

Research Article

Carotenoprotein from by-product of banana shrimp (*Penaeus merguensis*) extracted using protease from viscera of rainbow trout: antiradical and angiotensin I-converting enzyme inhibitory activity

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Abstract

Carotenoprotein as a marine bioactive compound was recovered from banana shrimp by-product using protease from rainbow trout viscera at various levels (5 and 15 units/g shrimp shell). The yield of carotenoprotein increased with increasing enzyme concentration up to 51.6%, which was consistent with increasing degree of hydrolysis. Protein and lipid content of the hydrolysate was 79.6 and 9.4 percent, respectively. The hydrolysate had a high nutritional value with a high amount of essential amino acids (465 mg/g) compared to the control (254 mg/g). The carotenoprotein exhibited a dose-dependent increase in antiradical activity measured by two in vitro assays, with an efficiency of 94 and 57 percent for DPPH and ABTS scavenging activities, respectively. Protease-extracted carotenoprotein showed higher ACE inhibiting effect than the control at different concentrations ($p < 0.05$). The results from this study showed that carotenoprotein from banana shrimp have both radical scavenging activity and antihypertensive properties and a high content of essential amino acids, and could be good source for value-added nutritive food ingredients.

Keyword: Rainbow trout viscera, Protease, Banana shrimp, Carotenoprotein, Radical scavenging, Activity, ACE inhibitory

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Introduction

Shrimp has become an important product in the world fishery economy, both due to its volume (about 20% of the world's seafood market) and wide geographical distribution. Annual production of shrimp fishery and aquaculture in the world is over 8 million tons (FAO, 2017). Depending on species and processing methods, solid waste (head, legs, shell) generation during processing of shrimps ranges from 50 to 60% of total body weight (Saini *et al.*, 2018). Typically, these by-products are discarded to the environment without any use or treatment (Prameela *et al.*, 2017). Shrimp by-products could be interesting source of chitin, protein, other nutritive components and carotenoids with functional or biological properties, including antiradical activity (Nwe *et al.*, 2014; Senphan *et al.*, 2014; Hajji *et al.*, 2018). Converting shrimp by-product to value added compounds can help solve the problem of its disposal and create extra revenue, which can contribute to develop seafood-based economy. Generally carotenoids in crustaceans are bound to a high-density lipoprotein. These compounds, which have a stable complex structure, are called carotenoprotein (Shahidi and Brown, 1998). Today there is an increasing interest in the use of carotenoprotein in food industry because of its natural origin, non-toxicity and being a vitamin A precursor (Hamdi *et al.*, 2017, 2018). Different methods/techniques have been used to extract bioactive products such

as carotenoid and carotenoprotein from crustacean by-products. These include organic solvents (Hamdi *et al.*, 2018), fermentation (Cremades *et al.*, 2001; Bueno-Solano *et al.*, 2009), vegetable oils (Sowmya and Sachindra, 2012), and supercritique fluid extraction (Razi Parjikolaei *et al.*, 2017). Enzymatic hydrolysis using enzymes from different sources has also been investigated as an alternative method for extracting bioactive compounds (Chakrabarti, 2002; Thiansilakul *et al.*, 2007; Babu *et al.*, 2008; Kishimura *et al.*, 2008; Senphan *et al.*, 2014; Sila *et al.*, 2014; Poonsin *et al.*, 2018). To reduce the cost of commercially available enzymes, one possibility is to use protease extracted from sources such as fish viscera for carotenoprotein extraction. Alkaline proteases extracted from fish digestive system (intestine, pyloric caeca, etc.) cleave peptide bonds (Kishimura *et al.*, 2008; Poonsin *et al.*, 2018) and via hydrolysis process can improve specific properties of protein, such as amino acid content, antiradical and antihypertensive activities compared to the raw material. The aim of the present study was to determine amino acid profile, functional properties, and bioactive potential (radical scavenging activity and antihypertensive activities) of carotenoprotein extracted from banana shrimp with partially purified protease from rainbow trout (*Oncorhynchus mykiss*) digestive system. The research aims to increase the value of seafood side streams and reduce environmental problems.

Materials and methods

Chemicals/enzyme

2,2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) di-ammonium salt (ABTS), 2,4,6-Tris(2-pyridyl)-s-triazine and 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 3-(2-pyridyl)-5-6-diphenyl-1,2,4-triazine-4',4''-disulphonic acid sodium salt (ferrozine) were reagent grade and obtained from Sigma (St. Louis, MO, USA). All other chemicals were purchased from Merck Co. (Darmstadt, Germany).

Preparation of shells from Banana shrimp

Shrimp processing by-product (including heads, shells and tails) of banana shrimp (*Penaeus merguensis*) were provided fresh by a local shrimp processing plant in Hormozgan Province, Iran. Samples were transported on ice (sample to ice ratio 1:2) to Seafood Processing Laboratory in marine sciences department at Tarbiat Modares University of Iran within 3h after production. The by-products were dried by flowing hot air (Memmert, Schwabach, Germany) for 6h at 60°C, and eventually turned into small particles (0.1 mm) using a Panasonic MX-GX1571 blender (Japan) and kept in dark (4°C).

Extraction of alkaline protease from rainbow trout digestive tract

Sample preparation

Rainbow trout with an average weight of 650g was purchased from a fish farm in Mazandaran Province, Iran. The fish were immediately eviscerated and the

digestive system, including intestine and pyloric caeca were separated and used for protease extraction. The samples were cut into pieces and homogenized with three volumes of cold acetone to remove the fat. The homogenate was filtered on Whatman No. 4 paper and the residue was dried under vacuum condition at room temperature for 6h. To prepare the protease extract, the acetone-dried residue was mixed with 10 mM tris-buffer (pH 8.0) at a ratio of 1:30 (w/v) and shaken for 3h at 4°C. The alkaline crude protease extract (CPE) was recovered using a refrigerated centrifuge (Hettich, Universal 320R, Germany) centrifuging the homogenate at 7250g for 30 min (Senphan *et al.*, 2014).

Ammonium sulfate precipitation

The CPE was subjected to ammonium sulfate precipitation at 40-60% w/v saturation by slow continuous stirring in a cold room (Michail *et al.*, 2006). Initial experiments in our laboratory showed that using 40-60% saturation resulted in enzyme with higher purity and efficiency compared to other ranges of saturation (0-20%, 20-40% and 60-80%). After dialyzing against extraction buffer, the precipitate was lyophilized and referred to as partially purified alkaline protease.

Protease activity assay

Activity of the alkaline protease was measured as described by Lassoued *et al.* (2015), using casein as the substrate. The assay was performed at pH 8 and

55°C. A blank was similarly conducted except that the enzyme was added immediately after addition of trichloroacetic acid (5% w/v). One unit of enzymatic activity was defined as the amount of enzyme capable of releasing 1 μmol of tyrosine per min per mg soluble protein of the protease extract. The protein content was determined by the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as a standard. All measurements were run in triplicate.

Extraction of the carotenoprotein

Enzymatic extraction of carotenoprotein was performed according to a slightly modified version of Simpson and Haard method (1985). Ground shrimp by-products (50g) were blended with five volumes of water (pH 8.0) and pre-incubated at 55°C for 20 min prior to

adding crude protease extract. Two levels of protease activity (5 and 15 units/g shrimp shell) were used. The mixture was shaken in a shaker at 200 rpm and 55°C for 120 min. The mixture was heated by microwave for 5 min at temperature $>90^\circ\text{C}$ to inactivate enzyme and centrifuged at 8000g for 10 min. The supernatant was lyophilized (Christ, Alpha 1-2 LDplus, Australia) and the dry matter was referred to as carotenoprotein. The control was made in the same way, but without addition of enzyme.

Determination of the degree of hydrolysis

The degree of hydrolysis (DH), defined as the ratio (%) of α -amino nitrogen with respect to total nitrogen of carotenoprotein, was determined using formol titration method as describe by Taylor (1957):

$$\text{DH (\%)} = \frac{(\text{B2} - \text{B1}) \times \text{atomic weight of nitrogen}}{\text{amino nitrogen assayed by formol titration} \times \text{TN}} = \frac{\text{AN} \times 100}{\text{TN}}$$

B1 = ml 0.1 N-NaOH consumed by sample at certain time.

B2= ml 0.1 N-NaOH consumed by blank.

TN= total nitrogen assayed by micro Kjeldahl method.

Determination of chemical composition

Moisture and ash content were determined after heating in an oven at 103 and 550°C, respectively, until constant weight. Total protein was measured by Kjeldahl method (AOAC, 1990) and the lipid content was determined by the Soxhlet method. Chitin content was determined according to the method of Spinelli *et al.* (1974) as modified by Simpson and

Haard (1985). Dried shrimp waste (2g) was mixed with 30 mL of 2% (w/v) NaOH at 25°C for 6h. After filtration, the residue was shaken with 15 mL of 1M HCl for 30 min at 25°C, filtered and washed with deionized water and then homogenized with cold acetone (-20°C) for 3 min to remove the pigment. The mixture was then washed and dried at 60°C for 24h using an oven (Memmert,

Schwabach, Germany) and the dried matter was referred to as “chitin”.

Total carotenoids

Total carotenoid content was

determined according to Saito and Regier (1971) and Simpson and Haard (1985), with slight modification. The carotenoid content was calculated as astaxanthin using the equation given by Saito and Regier (1971):

$$\text{Carotenoid content } (\mu\text{g/g sample}) = \frac{\text{A468} \times \text{volume of extract} \times \text{dilution factor}}{0.2 \times \text{weight of sample used in gram}}$$

Where 0.2 is the A468 of 1 g/ml standard astaxanthin.

Determination of colour

Spectral reflectance of samples was measured using a colourimeter (micromatch spectrophotometer 181/3, Sheen Instruments, UK) calibrated against black and white tiles. CIE L*, a* and b* system was used to determine colour parameters values where L* defines lightness and varies between 0 (absolute black) and 100 (absolute white), a* measures greenness (+a*) or redness (-a*) and b* measures blueness (+b*) and yellowness (-b*). Colour of the sample was calculated using the following equation:

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Amino acid analysis

Amino acid composition of extracted carotenoprotein was determined according to the method of Gehrke *et al.* (1985). The samples were hydrolyzed in 6M HCl for 24h at 110°C and analyzed using HPLC (Knauer, Germany) with a fluorescence detector (RF-530, Knauer). Then samples were derivatized with o-phthalaldehyde (OPA) and analyzed using a C18

column (Knauer) at a flow rate of 1 mL/min with fluorescence detector (RF-530, Knauer, Germany).

Molecular weight

Molecular weight distribution of the carotenoprotein was determined by fast protein liquid chromatography system (ÄKTA, Amersham Biosciences, Sweden) on a Superdex™ 10/300 GL column at a flow rate of 0.5 mL/min, which separates the sample with a molecular weight range from 100 to 7000 g/mol. A molecular-weight calibration curve was plotted from the average elution volume of the following standards: Cytochrome c (12327 Da), Aprotinin (6511 Da), Vit B12 (1355 Da) and Cysteine (121.16 Da). The chromatographic data were recorded and analyzed by Unicorn software.

Antiradical activity

Different concentrations (0.25, 0.5, 1.0 and 2.5 mg/mL) of the carotenoprotein were prepared for determination of radical scavenging activity.

DPPH radical-scavenging capacity

The DPPH radical scavenging activity was determined according to the method of Thiansilakul *et al.* (2007). The sample (1.5 mL) was mixed with 1.5 mL of 0.15 mM DPPH in 95% methanol and allowed to stand in dark at room temperature for 30 min. Absorbance of the resulting solution

was measured at 517 nm using a spectrophotometer. A blank was made in the same way except that distilled water was used instead of carotenoprotein. For the control sample, ethanol was used instead of DPPH ethanol solution. Radical scavenging ability (RSA, %) was calculated as follows:

$$\text{RSA \%} = (\text{Absorbance}_{\text{blank}} - (\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{control}})) \times 100$$

ABTS radical scavenging activity

ABTS radical scavenging activity was evaluated as described by Senphan *et al.* (2014). The working solution was prepared by mixing equal quantities of 7.4 mM ABTS solution and 2.6 mM

potassium persulphate solution. After 12h, sample (150 μL) was mixed with 2850 μL of ABTS solution. Absorbance was then measured at 734 nm using spectrophotometer after 2h. The activity was calculated as follows:

$$\text{RSA \%} = (\text{Absorbance}_{\text{blank}} - (\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{control}})) \times 100$$

ACE-inhibiting activity

Angiotensin-converting enzyme (ACE) inhibitory activity of sample was investigated by method of Sentandreu and Toldrá (2006). This procedure is based on the reaction between ACE and o-aminobenzoylglycyl-p-nitro-L-phenylalanyl-L-proline (Abz-Gly-Phe-(NO₂)-Pro) as a fluorescent substrate. The fluorescence was measured using excitation and emission wavelengths of 355 and 405 nm, respectively (Fluorescence Spectrometer 3000, Perkin Elmer, UK).

differences. Duncan's test was used for comparison of means to determine the significant difference between samples within 95% confidence interval using SPSS version 22.0 (SPSS Statistical Software, Inc., Chicago, IL, USA).

Results*Chemical composition and colour*

Protein was the major component (on a dry matter basis) both in shrimp by-product and in carotenoprotein (CP) followed by chitin, ash and lipid. Protein content of CP (79.6%) was higher than that in shrimp by-product (52.5%, $p < 0.05$). Furthermore, lipid content of carotenoprotein was significantly higher than that in shrimp shell. Colour values of samples are shown in Table 1.

Statistical analysis

All experiments were conducted with three replicates. The obtained data were subjected to one-way analysis of variance (ANOVA) to determine

Table 1: Proximate composition of banana shrimp by-product and carotenoprotein recovered with rainbow trout proteases, mean \pm standard deviation.

Compositions	Shrimp by-product	Carotenoprotein
Protein (% dry wt)	52.48 \pm 0.15	79.57 \pm 0.36
Fat (% dry wt)	4.35 \pm 0.01	9.39 \pm 0.90
Ash (% dry wt)	18.40 \pm 1.07	11.22 \pm 0.42
Chitin (% dry wt)	27.01 \pm 0.96	0.56 \pm 0.00
Carotenoid (μ g/g)	16.54 \pm 1.09	55.96 \pm 0.00
Colour		
L*	52.7 \pm 3.32	34.9 \pm 1.01
a*	10.1 \pm 0.65	19.9 \pm 0.50

Amino acid composition

Amino acid profile of carotenoprotein extracted with or without rainbow trout protease treatment is shown in Table 2. Carotenoprotein obtained from banana shrimp by-product had higher total amino acid (978 mg/g) and total essential amino acid (465 mg/g) contents than those found in the control (637 and 254 mg/g, $p < 0.05$).

Molecular weight

Molecular weight distribution profile of carotenoprotein obtained with or without protease from rainbow trout viscera is shown in Figure 1.

Antiradical activity of carotenoprotein

DPPH and ABTS radical scavenging activity of lyophilized carotenoprotein is presented in Figure 2. As described for the DPPH assay, ABTS radical scavenging activity gradually increased as carotenoprotein concentration increased from 0.25 to 2.5 mg/mL. At 2.5 mg/mL, ABTS radical scavenging activity was significantly higher than that in the control (13% higher, $p < 0.05$).

Table 2: Amino acid profiles of shrimp carotenoprotein (mg/g of carotenoprotein).

Amino acid	Carotenoprotein	
	Control	Trout protease extracted
Asp	77.92	122.43
Glu	95.48	149.18
Asn	0.41	0.58
His ^A	16.34	31.87
Ser	40.08	54.19
Gln	1.09	0.19
Gly/Arg	43.68	62.60
Thr ^A	34.41	57.54
Tyr	88.03	128.57
Ala	13.41	22.22
Aba	22.29	15.70
Met ^A	12.46	27.89
Val ^A	32.20	62.79
Phe ^A	41.77	75.41
Ile ^A	30.26	63.67
Leu ^A	53.89	94.29
Lys ^A	33.54	58.39
Total amino acids	637.26	1027.51
Total EAA ^B	254.87	471.85
Total NEAA ^C	382.39	555.66

^A Essential amino acids in adults

^B Essential amino acids

^C Non-essential amino acids

ACE-inhibiting activity

ACE inhibitory activity of carotenoprotein at different concentrations is shown in Figure 3. The results showed that carotenoprotein recovered with rainbow trout proteases had significantly higher ACE inhibitory effect compared to that in the control, reaching an IC₅₀ value of 3.87 mg/mL.

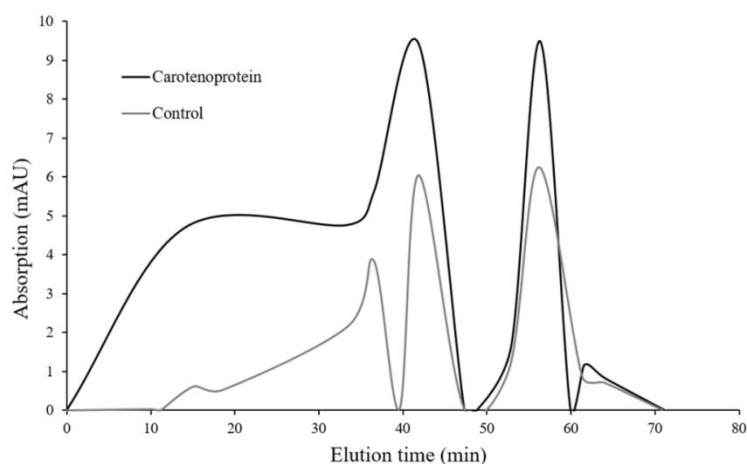


Figure 1: Molecular weight distribution profile of carotenoprotein recovered with or without rainbow trout protease.

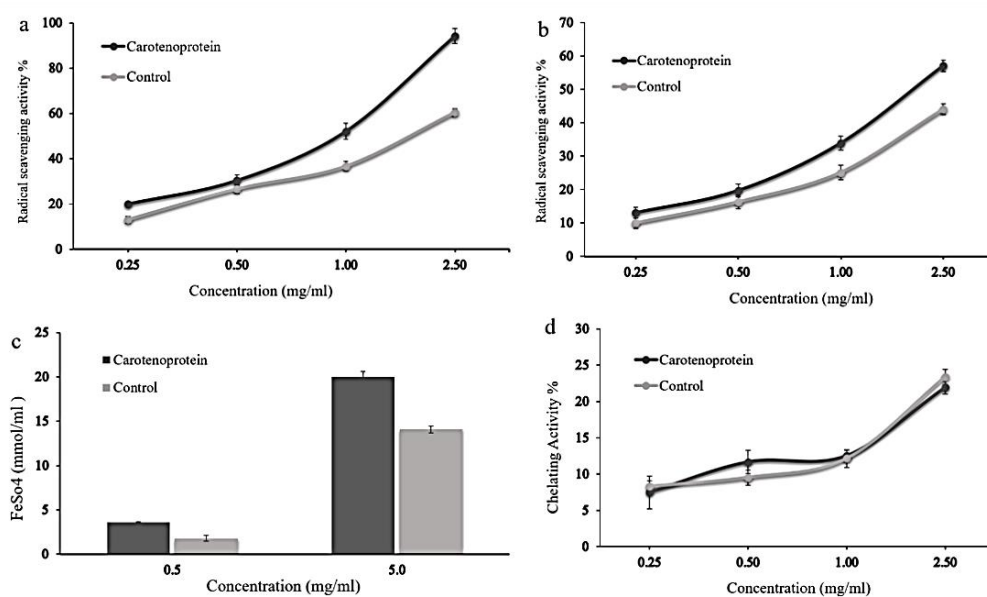


Figure 2: Antiradical activities of carotenoprotein recovered from banana shrimp by-product at different concentrations. (a) DPPH radical scavenging activity (b) ABTS radical scavenging activity. Error bars show standard deviation.

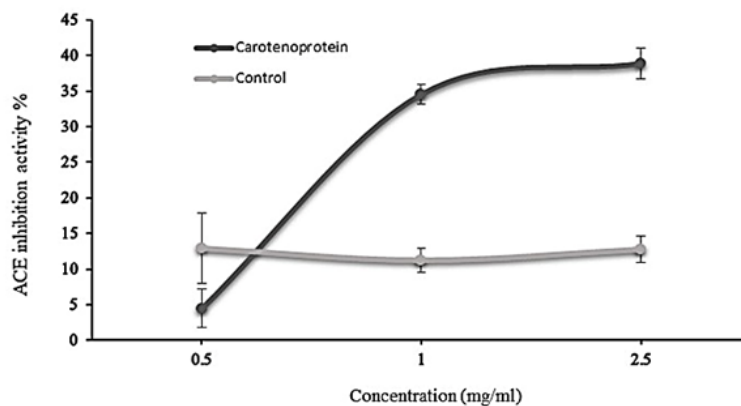


Figure 3: ACE inhibitory activities of carotenoprotein recovered from banana shrimp by-product at different concentrations.

Discussion

Effect of protease concentration on carotenoprotein recovery from shrimp by-product

At the same hydrolysis time, no significant difference was found between the yield of carotenoprotein at a protease concentration of 5 unit/g and that of the control (41.4 and 36.2%, respectively) but a higher yield was obtained when the fish protease concentration was increased to 15 unit/g (51.6 %, $p<0.05$). In addition, the effect of enzyme concentration on DH of carotenoprotein showed the same trend with an increase in enzyme level from 5 to 15 unit/g. Senphan *et al.* (2014) reported an increase in shrimp enzyme concentration (5 to 30 units/g shrimp shells), resulted in a significant increase in extracted protein from pacific white shrimp shells (*Penaeus vannamei*, $p<0.05$). Also, Tunisian barbel (*Barbus callensis*) proteases (1.0 units/g protein) yielded less than 12% recovery of carotenoprotein from deep-water pink shrimp (*Parapenaeus longirostris*) processing waste after 8h of hydrolysis (Sila *et al.*, 2012). Overall, the results indicated that rainbow trout protease had a dose-dependent ability to increase yield recovery and DH value from shrimp processing by-product. Senphan *et al.* (2014) found that actin, totally disappeared in Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) when protease from hepatopancreas was used.

Chemical composition

Protein content of CP (79.6%) was higher than that in shrimp by-product (52.5%, $p<0.05$). Furthermore, lipid content of carotenoprotein was significantly higher than that in shrimp shell but ash and chitin contents of carotenoprotein was lower than those in the by-product ($p<0.05$). Senphan *et al.* (2014) reported protein content in Pacific white shrimp waste to be 43.89%. As shown in Table 1, the results in our study showed that extracted carotenoprotein had a higher protein content than that in freeze-dried carotenoprotein recovered from shrimp shell with the treatment of barbel trypsin (71.09% protein and 16.47% lipid, Sila *et al.*, 2012). Ash and chitin content of carotenoprotein were similar to those reported by Chakrabarti (2002). Accordingly, protease from rainbow trout digestive system could effectively hydrolyze banana shrimp by-product to facilitate release of protein as main component and co-extract fat or lipo-protein, while insoluble undigested non-protein substances were retained.

The colour of carotenoprotein

Colour has a major effect on overall acceptability of a product. Carotenoprotein had a dark orange colour, with a*-value of 19.9 and was both more red, darker ($L^*= 34.9$) and more yellow ($b^*= 29.2$) than shrimp by-product ($p<0.05$, Table 1.). Natural astaxanthin is usually found either bound to proteins, or esterified with fatty acids in diester, monoester and free forms (Armenta and Guerrero-

Legarreta, 2009). Higher a* and b* values in the samples showed that astaxanthin with protein bound to it was recovered to high level from banana shrimp by-product.

Amino acid composition

The nutritional value of a food depends on the type and amount of amino acids available for bodily functions (Bueno-Solano *et al.*, 2009). Carotenoprotein obtained from banana shrimp by-product had higher total amino acid (978 mg/g) and total essential amino acid (465 mg/g) contents than those found in the control (637 and 254 mg/g, $p < 0.05$, Table 2). Both carotenoproteins obtained with fish enzyme and that of the control had high content of glutamate (131.41 and 95.48 mg/g), aspartate (113.77 and 77.92 mg/g) and tyrosine (114.51 and 88.03 mg/g), respectively. These results are in agreement with the results of Yuan *et al.* (2018) on hydrolysates from oriental shrimp (*Penaeus chinensis*) with commercial trypsin. Likewise, Klomkiao *et al.* (2009) found that the carotenoprotein extracted from black tiger shrimp (*Penaeus monodon*) waste treated with the aid of Bluefish proteases was also rich in these amino acids.

Molecular weight

The molecular weight distribution profile of the carotenoprotein obtained with or without protease from rainbow trout viscera is shown in Figure 1. Analysis of peptide size distribution indicated that the major part of the

samples was composed of different peptides with molecular weight ranging from approx. 300 to 3000 Da. As expected, the protein in the sample that was recovered with the aid of protease was more hydrolysed with a higher amount of small peptides than carotenoprotein recovered without enzyme ($p < 0.05$). Slizyte *et al.* (2016) found that peptides in FPH of salmon backbones had molecular weight between approx. 5500 to 6500 Da. Small peptides have a high potential for absorption in intestine (Roberts *et al.*, 1999).

Antiradical activity of carotenoprotein DPPH radical-scavenging capacity

Protease-extracted carotenoprotein showed increasingly DPPH free radical scavenging activity with increasing concentration (Fig. 2a). The activity was significantly higher than that of the control (non-hydrolyzed) at the higher concentrations ($p < 0.05$). At concentration of 2.5 mg/mL, scavenging activity of control was 60.4%, whereas that of the carotenoprotein was 94.2%. These results are in accordance with those reported by Senphan *et al.* (2014) and Sila *et al.* (2014) on carotenoprotein. In addition, both the DH and type of enzyme may affect hydrogen-donating ability (Shavandi *et al.*, 2017). As shown in Table 2, the carotenoprotein extracted by means of rainbow protease has a high content of tyrosine, methionine, lysine and histidine that possibly contribute to antiradical activity (Chen *et al.*, 1996).

ABTS radical scavenging activity

Protease-extracted carotenoprotein has higher ABTS scavenging activity than the control sample ($p < 0.05$). This is presumably due to enzymatic hydrolysis of banana shrimp protein, resulting in production of various antioxidative peptides (Kittiphattanabawon *et al.*, 2012). As described for the DPPH assay, ABTS radical scavenging activity gradually increased as carotenoprotein concentration was increased from 0.25 to 2.5 mg/mL. At 2.5 mg/mL, the ABTS radical scavenging activity was significantly higher than that of the control (13% higher, $p < 0.05$). Antiradical activity of carotenoprotein is due to presence of antiradical peptides, as well as non-protein antiradical compounds such as carotenoids (55.96 $\mu\text{g/g}$ sample) which act as electron donors reacting with free radicals (such as ABTS and DPPH) leading to formation of more stable products and terminating radical chain reactions.

ACE-inhibiting activity

The results showed that carotenoprotein recovered with rainbow trout proteases had significantly higher ACE inhibitory effect compared to that of the control, reaching an IC₅₀ value of 3.87 mg/mL. As the same shrimp by-product was used for extraction of carotenoproteins, the difference in ACE inhibitor activity is suggested to be due to the ability of proteases to break down the protein of raw material into ACE-inhibiting peptides (Slizyte *et al.*, 2016). There

was no significant difference between the ACE inhibitory activity of 100 and 250 $\mu\text{g/mL}$. ACE inhibitory activity of hydrolysates depends on the cumulative effect of different peptide chains instead of a single bioactive peptide (Ambigaipalan and Shahidi 2017). Several ACE inhibitory peptides have been recovered from different protein sources (Bougatef *et al.*, 2008; Geirsdottir *et al.*, 2011; Lin *et al.*, 2012). IC₅₀ value for banana shrimp by-product hydrolysate is lower than hydrolysates from frozen shrimp (Ambigaipalan and Shahidi 2017), defatted salmon backbones (Slizyte *et al.*, 2016) with an IC₅₀ more than 4.8 mg/mL, whereas it is higher than those of wild and farmed cod hydrolysates (Jensen *et al.*, 2013).

Rainbow trout protease was found to be efficient in hydrolysis and recovery of carotenoprotein complexes from banana shrimp by-product with a maximum yield of 51.6%. The enzyme-aided hydrolysate had protein, fat, and pigment content that were higher than that of the shrimp by-product. It also had good nutritional profiles due to high contents of essential amino acids. The hydrolysate from banana shrimp by-product also showed in vitro radical scavenging activity and ACE-inhibiting effect with IC₅₀ of 3.87 mg/mL. The carotenoprotein exhibited potential bioactive properties that could be a potential source of natural antiradical and value-added nutritive ingredients in various foods, which may offer a new field for improved use of shrimp processing by-product.

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References

- Ambigaipalan, P. and Shahidi, F., 2017.** Bioactive peptides from shrimp shell processing discards: Antioxidant and biological activities. *Journal of Functional Foods*, 34, 7-17. DOI: 10.1016/j.jff.2017.04.013.
- AOAC, 1990.** Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Rockville, USA. Available at: <https://www.cabdirect.org/cabdirect/abstract/19720492404>.
- Armenta, R.E. and Guerrero-Legarreta, I., 2009.** Amino acid profile and enhancement of the enzymatic hydrolysis of fermented shrimp carotenoproteins. *Food Chemistry*, 112(2), 310-315. DOI: 10.1016/j.foodchem.2008.05.075.
- Babu, C.M., Chakrabarti, R. and Surya Sambasivarao, K.R., 2008.** Enzymatic isolation of carotenoid-protein complex from shrimp head waste and its use as a source of carotenoids. *LWT-Food Science and Technology*, 41(2), 227-235. DOI: 10.1016/j.lwt.2007.03.006.
- Bougatef, A., Nedjar-Arroume, N., Ravallec-Plé, R., Leroy, Y., Guillochon, D., Barika, A. and Nasri, M., 2008.** Angiotensin I-converting enzyme (ACE) inhibitory activities of sardinelle (*Sardinella aurita*) by-products protein hydrolysates obtained by treatment with microbial and visceral fish serine proteases. *Food Chemistry*, 111(2), 350-356. DOI: 10.1016/j.foodchem.2008.03.074.
- Bueno-Solano, C., López-Cervantes, J., Campas-Baypoli, O.N., Lauterio-García, R., Adam-Bante, N.P. and Sánchez-Machado, D.I., 2009.** Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products. *Food Chemistry*, 112(3), 671-675. DOI: 10.1016/j.foodchem.2008.06.029.
- Chakrabarti, R., 2002.** Carotenoprotein from tropical brown shrimp shell waste by enzymatic process. *Food Biotechnology*, 16(1), 81-90. DOI: 10.1081/FBT-120004202.
- Chen H.M., Muramoto, K., Yamauchi, F. and Nokihara, K., 1996.** Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. *Journal of Agricultural and Food Chemistry*, 44(9), 2619-2623. DOI: 10.1021/jf950833m.
- Cremades, O., Ponce, E., Corpas, R., Gutierrez, J.F., Jover, M., Alvarez-Ossorio, M.C., Parrado, J. and Bautista, J., 2001.** Processing of crawfish (*Procambarus clarkii*) for the preparation of carotenoproteins and chitin. *Journal of Agricultural and Food Chemistry*, 49(11), 5468-5472. DOI: 10.1021/jf0104174.

- FAO, 2017.** *Fishstat plus: universal software for fishery statistical time series.*, Food and Agriculture Organization of the United Nations Fisheries Department, Fishery Information, Data and Statistics Unit, Rome, Italy. Available at: <http://www.fao.org/fishery/statistics/en>.
- Gehrke, C.W., Wall, L.L., Absheer, J.S., Kaiser, F.E. and Zumwalt, R.W., 1985.** Sample preparation for chromatography of amino acids: acid hydrolysis of proteins. *Journal of Association of Official Analytical Chemists*, 68(5), 811-821. DOI: 10.1093/jaoac/68.5.811.
- Geirsdottir, M., Sigurgisladottir, S., Hamaguchi, P.Y., Thorkelsson, G., Johansson, R., Kristinsson, H.G. and Kristjansson, M.M., 2011.** Enzymatic hydrolysis of blue whiting (*Micromesistius poutassou*); functional and bioactive properties. *Journal of Food Science*, 76(1), 14-20. DOI: 10.1111/j.1750-3841.2010.01877.x.
- Hajji, S., Younes, I., Affes, S., Boufi, S. and Nasri, M., 2018.** Optimization of the formulation of chitosan edible coatings supplemented with carotenoproteins and their use for extending strawberries postharvest life. *Food Hydrocolloids*, 83, 375-392. DOI: 10.1016/j.foodhyd.2018.05.013.
- Hamdi, M., Hammami, A., Hajji, S., Jridi, M., Nasri, M. and Nasri, R., 2017.** Chitin extraction from blue crab (*Portunus segnis*) and shrimp (*Penaeus kerathurus*) shells using digestive alkaline proteases from *P. segnis* viscera. *International Journal of Biological Macromolecules*, 101, 455-463. DOI: 10.1016/j.ijbiomac.2017.02.103.
- Hamdi, M., Nasri, R., Dridi, N., Mousa, H., Ashour, L. and Nasri, M., 2018.** Improvement of the quality and the shelf life of reduced-nitrites turkey meat sausages incorporated with carotenoproteins from blue crabs shells. *Food Control*, 91, 148-159. DOI: 10.1016/j.foodcont.2018.03.048.
- Jensen, I.J., Larsen, R., Rustad, T. and Eilertsen, K.E., 2013.** Nutritional content and bioactive properties of wild and farmed cod (*Gadus morhua* L.) subjected to food preparation. *Journal of Food Composition and Analysis*, 31(2), 212-216. DOI: 10.1016/j.jfca.2013.05.013.
- Kishimura, H., Klomklao, S., Benjakul, S. and Chun, B.S., 2008.** Characteristics of trypsin from the pyloric ceca of walleye pollock (*Theragra chalcogramma*). *Food Chemistry*, 106(1), 194-199. DOI: 10.1016/j.foodchem.2007.05.056.
- Kittiphattanabawon, P., Benjakul, S., Visessanguan, W. and Shajidi, F., 2012.** Gelatin hydrolysate from blacktip shark skin prepared using papaya latex enzyme: Antioxidant activity and its potential in model systems. *Food Chemistry*, 135(3), 1118-1126. DOI: 10.1016/j.foodchem.2012.05.080.
- Klomklao, S., Benjakul, S., Visessanguan, W., Kishimura, H.**

- and Simpson, B.K., 2009.** Extraction of carotenoprotein from black tiger shrimp shells with the aid of bluefish trypsin. *Journal of Food Biochemistry*, 33(2), 201-217. DOI: 10.1111/j.1745-4514.2009.00213.x.
- Lassoued, I., Hajji, S., Mhamdi, S., Jridi, M., Bayouhd, A., Barkia, A. and Nasri, M., 2015.** Digestive alkaline proteases from thornback ray (*Raja clavata*), Characteristics and applications. *International Journal of Biological Macromolecules*, 80, 668-675. DOI: 10.1016/j.ijbiomac.2015.07.038.
- Lin, L., Lv, S. and Li, B., 2012.** Angiotensin-I-converting enzyme (ACE)-inhibitory and antihypertensive properties of squid skin gelatin hydrolysates. *Food Chemistry*, 131(1), 225-230. DOI: 10.1016/j.foodchem.2011.08.064.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951.** Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, 193(1), 265-275. DOI: 10.1016/S0021-9258(19)52451-6.
- Michail, M., Vasiliadou, M. and Zotos, A., 2006.** Partial purification and comparison of precipitation techniques of proteolytic enzymes from trout (*Salmo gairdnerii*) heads. *Food Chemistry*, 97(1), 50-55. DOI: 10.1016/j.foodchem.2005.03.022.
- Nwe, N., Furuike, T. and Tamura, H., 2014.** Isolation and characterization of chitin and chitosan from marine origin. Advances in Food and Nutrition Research. In: *Marine carbohydrates: fundamentals and applications*, Part A. Kim S.K. editor, Elsevier Inc., Houston, USA, 72, 1-15. DOI: 10.1016/B978-0-12-800269-8.00001-4.
- Poonsin, T., Simpson, B.K., Benjakul, S., Visessanguan, W., Yoshida, A. and Klomkiao, S., 2018.** Carotenoprotein from Pacific white shrimp (*Litopenaeus vannamei*) shells extracted using trypsin from albacore tuna (*Thunnus alalunga*) spleen: Antioxidant activity and its potential in model systems. *Journal of Food Biochemistry*, 42(2), e12462. Published online, DOI: 10.1111/jfbc.12462.
- Prameela, K., Venkatesh, K., Immandi, S.B., Kasturi, A.P.K., Krishna, C.R. and Mohan, C.M., 2017.** Next generation nutraceutical from shrimp waste: The convergence of applications with extraction methods. *Food Chemistry*, 237: 121-132. DOI: 10.1016/j.foodchem.2017.05.097.
- Razi Parjikolaie, B., Errico, M., El-Houri, R.B., Mantell, C., Fretté, X.C. and Christensen, K.V., 2017.** Process design and economic evaluation of green extraction methods for recovery of astaxanthin from shrimp waste. *Chemical Engineering Research and Design*, 117, 73-82. DOI: 10.1016/j.cherd.2016.10.015.
- Roberts, P.R., Burney, J.D., Black, K.W. and Zaloga, G.P., 1999.** Effect of chain length on absorption of biologically active peptides from the gastrointestinal tract. *Digestion*, 60(4), 332-337. DOI:

- 10.1159/000007679.
- Saini, R.K., Moon, S.H. and Keum, Y.S., 2018.** An updated review on use of tomato pomace and crustacean processing waste to recover commercially vital carotenoids. *Food Research International*, 108, 516-529. DOI: 10.1016/j.foodres.2018.04.003.
- Saito, A. and Regier, L.W., 1971.** Pigmentation of brook trout (*Salvelinus fontinalis*) by feeding dried crustacean waste. *Journal of the Fisheries Research Board of Canada*, 28(4), 509-512. DOI: 10.1139/f71-071.
- Senphan, T., Benjakul, S. and Kishimura, H., 2014.** Characteristics and antioxidative activity of carotenoprotein from shells of Pacific white shrimp extracted using hepatopancreas proteases. *Food Bioscience*, 5, 54-63. DOI: 10.1016/j.fbio.2013.11.004.
- Sentandreu, M.A. and Toldrá, F., 2006.** A fluorescence-based protocol for quantifying angiotensin-converting enzyme activity. *Nature Protocols*, 1(5), 2423-7. DOI: 10.1038/nprot.2006.349.
- Shahidi, F. and Brown, J.A., 1998.** Carotenoid pigments in seafoods and aquaculture. *Critical Reviews in Food Science and Nutrition*, 38(1), 1-67. DOI: 10.1080/10408699891274165.
- Shavandi, A., Hu, Z., Teh, S.S., Zhao, J., Carne, A., Behkit, A., El-Din, A. and Bekhit, A., 2017.** Antioxidant and functional properties of protein hydrolysates obtained from squid pen chitosan extraction effluent. *Food Chemistry*, 227, 194-201. DOI: 10.1016/j.foodchem.2017.01.099.
- Sila, A., Nasri, R., Jridi, M., Balti, R., Nasri, M. and Gougatef, A., 2012.** Characterisation of trypsin purified from the viscera of Tunisian barbel (*Barbus callensis*) and its application for recovery of carotenoproteins from shrimp wastes. *Food Chemistry*, 132(3), 1287-1295. DOI: 10.1016/j.foodchem.2011.11.105.
- Sila, A., Sayari, N., Balti, R., Matinez-Alvarez, O., Nedjar-Arroume, N., Moncef, N. and Bougatef, A., 2014.** Biochemical and antioxidant properties of peptidic fraction of carotenoproteins generated from shrimp by-products by enzymatic hydrolysis. *Food Chemistry*, 148, 445-452. DOI: 10.1016/j.foodchem.2013.05.146.
- Simpson, B.K. and Haard, N.F., 1985.** The use of proteolytic enzymes to extract carotenoprotein from shrimp wastes. *Journal of applied Biochemistry*, 7(3), 212-222.
- Slizyte, R., Rommi, K., Mozuraityte, R., Eck, P., Five, K. and Rustad, T., 2016.** Bioactivities of fish protein hydrolysates from defatted salmon backbones. *Biotechnology Reports*, 11, 99-109. DOI: 10.1016/j.btre.2016.08.003.
- Sowmya, R. and Sachindra, N.M., 2012.** Evaluation of antioxidant activity of carotenoid extract from shrimp processing byproducts by in vitro assays and in membrane model

system. *Food Chemistry*, 134(1), 308-314. DOI: 10.1016/j.foodchem.2012.02.147.

Spinelli, J., Lehman, L. and Wieg, D., 1974. Composition, processing, and utilization of red crab (*Pleuroncodes planipes*) as an aquacultural feed ingredient. *Journal Fisheries Research Board of Canada*, 31(6), 1025-1029. DOI: 10.1139/f74-115.

Taylor, W.H., 1957. Formol titration: An evaluation of its various modifications. *The Analyst*, 82, 488-498. DOI: 10.1039/AN9578200488.

Thiansilakul, Y., Benjakul, S. and Shahidi, F., 2007. Antioxidative activity of protein hydrolysate from round scad muscle using alcalase and flavourzyme. *Journal of Food Biochemistry*, 31(2), 266-287. DOI: 10.1111/j.1745-4514.2007.00111.x.

Yuan, G., Li, W., Pan, Y., Wang, C. and Chen, H., 2018. Shrimp shell wastes: Optimization of peptide hydrolysis and peptide inhibition of α -amylase. *Food Bioscience*, 25, 52-60. DOI: 10.1016/j.fbio.2018.07.008.