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

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Received: January 2020
Accepted: March 2021

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-picyrylhydrazyl (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 merguiensis*) 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°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 described by Taylor (1957):

\[
DH (%) = \frac{(B_2 - B_1) \times \text{atomic weight of nitrogen}}{\text{amino nitrogen assayed by formol titration} \times TN} = \frac{AN \times 100}{TN}
\]

- 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):

\[
\text{Carotenoid content (µg/g sample)} = \frac{A_{468} \times \text{volume of extract} \times \text{dilution factor}}{0.2 \times \text{weight of sample used in gram}}
\]

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

**Determination of colour**

Spectral reflectance of samples was measured using a colorimeter (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 SuperdexTM 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:

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

**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:

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

**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).

**Statistical analysis**
All experiments were conducted with three replicates. The obtained data were subjected to one-way analysis of variance (ANOVA) to determine 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.
Table 1: Proximate composition of banana shrimp by-product and carotenoprotein recovered with rainbow trout proteases, mean ± standard deviation.

<table>
<thead>
<tr>
<th>Compositions</th>
<th>Shrimp by-product</th>
<th>Carotenoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (% dry wt)</td>
<td>52.48±0.15</td>
<td>79.57±0.36</td>
</tr>
<tr>
<td>Fat (% dry wt)</td>
<td>4.35±0.01</td>
<td>9.39±0.90</td>
</tr>
<tr>
<td>Ash (% dry wt)</td>
<td>18.40±1.07</td>
<td>11.22±0.42</td>
</tr>
<tr>
<td>Chitin (% dry wt)</td>
<td>27.01±0.96</td>
<td>0.56±0.00</td>
</tr>
<tr>
<td>Carotenoid (µg/g)</td>
<td>16.54±1.09</td>
<td>55.96±0.00</td>
</tr>
</tbody>
</table>

**Colour**

<table>
<thead>
<tr>
<th></th>
<th>Shrimp by-product</th>
<th>Carotenoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>52.7±3.32</td>
<td>34.9±1.01</td>
</tr>
<tr>
<td>a*</td>
<td>10.1±0.65</td>
<td>19.9±0.50</td>
</tr>
</tbody>
</table>

**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, \( p < 0.05 \)).

**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.

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

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

* Essential amino acids in adults
* Essential amino acids
* Non-essential amino acids
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 \textit{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 (\textit{Penaeus vannamei}, \( p<0.05 \)). Also, Tunisian barbel (\textit{Barbus callensis}) proteases (1.0 units/g protein) yielded less than 12% recovery of carotenoprotein from deep-water pink shrimp (\textit{Parapenaeus longirostris}) processing waste after 8h of hydrolysis (Sila \textit{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 \textit{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 \textit{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 \textit{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, Klomklaao 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 µg/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 µg/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.
Acknowledgement
Authors gratefully acknowledge Iran National Science Foundation (INSF) for financial support for this project.

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