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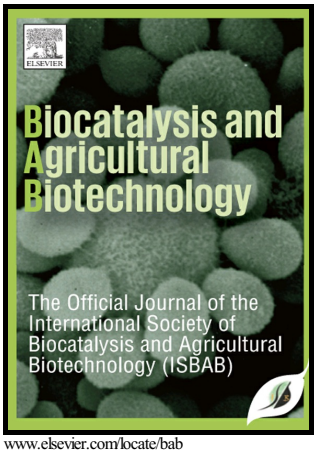

www.elsevier.com/locate/bab

PII: S1878-8181(18)30681-9
DOI: https://doi.org/10.1016/j.bcab.2018.12.013
Reference: BCAB948

To appear in: *Biocatalysis and Agricultural Biotechnology*

Received date: 9 October 2018
Revised date: 5 December 2018
Accepted date: 11 December 2018

Cite this article as: Pakteera Sripokar, Soottawat Benjakul and Sappasith Klomklao, Antioxidant and functional properties of protein hydrolysates obtained from starry triggerfish muscle using trypsin from albacore tuna liver, *Biocatalysis and Agricultural Biotechnology,* https://doi.org/10.1016/j.bcab.2018.12.013

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To be submitted to

Biocatalysis and Agricultural Biotechnology

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Abstract

Protein hydrolysates from starry triggerfish (*Abalistes stellaris*) muscle with a degree of hydrolysis (DH) of 60% were prepared using trypsin from albacore tuna (*Thunnus alalunga*) liver. The hydrolysates were investigated for antioxidant activity and functional properties. Antioxidant activities including DPPH (2, 2-diphynyl-1-picrylhydrazyl) radical scavenging activity, ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity, ferric reducing antioxidant power (FRAP) and metal chelating activity of hydrolysate samples were dose dependent. After being subjected to gastrointestinal model systems, the DPPH radical scavenging activity and metal chelating activity of the hydrolysates increased, especially in the duodenal condition, suggesting the enhancement of those activities of hydrolysates after ingestion. For functional properties, hydrolysis by the trypsin increased the solubility of hydrolysates to above 72.8% over a wide pH range. The hydrolysates possessed interfacial properties, which were governed by their concentrations. An increase in concentration of up to 2.0% (w/v) favoured the emulsifying activity index (EAI) and emulsion stability index (ESI), while a further increase to 3.0% (w/v) diminished emulsifying properties. Foam expansion and foam stability increased as the protein concentration increased. Therefore, the results of the present study suggest that starry triggerfish muscle can effectively be converted to protein hydrolysates, and the hydrolysates could be a potential ingredient in functional food as well as natural antioxidants in lipid food systems.

Keywords: Protein hydrolysates, Starry triggerfish, Antioxidant, Functional properties, Proteolysis, Trypsin

1. Introduction

Protein demand is growing; driven by a rising population, changing food preferences and a growing recognition of the importance of protein as a key ingredient for health and nutrition. The supplementation of dietary protein has been becoming more popular, especially for people on restrictive diets, athletes and the elderly (Egerton et al., 2018). Worldwide, fish protein hydrolysates (FPH) has been one of the most researched fish products of the last decade. The greater attention emerging towards FPH is due to their bioactive characteristics and growing global market. Previous studies of FPH have shown that, when added to food, they can contribute to water holding, emulsification and texture properties (Halim et al., 2016). Increased solubility is a frequently reported and valued property of FPH (Benjakul and Yarnpakdee, 2014). Bioactive characteristics such as antioxidation, antihypertension, antibacterial and antiprolifiration have also been reported (Halim et al., 2018; Khositanon et al., 2018; Singh and Vij, 2018; Song et al., 2016; Senphan and Benjakul, 2014; Khantaphant et al., 2011). Antioxadant characteristics can be an important feature for food preservation and providing potential health benefits (Phanturat et al., 2010). Numerous peptides derived from hydrolyzed fish protein have been shown to have antioxidant activities such as protein hydrolysates from the muscle of ornate threadfin bream (Nalinanon et al., 2011), brownstripe red snapper (Khantaphant et al., 2011), toothed ponyfish (Klomklao et al., 2013), seabass (Senphan and Benjakul, 2014), whole tilapia waste (Tejpal et al., 2017), blue whiting (Egerton et al., 2018) and eel (Halim et al., 2018). FPH can be used in food systems, comparable to other pertinent protein hydrolysates (Kristinsson and Rasco, 2000). The use of enzyme technologies for the recovery and modification of protein has led to the production of a broad spectrum of food ingredients and industrial products (Kristinsson and Rasco, 2000).

Enzymatic hydrolysis of food proteins is an efficient way to recover potent bioactive peptides (Thiansilakul et al., 2007). Proteases from different sources are commonly employed to obtain a more selective hydrolysis due to their specificity for peptide bonds adjacent to certain amino acid residues (Nalinanon et al., 2011; Klomklao et al., 2010).

Thailand is the world's largest producer and exporter of canned tuna. Albacore tuna is one of the most commercially important tuna species for the Thai tuna industry (Klomklao and Benjakul, 2018). Because of a large amount of tuna viscera, especially liver, discarded during processing, it can serve as a promising source of trypsin for further use. Trypsin (EC 3.4.21.4) recovery from tuna liver is an approach to minimize the economic and ecological problems of the manufacturing discard. Furthermore, FPH with bioactivity prepared with the aid of fish proteases can be obtained as a new value-added product with high market value (Khantaphant and Benjakul, 2008). Trypsin from albacore tuna liver was recently partitioned, purified and characterized (Sripokar et al., 2016a; Klomklao and Benjakul, 2018).

The starry triggerfish (*Abalistes stellaris*), or flat-tailed triggerfish, belongs to the order Tetraodontiformes and is a member of the Balistidae family (Sripokar et al., 2016b). This fish is used primarily for fish meal and are not considered palatable for direct human consumption in Thailand. To increase the value of this fish species, the production of new value-added products such as protein hydrolysates, with nutritive value and bioactivity, can pave the way for its full utilization. The use of trypsin from albacore tuna liver, for hydrolysate production, could lower the cost of commercial proteases. Based on our previous study, optimum conditions for starry triggerfish muscle hydrolysis using trypsin from albacore tuna liver were 5.5% trypsin, at 55 °C for 40 min reaction time and a fish muscle/buffer ratio of 1:3 (w/v). However, no information regarding the antioxidative activity and biochemical properties of starry triggerfish hydrolysates treated with the trypsin from albacore tuna liver has been reported. Therefore, the objectives of the present investigation

were to study the functionalities and antioxidant properties of protein hydrolysates prepared from starry triggerfish muscle using the trypsin from albacore tuna liver.

2. Materials and Methods

2.1. Chemicals

L-leucine, 2,4,6-trinitrobenzenesulfonic acid (TNBS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 3-(2-pyridyl)-5-6-diphenyl-1,2,4-triazine-4',4''-disulphonic acid sodium salt (ferrozine) and bovine serum albumin were procured from Sigma Chemical Co. (St. Louis, MO, USA.). Tris (hydroxymethyl) aminomethane (Tris), sodium sulfite, potassium persulphate, ferrous chloride and Folin-Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS) were procured from Bio-Rad Laboratories (Hercules, CA, USA). Pepsin from porcine gastric mucosa (EC 3.4.23.1) and pancreatin from porcine pancreas were procured from Sigma–Aldrich, Inc. (St. Louis, MO, USA). All of the chemicals used were of analytical grade.

2.2. Fish sample preparation

Liver of albacore tuna (*Thunnus alalunga*) were obtained from Tropical Canning (Thailand) Public Co. Ltd., Hat Yai, Songkhla. The samples were packed in polyethylene bags, kept in ice with a sample/ice ratio of 1:3 (w/w) and transported to the research laboratory within 2 h. Pooled internal organs were separated and only the liver was collected. Liver was cut and homogenized into powder in three volumes of acetone at -20 °C for 30 min according to the method of Klomklao et al. (2007). The homogenate was filtrated in vacuo on Whatman No. 4 filter paper (Whatman International Ltd., Maidstone, UK). The residue obtained was then homogenized in two volumes of acetone at -20 °C for 30 min. The residue was left at room temperature until dried and free of acetone odor.

Starry triggerfish (*Abalistes stellaris*) with a length of 30-35 cm were purchased from a dock in Trang, Thailand. The fish, off-loaded approximately 18-24 h after capture, were placed on ice at a fish/ice ratio of 1:2 (w/w) and transported to the research laboratory within 2 h. Upon arrival, the fish were filleted and the ordinary muscle was collected and ground to uniformity. A portion of mince (500 g) was placed in a polyethylene bag and stored at -20 °C until needed.

2.3. Trypsin preparation and activity assay

To prepare the albacore tuna trypsin extract, liver powder was suspended in 50 mM Tris-HCl buffer, pH 8.5, containing 0.2% Brij 35 at a ratio of 1:9 (w/v) and stirred continuously at 4 °C for 30 min. The suspension was centrifuged at 5,000×g at 4 °C for 30 min using a Sorvall Modell RC-B Plus centrifuge (Newtown, CT, USA). The supernatant was collected and referred to as "albacore tuna trypsin".

Albacore tuna trypsin was determined for trypsin activity using N^{α}-p-Tosyl-L-arginine methyl ester hydrochloride (TAME) as a substrate, as described by Klomklao and Benjakul (2018). Enzyme solution with an appropriate dilution (20 µl) was mixed with 3.0 ml of 1 mM TAME in 10 mM Tris-HCl buffer, pH 8.0 and incubated at 30 °C for 20 min. Production of *p*-tosyl-arginine was measured by monitoring the increment in absorbance at 247 nm. One unit of activity was defined as the amount causing an increase of 1.0 in absorbance at 247 nm per min.

2.4. Production of protein hydrolysates from starry triggerfish muscle

Starry triggerfish mince (20 g) was mixed with 0.1 M glycine-NaOH buffer, pH 8.5 at a ratio of 1:3 (w/v) and pre-incubated at 55 °C for 10 min (Klomklao et al., 2013; Sripokar et al., 2016b). The enzyme hydrolysis was started by adding albacore tuna trypsin at a level

of 5.5% (w/w). The reaction was conducted at pH 8.5 and 55 °C for 40 min. After 40 min of hydrolysis, the enzyme was inactivated by heating at 90 °C for 15 min in a water bath (Memmert, Schwabach, Germany). The mixture was then centrifuged at 5,000×g at 4 °C for 10 min. The supernatant was then collected and lyophilized using a Dura-TopTM lp freeze-dryer (FTS systems Inc., Stone Ridge, NY, USA). The freeze-dried protein hydrolysates obtained were subjected to analyses.

2.5. Determination of α-amino acids and DH

The α-amino acid content and DH was measured according to the method of Klomklao et al. (2013). To properly diluted hydrolysate samples (125 µl), 2.0 ml of 0.2 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 sulfite. The mixtures were cooled at room temperature for 15 min. The absorbance was read at 420 nm and α-amino acid was expressed in term of L-leucine. DH was calculated as follows:

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

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 starry triggerfish muscle homogenate. L_{max} is total α-amino acid in the original starry triggerfish muscle homogenate obtained after acid hydrolysis with 6 N HCl at 100 °C for 24 h.

2.6. Determination of antioxidative activity

The starry triggerfish protein hydrolysates with 60% DH was dissolved in distilled water at obtained concentrations of 5, 10, 20, 30 and 40 mg hydrolysates/ml. Antioxidant activity was determined as follows.

2.6.1. DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Nalinanon et al. (2011) and Laywisakul et al. (2017) with a slight modification. For the 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 dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The blank was prepared in the same manner, except that the distilled water was used instead of the sample. DPPH radical scavenging activity was calculated according to the following equation (Yen and Wu, 1999):

DPPH radical scavenging activity = $(1- (A_{517}$ of sample/A_{517} of control)) × 100

2.6.2. ABTS radical scavenging activity

ABTS radical scavenging activity was determined by ABTS assay, as described by Binsan et al. (2008). The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 50 ml methanol, in order to obtain an absorbance of 1.1±0.02 units at 734 nm using a spectrophotometer. Fresh ABTS solution was prepared for each assay. The sample (150 μl) was mixed with 2,850 μl of ABTS solution and the mixture was left at room temperature for 2 h in the dark. The absorbance was then measured at 734 nm using a spectrophotometer. The blank was prepared in the same manner, except that distilled water was used instead of the

sample. ABTS radical scavenging activity was calculated according to the following equation:

$$\text{ABTS radical scavenging activity} = (1 - (A_{734} \text{ of sample}/A_{734} \text{ of control})) \times 100$$

2.6.3. Ferric reducing antioxidant power (FRAP) assay

FRAP was assayed according to the method of Benzie and Strain (1996). Stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3 6H_2O$ solution. A working solution was prepared fresh by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3 6H_2O$ solution. The mixed solution was incubated at 37 °C for 30 min and was referred to as the FRAP solution. A sample (150 μl) was mixed with 2,850 μl of FRAP solution and kept for 30 min in the dark. The ferrous TPTZ complex (colored product) was measured by reading the absorbance at 593 nm. Increased absorbance of the reaction mixture indicates the increasing ferric reducing antioxidant power.

2.6.4. Metal chelating activity

The chelating activity on Fe^{2+} was determined using the method of Boyer and McCleary (1987) with a slight modification. The 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 at room temperature for 20 min. The absorbance was then measured at 562 nm. The blank was conducted in the same manner but distilled water was used instead of the sample. The chelating activity was calculated as follows:

$$\text{Chelating activity} = (1 - (A_{562} \text{ of sample}/A_{562} \text{ of control})) \times 100$$

2.7. pH and thermal stability of antioxidant peptides

Starry triggerfish protein hydrolysates were dissolved in distilled water to obtain the concentration of 40 mg protein/ml. The 5 ml of sample solutions were adjusted to pHs 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 with 1 or 6 M HCl and 1 or 6 M NaOH and incubated at room temperature for 30 min. The pHs of sample solutions were then adjusted to 7.0 with 1 M phosphate buffer. The final volume of all solutions was brought up to 20 ml using distilled water. The residual antioxidant activities were determined using DPPH and metal chelating assay.

To determine thermal stability, starry triggerfish protein hydrolysate at a concentration of 40 mg hydrolysates/ml were prepared using distilled water as a medium. The 5 ml of sample solutions were transferred into a screw-capped test tube. The tube was capped tightly and placed in a boiling water bath (100 °C) for 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. The treated samples were suddenly cooled in iced water. The sample without incubation (25 °C) was used as the control. The residual antioxidant activities were determined using DPPH and metal chelating assay.

2.8. Stability in gastrointestinal tract model system

Gastrointestinal tract model system was prepared according to the method of Lo et al. (2006) with a slight modification. The hydrolysate was dissolved in distilled water to obtain a concentration of 0.5 g/ml. The solution was adjusted to pH 2.0 with 1 M HCl and pepsin dissolved in 0.1 M HCl was added to obtain the final concentration of 40 g pepsin/kg hydrolysate. The mixture was incubated at 37 °C for 1 h with a continuous shaking (Memmert Model SV 1422, Schwabach, Germany). Thereafter, the pH of the reaction mixture was raised to 5.3 with 1 M NaOH before the addition of 20 g pancreatin/kg hydrolysates. Subsequently, the pH of the mixture was adjusted to 7.5 with 1 M NaOH. The

mixture was incubated at 37 °C for 3 h with a continuous shaking. The digestion was terminated by submerging the mixture in boiling water for 10 min. During digestion, the mixture was randomly taken at 0, 15, 30, 60, 90, 120, 150, 180 and 210 min for determination of DPPH radical scavenging activity and metal chelating activity.

2.9. Functional properties of protein hydrolysates

2.9.1. Solubility

To determine solubility of the hydrolysates, starry triggerfish hydrolysate samples (200 mg) were dispersed in 20 ml of deionized water and the pH of the mixture was adjusted to 3, 5, 7 and 9 with either 1 M HCl or 1 M NaOH. The mixture was stirred at room temperature for 30 min. The volume of solutions was made up to 25 ml by distilled water, previously adjusted to the same pH as the sample solution, prior to centrifugation at 5,000×g for 15 min. Protein content in the supernatant was determined using the Lowry method (Lowry et al., 1951), with bovine serum albumin as a standard. Total protein content in the same was determined after solubilization of the sample in 0.5 M NaOH. Solubility of the hydrolysates was calculated as follows:

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

2.9.2. Emulsifying properties

The emulsifying activity index (EAI) and the emulsion stability index (ESI) were used to measure the emulsifying properties of protein hydrolysate. EAI and ESI were determined according to the method of Pearce and Kinsella (1978) with a slight modification. Soybean oil (2 ml) and protein hydrolysate solutions (0.5%, 1.0%, 2.0% and 3.0%, 6 ml) were homoginized (Model T25 basic; IKA Labortecnik, Selangor, Malaysia) at a speed of 20,000 rpm for 1 min. An aliquot of the emulsion (50 μl) was pipette from the middle portion

of the container at 0 and 10 min after homogenization and subsequently diluted 100-fold using 0.1% SDS solution. The mixture was mixed thoroughly for 10 s using a vortex mixer (G-560E, Vortex-Genie 2, Scientific Industries, Inc., Bohemia, NY). Absorbance at 500 nm of the resulting dispersion was measured using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). EAI and ESI were calculated by the following formulae:

$$EAI\ (m^2/g) = (2 \times 2.303 \times A \times DF) / l\phi C$$

where A is A_{500}, DF is dilution factor (100), l is path length of cuvette (m), ϕ is oil volume fraction and C is concentration of hydrolysates in aqueous phase (g/m^3);

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

where ΔA is $A_0 - A_{10}$ and Δt is 10 min.

2.9.3. Foaming properties

Foam expansion (FE) and foam stability (FS) of hydrolysate solutions were determined according to the method of Shahidi et al. (1995) with a slight modification. Hydrolysate solutions (20 ml) with 0.5%, 1.0%, 2.0% and 3.0% concentrations were transferred into a 100-ml cylinder. The solutions were homogenized at 13,400 rpm for 1 min at room temperature. The samples were allowed to stand for 0, 30 and 60 min. FE and FS were then calculated using the following equations:

$$FE\ (\%) = (V_T/V_0) \times 100$$

$$FS\ (\%) = (V_t/V_0) \times 100$$

where V_T is total volume after whipping; V_0 is the original volume before whipping and V_t is total volume after leaving at room temperature for different times (30 and 60 min).

2.10. Statistical analysis

Experiments were run in triplicate using three different lots of samples. All data were subjected to analysis of variance (ANOVA) and differences between means were evaluated by Duncan's multiple range test (Steel and Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Antioxidative activities

3.1.1. DPPH radical scavenging activity

The relatively stable DPPH radical has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and thus to evaluate the antioxidant activity (Jao and Ko, 2002). The DPPH radical scavenging activity of protein hydrolysates from starry triggerfish muscle prepared using albacore tuna trypsin with the DH of 60% was measured at different concentrations (0, 5, 10, 20, 30 and 40 mg/ml) and the results are shown in Fig. 1a. The DPPH radical scavenging activity increased as the concentration of protein hydrolysates increased up to 20 mg/ml ($p<0.05$). However, no differences in activity were observed as concentration increased from 30 to 40 mg/ml. DPPH is a stable free radical that exhibits a maximal absorbance at 517 nm in ethanol. When DPPH encounters a proton-donating substance, such as an antioxidant, the radical is scavenged. The color is changed from purple to yellow and the absorbance is decreased (Khantaphant and Benjakul, 2008). Hence, starry triggerfish hydrolysates obtained could donate hydrogen atom to free radicals and become more stable diamagnetic molecule, leading to the termination of the radical chain reaction (Khantaphant and Benjakul, 2008). Nevertheless, the efficiency in hydrogen donation of peptides produced was governed by their concentration.

3.1.2. ABTS radical scavenging activity

ABTS radical assay determine the antioxidative activity, in which the radical is quenched to form ABTS-radical complex (Khantaphant and Benjakul, 2008). With increasing concentration, protein hydrolysate showed increased ABTS radical scavenging activity ($p<0.05$) (Fig. 1b). The highest ABTS radical scavenging activity was found in hydrolysate with 40 mg/ml (47%). ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating compounds (scavengers of aqueous phase radicals) and of chain-breaking antioxidants (scavenger of lipid peroxyl radicals) (Binsan et al., 2008). The amino acid sequence in peptides might affect the antioxidant activity. The ABTS radical is relatively stable and is readily decreased by antioxidants (Klomklao et al., 2013). With high ABTS radical scavenging activity, it was postulated that antioxidative compounds were most likely hydrophilic.

3.1.3. FRAP

FRAP of starry triggerfish protein hydrolysates was investigated at different concentrations ranging from 0 to 40 mg/ml and the results are illustrated in Fig 1c. FRAP increased significantly with the increase in the concentration of hydrolysates. The greater reducing power indicated that hydrolysates could donate an electron to free radicals, leading to the prevention or retardation of propagation (Klomklao et al., 2013). Results obtained were in agreement with the published reports (Intarasirisawat et al., 2012; Tejpal et al., 2017). FRAP is generally used to measure the capacity of a substance in reducing TPTZ-Fe(III) complex to TPTZ-Fe(II) complex (Binsan et al., 2008). In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound (Thiansilakul et al., 2007). The results suggested that antioxidative compounds in protein hydrolysates tested showed higher FRAP when higher concentrations

were used. The differences in FRAP might be governed by peptides in the hydrolysates. The reducing power of starry triggerfish protein hydrolysate could be used to reduce DNA damage, mutagenesis, carcinogenesis and inhibition of pathogenic bacterial growth (Gulcin et al., 2010).

3.1.4. Metal chelating activity

As the concentration increased up to 20 mg/ml, chelating activity on Fe^{2+} of starry triggerfish protein hydrolysates increased ($p<0.05$) (Fig. 1d). However, no changes of metal chelating activity of hydrolysates with a concentration of more than 20 mg/ml was observed. Peptides in hydrolysates could chelate the prooxidants, leading to decreased lipid oxidation (Klomklao et al., 2013). The chelation of Fe^{2+} represents the ability of hydrolysates in metal chelating (Nalinanon et al., 2011). Ferrozine quantitatively forms complexes with Fe^{2+} ion. In the presence of chelating agents the complex formation is disrupted, affecting the decrease in color formation (Thiansilakul et al., 2007). The chelation of transition metal ions by an antioxidant or antioxidative peptide would reduce the availability of pro-oxidative metal ions, and could also by this means reduce lipid oxidation (Thammarat et al., 2015). From the results, hydrolysates from starry triggerfish muscle treated with trypsin from albacore tuna liver had a potential chelating ability toward iron and the metal chelating activity of starry triggerfish protein hydrolysates was dose dependent.

3.2. Functional properties of protein hydrolysates

3.2.1. Solubility of the hydrolysates

The solubility of the hydrolysates of the lyophilized protein hydrolysates from starry triggerfish muscle obtained from trypsin from albacore tuna liver was determined at pH 3.0, 5.0, 7.0 and 9.0 and the results are shown in Fig 2. All hydrolysates were soluble over a wide pH range, in which more than 72% solubility was obtained. The minimum solubility

values were presented at pH 5.0 (72.8% solubility) and maximum solubility values at pH 3.0 (94.0% solubility) were observed ($p<0.05$). The change in solubility can be attributed to the net charge of the amino acid residues after the hydrolysis process, which increases as the pH moves away from the isoelectric point, promoting the aggregation of hydrophobic interaction (Taheri et al., 2013). The solubilities of protein hydrolysates were quite low at pH 4 as reported in salmon by products (Gbogouri et al., 2004) and yellow stripe (Klompong et al., 2007). The same result was also reported in silver carp (Dong et al., 2008) which showed decrease in solubility at the pH 4-5 and drastically increased with increase in pH. The result suggested that near pH 4-5 proteins hydrolysates with high molecular weight (MW) remaining after hydrolysis were precipitated at this pH, which was close to the isoelectric point (pI). The pH affects the charge on the weakly acidic and basic side chain groups and hydrolysates generally show low solubility at their isoelectric points (Naqash and Nazeer, 2013). Solubility is one of the most important functional properties of a protein and can be increased by the hydrolysis process (Klompong et al., 2007). 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). Enzymatic hydrolysis potentially affects the molecular size and hydrophobicity, as well as polar and ionizable groups of protein hydrolysates (Klompong et al., 2007). The balance of hydrophilic and hydrophobic forces of peptides is another crucial influence on solubility (Gbogouri et al., 2004).

3.2.2. Emulsifying properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) of hydrolysates from starry triggerfish muscle with different concentrations (0.5%, 1.0%, 2.0% and 3.0% w/v) are shown in Fig 3. Both EAI and ESI were concentration-dependent (Fig 3a,

3b). An increase in concentration favoured emulsifying activity up to 2.0% (w/v) concentration, while a further increase in concentration at 3.0% (w/v) reduced emulsifying activity (Fig 3a). Similarly, the initial increase in concentration favoured increase emulsion stability up to 2.0%, after which a decline was observed with a further increase in concentration (Fig 3b). The initial increase in concentration of hydrolysates facilitated enhanced interaction between the oil phase and the aqueous phase. However, as the concentration increased, a point was reached where a further increase in concentration led to an accumulation of proteins in the aqueous phase. This development resulted in a decrease of emulsifying activity (Lawal, 2004). Emulsion stability diminished after 2.0%, presumably due to an increase in protein-protein interaction at the expense of protein and peptide-oil interaction. Lin and Chen (2006) proposed that the emulsification process includes two steps: (1) deformation and disruption of droplets which increase the specific surface area of emulsion and (2) stabilization of this newly-formed interface by emulsifier or surfactant. Protein hydrolysates are surface-active materials and promote an oil-in-water emulsion due to their hydrophilic and hydrophobic groups and their charge (Kristinsson and Rasco, 2000; Klomklao et al., 2013). From the results, emulsifying characteristics of hydrolysates from starry triggerfish muscle with 60% DH were governed by concentration employed.

3.2.3. Foaming properties

Foam expansion and foam stability of starry triggerfish protein hydrolysates at various concentrations (0.5%, 1.0%, 2.0% and 3.0%) are depicted in Table 1. Foam expansion at 0 min after whipping indicated the foam abilities of protein hydrolysates, which increased from 128.89% to 208.89% when hydrolysate concentrations increased from 0.5% to 3.0% ($p<0.05$). Sanchez and Panito (2005) reported that an increase in protein concentration resulted in a higher rate of diffusion. Foam expansion after whipping was

monitored for 30 and 60 min to indicate the foam stability of protein hydrolysates. Formation of foam is governed by three factors, including transportation, penetration and reorganization of molecules at the air-water interface (Klomklao et al., 2013). Foam expansion after whipping for 30 and 60 min was monitored to indicate the foam stability of starry triggerfish protein hydrolysates. Foam stability was increased with increasing concentration of the hydrolysates. The results suggest that foam stability of the hydrolysates from starry triggerfish muscle was improved by increasing concentration. A similar result was also reported in protein hydrolysates from toothed ponyfish muscle produced with viscera extract from hybrid catfish (Klomklao et al., 2013). 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 et al., 1994).

3.3. pH and thermal stability of antioxidant peptides

The stability of bioactive peptides to pH is an important criteria for gastrointestinal (GI) stability, because food encounters different pH at different digestion stages. Gastric pH is considered among one of the important factors affecting the survival of bioactive molecules during their passage through the stomach (Singh and Vij, 2018). The pH in the human stomach ranges from 2 to 5, and food will take at least 2 h to pass through stomach after ingestion (Plessas et al., 2017). On the other hand, bile in the large intestine maintains an almost neutral pH (Plessas et al., 2017). The influences of pH on the stability of antioxidant peptides are depicted in Fig. 4a. DPPH radical scavenging activity and metal chelating activity of the antioxidant peptide remained constant over the pH range of 1-10. At pH 11, DPPH radical scavenging activity and metal chelating activity slightly decreased ($p<0.05$). The results suggested that antioxidant peptides exhibiting DPPH radical scavenging

activity and metal chelating activity might lose their activity to some extent at high pH. Due to the stability over a wide pH range, antioxidant peptides from the muscle of starry triggerfish have potential for application in any food system at extreme pH.

Thermal stability of bioactive peptides are important because food products undergo several heat treatments before they reach the market. Generally, thermal treatment can cause protein denaturation, association, and aggregation (Singh and Vij, 2018). Thermal stability of antioxidant activity of the starry triggerfish protein hydrolysates with 60% DH as monitored by DPPH radical scavenging activity and metal chelating activity assay is shown in Fig. 4b. DPPH radical scavenging activity and metal chelating activity of hydrolysate were stable when heated at 100 °C up to 180 min, where activities of 100% were retained. In general, proteins are heat sensitive, which can lead to their aggregation. However, it has been reported that low molecular weight peptides are heat-stable (Nalinanon et al., 2011). Smaller size peptides were more stable to aggregation at high temperatures (Zayas, 1997). These results indicate that starry triggerfish protein hydrolysates with 60% DH could be incorporated in cooked food systems without a significant loss of their antioxidant activities.

3.4. Changes in antioxidative activity in gastrointestinal tract model systems (GIMs)

GIMs was used to simulate the ingestion system of human body and the remaining antioxidative activities of starry triggerfish protein hydrolysates were monitored (Fig. 5). When protein hydrolysates were orally administrated, their bioactive peptides should be resistant to hydrolysis by digestive proteases in order to be adsorbed and reach the target organ to function as an antioxidant (Burkitt, 2001). Starry triggerfish protein hydrolysates showed a slight increase in DPPH radical scavenging activity and metal chelating activity during pepsin digestion ($p<0.05$). Pepsin might hydrolyze starry triggerfish protein hydrolysates to some degrees, yielding the new peptides with DPPH radical scavenging

activity and metal chelating activity. The further increases in DPPH radical scavenging and metal chelating activities were also found in the intestinal simulated system up to 150 min. Thereafter, no changes in DPPH radical scavenging and metal chelating activities were found during 150 and 210 min of incubation ($p>0.05$). The result suggested that pancreatin might cleave the peptides to some degrees, leading to the release of new antioxidative peptides. This could enhance the antioxidative activities of protein hydrolysates. Generally, gastrointestinal tract actually leads to the generation of more potent bioactive peptides (Megías et al., 2009). Khantaphant et al. (2011) found increased antioxidative activity of protein hydrolysates from the muscle of brownstripe red snapper using flavourzyme after being ingested in the simulated model system. Senphan and Benjakul (2014) also reported that when the hydrolysates from seabass skin prepared using ammonium sulphate precipitated fraction from Pacific white shrimp hepatopancreas with 40% DH was subjected to GIMs, ABTS radical scavenging activity and chelating activity increased, especially in the duodenal condition. The antioxidative activities of protein hydrolysates after incubation in GIMs were dependent on peptides in the hydrolysates, in terms of size, amino acid composition and sequence, which could be targeted by digestive proteases (Megías et al., 2009). From the results, the antioxidative activity of starry triggerfish hydrolysates could be preserved after treatment with these gastrointestinal enzymes in GIMs. Therefore, the antioxidative activities of starry triggerfish hydrolysates were more likely preserved after digestion in the real gastrointestinal tract of the human body.

4. Conclusion

The protein hydrolysates derived from starry triggerfish using trypsin from albacore tuna liver appear to be good sources of desirable peptides. The protein hydrolysates could be used as an emulsifier and as a foaming agent with antioxidant activities. Hence, starry

triggerfish protein hydrolysates can be used in food systems as a natural additive possessing antioxidative properties. Furthermore, based on pH and thermal stabilities as well as GI digestion, antioxidant peptides in protein hydrolysates can be incorporated as a multifunctional ingredient into foods.

5. Acknowledgments

This research was supported by the Thailand Research Fund and Thaksin University for Project No. RSA6080044 to Dr. Sappasith Klomklao.

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Table 1 Foaming properties of starry triggerfish protein hydrolysate at varying concentrations

Hydrolysate concentrations (%, w/v)	FE* (%)	FS* (%)	
		30 min	60 min
0.5	128.89±1.92a**	118.89±1.92a	97.78±3.85a
1.0	147.78±1.94b	131.48±1.70b	115.56±5.09b
2.0	187.78±1.92c	154.44±1.92c	122.20±8.39bc
3.0	208.89±10.18d	170.00±8.82d	130.00±3.33c

*Mean ± SD from triplicate determinations.

**Different letters in the same column indicate significant differences ($p<0.05$).

Figure Legends

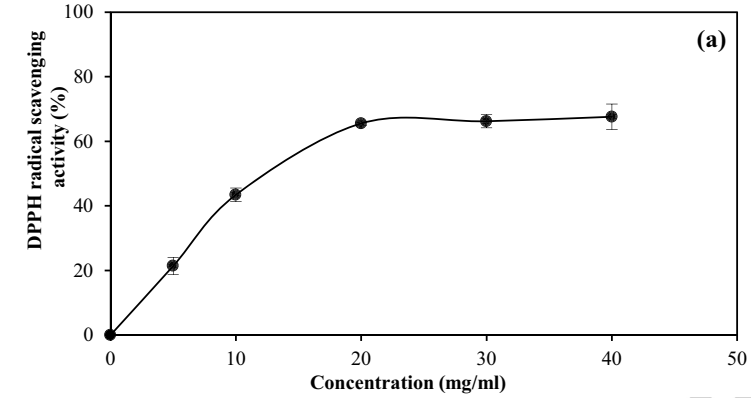
Fig 1. DPPH radical (a) and ABTS radical (b) scavenging activity, ferric reducing antioxidant power (c) and metal chelating activity (d) of starry triggerfish protein hydrolysate at different concentrations. Bars represent the standard deviation from triplicate determinations.

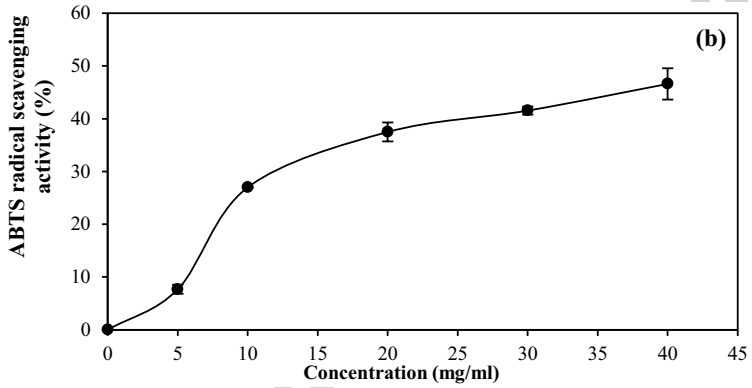
Fig 2. Solubility of starry triggerfish protein hydrolysates at various pHs. Bars represent the standard deviation from triplicate determinations.

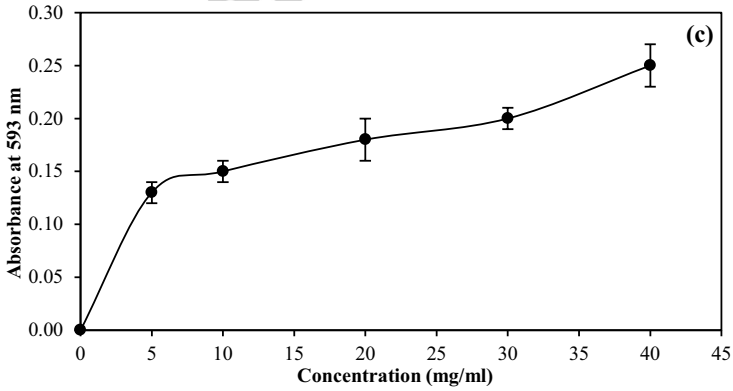
Fig 3. Effect of concentration of starry triggerfish protein hydrolysate on emulsifying activity index (a) and emulsion stability index (b). Bars represent the standard deviation from triplicate determinations.

Fig 4. Effect of pH (a) and heating time (b) on DPPH radical scavenging activity and metal chelating activity of starry triggerfish protein hydrolysate. Bars represent the standard deviation from triplicate determinations.

Fig 5. Antioxidative activities of hydrolysate from starry triggerfish protein hydrolysate in gastrointestinal tract model system. Bars represent the standard deviation from triplicate determinations.







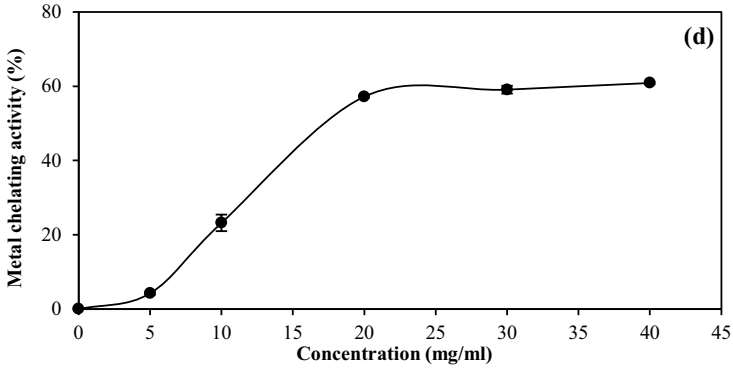
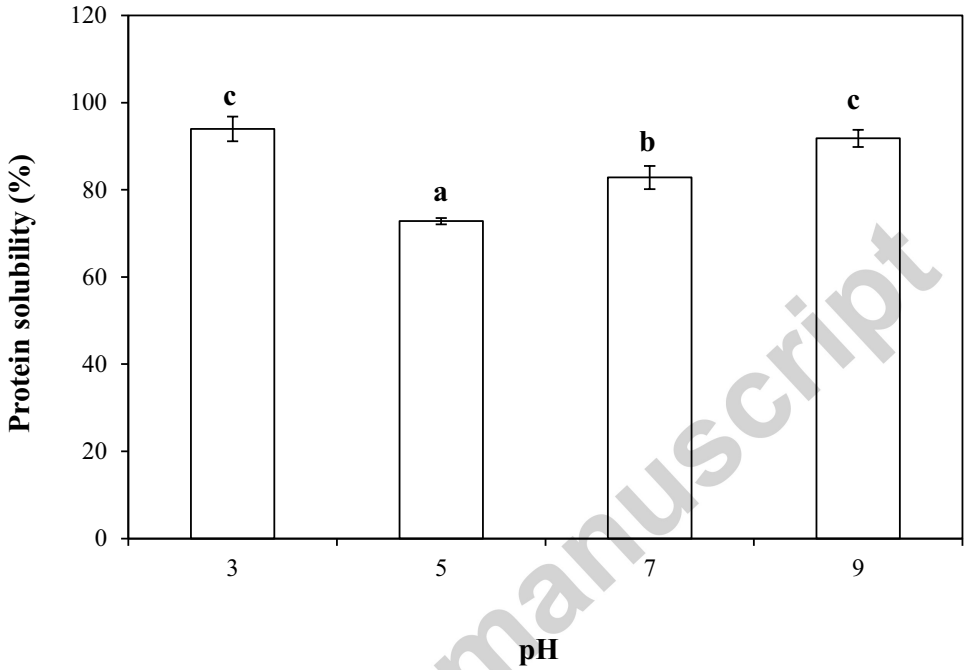


Fig. 1



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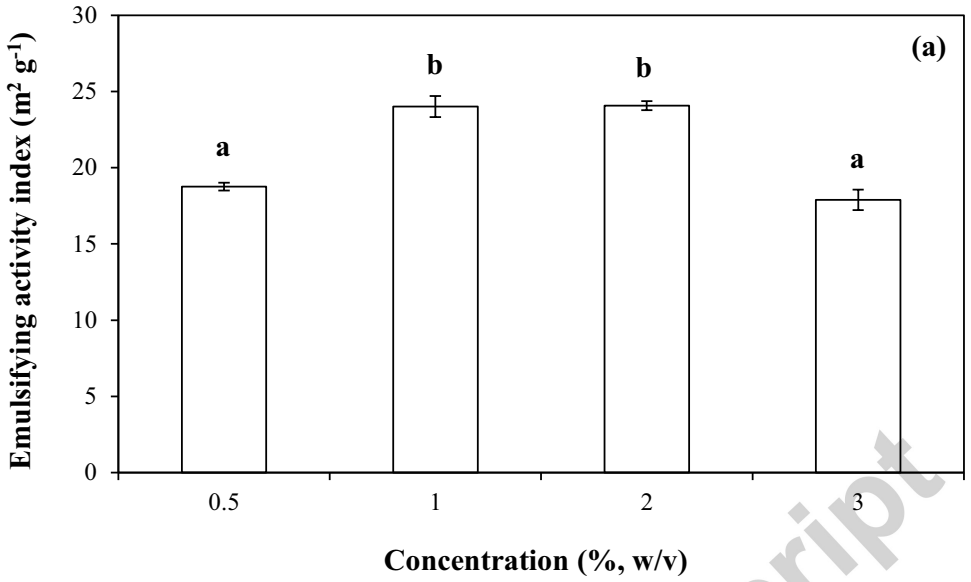

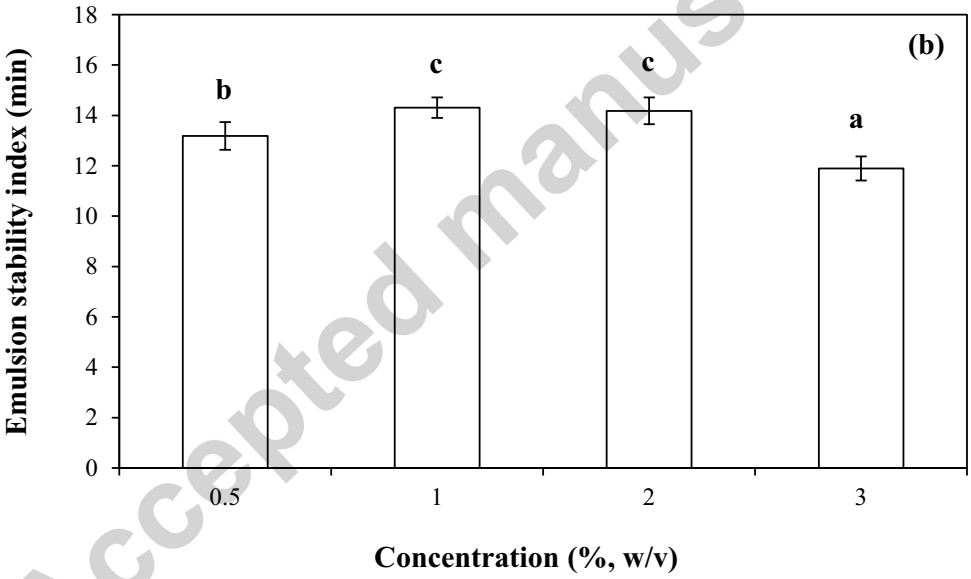

Fig. 3.

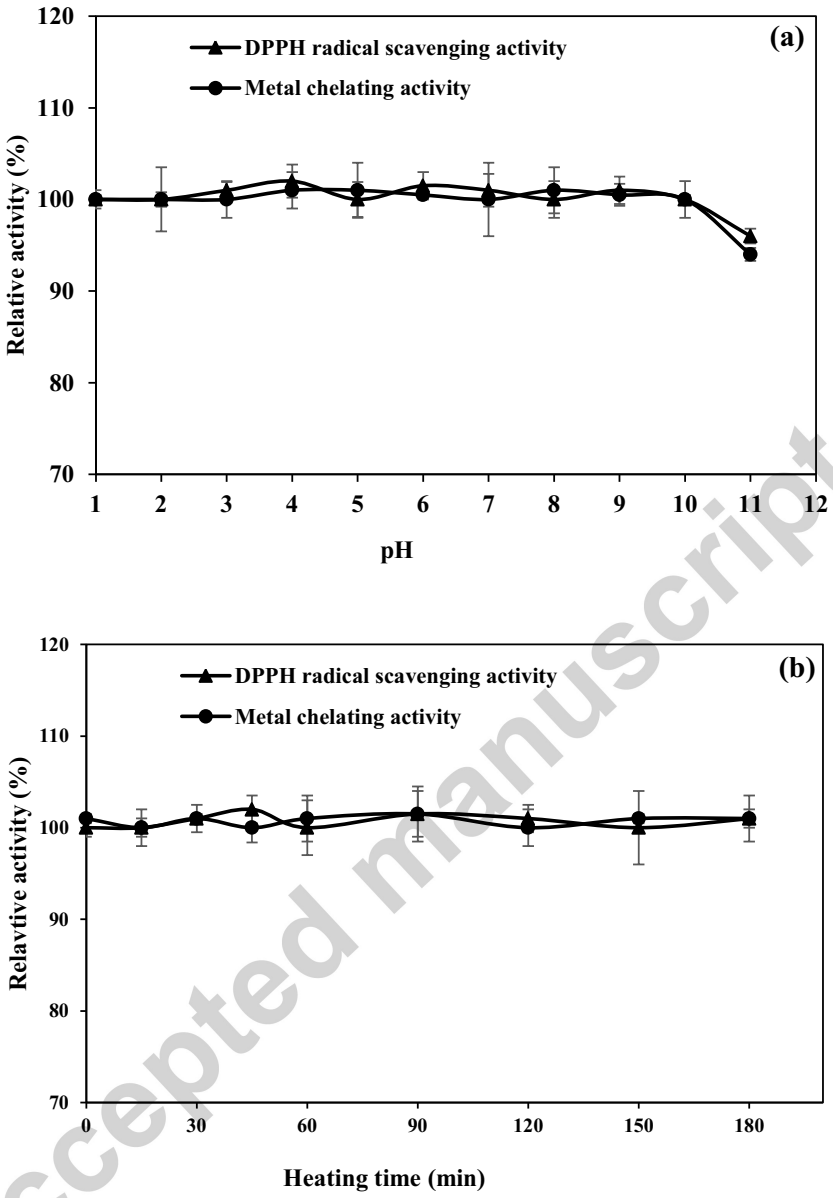

Fig. 4.

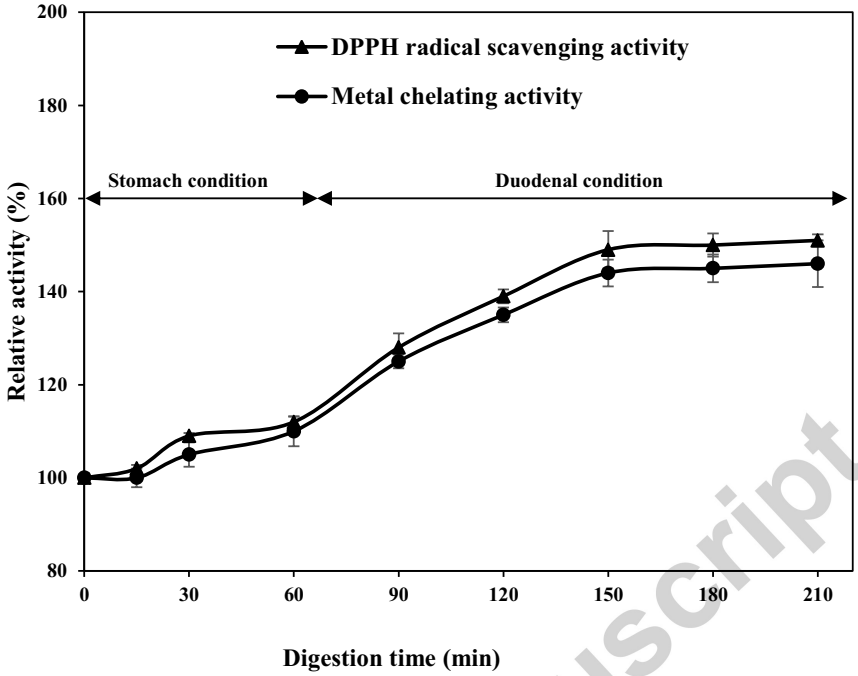

Fig. 5.

Highlights

- Starry triggerfish hydrolysates possessed antioxidative activities.
- The antioxidant activity was increased after pepsin and pancreatin treatments.
- The solubility was between 72.8% and 94.0%.
- The hydrolysates could potentially be useful as a functional food ingredient.