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Comparison between gelatines extracted from mackerel and blue whiting bones after different pre-treatments

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Abstract

Gelatines were extracted from mackerel and blue whiting bones after chemical or enzymatic pre-treatments and their functional properties (solubility, foaming and emulsifying properties) were analysed. The pre-treatment significantly ($p<0.05$) affected the composition and the functional properties of the extracted gelatines. The amino acid analyses showed that chemically pre-treated bone gelatines had higher imino acids (proline and hydroxyproline) contents compared to those extracted after the enzymatic pre-treatment, regardless of the fish species. It was observed that all gelatines had higher solubility at low pH with a maximum value observed at pH 2. A significant effect of ionic strength was observed. Increasing the NaCl concentration to more than 1% resulted in a significant decrease of the solubility. Mackerel bone gelatines showed lower foaming capacity (FC) and higher foaming stability (FS) than blue whiting bone gelatines. Increasing the concentration of gelatine decreased the emulsifying activity (EAI) but increased the stability (ESI) indices. The use of enzymes in the pre-treatment process generated gelatines with significantly ($p<0.05$) higher EAI and ESI.

Key words: mackerel, blue whiting, bones, gelatines.

1. Introduction

Every year, significant amounts of waste are generated by the fish processing industries. These wastes are regarded as low quality products and are discarded or in the best case scenario processed into fishmeal and pet food (Kim & Mendis, 2006). Fish waste is costly to dispose of and is typically discarded overboard in case of onboard processing or buried to landfill for the on-shore processing.

Environmental legislation has contributed to the introduction of sustainable waste management practices in the European Union. The European Directive 1999/31/EC on the landfill of waste (Council Directive, 1999) and the Regulation (EC) No 1774/2002 restrict the disposal of untreated organic waste not intended for human consumption. Therefore more sustainable alternatives are needed. Recycling fish waste is of interest from an environmental point of view by reducing the organic contaminant charge. Recent advances in fish waste management have resulted in their examination as a source of ingredients with a potential application to the food industry. Underutilised fish species along with fish processing discards may be potential sources of bioactive and functional ingredients such as gelatine (Shahidi, 1994).

Gelatine is a biopolymer produced by extraction and hydrolysis of fibrous, insoluble collagen. Sources for fish collagen can be fish skin, bones, scales or connective tissue (Kim & Mendis, 2006). The industrial process of gelatine manufacture involves either an acid or alkaline pre-treatment followed by extraction with warm water. The heat denaturation converts collagen into gelatine. Further clarification steps include filtration, concentration, drying and milling (Schrieber & Gareis, 2007). The quality of gelatine preparation depends on its physicochemical

properties, which are influenced not only by the species or tissue from which it is extracted, but also by the severity of pre-treatment and extraction process.

Atlantic mackerel (*Scomber scombrus*) is a pelagics species and is abundant in cold and temperate shelf areas such as the North Atlantic Ocean (Collette & Nauen, 1983). The world catch of mackerel was estimated around 566 thousand tonnes in 2007 (FAO, 2009). Blue whiting (*Micromesistius poutassou*) is a typical lean species and belongs to the *Gadidae* family along with cod and haddock. Blue whiting is an underutilised fish with a global catch estimated to be about 1.7 thousand tonnes in 2007 (FAO, 2009).

In this study, mackerel and blue whiting, models for oily and white fish, respectively, were investigated for gelatines extraction. The effect of the pre-treatment of fish bones on the composition and functional properties of gelatines was evaluated.

2. Materials and methods

2.1 Materials

Atlantic mackerel (*Scomber scombrus*) caught in early March 2007, were kindly provided by Bord Iascaigh Mhara (BIM, Ireland). Blue whiting (*Micromesistius poutassou*) caught in January 2008, were provided by Donegal Seafood. Both fish were caught in the area FAO 27 (Atlantic, Northeast). The average weights for mackerel and blue whiting were 277 g and 116.8 g, respectively. Fillets were manually removed after beheading and evisceration of fish, the remaining meat was separated from the frame using a knife. Bones and scales were cut manually into small pieces (1 to 2 cm length) using scissors. The bones were divided into batches and kept in the freezer at - 20 °C for less than one month before use. All chemicals used were analytical grade.

2.2 Enzymes

Flavourzyme is a fungal protease/peptidase complex obtained from *Aspergillus oryzae*. Flavourzyme had a declared activity of 500 leucine aminopeptidase units (LAPU)/g), where one LAPU is defined as the amount of enzyme which hydrolyzes 1 μmol of L-Ieucine-p-nitroanilide per minute. Alcalase is an alkaline enzyme produced by *Bacillus licheniformis*. Alcalase had an activity of 2.4 Anson units (AU)/g, where one Anson unit is defined by Aspmo *et al.* (2005) as the amount of enzyme that releases 1.0 mEq of tyrosine from urea-denatured hemoglobin per minute. Flavourzyme was produced by DSM Nutritional Products, Inc. (Kaiseraugst, Switzerland) and Alcalase was produced by Novozyme Co. (Copenhagen, Denmark). Both enzymes were supplied by Sigma-Aldrich, Inc. (Dublin, Ireland).

2.3 Proximate analysis of fish bones

The proximate analysis was carried out according to the procedures of the Association of Official Analytical Chemists (AOAC, 2000). Protein content was determined by the Kjeldahl method using an automatic Kjeldahl system (Gerhardt, Bonn, Germany). The protein content was calculated by using a conversion factor of 6.25. Moisture was determined by drying the sample using an oven (Qualivac, Greenfield Oldham, UK) until reaching a constant weight (at 100 °C for 18 hours) and ash by incineration in a muffle furnace (Carbolite, Bamford Sheffield, England) at 550 °C for 4 hours. Lipid content was determined according to the Bligh and Dyer method (Bligh and Dyer, 1959). All analyses were performed in triplicate.

2.4 Extraction of gelatine

Different pre-treatment methods were used to extract gelatines from mackerel and blue whiting bones.

2.4.1 Pre-treatment

2.4.1.1 Chemical pre-treatment:

Bones (250 g) used for gelatine extraction were treated with 0.1 N NaOH at a ratio of 1/3 (w/v) for 30 min and this step was repeated 3 additional times to eliminate non-collagenous proteins and fat.

2.4.1.2 Enzymatic pre-treatment:

Fish bones (250 g) were mixed with 0.1 M potassium phosphate buffer (pH 8) at a ratio of 1/3 (w/v) then heat treated in a microwave oven model R-244 (Sharp Electronics Ltd, Uxbridge, UK) for 5 min to inactivate the endogenous enzymes. After air cooling, Flavourzyme or Alcalase were added at an enzyme/substrate ratio of 0.1% (v/w). The bones were hydrolysed for 4 h at 50 °C with continuous shaking at 150 rpm using a Gallenkamp orbital incubator (AGB, Dublin, Ireland). After hydrolysis the samples were heat treated in the microwave oven for 5 min to inactivate the enzymes. The mixture was allowed to cool down and filtered through a 1 mm pore size sieve to separate the bones from the protein hydrolysates. The clean bones were collected and demineralised.

2.4.2 Demineralisation and gelatine extraction

Fish bones were demineralised at room temperature for 18 hours using 0.25 N HCl (1/3, w/v). The demineralised bones were washed under running water from a tap to remove the acid.

2.4.3 Gelatine extraction

The pre-treated bones were mixed with distilled water (at a ratio of 1/3, w/v) and gelatine was extracted in a water bath at 45 °C for 18 hours. All extraction steps were done with continuous stirring at 150 rpm. Extracted gelatine was filtered using a Whatman No.4 filter paper (Whatman, Maidenstone, England). Gelatine was then evaporated under vacuum at 45 °C using a Büchi Rotavapor model R-210 fitted with a Büchi temperature-controlled water bath model B-491 and Büchi vacuum system model V-700 (Büchi UK Ltd., Oldham, UK), freeze dried (Labconco corporation, Kansas City, MO, USA) and ground. Gelatine extraction was done in triplicate for each fish species and repeated for 3 batches. Gelatine extraction yield was calculated as g of dry gelatine per 100 g of bones.

2.5 Protein pattern of fish gelatines

The electrophoresis procedure was carried out according to the method described by Khiari *et al.* (2011). Gelatine solutions (5 mg/mL) were prepared in distilled water at 60 ºC and then diluted to a final concentration of 2 mg/mL with sample buffer containing β-mercaptoethanol (Sigma, Dublin, Ireland). Gelatine samples were heated to 85 ºC for 10 min to denature the proteins. Samples and molecular weight marker (10 µL each) were loaded onto SDS-PAGE having a 4% stacking gel and 10% resolving gel according to Laemmli (1970), the analysis was run in an Atto Dual Mini-slab Size Electrophoresis System AE-6450 (Atto Corporation, Tokyo, Japan) at a constant current of 25 mA/gel. Protein bands were stained with Coomassie Brilliant Blue R250. The gel was de-stained using a mixture of isopropanol, acetic acid and distilled water (12:10:78, v/v/v).

The molecular weight marker (Sigma, Dublin, Ireland) contained a lyophilised mixture of six proteins: bovine carbonic anhydrase (29 kDa), egg albumin (45 kDa), bovine albumin (66 kDa), phosphorylase B from rabbit (97.4 kDa), β-galactosidase from *E. coli* (116 kDa) and myosin from rabbit muscle (200 kDa).

2.6 Amino acids analysis

The amino acid profile of gelatines was determined according to the method described by Khiari *et al.* (2011). Briefly, 10 µg of gelatine were subjected to hydrolysis for 24 h at 110 °C, with 6 M HCl containing 0.1 % phenol in vacuum-sealed hydrolysis vials. Norleucine (Sigma, Madrid, Spain) was added as an internal standard. The amino acid composition was analysed using a cation exchange Biochrom 20 amino acid analyzer (Pharmacia Biotech, Ltd., Cambridge, England) with postcolumn derivatisation with ninhydrin. All amino acids were detected at an absorbance of 570 nm, except for proline and hydroxyproline which were measured at 440 nm. Tryptophan and cysteine are completely lost by acid hydrolysis whereas methionine can be destroyed to varying degrees (Lourenço *et al.*, 2002). Cysteine was determined as cysteic acid by oxidation of the protein with formic acid prior to hydrolysis, according to Hirs (1967). Results were averaged and presented as grams (g) per 100 g amino acids. The amino acid analysis was done in the Service of Protein Chemistry at the Centro de Investigaciones Biologicas (CSIC, Madrid).

2.7 Protein solubility

The effect of pH and ionic strength on the solubility of gelatines was determined according to the method of Montero *et al.* (1991), with some modifications.

2.7.1 Effect of pH on gelatine solubility

Gelatine samples were first dissolved in distilled water to a final concentration of 0.3% (w/v, protein content). Eight mL of the gelatine solutions were added to a glass test tube and the pH was adjusted ranging from 2.0 to 12.0 with either 1 M HCl or 1 M NaOH using an Orion pH meter Model 420A (Orion Research Inc, Beverly, MA. USA). The pH meter was calibrated at pH 4.0, 7.0 and 10.0 before the analysis. The final volume was then adjusted to 10 mL by distilled water having the same pH as the gelatine solution. Samples were centrifuged at 9,000×g for 15 min at 5 ºC. Protein content of the clear supernatant was determined according to the Biuret assay as described by Gornall *et al.* (1949) and using bovine serum albumin (BSA, Sigma-Aldrich, Inc., Dublin, Ireland) as a reference protein on a weight basis. Relative solubility was calculated in comparison with that obtained at the pH giving the highest solubility.

2.7.2 Effect of NaCl on gelatine solubility

Gelatines were dissolved in 50 mM potassium phosphate buffer at pH 7.5 to a final concentration of 0.6% (w/v, protein content). Five mL of gelatine solution were mixed with 5 mL of NaCl in 50 mM phosphate buffer at pH 7.5 at various concentrations (0, 2, 4, 6, 8, 10 and 12% (w/v)). The mixture was stirred continuously using a magnetic-stirrer device for 30 min at 5 °C, followed by centrifugation at 9,000×g for 15 min at 5 ºC. Protein content of the clear supernatant was determined as described above. Relative solubility was calculated in comparison with that obtained at the NaCl concentration giving the highest solubility.

2.8 Foaming properties

Foaming properties including foaming capacity (FC) and foam stability (FS) were determined by the method of Fernandez & Macarulla (1997) with minor modifications. Gelatine solutions were prepared in 50 mM phosphate buffer at pH 7.5 to a final concentration of 0.3% (w/v, in protein content). Five mL of each sample were homogenized with an Ultra-Turrax homogenizer, model T 25 (IKA Works, Inc. Staufen, Germany) at 23,000 rpm for 1 min. FC was calculated as the percent increase in volume of the protein dispersion upon mixing, while FS was estimated as the percentage of foam remaining after 15 min.

2.9 Emulsifying properties

The emulsifying properties of gelatine samples were determined by the method of Pearce & Kinsella (1978) with some modifications. Different concentrations of gelatine solution were used. Gelatines were first dissolved in 50 mM potassium phosphate buffer containing 0.3 M NaCl at pH 7.5 to the final concentration of 0.05, 0.1 or 0.2% (w/v, in protein content). Then 2.0 mL of commercial sunflower oil was mixed with 8.0 mL of each gelatine solution. The mixture was vortexed in a plastic tube at 25°C and homogenized at 23,000 rpm for 1 minute. An aliquot (50 µL) of emulsion was diluted in 5 mL sodium dodecyl sulfate (SDS) solution (0.1%, w/v) and the absorbance was measured at 500 nm (Milton Roy Spectonic 1201, Rochester, NY, USA). To estimate the emulsion stability, the emulsions were left for 15 min at 25 °C and then 50 µL of the emulsion were diluted in 5 mL SDS solution (0.1%, w/v) and the absorbance was measured at 500 nm. The emulsifying activity and emulsion stability were expressed as indexes.

EAI (m^2/g) was defined as:

$$EAI(m^2/g) = \frac{2 \times 2.303}{C \times \phi \times 10^4} \times A_{500} \times Dilution$$

Where; A_{500} represents the absorbance at 500 nm, C the protein concentration (g/mL) before emulsification and Φ the oil volume fraction (v/v) of the emulsion (i.e. the volume of emulsion droplets divided by the total volume of the emulsion, Φ=0.2).

ESI (%) was calculated as the ratio of the turbidity measured at 500 nm of the emulsion at time zero (A_0) and after 15 min (A_{15}) (Agyare *et al.*, 2009).

$$ESI(\%) = 100 \times \frac{A_{15}}{A_0}$$

2.10 Statistical analyses

ANOVA (Multifactor and one-way) was used to find differences between treatments. Means were compared by significant difference (LSD) test, at a significance level of $p<0.05$ using the Statgraphics Centurion XV software (version 15.1.02; StatPoint, Inc., Warrenton, VA, USA). Three independent trials were carried out.

3. Results and discussion

3.1 Characterisation of fish bones and gelatine extraction yield

Mackerel and blue whiting bones had similar protein (19.8 and 19.5%, respectively) and moisture (64.9 and 64.2%, respectively) contents. The ash content was considerably high for both fish bones (8.9 and 16.0% for mackerel and blue whiting, respectively) mainly due to the high content of minerals. The fat content of mackerel bones was significantly ($p<0.05$) higher than blue whiting bones (5.5 and

279 0.8%, respectively), which could be due to the variation among the species (fatty and
280 lean fish).

281 The gelatine extraction yields varied depending on the pre-treatment used for
282 mackerel and blue whiting bones (Table 1). Regardless of fish, gelatine extracted
283 from chemically pre-treated bone, showed the lowest yield. Yields of 2.5 and 1.0%
284 were observed for mackerel and blue whiting respectively. Bones pre-treated
285 enzymatically generated significantly ($p<0.05$) highest yields (~3.9 and 1.8%, for
286 mackerel and blue whiting, respectively).

287

288 **3.2 Protein pattern of fish gelatines**

289 The electrophoretic (SDS-PAGE) profiles of the various gelatine preparations
290 are shown in Figure 1.

291 The gel electrophoresis of chemically pre-treated fish bone gelatines (Figure 1
292 A & B, lane 4) showed the presence of the three bands (one β chain and two α chains).
293 These three chains are characteristics of type I gelatine. The β chain seems to be lower
294 or entirely absent in bone gelatines extracted after the enzymatic pre-treatment (Figure
295 1 A & B, lane 2 & 3), which could be due to the combined effect of enzymatic and
296 chemical hydrolysis of the gelatines as a consequence of the demineralisation step.

297 The presence of low molecular weight proteins was observed in gelatines
298 extracted after the enzymatic pre-treatment. This may indicate a partial hydrolysis of
299 gelatine during extraction (Giménez *et al.,* 2005).

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3.3 Amino acid profile

The amino acid composition of gelatine from mackerel and blue whiting bones, expressed as percentage of total amino acid, is shown in Table 2. Different profiles were observed for fish bone gelatines depending on the pre-treatment. Mackerel bone gelatine, pre-treated with sodium hydroxide, had high glycine content (21.3%). Gelatines extracted after the enzymatic pre-treatment of mackerel bones, showed significantly different amino acid profile. The glycine content was half of that observed for chemically pre-treated bone gelatines (~11.5%). Blue whiting bone gelatines, pre-treated with sodium hydroxide and Alcalase, had similar glycine content (~20%). However, lower glycine content was observed with Flavourzyme pre-treated blue whiting bone gelatines (17%).

For both fish, significant ($p<0.05$) differences among the content of hydrophobic amino acids (Ala, Val, Ile, Leu, Met, Phe, Tyr and Cys) were observed for enzymatically pre-treated bone gelatines compared to chemically pre-treated bone gelatines. Low content of imino acids (proline and hydroxyproline) were observed for both fish bone gelatines, pre-treated enzymatically, compared to chemically pre-treated fish bone gelatines. The imino acid content of chemically pre-treated bone gelatines was similar to that observed for Japanese sea bass caudal fin (Nagai, 2004), black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*) bone collagens (Ogawa *et al.*, 2003). The difference among proline and hydroxyproline contents of mackerel bone gelatines may affect the rheological properties of the gelatines. Gelatines with low proline and hydroxyproline level usually show lower melting point and weaker gel network (Gilsenan & Ross-Murphy, 2000; Johnston-Banks, 1990).

Regardless of the pre-treatment, tryptophan was not detected in any gelatines but cysteine was present in low levels. This may indicate some contaminations by non-collagenous protein (Morimura *et al.*, 2002).

3.4 Protein solubility

The effect of pH and NaCl on the relative solubility of gelatines from mackerel and blue whiting bones are shown in Figure 2.

All the gelatines, regardless of the pre-treatment used, showed similar pH behaviour. The solubility was higher at low pH, with a maximum at pH 2. The lowest solubility was observed close to neutral pH (Figure 2 A & B). Similar results were also reported by Aewsiri *et al.* (2008) for gelatines from precooked tuna fin.

The effect of NaCl on the relative solubility is depicted in Figure 2 (C & D). In general, the solubility of gelatines decreased gradually with increasing concentration. The decrease in solubility with the increase of NaCl concentration is probably due to the increase of the hydrophobic interactions and to the competition of ionic salts for water (Vojdani, 1996).

The higher solubility of gelatines, from both fish bones pre-treated enzymatically, compared to those pre-treated chemically could be due to the presence of lower molecular weight peptides formed during the extraction process (enzymatic hydrolysis and demineralisation step). The hydrolysis generally cleaves peptides with more polar residues that may interact with water molecule through hydrogen bonds and results in an increase in solubility (Gbogouri *et al.*, 2004).

3.5 Foaming capacity and stability

Foams are complex two-phase colloidal systems which contain at least a continuous liquid phase and a gas phase dispersed as bubbles or air cells. The

properties of foams determine their industrial applications. In the food industry, the determination of foaming properties has a significant impact on the processing and the quality of some products (Exerowa & Kruglyakov, 1998).

The foaming capacity (FC) of gelatines, extracted from mackerel and blue whiting bones, varied significantly ($p<0.05$) depending on the source and the pre-treatment. Regardless of the pre-treatment, gelatines from blue whiting bones showed higher FC than mackerel bone gelatines. Mackerel bone gelatines showed similar values for FC (~38 %, Figure 3 A) with no significant ($p>0.05$) differences between pre-treatments. Gelatines extracted from blue whiting bones, after enzymatic pre-treatment, had significantly ($p<0.05$) higher FC than chemically pre-treated bones (Figure 3 B). All mackerel bone gelatines, regardless of the pre-treatment used had very high foaming stability (Figure 3 C). Blue whiting bone gelatines had lower FS than mackerel bone gelatines. The chemical pre-treatment of blue whiting bones resulted in gelatines with significantly ($p<0.05$) lower FS than enzymatic pre-treatment.

Foaming properties (foaming capacity and stability) of a protein, including gelatine, might be influenced by the source, intrinsic properties, the compositions and conformations of the protein in solution (Wilde & Clark, 1996; Zayas, 1997). The process of foam formation depends largely on the protein adsorption kinetics at the air-water interface (Phillips *et al.*, 1994). The higher FC observed with enzymatically pre-treated bone gelatines may be due to the higher amount of hydrophobic amino acid residues (Ala, Val, Ile, Leu, Met, Phe, Tyr and Cys) compared to chemically pre-treated bone gelatines (Table 2). The foaming agent, having an amphiphilic property, adsorbs at the air-water interface and orients itself in such a way that the lipophilic group orients towards the non-polar phase and the hydrophilic group towards the

aqueous phase. This phenomenon reduces the surface tension allowing the formation of the foam (Liceaga-Gesualdo & Li-Chan, 1999). The lower FS observed with chemically pre-treated mackerel and blue whiting bone gelatines compared to those extracted after the enzymatic pre-treatment of bones, could be due to the lower percentage of negatively charged amino acids (Asp and Glu). On average, the enzymatic pre-treated bone gelatines had 21% negatively charged amino acids compared to 16% from chemically pre-treated bone gelatines. Higher content of negatively charged amino acids, observed with enzymatically pre-treated bone gelatines, may have prevented the neutralisation of charge in gelatine molecules and enhanced the FS.

3.6 Emulsifying capacity and stability

Emulsions are a major component of many foods and their properties (emulsifying capacity and stability) play an important role in the formulation of food products (Spyropoulos *et al.*, 2011). Emulsion activity index (EAI) and emulsion stability index (ESI) for gelatines from mackerel and blue whiting bones at different protein concentrations (0.05, 0.1 and 0.2%) are shown in Table 3.

3.6.1 Emulsifying capacity

The emulsifying activity index (EAI), a measurement of the area of interface stabilized per unit weight of protein (m^2/g) relates to the ability of a protein to coat an interface (Pearce & Kinsella, 1978). The results showed that the increase of the concentration of gelatine solution decreased the emulsifying activity (EAI). Similar results were reported by Binsi *et al.* (2009) for gelatine from skin of bigeye snapper. The protein concentration is an important parameter that affects the emulsifying activity. Low protein concentration favours higher EAI, due to the ability of the

protein to diffuse and adsorb at the oil-water interface (Cheftel *et al.,* 1985). While at high protein concentration, the diffusion is limited as a result of the activation energy barrier (Phillips, 1981).

For all the concentrations studied (0.05, 0.1 and 0.2%), gelatines extracted from mackerel and blue whiting bones after pre-treatment with Alcalase and Flavourzyme showed significantly ($p<0.05$) highest EAI, while the chemical pre-treatment gave gelatines with the lowest EAI (Table 3). This possibly resulted from the difference in the intrinsic properties, composition and conformation among the different gelatines (Cheftel *et al.,* 1985). Mechanisms of the emulsification process of gelatines are correlated to the adsorption ability at the surface of freshly formed oil droplets during homogenization and formation of a protective membrane that prevents droplets coalescence. According to Rahali *et al.* (2000), the degree of insertion of peptides in the interfacial layer mostly depends on the alternative distribution of hydrophobic and charged amino acids. The flexibility of protein (or peptide) structure may be an important structural factor governing the emulsification (Kato *et al.,* 1985). It is also known that protein solubility plays an important role in emulsification because rapid migration and adsorption at the interface are critical (Chobert *et al.,* 1988).

3.6.2 Emulsifying stability

For all gelatines, regardless of the source and the pre-treatment, a positive correlation between the protein concentration and the ESI was found (increasing the concentration of gelatine solutions increased the ESI). Similar results were previsouly observed for whey proteins (Hung & Zayas, 1991). High protein concentrations result

in higher viscosity of the dispersion. This usually leads to a better emulsion stability probably by reducing the coalescence rate (Sajjadi, 2007).

For both fish, gelatines extracted after the enzymatic pre-treatment of bones showed significantly ($p<0.05$) higher ESI compared to those extracted after the chemical pre-treatment (Table 3). As discussed before, gelatines from mackerel and blue whiting bones, pre-treated with Flavourzyme and Alacalse, had significantly higher hydrophobic amino acid residues. According to Giménez *et al.* (2009), a higher content in hydrophobic amino acid residues results in an effective distribution of hydrophilic/hydrophobic amino acids which leads to an improvement of the emulsifying properties of gelatines. Hence, the higher content of hydrophobic amino acid residues, observed with enzymatically pre-treated bone gelatines, may have resulted in better EAI and ESI in comparison to chemically pre-treated bone gelatines.

4. Conclusion

The composition and the functional properties of mackerel and blue whiting bone gelatines were affected by the pre-treatment used in the extraction process. The enzymatic pre-treatment of fish bones produced gelatines with higher content of hydrophobic amino acid residues. The presence of these amino acids had a positive effect on the functional properties of the gelatines. In general, higher foaming and emulsifying properties were observed with enzymatically pre-treated bone gelatines.

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Figure Captions

Figure 1 SDS-PAGE patterns of mackerel (A) and blue whiting (B) bone gelatines. Lane 1: Molecular weight marker (MW. 30,000 - 200,000); lane 2: gelatine from bone pre-treated with Flavourzyme; lane 2: gelatine from bone pre-treated with Alcalase; lane 4: gelatine from bone pre-treated with NaOH.

Figure 2 Foaming capacity (FC) and stability (FS) of gelatines from mackerel (A & C) and blue whiting (B & D) bones extracted using different pre-treatments.

Figure 3 Relative solubility of gelatines from mackerel (A & C) and blue whiting (B & D) bones extracted using different pre-treatments. Solubility in the pH range 2 – 12 (A & B) and solubility as function of NaCl concentration (C & D).

Table Captions

Table 1 Yield of gelatine extraction.

Table 2 Average amino acid composition (g/100 g amino acids) of gelatines extracted from mackerel and blue whiting bones using different pre-treatments.

Table 3 Emulsifying activity (EAI) and stability (ESI) indexes of gelatines from mackerel and blue whiting bones extracted using different pre-treatments at different concentrations (0.05, 0.1 and 0.2%).

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653 Figure 1
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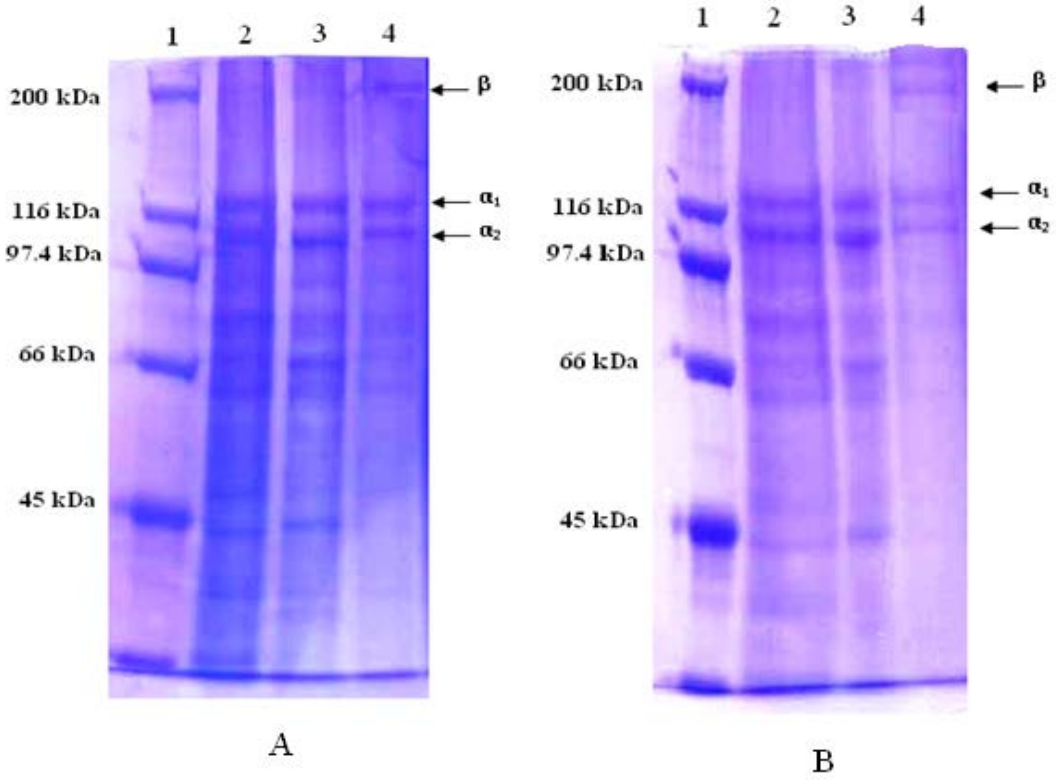

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Figure 2

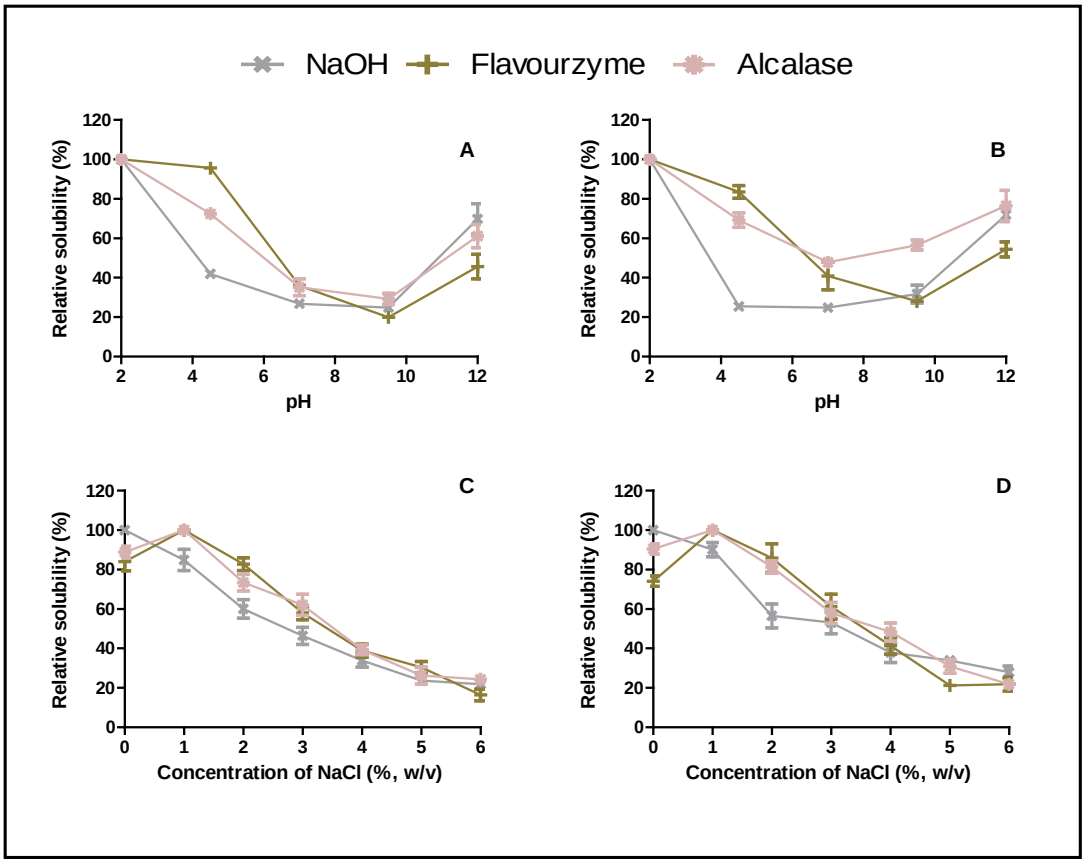

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Figure 3

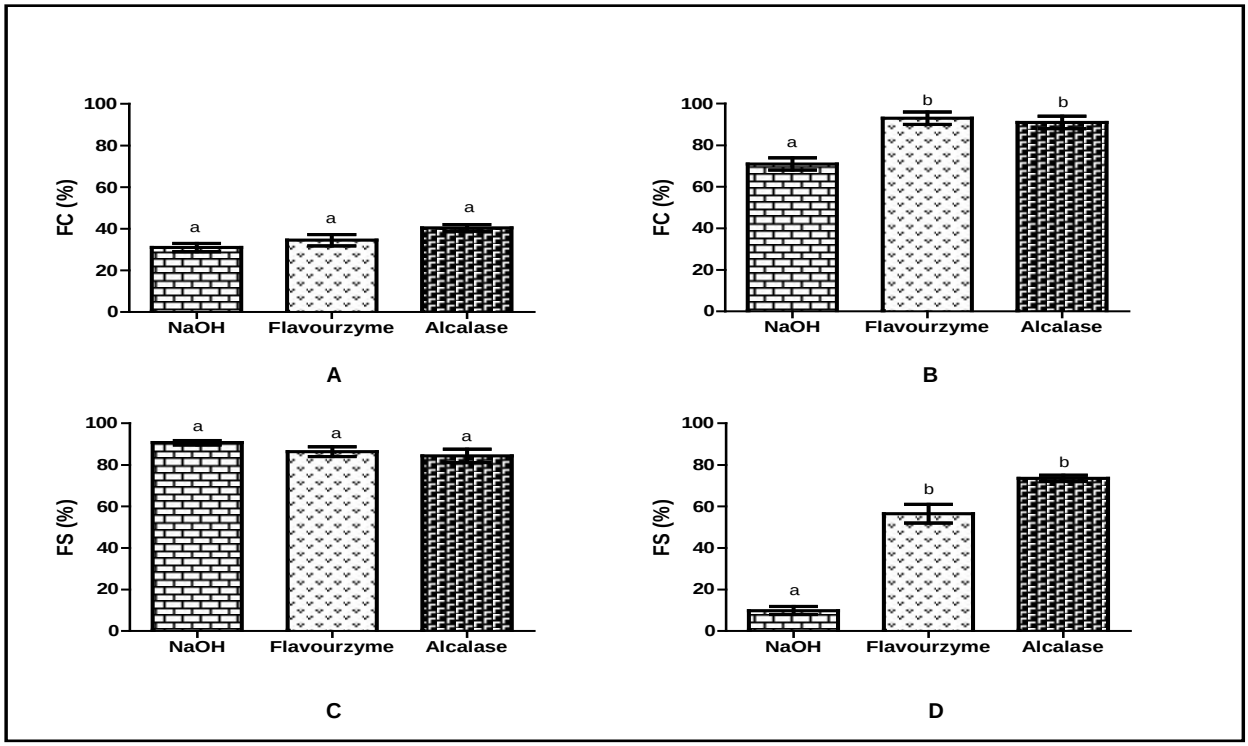

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Table 1

Pre-treatment	Mackerel	Blue whiting
NaOH	2.5±0.1^{aB}	1.0±0.1^{aA}
Alcalase	3.7±0.2^{bB}	1.8±0.2^{bA}
Flavourzyme	4.0±0.1^{bB}	1.9±0.2^{bA}

Pre-treatment: NaOH: alkaline; Alcalase and Flavourzyme. Values are given as mean ± standard deviation. Different lower case letters in the same column indicate significant ($p<0.05$) differences between pre-treatments. Different upper case letters in the same row, within the same pre-treatment (i.e. chemical or enzymatic), indicate significant ($p<0.05$) differences between fish species.

Table 2

Amino acids	Content (g/100 g amino acids)					
	Mackerel			Blue whiting		
	NaOH	Alcalase	Flavourzyme	NaOH	Alcalase	Flavourzyme
Asp	6.1	8.1	7.8	6.5	8.7	8.4
Thr	2.8	3.8	3.7	3.0	2.9	3.3
Ser	4.8	4.6	4.4	5.6	5.3	5.4
Glu	9.8	13.5	13.3	10.5	10.9	11.7
Gly	21.3	11.4	11.6	20.7	20	17.3
Ala	9.0	7.0	7.3	9.1	8.8	8.3
Cys	0.8	1.2	1.2	0.1	0.2	0.2
Val	2.3	4.0	3.7	2.3	2.5	2.8
Met	2.1	2.6	2.6	2.4	2.5	2.7
Ile	1.3	3.0	2.5	1.4	1.6	2.0
Leu	3.7	6.2	6.0	2.9	3.4	4.1
Tyr	1.1	2.7	2.3	0.7	1.1	1.5
Phe	2.6	3.3	3.1	2.4	2.4	2.7
His	1.2	2.3	2.3	1.6	1.6	1.8
Lys	4.1	6.7	7.0	3.7	4.1	4.8
Arg	8.4	7.3	7.5	8.6	8.8	8.4
Pro	10.3	6.9	7.7	9.7	9.4	8.4
Hyp	8.3	5.4	6.0	8.5	5.7	6.1

Pre-treatment: NaOH: alkaline; Alcalase and Flavourzyme. The amino acid composition was performed in triplicate and data correspond to mean values.

Table 3

		EAI			ESI		
		0.2%	**0.1%**	**0.05%**	**0.2%**	**0.1%**	**0.05%**
Mackerel	**NaOH**	8.3 ± 1.5^{aA}	10.7 ± 1.9^{aB}	15.3 ± 2.4^{aB}	35.2 ± 3.6^{bB}	26.2 ± 3.7^{bB}	13.1 ± 2.4^{aA}
	Alcalase	22.6 ± 3.4^{cA}	35.7 ± 3.1^{cB}	36.5 ± 2.5^{bB}	54.4 ± 4.5^{cB}	53.7 ± 2.3^{cB}	24.9 ± 0.9^{bA}
	Flavourzyme	23.1 ± 2.7^{cA}	30.0 ± 0.8^{cB}	35.0 ± 5.5^{bB}	52.8 ± 2.8^{cB}	48.1 ± 1.6^{cB}	24.7 ± 1.4^{bA}
Blue whiting	**NaOH**	16.4 ± 1.2^{bA}	24.6 ± 2.9^{bB}	39.3 ± 4.8^{bC}	17.9 ± 2.8^{aB}	11.4 ± 2.2^{aA}	9.3 ± 2.8^{aA}
	Alcalase	19.8 ± 0.7^{cA}	33.5 ± 2.7^{cB}	62.1 ± 3.5^{cC}	37.5 ± 3.4^{bB}	32.6 ± 3.4^{bB}	10.4 ± 2.7^{aA}
	Flavourzyme	20.8 ± 2.5^{cA}	35.6 ± 2.4^{cB}	54.6 ± 2.4^{cC}	40.8 ± 5.0^{bC}	27.7 ± 3.1^{bB}	13.6 ± 1.5^{aA}

Pre-treatment: NaOH: alkaline; Alcalase and Flavourzyme. Values are given as mean ± standard deviation. Different lower case letters in the same column indicate significant ($p<0.05$) differences between pre-treatments. Different upper case letters in the same row, within the same parameter (i.e. EAI or ESI), indicate significant ($p<0.05$) differences between concentrations.