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Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*)

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Abstract

Composition, functional properties and antioxidative activity of a protein hydrolysate prepared from defatted round scad (*Decapterus maruadsi*) mince, using Flavourzyme, with a degree of hydrolysis (DH) of 60%, were determined. The protein hydrolysate had a high protein content (48.0%) and a high ash content (24.56%). It was brownish yellow in colour ($L^* = 58.00$, $a^* = 8.38$, $b^* = 28.32$). The protein hydrolysate contained a high amount of essential amino acids (48.04%) and had arginine and lysine as the dominant amino acids. Na^+ was the predominant mineral in the hydrolysate. The protein hydrolysate had an excellent solubility (99%) and possessed interfacial properties, which were governed by their concentrations. The emulsifying activity index of the protein hydrolysate decreased with increasing concentration ($p < 0.05$). Conversely, the foaming abilities increased as the hydrolysate concentrations increased ($p < 0.05$). During storage at 25 °C and 4 °C for 6 weeks, the antioxidative activities and the solubility of round scad protein hydrolysate slightly decreased ($p < 0.05$). Yellowness (b^*-value) of the protein hydrolysate became more intense as the storage time increased but the rate of increase was more pronounced at 25 °C than at 4 °C.

Keywords: Fish protein hydrolysate; Hydrolysis; Functional property; Antioxidative activity; Round scad

1. Introduction

Protein-rich byproducts from the seafood industry, especially dark-fleshed fish, have limited uses due to their dark colour, susceptibility to oxidation and off-flavour. As a sequence, they are discarded or processed into low market-value products, such as fish meal and fertiliser. The application of enzyme technology to recover modified seafood protein may produce a broad spectrum of food ingredients or industrial products (Kristinsson & Rasco, 2000a). Recent advances in biotechnology have also demonstrated the capacity of enzymes to produce novel food products, modified foodstuffs and improved waste management (Benjakul & Morrissey, 1997; Sathivel et al., 2003). Flavourzyme (endo- and exopeptidase enzyme) has been used to produce protein hydrolysates with acceptable functional properties (Kristinsson & Rasco, 2000b). Hydrolysates produced under controlled conditions yield desirable functional properties, high nutritional value and reduced bitterness (Kristinsson & Rasco, 2000a, 2000b; Liceaga-Gesualdo & Li-Chan, 1999). The variables affecting the enzymatic process have been reported to be protease specificity and concentration, temperature, pH, nature of the proteinaceous substrate and degree of hydrolysis (DH) attained (Adler-Nissen, 1977). Fish protein hydrolysates, obtained by controlled enzymatic hydrolysis, are among the best protein hydrolysates in terms of nutritional properties, balanced amino acid composition and high digestibility (Kristinsson & Rasco, 2000a), but are mainly used for animal nutrition because of their bitter flavour and fishy odour. Numerous approaches have been used in the debittering of food pro-

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tein hydrolysates, including selective separation or extraction, masking of bitter taste, enzymatic hydrolysis of bitter peptides and the plastein reaction (FitzGerald & O'Cuinn, 2006; Saha & Hayashi, 2001). Enzymatic hydrolysis of food proteins is an efficient way to recover potent bioactive peptides. Fish protein hydrolysates have been shown to have potential for nutritional or pharmaceutical applications (Amarowicz & Shahidi, 1997; Wu, Chen, & Shiau, 2003; Wergedahl et al., 2004).

Round scads are the prevalent pelagic fish in Thailand, with an estimated annual harvest of 104,000 metric tons per year (Department of Fisheries, 2002). However, human consumption is still limited. Therefore, the production of new value-added products such as protein hydrolysates, with nutritive value, as well as good functional properties, can pave the way for full utilization of this species. Recently, round scad protein hydrolysate with antioxidative activity has been successfully produced (Thiansilakul, Benjakul, &Shahidi, accepted for publication). Protease used and DH directly affect the antioxidative activity of protein hydrolysate (Sathivel et al., 2003; Wu et al., 2003). However, no information regarding the functionalities and storage stability of round scad protein hydrolysate has been reported. The objectives of this investigation were to study the compositions, functional properties and antioxidative activities of round scad protein hydrolysate prepared by hydrolysing fish meat with Flavourzyme and to elucidate its stability during extended storage.

2. Materials and methods

2.1. Chemicals and enzyme

L-Leucine, 2,4,6-trinitrobenzenesulphonic acid (TNBS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 3-(2-pyridyl)-5-6-diphenyl-1,2,4-triazine-4′,4″-disulphonic acid sodium salt (ferrozine) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium sulphite and ninhydrin were obtained from Riedel-deHaën (Seelze, Germany). Ferric chloride and ferrous chloride were procured from Merck (Damstadt, Germany). All chemicals were of analytical grade. Flavourzyme 500 l was provided by Novozymes (Bagsvaerd, Denmark).

2.2. Fish sample preparation

Round scad (*Decapterus maruadsi*) were purchased from the dock in Songkhla, Thailand. The fish, off-loaded approximately 18–24 h after capture, were placed in ice at a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, within 2 h. Upon the arrival, fish were filleted and the ordinary muscle was collected and ground to uniformity. A portion of mince (100 g) was placed in a polyethylene bag and stored under vacuum at −20 °C until used.

2.3. Production of round scad protein hydrolysates

Round scad mince was mixed with isopropanol in a ratio of 1:2 (w/v) and heated at 70 °C for 30 min. The solvent was drained off and the defatted mince was rinsed twice with five volumes of distilled water and then centrifuged at 3000*g* at 4 °C for 15 min, using a Sorvall Model RC-5B Plus centrifuge (Newtown, CT, USA). The resulting isopropanol-defatted mince was mixed with distilled water in a ratio of 1:2 (w/v) and homogenised at a speed of 13,000 rpm for 1 min using an IKA labortechnik homogeniser (Selangor, Malaysia). The homogenate was mixed with McIlvaine buffer (0.2 M sodium dihydrogen phosphate and 0.1 M sodium citrate) (pH 7.0) in a ratio of 1:1 (w/v) and pre-incubated at 50 °C for 10 min. The enzymatic hydrolysis was started by adding Flavourzyme in the amount required to gain 60% DH, as described by Benjakul and Morrissey (1997). After 1 h of hydrolysis, the enzyme was inactivated by heating at 90 °C for 15 min in a water bath (Model W350, Memmert, Schwabach, Germany). The mixture was then centrifuged at 2000*g* at 4 °C for 10 min using a Sorvall Model RC-5B Plus centrifuge (Newtown, CT, USA) and the supernatant was collected. Round scad protein hydrolysate was freeze-dried using a Dura-Top™μp freeze-dryer (FTS systems Inc., Stone Ridge, NY, USA). The freeze-dried round scad protein hydrolysate obtained was subjected to analyses and stability study.

2.4. Determination of α-amino acids and DH

The α-amino acid content was determined according to the method of Benjakul and Morrissey (1997). To diluted protein hydrolysate samples (125 μl), 2.0 ml of 0.2125 M phosphate buffer, pH 8.2 and 1.0 ml of 0.01% TNBS solution were added. The solution was mixed thoroughly and placed in a temperature-controlled water bath (Model W350, Memmert, Schwabach, Germany) at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulphite. The mixtures were cooled at room temperature for 15 min. The absorbance was read at 420 nm and α-amino acid was expressed in terms of L-leucine. DH was calculated as follows (Benjakul & Morrissey, 1997):

$$DH = [(L_t - L_0)/(L_{max} - L_0)] \times 100$$

where L_t is the amount of α-amino acid released at time t. L_0 is the amount of α-amino acid in the original round scad muscle homogenate. L_{max} is total α-amino acid in the original round scad muscle homogenate obtained after acid hydrolysis (6 M HCl at 100 °C for 24 h).

2.5. Proximate analysis

Moisture, protein, fat and ash contents were determined according to the methods of AOAC (2000), numbers 950.46, 928.08, 960.39 and 920.153, respectively. The contents were expressed on a wet weight basis.

2.6. Amino acid analysis

Amino acids in the freeze-dried hydrolysate were determined according to the method of Cohen and Michaud (1993). Hydrolysate (10 μg) was dissolved in 10 mM HCl (10 μl) and treated with 0.2 M borate buffer pH 9.3 (30 μl). The sample solution was reacted with 10 mM 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (10 μl) to form stable unsymmetric urea derivatives. Amino acids were separated by reverse phase HPLC (AccQ Tag column, Waters, Milford, MA, USA) at 37 °C, using gradient mobile phase: deionized water, acetonitrile and eluent A (sodium acetate, phosphoric acid, triethylamine) and detected by a UV detector (Waters 486, Milford, MA, USA) at 254 nm and fluorescence detector (Jasco FP-920, Great Dunmow, Essex, UK) with excitation wavelength at 250 nm and emission wavelength at 395 nm. The amount of amino acids was calculated, based on the peak area in comparison with that of standard. The amino acid content was expressed as a percentage of total amino acids in the sample.

2.7. Determination of mineral contents

Analyses of iron (Fe), copper (Cu), manganese (Mn), cadmium (Cd), nickel (Ni), magnesium (Mg), sodium (Na), phosphorus (P), potassium (K), calcium (Ca), and sulphur (S) contents in freeze-dried hydrolysate were carried out using the inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Model 4300 DV, Perkin Elmer, Shelton, CT, USA) according to the method of AOAC (1999). Sample (4 g) was mixed well with 4 ml of 70% nitric acid. The mixture was heated on the hot plate until digestion was completed. The digested sample was transferred to a volumetric flask and the volume was made up to 10 ml with deionised water. The solution was then subjected to analysis. Flow rates of argon to plasma, auxiliary and nebuliser were maintained at 15, 0.2, and 0.8 l/min, respectively. Sample flow rate was set at 1.5 ml/min. The concentration of mineral was calculated and expressed as mg/kg sample.

2.8. Colour measurement

The colour of freeze-dried hydrolysate was measured by Hunter lab colour meter and reported by the CIE system. L^*, a^* and b^* parameters indicate lightness, redness and yellowness, respectively.

2.9. Determination of functional properties

2.9.1. Solubility

Nitrogen solubility index (NSI) was used to determine the solubility of protein hydrolysate, following the procedure of Morr (1985) with a slight modification. The protein hydrolysate (0.5 g) was dispersed in 50 ml of 0.1 M sodium chloride (pH 7.0). The mixture was stirred at room temperature for 1 h and centrifuged at 2560*g* for 30 min, using a Sorvall Model RC-5B Plus centrifuge (Newtown, CT, USA). The supernatant was filtered through Whatman paper No. 1 and the nitrogen content in the total fraction and in the soluble fraction was analysed by the Kjeldahl method (2000). The nitrogen solubility index (NSI) was calculated as follows:

$$\text{NSI } (\%) = (A/B) \times 100$$

where A is protein content in supernatant and B is total protein content in sample.

2.9.2. Emulsifying properties

The emulsifying activity index (EAI) and the emulsion stability index (ESI) were determined according to the method of Pearce and Kinsella (1978) with a slight modification. Soybean oil (10 ml) and 30 ml of protein hydrolysate solution at different concentrations (0.1%, 0.5%, 1% and 3% w/v) were mixed and homogenised at a speed of 20,000 rpm for 1 min, using an IKA labortechnik homogeniser (Selangor, Malaysia). Aliquots of the emulsion (50 μl) were pipetted from the bottom of the container at 0 and 10 min after homogenisation and diluted 100-fold using 0.1% SDS solution. The absorbance of the diluted solution was measured at 500 nm. 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:

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

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

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

2.9.3. Foaming properties

Foaming ability and foam stability of protein hydrolysate were tested according to the method of Shahidi, Xiao-Qing, and Synowiecki (1995). Twenty millilitres of protein hydrolysate solution (0.1%, 0.5%, 1% and 3% w/v) were homogenized in a 50 ml cylinder at a speed of 16,000 rpm to incorporate the air for 1 min. The total volume was measured at 0, 0.5, 5, 10, 40 and 60 min after whipping. Foaming ability was expressed as foam expansion at 0 min, while foam stability was expressed as foam expansion during 60 min. Foam expansion was calculated according to the following equation (Sathe & Salunkhe, 1981):

$$\text{Foam expansion } (\%) = [(A - B)/B] \times 100$$

where A = volume after whipping (ml) at different times and B = volume before whipping (ml).

2.10. Determination of antioxidative activities

2.10.1. DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined as described by Wu et al. (2003) with a slight modification.

To diluted sample (1.5 ml), 1.5 ml of 0.15 mM DPPH in 95% ethanol were added. The mixture was then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the resultant solution was read at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The blank was prepared in the same manner, except that distilled water was used instead of sample. The scavenging effect was calculated as follows:

$$\text{Radical-scavenging activity } (\%) = [(B - A)/B] \times 100$$

where A is A_{517} of sample and B is A_{517} of the blank.

2.10.2. Reducing power

The reducing power was determined according to the method of Wu et al. (2003) with a slight modification. Diluted sample (1 ml) was mixed with 1 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The mixtures were incubated at 50 °C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. To an aliquot (1 ml) of reaction mixture, 1 ml of distilled water and 200 μl of 0.1% $FeCl_3$ were added. The absorbance of the resultant solution was read at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.10.3. Chelating activity on Fe^{2+}

The chelating of Fe^{2+} was measured by the method of Boyer and McCleary (1987) with a slight modification. Diluted sample (4.7 ml) was mixed with 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine. The reaction mixture was allowed to stand for 20 min at room temperature. The absorbance was then read at 562 nm; the blank was prepared in the same manner except that distilled water was used instead of the sample. The chelating activity was calculated as follows:

$$\text{Chelating activity } (\%) = [(B - A)/B] \times 100$$

where A is A_{562} of sample and B is A_{562} of the blank.

2.11. Study on the stability of round scad protein hydrolysate during storage

The freeze-dried hydrolysate (2.5 g) was kept in the amber vial and closed tightly with a screw-cap. After storage at 4 and 25 °C for 0, 1, 2, 4 and 6 weeks, the samples were taken for analyses of antioxidative activities and solubility as described previously. Prior to colour measurement, the samples were dissolved in water to obtain the concentration of 10 mg/ml.

2.12. Statistical analysis

All experiments were carried out in triplicate. Analysis of variance (ANOVA) was performed and mean comparisons were run by Duncan's multiple range test (Steel & Torrie, 1980). Analysis was performed using a SPSS package (SPSS 10.0 for windows, SPSS Inc, Chicago, IL).

3. Results and discussion

3.1. Proximate analysis

The proximate composition of the freeze-dried round scad protein hydrolysate is shown in Table 1. The protein hydrolysate had a high protein content (69.0%) and could be an essential source of proteins. The high protein content was a result of the solubilisation of protein during hydrolysis, the removal of insoluble undigested non-protein substances and the partial removal of lipid after hydrolysis (Benjakul & Morrissey, 1997). The percentage of solubilised protein was found to depend on the amount of lipids in the raw material. Raw material containing the highest amount of lipids gave the lowest percentage of solubilised protein (Šližyte, Daukšas, Falch, Storrø1, & Rustad, 2005). Defatting of round scad mince with isopropanol was carried out prior to hydrolysis and the removal of the fat layer after hydrolysis resulted in a low lipid content in the resulting protein hydrolysate (0.15%). During the hydrolysis process, the muscle cell membranes tend to round up and form insoluble vesicles, leading to the removal of membrane structured lipids (Shahidi et al., 1995). A reduced lipid content was reported in the protein hydrolysate from salmon (Gbogouri, Linder, Fanni, & Parmentier, 2004), capelin (Shahidi et al., 1995) and herring (Liceaga-Gesualdo & Li-Chan, 1999; Sathivel et al., 2003). From the result, the protein hydrolysate had a high ash content (24.56%), most likely caused by the use of McIlvaine buffer required for pH adjustment and pH control of enzymatic hydrolysis. A high ash content has been recognised as a drawback of protein hydrolysate, making applications limited (Picot et al., 2006; Shahidi et al., 1995; Šližyte et al., 2005).

3.2. Amino acid composition

The amino acid compositions of the freeze-dried round scad protein hydrolysates are presented in Table 2. Protein hydrolysate was rich in arginine, lysine, histidine and leucine, which accounted for 14.0%, 13.9%, 11.2% and 10.1% of the total amino acids, respectively. From the result, the protein hydrolysate had an essential amino acid/non-essential amino acid ratio of 0.92. Fish and shellfish have been reported to contain the high essential amino acid/non-essential amino acid ratio (Iwasaki & Harada, 1985). Therefore, the obtained protein hydrolysate could possibly be a dietary protein supplement to poorly balanced dietary pro-

Table 1
Proximate composition of round scad protein hydrolysate

Composition	Content (%)[a]
Moisture	8.75 ± 0.40
Protein	69.0 ± 3.57
Lipid	0.15 ± 0.03
Ash	24.56 ± 2.56

[a] Means ± SD from triplicate determinations.

Table 2
Amino acid composition of round scad protein hydrolysate

Amino acids	Content (%)
Asp + Asn	2.04
Ser	8.16
Glu + Gln	3.47
Gly	1.49
His	11.2
Arg	14.0
Thr[a]	5.09
Ala	5.31
Pro	0.51
Cys–S–S–Cys	0.69
Tyr	5.20
Val[a]	6.77
Met[a]	4.51
Lys[a]	13.9
Ile[a]	3.15
Leu[a]	10.1
Phe[a]	4.52

[a] Essential amino acids.

teins. Fish byproducts have a high percentage of essential amino acids and can be used to produce nutritious products (Shahidi, 1994). Shahidi et al. (1995) reported that capelin hydrolysate had a similar amino acid profile to that of the raw material. Nevertheless, the sensitive amino acids, such as methionine and tryptophan, were present in smaller amounts after hydrolysis and decolourisation of hydrolysate by charcoal. From the results, round scad protein hydrolysate contained a low level of proline (0.51%). Nevertheless, it might contribute to the bitterness of the hydrolysate. The presence of proline residues in the centre of the peptides contributes to the bitterness. Thus the peptidase, which can cleave the hydrophobic amino acids and proline, is capable of debittering protein hydrolysate (Capiralla, Hiroi, Hirokawa, & Maeda, 2002). Additionally, the protein hydrolysate consisted of hydrophobic amino acids, such as leucine (10.1%), isoleucine (3.16%), phenylalanine (4.52%), and valine (6.77%). Bitterness of protein hydrolysates is associated with the release of peptides containing hydrophobic amino acid residues (FitzGerald & O'Cuinn, 2006). Bitterness was one of the main contributors to off-flavour of fish hydrolysate. Hevia and Olcott (1977) reported that bitter peptide from ficin-treated fish concentrate contained glycine, isoleucine, phenyalanine and valine.

Recently, a protein hydrolysate from round scad has been reported to exhibit antioxidative activity (Thiansilakul et al., accepted for publication). Amino acids in round scad protein hydrolysate are possibly involved in antioxidative activity. Amino acids have been known to exhibit antioxidant activity; tryptophan and histidine showed high antioxidative activity in comparison with methionine, cysteine, glycine and alanine (Riisom, Sims, & Fiorti, 1980). Antioxidative activity of histidine or a histidine-containing peptide may be attributed to the chelating and lipid radical-trapping ability of the imidazole ring, whereas the tyrosine residue in the peptide may act as a potent hydrogen donor (Je, Park, & Kim, 2005; Uchida & Kawakishi, 1992). Generally, aromatic amino acids are considered to be effective radical-scavengers, because they can donate protons easily to electron-deficient radicals. At the same time, their antioxidative stability can remain via resonance structures (Rajapakse, Mendis, Jung, Je, & Kim, 2005). From the results, round scad protein hydrolysate had a high nutritional value, based on its amino acid profile.

3.3. Mineral content

The freeze-dried round scad protein hydrolysate consisted of different minerals at different levels, as shown in Table 3. Na, K, Ca and Mg were found at high concentrations while Ni was not detectable. The high content of Na (16.0%) was associated with the use of McIlvaine buffer for the hydrolysate preparation. The apparent metal ions could act as pro-oxidants in the hydrolysate. Transition metal ions, particularly Cu and Fe, are known to be major catalysts of oxidation (Thanonkaew, Benjakul, & Visessanguan, 2006). Sathivel et al. (2003) reported that K, Mg, P, Na, S and Ca were abundant in herring and herring byproduct hydrolysates and varied with the substrate used. Fish protein hydrolysates usually contain a moderate NaCl content due to salting for conservation or pH adjustments during the pH shift process. However it has limited application (Picot et al., 2006).

3.4. Colour

During hydrolysis, round scad mince turned brownish. As a result, the freeze-dried protein hydrolysate was brownish yellow in colour ($L^* = 58.00$, $a^* = 8.38$, $b^* = 28.32$). Additionally, Flavourzyme with a dark colour also contributed to the brownish colour of the resulting hydrolysate. From the study of Sathivel et al. (2003), it was reported that the colour of whole herring and herring byproduct hydrolysates, prepared using Alcalase, varied with substrates. Herring gonad hydrolysate was the darkest ($L^* = 74.6$) and

Table 3
Mineral content of round scad protein hydrolysate

Minerals	Content (mg/kg)
Fe	4.28
Cu	2.26
Mn	0.95
Cd	0.10
Ni	ND[a]
Mg	245
P	12.8
K	0.42[b]
Ca	715
S	0.69
Na	16.0[b]

[a] ND: Not detectable or below detection limit.
[b] Expressed as % (w/w).

most yellowish ($b^* = 18$), whereas whole herring hydrolysate was the lightest ($L^* = 89.4$) and least yellowish ($b^* = 8.0$). Dark-fleshed fish, such as sardine and mackerel, contained a high amount of myoglobin, which is susceptible to oxidation (Chaijan, Benjakul, Visessanguan, & Faustman, 2004). The dark colour of fish protein hydrolysate was probably from the oxidation of myoglobin and the melanin pigment of the raw material (Benjakul & Morrissey, 1997). Therefore, the varying colour of fish protein hydrolysate depended on the composition of the raw material and the hydrolysis condition. The round scad used is a pelagic dark-fleshed fish. As a result, the brownish-yellow colour of hydrolysate was most likely caused by pigments in the muscle.

3.5. Functional properties

3.5.1. Solubility

Nitrogen solubility index (NSI) of the freeze-dried round scad protein hydrolysate was 99%. NSI has been used to determine protein solubility, mainly caused by the dispersion of protein in the solvent (Cheftel, Cuq, & Lorient, 1985). Solubility is one of the most important physicochemical and functional properties of protein hydrolysates (Kinsella, 1976; Kristinsson & Rasco, 2000a). Good solubility of proteins is required in many functional applications, especially for emulsions, foams and gels. Soluble proteins provide a homogeneous dispersibility of the molecules in colloidal systems and enhance the interfacial properties (Zayas, 1997). From the results, it was suggested that the high solubility of protein hydrolysate was due to the size reduction and the formation of smaller, more hydrophilic and more solvated polypeptide units (Adler-Nissen, 1986; Cheftel et al., 1985). In addition, insoluble protein fractions were removed by centrifugation before the protein hydrolysate was freeze-dried. The high nitrogen solubility of protein hydrolysate indicates potential applications in formulated food systems by providing attractive appearance and smooth mouthfeel to the product (Peterson, 1981).

3.5.2. Emulsifying properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) of round scad protein hydrolysates at various concentrations (0.1%, 0.5%, 1.0% and 3.0%) are shown in Table 4. EAI of protein hydrolysates decreased with increasing concentrations ($p < 0.05$). The decrease in ESI of protein hydrolysates was observed when hydrolysate concentration increased up to 0.5% ($p < 0.05$). However, no differences in ESI were noticeable when the hydrolysate concentrations were above 0.5% ($p > 0.05$). EAI estimates the ability of the protein to aid in the formation and stabilisation of newly-created emulsion by giving units of area of the interface that is stabilised per unit weight of protein; this is determined by the turbidity of the emulsion at a wavelength of 500 nm (Pearce & Kinsella, 1978). Protein hydrolysates are surface-active materials and promote an oil-in-water emulsion because of their hydrophilic and hydrophobic groups and their charge (Gbogouri et al., 2004; Kristinsson & Rasco, 2000a). The dependence of emulsifying activity on the concentration of protein has been explained by adsorption kinetics (Kinsella, 1976). At low protein concentrations, protein adsorption at the oil–water interface is diffusion-controlled. At high protein concentration, the activation energy barrier does not allow protein migration to take place in a diffusion-dependent manner that leads to accumulation of proteins in the aqueous phase. This resulted in the decrease in EAI of protein hydrolysate. ESI also decreased with increasing hydrolysate concentration. The increase in protein–protein interaction resulted in a lower protein concentration at the interface (Lawal, 2004). Thus, a thinner film stabilising the oil droplet was postulated.

Table 4
Emulsifying properties of round scad protein hydrolysates at various concentrations

Hydrolysate concentrations (%)	Emulsifying activity index[A] (m^2/g)	Emulsion stability index[A] (min)
0.1	37.28 ± 1.88^{aB}	35.89 ± 6.65^{a}
0.5	23.67 ± 0.06^{b}	11.96 ± 0.22^{b}
1.0	13.38 ± 0.02^{c}	12.09 ± 0.28^{b}
3.0	5.31 ± 0.02^{d}	12.32 ± 0.04^{b}

[A] Means ± SD from triplicate determinations.
[B] Different superscripts in the same column indicate significant differences ($p < 0.05$).

3.5.3. Foaming properties

Foam expansions of round scad protein hydrolysates at various concentrations (0.1%, 0.5%, 1% and 3%) are depicted in Fig. 1. Foam expansion at 0 min after whipping indicated the foam abilities of protein hydrolysates, which increased from 23.33% to 70% when hydrolysate concentrations increased from 0.1% to 3% ($p < 0.05$). Sánchez and Patino (2005) revealed that an increase in protein concentration resulted in a higher rate of diffusion. Foam expansion after whipping was monitored for 60 min to indicate the foam stability of protein hydrolysates. Within the first

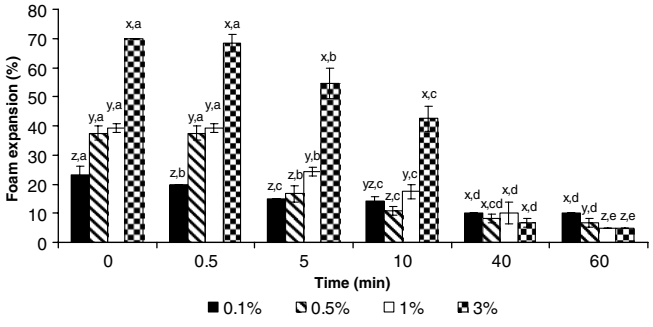

Fig. 1. Foaming properties of round scad protein hydrolysates at various concentrations. Bars represent the standard deviation from triplicate determinations. Different letters a, b, c, d and e within the same concentration indicate significant differences ($p < 0.05$) and different letters x, y and z within the same holding time indicate significant differences ($p < 0.05$).

10 min, the protein hydrolysate with a concentration of 3% showed the highest foam stability ($p < 0.05$). Thereafter, slight differences in foam expansion were observed among hydrolysates with different concentrations. Lawal (2004) pointed out that an increase in foam stability with increasing concentration was a result of formation of stiffer foams. Foaming properties are physicochemical characteristics of proteins, allowing them to form and stabilise foams. In the case of surfactants, the stability of foams is a consequence of the well-ordered orientation of the molecules at the interface, where the polar head is located in the aqueous phase and the hydrophobic chain faces the apolar component (Sánchez-Vioque, Bagger, Rabiller, & Guéguen, 2001).

To improve the functional properties of proteins, enzymatic modification has been extensively employed. Flavourzyme has been used to produce a protein hydrolysate with acceptable functional properties (Kristinsson & Rasco, 2000b). The peptides produced by enzymatic hydrolysis have smaller molecular sizes and less secondary structure than have the original protein (Jeon, Byun, & Kim, 1999). The functional properties of hydrolysed proteins are governed mainly by their molecular weight (Adler-Nissen, 1986) and hydrophobicity (Turgeon, Gauthier, Molle, & Leonil, 1992). Generally, fish protein hydrolysates may be expected to have increased solubility and significant changes in foaming, water-holding, fat adsorption and emulsifying properties, compared to those of native proteins or common food protein ingredients (Gbogouri et al., 2004; Sathivel et al., 2003).

3.6. Antioxidative activities

Lipid oxidation deteriorates flavour, colour, and nutritional quality of foods (Nawar, 1996), and effective natural antioxidants play an important role in the food industry. Protein hydrolysates from herring byproducts, capelin, yellowfin sole frame and Alaska pollack frames have been reported to exhibit antioxidative activity (Amarowicz & Shahidi, 1997; Je et al., 2005; Jun, Park, Jung, & Kim, 2004; Sathivel et al., 2003). The antioxidative activities of round scad protein hydrolysate were determined through the DPPH radical-scavenging activity, reducing power and Fe^{2+}-chelating ability, with the values of 59.9%, 1.01% and 58.2%, respectively. $DPPH^{\cdot}$ is a stable free radical that shows maximal absorbance at 517 nm in ethanol. When $DPPH^{\cdot}$ encounters a proton-donating substance, the radical is scavenged by changing colour from purple to yellow and the absorbance is reduced (Shimada, Fujikawa, Yahara, & Nakamura, 1992). For the reducing power assay, the presence of reductants (antioxidants) in tested samples results in reducing Fe^{3+}/ferricyanide complex to the ferrous form. The Fe^{2+} can therefore be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The results suggested that round scad protein hydrolysates possibly contained amino acids or peptides, which functioned as electron donors and could react with free radicals to form more stable products. The chelating of ferrous ions was used to determine the ability of hydrolysates in metal-chelating activity. Ferrozine can quantitatively form complexes with Fe^{2+} ion. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease of colour formation. The results indicated that protein hydrolysates had a pronounced capacity for iron binding. Transition metals, such as Fe, Cu and Co, in foods affect both the speed of autoxidation and the direction of hydroperoxide breakdown to volatile compounds (Nawar, 1996). Thus, protein hydrolysate likely prevented the lipid oxidation via radical-scavenging activity and metal-chelating ability.

3.7. Stability of round scad protein hydrolysate during storage

3.7.1. Antioxidative activities

The stability of antioxidative activity in freeze-dried round scad protein hydrolysate during storage at 25 °C and 4 °C is depicted in Fig. 2. Slight decreases in DPPH radical-scavenging activity of protein hydrolysate under both storage conditions were observed within the first week of storage ($p < 0.05$) and no marked changes were observed thereafter ($p < 0.05$) (Fig. 2(a)). However, no changes in reducing power or metal-chelating activity of the protein hydrolysate (under both storage conditions) were found within the first 2 weeks of storage ($p > 0.05$). Subsequently, slight decreases were noticeable ($p < 0.05$) (Fig. 2(a) and (b)). This might be due to the destruction of antioxidative compounds as the storage time increased, leading to some losses in antioxidative activity. From the results, protein hydrolysate was more stable when stored at 4 °C than at 25 °C.

3.7.2. Solubility

The solubility of the freeze-dried round scad protein hydrolysate was expressed as a percentage of soluble nitrogen compounds with respect to total nitrogen in each sample. Changes in solubility of the protein hydrolysate during storage at 25 °C and 4 °C are shown in Fig. 3. During 6 weeks of storage, the solubility of protein hydrolysate stored at both temperatures slightly decreased ($p < 0.05$). No substantial differences in solubility were observed between hydrolysates kept at 4 °C and 25 °C throughout the storage of 6 weeks. The decrease in solubility might be due to the aggregation of those peptides with the concomitant formation of a larger aggregate with the lowered solubility.

3.7.3. Colour

During storage, the hydrolysate was taken and dissolved to obtain the concentration of 10 mg/ml and the colour of resulting solution was determined (Table 5). No changes in L^*, a^* and b^* values of the solution of protein hydrolysate stored at 4 °C were observed up to 6 weeks of storage ($p > 0.05$). Conversely, significant changes in a^* and b^* values of the solution of protein hydrolysate stored at 25 °C

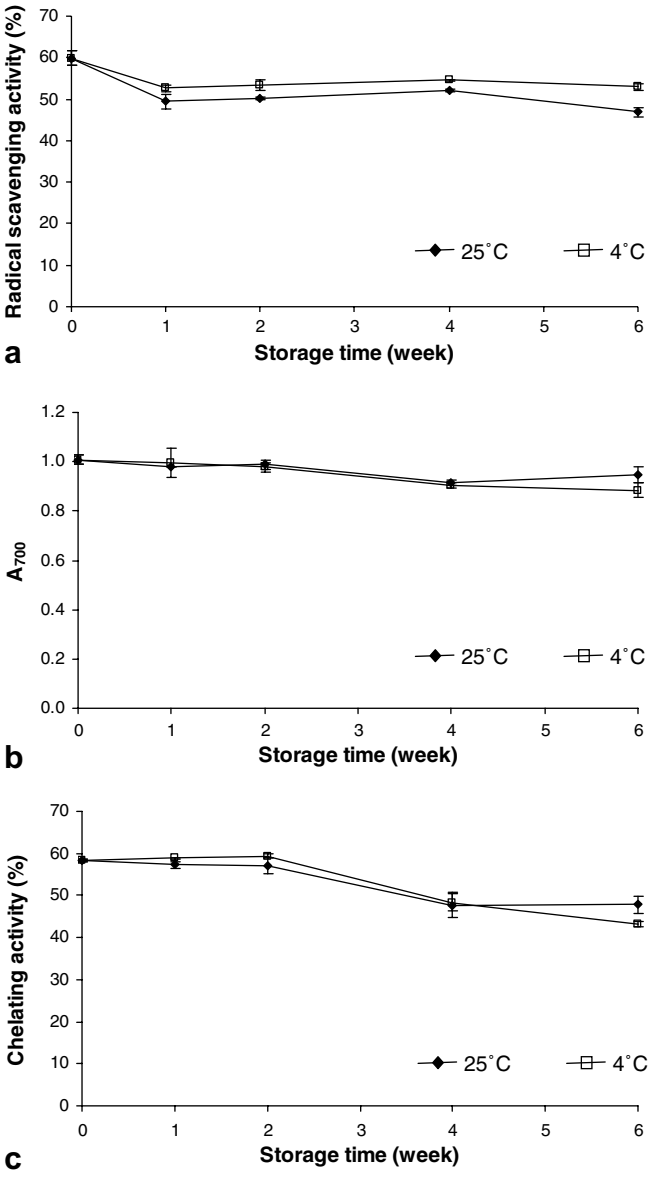

Fig. 2. Changes in DPPH radical-scavenging activity (a), reducing power (b) and chelating activity (c) of round scad protein hydrolysate during storage at 25 °C and 4 °C for 6 weeks. Bars represent the standard deviation from triplicate determinations.

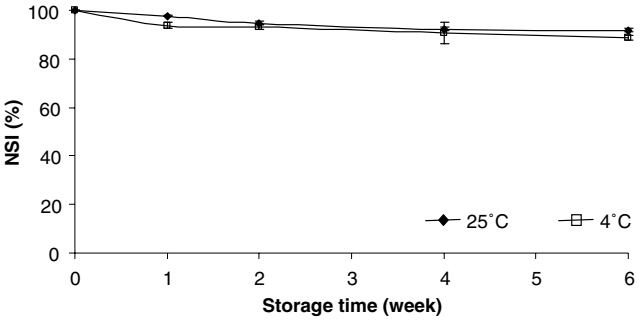

Fig. 3. Changes in solubility (nitrogen solubility index: NSI) of round scad protein hydrolysate during storage at 25 °C and 4 °C for 6 weeks. Bars represent the standard deviation from triplicate determinations.

were noticeable at week 6. The increase in b^* value indicated an increase in yellowness of samples during storage at 25 °C. Hoyle and Merritt (1994) reported that herring hydrolysates had decreases in lightness scores and increases in b values, which indicated darkening during storage. The formation of brown pigments might result from aldol condensation of carbonyls produced from lipid oxidation upon reaction with basic groups in proteins via Maillard reaction. The Maillard reaction is a non-enzymatic browning reaction and mainly contributes to the darkening of food products (Van Boekel, 1998). From the result, it appears that the colour of freeze-dried protein hydrolysate was stable for 6 weeks when stored at 4 °C and the changes in colour were more pronounced when storage temperature increased.

4. Conclusion

The protein hydrolysate derived from round scad may potentially serve as a good source of desirable quality peptides and amino acids. It could be used as an emulsifier and as a foaming agent with antioxidative activities. It was quite stable during storage at both 25 °C and 4 °C. Therefore, round scad protein hydrolysate can be used as a natural additive, possessing functionalities and antioxidative properties in food systems. However, the bitterness of protein hydrolysate should be evaluated and the appropriate debittering should be applied in order to obtain a fish

Table 5
Changes in L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values[A] of the solution (10 mg/ml) prepared from round scad protein hydrolysate stored at 25 °C and 4 °C for different times

Storage temperature (°C)	Colour parameter	Storage time (week)				
		0	1	2	4	6
25	L^*	12.50 ± 0.79^{ax}[B]	14.53 ± 1.66^{ax}	13.55 ± 1.54^{ax}	14.01 ± 3.16^{ax}	13.44 ± 2.30^{ax}
	a^*	-1.00 ± 0.15^{ax}	-1.15 ± 0.05^{ay}	-1.23 ± 0.01^{ax}	-1.19 ± 0.17^{ay}	-1.56 ± 0.23^{by}
	b^*	0.45 ± 0.13^{bx}	0.89 ± 0.06^{bx}	0.39 ± 0.06^{bx}	0.49 ± 0.72^{bx}	1.59 ± 0.15^{ax}
4	L^*	12.50 ± 0.79^{ax}	13.47 ± 0.61^{ay}	12.72 ± 0.81^{ay}	11.83 ± 1.38^{ay}	12.29 ± 0.43^{ay}
	a^*	-1.00 ± 0.15^{ax}	-0.99 ± 0.09^{ax}	-1.24 ± 0.22^{ax}	-1.02 ± 0.14^{ax}	-1.35 ± 0.27^{ax}
	b^*	0.45 ± 0.13^{ax}	0.37 ± 0.17^{ay}	0.36 ± 0.26^{ax}	0.08 ± 0.85^{ax}	0.76 ± 0.07^{ay}

[A] Means ± SD from triplicate determination.
[B] Different superscripts in the same row, a and b, indicate significant differences ($p < 0.05$). Different superscripts in the same column, x and y, within the same colour parameter indicate significant differences ($p < 0.05$).

protein hydrolysate with the sensorial acceptability for further applications.

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