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Characterization of structural and functional properties of fish protein hydrolysates from surimi processing by-products

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ABSTRACT

Structural and functional properties of fish protein hydrolysates with different degrees of hydrolysis (DH) from surimi processing by-products, prepared by Protamex and Alcalase, were evaluated. As the DH increased, the zeta potentials of the hydrolysates increased ($p > 0.05$). The surface hydrophobicity of the hydrolysates was significantly affected by DH ($p < 0.05$). A wide variety of peptides were obtained after hydrolysis by Protamex and Alcalase. The hydrolysate with DH 10%, prepared by Protamex, contained more large protein molecules than did the others. Hydrolysis by both enzymes increased solubility to more than 65% over a wide pH range (pH 2–10). The interfacial activities of hydrolysates decreased with increasing DH ($p < 0.05$). The hydrolysate with DH 10%, prepared by Protamex, exhibited the best interfacial properties among all of the samples. Thermal properties were also affected by the hydrolysis. The results reveal that structures and functionalities of the hydrolysates were determined both by DH and enzyme type employed.

1. Introduction

Surimi processing by-products (including fish meat leftover on bones, head, skin, and viscera, and accounting for about 60–70% of the fish weight), contain approximately 20–30% of protein (Torres, Chen, Rodrigo-Garcia, & Jaczynski, 2007). Most of them are currently discarded as an industrial solid waste or underutilized as animal feed or fertilizer (Gehring, Gigliotti, Moritz, Tou, & Jaczynski, 2011). In China, silver carp (*Hypophthalmichthys molitrix*) is the main freshwater species for surimi processing, with an estimated annual consumption of 3,524,800 metric tons, with processing by-products comprising more than 65% or 2,291,120 metric tons of waste (Ministry of Agriculture of the People's Republic of China, 2006). Annual global production is nearly 4.2 million metric tons in the Asia–Pacific region (Naseri, Rezaei, Moieni, Hosseni, & Eskandari, 2010). Therefore, utilisation of surimi processing by-products (such as the recoveries of proteins from the by-products) for subsequent use in human foods is very important for the economic viability and increase of add-value of the aquatic foods industry.

Controlled enzymatic hydrolysis of protein-rich fish wastes is believed to be a better way to transform wastes into products. The hydrolysates produced have functional or biological properties and are appropriate for different applications, compared to those of native proteins or common food protein ingredients (Gbogouri, Linder, Fanni, & Parmentier, 2004; Kristinsson & Rasco, 2000; Suthasinee, Sittiwat, Manop, & Apinya, 2005). Thus, the hydrolysis of surimi processing by-products can reduce the costs of surimi production. Moreover, the resource waste and environment pollution associated with disposal could be minimised.

Nowadays, numerous *in vitro* studies have already focussed on the bioactivity of fish protein hydrolysates (Khantaphant & Benjakul, 2008; Klompong, Benjakul, Kantachote, & Shahidi, 2007; Raghavan & Kristinsson, 2009; Theodore, Raghavan, & Kristinsson, 2008; Thiansilakul, Benjakul, & Shahidi, 2007; Wu, Chen, & Shiau, 2003), whereas studies on the relationships of molecular structures to functional properties have been limited. The latter play a significant role in the application of hydrolysates as binders, emulsifiers, gelling agents or nutritional supplements (Sathivel et al., 2004). Generally, the molecular characteristics of fish protein hydrolysates, such as molecular weight (Adler-Nissen, 1986), hydrophobicity (Turgeon, Gauthier, Mollé, & Léonil, 1992) and polar groups of the hydrolysate (Kristinsson & Rasco, 2000) directly affect the functional properties and uses as food ingredients (Kristinsson & Rasco, 2000).

To date, little information regarding the structures and functional properties of protein hydrolysates from surimi processing (with silver carp) by-products is available. A better understanding of the structural and functional properties of the hydrolysates would be essential for the control of their properties during processing and application. Due to the high production of surimi processing by-products every year, the investigation could be significantly useful to improve the economic value of the aquatic

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foods industry. For the above purpose, the objectives of the present study were to prepare fish protein hydrolysates with different degrees of hydrolysis (DH), using commercial proteinase (Protamex and Alcalase) and to (i) examine the influences of the hydrolysis on the structural changes of fish protein by zeta potential, surface hydrophobicity and high performance size exclusion chromatography (SEC-HPLC) tests and (ii) characterise their functionality in terms of solubility, emulsifying, foaming and thermal properties.

2. Materials and methods

2.1. Materials

Surimi processing (with silver carp (*H. molitrix*)) by-products, including fish meat leftover on bones, head, skin, and viscera, was supplied by Hunan Yiyang Yihua Aquatic Products Co., Ltd. The company has been certified as exporting aquatic products by the European Economic Community and American Food and Drug Administration. Its main products are fresh-water fish surimi, surimi products, and fillets. The supplied by-products were ground into uniformity with ice and sealed in polyethylene bags and stored at −40 °C until used. Protamex (120,000 U/g) and Alcalase (200,000 U/g) were obtained from Novozymes China Inc. (Suzhou, Jiangsu). All other reagents and chemicals were of analytical grade.

2.2. Preparation of protein hydrolysates

The ground by-products were defatted with isopropanol (1:5, g:ml) for 1 h at 30 °C with continuous stirring. The supernatants were recovered using a Buchner funnel and then air-dried at room temperature.

The defatted materials were suspended in distilled water (3%, w/v) and homogenised at a speed of 10,000 rpm for 1 min using a T10 homogenizer (IKA, Germany). The homogenates were pre-incubated at each optimal temperature for 30 min prior to enzymatic hydrolysis. The homogenates were hydrolysed by Protamex and Alcalase to the same DH (10–30%) in bioreactors under optimal enzyme conditions (pH 7.0 and 50 °C for Protamex; pH 8.5 and 60 °C for Alcalase). The hydrolysis reactions were started by the addition of Protamex and Alcalase at a level of 2400 and 3000 (U/g, enzyme/substrate), respectively, and the DH of the hydrolysates was determined, using the pH-stat method (Adler-Nissen, 1986). The pH values of the mixtures were maintained constant during hydrolysis, using 1 M NaOH. Once the desired DH was reached, the pH of the sample solution was adjusted to 7.0 and then the solution was heated at 90 °C for 10 min to inactivate the proteases. The hydrolysates were centrifuged at a speed of 10,000 rpm at 4 °C for 15 min to separate insoluble and soluble fractions. Finally, the supernatants were dialyzed at 4 °C for 24 h, freeze-dried, and then stored at 4 °C. In the present study, the DHs of the hydrolysates were as follows: Protamex DH 10 ± 0.28%, Protamex DH 20 ± 0.35%, Protamex DH 30 ± 0.50%, Alcalase DH 10 ± 0.19%, Alcalase DH 20 ± 0.31% and Alcalase DH 30 ± 0.46%. Each difference of the DH prepared by Protamex and Alcalase was not significant ($p > 0.05$). Therefore, DH 10%, 20% and 30% were used for the experiments.

2.3. Determination of structures

2.3.1. Zeta potential measurements

Zeta potentials of hydrolysates with different DHs were determined, using a Zetasizer 2000 (Malvern Instruments, Southborough, UK). The samples were diluted by a factor of 10^5 with distilled water and then injected into the apparatus. The averages of five measurements were reported as zeta potentials.

2.3.2. Surface hydrophobicity measurements

Surface hydrophobicities of hydrolysates with different DHs were determined, using the fluorescence probe, 1-anilino-8-naphthalene-sulfonate (ANS), as described by Kato and Nakai (1980). 40 μl of 8 mM ANS were added to the samples with a concentration ranging from 0.005 to 1 mg/ml. The relative fluorescence intensities (RFI) of the samples were measured, using a 650-60 spectrometer (Hicathi, Tokyo, Japan) at 365 and 484 nm as the excitation and emission wavelengths, respectively. The initial slope of the RFI against hydrolysate concentration (mg/ml) was calculated by linear regression analysis and reported as an index of surface hydrophobicity of hydrolysate.

2.3.3. Determination of molecular weight distributions

The molecular weight distributions of hydrolysates with different DHs were estimated by high performance size-exclusion chromatography (SEC-HPLC). Various samples were first solubilised using 0.1 M Na_2SO_4 in 0.1 M sodium phosphate buffer (pH 6.7). The suspensions were centrifuged at a speed of 10,000 rpm for 15 min and the supernatants were filtered through cellulose acetate membranes with pore size of 0.45 μm (Merck, Germany) to remove any insoluble particles. A Shimadzu liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) equipped with a TSKgel 2000 SWXL column (30 mm i.d. × 7.8 mm, Tosoh, Tokyo, Japan) and a Shimadzu ultraviolet detector were used. The hydrolysates were applied to the column and eluted at a flow rate of 1 ml/min and monitored at 220 nm at 25 °C. A molecular weight calibration curve was prepared from average retention times of following standards: bovine serum albumin (Mw: 67,000 Da), peroxidase (Mw: 40,200 Da), ribonuclease A (Mw: 13,700 Da), glycine tetramer (Mw: 246 Da) and p-aminobenzoic acid (Mw: 137.14 Da) (Sigma Co., St. Louis, MO, USA).

2.4. Determination of functional properties

2.4.1. Solubility

The hydrolysates with different DHs (100 mg) were dispersed in 10 ml of distilled water and pHs of the solutions were adjusted to 2.0, 4.0, 7.0 and 10.0 with 1 M HCl and 1 M NaOH. Each solution was magnetically stirred for 1 h at 25 °C. The solutions were centrifuged at a speed of 3000 rpm for 10 min, and the soluble fractions were collected. Then the protein contents in the supernatants were determined according to the method of Lowry, Rosebrough, Farr, and Randall (1951):

$$\text{Solubility } (\%) = \frac{\text{protein content in supernatant}}{\text{total protein content in sample}} \times 100\%. \quad (1)$$

2.4.2. Emulsifying properties

Emulsifying properties of the hydrolysates with different DHs, including emulsifying activity index (EAI) and emulsion stability index (ESI), were determined according to the method of Pearce and Kinsella (1978) with slight modifications. 30 ml portions of 2 mg/ml of each hydrolysate solution were homogenised in a mixer at high speed and 10 ml of soybean oil was added and the pH value of each sample was adjusted to 2.0, 4.0, 7.0 and 10.0. The mixtures were homogenised using a homogenizer (IKA, Germany) at a speed of 10,000 rpm for 1 min. 50 μl of the emulsion was pipetted from the bottom of the mixture at 0 and 10 min after homogenisation and diluted to 5 ml with 0.1% (w/v) dodecyl sulfate sodium salt (SDS). The absorbance of the diluted solution was measured at 500 nm, using a UV2600 spectrophotometer (UNICO Instruments, Shanghai, China). The absorbances (A_0 and A_{10}) were used to calculate the EAI and ESI: