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

Zied Khiari [a,*], Daniel Rico [b], Ana Belen Martin-Diana [b], Catherine Barry-Ryan [a]

[a] School of Food Science and Environmental Health, Dublin Institute of Technology (DIT), Cathal Brugha Street, Dublin 1, Ireland
[b] Agricultural Technological Institute of Castilla and Leon, Government of Castilla and Leon, Finca Zamaduenas, Valladolid, Spain

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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, for both 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 index (EAI) but increased the stability index (ESI). The use of enzymes in the pre-treatment process gave gelatines with significantly ($p < 0.05$) higher EAI and ESI.

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 in landfills with on-shore processing.

Environmental legislation has contributed to the introduction of sustainable waste management practises 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. Recent advances in fish waste management have resulted in it being considered as a source of ingredients with potential applications 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 the 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 the 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 bones serving as models for oily and white fish, respectively, were investigated for gelatine 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 (*S. scombrus*) caught in early March 2007 were kindly provided by Bord Iascaigh Mhara (BIM, Dublin, Ireland). Blue

* Corresponding author. Present address: Department of Agricultural Food & Nutritional Science (AFNS), 4-10, Agriculture/Forestry Centre, University of Alberta, Edmonton, AB, Canada T6G 2P5. Tel.: +1 780 492 4614.
E-mail address: khiari@ualberta.ca (Z. Khiari).

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whiting (*M. poutassou*) caught in January 2008 were provided by Donegal Seafood (Donegal, Ireland). Both fish were caught in the area FAO 27 (Atlantic, Northeast). The average weights for mackerel and blue whiting were 277 and 117 g, respectively. Fillets were manually removed after beheading and evisceration of fish, the remaining meat was separated from the frame using a knife. Bones were cut manually into small pieces (1–2 cm length) using scissors. The bones were divided into batches and kept in the freezer at −20 °C for less than 1 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 hydrolyses 1 μmol of L-leucine-p-nitroanilide per min. 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, Horn, and Eijsink (2005) as the amount of enzyme that releases 1.0 mEq of tyrosine from urea-denatured haemoglobin per min. Flavourzyme was prepared by DSM Nutritional Products, Inc. (Kaiseraugst, Switzerland) and Alcalase was prepared 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 (AOAC method 984.13) was determined using the Kjeldahl method using an automatic Kjeldahl system (Gerhardt, Bonn, Germany). The protein content was calculated by using a conversion factor of 6.25 for the bone protein. Moisture (AOAC method 927.05) was determined by drying the sample using an oven (Qualivac, Greenfield Oldham, UK) until reaching a constant weight (at 100 °C for ~18 h). Ash (AOAC method 942.05) was determined by incineration in a muffle furnace (Carbolite, Bamford, Sheffield, England) at 550 °C for 4 h. Lipid content was determined according to the Bligh and Dyer method (Bligh & 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 methods

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 three 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 and filtered through a 1 mm pore size Fisherbrand U.S. Standard Brass Test Sieve (Thermo Fisher Scientific Inc., Newington, NH, USA) to separate the bones from the protein hydrolysates. The clean bones were collected and demineralised.

2.4.2. Demineralisation and gelatine extraction

Chemically and enzymatically pre-treated fish bones were demineralised at room temperature for 18 h using 0.25 N HCl (1/3, w/v). The demineralised bones were washed under running tap water for 15 min to remove the acid.

2.4.3. Gelatine extraction

The pre-treated bones were mixed with distiled water (at a ratio of 1/3, w/v) and gelatine was extracted at 45 °C for 18 h using a Sotax A6 dissolution test rate apparatus (Sotax AG, Basel, Switzerland). 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 three batches of each species. Gelatine extraction yield was calculated as g of dry gelatine per 100 g of initial untreated bones.

2.5. Protein patterns of fish gelatines

The electrophoresis procedure was carried out according to the method described by Khiari, Rico, Martin-Diana, and Barry-Ryan (2011). Gelatine solutions (5 mg/mL) were prepared in distiled water at 60 °C and then diluted to a final concentration of 2 mg/mL with sample buffer containing β- mercaptoethanol (Sigma–Aldrich, Inc.). Gelatine samples were heated to 85 °C for 10 min to denature the proteins. Samples and molecular weight markers (10 μL each) were loaded onto SDS–PAGE having a 4% stacking gel and 10% resolving gel according to the method of 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 for 1 h with Coomassie Brilliant Blue R250 (Sigma–Aldrich, Inc.). The gel was de-stained using a mixture of isopropanol, acetic acid and distiled water (12:10:78, v/v/v) using six changes for a total of ~4 h.

The molecular weight markers (Sigma–Aldrich, Inc.) 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 *Escherichia coli* (116 kDa) and myosin heavy chain 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 hydrolysed for 24 h at 110 °C, with 6 M HCl containing 0.1% phenol in vacuum-sealed hydrolysis vials. Norleucine (Sigma–Aldrich, Inc., Madrid, Spain) was added as an internal standard. The amino acid composition was analysed using a cation exchange Biochrom 20 amino acid analyser (Pharmacia Biotech, Ltd., Cambridge, England) with postcolumn derivatisation with ninhydrin. All amino acids were measured 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, Barbarino, De-Paula, Pereira, & Marquez, 2002). Cysteine was determined as cysteic acid by oxidation of the protein with formic acid prior to hydrolysis, according to Hirs (1967). Results