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Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type

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Abstract

Antioxidative activity and functional properties of protein hydrolysates from yellow stripe trevally (*Selaroides leptolepis*) meat, hydrolyzed by Alcalase 2.4L (HA) and Flavourzyme 500L (HF) with different degrees of hydrolysis (DH) were investigated. As the DH increased, DPPH radical-scavenging activity and reducing power of HA decreased ($p < 0.05$) but no differences were observed for HF ($p > 0.05$). Metal chelating activity of both HA and HF increased with increasing DH ($p < 0.05$). HF generally had a higher ($p < 0.05$) chelating activity than had HA at the same DH tested. At low DH (5%), HA exhibited a better DPPH radical-scavenging activity while, at high DH (25%), HF had a higher ($p < 0.05$) reducing power. For the functional properties, hydrolysis by both enzymes increased protein solubility to above 85% over a wide pH range (2–12). When the DH increased, the interfacial activities (emulsion activity index, emulsion stability index, foaming capacity, foam stability) of hydrolysates decreased ($p < 0.05$), possibly caused by the shorter peptide chain length. At the same DH, the functionalities of protein hydrolysate depended on the enzyme used. The results reveal that antioxidative activity and functionalities of protein hydrolysates from yellow stripe trevally meat were determined by the DH and by the enzyme type employed.

Keywords: Fish protein hydrolysate; Antioxidative activity; Functional property; Hydrolysis; Yellow stripe trevally

1. Introduction

Fish processing by-products and the under-utilized species are commonly recognized as low-value resources with negligible market value. Additionally, inappropriate disposal is a major cause of environmental pollution. Hydrolysis processes have been developed to convert under-utilized fish and fish by-products into the marketable and acceptable forms (Gildberg, 1993; Quaglia & Orban, 1987), which can be widely used in food rather than as animal feed or fertilizer (Benjakul & Morrissey, 1997).

Functional properties of protein can be improved by enzymatic hydrolysis under controlled conditions (Quaglia & Orban, 1990). Hydrolysis potentially influences the molecular size, hydrophobicity and polar groups of the hydrolysate (Adler-Nissen, 1986; Kristinsson & Rasco, 2000). The characteristics of hydrolysate directly affect the functional properties and the uses as food ingredients (Kristinsson & Rasco, 2000). Hydrolysate has an excellent solubility at high degree of hydrolysis (Gbogouri, Linder, Fanni, & Parmentier, 2004; Quaglia & Orban, 1987; Shahidi, Han, & Synowiecki, 1995). High solubility of fish protein hydrolysate over a wide range of pH is a substantially

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useful characteristic for many food applications. Furthermore, it influences the other functional properties, such as emulsifying and foaming properties (Gbogouri et al., 2004; Kristinsson & Rasco, 2000). However, a very high degree of hydrolysis can have enormously negative effects on the functional properties (Kristinsson & Rasco, 2000). Greater emulsifying capacity and emulsion stability were noticeable when DH was low for salmon byproduct hydrolysate (Gbogouri et al., 2004) and sardine hydrolysate (Quaglia & Orban, 1990). Shahidi et al. (1995) also reported good foaming properties for capelin protein hydrolysates prepared by Alcalase at low DH.

Apart from their functionalities, protein hydrolysates from different sources, such as whey, soy protein (Pena-Ramos & Xiong, 2003), egg-yolk (Sakanaka, Tachibana, Ishihara, & Juneja, 2004), prawn (Suetsuna, 2000), tuna cooking juice (Jao & Ko, 2002), yellowfin sole frame (Jun, Park, Jung, & Kim, 2004), Alaska Pollack frame (Je, Park, & Kim, 2005), herring (Sathivel et al., 2003), mackerel (Wu, Chen, & Shiau, 2003) and capelin (Amarowicz & Shahidi, 1997), have been found to possess antioxidant activity. Levels and compositions of free amino acids and peptides were reported to determine the antioxidant activities of protein hydrolysates (Wu et al., 2003). However, there is a little information regarding protein hydrolysates from the meat of yellow stripe trevally (*Selaroides leptolepis*) and their antioxidative activity and functional properties as affected by DH and enzymes used. Therefore, this study aimed to produce a protein hydrolysate from the meat of yellow stripe trevally, an underutilized species, with different DHs using two different proteinases and to study their antioxidative activities and functional properties.

2. Materials and methods

2.1. Chemicals and enzymes

Both 2,2-diphenyl-1-picryhydrazyl (DPPH) and 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine) were purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) was procured from Kanto Chemical Co., Inc. (Tokyo, Japan). Alcalase 2.4L and Flavourzyme 500L were obtained from Novo Nordisk (Bagsvaerd, Denmark) and East Asiatic Company Ltd. (Bangkok, Thailand), respectively.

2.2. Fish sample preparation

Yellow stripe trevally (*S. leptolepis*), 65 g/fish, off-loaded approximately 24–36 h after capture, were obtained from the fishing port in Satul province along the coast of the Andaman Sea. The fish were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the laboratory. Upon arrival, fish were washed and the meat was separated manually. The meat was minced, using a grinder with 0.4 cm diameter holes. The mince was stored in plastic bags in ice until used.

The mince was defatted according to the method of Sikorski and Naczk (1981) with a slight modification. The mince was mixed with isopropanol at a ratio of 1:4 (w/v), homogenized and allowed to stand at room temperature (30–32 °C) for 50 min. The supernatant was drained and the residue was defatted at 75 °C for 90 min using isopropanol at a ratio of 1:4 (w/v). The supernatant was then removed and the precipitate was air-dried at room temperature (30–32 °C). The proximate composition AOAC (2000), as well as the solubility (Xiong, Lou, Wang, Moody, & Harmon, 2000), of both mince and defatted mince, were determined.

2.3. Production of protein hydrolysates

Mince (30 g), or defatted mince (7 g), was suspended in 120 ml and 143 ml of distilled water, respectively, in order to obtain the same protein content. The mixtures were pre-incubated at 50 or 60 °C for 20 min prior to enzymatic hydrolysis using Flavourzyme and Alcalase, respectively. The hydrolysis reaction was started by the addition of the enzyme (Alcalase or Flavourzyme) at levels of 0.25, 0.5, 1, 2.5, 5, 7.5 and 10% (w/w). The reaction was conducted at pH 8.5, 60 °C for Alcalase and at pH 7.0, 50 °C for Flavourzyme for up to 20 min, using the pH-stat method, as described by Adler-Nissen (1986). The pH of the mixture was maintained constant during hydrolysis using 2 M NaOH. DH was then calculated as follows:

$$\mathrm{DH}(\%) = \frac{BN_b}{M_p \alpha h_{tot}} \times 100$$

where B is the amount of alkali consumed (ml), N_b is the normality of alkali, M_p is the mass of the substrate (protein in grammes, % $N \times 6.25$), $1/\alpha$ is the calibration factors for pH-stat, and h_{tot} is the content of peptide bonds (Adler-Nissen, 1986).

At designated DHs, the enzymatic reaction was terminated by placing the samples in a water bath at 90 °C for 15 min with occasional agitation. The samples were cooled and the pH values of samples were then adjusted to 7.0 with 6 M HCl or 1 M HCl. Hydrolysates were centrifuged at 2000*g* for 10 min, using a Biofuge primo centrifuge (Sorvall, Hanau, Germany). Supernatants obtained were freeze-dried using a freeze-dryer (Dura-stop, New York, NY, USA).

Log_{10} (enzyme concentration) vs. DH was plotted. From the regression equation, the enzyme concentrations required to hydrolyze yellow stripe trevally mince to obtain the desired DHs (5%, 15%, 25%) were calculated.

2.4. Proximate analysis

Moisture, protein and fat were determined according to the method of AOAC (2000). The protein and fat contents were expressed on a dry weight basis.

2.5. Determination of antioxidative activities

2.5.1. 2,2-Diphenyl-1-picryhydrazyl (DPPH) radical-scavenging activity

DPPH radical-scavenging activity was measured, using the method of Yen and Wu (1999). HA or HF with different DHs were dissolved in distilled water to obtain a concentration of 40 mg protein/ml. To 4 ml of sample solutions, 1.0 ml of 0.2 mM DPPH was added and mixed vigorously. After incubating for 30 min, the absorbance of the resulting solutions was measured at 517 nm using a spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan). The control was conducted in the same manner, except that distilled water was used instead of sample. DPPH radical-scavenging activity was calculated according to the following equation (Yen & Wu, 1999):

$$\text{DPPH radical-scavenging activity (\%)} = \left(1 - \frac{A_{517}\text{ of sample}}{A_{517}\text{ of control}}\right) \times 100$$

2.5.2. Reducing power

Reducing power was determined by the method of Oyaiza (1986). The sample solution (0.5 ml, 40 mg protein/ml) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. An aliquot (2.5 ml) of 10% trichoroacetic acid was added to the mixture, followed by centrifugation at 3000 rpm (Hettich mikro 20, Tuttlingen, Germany) for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 2.5 ml of 0.1% ferric chloride and the absorbance was read at 700 nm. Increased absorbance of the reaction mixture indicates increasing reducing power.

2.5.3. Metal-chelating activity

The chelating activity on Fe^{2+} was determined, using the method of Decker and Welch (1990). One millilitre of sample solution (40 mg protein/ml) was mixed with 3.7 ml of distilled water. The mixture was then reacted with 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) for 20 min at room temperature. The absorbance was read at 562 nm. The control was prepared in the same manner except that distilled water was used instead of the sample. Chelating activity (%) was then calculated as follows (Decker & Welch, 1990):

$$\text{Chelating activity (\%)} = \left(1 - \frac{A_{562}\text{ of sample}}{A_{562}\text{ of control}}\right) \times 100$$

2.6. Determination of functional properties

2.6.1. Solubility

To determine protein solubility, 200 mg of protein hydrolysate sample were dispersed in 20 ml of deionized water and pH of the mixture was adjusted to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 with 1 or 6 N HCl and 1 or 6 N NaOH. The mixture was stirred at room temperature for 30 min and centrifuged at 7500*g* for 15 min. Protein contents in the supernatant were determined using the Biuret method (Robinson & Hodgen, 1940). Total protein content in the sample was determined after solubilization of the sample in 0.5 N NaOH. Protein solubility was calculated as follows:

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

2.6.2. Emulsifying properties

Emulsifying properties were determined according to the method of Pearce and Kinsella (1978). Vegetable oil (10 ml) and 30 ml of 1% protein solution were mixed and the pH was adjusted to 2, 4, 6, 8 and 10. The mixture was homogenized using a homogenizer (Polytron, Luzern, Switzerland) at a speed of 20,000 rpm for 1 min. An aliquot of the emulsion (50 μl) was pipetted from the bottom of the container at 0 and 10 min after homogenization and mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution. The absorbance of the diluted solution was measured at 500 nm using a spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan). The absorbances measured immediately (A_0) and 10 min (A_{10}) after emulsion formation were used to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI) as follows (Pearce & Kinsella, 1978):

$$\text{EAI (m}^2\text{/g)} = \frac{2 \times 2.303 \times A_{500}}{0.25 \times \text{ protein weight (g)}}$$

$$\text{ESI (min)} = A_0 \times \Delta t / \Delta A$$

where $\Delta A = A_0 - A_{10}$ and $\Delta t = 10$ min

2.6.3. Foaming properties

Foaming capacity and stability of yellow stripe trevally protein hydrolysate were determined according to the method of Sathe and Salunkhe (1981). Twenty millilitres of 0.5% sample solution were adjusted to pH 2, 4, 6, 8 and 10, followed by homogenization at a speed of 16,000 rpm, using a homogenizer (IKA Labortechnik, Selangor, Malaysia) to incorporate the air for 2 min at room temperature. The whipped sample was immediately transferred into a 25 ml cylinder and the total volume was read after 30 s. The foaming capacity was calculated according to the following equation (Sathe & Salunkhe, 1981):

$$\text{Foaming capacity (\%)} = \frac{A - B}{B} \times 100$$

where A is the volume after whipping (ml),

B is the volume before whipping (ml)

The whipped sample was allowed to stand at 20 °C for 3 min and the volume of whipped sample was then recorded. Foam stability was calculated as follows:

$$\text{Foam stability}\ (\%) = \frac{A - B}{B} \times 100$$

where A = volume after standing(ml),
B = volume before whipping (ml).

2.7. Statistical analysis

One-way ANOVA was used and mean comparison was performed by Duncan's multiple range test (Steel & Torrie, 1980). Statistical analysis was carried out using the SPSS statistic programme (Version 10.0) for Windows (SPSS Inc. Chicago, IL).

3. Results and discussion

3.1. Effect of defatting on composition and hydrolysis of yellow stripe trevally meat

Chemical compositions of whole mince and defatted mince are shown in Table 1. Defatted mince had a much lower fat content (0.67%) than had whole mince (3.23%). Coincidentally, a higher protein content was noticeable in the defatted mince. Due to the drying process after defatting, the moisture of the defatted sample was removed to a large extent. The result was in agreement with that of Sikorski and Naczk (1981), who found that isopropanol could remove the fat in fish muscle effectively prior to hydrolysis. Nevertheless, the solubility of defatted mince in KCl was lowered. During isopropanol extraction, proteins are vulnerable to solvent as well as to heat treatment (Mutilangi, Panyam, & Kilara, 1996), leading to the exposure of the hydrophobic domain and aggregation of protein (Sikorski & Naczk, 1981). Isopropanol and other alcohols have been known to compete with protein in water binding. As a result, water was more removed from protein molecules in the presence of solvent (Hoyle & Merritt, 1994; Sikorski & Naczk, 1981). Mutilangi et al. (1996) reported a decrease in solubility of whey protein concentrate subjected to heat treatment. Protein denaturation was a two-stage process initiated by disruption of secondary and tertiary structures, leading to unfolding (Townsend & Nakai, 1983) and aggregation or coagulation (Mutilangi et al., 1996). At high temperature, conformational change was irreversible and polymerization, by the formation of intermolecular disulphide bonds, occurred. The aggregation of the denatured molecules is mediated by hydrophobic and sulfhydryl-disulphide interchange reactions (Wong, 1989). From the result, it appears that the defatting process caused denaturation of protein, as evidenced by decreased solubility. Myofibrillar proteins are soluble in high salt solutions (Wu & Smith, 1987). Therefore, aggregation of proteins might be associated with the lower solubility in KCl.

Table 1
Chemical compositions and properties of yellow stripe trevally mince and defatted mince[a]

Compositions/property (%)	Mince	Defatted mince
Protein[b]	84.2 ± 1.07	96.8 ± 1.02
Fat[b]	3.23 ± 0.27	0.67 ± 0.07
Moisture	79.67 ± 0.52	24.73 ± 0.27
Solubility	45.07 ± 1.96	19.56 ± 0.35

[a] Mean ± SD from triplicate determinations.
[b] Dry weight basis.

Hydrolysis of mince and defatted mince, using Alcalase or Flavourzyme, was carried out by the pH-stat method (Fig. 1). Rapid hydrolysis was observed within the first 3 min. Thereafter, a slower rate of hydrolysis was found up to 20 min (Fig. 1). The typical hydrolysis curves were also reported for Pacific whiting solid wastes (Benjakul & Morrissey, 1997), herring (Liceaga-Gesualdo & Li-Chan, 1999), salmon fillet muscle (Kristinsson & Rasco, 2000), salmon (Gbogouri et al., 2004), capelin (Shahidi et al., 1995) and sardine (Quaglia & Orban, 1987). At the same time of hydrolysis, higher DH was observed for the HA or HF with higher amounts of added enzymes. The result indicates that peptide bonds were more likely cleaved in the presence of a higher amount of enzyme, both Alcalase and Flavourzyme.

When comparing DH of HA or HF produced from whole mince and defatted mince, the hydrolysates obtained from mince possessed a higher DH than did those derived from defatted mince ($p < 0.05$). This result was in accordance with Hoyle and Merritt (1994) who found that protein hydrolysates produced from defatted herring had a lower DH than had those from the original herring. Basically, the enzyme interacts rapidly with the insoluble protein particles, and then polypeptide chains that are loosely bound to the surface are hydrolyzed. The more compacted core proteins are cleaved more slowly (Benjakul & Morrissey, 1997). The proteins in defatted mince were most likely denatured, as indicated by a decreased solubility (Table 1). As a consequence, the protein substrates were less susceptible to hydrolysis by the added enzyme. Hoyle and Merritt (1994) found that denatured fish protein possessed poor wettability, thereby reducing the dispersibility and hence accessibility of enzyme to the substrate. During the defatting process, endogenous proteinases in fish muscle might undergo denaturation. The high temperatures used in the defatting process might inactivate endogenous enzymes and hence reduce the rate of hydrolysis, thus leading to a lower DH in hydrolysate from defatted mince. Endogenous enzymes, such as cathepsins in raw fish muscle have been hypothesized to provide an additional proteolytic effect to commercial enzymes during hydrolysis (Mackie, 1982; Ting, Montgomery, & Anglemier, 1968).

With the same protein substrate and the same amount of enzyme, HA showed a higher DH than did HF over the entire hydrolysis period. The higher DH observed with HA indicates higher proteolytic activity of Alcalase toward yellow stripe trevally muscle proteins, compared to Flavourzyme. Generally, alkaline proteases, including Alcalase, exhibit higher activities than do acid or neutral

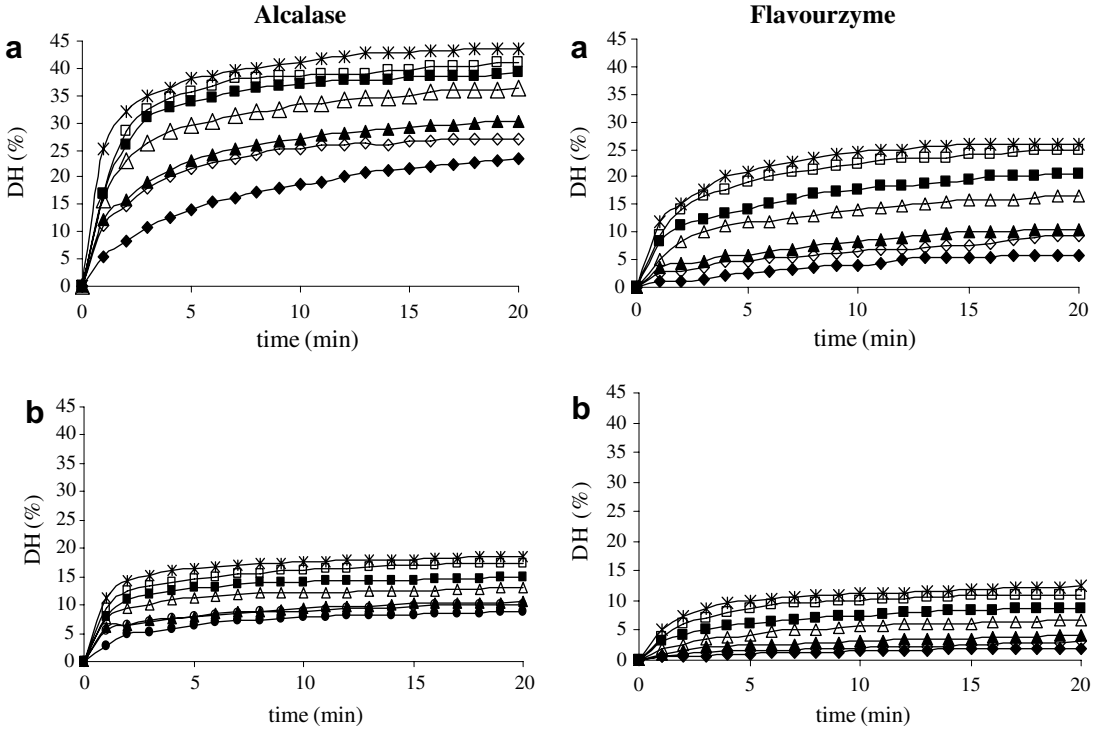

Fig. 1. Degree of hydrolysis (DH) of yellow stripe trevally mince (a) and defatted mince (b) during hydrolysis with Alcalase or Flavourzyme at different concentrations (0.25% (◆), 0.5% (◇), 1% (▲), 2.5% (△), 5% (■), 7.5% (□), 10% (*)).

proteases such as Flavourzyme (Rebeca, Pena-vera, & Diaz-Castaneda, 1991). Therefore, the susceptibility, to hydrolysis, of yellow stripe trevally muscle proteins depends on the type of enzyme used.

When $\log_{10}$ (enzyme concentration) and DH were plotted, a linear relationship was observed (Fig. 2). Similar results were reported by Benjakul and Morrissey (1997) for Pacific whiting solid wastes and by Cheftel, Ahern, Wang, and Tannenbaum (1971) for fish protein concentrate. From this regression, the exact concentration of enzyme required to hydrolyze yellow stripe trevally mince to obtain the specific DH could be calculated. Due to the poor hydrolysis of defatted mince, the original mince without any fat removal was used in further studies.

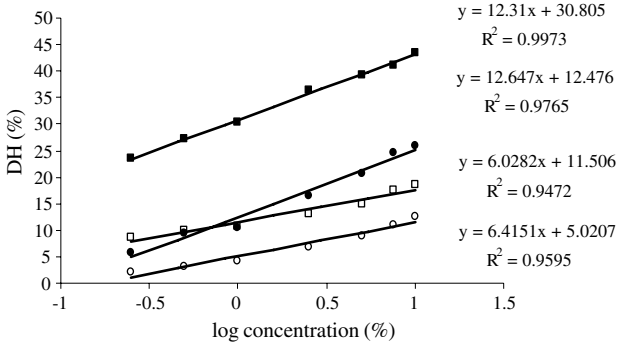

Fig. 2. Relationship between DH and log enzyme concentration of Alcalase in yellow stripe trevally mince (■) and defatted mince (□) and of Flavourzyme in yellow stripe trevally mince (●) and defatted mince (○). Different amounts of enzyme were added to the homogenate of mince or defatted mince. The reaction was run for 20 min at pH 8.5, 60 °C and pH 7, 50 °C for Alcalase and Flavourzyme, respectively.

3.2. Effect of DH and enzyme type on antioxidative activity of yellow stripe trevally protein hydrolysate

3.2.1. DPPH radical-scavenging activity

DPPH radical-scavenging activities of HA and HF with different DHs are depicted in Fig. 3. HA with 5% DH exhibited the highest DPPH radical-scavenging activity ($p < 0.05$). As the DH increased, DPPH radical-scavenging activity of HA decreased ($p < 0.05$). Nevertheless, no differences ($p > 0.05$) were observed for HF with different DH, ranging from 5% to 25%. Additionally, no differences ($p > 0.05$) in DPPH radical-scavenging activity were found between HA and HF having DH of 15% and 25%. However, at low DH (5%), HA exhibited a better DPPH radical-scavenging activity than did HF. Antioxidative activity of protein hydrolysates depends on the proteases (Jun et al., 2004) and hydrolysis conditions employed (Jao & Ko, 2002; Jun et al., 2004; Pena-Ramos & Xiong, 2003). During hydrolysis, a wide variety of smaller peptides and free amino acids is generated, depending on enzyme specificity. Changes in size, level and composition of free amino acids and small peptides affect the antioxidative activity (Wu et al., 2003). Jun et al. (2004) reported that yellowfin sole hydrolysate, prepared using pepsin at lowest DH (22%), had a higher antioxidative activity, than had those produced using other enzymes, such as Alcalase,

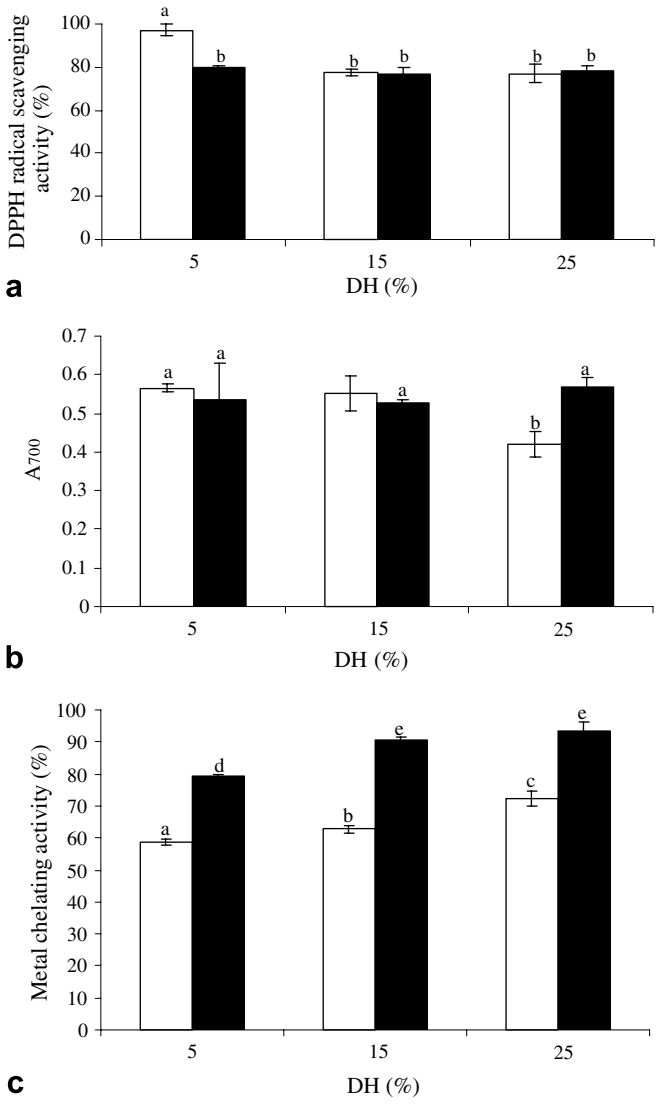

Fig. 3. DPPH radical-scavenging activity (a) reducing power (b) and metal-chelating activity (c) of yellow stripe trevally protein hydrolysate produced using Alcalase (□) and Flavourzyme (■) with different DHs. Bars represent standard deviations from triplicate determinations. Different letters within the same parameter indicate the significant differences ($p < 0.05$).

α-chymotrypsin, papain, pepsin, Pronase E, Neutrase and trypsin. The molecular mass of the antioxidant was identified as 13 kDa. Wu et al. (2003) found that mackerel hydrolysate, with molecular weight of approximately 1400 Da, possessed a stronger antioxidant activity than did those with the molecular weights of 900 and 200 Da. DPPH is a stable free radical with an absorbance maximum at 517 nm in ethanol. When DPPH encounters a proton-donating substance, such as an antioxidant, the radical is scavenged and the absorbance is reduced (Shimada, Fujikawa, Yahara, & Nakamura, 1992). The result reveals that the yellow stripe trevally hydrolysates potentially contained substances which were electron donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction.

3.2.2. Reducing power

As the DH increased, the reducing power of HA decreased ($p < 0.05$), but there were no differences ($p > 0.05$) for HF with different DH. No changes ($p > 0.05$) in reducing power were found between HA and HF with DH of 5% and 15%. However, at high DH (25%), HF showed a higher reducing power than did HA ($p < 0.05$). From this result, it appears that protein hydrolysate from yellow stripe trevally meat could function by donating electrons to the free radicals. Moreover, the reducing power of yellow stripe trevally protein hydrolysate was found to depend on the DH and enzyme used.

3.2.3. Metal-chelating activity

Metal-chelating activity of both HA and HF increased with increasing DH ($p < 0.05$). At the same DH, HF showed a higher chelating activity than did HA ($p < 0.05$). A higher degree of cleavage of peptide bonds rendered hydrolysates with higher metal-chelating activities. Hydrolyzed protein from capelin was also found to possess antioxidant activity (Amarowicz & Shahidi, 1997). Therefore, increased metal-chelating activity could be increased through hydrolysis with certain enzymes. Peptides in hydrolysates could chelate the prooxidants, leading to decreased lipid oxidation. Transition metals, such as Fe, Cu, Co, in foods affect both the rate of autoxidation and breakdown of hydroperoxide to volatile compounds. Transition metal ions react very quickly with peroxides by acting as one-electron donors to form alkoxyl radical (Gordon, 2001). Therefore, chelation of transition metal ions by antioxidant or antioxidative peptide would retard the oxidation reaction (Sherwin, 1990).

From the results presented here, the peptides in HA and HF could act both as primary and secondary antioxidants. Size and sequence of amino acids in the resulting peptides most likely determine the antioxidant activity of protein hydrolysates (Chen, Muramoto, Yamaguchi, Fujimoto, & Nokihara, 1998).

3.3. Effect of DH and enzyme type on functional properties of yellow stripe trevally protein hydrolysate

3.3.1. Solubility

The solubilities of HA and HF with different DH in the pH range of 2–12 are shown in Fig. 4. All hydrolysates were soluble over a wide pH range with more than 85% solubility. In general, the degradation of proteins to smaller peptides leads to more soluble products (Chobert, Bertrand-Harb, & Nicolus, 1988; Gbogouri et al., 2004; Linder, Fanni, & Parmentier, 1996). Both HA and HF, with high DH, had higher solubilities than had those possessing lower DH. This lends further support to the findings of Gbogouri et al. (2004), Shahidi et al. (1995) and Quaglia and Orban (1987) who reported that hydrolysates had an excellent solubility at high degrees of hydrolysis. The balance of hydrophilic and hydrophobic forces of peptides is another crucial influence on solubility increments (Gbogo-

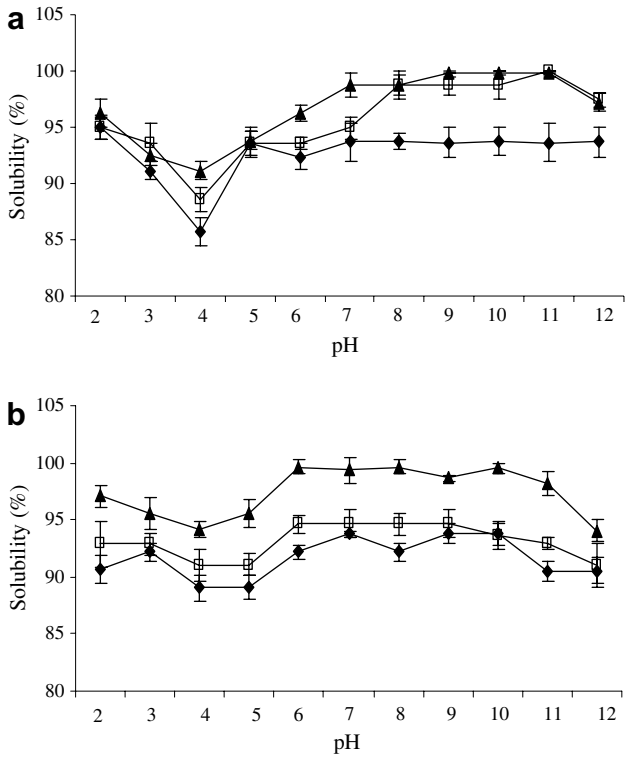

Fig. 4. Solubility of yellow stripe trevally protein hydrolysates prepared using Alcalase (a) and Flavourzyme (b) with different DHs as influenced by pHs: DH 5% (◆), 15% (□) and 25% (▲). Bars represent standard deviations from triplicate determinations.

uri et al., 2004). Enzymatic hydrolysis potentially affects the molecular size and hydrophobicity, as well as polar and ionizable groups of protein hydrolysates (Mutilangi et al., 1996; Turgeon & Gauthier, 1990). The smaller peptides from myofibrillar proteins are expected to have proportionally more polar residues, with the ability to form hydrogen bonds with water and augment solubility (Gbogouri et al., 2004). As a consequence, hydrolysates with smaller peptides, i.e. higher DH, were more soluble.

The solubilities of HA and HF were quite low at pH 4, whereas solubilities above 90% were noticeable at other pHs tested. Salmon byproduct hydrolysates also showed the lowest solubility at pH 4 (Gbogouri et al., 2004). The pH affects the charge on the weakly acidic and basic side-chain groups and hydrolysates generally show low solubility at their isoelectric points (Chobert et al., 1988; Linder et al., 1996). Solubility variations could be attributed to both net charge of peptides, that increase as pH moves away from pI, and surface hydrophobicity, that promotes the aggregation via hydrophobic interaction (Sorgentini & Wagner, 2002). Due to the high solubility of the muscle hydrolysate over a wide pH range, it was presumed that products had a low molecular weight and were hydrophilic in nature (Sorgentini & Wagner, 2002).

3.3.2. Emulsifying properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) of both HA and HF with various DH (5%, 15%, 25%) are shown in Fig. 5. EAI and ESI of both HA and HF decreased ($p < 0.05$) with increasing DH. At low DH (5%), hydrolysates exhibited strong emulsifying properties. With a limited degree of hydrolysis, the hydrolysates have exceptional emulsifying activity and stability (Kristinsson & Rasco, 2000). Higher contents of larger molecular weight peptides or more hydrophobic peptides contribute to the stability of the emulsion (Mutilangi et al., 1996). On the other hand, excessive hydrolysis brings about the loss of emulsifying properties (Gbogouri et al., 2004; Kristinsson & Rasco, 2000; Mahmoud, Malone, & Cordle, 1992; Quaglia & Orban, 1990). The peptides with low molecular weight may not be amphiphilic enough to exhibit good emulsifying properties (Chobert et al., 1988). The mechanism to generate the emulsion system is attributed to the adsorption of peptides on the surface of freshly formed oil droplets during homogenization and the formation of a protective membrane that inhibits coalescence of the oil droplet (Dickinson & Lorient, 1994). Hydrolysates are surface-active materials and promote oil-in-water emulsion because of their hydrophilic and hydrophobic groups with their associated charges (Gbogouri et al., 2004; Rahali, Chobert, Haertle, & Gueguen, 2000). Thus, hydrolysates with a higher DH had poorer EAI and ESI due to their small peptide size. Small peptides migrate rapidly and adsorb at the interface, but show less efficiency in decreasing the interface tension since they cannot unfold and reorient at the interface like large peptides to stabilize emulsions (Gbogouri et al., 2004; Rahali et al., 2000). Apart from peptide size, amphiphilicity of peptides is important for interfacial and emulsifying properties. Rahali et al. (2000) analyzed amino acid sequence at an oil/water interface and concluded that amphiphilic character was more important than was peptide length for emulsion properties. The flexibility of protein or peptide structure may also be a vital factor governing the emulsifying properties (Kato, Komatsu, Fujimoto, & Kobayashi, 1985).

When considering the effect of pH on EAI and ESI, the lowest EAI and ESI were found at pH 4, with coincidental decrease in solubility (Fig. 5). Since the lowest solubility occurred at pH 4, peptides could not move rapidly to the interface. Additionally, the net charge of peptide could be minimized at pH 4. The higher EAI of hydrolysates accompanied their higher solubility (Mutilangi et al., 1996). Hydrolysates with high solubility can rapidly diffuse and adsorb at the interface.

At the same DH, HF had a better EAI than had HA. Conversely, HA showed a higher ESI than did HF. EAI and ESI generally increased as pH moved away from pH 4. This effect was more pronounced with HA, thus suggesting that the sequence and composition of amino acids in peptide between HA and HF might be different, leading to varying charge of the resulting peptides at a particular pH. Emulsifying properties were influenced by specificity of enzyme (Gauthier, Paquin, Pouliot, & Turgeon, 1993).

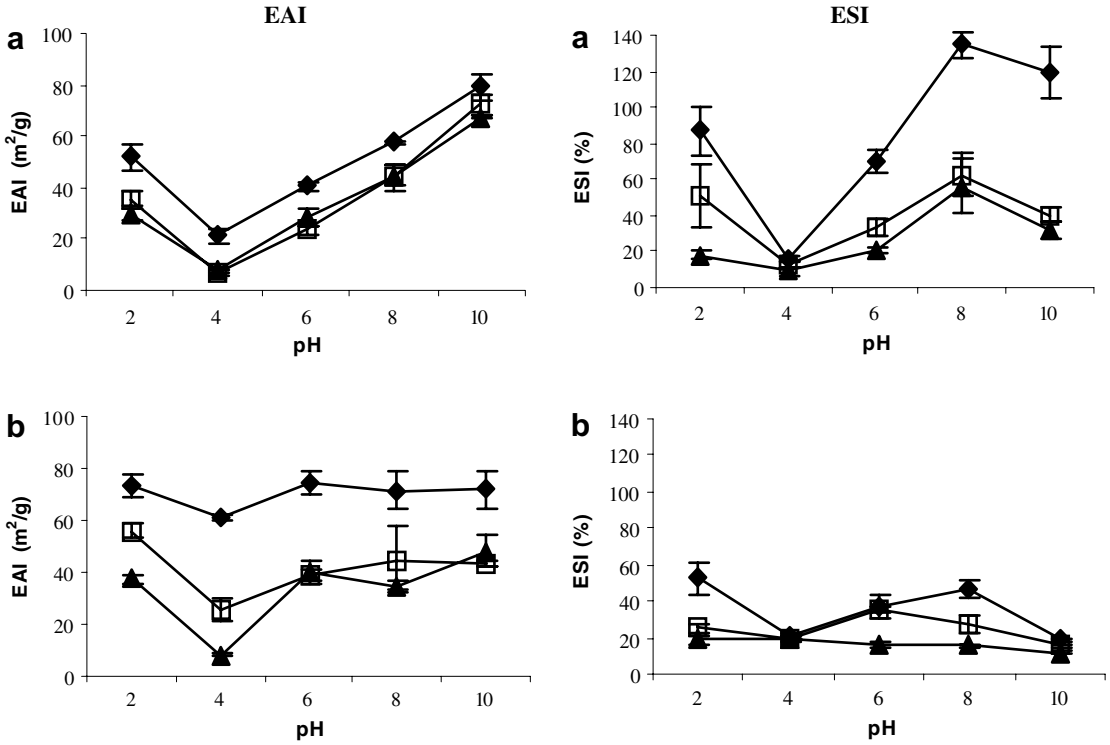

Fig. 5. Emulsifying activity index (EAI) and emulsion stability index (ESI) of yellow stripe trevally protein hydrolysates prepared using Alcalase (a) and Flavourzyme (b) with different DHs as influenced by pHs: DH 5% (◆), 15% (□) and 25% (▲). Bars represent standard deviations from triplicate determinations.

3.3.3. Foaming properties

As DH increased, both HA and HF displayed a lower foaming capacity and foam stability ($p < 0.05$) (Fig. 6). Shahidi et al. (1995) reported good foaming properties for capelin protein hydrolysates at low DH (12%). Further hydrolysis could reduce the foaming stability since the more microscopic peptides do not have the strength needed to maintain a stable foam (Shahidi et al., 1995). High molecular weight peptides are generally positively related to foam stability of protein hydrolysates (van der Ven, Gruppen, de Bont, & Voragen, 2002). Hydrophobicity of unfolded proteins has been shown to correlate with foaming characteristics (Townsend & Nakai, 1983). Foam formation is governed by three factors, including transportation, penetration and reorganization of molecules at the air–water interface. To exhibit good foaming, a protein must be capable of migrating rapidly to the air–water interface, unfolding and rearranging at the interface (Halling, 1981). Dickinson (1989) and Mutilangi et al. (1996) suggested that the foaming capacity of protein was improved by making it more flexible, by exposing more hydrophobic residues and by increasing capacity to decrease surface tension. For the adsorption at the air–water interface, molecules should contain hydrophobic regions (Mutilangi et al., 1996). Foam stability depends on the nature of the film and reflects the extent of protein–protein interaction within the matrix (Mutilangi et al., 1996). Foam stability is enhanced by flexible protein domains, which enhance viscosity of the aqueous phase, protein concentration and film thickness (Phillips, Whitehead, & Kinsella, 1994).

The foaming properties of both HA and HF hydrolysates were affected by pH. Foaming capacity tended to decrease at pH 4. The foaming capacity of HA reached a maximum at pH 6 with a slight decrease at alkaline pH. Therefore, net charge should influence the adsorption of the proteins at the air–water interface. When the net charge was increased, the foaming property was enhanced (Sorgentini & Wagner, 2002; Townsend & Nakai, 1983). The lowest foaming properties of proteins also coincided with the lowest solubilities at their isoelectric pH (Pearson, 1983). For foam stability, the lowest value was found at pH 4 for both HA and HF. A decreased foam stability was also observed in HF with DH of 15% and 25% at pH 10. The low foam stability was concomitant with the low solubility at pH 4. Protein solubility makes an important contribution to the foaming behaviour of protein. The pH of the dispersing medium dramatically influences foaming properties, especially foam stability (Townsend & Nakai, 1983). Foam stability depends principally on the nature of the film and reflects the extent of protein–protein interaction within the matrix (Mutilangi et al., 1996). The decreased foam stability at very acidic or alkaline pH might be due to the repulsion of peptides via ionic repulsion.

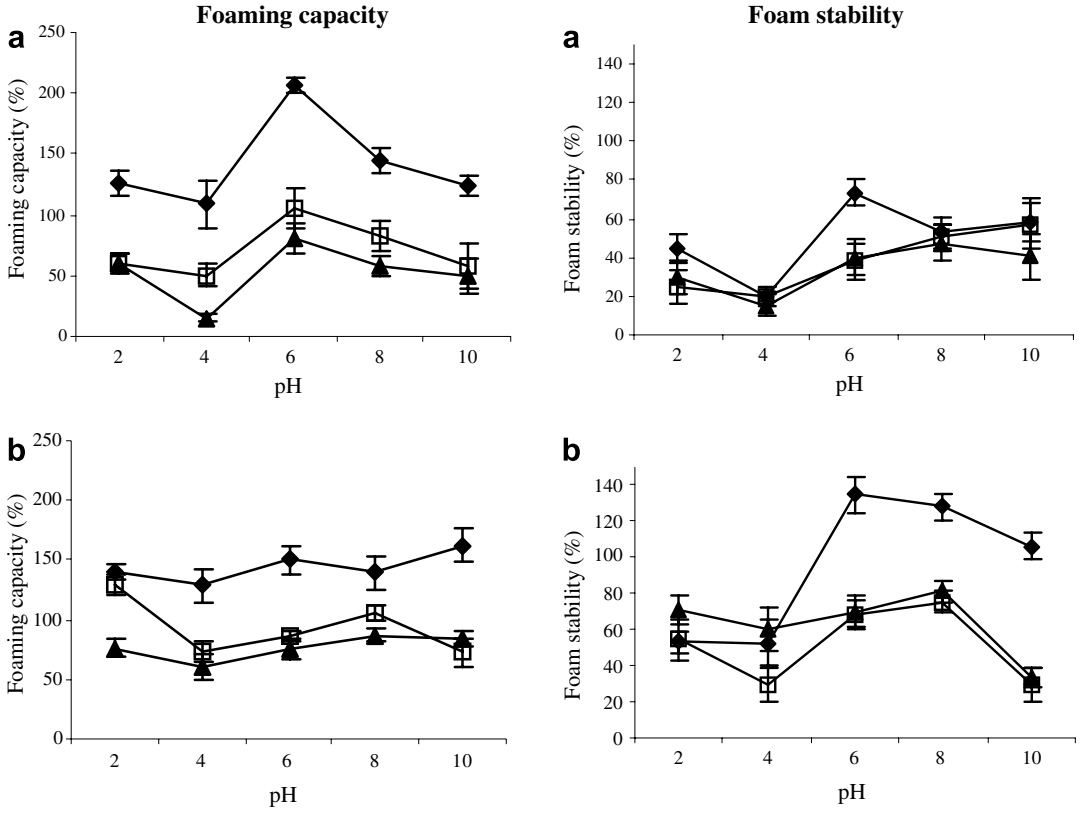

Fig. 6. Foaming capacity and foam stability of yellow stripe trevally protein hydrolysates prepared using Alcalase (a) and Flavourzyme (b) with different DHs as influenced by pHs: DH 5% (◆), 15% (□) and 25% (▲). Bars represent standard deviations from triplicate determinations.

At the same DH, HF exhibited a superior foam stability to HA ($p < 0.05$). Therefore, the size and charge of peptides may be different for hydrolysates produced by different enzymes. HF most likely contained larger peptides which could form flexible films around the air bubbles, as evidenced by a higher foam stability.

4. Conclusions

Antioxidant activity of protein hydrolysates from yellow stripe trevally meat varied with DH and enzyme used. Emulsifying and foaming properties of the protein hydrolysates were also dictated by both factors. Additionally, the functionality of hydrolysates was affected by pH. Therefore, yellow stripe trevally protein hydrolysate can be used in food systems as a natural additive possessing antioxidative properties.

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