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Functional properties of fish protein hydrolysates from Pacific whiting (*Merluccius productus*) muscle produced by a commercial protease

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Abstract

Pacific whiting (*Merluccius productus*) muscle was used to produce hydrolysates with 10%, 15% and 20% degree of hydrolysis (DH) using the commercial protease Alcalase® and were characterized at pH 4.0, 7.0 and 10 according their solubility, emulsifying and foaming properties. Protein recovered in soluble fractions increased proportionally with the hydrolytic process, yielded 48.6 ± 1.9, 58.6 ± 4.1 and 67.8 ± 1.4 of total protein after 10%, 15% and 20% DH, respectively. Freeze-dried hydrolysates presented almost 100% solubility ($p > 0.05$) at the different pHs evaluated. Emulsifying properties (EC, EAI and ESI) were not affected by DH as most samples showed similar ($p > 0.05$) results. Higher EC ($p \leqslant 0.05$) than sodium caseinate, used as control, were obtained at pH 4 for most hydrolysates. Hydrolysates showed very low foaming capacity not affected by pH; but foam stability was equal or even better ($p > 0.05$) than bovine serum albumin (BSA), except at pH 4.0. Results suggest that hydrolysates from Pacific whiting muscle can be produced with similar or better functional properties than the food ingredients used as standards.
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Keywords: Proteolytic activity; Pacific whiting; Foaming capacity; Emulsifying capacity; Fish protein hydrolysates; Functional characterization

1. Introduction

Pacific whiting (*Merluccius productus*), from the Gulf of California, is an abundant species with stocks reported to be of about 100,000 tonnes (Casas-Valdez, 2004). However, it is considered an under-utilized species due to poor handling infrastructure available for their catch, and most important, owing to its high tendency to become parasitized with myxosporidia (*Kudoa paniformis* and/or *K. thyrsitis*). Prevention and control of fish parasitization is difficult because methods of transmission are not yet conclusive. Although it has been reported that their presence do not present a human health risk (Alvarez-Pellitero & Sitja-Bobadilla, 1993), recent studies found certain immunological response by its consumption suggesting that might possess a human risk (Martinez de Velasco, Rodero, Chivato, & Cuellar, 2007); however, no conclusive relationship with pathological disorders have been found. Yet, parasite presence have been linked to high levels of proteolytic activity in the muscle (Erickson, Gordon, & Anglemier, 1983; Kudo, Barnett, & Nelson, 1987) promoting different muscle changes such as muscle filament degradation, loss of texture and myofibrillar protein functionality (Jiang, 2000) limiting their use for production of gel type products by the seafood industry (Martinez de Velasco, Rodero, Zapatero, & Cuellar, 2002), thus affecting its marketability (Adlerstein & Dorn, 1998).

A method that has been suggested as an alternative to overcome this problem is the enzymatic modification for the production of protein hydrolysates with functional characteristics than can be used not only as animal feed or silage but for human consumption (i.e., food additives and nutraceuticals) (Liceaga-Gesualdo & Li-Chan, 1999). Controlled enzymatic hydrolysis of proteins produces a series of small polypeptides which can modify and even

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improve the functional characteristics of proteins (Haard, 2001) for different applications.

In a preliminary study, we produced a functional fish protein hydrolysate (FPH) from Pacific whiting (*Merluccius productus*) by using endogenous proteolytic activity. Thus, a step forward was to produce FPH from this species by a different meaning. Therefore, the objective of the present study was to produce several FPH with different degrees of hydrolysis (DH) using the commercial proteinase Alcalase®, characterizing their functionality in terms of solubility, emulsifying and foaming properties.

2. Materials and methods

2.1. Fish sample

Pacific Whiting (*Merluccius productus*) was harvested off the Gulf of California by a commercial fishing vessel from the fishing camp "El Desemboque del Seri", Sonora, México. Fish sample, with no evident signs of parasites (as described in Mazorra-Manzano, Pacheco-Aguilar, Ramírez-Suárez, & García-Sánchez, 2008) were used in the study. Edible portions were obtained by means of a mechanical deboner and divided in sublots (3) in polyethylene bags (500 g) and kept frozen at −20 °C until used.

2.2. Preparation of fish protein hydrolysates (FPH)

Alcalase® 0.6 L (Novozymes, Bagsværd, Denmark) was used for FPH production. Conditions used were as follows: enzyme preparation was added at 1.0%, 1.5%, and 3.0% (v/w; enzyme/protein) to muscle homogenate containing 8% (w/w) protein, pH 8.0 and 50 °C with the purpose of getting 10%, 15% and 20% degree of hydrolysis (DH), respectively; all in a pre-established time of no more than 2 h. The pH stat technique (Adler-Nissen, 1986) was used to control the DH, by adding 1 N NaOH using a Mettler DL25 autotitrator (Mettler Instrument Co., Hightstown, NJ). DH was calculated from the following equation, based on the amount of base consumed during proteolysis

$$\mathrm{DH} = V_B N_B (1/\alpha) \times (1/\mathrm{MP}) \times (1/h_{tot}) \times 100$$

where V_B = base consumption (mL); N_B= base normality; α = dissociation degree = $10^{pH-pK}/(1+10^{pH-pK})$; MP = mass of protein substrate (g); h_{tot} = total number of peptide bonds (meq α-amino/g protein); Mean pK used for DH determinations was 7.61 as previously calculated at 50 °C and described in next section. Hydrolytic reaction was stopped by raising the homogenate temperature to 80 °C and held for 10 min. Then, mixture was cooled down to room temperature and centrifuged at 9500*g* for 30 min at 20 °C, in a refrigerated Beckman Model J2-21 centrifuge (Beckman Instruments Inc. Palo Alto, CA). The soluble fraction was freeze dried in a Labconco freeze-drier 77530 Model (Labconco Corporation. Kansas City, MO). Dry product was vacuum-packed in polyethylene bags and maintained at −20 °C until used. The hydrolytic process yield was obtained by the relationship of total soluble protein recovered after centrifugation respect to the total protein content of unhydrolysed homogenate measured by Lowry, Rosebrough, Farr, and Randall (1951).

2.3. Degree of hydrolysis determination (DH) and pK calculation

The DH was determined by the 2,4,6-trinitrobenzene sulphonic acid (TNBS) technique described by Adler-Nissen and Olsen (1979) with modifications: A mixture of 1 mL of hydrolysate and 10 mL of 10% (w/v) SDS was heated at 80 °C for 30 min and diluted with distilled water to a final concentration of 1% (w/v) SDS. From this solution, 0.25 mL were taken for free amino groups quantification using the TNBS technique with L-leucine (Sigma Chemical Co., St. Louis, MO) as standard at 0.06, 0.13, 0.25, 0.5, 1, 2 and 4 μM/mL. Absorbance readings were done at 340 nm using a Lambda 3B UV/VIS spectrophotometer (Perkin-Elmer Co. Norwalk, CT). Data were transformed to meq α-amino/g of protein (or hydrolysis equivalents, h). To calculate the DH a total hydrolysis equivalent (h_{tot}) of 8.6 meq α-amino/g of protein was considered as suggested by Adler-Nissen (1986) for a fish protein concentrate, where $\%\mathrm{DH} = h/h_{tot}(100)$.

The pH-stat calibration implies mean pK determination of protein hydrolysates; thus it was determined as given by Adler-Nissen (1986). Briefly, muscle homogenate containing 8% (w/w) protein, adjusted at pH 8.0, 8.5, and 9.0, was heated at 50 °C in a water bath. Afterward, an enzyme preparation was added at 2% (v/w; enzyme/protein). During hydrolysis, pH was maintained using a Mettler DL25 autotitrator (Mettler Instrument Co., Hightstown, NJ). Base consumption and free amino groups increments were determined at 5, 10, 15, 30, 45, 60, 90, and 120 min. meq of NaOH/g of protein consumed were related with meq of free amino groups/g of protein and, according to slopes obtained at different pH tested (data not shown), mean pK was calculated as follows:

$$\mathrm{pK} = \mathrm{pH}_2 + \log(m_1 - m_2) - \log(10^{\mathrm{pH}_2 - \mathrm{pH}_1} \times m_2 - m_1)$$

where $\mathrm{pH}_2 > \mathrm{pH}_1$; m_1 and m_2 are the slopes at pH_1 and pH_2, respectively.

2.4. Analytical methods

2.4.1. Proximate analysis

Moisture, protein, fat and ash of FPH were determined following the AOAC. (1993) methodologies (Sec 950.46, 938.08, 960.39 and 955.04, respectively).

2.4.2. SDS–PAGE analysis

Sample preparation was carried out as follows: 200 L of protein soln (10 mg/mL) were treated with 900 L of cold acetone to induce protein precipitation. After 2 h at

−80 °C, sample was centrifuged at 4900*g* for 5 min in an IECCentra-4B centrifuge (International Equipment Co., Meedham Hights, MA). Supernatant was discarded and precipitated polypeptides were re-dissolved in 100 L of deionized water (20 g/L protein concentration), mixed with 2× sample buffer (4% (w/v) SDS, 20% (v/v) glycerol, 10% (v/v) β-mercaptoethanol, 0.125 M Tris, pH 6.8) and heat treated at 100 °C for 5 min). SDS–PAGE analysis was performed accordingly to Laemmli (1970). A 20% (w/v) acrylamide separating gel (width × height × thickness = 100 × 50 × 1.5 mm), with a 10% (w/v) acrylamide stacking gel, were used. Aliquots of 40 μg of protein per lane were loaded onto the acrylamide gel. Broad range molecular weight protein markers (6.5–200 kDa) (Bio-Rad Laboratories Inc. Hercules, CA) were used as standards. Gels were analyzed by a Turbo Pascal computer program (Borland International, 1982, 5.5 version) which consisted in scanning a black and white image of the gel with the imagestar program Microteck scanner (Hewlett Packard, San Jose, CA).

2.5. Functional properties of FPH

To observe FPH functionality at various pHs, FPH powders were dissolved in water and pH adjusted to 4.0, 7.0 and 10.0 using either 0.1 N of NaOH or HCl accordingly.

2.5.1. Peptide solubility (PS)

PS was carried out according to Chobert, Bertrand-Hard, and Nicholas (1988) with slight modifications. The hydrolysate (0.5 g) was dissolved in distilled water and adjusted to 20 mL after pH adjustment. Protein content was determined by microKjeldahl (AOAC, 1993) before (whole solution) and after (supernatant) centrifugation at 4000*g* for 30 min at 20 °C using a Beckman J2-21 centrifuge. Results were reported as % protein solubility.

2.5.2. Emulsifying Properties

Emulsifying capacity (EC) was determined according to Webb, Ivey, Craig, Jone, and Monroe (1970) with slight modifications. Briefly, 50 mL of 0.3% (w/v) protein solution were homogenized in a mixer at high speed, while adding edible corn oil with a 500 mL separator funnel at a constant flux of 1.1 mL/s. Oil addition was stopped when a change in the emulsion type was noted. Oil consumption (at inversion phase) was quantified by weight difference and the EC reported as gram of oil emulsified/50 mL of 0.3% protein solution. Emulsifying activity index (EAI) was determined according to Pearce and Kinsella (1978) with slight modifications. Briefly, in a 50 mL polyallomer tube (Nalge Co., Rochester, NY), 9 mL of 0.1% (w/v) protein solution were homogenized with 3 mL of edible corn oil using a Tissumizer Tekmar homogenizer (Tekmar Co. Cincinnati, OH) for 30 s at velocity 50, regulated with a regulator TR-10 (Tekmar Co. Cincinnati, OH). After one minute of emulsion formed, 50 μL were taken from the bottom of the tube and diluted to 10 mL with 0.1% (w/v) SDS (1:200 dilution). The dilution was mixed by gentle inversion of the tube and the absorbance was read at 500 nm using a spectrophotometer (Lambda 3B UV/VIS; Perkin-Elmer Co. Norwalk, CT). The EAI was calculated using the following formula:

$$\text{EAI } (\text{m}^2/\text{g}) = (2.303 \times 2 \times \text{dil} \times A)/(c \times \phi \times 10{,}000),$$

where dil = dilution factor, 200; A = absorbance at 500 nm;c = protein concentration (g/mL), 0.001; φ = disperse phase volume fraction = 0.25.

Emulsion stability index (ESI) was determined as described by Pearce and Kinsella (1978) with slight modifications. Briefly, exactly 3 min after emulsion was prepared for EAI, 5 mL were taken from the bottom of the tube and transferred to a glass tube. After 24 h at room temperature, a 1:200 dilution was prepared with 0.1% (w/v) SDS and absorbance was read at 500 nm. The difference of EAI at 0 and 24 h was reported as % ESI using the next formula:

$$\%\text{ESI} = 100 - [\text{EAI}_{(t=0)} - \text{EAI}_{(t=24\text{h})}/\text{EAI}_{(t=0)}] \times 100$$

Sodium caseinate (SCA) (Sigma Chemical Co. St. Louis, MO) was used as a standard for comparison against emulsifying properties of FPH.

2.5.3. Foaming properties

Foaming capacity (FC) was determined by the aeration method proposed by Waniska and Kinsella (1979), with slight modifications. A graduated empty glass chromatography column (30 cm long × 1 cm i.d.) with a fixed filter in the bottom (BioRad Laboratories, Richmond, CA) was used. Five milliliters of 0.3% (w/v) protein solution were put in the column and connected to an autotitrator Mettler DL25 (Mettler Instrument Co. Hightstown, NJ) in order to flush 10 mL of air at a 40 mL/min constant flux. FC was calculated by dividing the volume of formed foam by the volume of solution used to form the foam. FC was reported as mL of formed foam/mL of 0.3% protein solution.

Foam Stability (FS) was evaluated by the "drained liquid" method used by Waniska and Kinsella (1979) with slight modifications. The total volume of liquid that was forming part of foam after a minute of being produced was considered as 100%; the volume of liquid drained after 5 min resting was monitored. Data was taken as % retained liquid over time. Foaming properties of FPH were compared with standard bovine serum albumin (BSA) (Sigma Chemical Co. St. Louis, MO).

Statistical analysis. A completely randomized factorial (3 × 3) designed was used. Main effects were degree of hydrolysis (DH) and pH. Data was analyzed with two way ANOVA. When statistical differences among means were found, Duncan's multiple range tests was used for multiple comparison of means. For all cases, significance level was set at $p \leqslant 0.05$. Three replicates were carried out for each treatment. A linear regression was carried

out to describe a possible relationship between DH and protein recovery.

3. Results and discussion

3.1. Degree of hydrolysis (DH) and pK

Protein hydrolysate production with controlled DH requires pH stat calibration which in turn involves mean pK determination in order to have a better hydrolytic process control. Thus, mean pK obtained under experimental conditions at 50 °C was 7.61 ± 0.01, according to resulting slopes (see Section 2.3). This value was used to estimate base consumption at constant pH of 8.0 required to obtain 10%, 15% and 20% DH. The pK for *Merluccius productus* muscle was higher that the one reported for soy protein (pK = 7.61 for Pacific whiting muscle protein *vs.* 7.1 for soy protein at 50 °C), mainly due to amino acid composition of proteins (Adler-Nissen, 1986). In addition, pK increased parallel to DH; for soy protein, pK corresponded to <12% DH, in contrast to the 20% DH obtained in the present study. This discrepancy makes necessary a pK calculation for each protein source to be hydrolyzed, thus getting better DH results if pH stat methodology is going to be used.

Additionally, DH is an important factor highly related with the hydrolytic process yield (Adler-Nissen, 1986). Results showed that as DH increased so did the soluble protein ($p \leqslant 0.05$) (Table 1). Higher DH produces lower molecular weight polypeptides thus increasing their solubility as shown by the 20% DH, which recovered almost 70% of protein. Linear regression analysis showed a highly significant dependency between variables (DH and Protein Recovery) (PR = 1.92 DH + 29.53, $R^2 = 0.999$; $p \leqslant 0.05$).

3.2. Analytical methods

3.2.1. Proximate analysis

Proximate composition of soluble fractions of freeze-dried FPHs is displayed in Table 2. Moisture, protein and ash content of Pacific whiting ground muscle (Control) did not show a remarkable variation between samplings, giving percent values of 82.6 ± 1.0, 16.1 ± 0.9 and 1.1 ± 0.05, respectively. FPH powders (from all hydrolysates) had a whitish to light yellow colour appearance with minimal fishy odour and taste. Respect to protein contents, similar results were reported by other authors for different fish and degree of hydrolysates (Gbogouri, Linder, Fanni, & Parmentier, 2004; Kristinsson & Rasco, 2000; Sathivel et al., 2003). The high ash content of samples should be attributed to the addition of alkali required for pH adjustment (pH 8.0) and its control during the hydrolytic process. FPH presented a low fat content which is in accordance with the Pacific whiting (*Merluccius productus*) lean muscle (Pacheco-Aguilar, Crawford, & Lampila, 1989).

Table 1
Degree of hydrolysis (DH) effect over protein recovery on the hydrolysates soluble fraction produced from Pacific whiting (*Merluccius productus*)

DH (%)	Protein recovery (%)
10	48.6 ± 1.9[a]
15	58.6 ± 4.1[b]
20	67.8 ± 1.4[c]

Values are mean ± SD of three replicates. Values with different letter are statistically different ($p \leqslant 0.05$).

Table 2
Proximate composition (%) of lyophilized fish protein hydrolysates produced from Pacific whiting (*Merluccius productus*)

	10% DH	15% DH	20% DH
Water content	3.6 ± 1.9[a]	3.2 ± 0.1[a]	2.8 ± 0.8[a]
Protein	88.6 ± 0.3[a]	88.4 ± 0.3[a]	85.6 ± 0.3[b]
Lipids	0.1 ± 0.1[a]	0.2 ± 0.2[a]	0.3 ± 0.1[a]
Ash	11.9 ± 0.1[a]	11.7 ± 0.4[a]	11.9 ± 0.4[a]

Values in the same line with same letter are statistically equal ($p > 0.05$). All % dry basis.

3.2.2. SDS–PAGE analysis

In order to observe the effect of hydrolysis over the *Merluccius productus* muscle proteins, samples were subjected to electrophoresis. It is important to mention that samples in the study represent just the soluble fraction obtained after the hydrolytic process and that the amount of these fractions increased proportionally to DH, as was discussed before (Table 1). It is expected that this polypeptides came from the hydrolysis of major protein present in the muscle (i.e., myosin heavy chain: 200 kDa, actin: 45 kDa, etc.) before the hydrolytic process. Hydrolysates showed a unique smearing band at the bottom of the gel below 20 kDa (Electrophoresis gel not shown). Smearing bands started at 19, 12, and 10 kDa for 10%, 15% and 20% DH, respectively, which indicate a progressive hydrolysis of proteins in the soluble fraction also occurred. A relationship of molecular weight and functional properties of polypeptides present in hydrolysates is discussed in the next section.

3.3. Functional properties

Most protein ingredients have a particular functional characteristic, therefore imparting special attributes to food products. However, functionalities such as emulsifying, foaming, thickening and gelling are often affected by their solubility (Damodaran, 1996). Sometimes, intact proteins lacking of these properties require modification such as enzymatic hydrolysis (Haard, 2001). Soluble peptides, thus obtained, can contribute in emulsion and foaming enhancing characteristics (Raymundo, Empis, & Sousa, 2000). Hence, solubility, emulsifying and foaming functionalities of hydrolysates produced in this study were evaluated and compared against common food proteins such as sodium caseinate (SCA) and bovine serum albumin

(BSA), both with excellent emulsifying and foaming properties, respectively (Hake & Kinsella, 1989; Townsend & Nakai, 1983; Waniska & Kinsella 1979).

3.3.1. Peptide solubility (PS)

As mentioned before, PS plays one of the most important physicochemical and functional properties of hydrolysates. Solubility of all hydrolysates produced were over 97% at three pHs evaluated, finding not significant difference ($p > 0.05$) among samples. Similar results were reported by Kristinsson and Rasco (2000) using commercial enzymes for 10 and 15% DH of Atlantic salmon (*Salmo salar*) muscle. This high solubility of hydrolysates was due to generation of low molecular weight peptides as discussed above. Solubility of hydrolyzed protein in a broad pH range is one of the more desirable physicochemical and functional properties from which derived the rest of the functionalities (emulsifying and foaming) in a food system (Raymundo et al., 2000).

3.3.2. Emulsifying properties

Fig. 1 shows the emulsifying capacity (EC) of FPH produced *vs.* sodium caseinate (SCA). When comparing samples against control (SCA), results indicated that EC was affected significantly ($p \leqslant 0.05$) by DH and pH, except at pH 4, where DH showed higher values ($p \leqslant 0.05$) than SCA. However, when looking only at samples, most of them were statistically equal ($p > 0.05$) at their respective pH. Furthermore, results indicated that pH 10 had less effect over EC as all samples showed the highest values ($p \leqslant 0.05$) at this pH.

Hydrolysis of proteins may have a positive effect up to a certain point as extensive hydrolysis may result in a drastic loss of emulsifying properties. Although small peptides diffuse to, and absorb fast at the interface, they are less efficient in reducing the interfacial tension due to lack of unfolding and reorientation at the interface as large peptides do (Gbogouri et al., 2004). At high pH (i.e., 10) structural unfolding of polypeptides, resulting from the negative charges generated, can cause repulsion and probably allow a better orientation of them at the interface. This could result in a more efficient exposition of hydrophilic and hydrophobic residues in these peptides, promoting a major interaction at the oil-in-water (O:W) interface (Flinger & Mangino, 1991).

Fig. 2 shows the emulsifying activity index (EAI) of hydrolysates produced compared to the control. Higher indices represent finer and higher number of dispersed fat globules, implying also a polypeptide ability of being absorbed at the O:W interface thus covering a mayor interfacial area. Results indicate that pH is a major factor to be considered in the evaluation of this characteristic, as it is evident the highest ($p \leqslant 0.05$) EAI of hydrolysates at pH 10, independent of DH. No difference was found ($p > 0.05$) in EAI of 15% and 20% DH at pH 4 and 7, respectively. However, EAI of 10% DH was higher ($p \leqslant 0.05$) than control protein (SCA) at pH 4. Results can be explained by the pH effect in the polypeptide molecular repulsion as discussed in EC analysis.

With respect to ESI (Fig. 3), a similar behavior was found for each DH, yet similar to SCA (pH 7 and 10). This property showed a tendency to increase at higher pHs. This effect was more pronounced at 15% and 20% DH. Several studies (Gbogouri et al., 2004; Klompong, Benjakul, Kantachote, & Shahidi, 2006; Kristinsson & Rasco, 2000) have reported that emulsion stability is affected as size of peptides decrease. However, our results do not agree with reported ones. This discrepancy can result from differences in amino acid composition and its distribution in the polypeptides produced. In this respect, Waniska and Kinsella (1979) reported that protein (as well as peptides) interfacial development is influenced by the amino acid composition and structural conformation of the polypeptide. On the other hand, Klompong et al. (2006) showed the same tendency as found in the present work.

Thus, it can be inferred that pH 10.0 generated higher protein/peptide unfolding and hydrophobic regions

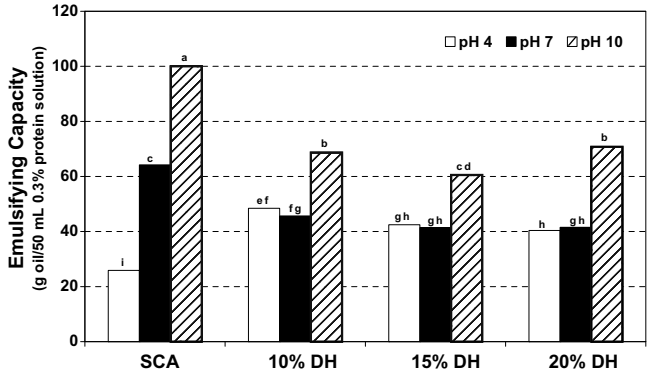

Fig. 1. Emulsifying capacity (EC) of hydrolysates produced from Pacific whiting (*Merluccius productus*) and its comparison with sodium caseinate (SCA) as a function of pH. DH = degree of hydrolysis. Results are the means from three replicates. Different letters show significant differences ($p \leqslant 0.05$).

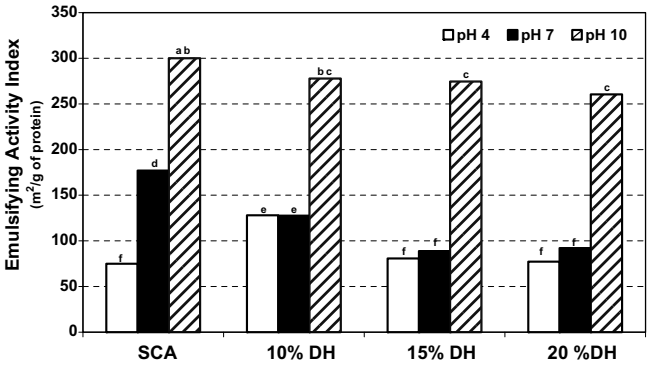

Fig. 2. Emulsifying activity index (EAI) of hydrolysates produced from Pacific whiting (*Merluccius productus*) and its comparison with sodium caseinate (SCA) as a function of pH. DH = degree of hydrolysis. Results are the means from three replicates. Different letters show significant differences ($p \leqslant 0.05$).

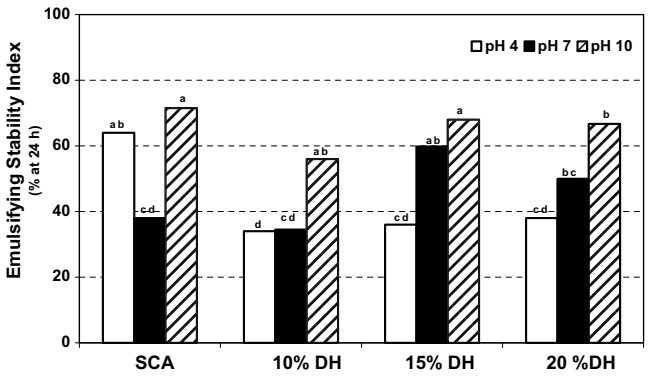

Fig. 3. Emulsifying stability index (ESI) of hydrolysates produced from Pacific whiting (*Merluccius productus*) and its comparison with sodium caseinate (SCA) as a function of pH. DH = degree of hydrolysis. Results are means from three replicates. Different letters show significant differences ($p \leqslant 0.05$).

exposition, therefore facilitating a better orientation at the O:W interface as shown by emulsifying properties. Results also suggest that at pH 10, molecular repulsion generated among protein/polypeptides due to negative charge density produced resulted in a more stable and cohesive interface, thus retarding emulsion coalescence. Additionally at pH 4.0 the FPH behaved with better EC and EAI than the SCA, due to possible decrease in solubility presented by SCA at this pH (Fox, 2001).

3.3.3. Foaming properties

The foaming ability of hydrolysates produced from Pacific whiting was compared against BSA. Neither hydrolysis nor pH improved such functionality. All results were lower ($p \leqslant 0.05$) than BSA thus displaying less foam volume produced per mL of protein solution. BSA foaming ability was higher than hydrolysates with values of 5.5, 5.2 and 9.6 *vs.* 2.8, 2.9 and 3 mL of foam/mL protein solution at pH 4, 7 and 10, respectively. A protein needs several attributes to present good foamibility such as: (a) it needs to be absorbed into the interface and reorganize its structure (surface denaturation) rapidly (Wilde & Clark, 1996) and (b) to have certain molecular characteristics such as good balance of surface and molecular hydrophobicity, net charge and charge distribution, among other factors (Damodaran, 1997). It seems peptides in hydrolysates produced in the present study were not absorbed and denatured efficiently to reduce the interfacial tension enough and form the viscoelastic film required for a good foaming agent (Dickinson & McClements, 1996) contrary to what the malleable, flexible globular protein BSA showed. Certainly, Adler-Nissen (1986) reported that enzymatic hydrolysis of proteins results in an improvement of foaming characteristics, but this premise might have its limitations, i.e., size, composition and net charge of peptides.

The pH had a major effect on foam stability (FS) of hydrolysates (Fig. 4), which were less stable ($p \leqslant 0.05$) than BSA at pH 4 (Fig. 4a), thus reaching their foam half life in

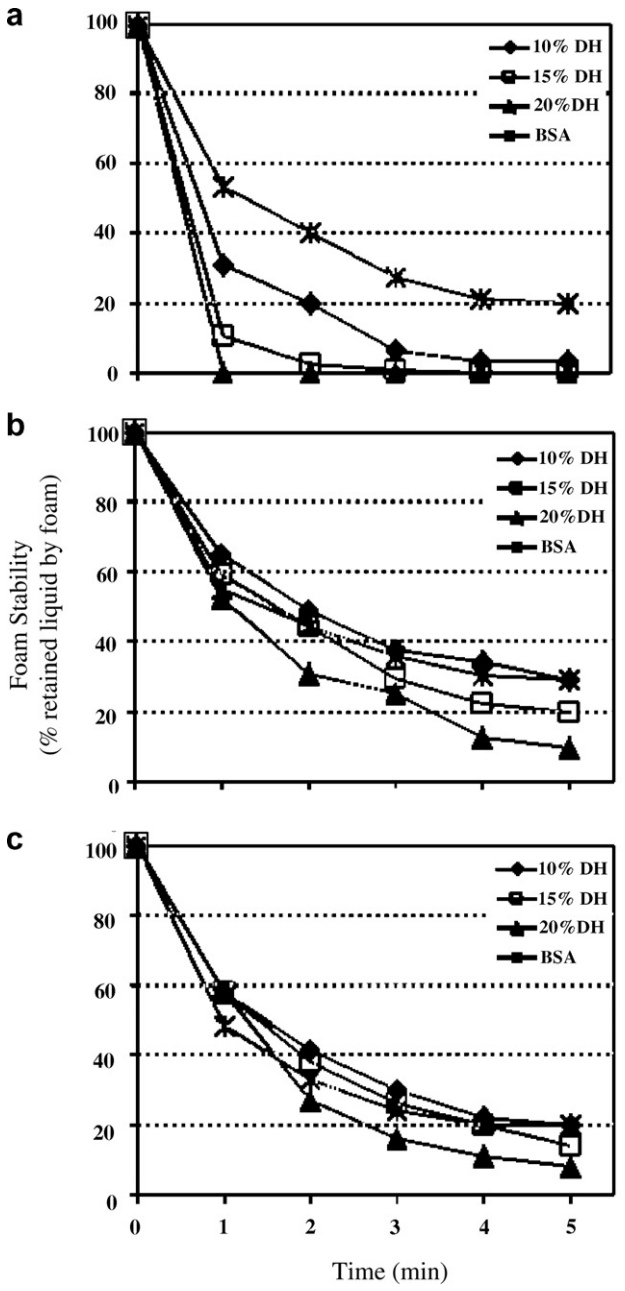

Fig. 4. Foam stability (FS) of hydrolysates produced from Pacific whiting (*Merluccius productus*) and its comparison with bovine serum albumin (BSA) as a function of pH and time: (a) pH 4, (b) pH 7 and (c) pH 10, all left still at 25 °C. Results are means of three replicates.

less than a minute. However, at pH 7 and 10 (Fig. 4b and c, respectively), 10% and 15% DH resulted in the same or even better FS ($p > 0.05$) than BSA. The effect of peptide size (thus DH) and pH over FS can be observed clearly as 10% DH showed higher FS than 15%, and this last one higher than 20% DH. Results are conclusive with respect to the good characteristics shown by hydrolysates as foam stabilizers, having similar or inclusive higher stabilities than BSA. A similar effect of pH over FS was reported by Klompong et al. (2006) where the lowest FS values were shown at pH 4 with similar tendencies at pH

7 and 10. Solubility, as well as size and interaction of proteins/peptides at the formed film, play an important role in foam stability. In a preliminary study, where a hydrolysate (5% DH) were produced by using the endogenous muscle proteolytic activity, the FS was higher at all pH than BSA, corroborating the importance of size (and probably charge) of polypeptides produced over the stability of a foam. Thus, lower molecular weight peptides produced in this study, promoted less stability. However, according to Damodaran (1996), FC and FS are influenced by two different sets of molecular properties of protein/peptides that are often antagonistic. While the first is affected by rate of absorption, flexibility, and hydrophobicity, the last depends on the viscoelastic nature of the film.

4. Conclusions

Results in the present study showed that hydrolysates produced from Pacific whiting (*Merluccius productus*) muscle can be used as food ingredients or additives to impart a desire characteristic to food products or increase food storage stability, acting as emulsifying, foaming or dispersing agents, in sausages, mayonnaise, salad dressings, beverages, creams, etc., all these in a broad pH range. Under a specific degree of hydrolysis and pH, hydrolysates produced could substitute functional compounds satisfactorily, such as sodium caseinate, and bovine serum albumin, commonly utilized in food formulations. Results also showed that the effect of degree of hydrolysis as well as pH affected some properties, being the pH the most relevant in terms of functionality. Overall results demonstrate that hydrolysates produced in the present study have good functional properties indicating their possible use in different food systems however, further research including real food systems are recommended. The use of commercial enzymes for production of highly functional hydrolysates from marine species of low commercial value can be a feasible technology to make the most of a vast underutilized resource (such as the Pacific whiting) and use it as a food ingredient for direct human consumption.

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