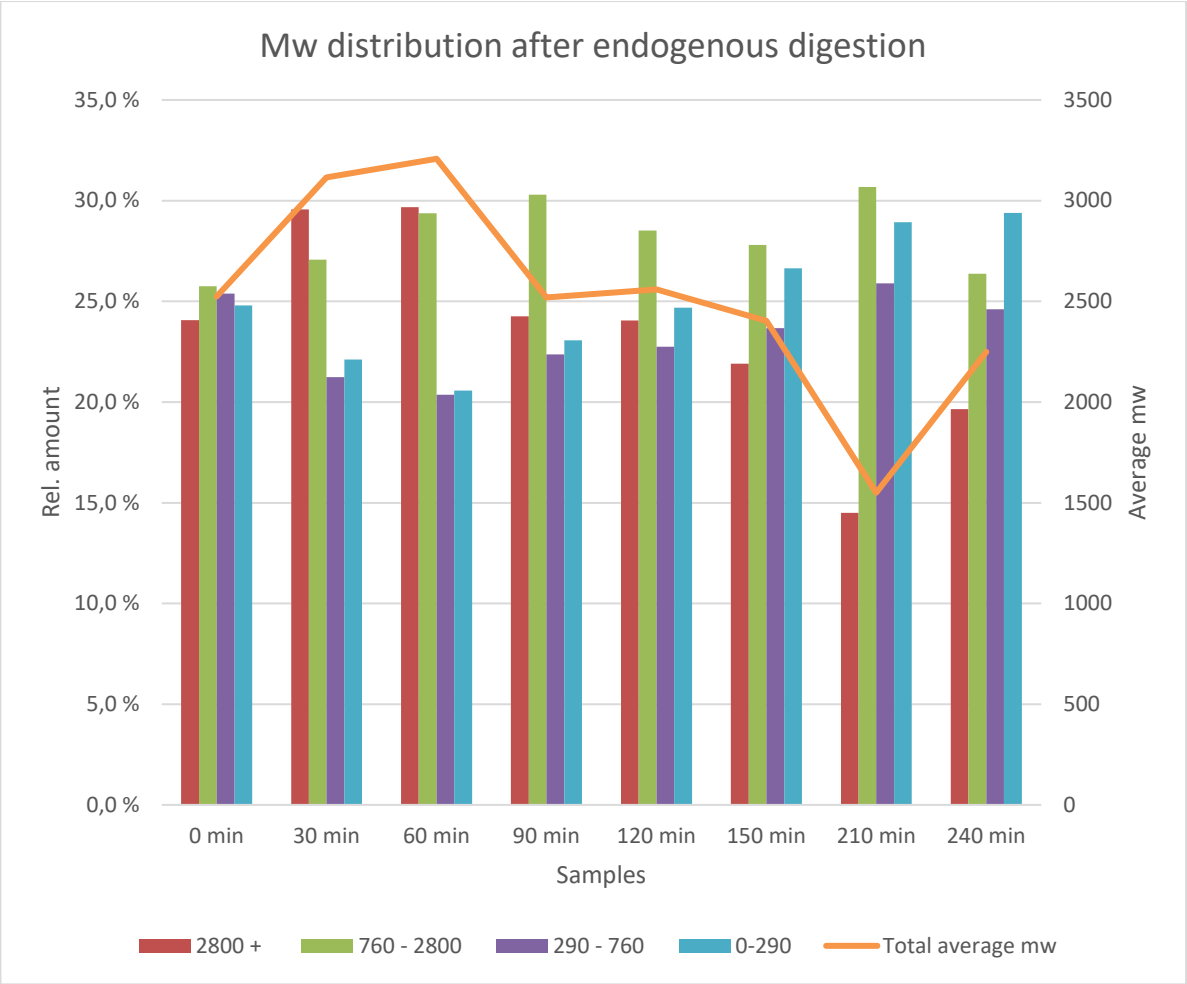

Figure 10 mw distribution of hydrolysate of Mp after four h hydrolysis. samples taken every 30 min. The green trace is average mw.

Sensory evaluation of filtered hydrolysates

To achieve a product that may be of commercial interest as human food it may be of interest to remove or enhance certain sensory attributes present in the products. This can be performed by filtering at nano-, ultra- or micro-levels dependent on what components to enhance or remove. An experiment was conducted with this focus as Mueller's pearlside hydrolysates are good candidates for taste modification. The radar charts in figures 11 and 12 display the sensory effects filtration and diafiltration (marked as 3x in the figures) had and what differences could be detected between the different enzymes. The scale went from 1 – no taste, to 5 – extreme taste. The effect of filtration (comparing the two figures overall) seems to be quite clear and undivided in that there is a more accentuated taste of fish in the concentrates and of salt in the filtrates. The more ambiguous results seem to indicate that there are also perhaps more pungent tastes in the filtrates. Interestingly, the taste of sweetness is by average the same in both filtrates and concentrates.

The effect of diafiltration (comparing green and purple traces in both figures) is more uncertain. One difference stands out however, visible in fig 11, namely that the pungent and stale tastes seem increased after diafiltration. It is unexpected that these tastes accumulate with increased filtration steps. In fig 12 no clear distinction can be made between the two. This feature is more generally applicable to fig 12 as well: that the four samples are less scattered in the figure and keep more in line with each other.

Enzymes (comparing different colour traces in both figures) seem to play a smaller role in the sensory attributes than the filtration does, especially as mentioned earlier in the concentrates. Exocut-TR L seem to create a sweeter taste in the filtrate and Endocut-07L somewhat higher in taste of fodder than the rest.

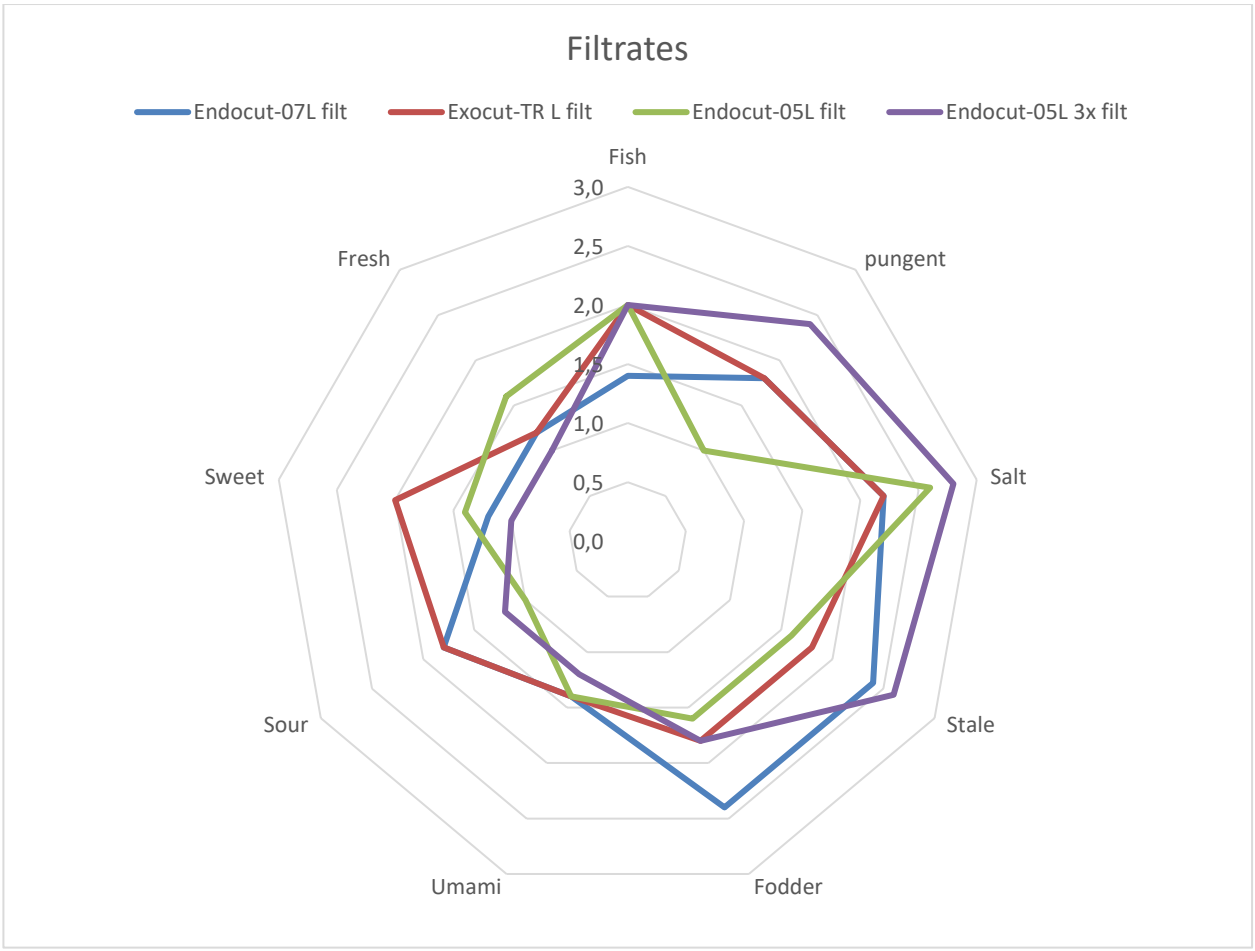

Figure 11 radar chart depicting the filtrates sensory attributes and average score as performed by 7 semi-trained judges.

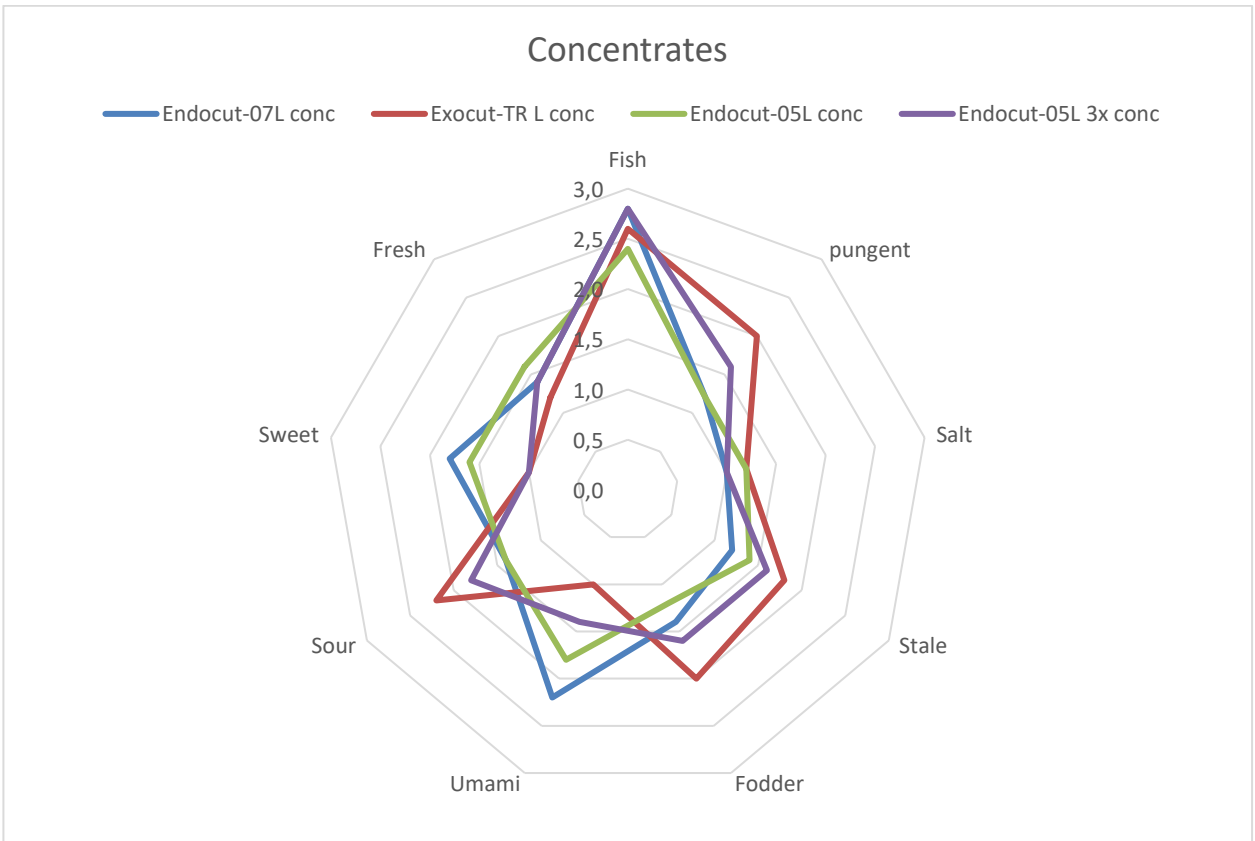

Figure 12 radar chart depicting the concentrates sensory attributes and average score as performed by 7 semi-trained judges.

Analysis of undesirable products in raw and processed biomass

Mesopelagic biomasses and mesopelagic species from the North Atlantic Ocean/Norwegian Sea and their processed products have been analysed for undesirables (e.g. dioxins, PCBs, PBDE, organochlorine pesticides, metals metalloids) and nutrients (e.g. vitamins, lipids classes and composition, essential elements). The levels of undesirables and nutrients in common mesopelagic species from Norwegian fjords have been published [1] and a theoretical processing scenario was applied assuming that these species are being processed to fish meal or oil [2-3]. In addition to the theoretical processing scenario, real measured processing factors have been established for the production of fish oil and meal, that predict the levels of undesirables in fish meal and oil based on catch biomass levels [2]. In addition, the relative loss of undesirables in the waste water phase during processing mesopelagic biomasses are established [2]. Finally analytical methods for plastics in these matrixes have been developed.

Main findings regarding nutrients

We analysed the nutrient composition in six of the most abundant mesopelagic species and evaluated their potential contribution to food and feed security [3]. The six species make up a large part of the mesopelagic biomass in deep Norwegian fjords. Several of the analysed mesopelagic species, especially the fish species *Benthosema glaciale* and *Maurolicus muelleri* (Mueller's pearlside), were nutrient dense, containing a high level of vitamin A1, calcium, selenium, iodine, eicopentaenoic acid (EPA), and docosahexaenoic acid (DHA)

Table 3 example of overview of essential vitamins and trace elements in mesopelagic species from Norwegian fjords.

Species	*n*	Vitamin A1 µg/100g	Iodine µg/100g (min–max)	Calcium mg/100g (min–max)	Potassium mg/100g (min–max)	Magnesium mg/100g (min–max)	Phosphorus mg/100g (min–max)	Sodium mg/100g (min–max)	Selenium µg/100g (min–max)	Zinc mg/100g (min–max)	Iron mg/100g (min–max)
B. glaciale	7	1633 ± 356 (1300–2300)	43 ± 6 (30–49)	500 ± 47 (420–550)	258 ± 51 (160–300)	67 ± 12 (52–89)	383 ± 60 (280–440)	385 ± 108 (300–600)	61 ± 9 (47–72)	0.8 ± 0.1 (0.7–1.0)	1.08 ± 0.44 (0.61–1.83)
Maurolicus muelleri	4	1020 ± 395 (480–1400)	27 ± 14 (16–47)	543 ± 60 (480–600)	227 ± 6 (220–230)	61 ± 8 (54–70)	400 ± 10 (390–410)	380 ± 69 (340–460)	44 ± 8 (34–52)	1.1 ± 0.1 (1.1–1.2)	1.56 ± 0.05 (1.50–1.60)
M. norvegica	4	63.3 ± 15.3 (50.0–80.0)	119 ± 42 (85–180)	658 ± 57 (590–730)	358 ± 33 (320–390)	163 ± 13 (150–180)	368 ± 22 (340–390)	495 ± 124 (360–660)	101 ± 41 (71–160)	1.0 ± 0.1 (0.9–1.1)	2.15–1.39 (0.98–4.00)
Pasiphaea sp.	3	11.0 ± 1.0 (10.0–12.0)	46 ± 4 (42–50)	633 ± 211 (410–830)	283 ± 110 (160–370)	83 ± 29 (53–110)	333 ± 119 (200–430)	337 ± 107 (220–430)	43 ± 21 (23–65)	0.9 ± 0.3 (0.6–1.1)	0.39 ± 0.30 (0.19–0.74)
Eusergestes arcticus	4	34.5 ± 29.6 (6.0–60.0)	117 ± 6 (110–120)	532 ± 88 (460 -660)	358 ± 22 (300–420)	378 ± 22 (350–400)	377 ± 22 (350–400)	363 ± 51 (300–420)	52 ± 17 (38–76)	1.8 ± 0.9 (1.0–3.1)	0.32 ± 0.11 (0.23–0.45)
Periphylla periphylla	2	0.3 (0.15–0.45)	2.3 (2–2.5)	43 (42–44)	83 (80–86)	105 (110–110)	12.3 (9.6–15.0)	1000 (1000–1000)	3.9 (3.4–4.4)	0.1 (0.1–0.1)	0.04 (0.04–0.05)
*M. * poutassou*	10	2370 (1000–4500)	23 (19–34)	429 (198–785)	264 (233–282)	64 (55–72)	309 (222–517)	425 (373–466)	62 (60–64)	1.1 (1.0–1.2)	1.75 (1.50–2.00)

Data are expressed as mean ± standard deviation, and minimum and maximum values in brackets. *n* = number of composite samples. * Measurements on individual samples, data from https://sjomatdata.hi.no/#search/.

Main findings regarding trace elements

Trace elements (i.e., arsenic, cadmium, mercury, and lead), organic pollutants (i.e., dioxins, furans, dioxin-like polychlorinated biphenyls, and polybrominated flame-retardants), and potentially problematic lipid compounds (i.e., wax esters and erucic acid) were analysed and compared to existing food and feed maximum levels and intake recommendations [1]. While most undesirables were low considering European food legislation, we identified a few potential food safety issues regarding high levels of fluoride in Northern krill, wax esters in glacier lanternfish, and long-chain monounsaturated fatty acids in silvery lightfish. Our estimates in processed biomass indicated high levels of undesirable trace elements in the protein fraction, frequently exceeding the maximum levels for feed ingredients. However, in fish meal, almost no exceedances were seen. In the oil fraction, dioxins and furans were above the maximum levels, given for food and feed ingredients

Table 4 Example of overview of fatty acids, wax esters, erucic acid and cetoleic acid in mesopelagic fish species caught in Norwegian fjords (from Wiech et al. 2020).

Species	Fatty acids	Fatty alcohols	Wax esters	Erucic acid 22:1 (n-9)	Cetoleic acid 22:1 (n-11)	Total fat content
	[µg/100 µg w.w.]		% Of fatty acids	[µg/100 µg w.w.]		
	Mean ± SD /(Min-Max)					
Benthosema glaciale	6.8 ± 1.8 (3.1 - 7.8)	4.2 ± 1.2 (1.8 - 5.1)	76	0.05 ±0.02 (0.02-0.07)	0.78 ±0.24 (0.26-1.07)	13.7 ± 3.7 (6.1–16.0)
Maurolicus muelleri	14.5 ± 7.9 (5.3 - 21.1)	0.03 ± 0.01 (0.02 - 0.05)	< 0.5	0.12±0.08 (0.03-0.20)	3.1±1.8 (0.7-4.6)	17.8 ± 8.1 (7.1–24.7)
Meganyctiphanes norvegica	4.2 ± 0.8 (3.3 - 4.9)	0.07 ± 0.02 (0.06 - 0.09)	< 1.5	0.03±0.02 (0.002-0.05)	0.26±0.22 (0.012-0.54)	5.5 ± 0.6 (4.9–5.9)
Pasiphaea spp.	3.7 ± 1.8 (2.4 - 5.7)	0.02 ± 0.01 (0.01 - 0.03)	< 0.5	0.03±0.02 (0.013-0.05)	0.20±0.15 (0.19-0.29)	5.4 ± 2.7 (3.3–8.4)
Eusergestes arcticus	5.3 ± 2.1 (2.6 - 7.8)	2.4 ± 1.0 (1.1 - 3.3)	46	0.04±0.02 (0.01-0.05)	0.52±0.23 (0.01-0.05)	9.4 ± 3.1 (4.9–12.1)
Periphylla periphylla	0.19 (0.15 - 0.22)	0.04 (0.01 - 0.08)	22	0.003 ± 0.001 (0.001-0.003)	0.027 ± 0.011 (0.011-0.035)	0.45 (0.34–0.56)

Main findings on processed biomasses

Commercial mixed mesopelagic biomasses caught at the North-East Atlantic were processed into the marine feed ingredients fish meal and fish oil [2]. Our measurements on processed mesopelagic biomasses show that only fluoride exceeds legal feed safety limits. Due to high levels of fluoride in crustaceans, their catch proportion will dictate the fluoride level in the whole biomass and can be highly variable. Processing factors are established that can be used to estimate the levels of undesirables in mesopelagic aquafeed ingredients from highly variable species biomass catches. Levels of most the studied undesirables (dioxins, PCBs, organochlorine pesticides, brominated flame retardant, metals, metalloids) were generally low compared to aquafeed ingredients based on pelagic fish.

Outcomes

1. Scientific publications in journal “Foods” impart factor 5.561
2. Overview over nutritional composition in six of the most abundant mesopelagic species and evaluated their potential contribution to food and feed security, indicating these to be a good source for “marine” vitamins and essential trace elements
3. Overview over the level of undesirables in six of the most abundant mesopelagic species with regards to food safety, indicating few potential food safety issues regarding metals and persistent organic pollutants. However, high levels of fluoride in Northern krill, wax esters in glacier lanternfish, and long-chain monounsaturated fatty acids in silvery lightfish might be issues.
4. Overview over processing factors when commercial mixed mesopelagic biomasses are processed into fish meal and oil, with fluoride as a potential feed safety issue depending on the degree of krill as by-catch and used legislation.

Bioactivity studies of generated silage, aqueous extracts and hydrolysates

Bioactivity screening of silages, aqueous extracts and hydrolysates is the responsibility of partners Teagasc within Task 3.5 of WP3. Biomass for bioactivity assessment was supplied by Nofima (Norwegian trawl) and AZTI (Spanish trawls) initially. In addition, Teagasc using different biomass supplied by the Marine Institute Ireland, generated hydrolysates. Bioactivities of all three "product types" (silage, aqueous extracts and hydrolysates) supplied by AZTI, Nofima or Teagasc was assessed using validated, *in vitro* bioassays. The aim of this task was to determine mesopelagic biomass with potential to impact positively on human (and/or animal health) with the end goal being development of a commercial product for use as a functional food or nutraceutical product. Health targets for any potential product include heart health, antioxidant activity, gut health, mental health and sarcopenia. Samples received from Nofima by Teagasc consisted of twelve hydrolysates labelled as M1-M4, K1-K4 and C1-C4 generated using four different enzymes namely: 1= Alcalase®; 2= Endocut 01®; 3 = Endo and 4 = FoodproPNL®. The second set of samples were obtained from three different trawls, which were carried out in the three different fishing stations (4, 6, 7). The Supplied biomass consisted mainly of Mueller's pearlside. Catch from station 7 contained Krill and Mueller's pearlside. Biomass was processed in three different ways:

- Fishmeal process: LATH-2-26
- Enzymatic hydrolysis: LATH-2-27
- Silage: LATH-2-25

Each process produced three different fractions: an aqueous fraction (water soluble peptides), a solid fraction and an oil fraction. Each sample was labelled as in the following example: LATH-2-26-H4 aqueous phase (Teagasc labels: AQ1- AQ9/ Lip 1- Lip 9/ Solid1-Solid 9). Proximate analysis using AOAC methods to determine protein, ash and lipid content of all extracts was carried out. All received fractions were screened for potential bioactivities using the suite of bioassays available at Teagasc. Assays carried out included the Angiotensin-I-converting enzyme (ACE-1; EC 3.4.15.1) inhibition assay, which was used to detect the potential of extracts to maintain normotensive blood pressure, and the dipeptidyl peptidase IV (DPP-IV; EC 3.4.14.5) inhibition assay used to detect extract potential to regulate and prevent type-2-diabetes. Samples were also screened using assays to detect bioactives for maintenance of mental health - the Acetylcholinesterase inhibition assay (AChE; EC 3.1.1.7) and antioxidant assays based on hydrogen electron transfer (HAT) methods and single electron transfer (SET) methods. The cyclooxygenase enzyme (COX; EC 1.14.99.1) inhibition assay was also used to detect potential anti-inflammatory activity in the samples supplied.

Hydrolysates and fractions with potential to help maintain normal blood pressure and prevent type-2-diabetes were identified from samples supplied by Nofima. These were samples M1 and C3 (both hydrolysates supplied by Nofima) and an aqueous extract sample 1 corresponding to sample LATH-2-25-H4-aq. ACE-1 IC_{50} values were determined. Moreover, the ability of samples supplied by Nofima to inhibit enzymes including AChE and the antioxidant activity of the extracts was determined but little bioactivity was observed. Fractions C1, M1 and Aq1 were further characterised using mass spectrometry and their peptide contents identified. 135 peptides were identified in fraction A1: 83 peptides in fraction Q1 and > 500 in fraction C3. Using *in silico*

analysis, three peptides were identified as having potential to be bioactive according to Peptide Ranker. These peptides were GPLGPLGPLGPLGPLGPLGP (20 amino acids long, derived from AQ1 sample, 0.95 Peptide Ranker), generated from a collagen type IV protein; FPGPFGPLGTPGPFG (15 amino acids long, derived from C3 sample, 0.950 peptide ranker) from an uncharacterised protein and ALLVADFGLQVSYDWNWR (18 amino acids long, derived from M1 sample, 0.728 peptide ranker) from an uncharacterised protein.

Results

Proximate composition

The proximate compositions of received extracts are shown in figure 13. Protein, ash and lipid content of supplied fractions was determined using AOAC Method 968.06, 15th Edition-Dumas method using Leco FP628 for protein; ash was determined in a furnace at 700°C for 3 hours and lipid content was determined using AOAC Method 2008.06 using an Oracle rapid NMR fat analyser.

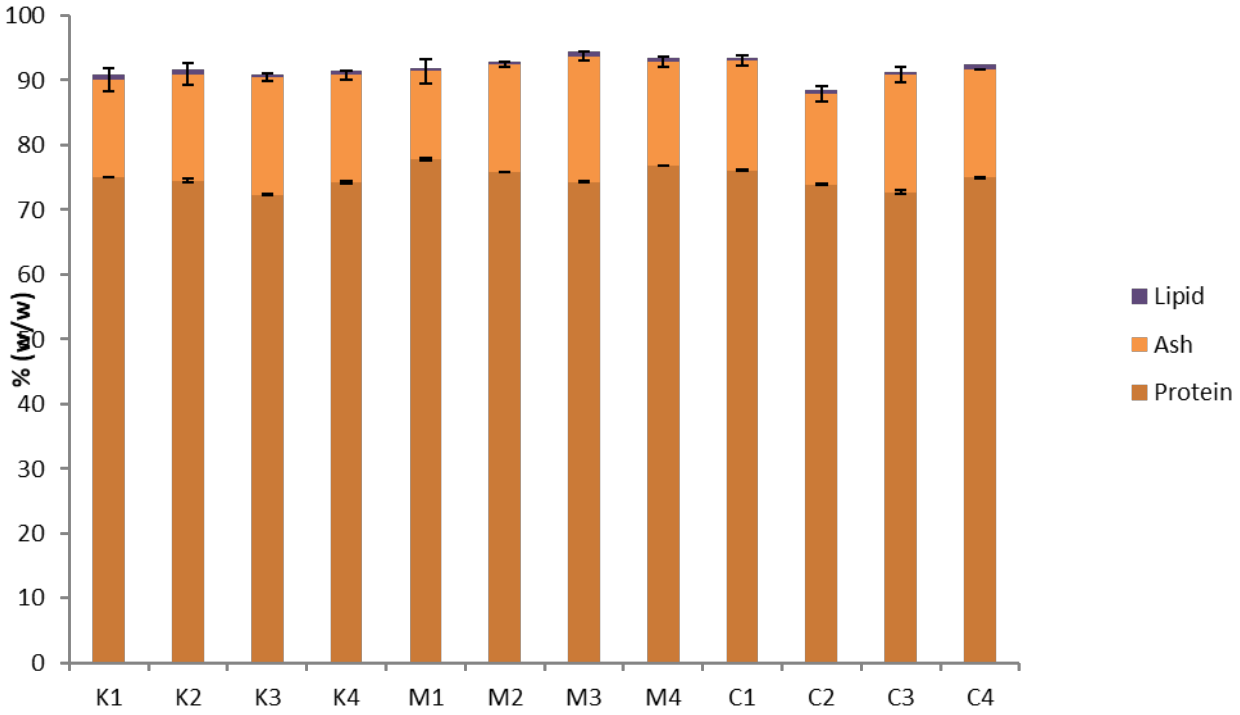

Figure 13 Protein, ash and lipid content of Nofima hydrolysate samples generated using four different enzymes (n=3)

Ash content ranged from 14.99 (K1) to 18.12% (C4) of dry matter. Protein content was ~75% of dry matter for all samples analysed. The lipid content of fractions was negligible.

ACE-1 inhibitory activity

Hydrolysate samples were screened for ACE-1 inhibitory activities using the ACE-1 colorimetric inhibition assay. In brief, 20µL of each sample aqueous solution at a concentration of 1 mg/mL was added to 20µL substrate and 20µL enzyme working solution in triplicate in a 96 well plate. Captopril© was used as a positive control. Samples were incubated at 37 °C for 1 h. A 200µL of indicator working solution was then added to each well, and subsequent incubation at room temperature was carried out for 10 min. Absorbance at 450 nm was read using a FLUOstarOmega microplate reader (BMG LABTECH GmbH, Offenburg, Germany). The percentage of inhibition was calculated using the following equation:

% ACE-I inhibition = 100% Initial activity - Inhibitor × 100/ 100% Initial activity

Figure 14 shows the percentage ACE-1 inhibition by hydrolysates when assayed in aqueous solutions at a concentration of 1 mg/ml. As shown in figure 14, hydrolysates K1 (Krill hydrolysed with Alcalase®); M4 (Muelleri hydrolysed with FoodproPNL) and C3 (A combination of Muelleri and Krill hydrolysed with enzyme labelled Endo) inhibited the ACE-1 enzyme by 88.27%, 89.56% and 86.26%, respectively when assayed at a concentration of 1 mg/ml compared to the commercial control Captopril® which was assayed at a concentration of 0.05 mg/ml and inhibited ACE-1 by 93.83%.

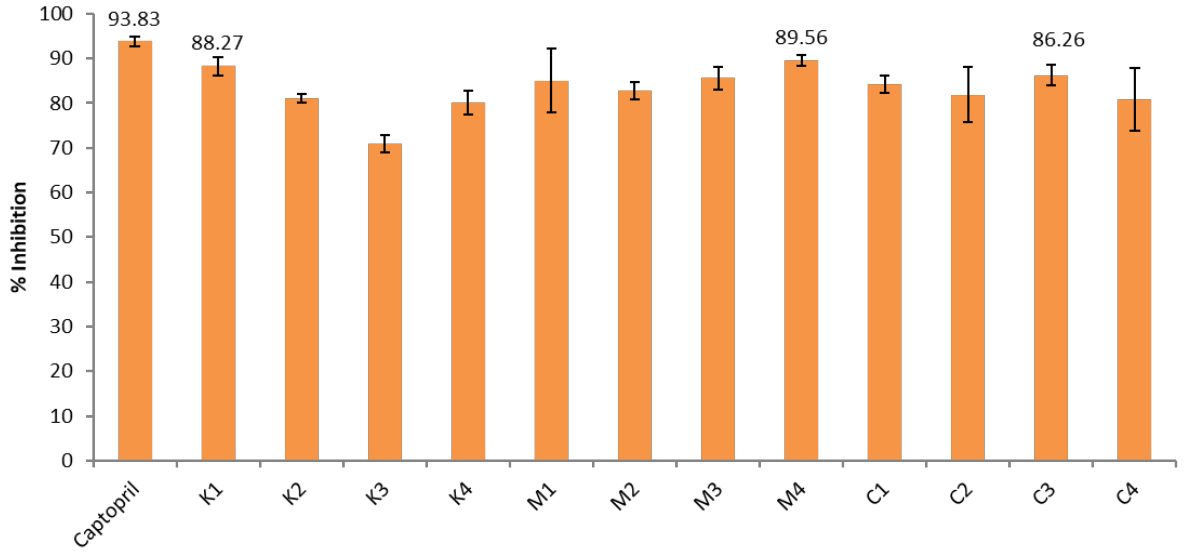

Figure 14 ACE-1 inhibitory activity by Nofima generated hydrolysates assayed at a concentration of 1 mg/ml compared to the commercial control Captopril® (n=3)

The ACE-1 inhibitory activities of the aqueous extracts gathered from the trawl and labelled AQ1-9 determined using this assay and compared to Captopril®. Results are shown in figure 15.

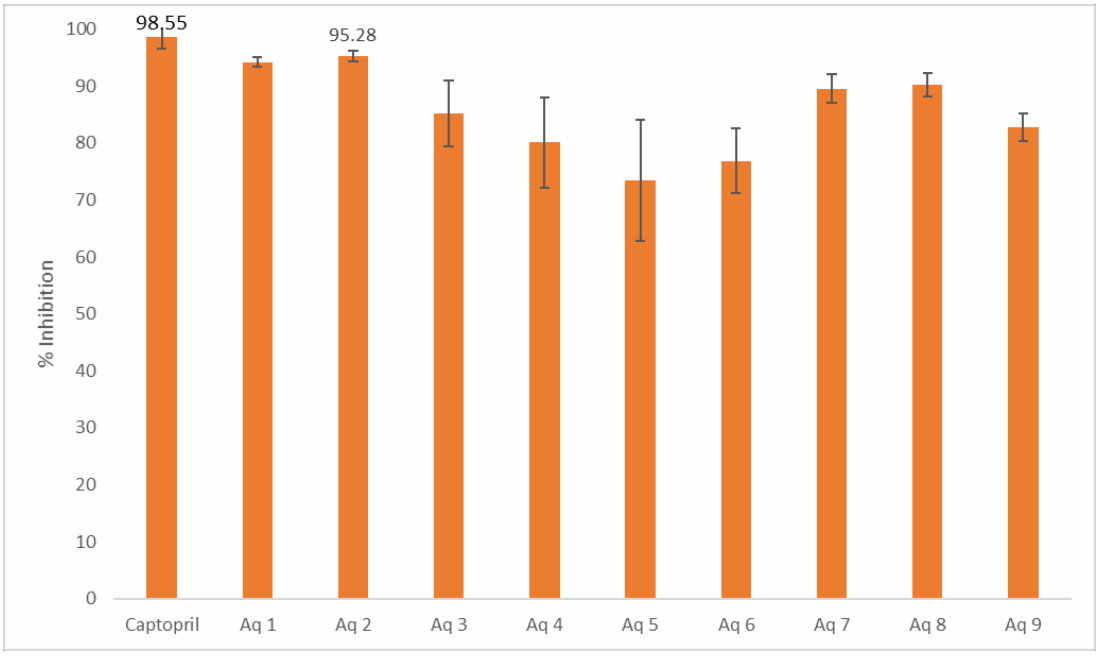

Figure 15 ACE-1 inhibition by aqueous extracts (n=3)

Aqueous fraction 2 (Aq 2) inhibited ACE-1 by 95.28% at a concentration of 1 mg/ml compared to the control Captopril ®. Aq 2 corresponds to the aqueous fraction of sample labelled LATH-2-25-H4-aq. It was not possible to assay lipid fractions from these trawls or indeed the solid biomass, as they were insoluble in solvents used for the ACE-1 assay. The concentration of samples M1, C3 (both hydrolysates) and Aq1 that inhibited ACE- 1 by 50% (IC_{50} value) were determined (figures 16-18)

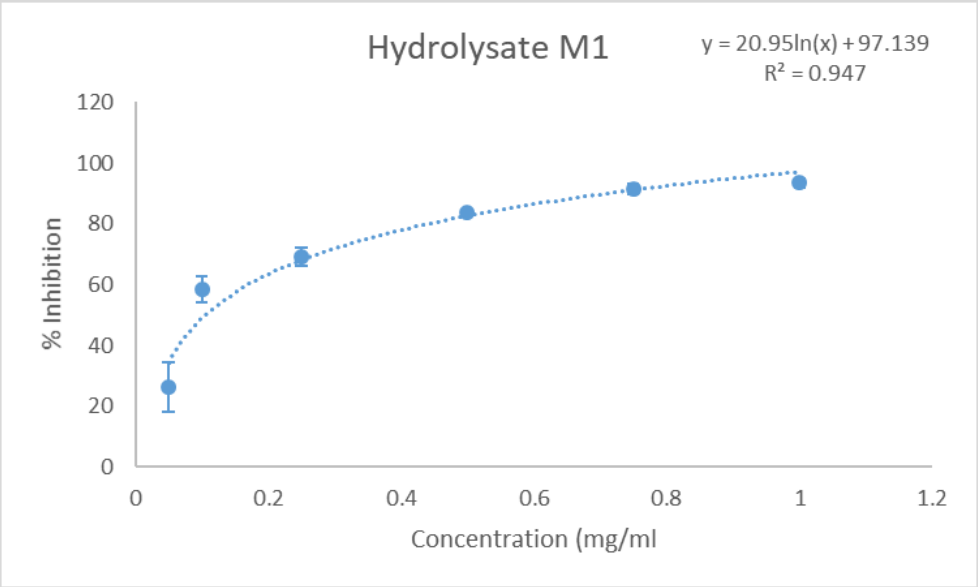

Figure 16 IC_{50} values for hydrolysate M1

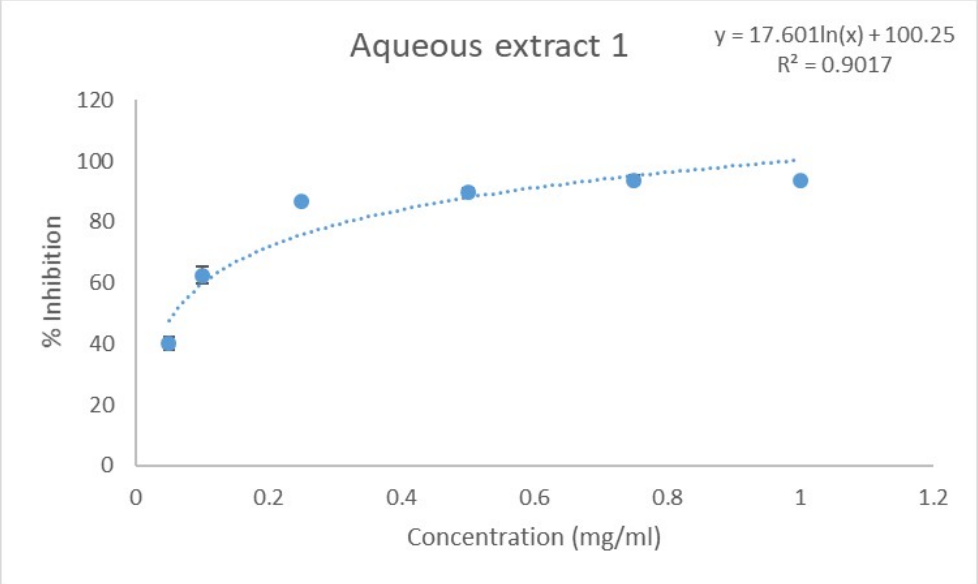

Figure 17 IC_{50} values for sample Aq

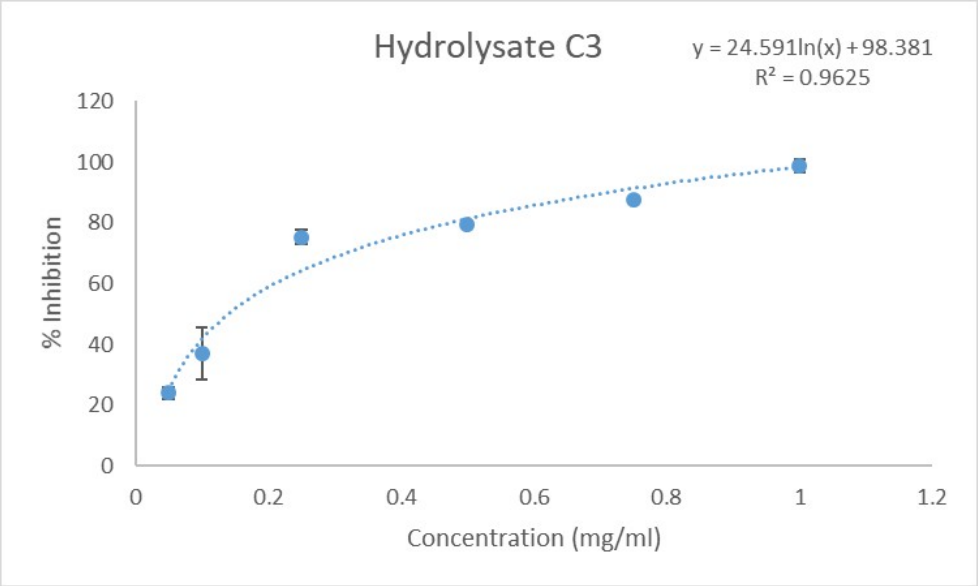

Figure 18 IC_{50} values for hydrolysate C3

The hydrolysate generated using FoodPro PNL of Mueller's pearlside inhibited ACE-1 by greater than 85% when assayed at a concentration of 1 mg/ml compared to the positive control Captopril(C) and had and ACE-1 IC_{50} value of 0.1 mg/ml. Characterisation of this hydrolysate in terms of amino acid and peptides is underway.

Dipeptidyl peptidase IV inhibition

The DPP-IV inhibitory activities of the hydrolysates and samples were determined using a DPP-IV inhibitor assay where the positive control was Sitagliptin – a commercial type-2-diabetes preventative drug. The percentage inhibition of DPP-IV was calculated as follows:

% Inhibition of DPP-IV = (RFU DPP-IV activity − RFU Inhibitor)/ (RFU DPP−IV activity) × 100

Where: RFU DPP-IV activity is the fluorescence (measured in relative fluorescence units (RFU)) of the measured without any inhibitor/test fraction, and RFU Inhibitor is the RFU measured in the presence of the Sitagliptin or the test hydrolysate or fraction. Figure 17 shows the DPP-IV inhibitory activities of hydrolysates and aqueous fractions supplied by Nofima to Teagasc. Samples were assayed at a concentration of 1 mg/ml compared to the positive control Sitagliptin, which was assayed at a concentration of 0.05 mg/ml.

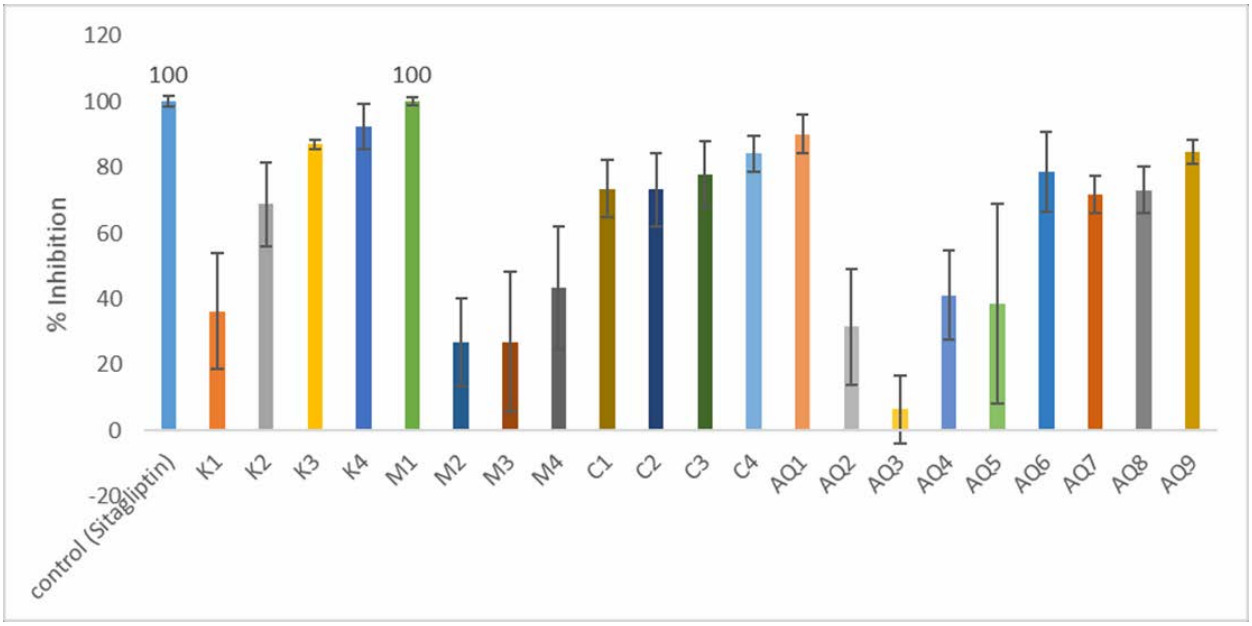

Figure 19 DPP-IV inhibitory activities of hydrolysates and fractions supplied by Nofima (n=3)

As shown in figure 19 several hydrolysates and aqueous extracts inhibited DPP-IV by greater than 50% when assayed at a concentration of 1 mg/ml. Hydrolysate M1 and AQ1 were selected for further characterisation work using mass spectrometry as these samples inhibited DPP-IV by 100% and 90.08% respectively, when assayed in triplicate.

Acetylcholinesterase inhibition assay

Acetylcholinesterases (AChEs) are enzymes that hydrolyse the neurotransmitter acetylcholine (Ache) to acetate and choline. AChE is located at the synaptic cleft and functions to terminate synaptic transmission by catalysing the breakdown of Ache allowing cholinergic neurons to return to a resting state after activation. Changes in AChE activity may result from exposure to certain insecticides, which act as cholinesterase inhibitors. Inhibitors of AChE are also used to treat certain conditions such as dementia. AChE inhibitory activity of hydrolysates and samples supplied by Nofima was determined for hydrolysates (sample set 1) and oil fraction samples from silages, fishmeal and hydrolysates (sample set 2) made by Nofima from samples caught from three different trawls (LATH -2 -25 to LATH-2-27 oil fractions). These samples are labelled L1 to L9 in figures 20 and 21.

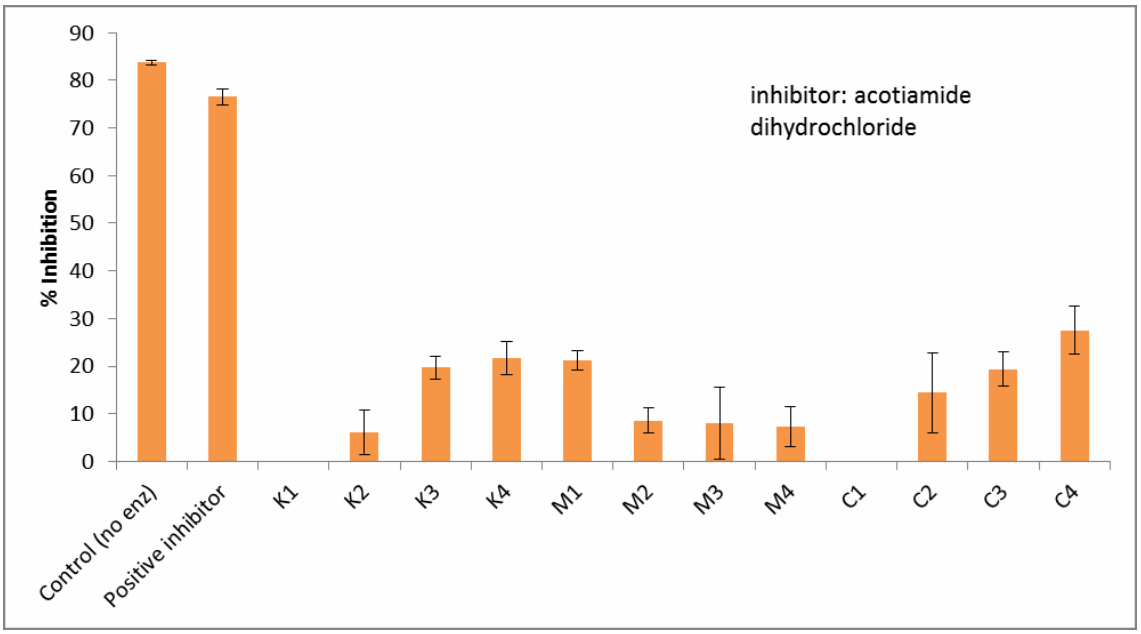

Figure 20 AChE inhibitory activities of hydrolysate samples supplied by Nofima assayed at a concentration of 1 mg/ml (n=3)

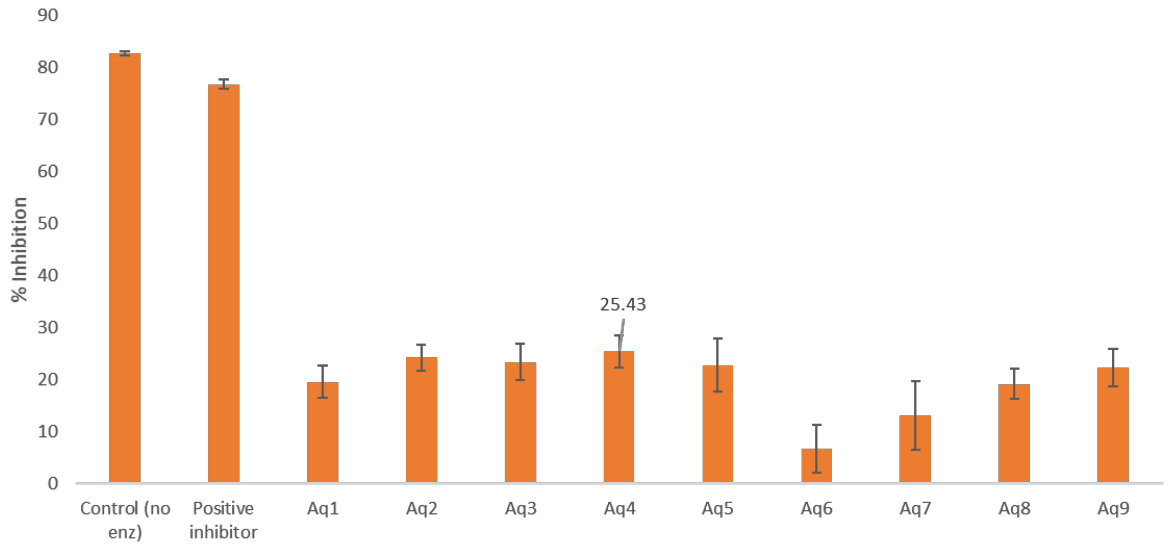

Figure 21 AChE inhibitory activities of samples supplied by Nofima assayed at a concentration of 1 mg/ml (n=3)

Samples assayed inhibited AChE by between 20-27% when assayed at concentration of 1 mg/ml and further screening for AChE inhibitory activities were not carried out due to the values obtained at 1mg/ml.

Analysis of lipid samples

A GC-MS method was established to determine the fatty acid methyl esters (FAME) present in lipid samples supplied by Nofima. Results are shown in figure 22. Palmitic acid was detected in all samples along with EPA.

		mg fatty acid/g lipid								
Name (methyl ester)	Short Name	L1	L2	L3	L4	L5	L6	L7	L8	L9
Butyric acid	C4:0	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Caproic acid	C6:0	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Caprylic acid	C8:0	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Capric acid	C10:0	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Undecanoic acid	C11:0	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Lauric acid	C12:0	0.14	0.00	0.28	0.08	0.00	0.00	0	0	0
Tridecanoic acid	C13:0	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Myristic acid	C14:0	40.14	56.77	52.75	46.31	50.44	58.31	43.54851295	50.70543	55.77591
Myristoleic acid	C14:1	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Pentadecanoic acid	C15:0	2.13	1.73	1.82	2.04	1.90	1.07	0.11824375	1.51317	0
cis-10-Pentadecenoic acid	C15:1	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Palmitic acid	C16:0	173.36	203.16	155.97	198.66	203.40	175.75	221.3090844	237.7634	174.3782
Palmitoleic acid	C16:1	24.36	27.59	17.20	26.80	23.68	19.78	26.57663335	23.40331	20.15472
Heptadecanoic acid	C17:0	47.48	50.00	43.48	40.19	36.01	37.59	40.00	35.71	47.62
cis-10-Heptadecenoic acid	C17:1	0.42	0.00	0.26	0.37	0.15	0.00	0	0	0
Stearic acid	C18:0	8.13	7.32	6.02	6.86	5.85	4.74	5.307163651	6.354739	3.398899
Elaidic acid	C18:1 t	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Oleic acid	C18:1 c	68.42	73.61	58.41	73.72	67.12	64.13	76.28402509	71.1668	68.25605
Linolelaidic acid	C18:2 t	0.00	0.00	0.01	0.10	0.00	0.00	0	0	0
Linoleic acid	C18:2 c	5.78	6.48	7.42	5.31	4.88	7.04	4.460250293	5.5813	7.505203
γ-Linolenic acid	C18:3 n6	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
α-Linolenic acid	C18:3 n3	4.71	6.94	6.31	4.59	6.11	6.74	3.142762822	6.136556	6.137945
Arachidic acid	C20:0	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
cis-11-Eicosenoic acid	C20:1	0.42	41.78	1.47	0.51	0.47	1.18	0	0.077866	0.074273
cis-11,14-Eicosadienoic acid	C20:2	0.00	0.00	0.00	0.01	0.00	0.00	0	0	0
Henicosanoic acid	C21:0	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
cis-8,11,14-Eicosatrienoic acid	C20:3 n6	0.00	0.00	0.00	0.24	0.00	0.00	0	0	0
Arachidonic acid	C20:4 n6	0.34	0.00	0.00	0.00	0.00	0.00	0	0	0
cis-11,14,17-Eicosatrienoic acid	C20:3 n3	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
cis-5,8,11,14,17-Eicosapentaenoic	C20:5 n3 (EPA)	54.70	51.53	41.51	44.64	46.60	40.27	62.75141802	44.34077	46.06017
Behenic acid	C22:0	0.00	0.00	0.00	32.30	25.57	0.00	20.51084901	28.87069	20.13253
Erucic acid	C22:1	48.15	0.00	99.85	58.54	97.90	135.81	64.15713814	119.8848	159.1731
cis-13,16-Docosadienoic acid	C22:2 n6	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Tricosanoic acid	C23:0	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
Lignoceric acid	C24:0	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0
cis-4,7,10,13,16,19-Docosahexaenoic acid	C22:6 (DHA)	0.00	129.15	0.00	0.00	0.00	0.00	0	0	0
Nervonic acid	C24:1	6.61	4.42	3.30	83.66	92.14	1.42	1.525764533	122.6595	108.6438
TFA		437.8025758	610.47	452.59	584.74	626.20	516.23	529.691846	718.4584	669.6908
SFA		223.904697	268.98	216.83	286.25	287.15	239.87	290.7938537	325.2074	253.6856
UFA		213.8978788	341.49	235.75	298.49	339.06	276.36	238.8979923	393.2509	416.0053
MUFA		148.3721212	147.40	180.52	243.79	281.47	222.31	168.5435611	337.1923	356.302
PUFA		65.52575758	194.09	55.26	54.90	57.58	54.05	70.35443114	56.05862	59.70332
omega 6		6.12	6.48	7.44	5.66	4.88	7.04	4.46	5.58	7.51
omega 3		59.41	187.61	47.82	49.23	52.70	47.01	65.89	50.48	52.20
omega 6:omega 3 ratio		0.102952803	0.034534861	0.155532195	0.11505673	0.092593113	0.14969949	0.067688076	0.11057	0.143783

Figure 22 FAME analysis of lipid sample fractions (L1-L9 (received from Nofima)

Irish and Spanish samples

Samples of Mueller's pearlside and Blue whiting from blue whiting and WESPAS surveys were received at Teagasc from the Marine Institute on 22/10/2021. The proximate nutritional composition of the raw biomass was determined using AOAC methods as described earlier. Results are shown in figure 23. In addition, four hydrolysates generated from mesopelagic biomass using the enzymes Corolase PP, Bromelain, Rohalase and MaxiPro were supplied to Teagasc by Nofima for assessment in May 2022. Moreover, AZTI supplied 12 hydrolysate samples to Teagasc in April 2022. These samples were:

MMD02 & MME02 (generated using Alcalase 2.4 L FG); MMD06 and MME06 (generated using Papain); MMD10 and MME10 (generated using Bromelain); MMA058 and MMD14 (generated using Papain and Bromelain); MMB010 and MMD18 (generated using Protamex enzyme) and MMB034 and MMC019 (Endogenous enzymes).

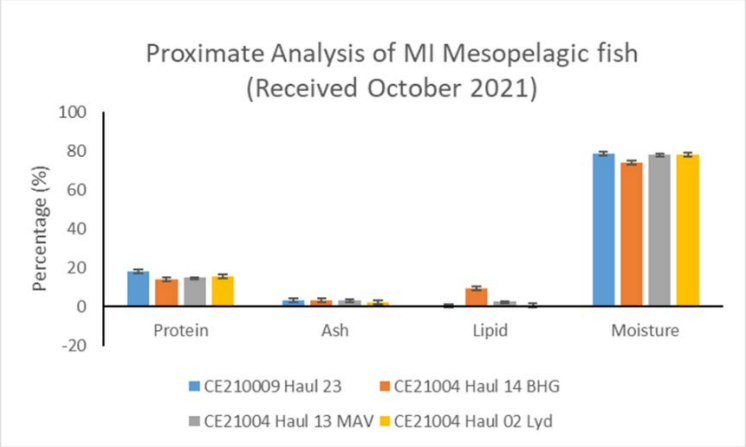

Figure 23 Proximate compositional analysis of mesopelagic biomass received from the MI (Ireland) October 2021

The heart health benefits of hydrolysates generated from Irish supplied biomass using the enzyme Alcalase® was also determined. Figure 24 shows the ACE-1 inhibitory effect of the hydrolysates when tested at a concentration of 1 mg/mL compared to a positive control, Captopril© which is a commercially available ACE-1 inhibitory drug.

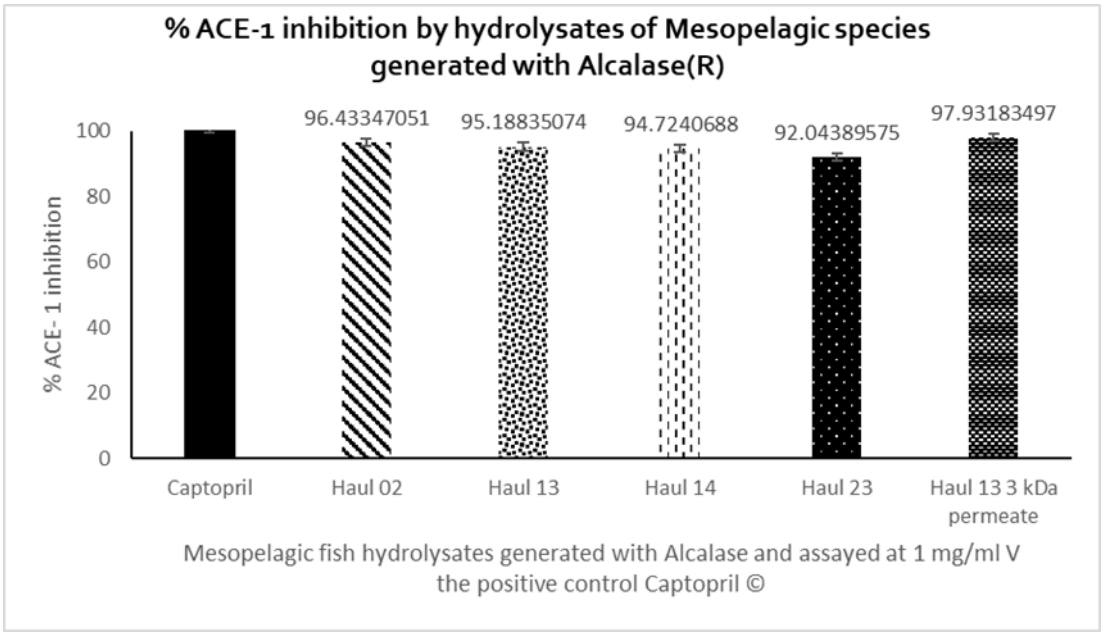

Figure 24 ACE-1 inhibitory activity of hydrolysates generated from mesopelagic fish supplied by Irish MI to Teagasc

The ability of Irish, Norwegian and Spanish hydrolysates generated from mesopelagic fish using enzymes to inhibit enzymes important in the development of inflammation like the cyclooxygenase enzymes (COX-1 and COX-2) was also determined (figures 25 and 26).

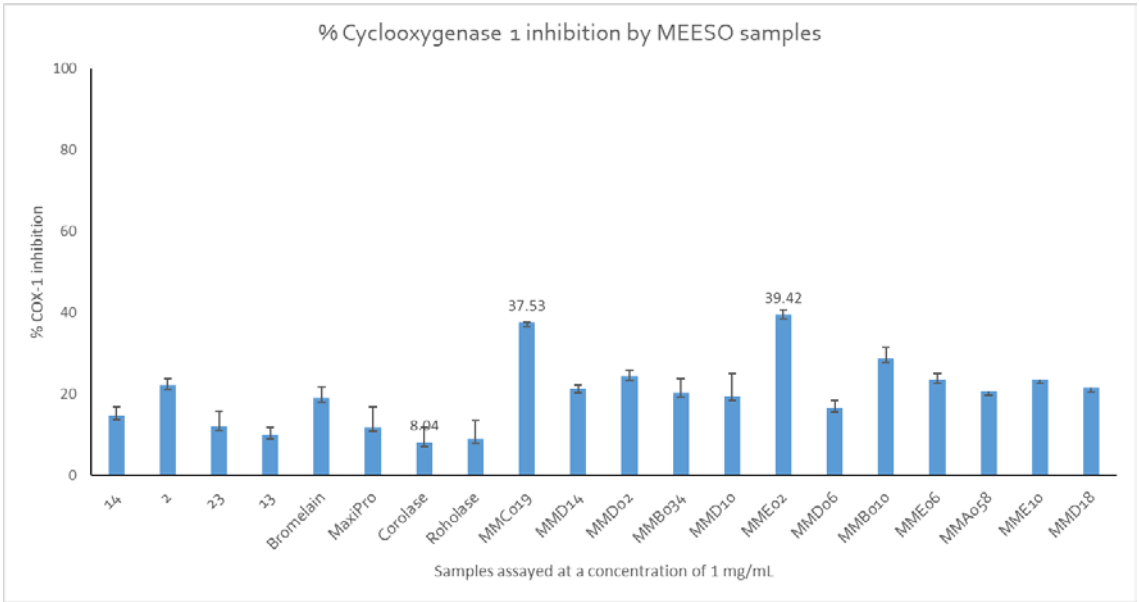

Figure 25 COX-2 inhibitory activity of hydrolysates generated from mesopelagic fish supplied by Irish MI to Teagasc (14, 2, 23, 13), AZTI (MMC019 – MMD18) and Nofima (Bromelain, MaxiPro, Corolase and Rohalase) samples.

This project has received funding from the EU H2020 research and innovation programme under Grant Agreement No 817669

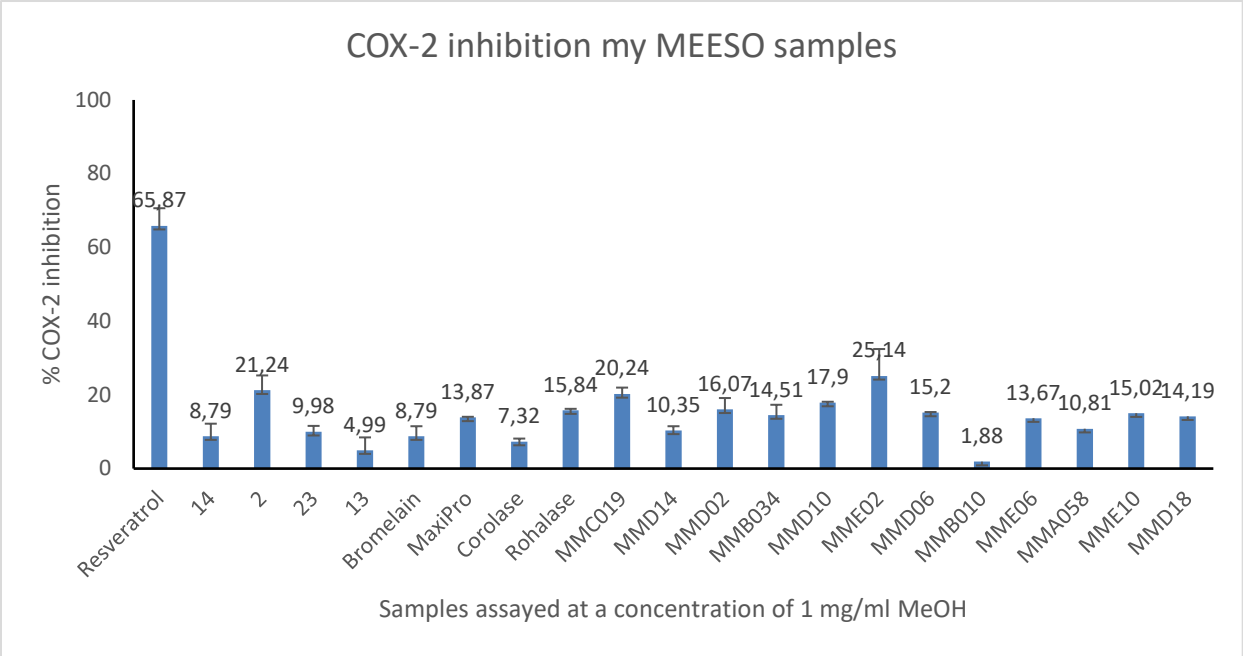

Figure 26 COX-2 inhibitory activity of hydrolysates generated from mesopelagic fish supplied by Irish MI to Teagasc (14, 2, 23, 13), AZTI (MMC019 – MMD18) and Nofima (Bromelain, MaxiPro, Corolase and Rohalase) samples

The antioxidant potential of the generated hydrolysates was determined using the ABTS assay and results are shown in figure 27.

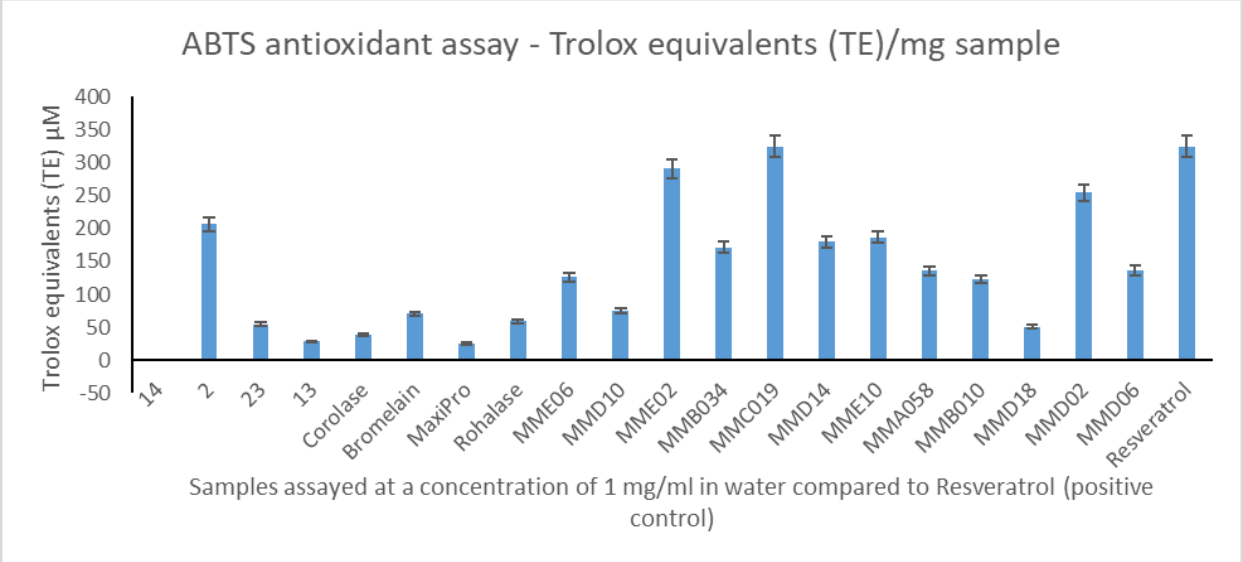

Figure 27 The antioxidant potential of Irish, Spanish and Norwegian hydrolysates relative to the positive control resveratrol when assayed at a concentration of 1 mg/mL. Results are expressed in terms of Trolox equivalents (TE) µM.

The ability of hydrolysates to inhibit DPP-IV was also determined and compared to the positive control and drug Sitagliptin (figure 28).

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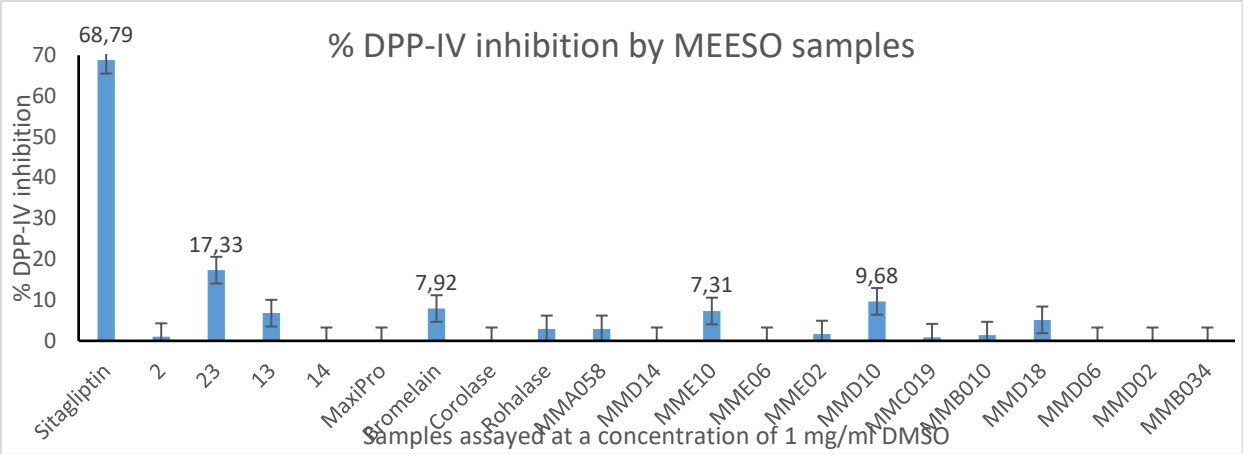

Figure 28 The ability of Irish, Spanish and Norwegian hydrolysates to inhibit DPP-IV and impact prevention of T2D. Results are expressed relative to the commercial DPP-IV inhibitory drug Sitagliptin. All samples assayed at a concentration of 1 mg/mL in DMSO.

Fractions showing promise for use as nutraceuticals in food or feed include MME02 and MMC019 (COX inhibition ~ and potential anti-inflammatory and pain prevention use and antioxidant activity relevant to resveratrol); all Irish hydrolysates in relation to ACE-1 inhibition (benefits for blood pressure and heart health) and peptides characterised from these "products".

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Industry workshop

"Building our knowledge of the mesopelagic zone", which was organised by MEESO in conjunction with the Irish Sea Fisheries Board (BIM), was held in Limerick City, Ireland on the 25th of March 2022. The industry-focused seminar sought to communicate progress on the key themes within MEESO and provide a view on our current understanding of the sustainable development potential of mesopelagic resources and potential commercialisation paths.

In total there were ten 20-minute presentations with 5 minutes between speakers for audience questions (entire seminar available at https://www.youtube.com/watch?v=T2HMfoygRVw). The first stage of the seminar focused on modelling, trawl technologies, and sampling for biomass estimation. Specifically, Dr Douglas C. Speirs (University of Strathclyde, Scotland) presented on the modelling of mesopelagic fish and their ecosystems and the trade-offs for the main models that are available. This was followed by Dr. Webjørn Melle's (Institute of Marine Research, Norway) overview on the new developments of acoustic and trawl technologies for biomass estimation of mesopelagics, with some of the progress and limitations well described. Prof David Reid (Marine Institute, Ireland) detailed the acoustic survey outputs from MEESO's scientific surveys and Dr Eduardo Grimaldo (SINTEF, Norway) presented a practical account of how to estimate and mitigate potential catch losses through mesh selection options in mesopelagic trawls. Richard Curtin (BIM, Ireland) provided an interesting example of ecosystem-based management case study of Antarctic krill fishery and the lessons that can be learned for management of other mesopelagic resources.

Following a brief break for refreshments and networking the focus shifted initially towards industry's (Frank Trearty, Pelagia) view on the commercial development potential of mesopelagics, outlining opportunities, challenges, and enablers required. The next stage of the seminar provided technical detail on the quality, sensory, food safety and product development potential from mesopelagics. Dr Runar G. Solstad (Nofima, Norway) described the sensory developments of Mueller's pearlside" and its implications on product quality. Dr. Lars Thoresen (Nofima, Norway) then presented on the different processing methods trialled for mesopelagics and the pros and cons of each. Dr Maria Hayes, (Teagasc, Ireland) presented a detailed account of the health beneficial ingredients from mesopelagic fish based on laboratory assessments and some of the associated challenges and opportunities the findings present.

The final presentation on the economic implications of fishing mesopelagics was provided by "Dr Rolf A. Groeneveld (Wageningen University, The Netherlands), and took account of the catch yields and market conditions required given the potential high costs of targeting mesopelagics. At the end of the seminar there was an open-panel discussion where attendees from the floor or virtually had the opportunity to ask questions. This was a very productive part of the seminar where there was a wide diversity of interesting questions and the fact that there was breadth of expertise present lent to the comprehensive answers and feedback provided. In total there were over 30 attendees and an almost equal split between in-person and virtual. The presentation slide decks, and associated recordings were communicated to attendees following the seminar. Overall, feedback was very positive.

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Future work

Mesopelagic biomass will be further investigated for food and feed use. Results from laboratory scale processing indicate that the biomass that was analysed (consisting of mainly Mueller's Pearlside and Northern krill) is suitable for fishmeal and silage production, as well as enzymatic hydrolysis aimed at protein products for human consumption. Silage is particularly attractive for on-board processing due to its simplicity and minimal energy requirements. Therefor future work will focus on optimization of the silage process. Time-course silage experiments will be conducted to discover optimal times, temperature, and acid concentration. Concentration of water-soluble protein, free fatty acids, biogenic amines, as well as oxidation parameters will be monitored.

Hauls from additional locations and seasons will be analysed to provide an overview of exploitable biomass for a commercial fishery and how it may be optimally processed.

A more thorough characterisation of bioactive fractions in terms of amino acid sequences is planned.

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Conclusion

This preliminary work bears the mark of still being early stage in a relatively unexploited fishery. A few conclusions can still be drawn from this work.

- Enzymes seem to play a smaller role in the sensory attributes than the filtration does, but even after filtration there is still a prominent fish-taste or salty taste.
- The fish meal has been deemed suitable for feed inclusion, however neither the meal nor the silage is suitable for human consumption, and one must beware of potentially high oxidation levels.
- Levels of most of the studied undesirables (dioxins, PCBs, organochlorine pesticides, brominated flame retardant, metals, metalloids) were generally low compared to aquafeed ingredients based on pelagic fish.
- While most undesirables were low considering European food legislation, we identified a few potential food safety issues regarding high levels of fluoride in Northern krill, wax esters in glacier lanternfish, and long-chain monounsaturated fatty acids in silvery lightfish. Our estimates in processed biomass indicated high levels of undesirable trace elements in the protein fraction, frequently exceeding the maximum levels for feed ingredients. However, in fish meal, almost no exceedances were seen. In the oil fraction, dioxins and furans were above the maximum levels, given for food and feed ingredients
- Several of the analysed mesopelagic species, especially the fish species *Benthosema glaciale* and *Maurolicus muelleri* (Mueller's pearlside), were nutrient dense, containing a high level of vitamin A1, calcium, selenium, iodine, eicopentaenoic acid (EPA), and docosahexaenoic acid (DHA)
- In terms of use as a human food source or functional foods, the mesopelagic products (hydrolysates and aquatic fractions) show potential for use as functional foods for use in prevention of diseases associated with metabolic syndrome including high blood pressure or hypertension, type 2 diabetes (T2D) and inflammation. The Organoleptic properties, safety and efficacy of the developed hydrolysate products is still under assessment.
- Fractions showing promise for use as nutraceuticals in food or feed include MME02 and MMC019 (COX inhibition ~ and potential anti-inflammatory and pain prevention use and antioxidant activity relevant to resveratrol); all Irish hydrolysates in relation to ACE-1 inhibition (benefits for blood pressure and heart health) and peptides characterised from these "products".

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