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Biochemical and Functional Properties of Sardinella (*Sardinella aurita*) By-Product Hydrolysates

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Summary

Fish protein hydrolysates (FPHs) with different degrees of hydrolysis (DH of 6.62, 9.31 and 10.16) were prepared from heads and viscera of sardinella (*Sardinella aurita*) by treatment with Alcalase®. The liquid hydrolysates were spray dried. All spray dried by-product hydrolysates contained 73 to 75 % of proteins and low content of lipids. Reversed-phase HPLC profiles showed that low DH hydrolysates contained high hydrophobic peptides and the extent of hydrolysis resulted in an increase of fish hydrophilic peptides. Some functional properties of FPHs were assessed and compared with those of casein or the undigested sardinella protein. Solubility increased while emulsifying capacity and whippability decreased with the increase in the degree of hydrolysis. The product, with a DH of 10.16 %, had an excellent solubility (100 %) over a pH range of 6.0–10.0. The antioxidant activity of the hydrolysates was also tested. All FPHs exhibited more than 50 % inhibition of linoleic acid peroxidation. The antioxidant activity of FPH with 10.16 % DH was about 73 % of that of α-tocopherol, a natural antioxidative agent.

Key words: fish protein hydrolysates, heads and viscera, sardinella by-product, functional properties, *Sardinella aurita*, antioxidant activity

Introduction

In 2002, the catch of sardinella in Tunisia was about 13 300 tonnes (*1*). During processing, solid wastes including heads and viscera are generated and can contain 30 % of the original material. These wastes, which represent an environmental problem to the fishing industry, constitute an important source of protein. Traditionally, this material has been converted to powdered fish flour for animal feed (2).

Novel means of processing are required to convert the underutilized wastes and by-products into more marketable and acceptable form. One alternative is to produce fish powders or fish protein hydrolysates that may be used as carbon and/or nitrogen source for biomass and metabolite production. The use of fish protein hydrolysates for maintaining the growth of different microorganisms has received a great attention (*3–6*). Several studies revealed that fish protein hydrolysates performed effectively as a nitrogen source for microbial growth (*7*) and enzyme production (*8,9*).

Utilizing proteolytic enzymes, fish protein hydrolysates (FPHs) can be prepared in controlled conditions with peptides having new and/or improved properties that may be used as food ingredients. Many enzymes have been described to be interesting in hydrolysis of fish proteins (papain, Alcalase®, Neutrase®, Flavourzyme®, Protamex®, *etc.*) (*10*). However, the substrate is one of the major factors influencing the hydrolysis (*11*). Kristinsson and Rasco (*12*) mentioned that Alcalase® was the most desirable enzyme in hydrolyzing fish proteins. The enzymatic hydrolysis of native proteins improved its functional properties, including solubility, emulsifying and foaming characteristics (*13*) and offered interesting

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opportunities for food applications. Functional properties can be defined as the overall physicochemical properties of proteins in food systems during processing, storage and consumption (*14*). The changes in functional properties of native proteins are related to peptides and free amino acids produced by enzymatic hydrolysis, which are mainly characterized by a lower molecular mass, exposure of hydrophobic groups and by an increased number of ionic groups (*15*).

In this paper, we will investigate some of the functional properties and antioxidant activity of Alcalase® FPHs at 6.62, 9.31 and 10.16 % DH (FPH1, FPH2 and FPH3, respectively) obtained from sardinella by-product by stopping hydrolysis reaction at different times.

Materials and Methods

Materials

Sardinella (*Sardinella aurita*) was obtained from a local supplier. The proteolytic enzyme used was Alcalase® (Novozymes®, Denmark). Alcalase® is a bacterial protease from *Bacillus licheniformis*.

Preparation of fish protein hydrolysate

The scheme for production of protein hydrolysates from sardinella heads and viscera is given in Fig. 1. Heads and viscera (500 g) were first minced then cooked to inactivate endogenous enzymes. The cooked heads and viscera sample was mixed with an equal amount of distilled water and homogenized in a Moulinex® blender for about 2 min. The pH of the mixture was adjusted to the optimum activity value for the enzyme. The hydrolysis was performed under the following conditions: pH=8.0, t=50 °C. The enzyme was added to the reaction to give an enzyme/substrate ratio of 727.26 U/g (units of enzyme by mass of sardinella heads and viscera). Protease activity was determined according to the Kembhavi *et al.* method (*16*). The pH of the mixture was maintained constant at pH=8.0 during the enzymatic reaction by continuous addition of 4 M NaOH solution to the reaction mixture. After the required digestion time the reaction was stopped by heating the solution at 80 °C for 20 min to inactivate the enzyme. The fish hydrolysate was centrifuged at 5000 × g for 20 min to separate insoluble and soluble fractions. Finally, the soluble phase was spray-dried in a Büchi® 190 mini spray dryer Labortechnik AG, Switzerland (inlet air t=160 °C, outlet air t=80 °C, p=3 bar, and flow rate v=500 mL/h). Samples were stored as hydrolyzed sardinella protein powder.

Determination of the degree of hydrolysis (DH)

The degree of hydrolysis (DH), defined as the percent ratio of the number of peptide bonds broken (N) to the total number of peptide bonds per mass unit (N_{total}), was in each case calculated from the volume of base consumed (*17*), as given below:

$$DH = \frac{V_B \times c_B}{m_P} \times \frac{1}{\alpha} \times \frac{1}{N_{total}} \times 100 \qquad /1/$$

where V_B is the volume of base consumed (in litre) to keep the pH constant during the reaction, c_B is the molarity of the base, m_P is the mass (kg) of protein (N × 6.25), and α is the degree of dissociation of the α-NH_2 groups released during hydrolysis expressed as $\alpha = \frac{10^{pH-pK}}{1+10^{pH-pK}}$; where pH and pK are the values at which the proteolysis was conducted. The total number of peptide bonds (N_{total}) in the fish protein concentrate was assumed to be 8.6 meq/g (*17*).

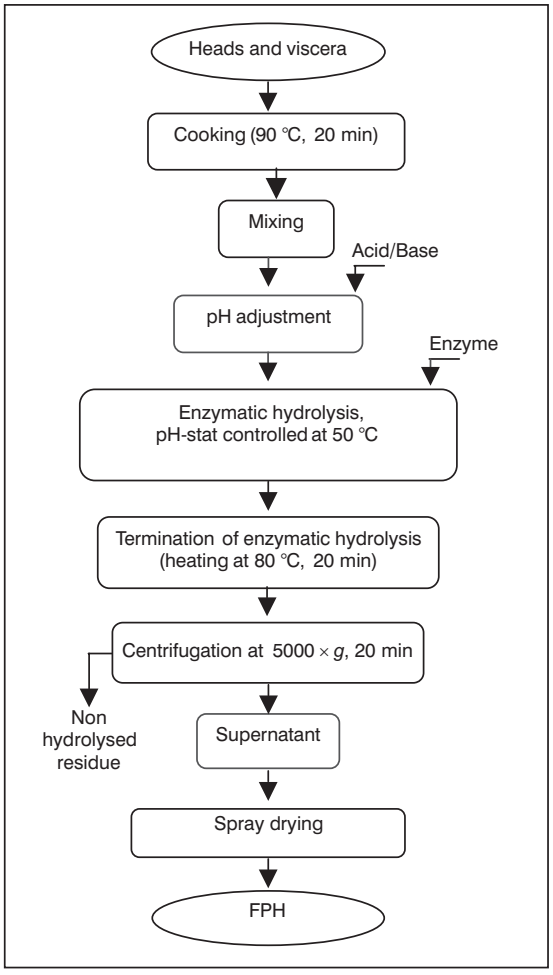

Fig. 1. A flow chart for the preparation of fish protein hydrolysates

Chemical analysis

The moisture and ash content were determined according to the AOAC standard methods 930.15 and 942.05, respectively (*18*). Total nitrogen content of FPHs was determined by using the Kjeldahl method. Crude protein was estimated by multiplying total nitrogen content by the factor of 6.25. Lipids were determined gravimetrically after Soxhlet extraction of dried samples with hexane. All measurements were performed in triplicate.

Molecular characterization of the hydrolysates by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on all samples using a 4 % stacking gel and 15 % acrylamide gel (*19*). Protein con-

tent of the samples was determined by Bradford method (*20*) using bovine serum albumin as a standard. The electrophoresis was performed in a water-cooled electrophoresis apparatus (Bio-Rad® instruments, USA) using a Consort® (Belgium) power supply.

The protein standard used was purchased from Sigma-Aldrich®, USA (albumin, bovine 66 000 Da; ovalbumin, chicken 45 000 Da; glyceraldehyde-3-phosphate dehydrogenase, rabbit 36 000 Da; carbonic anhydrase, bovine 29 000 Da; trypsinogen, bovine, PMSF-treated 24 000 Da; trypsin inhibitor, soybean 20 100 Da; and α-lactalbumin, bovine 14 200 Da).

Analysis of fish protein hydrolysates by reversed-phase high performance liquid chromatography

Peptides contained in FPHs were separated by reversed-phase high performance liquid chromatography (RP-HPLC), using a liquid chromatograph LC-10 (Shimadzu®, Japan) on a 250 × 8 mm Eurosphère-100 C18 column. The column was equilibrated with solvent A (0.1 % trifluoroacetic acid in ultra pure water) and peptides were eluted by a linear increase of solvent B (0.1 % trifluoroacetic acid in acetonitrile) from 0 % at 0 min to 80 % at 60 min. The flow rate was 0.4 mL/min. The elution was monitored at 214 nm.

Solubility

Solubility of FPHs and undigested fish protein was determined over a range of pH values from pH=3.0 to 10.0, as described by Tsumura *et al.* (*21*) with slight modifications. Briefly, 1 g of each sample was dissolved in 100 mL of distilled water and the pH was adjusted using 2 M HCl or 2 M NaOH solutions. The solution was stirred for 10 min at room temperature of (25±1) °C and then centrifuged at 8000 × *g* for 10 min. After appropriate dilution, the nitrogen content in the supernatant was determined by biuret method (*22*). The solubility of FPHs, defined as the amount of soluble nitrogen from the total nitrogen, was calculated as follows:

$$\text{Nitrogen solubility} = \frac{\text{Supernatant nitrogen concentration}}{\text{Sample nitrogen concentration}} \times 100 \qquad /2/$$

Solubility analysis was carried out in triplicate.

Emulsifying properties

Emulsifying capacity was determined as described by Kinsella (*23*). A volume of 5 mL of corn oil was mixed with 5 mL of a 5 % FPH in distilled water and homogenized at 20 000 rpm for 90 s at room temperature of (25±1) °C. The emulsion was poured into 10-mL graduated tubes and centrifuged at 2400 × *g* for 3 min. The volume of each fraction (oil, emulsion and water) was determined and the emulsifying capacity was expressed as mL of emulsified oil per g of FPH.

Fat absorption

The capacity of the FPH to absorb fat was determined as described by Shahidi *et al.* (*11*) and modified by Slizyte *et al.* (*24*). Samples (0.5 g) were mixed with 10 mL of corn oil in 50-mL centrifuge tube. The mixture was then kept for 30 min at room temperature of (25±1) °C while mixing every 10 min for 30 s. The mixture was then centrifuged for 25 min at 2000 × *g* and the volume of the supernatants was weighed. This test was performed in triplicate and fat absorption was expressed as the volume (mL) of fat absorbed by 1 g of protein hydrolysate. Fat adhesion to the walls in the tube was evaluated in an empty tube.

Foaming properties

The whippability of all hydrolysates was determined by the method of Watanabe *et al.* (*25*) with slight modifications. A mass of 0.25 g of sample of FPH was dispersed in 25 mL of distilled water. The mixture was adjusted to pH=4.0, 6.0, or 7.0 with 2 M HCl and then homogenized for 1 min with a Moulinex® R62 homogenizer at room temperature of (25±1) °C. The whipped sample was then poured into a graduated cylinder and the volume of the water that had drained from the foam phase was measured after 30 s. The whippability is expressed by the following equation:

$$\text{Whippability} = \frac{(\text{Total volume} - \text{Drainage volume})}{\text{Initial volume}} \qquad /3/$$

All results were means of triplicates.

Antioxidant activity

Inhibition of linoleic acid autoxidation

The oxidation of linoleic acid was conducted as described by Osawa and Namiki (*26*). A sample of FPH (1.3 mg) was dissolved in 10 mL of 50 mM phosphate buffer (pH=7.0), and added to a solution of 0.13 mL of linoleic acid and 10 mL of 99.5 % ethanol. The total volume was then adjusted to 25 mL with distilled water. The mixture was incubated in a 50-mL assay tube with a screw cap at (40±1) °C for 5 days in a dark room and the degree of oxidation was evaluated by measuring the ferric thiocyanate values according to the method of Mitsuta *et al.* (*27*). A total of 100 μL of the oxidized linoleic acid solution was added to 4.7 mL of 75 % ethanol, 0.1 mL of 30 % ammonium thiocyanate, and 0.1 mL of 20 mM ferrous chloride solution in 3.5 % HCl. After stirring for 3 min, the absorbance was measured at 500 nm. α-tocopherol, a natural antioxidative agent, was used as reference and distilled water as control. The antioxidative capacity of the inhibition of peroxide formation in linoleic acid system was expressed as follows:

$$\text{Inhibition} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100 \qquad /4/$$

Scavenging effect on DPPH free radical

The scavenging effect of the hydrolysates on α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical was measured according to Wu *et al.* (*28*). A volume of 1.5 mL of each sample was added to 1.5 mL of 0.1 mM DPPH in 95 % ethanol and the obtained mixture was shaken vigorously. After storage at room temperature for 30 min, the absorbance of the resulting solution was measured

at 517 nm. Lower absorbance represented higher DPPH scavenging activity. The scavenging effect is expressed as:

$$\frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100 \quad /5/$$

Statistical analysis

Statistical analyses were performed with Statgraphics ver. 5.1, professional edition (Manugistics Corp., USA) using ANOVA analysis. Differences were considered significant at $p<0.05$.

Results and Discussion

Preparation of fish protein hydrolysates

A flow diagram for the sardinella heads and viscera hydrolysates is shown in Fig. 1. In order to study the effect of the DH on the yield of protein recovery and the evolution of functional properties, hydrolysates having DH values of 6.62, 9.31 and 10.16 % were generated by treatment with Alcalase®. The yield of the spray-dried hydrolysates was 4.23 %.

The curve of hydrolysis of sardinella heads and viscera with Alcalase® 2.4 L after 3 h of incubation is shown in Fig. 2. The hydrolysis of the fish protein was characterized by an initial rapid phase, during which a large number of peptide bonds were hydrolyzed. The rate of enzymatic hydrolysis subsequently decreased, and then the enzymatic reaction reached the steady-state phase when no apparent hydrolysis took place. Similar results were found by Quaglia and Orban (*29*) with sardines, Kristinsson and Rasco (*30*) with Atlantic salmon, and Guérard *et al.* (*7*) with yellowfin tuna. The decrease in the reaction rate could be explained by the inhibition of the enzyme by the products formed at high degree of hydrolysis. Those products act as effective substrate competitors to the undigested or partially digested fish proteins.

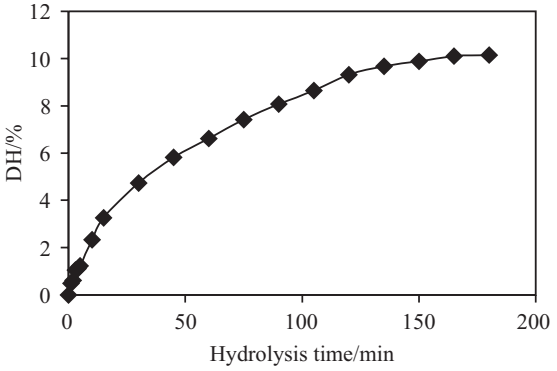

Fig. 2. Hydrolysis curve for sardinella heads and viscera with Alcalase® 2.4 L

Chemical composition

The chemical composition of FPHs and defatted undigested fish protein were determined and compared to that of a commercial peptone (tryptone N1, purchased from Organotechnie SA, France). As shown in Table 1, all hydrolysates and peptone had approximately the same composition. The protein and lipid contents of undigested fish proteins were significantly lower than those of hydrolysates.

Table 1. Composition of raw material and fish protein hydrolysates (FPH)

Hydrolysate	Composition/%			
	Protein	Lipids	Moisture	Ash
Defatted undigested fish proteins	58.25±2.16	5.42±1.23	5.88±1.12	25.23±2.23
FPH1	75.01±1.72	8.53±1.11	1.35±0.55	14.81±0.14
FPH2	72.99±1.82	10.21±1.58	2.83±0.42	13.06±0.13
FPH3	73.05±1.91	10.29±1.76	4.56±0.27	12.10±0.12
Tryptone (Organotechnie SA)	75.62±1.23	8.50±0.98	4.40±0.32	12.50±0.56

The three by-product hydrolysates contained higher protein content (73–75 %) and low moisture content (<5 %), which might significantly contribute to stability. However, since sardinella is a fatty fish, the FPHs had relatively high lipid content. The ash content of FPHs was 14.81, 13.06, and 12.10 % for FPH1, FPH2, and FPH3, respectively.

SDS-PAGE

The electrophoretic patterns of the sardinella hydrolysates, presented in Fig. 3, showed the presence of one major band with a molecular mass of less than 14.2 kDa, which is more intensive in FPH3 than in the other hydrolysates. The three hydrolysates also showed two bands in the range of 55 and 30 kDa, which may be the result of larger proteins from the starting material or proteins contained in raw material which are not totally hydrolyzed by the enzyme.

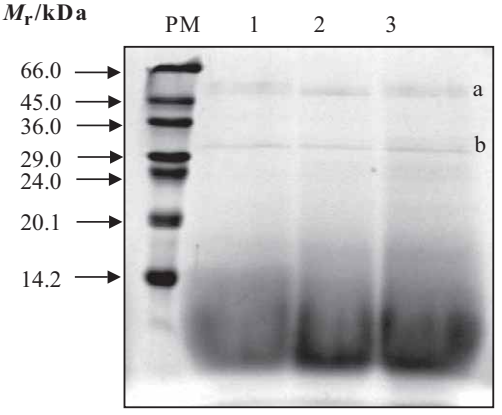

Fig. 3. SDS-PAGE profiles of FPHs: lane PM, peptide marker; lane 1, FPH1 (DH=6.62 %); lane 2, FPH2 (DH=9.31 %); and lane 3, FPH3 (DH=10.16 %)

Reverse phase-high performance liquid chromatography analysis

Wilding *et al.* (*31*) reported that the ratio of hydrophilic/hydrophobic peptides is the most important factor influencing functional properties such as whippability

and emulsifying activity. RP-HPLC is the most appropriate method to separate peptides in FPHs and it can give a good idea of the hydrophobic/hydrophilic peptide ratio (*32*). The retention of peptides depends on their size as well as their polarity. Fig. 4 shows an analysis of FPHs by RP-HPLC. A number of peaks are detectable by RP-HPLC illustrating the heterogeneous composition of the FPHs. From the RP-HPLC profiles a decrease of the hydrophobic peptides and an increase of the peptide material eluted earlier were noted as the degree of hydrolysis increased. The apparent peptide distribution of FPH1 was similar to that of FPH2. Both contain more late-eluting hydrophobic peptides than FPH3. However, extensively hydrolysed FPH3, which contains more hydrophilic peptides than the other hydrolysates, still has high concentration of late-eluting hydrophobic peptides.

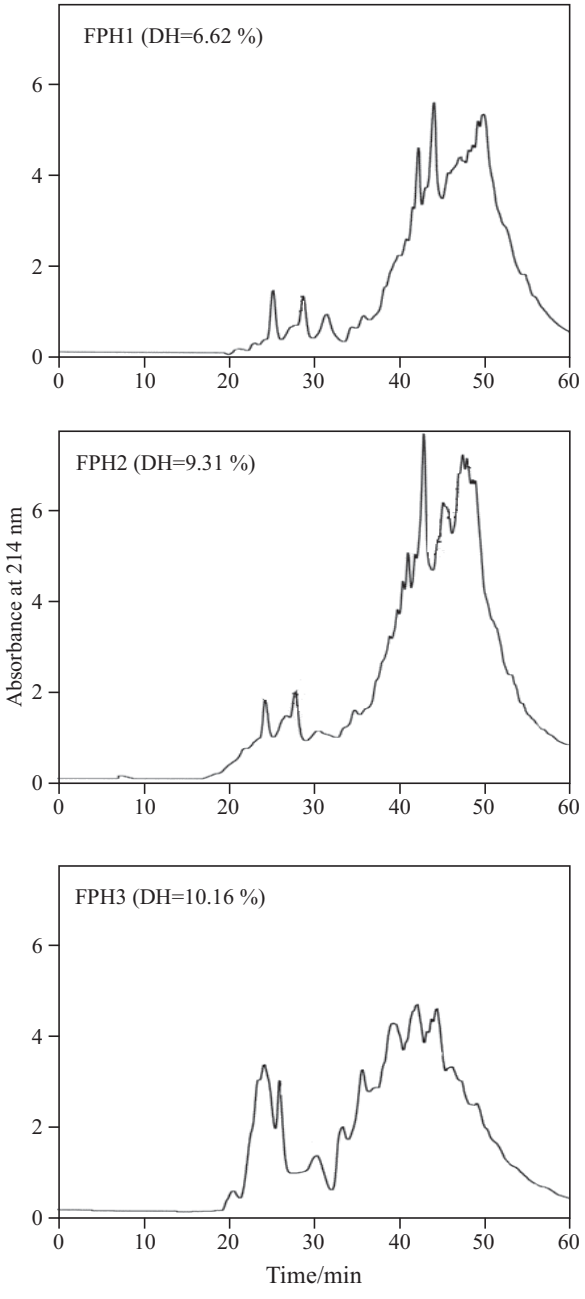

Fig. 4. RP-HPLC profiles of fish protein hydrolysates

Solubility

Solubility is one of the most important functional properties of protein and protein hydrolysates (*33*). Many of the other functional properties such as emulsification and foaming are affected by solubility (*31*). The solubility of FPHs and undigested fish proteins at pH ranging from 3.0 to 10.0 is shown in Fig. 5. The undigested fish proteins were less soluble than the hydrolysates, having a solubility of 4 and 50 % at pH=3.0 and 10.0, respectively. FPHs with different DH showed different solubility profiles at different pH conditions. Increasing the degree of hydrolysis increased the solubility of the total hydrolysates. High solubility of FPHs is due to cleavage of proteins into smaller peptides that usually have increased solubility (*13*). The difference in solubility observed among hydrolysates can be due to peptide length and the ratio of hydrophilic/hydrophobic peptides.

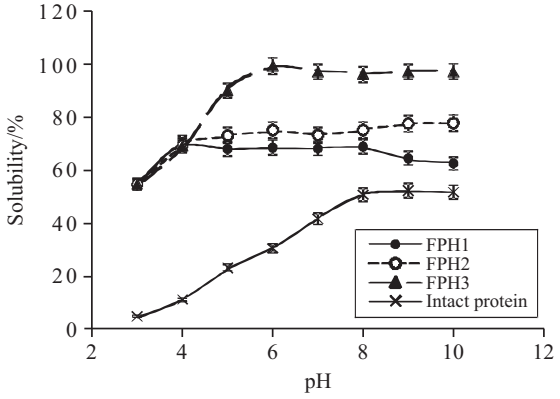

Fig. 5. Solubility of fish protein hydrolysates in water at different pH values

The lowest FPH solubility was achieved at pH=3.0. FPH solubility increased with the increase of pH values and reached 100 % at pH=6.0 in FPH3 (10.16 % DH). The hydrolysate with a high degree of hydrolysis (FPH3), which may have lower molecular mass, had excellent solubility in a pH value ranging from 6.0 to 10.0. This can be explained by the fact that hydrolysis exposes some of the hydrophobic groups to the surface. In addition, it converts some hydrophobic groups into hydrophilic ones by generating two end carbonyl and amino groups, as reported by Kristinsson and Rasco (*12*).

High nitrogen solubility of FPHs indicates potential applications in formulated food systems by providing attractive appearance and smooth mouthfeel to the product, as suggested by Petersen (*34*).

Foaming capacity

The foaming properties of the 3 hydrolysates and the undigested fish proteins were determined by measuring their whippability at pH values of 4.0, 6.0, and 7.0. The foaming capacity is improved upon limited proteolysis. As shown in Fig. 6, FPH at 6.62 % DH had a higher whippability, which was even better than the undigested protein. Foam formation decreased with increasing DH and also with increasing pH values, except for FPH2. Poor foaming properties of FPH3 can be explained by

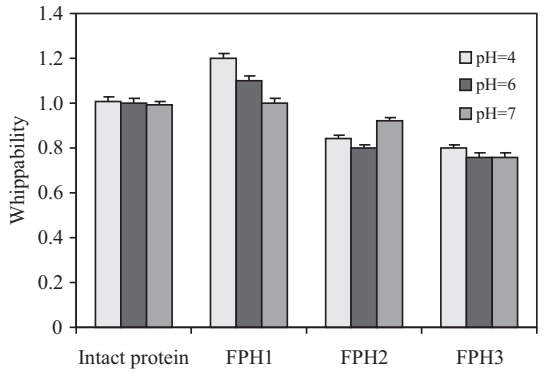

Fig. 6. Foaming activity of fish protein hydrolysates at pH=4.0, 6.0 and 7.0. Results are means of 3 replicates. Error bars represent standard deviation

the small size of peptides, which would hinder the formation of a stable film around the gas bubbles, and also by the apparition of hydrophilic peptides during extensive hydrolysis (Fig. 4). This is in line with previous findings reporting that a good cohesiveness of films is only reached with high molecular mass peptides or partially hydrolysed proteins (*35*).

Fat absorption

Fat binding capacity is an important functional characteristic of ingredients used in the meat and confectionery industries. Several factors may affect the ability of hydrolysates to bind fat, such as bulk density of the protein (*23*), degree of hydrolysis (*36*), and enzyme-substrate specificity (*37*). Kristinsson (*38*) reported that the fat absorption of salmon protein hydrolysate decreased with increasing DH. Sathivel *et al.* (*39*) investigated the functional properties of hydrolyzed herring and herring by-products and found comparable fat absorption to that of egg albumin.

Fat absorption of sardinella protein hydrolysates (Table 2) shows that there is no correlation between the fat absorption and DH. All FPHs exhibited moderate fat absorption, greater than undigested fish proteins. The hydrolysate at 9.31 % DH had the highest fat absorption capacity, while FPH1 had the lowest fat absorption capacity. Both FPH3 and FPH2 had greater ability ($p<0.05$) to bind corn oil than undigested fish protein and casein used as control. These results could be explained by the fact that hydrolysis can liberate some peptides from the native protein, which would enhance the flexibility of the peptides of FPH2. The extensive hydrolysis would break many peptide bonds, thus contributing to the decrease of the oil binding properties shown in FPH3.

Emulsifying capacity

The emulsifying capacity of the FPHs was compared with casein and undigested fish proteins. As shown in Table 3, the emulsifying capacity decreased with the increase in hydrolysis degree. The decrease in emulsifying activity of the extensively hydrolysed FPH is essentially due to the reduction of hydrophobicity of the hydrolysate, as shown in Fig. 4, and to changes in peptide size during hydrolysis. The emulsifying capacity of FPH1 was higher than casein and the undigested fish proteins, and was twofold higher than that of FPH3. Due to its high degree of hydrolysis, the FPH3 mainly consists of short peptides and free amino acids and only a limited concentration of surface-active large peptides. A direct relation between surface activity and peptide length was reported (*40*). The smaller peptides often have reduced emulsifying properties. Indeed, a peptide is required to have a minimum length of about 20 residues to possess good emulsifying and interfacial properties (*41*). Similar observations were reported by Quaglia and Orban (*42*) on the emulsifying capacity of Alcalase®-hydrolysed sardine protein. Their data showed a 1.5- to 2-fold reduction in the emulsifying capacity of the hydrolysates by increasing DH from 5 to 20 %.

Antioxidant activity

Lipid peroxidation is of great concern to the food industry because it leads to the development of undesirable off-flavours and potentially toxic reaction products (*43*). Many synthetic antioxidants have been used, but nowadays they are under strict regulation due to the potential health hazards caused by such compounds. Therefore, the search for natural antioxidants as alternatives to synthetic ones is of great interest among researchers. Many FPH from fish processing by-products such as FPH from yellowfin sole skin gelatine (*44*), cod frame protein (*45*) and Alaska pollock frame protein (*46,47*) have been shown to have antioxidative activity.

Table 2. Fat absorption of fish protein hydrolysates (FPH) compared to casein

	Hydrolysates				
	Intact protein	FPH1	FPH2	FPH3	Casein
$\frac{V(\text{oil})}{m(\text{FPH})}/(\text{mL/g})$	0.72±0.06	0.911±0.03	2.19±0.5	1.52±0.3	1.4±0.2

Table 3. Emulsifying capacity of fish protein hydrolysates at different DH

	Hydrolysates				
	Intact protein	FPH1	FPH2	FPH3	Casein
$\frac{V(\text{emulsified oil})}{m(\text{FPH})}/(\text{mL/g})$	16.7±0.4	20±0.18	15.2±0.47	10.8±0.3	17±0.8

Inhibition of linoleic acid autoxidation

In this study, we investigated the antioxidative activity of the three FPHs prepared from sardinella by-products and compared them to α-tocopherol, which has been widely used as a natural antioxidative agent. As shown in Table 4, the control (without antioxidant) had the highest absorbance value, indicating the highest degree of oxidation among samples, whereas the control with α-tocopherol had the lowest absorbance. All FPHs exhibited more than 50 % inhibition of linoleic acid peroxidation. However, this inhibition was smaller than α-tocopherol, providing about 80 % inhibition of linoleic acid peroxidation. The product with the highest DH (10.16 %) possessed more effective antioxidative activity (59 %) than FPH1 (51 %) and FPH2 (55.46 %). Je *et al.* (*47*) reported the antioxidative activity of a peptide isolated from Alaska pollock (*Theragra chalcogramma*) and showed that this peptide exhibited about 85 % inhibition of linoleic acid peroxidation.

Table 4. Antioxidant activity of by-product hydrolysates in linoleic acid autoxidation system measured by the ferric thiocyanate method

	Absorbance at 500 nm	Inhibition/%*
Control	2.250±0.010	0
FPH1	1.102±0.011	51.02±0.011
FPH2	1.002±0.010	55.46±0.010
FPH3	0.920±0.009	59.11±0.009
α-Tocopherol	0.550±0.005	80.00±0.005

Values represent means ± SE (*N*=3)
Control: without antioxidant

$$\text{*Inhibition} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

Scavenging effect on DPPH free radical

The antioxidant activity of the FPH3 (with the highest DH) was also tested by its ability to scavenge the DPPH free radical. The FPH3 shows an antioxidant activity of (41±2) %. This result is better than obtained by Wu *et al.* (*28*) with protein hydrolysates of mackerel (*Scomber austriasicus*), which have the scavenging effect of only 15.4 %.

Both results obtained by inhibition of linoleic acid autoxidation and scavenging effect on DPPH free radical suggest the presence of peptides with antioxidant activity in FPHs.

Conclusion

Hydrolysates having DH values of 6.62, 9.31 and 10.16 % were generated from sardinella by-products with Alcalase®. The functional properties and the antioxidative activity of the hydrolysates were investigated. Extensive enzymatic hydrolysis of fish protein hydrolysate resulted in a considerable increase in solubility and a reduction in the emulsifying capacity and whippability. The fish protein hydrolysed with Alcalase® resulted in a product that had excellent solubility over a wide pH range. Furthermore, all FPHs exhibited more than 50 % inhibition of linoleic acid peroxidation, suggesting the presence of peptides with antioxidative activity in FPHs. Further works should be done to isolate and characterize potent antioxidative peptides from sardinella fish protein hydrolysates.

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