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Nutritional and functional properties of fishmeal produced from fresh by-products of cod (*Gadus morhua L.*) and saithe (*Pollachius virens*)

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Abstract

Fresh by-products of whitefish such as cod and saithe is processed to fishmeal (FM) on-board seagoing Norwegian trawlers. The aim of this study was to document the properties of whitefish FM (WFM) protein with respect to, physicochemical and bioactive properties. Analysis of the proximate composition of representative seasonal WFM batches show that the production is robust without much variance. The mean protein (61.9 ± 1.2), fat (8.9 ± 1.1%), moisture (5 ± 1.2%) and ash content (22.4 ± 0.8%), reflect the use of lean and bony raw-material. The WFM has a low content of free amino acids (0.7%) and biogenic amines (< 1000 mg/kg) that confirm the high quality and freshness of the raw material. Amino-acid analysis identified the presence of all nutritionally essential amino acids. The WFM physicochemical properties was comparable to soy-bean meal (SBM) by analysis of solubility, water-holding capacity (WHC), the emulsion stability (ES). Proteolytic degradation of the WFM was used to demonstrate the presence of bioactive peptides with inhibiting activity against angiotensin-converting enzyme (ACE) activity, *in vitro*. Taken

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together, WFM produced from fresh by-products is an excellent protein source with attributes of interest beyond the aquafeed-market.

Keywords: Food science, Food analysis

1. Introduction

Fishmeal is an superior aqua-feed and pet-food ingredient due to the high content of protein and the nutritive value of lipids and other constituents (Cho and Kim, 2011). However, a stagnation of the global capture fisheries concurrent with the increased demand by the current aquaculture growth, limit the FM availability, drive the price and force the feed-industry to utilize FM sources more efficiently (Olsen and Hasan, 2012). The promising exploration of novel and cheaper proteins from insects and plants also contributes to a more unpredictable future market for FM producers (Olsen and Hasan, 2012). Norway is a major provider of whitefish originating from well-managed and stable sources of wild-caught species such as cod (*Gadus morhua)*, saithe (*Pollachius virens)* and haddock (*Melanogrammus aeglefinus).* The fish is processed by heading and gutting (HG) or filleting of the fresh fish on-board modern sea-going trawlers resulting in the production of fish offcuts and offals as by-products. Less than 45% of the available whitefish by-products are currently utilized for production purposes (Olafsen et al., 2014). However, compact FM factories are now implemented in newly contracted trawling vessels to enable conversion of fresh by-products to WFM. The WFM is produced on-board by cooking, pressing and drying of fresh by-products. On the contrary to the land-based FM industry, the press-liquid (stickwater) containing water-soluble proteins is currently not utilized due to space constraints and the energy cost of evaporation, resulting in a pure press-cake FM (Hall, 2010). The Common Fisheries Policy (CFP) reforms agreed by the European Union ministers include a discard ban where fishers will be required to land all fish. This landing obligation include all the by-products and are expected to be implemented by the CFP by 2019 (European Commission, 2013) hence forcing the industry to find better economical solutions to improve the current marginal profit of costly WFM production at-sea. The WFM has been approved for human consumption by Norwegian authority but is mainly sold as a moderately priced commodity product. The combined effects of population increase and increasing standards of living in developing countries are expected to create a high demand for animal-derived protein by 2050 (FAO, 2006). Thus new initiatives will be required to produce the necessary quantities of high quality (Boland et al., 2013). An important contribution to future protein nutrition of man could be helped by shifting marine protein up the value-chain to produce nutritious and health-promoting ingredients (Boland et al., 2013; Cashion et al., 2017). In concordance with the growing documentation of functional- and health-promoting properties of marine proteins and peptides of different sources the exploration of WFM as a protein source for

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the human consumption market seem mature (Ahn and Kim, 2013; Brooks et al., 2013; He et al., 2013). Better documentation of the WFM as a protein source could facilitate the development of new products and increase the profit margins for utilization of by-products (Brooks et al., 2013). Proteins from marine sources have promising properties as functional ingredients due to their physicochemical ability to promote film and foam formations, induce gel forming, adsorb oils and promote emulsification and bind water (Lordan et al., 2011). Gelatin isolated from collagene-rich, marine material, is for instance used as a food additive to increase the texture, water-holding capacity and stability in food products (Lordan et al., 2011). The current work therefore focus on the characterisation of chemical and physiochemical properties to extend the knowledge of WFM as a protein product. The WFM was analysed with respect to chemical quality parameters, amino-acids profiles and selected physiochemical properties like water holding capacity (WHC), emulsifying stability (ES) and solubility. The biological activity of WFM protein hydrolysates was analysed for inhibiting effect on the angiotensin-converting enzyme (ACE) before and after proteolysis.

2. Materials and methods

2.1. Materials

Representative WFM samples were collected from five batches produced during the winter months in the years 2012, 2013 and 2014. The WFM was produced on board the whitefish trawler F/T Havstrand equipped with FM factory capable of processing heads and viscera produced from HG cod (*Gadus morhua)* and saithe (*Pollachius virens)*. The raw material was processed by coarse grinding and steam-heated screw cooking at 90 °C for approximately 30–45 min. Then, the cooked raw material was pressed in a twin-screw press before the press-cake was dried in a rotational steam-heated dryer at 90–100 °C for approximately 30 min. The dried press-cake was milled to FM powder and packed in in 25 kg paper bags. The press liquid (stick water) containing fish solubles was discarded in the process. Ethoxyquin or other preservatives were not added during the production. Samples of the WFM were then stored at room-temperature until analysis. The experimental production of WFM 6 based on fish-heads as the only raw-materials source, were made by manually sorting out viscera before the raw-material was processed as described above. Soybean meal (SBM) was used as a reference in the functional assays and was purchased at a local health shop. The SBM contained per 100 g dry matter: 37 g protein 23.5 g fat and 27.9 g carbohydrates (Saltå Kvärn, Järna, Sweden).

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2.2. Chemicals

Lyophilized powder of rabbit lung angiotensin converting enzyme (ACE), substrate for ACE (FAPGG or N-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine), Protamex ® (Sigma P0029) and standard chemicals, were purchased from Sigma–Aldrich (St. Louis, MO).

2.3. Proximate chemical composition, amino acids and biogenic amines

Crude protein (6.25 × nitrogen) was analysed according to ISO 5983–2 (ISO, 2009) and the lipid content by American Oil Chemists' Society method Ba 3–38 (Brühl, 1997). Determination of moisture (water) was performed according to ISO 6496 (ISO, 1999) and total ash content according to ISO 5984 (ISO, 2002). The salt content was analysed using AOAC (2005) method 937.09 (AOAC, 2005). The amino acid profiles were determined by HPLC and acid hydrolysis according to ISO 13903 (ISO, 2005). The content of Tryptophane was analysed according to ISO 13904 (ISO, 2016), and the content of Cysteine, Cystine and Methionine according to the oxidative method ISO 13903 (ISO, 2005). Biogenic amines were analysed by the Dansyl method (Önal et al., 2013).

2.4. Solubility and water holding capacity (WHC)

WFM for physiochemical analysis were accurately weighted (5 g ± 0.1 g) into Erlenmeyer flasks and autoclaved water added to 100 ml. The flasks were shaken at 150 rpm for 15 min to ensure proper mixing of FM and water. The water-soluble fraction was collected after centrifugation at 5000 x g for 20 min and then supernatant filtered through a Whatman qualitative filter paper (5–8 μm) before analysis. Percentage solubility (g/100 ml) was determined by pipetting of 30 ml filtered supernatant into pre-weighted aluminium trays and determination of dry matter after heating overnight at 102 ± 4 °C. For the calculation of the % WHC, FM samples were accurately weighted (5 g ± 0.1 g) in pre-weighted Falcon tubes and water added to 50 g ± 0.1 g. The solutions were mixed for 5 min at 300 rpm/min and then centrifuged at 5000 x g for 10 min. The supernatant was decanted and percentage of water remaining in the pellet determined by weight (Bragadóttir et al., 2007).

2.5. Emulsion stability (ES)

0.5 g (± 0.05 g) WFM was weighed into 50 ml Falcon tubes. 10 ml 0.1 M NaCl and 10 ml pure Eldorado rapeseed oil (Unil AS, Oslo, Norway) was added to the tubes. The mixture was homogenized using Ultra Thurrax T18 (IKA-Werke GmbH & Co. KG, Germany) at 25000 rpm for 1 min. The resulting emulsions were poured into 100 ml graduated cylinders and incubated at room temperature before

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recording the emulsion and water phases after 15 min, 1 h and 24 h. ES (%) were determined according to a previous report (Geirsdottir et al., 2011).

2.6. Preparation of protein hydrolysates

10 g (± 0.1 g) of WFM was weighed into Erlenmeyer flasks and distilled water added to 100 g. The flasks were shaken at room temperature at 250 rpm for 15 min to dissolve water-soluble components before the temperature was adjusted to 55 °C in a water bath. Protamex (Novozymes A/S, Denmark), an enzyme where the activity is attributed to a Serine protease that cleave internal peptide bond in proteins, was selected for the hydrolysis. 0.1% of the enzyme (w/w, dry weight FM) was added to the flasks and the hydrolysis performed by gentle shaking (100 rpm) at 55 °C for 60 min. No adjustment of the pH was performed before or during the hydrolysis. 30 ml aliquots were transferred to 50 ml falcon tubes at time zero, 60 min and at 180 min and the protease was immediately inactivated in a boiling water bath for 10 min. Samples were centrifuged at 5000 x g for 20 min and the hydrolysate collected by careful pipetting of the supernatant. To remove WFM particles the hydrolysates were filtered through a Whatman qualitative filter paper (5–8 μm) before analysis. The protein concentration was determined by the bichinchoninic acid assay (BCA) using Bovine serum Albumin as standard protein (BCA-1, Sigma Chemicals). The Degree of Hydrolysis (% DH) was determined by the o-phthaldialdehyde (OPA) spectrophotometric method using aqueous serine, (0.1 g/L) as the reference standard (Church et al., 1985). Preparation of the OPA assay reagents and determination of the % DH were performed according to a modified protocol (Nielsen et al., 2001).

2.7. Determination of angiotensin I-converting enzyme (ACE) inhibitory activity

The ACE inhibitory activity was measured using commercial ACE from rabbit lung (Sigma Chemical Co, A6778) and the synthetic substrate peptide N-[3-(2-Furyl) acryloyl]-Phe-Gly-Gly (FA-PGG, Sigma Chemical Co 7131). ACE catalyse the cleavage of FA-PGG to furylacryloylphenylalanine (FAP) and glycileglycine and the reaction can be quantified by measuring the decrease in the absorbance at 340 nm. In 96 well Plates 10 μl (0.25U/ml) ACE was mixed with 150 μl 1.75 mM FA-PGG (dissolved in 50 mM Tris-Cl, pH 8 and 0.3 M NaCl) and 10 μl of protein hydrolysates. The activity was continuously monitored at 340 nm during incubation at 37 °C for 30 min to record changes in absorbance. As a control, the hydrolysate was replaced by deionized water in tubes containing FA-PGG and ACE. The ACE activity was calculated according to Shalaby et al., 2006.

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2.8. Statistical analysis

Unless otherwise stated, the results are presented as mean ± standard deviation (sd). Mean values were compared using one-way analysis of variance (ANOVA) SPSS Statistics 21.0 (SPSS Inc., Chicago, IL). The statistical significance level was set to $P < 0.05$.

3. Results and discussion

3.1. Proximate chemical composition

The proximate chemical composition of five WFM samples (WFM 1–5) collected during the 2012–2014 winter months, was analysed for total content of protein (N * 6.25), lipids, ash (minerals), water and NaCl (Table 1). The mean values for each constituent was calculated with their concomitant standard deviations to evaluate potential deviations within the seasonal production of WFM. The calculated standard deviations of WFM sample 1–5 was within 1–2% of the mean values of each constituent and suggest that the on board productions of FM are a robust process with minor raw-material variance. The calculated protein content (N * 6.25) was found to vary between 60.2 (WFM 3) to 64.5 resulting in a mean value > 62%. High quality FMs normally contain between 60–72% crude protein by weight (Cho and Kim, 2011) and this place the WFM in the lower end. A low protein content was not unexpected as it reflect high content of connective tissue in the by-products (Falch et al., 2006). Furthermore, the discarded stickwater holds water-soluble protein that potentially could contribute to the total protein content (Bechtel, 2008). The lipid content of FM can range from 4–20% depending on species used in the production (Miles and Chapman, 2012). The measured mean

Table 1. Proximate composition, % (g/100 g dry weight) of WFM batches from selected winter months.

WFM	Month-Year	Protein (N * 6.25)	Fat	Ash	Water	NaCl
1	12-2012	64.5	7.3	23.1	6.1	1.1
2	11-2013a	61.0	9.7	20.9	6.2	1.6
3	11-2013b	60.2	10.6	23.2	3.4	1.7
4	02- 2014	60.8	8.8	22.5	5.8	n.a
5	03-2014	62.9	8.3	22.4	3.7	n.a
	*mean ± sd	61.9 ± 1.8	8.9 ± 1.1	22.4 ± 0.8	5.0 ± 1.2	1.5 ± 0.3
–						
6**	12-2012	57.9	5.6	29.1	6.5	1.1

*The mean values ± standard deviation of WFM batch 1–5. **Sample 6 (WFM 6) was not included the calculations of the mean composition due to the biased raw-material content. n.a (not analysed).

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lipid content of 8.9% reflect that cod and saithe are lean species but also that viscera including lipid rich liver (Falch et al., 2006) significantly contribute to the content of the WFM. Considering that WFM is produced without addition of antioxidants a low lipid content is advantageous as less incidents of oxidations are likely to occur and as a result improved shelf-life over other FMs on the market. This is important as natural oxidation of fatty-acids in FM have been shown to affect nutritional quality (Opstvedt, 1975).

The mean content of minerals was 22.4% and a result of the high content of connective tissue and bones in the raw-material (Toppe et al., 2007). In a human consumption perspective the WFM could be considered a highly interesting source of calcium (Ca), phosphorus and magnesium that constitute the main minerals in fish bones (Toppe et al., 2007). This is supported by clinical trials documenting a good uptake of Ca from bone meal of cod (Malde et al., 2010). Considering the proximate composition of WFM6, an experimental production based on fish-heads, this was even more evident. Here, a very high mineral content (29.1%) was found along with a lower protein (57.9%) and fat content (5.6%) compared to the other WFM samples. The high mineral content combined with clearly suggest that FMs produced from fish-heads could be an excellent mineral source.

3.2. Analysis of protein quality by amino acid profiling

The content of amino-acids was analysed in WFM2 produced from viscera and fish-heads, and WFM6, produced from fish-head only (Table 2). All twelve amino acids considered to be nutritionally essential (EAA) were identified in both samples including Arginine (Arg), Aspartic acid (Asp), Cysteine (Cys), Histidine (His), Isoleucine (Iso), Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Threonine (Thr), Valine (Val) and Tryptophane (Trp). The calculated ratio of essential over non-essential AA was very similar for WFM 2 and WFM 6 suggesting that the protein is of comparable quality despite the differences of the raw-material basis. The determined total essential amino acid content (TEA) in the samples document a high-quality protein source as the daily requirement for indispensable amino acids could be covered WFM alone (FAO, 2013). Of the essential amino-acids Lys has received most attention given its nutritional importance and limited content in vegetable protein (Tome and Bos, 2007). The content of Lys was approx. 4.8 g/100 g dry matter in WFM 2 and 4.06 g/100 g dry matter in WFM6. A recommended daily intake of Lysine of 30 mg/kg/day has been suggested (FAO, 2013) and in theory, a healthy adult in the 50–90 kg range could cover this demand by consuming approx. 37–70 g WFM daily.

Nutritionally, novel classifications of amino-acids have recently been suggested labelling them either as conditionally essential or functional (Wu, 2013). Amino acids are considered conditionally essential (CEAA) when limited under special

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Table 2. Analysis of % (g/100 g dry matter) of amino acids (AA) and free amino-acids (FAA) in WFM samples produced from two different raw-material compositions.

Amino acid	WFM 2*	WFM 6**
AA		
Ala	3.76	3.74
Arg[EF]	4.00	3.85
Asp[F]	5.84	5.33
Cys[EF]	0.55	0.60
Glu[CF]	8.19	7.62
Gly[CF]	4.56	5.26
His[E]	1.38	1.25
Iso[E]	2.65	2.30
Leu[EF]	4.54	4.10
Lys[E]	4.78	4.06
Met[EF]	1.74	1.74
Phe[E]	2.57	2.57
Pro[CF]	2.85	3.14
Ser	2.99	2.98
Thr[E]	2.83	2.61
Trp[EF]	0.70	0.56
Tyr[EF]	2.07	2.10
Val[E]	3.00	2.70
TAA	59.00	56.37
TEA	30.81	28.44
E/A	0.52	0.50
–		
FAA		
Tau[CF]	0.29	0.24
TFAA	0.70	0.48

The summary of total amino acids (TAA), total essential amino acids (TEA), are shown. E/A, TEA: TAA. TFAA, total free amino-acids. [E] Essential amino acids. [F] Functional amino acids.[C] Conditionally essential amino acids. * WFM produced from viscera and heads. ** WFM produced from heads only.

physiological conditions in e.g premature infants or during metabolic disorders in adults. Functional amino acids participate in and regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of the organisms (Wu, 2013). Three of the protein-bound conditionally essential amino acids (Glu, Gly and Pro) suggested by Wu (2013) are present in WFM samples (Table 2). The fourth one Glutamine (Gln) is considered both

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conditionally essential and functional, but was not analysed in the experimental setup. Not surprisingly, the collagen associated amino-acids Gly and Pro (Toppe et al., 2007) were found in higher concentrations in WFM6 compared to WFM2 whereas Glu was lower. Considering the list of suggested functional amino acids in mammals (Wu, 2013) all but Gln were identified in the samples. Overall, these results suggest that WFM is an excellent source of nutritionally important amino-acids.

3.3. Free amino acids

A high score of free amino acids (TFAA) in the WFM 2 sample (0.70%) was observed compared to the WFM 6 sample (0.48%) (Table 2). The result hint at the activity of proteolytic enzymes present in the viscera that contribute to degradation of proteins. However, analysis of several samples will be required to confirm the apparent difference statistically. Taurine (Tau), is classified both as a CEAA and FAA, and is known to be involved in a range of physiological processes affecting human health (Bouckenooghe et al., 2006; Hosomi et al., 2012). In seafood the taurine content is high in general (Spitze et al., 2003). However, the processing of marine raw materials can promote loss of water soluble components including taurine (Dragnes et al., 2009a; Spitze et al., 2003). High concentrations of taurine was observed in both the analysed WFM samples with a content of 290 mg/kg (WFM 2) 240 mg/kg (WFM 6) and respectively. In comparison to the reported taurine concentrations in dried cod fillets of 375 $\pm$ 49 (Dragnes et al., 2009a) the content of taurine in WFM, is high. Interestingly, the result was obtained despite the fact that the stick-water containing water-soluble compounds such as FAAs, is discarded.

3.4. Biogenic amines

An important aspect of the on board production of fishmeal is the utilization of totally fresh raw material that in theory should ensure the highest quality possibly. It was therefore of interest to look at biogenic amines (BA) known to be associated with quality and freshness of the raw-materials and final products (Visciano et al., 2012). Factors that influence the content of BAs are storage temperature, handling practices, the presence of microorganisms with decarboxylase activity and the content of free amino acids (Prester, 2011). The analysed content of total BAs is shown in Table 3. The toxicological level of BAs depend among other factors on the presence of other amines and is therefore difficult to predict (Mišurcová et al., 2011). However, the total amount of BAs (TBA) indicate acceptable values compared to the suggested maximum content of 300 mg/kg (Shalaby, 1996) and 750 – 900 mg/kg (Prester, 2011). The major concern of BAs in fish is the level of histamine. The consumption of as low as 75 mg histamine has been reported to cause intoxication symptoms in healthy people, but an intake of about 1000 mg is

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Table 3. Biogenic amines (BA) in two selected WFM batches (mg/kg dry matter). TBA, total biogenic amines.

BA	WFM 2	WFM 6
–		
Tyramine	< 1	< 1
Putrescine	200.00	147.00
Cadaverine	7.51	4.54
Histamine	< 1	< 1
Tryptamine	< 5	< 5
2-Phenylamine	4.52	5.50
Spermidine	11.10	12.20
Spermine	4.92	7.66
TBA	235.05	183.90

required to promote severe intoxications (Rauscher-Gabernig et al., 2009). The content of histamine in the WFM samples are very low compared to other marine products (Shalaby, 1996). The higher content of BAs in WFM 2 compared to WFM 6 is in accordance with the observed higher content of FAA (Table 2).

3.5. Physicochemical properties

Physicochemical parameters of the WFM was analysed as dried marine proteins and peptides are known to contain interesting functional properties that are useful in many food applications (Freitas and Cortez-Vega, 2016). Few studies of the functional properties of FM proteins are available making comparisons challenging due to differences in methodology (Samuelsen et al., 2013; Sathivel et al., 2005). The determined solubility, WHC and % ES of WFM 1 to WFM 5 are shown in Table 4 where the results are compared to SBM. The solubility of the WFM dry matter (DM) was measured as described and results were calculated as g soluble FM per 100 g DM (Table 4). The mean values of WFM suggest that about 8.5% of the WFM compounds are water soluble compared to about 25% of the SBM compounds. This is somewhat lower compared to e.g. whole-meal produced from pelagic species reported to be in the 10–20% range (Ariyawansa, 2000). Analysis of the % WHC suggest that WFM is highly capable of water absorption (Table 4). The obtained results propose binding of water corresponding to more than twice its own DM and is comparable to values obtained for FM of e.g. pelagic species (Ariyawansa, 2000; Samuelsen et al., 2013).

The processing of FM include thermal treatments such as cooking of the raw-material and drying of the press-cake before grinding and thus the content of denatured

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Table 4. Functional properties of selected WFM batches compared to soy bean meal (SBM). Values for each batch are expressed as mean ± standard deviation (n = 3).

	% Solubility	WHC *	ES (%)		
Sample			0 hrs	2 hrs	24 hrs
WFM 1	7.27 ± 0.42 ^{H}	2.37 ^{F}	86.03 ± 1.97 ^{A}	76.07 ± 2.48 ^{B}	63.35 ± 7.42 ^{E}
WFM 2	9.41 ± 0.02 ^{H}	2.35 ^{F}	77.33 ± 8.95 ^{A}	68.07 ± 2.1^{C}	62.05 ± 4.03 ^{E}
WFM 3	9.22 ± 0.07^{H}	2.43 ^{F}	91.60 ± 5.48 ^{A}	85.97 ± 2.32 ^{D}	71.05 ± 5.59 ^{E}
WFM 4	8.48 ± 0.09 ^{H}	2.31 ^{F}	89.53 ± 4.14 ^{A}	80.17 ± 6.67 ^{B}	62.50 ± 5.94 ^{E}
WFM 5	8.02 ± 0.23 ^{H}	2.33 ^{F}	86.03 ± 2.42 ^{A}	74.10 ± 9.71 ^{B}	53.40 ± 4.81 ^{E}
mean ± sd	8.48 ± 0.016	2.38 ± 0.06	84.00 ± 7.00	75.5 ± 6.90	62.1 ± 5.70
SBM	25.10 ± 0.03^{G}	2.45 ^{F}	79.37 ± 5.37 ^{A}	70.23 ± 2.51 ^{B}	63.20 ± 2.40 ^{E}

Different superscript letters in the same column denotes a significant difference ($P < 0.05$). * g/g dry matter.

proteins are expected to be high. Still, WFM stands out as a protein source with better water holding capacities compared to results obtained for enzymatically derived hydrolysates of whitefish (Bragadóttir et al., 2007; Šližytė et al., 2009). The WHC of WFM is comparable to results obtained for the SBM. However, taking the different protein content of 61 g/100 g and 37.5 g/100 g into consideration, it is evident that the SBM have a higher water holding capacity binding per gram protein. Routine analysis of the content of water-soluble proteins by BCA protein assays indicated about 3.7% for WFM samples and about 5.5% for the SBM. The higher content of water soluble protein in the SBM likely contribute to the better score for % solubility and the WHC. % ES was recorded after 2 h and 24 h incubation and results compared to initial emulsion volumes (0 h) to record the rate of the decline. Based on the statistical analysis the average values of % ES obtained for WFM samples were comparable to values of SBM and each other at 0 h and 24 h incubation as no significant difference was calculated. Data obtained after two hours incubation suggested that % ES of WFM 3 was significantly different from SBM and that WFM 2 and WFM 3 were different from each other. However, as the calculated standard deviations vary these differences likely reflect a natural variation of % ES among the WFM samples due to minor differences in the proximate composition. pH was routinely measured in the solubilised WFM samples and typically resulted in values in the 6–7 range.

3.6. Determination of ACE-I-inhibitory activity

It was possible to detect *in vitro* ACE-inhibitory activity in hydrolysates of WFM as shown in Table 5. Inhibitory activity towards ACE after treatment of WFM with Protamex for 0 min, 60 min and 180 min was analysed and clearly increased with

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Table 5. Angiotensin converting enzyme (ACE) inhibitory effect of fishmeal hydrolysed with Protamex.

Hydrolysis time (min)	% DH	IC50 (μg/ml)
0	4.83 ± 0.57	1850 ± 0.01
60	38.67 ± 1.21	102.78 ± 0.12
180	51.27 ± 1.79	36.27 ± 0.06

Data are presented as the protein concentration (μg/ml) needed to reach $IC_{50\%}$ in a 1 mU ACE-assay. The corresponding degree of hydrolysis (% DH) for each timepoint, is shown. Data are presented as the mean ± the standard deviation (n = 3).

the determined degree of hydrolysis (% DH). The results suggest that the increasing inhibition with the time of proteolysis is likely due to the accumulation of biological active peptides. This is in concordance with previous reports of ACE-inhibitory activities identified in muscle and by-products of fish such as Alaska pollock, blue whiting and cod (Byun and Kim, 2001; Dragnes et al., 2009b; Geirsdottir et al., 2011; Jensen et al., 2014). The determined IC50 values reflect that the ACE-inhibitory activity was measured directly in the hydrolysates without any further concentration by e.g freeze drying of the peptides. This is evident from the results in Table 5 reporting a higher protein concentration in μg/ml needed to reach the IC50 compared to previous reports based on analysis of freeze dried hydrolysates (Byun and Kim, 2001; Dragnes et al., 2009b; Geirsdottir et al., 2011; Jensen et al., 2014). However, the main purpose of the measurement was fulfilled as the result demonstrate that biological activity is retained despite the multiple thermal treatments of raw-material proteins during the processing to WFM. This is interesting in a human consumption perspective where WFM could be considered a bioactive protein ingredient in a functional food setting (Shahidi and Ambigaipalan, 2015). However, further studies of bioactivities *in vitro* and in clinical trials would be necessary to clarify if WFM is a bioactive protein ingredient with impact on human health.

4. Conclusions

Freshly produced WFM was characterised for proximate chemical composition, biochemical parameters, selected physicochemical parameters and ACE-inhibitory activity. The WFM is of high-quality with a high protein and mineral content. All essential and functional amino acids are present documenting that WFM nutritionally, is a complete protein source. The protein quality was confirmed by a low content of free amino acids and biogenic amines acids suggesting minimal microbial activity and endogenous proteolytic enzyme activity in the raw material. The DM water holding capacity and the emulsifying properties of water soluble

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fraction resembles values obtained for SBM whereas the water solubility is significantly lower. Proteolysis of the WFM proteins was used to demonstrate the presence of compounds with inhibiting activity towards ACE, *in vitro*. Taken together, WFM proteins from fresh fish processing co-products have properties of interest to a human consumption market. Future characterisation of sensory and functional properties are vital before concluding on the potential for use in food matrixes. The potential for bioactive compounds should also be explored in more detail, preferably using enzymes simulating human digestion of proteins to mimic food intake.

Declarations

Author contribution statement

Ola Ween: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Janne Stangeland: Performed the experiments; Analyzed and interpreted the data.

Turid S. Fylling: Analyzed and interpreted the data.

Grete Hansen Aas: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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