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

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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.

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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, & Benjakul, 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, Molé, & Léon, 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...
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 protease (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 and minced, 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 560 U/g, 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 10² 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 hydrolysates.

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 Na2SO₄ 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): Solubility (%) = protein content in supernatant / total protein content in sample × 100%.

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 sulphate sodium salt (SDS). The absorbance of the diluted solution was measured at 500 nm, using a UV-2600 spectrophotometer (UNICO Instruments, Shanghai, China). The absorbances (A₀ and A₁₀) were used to calculate the EAI and ESI:
where \( A \) = absorbance at 500 nm; \( c \) = protein concentration (g/ml).

EAI (min) = \( \frac{A_0 \times 10}{A_0 - A_{10}} \)

2.4.3. Foaming properties

Foaming properties of the hydrolysates with different DHs, including foaming capacity and foam stability, were determined according to the method of Sathe and Salunkhe (1981) with slight modifications. 200 ml of 10 mg/ml hydrolysate solutions in 250 ml cylinder were adjusted to pH 2.0, 4.0, 7.0 and 10.0 and homogenised using homogenizer (IKA, Germany) at a speed of 10,000 rpm for 1 min to incorporate the air at 25 °C. The total volume after whipping was read immediately and used to calculate the foaming capacity, based on the following equation (Sathe & Salunkhe, 1981):

\[ \text{Foaming capacity} \% = \frac{A-B}{B} \times 100\% \]

where \( A \) is the volume after whipping (ml) and \( B \) is the volume before whipping (ml). The total volume of the whipped sample was recorded after standing for 10 min at 25 °C, and used to calculate the foam stability:

\[ \text{Foam stability} \% = \frac{A-B}{B} \times 100\% \]

where \( A \) is the volume after standing (ml) and \( B \) is the volume before whipping (ml).

2.4.4. Differential scanning calorimetry measurements

The net heat energy (enthalpy, \( \Delta H \)) and the maximum (\( T_{\text{max}} \)) and minimum (\( T_{\text{onset}} \)) temperatures for endothermic transitions of the hydrolysates with different DHs, as a function of temperature were determined using DSC (differential scanning calorimeter, Infinity Series F5010, Instrument Specialists, Inc., Spring Grove, IL, USA). 2.5 mg of each sample were prepared at pH 2.0, 4.0, 7.0 and 10.0 and homogenised using homogenizer (IKA, Germany) at a speed of 10,000 rpm for 1 min to incorporate the air at 25 °C. The total volume after whipping was read immediately and used to calculate the foaming capacity, based on the following equation (Sathe & Salunkhe, 1981):

\[ \text{Foaming capacity} \% = \frac{A-B}{B} \times 100\% \]

where \( A \) is the volume after whipping (ml) and \( B \) is the volume before whipping (ml).

Data show mean values (±SD) for five replicates. The different letters in columns indicate significant difference at \( p < 0.05 \).

3. Results and discussion

3.1. Structures of the hydrolysates with different DHs prepared by Protamex and Alcalase

3.1.1. Zeta potential observation

Zeta potentials of the hydrolysates with different DHs prepared by Protamex and Alcalase are shown in Table 1. The zeta potentials of the samples hydrolysed by both enzymes increased with increase of DH. Generally, a high absolute value of zeta potential generates a repulsive electrostatic force between the molecules, which is a key property of an aggregation resistant suspension. Therefore, it can be reasonably inferred that an increase of electrostatic repulsive forces between the hydrolysate molecules would favour an increase of their solubility and other solubility-related functional properties.

Besides, although the specificities of Protamex and Alcalase for peptide bonds adjacent to certain amino acid residues were quite different (Khantaphant & Benjakul, 2008), the difference of produced zeta potential after the hydrolysis of surimi processing by-products by the two enzymes was not significant (\( p > 0.05 \)). Thus, both Protamex and Alcalase could be efficient enzyme choices for preparing hydrolysates from surimi processing by-products.

3.1.2. Surface hydrophobicity

Surface hydrophobicity of the hydrolysates with different DHs prepared by Protamex and Alcalase was measured (Table 2). Changes in surface hydrophobicity mainly influence the interfacial properties of the hydrolysates. The results showed that the surface hydrophobicity of the hydrolysates was significantly affected by DH (\( p < 0.05 \)). Calderon de la Barca, Ruiz-Salazar, and Jara-Marini (2000) reported that proteolysis, due to shortening of peptide chains, is accompanied by gain or loss of hydrophobicity, which mainly depends on the nature of the hydrolysed protein and molecular weight of the formed peptides. In the present work, enzymatic hydrolysis by Protamex was accompanied by a decrease of surface hydrophobicity. The possible reason is that peptides released from protein in surimi processing by-products gradually adopted a conformation with hydrophilic groups exposed outward, while surface hydrophobicity of hydrolysates prepared by Alcalase increased along with the increasing DH. Liu, Kong, Xiong, and Xia (2010) found that enzymatic hydrolysis of porcine plasma protein by Alcalase was coupled with a decrease of surface hydrophobicity. Probably the difference of protein nature could explain the opposite tendency.

3.1.3. Molecular weight distribution

The calibration curve of five standard substances on a TSKgel G2000SWXL column is shown in Fig. 1 and this was obtained to

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protamex DH 10%</td>
<td>–22.5 (±1.1)b</td>
</tr>
<tr>
<td>Protamex DH 20%</td>
<td>–26.9 (±0.8)ab</td>
</tr>
<tr>
<td>Protamex DH 30%</td>
<td>–29.8 (±3.1)p</td>
</tr>
<tr>
<td>Alcalase DH 10%</td>
<td>–24.0 (±1.3)p</td>
</tr>
<tr>
<td>Alcalase DH 20%</td>
<td>–26.1 (±2.4)pb</td>
</tr>
<tr>
<td>Alcalase DH 30%</td>
<td>–29.2 (±0.9)pb</td>
</tr>
</tbody>
</table>

Data show mean values (±SD) for five replicates. The different letters in columns indicate significant difference at \( p < 0.05 \).

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface hydrophobicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protamex DH 10%</td>
<td>565.5 (±4.8)a</td>
</tr>
<tr>
<td>Protamex DH 20%</td>
<td>625.4 (±5.2)b</td>
</tr>
<tr>
<td>Protamex DH 30%</td>
<td>225.1 (±3.8)ab</td>
</tr>
<tr>
<td>Alcalase DH 10%</td>
<td>229.8 (±3.1)b</td>
</tr>
<tr>
<td>Alcalase DH 20%</td>
<td>262.1 (±2.4)bf</td>
</tr>
<tr>
<td>Alcalase DH 30%</td>
<td>393.1 (±3.5)b</td>
</tr>
</tbody>
</table>

Data show mean values (±SD) for three replicates. The different letters in columns indicate significant difference at \( p < 0.05 \).
interpret the results. The elution patterns (chromatograms not shown), corresponding to the hydrolysates prepared by Protamex and Alcalase with different DHs, displayed 6–7 major elution peaks at 220 nm. Comparing with standard molecular weights and considering the exclusion limit of the column, the major elution peaks corresponded to Mws of >150,000, 2468, 1360, 799, 502, 387, 226 and 138 Da, respectively. Relative proportions (%) of each peak are presented in Table 3. The hydrolysate with DH 10%, treated by Protamex, had the highest proportion of larger molecules (Mw > 150,000 Da), which was about 8.3%. Those of others ranged from 3.4% to 6.9%. Although the proportion of the larger molecules was lower, it is speculated that they were crucial to the functionality (such as the interfacial and gelling properties). The hydrolysate with DH 10%, treated by Protamex also contained peptides with Mw of 2468 Da, and the relative proportion reached 20.6%. Besides, the molecular weights of peaks of all the other hydrolysates were all lower than 1400 Da. These results suggested that hydrolysis by Protamex and Alcalase yielded a wide variety of peptides. Some studies have demonstrated that molecular weights of hydrolysates were closely related to the solubility (Dong et al., 2008; Gbogouri et al., 2004). Lee, Shimizu, Kaminogawa, and Yamauchi (1987) supported the conclusion that there is an optimum molecular size for peptides to be good emulsifiers. Slizyte et al. (2009) further found that the hydrolysates, which had the highest amount of peptides in the molecular weight range 80,000–1000 Da, displayed best emulsifying properties. Therefore, the peptides prepared by Protamex and Alcalase can also be considered as potential functional agents in food.

The present results also show that molecular weights of the hydrolysates with the same DH, produced by Alcalase, were generally lower than those by Protamex, which could be associated with the higher activities of the former (Klompong et al., 2007).

### Table 3

<table>
<thead>
<tr>
<th>Mw (Da)</th>
<th>&gt;150,000</th>
<th>2468</th>
<th>1360</th>
<th>799</th>
<th>502</th>
<th>387</th>
<th>226</th>
<th>138</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protamex DH 10%</td>
<td>8.3</td>
<td>20.6</td>
<td>29.5</td>
<td>17.0</td>
<td>–</td>
<td>5.9</td>
<td>12.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Protamex DH 20%</td>
<td>4.9</td>
<td>–</td>
<td>43.4</td>
<td>22.1</td>
<td>–</td>
<td>17.4</td>
<td>8.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Protamex DH 30%</td>
<td>4.7</td>
<td>–</td>
<td>40.8</td>
<td>27.3</td>
<td>5.8</td>
<td>11.6</td>
<td>10.9</td>
<td>3.1</td>
</tr>
<tr>
<td>Alcalase DH 10%</td>
<td>6.9</td>
<td>–</td>
<td>40.8</td>
<td>22.8</td>
<td>5.8</td>
<td>11.6</td>
<td>11.6</td>
<td>6.1</td>
</tr>
<tr>
<td>Alcalase DH 20%</td>
<td>4.7</td>
<td>–</td>
<td>41.7</td>
<td>18.8</td>
<td>2.5</td>
<td>9.6</td>
<td>13.7</td>
<td>7.4</td>
</tr>
<tr>
<td>Alcalase DH 30%</td>
<td>3.4</td>
<td>–</td>
<td>45.5</td>
<td>23.0</td>
<td>4.6</td>
<td>3.2</td>
<td>9.2</td>
<td>9.8</td>
</tr>
</tbody>
</table>

–: Not detected.
to form hydrogen bonds with water. The hydrolysates prepared by Alcalase at lower DH showed higher solubility. This lends further support to the finding that molecular weights of that hydrolysate were generally lower.

Some previous studies showed that the surface hydrophobicity of peptides was another crucial influence on the solubility of protein hydrolysate (Gbegbou et al., 2004; Klongpong et al., 2007). However, in the present study the effects of surface hydrophobicity on the solubility were less important than were the smaller size and charge group of the peptides produced during the hydrolysis process. For example, the surface hydrophobicity of the samples hydrolysed by Alcalase increased with increase of DH, while the solubility also increased.

The solubilities of protein hydrolysates prepared by both enzymes were relatively lower at about pH 4.0. The results of SEC-HPLC evidenced that some protein and/or peptides with high molecular weight (Mw) remained after hydrolysis. Such molecules could precipitate at this pH, which was close to the isoelectric point (pl) of fish proteins.

Due to the high solubility of the hydrolysates over a wide pH range, it was presumed that the hydrolysates with different DHs, prepared by Protamex and Alcalase, were good sources of protein and appropriate for many functional applications.

3.2.2. Emulsifying properties

EAI (m²/g) and ESI (min) of the hydrolysates with different DHs, prepared by Protamex and Alcalase as influenced by pHs are shown in Table 4. The hydrolysate with DH 10%, treated by Protamex, exhibited the best emulsifying properties among all the samples.

The hydrolysate with DH 10%, prepared by Protamex, showed lower solubility than did other samples. Thus, a higher proportion of larger molecular weight peptides and higher surface hydrophobicity, which were verified by the above characterisation of molecular structures, played more important roles in the emulsifying properties once the solubility of the sample reached a certain value. The results also showed that EAI and ESI of hydrolysates, prepared by both enzymes, decreased with increasing DH, and the difference of the emulsifying properties for the hydrolysates prepared by Protamex was significant (p < 0.05) with increasing DH. Both EAI and ESI of the hydrolysates with DH 20% and 30%, prepared by Alcalase, were better than those of samples with the same DH prepared by Protamex (p < 0.05). Thus, the size and molecular weight of the hydrolysates played the most significant roles in the emulsifying properties of the present hydrolysates. Lee et al. (1987) also reported that there was an optimum molecular size for peptides to be good emulsifiers. Moreover, the solubility, surface hydrophobicity and amino acid composition of the hydrolysates prepared by different enzymes may also be vital factors in governing the emulsifying properties.

When considering the effect of pH on EAI and ESI, the worst EAI and ESI were found at pH 4.0. It is probable that the pH value was close to the isoelectric point (pl) of fish proteins; therefore, some large molecules of the hydrolysates precipitated or the net charges of the large molecules were reduced, which led to the decrease of emulsifying properties. Similar results were also found in the study of Klongpong et al. (2007).

3.2.3. Foaming properties

Foaming capacity (%) and foam stability (%) of the hydrolysates with different DHs, prepared by Protamex and Alcalase as influenced by pHs, are shown in Table 5. The hydrolysate with DH 10% prepared by Protamex also exhibited the best foaming properties among all the samples (p < 0.05). As DH increased, the hydrolysates prepared by both enzymes displayed a lower foaming capacity and foam stability (p < 0.05). The results were in agreement with Klongpong et al. (2007) and Van der Ven, Gruppen, De Bont, and Voragen (2002). These authors also stressed that high molecular weight peptides are generally positively related to foam stability of protein hydrolysates, and surface hydrophobicity of unfolded proteins has also been shown to positively correlate with foaming characteristics.

The foaming properties of the hydrolysates were also affected by pH value. For foaming capacity and foam stability, the lowest value was found at pH 4.0 for all the samples, which coincided with the precipitation of the large protein molecules at their isoelectric pH. Klongpong et al. (2007) found that foaming capacity of protein hydrolysate of yellow stripe trevally, prepared by Alcalase and Flavourzyme, decreased at very acidic or alkaline pH due to the repulsion of peptides (via ionic repulsion). But, in the present study, higher foaming properties were found at pH 2.0, while the solubilities of the samples were lower at pH 2.0 than those at pH 7.0 and 10.0 (Fig. 2). Thus, it seems that the effects of composition and net charge of peptides, in hydrolysates produced in the present study, on the foaming properties outweighed that of solubility.

The hydrolysates with DH 20% and 30% prepared by Alcalase, exhibited foam properties superior to those of samples with the same DH prepared by Protamex (p < 0.05). Possibly, the differences of the surface hydrophobicity, size and charge of peptides could explain the difference in the foam properties.

3.2.4. Thermal properties

Table 6 compares the onset (T onset) and maximum (T max) temperatures for endothermic transitions, as well as the net heat energy (enthalpy, ΔH) required for the reaction to occur. Hydrolysates prepared by Protamex exhibited an endotherm with T max shifted to lower temperatures. A shift to lower transition temperature signified destabilization of protein structure and therefore led to lower energy required to denature the proteins. Interestingly, the T max values of hydrolysates prepared by Protamex were higher than those prepared by Alcalase, which

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Emulsifying activity index (EAI, m²/g) and emulsion stability index (ESI, min) of the hydrolysates with different DHs prepared by Protamex and Alcalase as influenced by pHs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EAI (m²/g)</td>
</tr>
<tr>
<td></td>
<td>pH 2.0</td>
</tr>
<tr>
<td>Protamex DH 10%</td>
<td>67.2 (±4.4)*</td>
</tr>
<tr>
<td>Protamex DH 20%</td>
<td>39.0 (±2.2)**</td>
</tr>
<tr>
<td>Protamex DH 30%</td>
<td>32.4 (±4.3)**</td>
</tr>
<tr>
<td>Alcalase DH 10%</td>
<td>57.6 (±4.2)**</td>
</tr>
<tr>
<td>Alcalase DH 20%</td>
<td>47.7 (±4.0)**</td>
</tr>
<tr>
<td>Alcalase DH 30%</td>
<td>37.9 (±2.1)**</td>
</tr>
</tbody>
</table>

Data show mean values (±SD) for three replicates. The different letters in columns indicate significant difference at p < 0.05.
with different DHs prepared by Protamex and Alcalase.

...hydrolysis to meet different application needs.

...gest that it might be possible to select specific forms of enzymatic

...structures and functional properties of the hydrolysates were

...from surimi processing by-products, prepared by Protamex and

...alkaline, \( \Delta H \)) for these transitions for the hydrolysates with different DHs prepared by Protamex and Alcalase.

...Data show mean values (±SD) for six replicates.

...The onset, \( T_{\text{onset}} \), and maximum, \( T_{\text{max}} \), temperatures for endothermic transitions and

...The enthalpy change of the hydrolysates, prepared by both en-

...The enthalpy change of the hydrolysates, prepared by both en-

...suggested that the former showed higher thermal stability than the latter.

...The enthalpy change of the hydrolysates, prepared by both en-

...4. Conclusion

...Structural and functional properties of fish protein hydrolysates from surimi processing by-products, prepared by Protamex and Alcalase with different DHs, were evaluated. The results reveal that structures and functional properties of the hydrolysates were determined by the DH and by the enzyme type employed. The structure–functionality relationships revealed by this study suggest that it might be possible to select specific forms of enzymatic hydrolysis to meet different application needs.

...Acknowledgements

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