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**Protein hydrolysates from Pacific white shrimp cephalothorax manufactured with
different processes: Compositions, characteristics and antioxidative activity**

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Abstract

Composition, characteristics and antioxidative activity of the hydrolysate from cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*) prepared using various hydrolysis processes were studied. Those processes consisted of autolysis (AU), hydrolysis using Alcalase at 0.5% (0.5A) or 1.0% (1.0A) and autolysis, followed by Alcalase hydrolysis at 0.5 and 1.0% (AU+0.5A and AU+1.0A). All hydrolysate samples had the higher protein content (86.04-89.24%) and lower amounts of ash (7.46-11.26%) and lipid (0.43-0.64%), compared to those of cephalothorax ($P<0.05$). The highest yield (54.04%) and protein recovery (84.15%) were observed in AU+1.0A sample, which had the maximum degree of hydrolysis (DH) (44.93%) ($P<0.05$). All hydrolysates had glutamic acid/glutamine (115.80-121.69 mg/g dry sample), aspartic acid/asparagine (84.04-90.28 mg/g dry sample), arginine (63.27-68.62 mg/g dry sample) and leucine (58.67-68.07 mg/g dry sample) as the dominant amino acids. Based on gel filtration chromatography, the hydrolysate with higher DH showed higher amount of smaller peptides with MW lower than 1,355 Da. When antioxidant activities of hydrolysates were determined, AU+1.0A sample had the highest ABTS radical scavenging activity, ferrous ion chelating activity and ORAC value, compared to others ($P<0.05$). However, the highest ferric reducing antioxidant activity and DPPH radical scavenging activity were obtained for 1.0A and AU samples, respectively ($P<0.05$). Furthermore, AU+1.0A sample showed higher protective effect against DNA damage induced by peroxyl radical than 1.0A and AU samples. Therefore, different hydrolysis processes directly affected the protein recovery, chemical composition and antioxidant activities of hydrolysate from the cephalothorax of Pacific white shrimp.

Keywords: Cephalothorax, Protein hydrolysate, Autolysis, Alcalase hydrolysis, Antioxidative activities

Statement of Novelty

The present study provides the newly developed process in utilization of cephalothorax using two-step hydrolysis, in which prior autolysis, followed by hydrolysis using Alcalase under the optimal condition was used. A new hydrolysis process potentially recovered protein from shrimp cephalothorax. The resulting hydrolysate with high yield exhibited the antioxidative activities with different modes of actions. It was also shown to prevent the DNA damage induced by radicals. Thus, the protein hydrolysate from aforementioned byproduct as the new functional ingredient can increase the revenue for the industry via the production of value added products with high nutrition. Additionally, it can reduce the disposal of byproducts, which may cause the environmental problem.

1. Introduction

Pacific white shrimp and its products have become economically important for Thailand [1]. It accounts for 90% of the global aquaculture shrimp production [2,1]. By the year 2012, frozen Pacific white shrimp and products were manufactured and exported over 500,000 tons, particularly to the USA and Japan. During shrimp processing, approximately 40-50% of its total weight are generated as by-products. Those contained 71.4% cephalothorax and 28.6% shell, which are rich in amino acids, peptides, proteins and other components [1,3]. Those leftovers generally have been transformed to animal feed and aquaculture diets [4]. Shrimp cephalothorax is an essential source of protein (50-65%, dry weight basis) and also serves as a source of lipid (11% dry weight basis), chitin (11% dry weight basis), enzymes and other nutritive components [5,4]. The utilization of shrimp cephalothorax by conversion to protein hydrolysate can be a promising approach to eliminate harmful environmental aspects as well as earn the revenue for shrimp processing industry [6].

Protein hydrolysates have attracted increasing interest as potential ingredients for many health-promoting functional foods due to biologically active peptides [5,7]. The recovery of proteins or peptides from shrimp waste by hydrolysis has been widely investigated [8,5,3,9-11]. Endogenous proteases were reported in hepatopancreas [12]. Under optimal condition, those proteases could autolysis [8]. Autolysis and enzymatic hydrolysis of cephalothorax depend on several factors including pH, temperature, time as well as enzyme/substrate ratio [6,1]. Cao et al. [8] reported that optimum autolysis condition for protein recovery of cephalothorax from *Penaens vannamei* included 50 °C, pH 7.85 and a substrate concentration at 23% (w/v), in which the highest degree of hydrolysis (45%) was obtained. The autolysis of shrimp head by gradual increase in temperature (40-60 °C) at 5 °C/30 min resulted in the higher protein recovery (87.4%), compared with autolysis performed at different single temperature (40, 50, and 60 °C) (43.6-73.6%) [5]. Additionally, a number of commercial proteases have been used for the production of protein hydrolysis from seafood processing by-products. Dey, Dora [6] suggested that protein recovery of hydrolysate from shrimp waste using Alcalase was higher than those using Neutrase, Protamex and Flavourzyme. Sila et al. [11] also documented that the protein hydrolysate from shrimp waste of deep-water pink shrimp was produced by Alcalase hydrolysis. Hydrolysate contained 80.8% protein, 2.74% lipid, 14.4% ash, 1.13% chitin and 1.08 µg carotenoid/g sample.

To maximize the hydrolysis of proteins from cephalothorax, autolysis could be exploited. The liberated protein or peptides more likely served as the proteinaceous substrate for subsequent hydrolysis by commercial proteases. The hydrolysates containing varying peptides from different hydrolysis processes might possess different compositions as well as bioactivities, especially antioxidant activity. Nevertheless, a little information regarding the prior autolysis in combination with subsequent enzymatic hydrolysis for production of

protein hydrolysate from shrimp cephalothorax exists. Thus, this study aimed to develop the hydrolysis process rendering the hydrolysate with increased yield and antioxidative activity.

2. Materials and methods

2.1 Chemicals

Alcalase from *Bacillus licheniformis* (20 unit/g dry matter) was obtained from Novozyme (Bagsvaerd, Denmark). 2,4,6-trinitrobenzenesulphonic acid (TNBS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt (ferrozine) 2,2'-azobis (2-methylpropionamidine) (AAPH) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Plasmid DNA (pUC 18) and SYBRTM Gold DNA Gel Stain was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). SephadexTM G-25, blue dextran and gel filtration calibration kit (vitamin B12, flavin adeninedinucleotide and glycine-tryrosine) were obtained from GE Healthcare (Uppsala, Sweden). All chemicals were of analytical grade.

2.2 Collection and preparation of cephalothorax from Pacific white shrimp

Cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*) were obtained from the Sea wealth frozen food Co., Ltd., Songkhla province, Thailand. Cephalothorax of shrimp with the size of 50–60 shrimp/kg (5 kg) were placed in a polyethylene bag. The bag was imbedded in a polystyrene box containing ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai,

Songkhla, within approximately 2 h. The sample was stored at -20 °C until used, but the storage time was not longer than 2 weeks.

2.3 Production of hydrolysate from shrimp cephalothorax

2.3.1 Preparation of cephalothorax

Frozen shrimp cephalothorax were ground using a blender (Phillips, Guangzhou, China) for 3 min. Ground sample was placed in a polyethylene bag and embedded in ice until used.

2.3.2 Hydrolysis of cephalothorax using different processes

Hydrolysis was performed following the method of Holanda, Netto [13] with a slight modification. Ground sample was mixed with distilled water at a ratio of 1:1 (w/v) and then homogenized at 11000 rpm for 2 min using a homogenizer model T25 digital (IKA®-Werke GmbH & CO.KG, Stanfen, Germany). The pH of homogenate was adjusted to 8.0 using either 1.0 M NaOH or 1.0 M HCl. Autolysis was conducted at 50 °C for 3 h in a temperature controlled water bath (Model W350, Memmert, Schwabach, Germany). Autolysis was terminated by submersing the mixture in hot water (90 °C) for 15 min.

For one-step hydrolysis processes, 0.5 or 1.0% Alcalase (w/w, solid content) was added into the homogenate (pH 8.0), pre-incubated at 60 °C for 15 min. The mixture was mixed well and hydrolysis was carried at 60 °C for 2 h. Enzyme inactivation was performed by subjecting the mixture to heating at 90 °C for 15 min.

For two-step hydrolysis processes, the homogenate (pH 8.0), was firstly incubated at 50 °C for 3 h for initial autolysis. After autolysis, the mixture was pre-incubated at 60 °C for 15 min. Subsequently, Alcalase (0.5 or 1.0% w/w) was added into the mixture. Hydrolysis was conducted at 60 °C for 2 h. Enzymatic reaction was terminated as previously described.

All the obtained mixtures were cooled down to room temperature (25 °C) using a running water. The mixtures were filtered with two layers of cheesecloth to remove the undigested carapace and pereopods (solid phase). The resulting filtrates were centrifuged at 4000xg at 4 °C using a refrigerated centrifuge model Avanti® J-E (Beckman Coulter, Inc., Palo Alto, CA, USA) for 15 min. Then, the supernatants were freeze-dried using a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark) for 72 h. The hydrolysate from autolysis was named “AU”, while those from one-step hydrolysis process with the aid of 0.5 and 1.0% Alcalase were referred to as “0.5A” and “1.0A”, respectively. The hydrolysates attained from two-step hydrolysis process using autolysis, followed by hydrolysis with 0.5 and 1.0% Alcalase were termed “AU+0.5A” and “AU+1.0A”, respectively. All the hydrolysate samples were then subjected to analyses.

2.4 Analyses

2.4.1 Proximate analysis

Ground cephalothorax and hydrolysate samples were analyzed for moisture, protein, fat and ash contents using the analytical method no. of 950.46, 920.153, 960.39 and 928.08, respectively [14]. The conversion factor used for calculation of protein content was 6.25. Chitin content was determined according to the method of Senphan et al. [9] with a slight modification. Samples (2 g) were mixed with 30 ml of 1.25 M NaOH at 100 °C for 3 h. The mixture was filtered under vacuum using a Whatman No.1 filter paper. The residue was shaken with 30 ml of 1 M HCl for 30 min at 25 °C, filtered and washed with distilled water. The washed residue was then homogenized with cold acetone (-20 °C) at a speed of 13,000 rpm using a homogenizer for 3 min to remove lipids and pigments. After washing with 3 volumes of distilled water, 30 ml of 0.5% NaOCl were mixed with the sample and stirred for 30 min at 25 °C. The mixture was then filtered and washed with distilled water. The residue

was dried at 60 °C for 24 h using an oven (Memmert, Schwabach, Germany). The dried matter referred to as “chitin” was weighed.

2.4.2 Yield and protein recovery

The yield of hydrolysate was calculated based on dry weight of initial shrimp cephalothorax after drying at 105 °C for 12 h in a hot air oven.

$$\text{Yield (\%)} = \frac{\text{weight of dry hydrolysate (g)}}{\text{weight of dried initial sample used (g)}} \times 100$$

Protein recovery of hydrolysate was calculated based on the initial amount of protein present in the initial cephalothorax as determined by the method of AOAC [14].

Protein recovery

$$(\%) = \frac{\text{protein content of supernatant (g/ml)} \times \text{volume of supernatant (ml)}}{\text{protein content of initial sample used (g/g)} \times \text{weight of initial sample used (g)}} \times 100$$

2.4.3 Determination of total carotenoid content

Total carotenoid content was determined according to the method of Senphan et al. [9] with a slight modification. After being extracted and properly diluted, the absorbance of samples was read at 468 nm. The content of carotenoid in hydrolysate sample was calculated using the equation given by Saito, Regier [15]:

$$\text{Total carotenoid (}\mu\text{g/g sample)} = \frac{A_{468} \times \text{volume of extract} \times \text{dilution factor}}{0.2 \times \text{weight of sample used (g)}}$$

where 0.2 is the A_{468} of 1 $\mu\text{g/ml}$ standard astaxanthin.

2.4.4 Amino acid analysis

Amino acid composition of the cephalothorax and hydrolysate samples was analyzed as described by Benjakul et al. [16]. The samples were hydrolyzed under reduced pressure in 4 M methanesulphonic acid containing 0.2% (v/v) 3-2(2-aminoethyl)indole at 115 °C for 24

h. The hydrolyzed samples were neutralized with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of 0.04 ml was applied to an amino acid analyzer (MLC-703; Atto Co., Tokyo, Japan).

2.4.5 Determination of degree of hydrolysis (DH)

DH of hydrolysate was determined according to the method of Benjakul, Morrissey [17]. Hydrolysate samples were determined for free amino group content using 2,4,6-trinitrobenzenesulfonic acid (TNBS). DH was calculated as follows:

$$DH = \left[\frac{L_H - L_0}{L_{\max} - L_0} \right] \times 100$$

where L_H corresponded to the amount of free amino acid in the hydrolysate. L_0 was the amount of free amino acid in original shrimp cephalothorax. $L_{\max}$ was the maximum amount of free amino acid in cephalothorax obtained after acid hydrolysis using of 6 M HCl. The hydrolysis was run at 105 °C for 24 h in an oil bath (BUCHI Labortechnik AG, Tokyo, Japan).

2.4.6 Determination of color

The color of hydrolysate powders (5 g) was measured by a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). L^* , a^* and b^* values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The colorimeter was warmed up for 10 min and calibrated with a white standard. Total difference in color (ΔE^*) was calculated according to the following equation [18]:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^* , Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of white plate standard ($L^* = 92.84$, $a^* = -1.26$ and $b^* = 0.47$).

2.4.7 Molecular weight distribution

Molecular weight distribution of hydrolysate samples was performed using a Sephadex G-25 gel filtration column (2.5 x 50 cm) (17-0032-01, GE Healthcare Bio-Science AB, Uppsala, Sweden). The sample (50 mg) was dissolved in distilled water (2 ml) and the mixture was loaded onto a column. After being loaded, the elution was performed using a ÄKTA chromatography system (ÄKTApurim plus, GE healthcare Bio-Science AB, Uppsala, Sweden) coupled with a fraction collector. Distilled water was used as eluent at a flow rate of 0.5 ml/min. The fractions of 3 ml were collected and their absorbance was recorded at 220 and 280 nm. Blue dextran (2,000,000 Da) was used for void volume measurement. The molecular weight (MW) markers, including insulin chain B (3495.89 Da), vitamin B12 (1355.4 Da), glycine-tyrosine (238.25 Da) and tyrosine (181.2 Da) were used. MW of fraction was estimated from the plot between available partition coefficient (K_{av}) and the logarithm of the molecular weight of the protein standards.

2.4.8 Determination of bitterness

Bitterness in the hydrolysates was examined by 5 female and 6 male panelists with the ages of 25 and 33. The panelists were trained using a caffeine as a standard for a period of one month, twice a week. The standard solutions at different concentrations (0, 25, 50 and 75 ppm) were presented. Distilled water was used to represent score of 0, while 75 ppm caffeine represented the score of 15. For evaluation, 15-cm line scale anchored from “none” to “intense” was used.

The hydrolysate samples, at a protein concentration of 2 g/100 ml, were served at ambient temperature coded with three-digit random number together with reference standard caffeine solution. Panelists then evaluated for bitterness of samples, compared to the

reference using a 15-cm line scale. Between samples, panelists were asked to eat a piece of un-salted cracker and rinse their mouths thoroughly with distilled water [19].

2.4.9 Determination of antioxidative activities

Prior to analysis, hydrolysates samples were dissolved in distilled water to obtain the concentration of 5 mg/ml. The solutions of hydrolysates were determined for antioxidative activities as follows: ABTS radical scavenging activity [20], DPPH Radical Scavenging Activity (DPPH) [7], ferrous ion chelating activity [7], ferric reducing antioxidant power (FRAP) Sae-leaw et al. [7] and oxygen radical absorbance capacity (ORAC) [7]. Activities were expressed as μmol Trolox (TE) equivalent/g sample, except for metal chelating activity, which was reported as μmol EDTA equivalent/g sample.

2.4.10 Measurement of inhibitory activity toward peroxyl radical induced supercoiled plasmid DNA strand scission

Peroxyl radical induced supercoiled plasmid DNA strand scission inhibitory activity was determined as described by Kittiphattanabawon et al. [21] with slight modifications. Supercoiled plasmid DNA (pUC 18) ($0.025 \mu\text{g}/\mu\text{l}$; $4 \mu\text{l}$) dissolved in 10 mM Tris-HCl containing 1 mM EDTA (pH 7.8) was mixed with $2 \mu\text{l}$ of different hydrolysates to obtain the final concentrations of 1, 3 and 5 mg/ml. To initiate the oxidation reaction, $4 \mu\text{l}$ of 10 mM AAPH were added. The mixture was incubated at 37°C for 1 h in the dark. The controls were prepared in the same manner by using distilled water instead of oxidants. After incubation, $2 \mu\text{l}$ of the loading dye (0.25% bromophenol blue, 50% glycerol) were added to the reaction mixture. Then, the mixture ($6 \mu\text{l}$) was loaded onto 1% agarose gel, and the DNA bands were stained with SYBR gold. Electrophoresis was conducted at 100 V for 50 min using a horizontal gel electrophoresis system (Mini-Sub[®] cell GT, Biorad, Hercules, CA,

USA) equipped with PowerPac™ basic power supply (Biorad, Hercules, CA, USA). The DNA Sub cell® bands were visualized under transillumination of UV light using Uvitec chemiluminescence Documentation System (Uvitec, Cambridge, UK).

2.5 Statistical analysis

All experiments were run in triplicate using three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out using the Duncan's multiple range test [22]. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Yield and protein recovery

Yield and protein recovery of the hydrolysates from cephalothorax prepared using different hydrolysis processes are shown in Table 1. Generally, yield of hydrolysate samples correlated well with protein recovery. The highest yield (54.04%) and protein recovery (84.15%) were found for AU-1.0A sample, compared to other hydrolysates ($P < 0.05$). Yield of hydrolysates prepared by one-step hydrolysis process was lower than that of hydrolysates prepared using two-step hydrolysis processes ($P < 0.05$). However, no difference in the yield between 1.0A and AU+0.5A samples was observed ($P > 0.05$). For hydrolysate prepared using single process, in which cephalothorax were hydrolyzed by Alcalase without prior autolysis, there were no differences in yield and protein recovery between 0.5A and 1.0A samples ($P > 0.05$). The result indicated that proteins of cephalothorax could be hydrolyzed to higher degree when two-step process including autolysis, followed by hydrolysis with 1.0% Alcalase was conducted. Shrimp cephalothorax contained hepatopancreas, which was an excellent source of proteases, especially trypsin and chymotrypsin [12]. Proteases in

hepatopancreas actively cleaved various proteinaceous substrates during autolysis [9]. In the present study, autolysis at 50 °C for 3 h used in the first step of hydrolysis resulted in the cleavage of proteins to some degree by endogenous proteases. Subsequently, the partially hydrolyzed protein substrates could be more effectively hydrolyzed by Alcalase in combination with endogenous proteases at the second step of hydrolysis, particularly when higher level of Alcalase was used. This was evidenced by the higher yield and protein recovery of AU+1.0A sample. Alcalase has been widely used in hydrolysate production because of its broad specificity with the high hydrolysis efficacy [7]. Karnjanapratum, Benjakul [23] reported that the higher hydrolysis of gelatin from unicorn leatherjacket skin was observed when autolysis was conducted before hydrolysis by papain from papaya latex. Therefore, the use of two-step hydrolysis process including autolysis at 50 °C for 3 h, followed by hydrolysis by 1.0% Alcalase for 2 h, could increase the cleavage of peptide bands in shrimp cephalothorax proteins. As a result, protein hydrolysate with the higher yield and protein recovery was obtained.

3.2 Degree of hydrolysis (DH)

DH of protein hydrolysates obtained from various hydrolysis processes is presented in Table 1. All hydrolysate samples had DH in the range of 35.31-44.93%. AU-1.0A sample showed the highest DH, compared with other samples ($P < 0.05$), while the lowest DH was found in AU sample (35.31%). Cao et al. (2008) documented that DH of hydrolysate from *Perna vannamei* was 45.06% when autolysis was conducted at pH 7.85, 50 °C for 3 h. In the present study, autolysis process was carried out at 50 °C and pH 8.0 for 3 h. When autolysis proceeded, proteins were cleaved by endogenous proteases including trypsin, chymotrypsin and elastase. This resulted in the release of peptides from protein matrix during hydrolysis [12]. When Alcalase was added after autolysis, DH continuously increased,

especially with increasing Alcalase levels ($P < 0.05$). The result reconfirmed that autolysis in conjunction with Alcalase effectively hydrolyzed proteins in shrimp cephalothorax. The partially hydrolyzed peptides or loosen protein matrix could favor the migration of Alcalase to substrate. Subsequently, the exposed substrates were readily available for hydrolysis by both endogenous proteases and Alcalase [23]. Overall, the increase in DH of hydrolysate was in accordance with the increases in yield and protein recovery (Table 1). Therefore, two-step hydrolysis showed the high efficacy in protein hydrolysis, particularly when Alcalase at 1.0% was used after autolysis.

3.3 Chemical compositions

Chemical compositions of the cephalothorax and its hydrolysates prepared by different processes are shown in Table 2. Cephalothorax of Pacific white shrimp comprised 58.43% protein, 15.75% ash and 17.26% lipid. The protein content of cephalothorax of Pacific white shrimp was higher than that of black tiger shrimp (*Penaeus monodon*), which was 52.3% [24] or 42.02% [6] and that of *Metapenaeus dobsoni* (40.06%) [25]. However, it was lower than that of Pacific white shrimp (*Penaeus vannamei*) (61.61%) as reported by Cao et al. [5]. Differences in shrimp species and the living environments were associated with varying chemical compositions [26]. Chitin and total carotenoid contents of cephalothorax were 8.32% and 150.58 $\mu\text{g/g}$ sample, respectively.

High protein contents (86.04-89.18%) of all the protein hydrolysates were observed. There were no differences in protein content among all hydrolysate samples ($P > 0.05$). The high protein content was a result of the solubilization of proteins during hydrolysis, the removal of insoluble undigested non-proteinaceous substances and the partial removal of lipid after hydrolysis [27]. All the hydrolysate samples had no difference in lipid contents ($P > 0.05$), except AU sample, which showed slightly lower content. It was noted that lipid

contents in all hydrolysates were much lower than that of cephalothorax. This indicated the effective removal of lipids from the hydrolysates. Lipid contents of all the hydrolysate were lower than that of hydrolysates from *Penaeus monodon* (2.4-3.0%) [6] and deep-water pink shrimp (2.74%) [11]. The low amount of lipid in the hydrolysate samples might lead to the stability towards lipid oxidation [11]. AU+0.5A and AU+1.0A samples had the higher ash content, compared with others ($P<0.05$). The higher ash content of hydrolysate samples was most likely caused by the release of mineral during the longer hydrolysis process. Cao et al. [5] reported that the most abundant mineral in protein hydrolysate was potassium, followed by calcium and magnesium. Although proteins could be effectively recovered from cephalothorax using two-step hydrolysis process involving autolysis with subsequent Alcalase hydrolysis, minerals were plausibly co-extracted.

Additionally, all hydrolysate samples had lower chitin content (0.67-1.08%) than shrimp cephalothorax (8.32%). It indicated that chitin was not liberated during hydrolysis by endogenous proteases or/and Alcalase. As a result, chitin was still retained in carapace or pereiopods. Carotenoid contents of protein hydrolysate samples were in the range of 3.37-6.11 $\mu\text{g/g}$ sample. Senphan et al. [9] reported that the carotenoid content in hydrolysate from shrimp shell increased with increasing enzyme levels. Carotenoproteins from Pacific white shrimp waste were more hydrolyzed with combination of protease and lipase, compared to those hydrolyzed by only protease [28]. When proteins associated with carotenoid, e.g. astaxanthin were hydrolyzed, carotenoids, which were lipid soluble, were more likely localized in lipid phase or pellet associated with lipids. As a consequence, lower carotenoid content was obtained in the resulting hydrolysates.

3.4 Amino acid composition

Amino acid compositions of the cephalothorax and hydrolysate from shrimp cephalothorax prepared by various hydrolysis processes are presented in Table 3. Cephalothorax of Pacific white shrimp consisted of glutamic acid/glutamine (54.36 mg/g dry sample), aspartic acid/asparagine (42.28 mg/g dry sample) and arginine (30.66 mg/g dry sample) as dominant amino acids. Lower contents of cysteine (0.12 mg/g dry sample), hydroxylysine (0.33 mg/g dry sample) and tryptophan (4.27 mg/g dry ample) were found in initial dried cephalothorax, compared with those of hydrolysates.

When cephalothorax was hydrolyzed, the major amino acids of all the hydrolysate samples were glutamic acid/glutamine, aspartic acid/asparagine, arginine and leucine, which accounted for 113.08-121.69, 85.53-90.28, 63.27-68.62 and 58.67-68.07 mg/g dry sample, respectively. The result was in accordance with Cao et al. [5] who reported that the abundant amino acids in hydrolysate from shrimp head of *Penaeus Vannamei* were glutamic acid, asparagine, and leucine. Sila et al. [11] also found glutamic acid/glutamine, aspartic acid/asparagine and alanine as predominant amino acids in hydrolysate from shrimp (*Parapenaeus longirostris*) by-products prepared using Alcalase. Nevertheless, negligible contents of cysteine (0.15-0.25 mg/g dry ample), hydroxylysine (0.96-1.21 mg/g dry sample) and tryptophan (9.28-9.64 mg/g dry sample) were observed in all the hydrolysates samples. Large amounts of isoleucine, leucine, lysine, phenylalanine, valine and threonine, essential amino acids for human being diet [6], were also found in all hydrolysates. Among all the hydrolysates, AU+1.0A sample (346.65 mg/g dry sample) had the highest essential amino acids, compared with others. Sila et al. [11] found that the hydrolysate from shrimp by-products had a high content of essential amino acids, such as arginine, lysine, histidine and leucine (53, 75, 22 and 34 residues per 1000 residues, respectively). Most amino acids of hydrolysate from *Penaens vannamei* heads prepared at different autolysis time were not

changed when hydrolysis time was higher than 3 h [8]. Those hydrolysates had a high content of essential amino acids and extremely high content of flavoring amino acids (glutamic acid, aspartic acid, glycine and alanine) [8]. Several amino acids, such as tyrosine, methionine, histidine, lysine, and tryptophan, may significantly contribute to the antioxidant activity of the hydrolysates [11]. The hydrophobic amino acids of all hydrolysate samples, including alanine, isoleucine, leucine, methionine, phenylalanine, proline, tyrosine and valine, were in the range of 358.83-377.56 mg/g dry sample. Nevertheless, hydrophobic amino acid residues in hydrolysates contributed to the bitterness of protein hydrolysate [29]. The results suggested that the hydrolysates from shrimp cephalothorax could serve as an excellent source of amino acids.

3.5 Color

The color has the direct influence on the appearance of a product, which has the impact on the overall acceptability of consumers. The color values of the hydrolysates from different hydrolysis processes expressed as L^* , a^* , b^* and ΔE^* are shown in Table 1. AU sample had the higher lightness (L^* -value) with lower redness (a^* -value) and yellowness (b^* -value), compared with other hydrolysate samples ($P < 0.05$). However, no differences in a^* -value were obtained between AU and 0.5A samples ($P > 0.05$). L^* -values of hydrolysate samples decreased when Alcalase was used in hydrolysis processes ($P < 0.05$). AU+1.0A sample had the lowest L^* -values but showed the highest a^* and b^* -values ($P < 0.05$). Higher a^* and b^* -values in hydrolysate were associated with the increase in carotenoid content during hydrolysis process as shown in Table 2. The difference in color was therefore governed by the different pigment contents [30]. Moreover, enzymatic browning reactions occurring during hydrolysis at temperature range of 50-60 °C plausibly contributed to the decrease in lightness, leading to darker color of protein hydrolysates. Two-step hydrolysis processes had

a longer hydrolysis time (5 h) than other processes (AU, 0.5A and 1.0A), leading to higher browning reaction. Generally, the hydrolysates obtained from two-step hydrolysis processes had a slightly higher redness and yellowness than those from one-step hydrolysis process. Overall, AU+0.5A and AU+1.0A samples showed the higher total difference in color (ΔE^* -value) along with higher b^* -values than others. Thus, the varying colors of the hydrolysates were more likely related with pigments present in hydrolysates. Additionally, it depended on the composition of the raw material and hydrolysis processes used.

3.6 Molecular weight distribution

Elution profiles of different shrimp cephalothorax hydrolysates on the Sephadex G-25 gel filtration chromatography are illustrated in Fig. 1. A_{220} was used to monitor peptide bonds, while A_{280} indicated the peptides or proteins, mainly containing aromatic amino acids. AU samples had three major peaks of both A_{220} and A_{280} , representing peptides having MW of 4350.8, 496, and 220 Da, respectively. The first peak of A_{220} and A_{280} peak, which contained peptides with MW of 8970-4350.8 Da, had lower peak area, compared with other fractions possessing lower MW. The result indicated that the hydrolysate had a larger proportion of peptides or proteins with MW lower than 1355 Da. The increase in DH (Table 1) of resulting hydrolysates was agreement with increasing peak area of fractions with low MW peptides. When Alcalase was used, the peak, especially the third peak, was slightly shifted to the lower MW. Coincidentally, the first peak of A_{280} was lower. This suggested that the hydrolysis was more pronounced with increasing Alcalase level used. For two-step hydrolysis (AU+0.5A and AU+1.0A) processes, the higher amount of small peptide fraction with MW lower than 220 Da was obtained, particularly when 1.0% Alcalase was used after autolysis (AU+1.0A). The result indicated that Alcalase could provide a hydrolysate with higher DH as indicated by the formation of smaller peptides. Two-step hydrolysis process thus showed higher efficacy

in hydrolysis. This was indicated by the higher yield with maximum DH when autolysis was conducted before the hydrolysis using Alcalase. Karnjanapratum, Benjakul [23] also documented that the varying MW profiles revealed the differences in the degree of protein hydrolysis, which was governed by the hydrolysis process and the enzyme used. Thus, the hydrolysis process and enzyme used directly affected the MW distribution of resulting hydrolysate samples.

3.7 Bitterness score

The bitterness scores were 4.16 ± 1.52 , 3.04 ± 1.63 , 2.66 ± 1.49 , 2.14 ± 1.55 and 2.12 ± 1.14 for AU, 0.5A, 1.0A, AU+0.5A and AU+1.0A samples, respectively. Caffeine standard solution (25 ppm) had the score of 5.44 ± 1.34 . AU+1.0A sample showed the lowest bitterness score, compared with others ($P < 0.05$). However, no difference in bitterness score between 0.5A, 1.0A, AU+0.5A and AU+1.0A samples were observed ($P > 0.05$). There was also no difference in bitterness between AU, 0.5A and 1.0A samples ($P > 0.05$). Bitterness might be associated with the formation of peptides containing hydrophobic amino acids such as valine, isoleucine, leucine, phenylalanine, tryptophan and tyrosine at C-terminal [29]. Bitterness was one of the main contributors to off-flavor of protein hydrolysate [27]. Although higher hydrophobic amino acids (377.56 mg/g dry sample) (Table 3) were present in AU+1.0A, low bitterness score was attained. Bitter taste of a peptide was governed by several factors such as DH, concentration and location of bitter taste residues and number of carbons on the R-group of branched chain amino acid [29]. When DH was increased, hydrophobic amino acids became more exposed. This led to the increased bitterness of hydrolysate [31]. Aspevik et al. [32] also found that the hydrophobic peptide fraction with MW between 500 and 2000 Da from Atlantic salmon showed positive correlation with bitterness and astringent flavor. However, two-step hydrolysis process with the highest DH (Table 1) (AU+0.5A and

AU+1.0A) had the lower bitterness, compared with the hydrolysate prepared using autolysis process alone (AU). This was plausibly governed by differences in amino acid sequences of hydrolysates. The hydrophobic amino acid residues in the peptides more likely determined the bitterness of hydrolysates [33]. Yarnpakdee et al. [29] reported that the two-step hydrolysis with Alcalase, followed by papain reduced the bitter taste of hydrolysate from Nile tilapia, compared to those produced using a single hydrolysis (Alcalase hydrolysis). Therefore, two-step hydrolysis process could reduce bitterness of hydrolysate.

3.8 Antioxidative activities of shrimp cephalothorax hydrolysate produced with different hydrolysis processes

3.8.1 ABTS radical scavenging activity

ABTS radical scavenging activities of all hydrolysates were in range of 443.87-857.89 $\mu\text{mol TE/g sample}$ (Table 4). The highest ABTS radical scavenging activity (857.89 $\mu\text{mol TE/g sample}$) was obtained for AU+1.0A sample ($P<0.05$). On the other hand, AU sample exhibited the lowest activity. In general, ABTS radical scavenging activities increased with increasing DH of resulting hydrolysates ($P<0.05$). Alcalase is endopeptidase capable of hydrolyzing proteins with broad specificity for peptide bonds. Proteins or peptides released during autolysis could be more cleaved to short chain peptides by Alcalase. Those peptides were able to scavenge ABTS radicals effectively. Senphan, Benjakul [1] reported that gelatin hydrolysates from seabass skin with DH ranging from 10% to 30% prepared using Alcalase had the increased ABTS scavenging activity with increasing DH. ABTS radical scavenging activity is used to determine the antioxidant activity of hydrogen-donating compounds (scavengers of aqueous phase radicals) [34]. Binsan et al. [20] reported that the water fraction of extracted Mungoong from cephalothorax of white shrimp showed the highest ABTS radical scavenging activity, compared with ethanol fraction. The result suggested that antioxidative

peptides in hydrolysate were most likely hydrophilic as indicated by high proportion of hydrophilic amino acids (Table 3). Thus, shrimp cephalothorax hydrolysates with higher DH had the higher ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction.

3.8.2 DPPH radical scavenging activity

Hydrolysate produced by autolysis process (AU sample) exhibited the highest DPPH radical scavenging activity ($P < 0.05$) (Table 4). However, there was no difference in DPPH radical scavenging activity between 0.5A and 1.0A samples ($P > 0.05$). Also, no differences in activity between AU+0.5A and AU+1.0A samples were found ($P > 0.05$). Overall, the increase in DH was shown to decrease DPPH scavenging radical activity of the resulting hydrolysates. Senphan, Benjakul [1] documented that hydrolysate prepared using Alcalase with DH of 10, 20 and 30% had no difference in DPPH radical scavenging activity. Sila et al. [11] reported that the hydrolysate from shrimp waste had the lower DPPH radical scavenging activity than BHA at the same concentration. The DPPH radical scavenging assay has been widely used to determine antioxidant properties of compounds. DPPH is a stable free radical, which can be reduced by a proton donating substrate such as an antioxidant, causing the decolorization of DPPH and reducing the absorbance at 517 nm in ethanol [11]. The decrease in DPPH radical scavenging activity of hydrolysate with higher DH (AU+0.5A and AU+1.0A) might be caused by the increased hydrophilicity of peptides. As a consequence, those peptides had lower ability to scavenge lipophilic DPPH radicals. DPPH radical scavenging assay is widely used for measurement of radical scavenging capacities of lipophilic antioxidant [35]. The result indicated that hydrolysates from cephalothorax of Pacific white shrimp contain peptides possessing the potential to prevent or retard lipid oxidation via a chain breaking

reaction. Nevertheless, capacity in hydrogen donation of peptides produced was governed by process used for hydrolysis.

3.8.3 Ferric reducing antioxidant power (FRAP)

All the samples had FRAP values in the range of 5.38-9.83 $\mu\text{mol TE/g sample}$. The highest FRAP was obtained in 1.0A sample ($P<0.05$) (Table 4). It was noted that hydrolysates from two-step process showed the lower FRAP than those from one-step process (0.5A and 1.0A) or autolysis (AU). No difference in the FRAP between AU+0.5A and AU+1.0A samples were observed ($P>0.05$). FRAP is commonly used to measure the capacity of substance in reducing TPTZ-Fe(III) complex to TPTZ-Fe(II) complex [20]. The result demonstrated that two-step hydrolysis processes, which rendered higher DH, might produce small peptides with low ability in reducing TPTZ-Fe(III) complex. FRAP of swollen seabass skin hydrolysate prepared using Alcalase increased with increasing DH up to 20%. However, no difference in FRAP of hydrolysates was noticeable when DH was higher than 20% [1]. Nevertheless, Karnjanapratum, Benjakul [36] reported that the increases in FRAP were in accordance with increasing α -amino group content of gelatin hydrolysate from unicorn leatherjacket skin. The higher reducing power indicated that the hydrolysate samples could donate the electron to free radicals to higher extent, leading to the prevention or retardation of propagation. Thus, the shrimp cephalothorax hydrolysate prepared by hydrolysis using 1% Alcalase contained high amounts of peptides, which potentially donated electron to free radicals, thereby terminating the chain reaction.

3.8.4 Ferrous ion chelating activity

AU+1.0A sample showed the highest ferrous chelating activity, while AU sample exhibited the lowest activity ($P<0.05$) (Table 4). The ferrous ion chelating activities of the

hydrolysates were 6.50, 11.56, 14.26, 15.24 and 17.95 $\mu\text{mol EDTA/g}$ sample for AU, 0.5A, 1.0A, AU-0.5A and AU+1.0A samples, respectively. The results of ferrous ion chelating activity were similar to those of ABTS radical scavenging activity (Table 4). The transition metals such as Fe, Cu and Co in foods participate in the formation of free radicals or reactive oxygen species, which directly affect both rates of autoxidation and breakdown of hydroperoxide to volatile compounds [1]. Thus, chelation of transition metal ions by certain peptides in hydrolysates could retard or interrupt the oxidation process. Ferrozine quantitatively forms complexes with the Fe^{2+} ion. In the presence of chelating agents, the complex formation is disrupted, resulting in the decrease in color formation [1]. The peptides present in the hydrolysates more likely had different metal ion chelating capacity, depending on the amino acid sequences and chain length of peptides [7]. Sila et al. [11] reported that the ferrous chelating capacity of the hydrolysate from shrimp (*Parapenaeus longirostris*) waste by Alcalase treatment was higher than that of butylated hydroxyanisole (BHA). However, gelatin hydrolysate from seabass skin prepared with different DHs (10% to 40%) had no differences in ferrous ion chelating activity [1]. In the present study, smaller peptides might be able to interact or bind with metal ion more effectively, compared with those with the larger sizes. Thus, the shrimp cephalothorax hydrolysates prepared under the optimal condition could act as the secondary antioxidant, which was able to chelate the prooxidative metals, leading to the decreased lipid oxidation.

3.8.5 Oxygen radical absorbance capacity (ORAC)

The ORAC values of hydrolysate prepared by different hydrolysis processes ranged from 339.90 to 391.81 $\mu\text{mol TE/g}$ sample (Table 4). The highest ORAC value was observed in AU+1.0A sample ($P < 0.05$), whereas the AU sample showed the lowest value ($P < 0.05$). However, no difference in ORAC values between AU+0.5A and AU+1.0A samples were

observed ($P>0.05$). There was no difference in ORAC values between 0.5A and 1.0A samples ($P>0.05$). The higher ORAC value of AU+1.0A was in accordance with the higher ABTS scavenging radical activity and ferrous ion metal chelating activity. ORAC is the assay, which determines the antioxidant activity of compounds by scavenging peroxy radical [7]. Nevertheless, ORAC assay is limited to measurement of chain breaking antioxidant capacity against only peroxy radicals [37]. Kittiphattanabawon et al. [38] reported that gelatin hydrolysates from blacktip shark skin prepared using papaya latex enzyme with 40% DH showed the highest ORAC value than those having DH of 10-30%. The result demonstrated that the hydrolysate prepared using two-step hydrolysis process, especially that using autolysis with subsequent hydrolysis by 1% Alcalase (AU+1.0A) had the highest ability in donating a hydrogen atom to the peroxy radical, compared with the others.

3.9 Inhibitory activity toward supercoiled plasmid DNA strand scission oxidation

The inhibition of supercoiled plasmid DNA strand scission oxidation induced by AAPH in the presence of hydrolysate from cephalothorax of Pacific white shrimp prepared by different hydrolysis processes is depicted in Fig. 2. The assay has been used to evaluate the antioxidant activity of hydrolysates, based on their protection of supercoiled DNA strand from scission by oxidative stressor into open circular or linear form [21]. The scission of supercoiled DNA strand took place and was converted to the open circular form when exposed to peroxy radicals [21]. The supercoiled DNA band of sample treated with AAPH was not detectable (lane CD). Peroxy radical of AAPH has a long half-life, thus having a greater affinity to diffuse into cells. This leads to more macromolecular damage [39]. Oxidative damage to DNA may occur at both the phosphate backbone and the nucleotide bases. There are a wide variety of modifications, including strand scission, sister chromatid exchange, DNA-DNA and DNA-protein cross-links as well as base modification [21].

In the presence of the selected hydrolysates (AU, 1.0A and AU+1.0A) at different levels (1, 3 and 5 mg/ml), the retention of supercoiled DNA strand increased with increasing amount of hydrolysates added. The highest retention of supercoiled DNA was obtained when hydrolysate at 5 mg/ml was incorporated. The result demonstrated that the hydrolysate from cephalothorax of Pacific white shrimp had a protective activity against DNA scission induced by peroxy radical. This might be associated with their ability to scavenge free radical and metal chelating activity as shown in Table 4. The result was in agreement with Kittiphattanabawon et al. [21] who documented that the ability to scavenge hydroxyl and peroxy radical and chelating activity of gelatin hydrolysate from blacktip shark skin contributed to the protective ability against DNA damage. Oxidative stress caused by reactive oxygen species (ROS), such as peroxy and hydroxyl radicals, led to damaged DNA, is involved in mutagenesis and carcinogenesis [40,41]. At the same concentration of hydrolysate incorporated, AU+1.0A had the higher inhibitory effect on DNA scission, compared to AU and 1.0A samples. The higher protective effect on DNA damage of AU+1.0A sample was related with higher ABTS radical scavenging activity and chelating activity (Table 4). Yarnpakdee et al. [39] reported that hydrolysate prepared by two-step processes from Nile tilapia showed higher protection effect against supercoiled DNA damage, compared to that prepared from the one step. Furthermore, Kittiphattanabawon et al. [21] also reported that the retention of supercoiled plasmid DNA induced by peroxy and hydroxyl radicals increased as DH of hydrolysate increased. Thus, the hydrolysates from cephalothorax of Pacific white shrimp could inhibit DNA oxidation induced by peroxy radical. It was suggested that hydrolysates could be used functional food ingredient to prevent oxidative stress.

4. Conclusion

The protein hydrolysate produced from cephalothorax of Pacific white shrimp could be used as potential source of nutritive ingredients or additives with high antioxidants. Hydrolysis processes directly influenced the chemical composition and antioxidative activities of the resulting hydrolysate. All hydrolysate samples had high protein content with low amount of lipid. Two-step hydrolysis process including autolysis, followed by Alcalase was an effective means to provide the peptides with small MW and less bitterness. Moreover, the hydrolysate showed high ABTS radical scavenging activity, metal chelating activity and ORAC. The hydrolysate also decreased peroxy radical-induced supercoiled plasmid DNA strand scission. Therefore, autolysis process followed by Alcalase hydrolysis was an efficient process for protein recovery of cephalothorax of Pacific white shrimp with high yield. The resulting hydrolysates with antioxidant activity could be used as functional ingredient or supplement.

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Table 1 Yield, protein recovery, degree of hydrolysis (DH) and color values of the hydrolysates from cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*) prepared by various hydrolysis processes

Samples	Yield (%)	Protein recovery (%)	DH (%)	Color values			
				L^*	a^*	b^*	ΔE^*
AU	46.46 $\pm$ 0.94d	73.55 $\pm$ 1.32d	35.31 $\pm$ 0.81e	78.75 $\pm$ 0.28a	1.42 $\pm$ 0.05c	19.05 $\pm$ 0.76c	23.82 $\pm$ 0.87c
0.5A	49.58 $\pm$ 0.41c	75.91 $\pm$ 1.63cd	37.61 $\pm$ 0.83d	78.13 $\pm$ 0.34b	1.25 $\pm$ 0.08c	17.34 $\pm$ 0.06d	23.28 $\pm$ 0.20c
1.0A	51.39 $\pm$ 0.46bc	77.91 $\pm$ 1.54c	40.53 $\pm$ 0.63c	77.00 $\pm$ 0.29c	1.95 $\pm$ 0.09b	21.58 $\pm$ 0.03b	25.13 $\pm$ 0.46b
AU+0.5A	52.00 $\pm$ 0.57b	81.18 $\pm$ 1.92b	42.28 $\pm$ 0.86b	76.94 $\pm$ 0.28c	1.99 $\pm$ 0.06b	22.54 $\pm$ 0.38a	27.06 $\pm$ 0.03a
AU+1.0A	54.04 $\pm$ 0.74a	84.15 $\pm$ 1.70a	44.93 $\pm$ 0.75a	76.17 $\pm$ 0.06d	2.41 $\pm$ 0.03a	21.83 $\pm$ 0.27a	27.09 $\pm$ 0.42a

Values are presented as mean $\pm$ SD ($n = 3$) (dry weight basic)

Different lowercase letters within the same column indicate significant difference ($P < 0.05$)

AU; autolysis at 50 °C for 3 h, 0.5A; 0.5% Alcalase for 2 h (55 °C, pH 8), 1.0A; 1.0% Alcalase for 2 h (55 °C, pH 8), AU+0.5A; autolysis for 3 h, followed by 0.5% Alcalase for 2 h (55 °C, pH 8), AU+1.0A; autolysis for 3 h, followed by 1.0% Alcalase for 2 h (55 °C, pH 8).

Table 2 Chemical compositions, chitin and total carotenoid contents of the cephalothorax and hydrolysates from shrimp cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*) prepared by various hydrolysis processes

Samples	Chemical composition (%)				Chitin (%)	Total carotenoid (µg/g)
	Moisture	Protein	Ash	Lipid		
Cephalothorax	75.86±.16	58.43±1.45	15.75±0.11	17.26±1.74	8.32±0.20	150.58±2.65
AU	5.11±0.09a	89.18±2.16a	7.46±0.20b	0.43±0.04b	0.67±0.06d	3.37±0.05d
0.5A	4.99±0.02b	89.24±2.09a	8.16±0.48b	0.56±0.06a	0.73±0.12cd	3.61±0.27d
1.0A	4.43±0.10c	88.23±1.38a	8.50±0.61b	0.58±0.02a	0.77±0.13cd	4.28±0.22c
AU+0.5A	4.17±0.04d	86.04±1.68a	10.59±1.37a	0.63±0.03a	0.98±0.1bc	5.47±0.23b
AU+1.0A	4.92±0.10b	86.84±2.04a	11.26±1.54a	0.64±0.09a	1.08±0.21a	6.11±0.30a

Values are presented as mean + SD (n = 3)

Different lowercase letters within the same column indicate significant difference (P<0.05). Caption: see Table 1.

Table 3 Amino acid compositions of the cephalothorax and hydrolysates of shrimp cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*) prepared by various hydrolysis processes

Amino acids (mg/g dry sample)	Samples					
	Cephalothorax	AU	0.5A	1.0A	AU+0.5A	AU+1.0A
Alanine	24.95	58.25	57.65	56.05	54.38	55.27
Arginine	30.66	65.95	68.62	64.44	63.27	65.92
Aspartic acid/asparagine	42.28	86.88	90.28	85.53	84.04	86.67
Cysteine	0.12	0.25	0.18	0.22	0.15	0.19
Glutamic acid/Glutamine	54.36	118.54	121.69	115.80	113.08	116.50
Glycine	25.86	59.79	62.24	59.25	57.97	59.01
Histidine	11.69	25.13	25.48	24.00	23.84	24.57
Isoleucine	17.05	43.03	38.55	39.93	39.44	41.29
Leucine	29.80	68.07	58.67	61.97	61.59	65.11
Lysine	29.90	64.27	65.74	62.47	61.17	62.20
Hydroxylysine	0.33	1.08	1.21	0.96	0.98	1.05
Methionine	9.65	2.41	19.37	19.92	19.48	20.52
Phenylalanine	20.65	45.85	42.63	43.12	43.19	44.56
Proline	23.90	54.36	54.69	53.67	51.92	51.17
Serine	18.92	40.22	41.97	40.28	39.56	40.22
Threonine	18.46	40.49	41.10	39.63	38.67	39.16
Tryptophan	4.27	9.28	9.34	9.29	9.64	9.51
Tyrosine	18.63	26.92	36.47	36.75	39.88	40.70
Valine	20.80	50.41	49.51	49.03	47.96	49.25
Total amino acid	402.28	861.19	885.39	862.32	850.22	872.87
Hydophobic amino acids	169.82	358.83	367.06	369.95	367.64	377.56
Essential amino acids	158.00	339.66	341.05	340.07	335.36	346.65

Caption: see Table 1.

Table 4 ABTS and DPPH radical scavenging activities, ferric reducing antioxidant power (FRAP), ferrous ion chelating activity and oxygen radical absorbance capacity (ORAC) of hydrolysates from cephalothorax of Pacific white shrimp prepared by different hydrolysis processes

Samples	ABTS ($\mu\text{mol TE/g sample}$)	DPPH ($\mu\text{mol TE/g sample}$)	FRAP ($\mu\text{mol TE/g sample}$)	Chelating ($\mu\text{mol EDTA/g sample}$)	ORAC ($\mu\text{mol TE/g sample}$)
AU	443.87 $\pm$ 30.16e	4.84 $\pm$ 0.26a	7.51 $\pm$ 0.08c	6.50 $\pm$ 0.12e	349.67 $\pm$ 11.21d
0.5A	611.71 $\pm$ 6.92d	4.13 $\pm$ 0.23b	8.51 $\pm$ 0.33b	11.56 $\pm$ 0.39d	343.27 $\pm$ 17.40bc
1.0A	693.79 $\pm$ 31.01c	3.96 $\pm$ 0.22b	9.83 $\pm$ 0.54a	14.26 $\pm$ 0.25c	358.47 $\pm$ 25.04bc
AU+0.5A	737.34 $\pm$ 6.94b	3.07 $\pm$ 0.35c	5.38 $\pm$ 0.22d	15.24 $\pm$ 0.82b	371.63 $\pm$ 7.83ab
AU+1.0A	857.89 $\pm$ 26.51a	2.69 $\pm$ 0.19c	5.40 $\pm$ 0.50d	17.95 $\pm$ 0.28a	391.81 $\pm$ 8.15a

Values are presented as mean + SD (n = 3)

Different lowercase letters within the same column indicate significant difference (P<0.05).

Caption: see Table 1.

Figure legend

Figure 1 Elution profile by Sephadex G-25 size exclusion chromatography of hydrolysates from cephalothorax of Pacific white shrimp prepared by different hydrolysis processes. Absorbance at 280 nm (filled circle), 220 nm (open circle). AU; autolysis at 50 °C for 3 h, 0.5A; 0.5% Alcalase for 2 h, 1.0A; 1.0% Alcalase for 2 h, AU+0.5A; autolysis for 3 h, followed by 0.5% Alcalase, AU+1.0A; autolysis for 3 h, followed by 1.0% Alcalase.

Figure 2 Agarose gel electrophoresis of DNA treated with peroxy radicals in the absence and presence of AU, 1.0A and AU+1.0A hydrolysate samples at different concentrations (1, 3 and 5 mg/ml). Caption: see Fig. 1. C and CD denote control (DNA alone) and control damage (DNA+oxidative stressors), respectively. 1, 3 and 5 denote DNA + radicals + hydrolysates at concentrations of 1, 3 and 5 mg/ml, respectively.

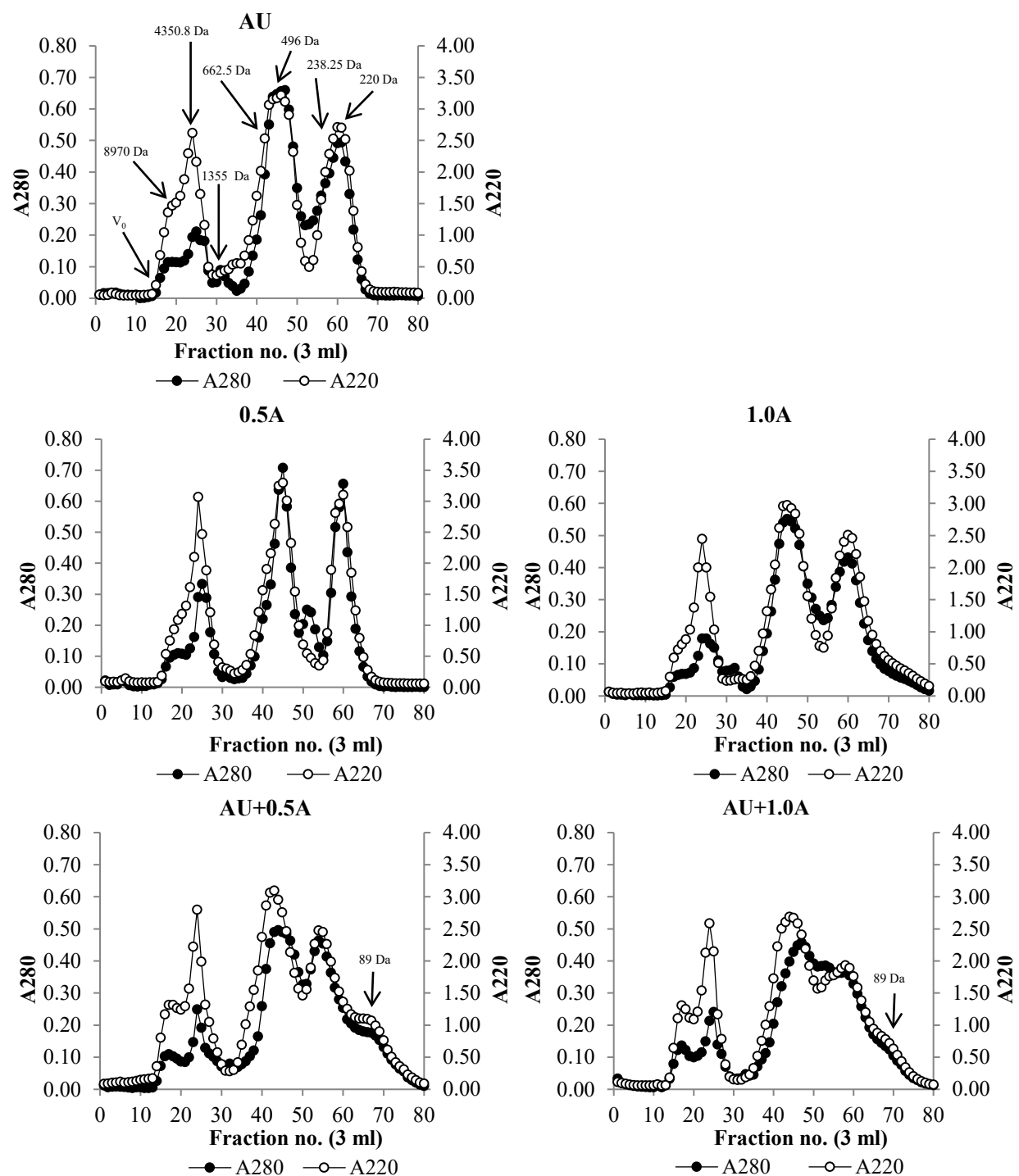


Figure 1

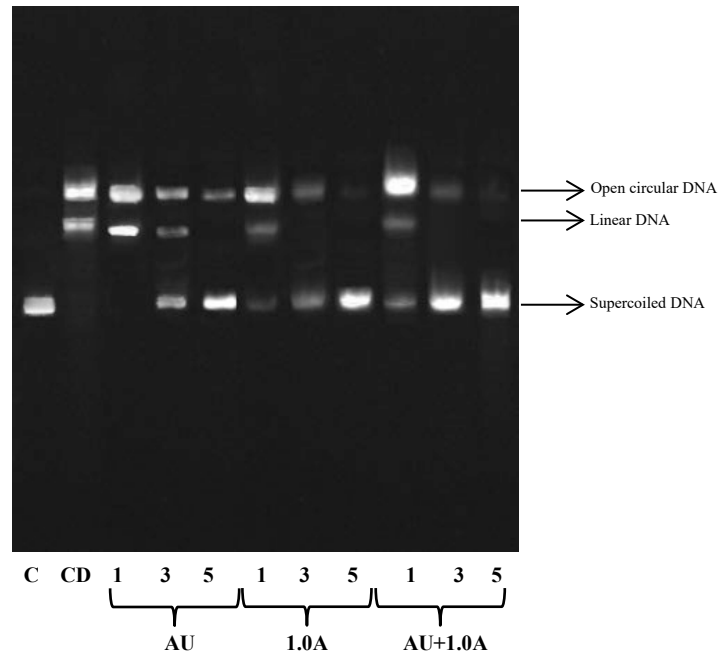


Figure 2