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Biochemical and antioxidant properties of peptidic fraction of carotenoproteins generated from shrimp by-products by enzymatic hydrolysis

Assaâd Sila [a,b], Nadhem Sayari [a], Rafik Balti [c], Oscar Martinez-Alvarez [d], Naima Nedjar-Arroume [b], Nasri Moncef [e], Ali Bougatef [f,*]

[a] Unité Enzymes et Bioconversion, National School of Engineering, PB 1173, 3038 Sfax, Tunisia
[b] Laboratoire de Procédés Biologiques, Génie Enzymatique et Microbien, IUT A Lille I, BP 179, 59653 Villeneuve d'Ascq Cedex, France
[c] Higher Institute of Applied Biology of Medenin, 4119 Médenine, Tunisia
[d] Institute of Food Science, Technology and Nutrition (ICTAN, CSIC), C/ José Antonio Novais 10, 28040 Madrid, Spain
[e] Laboratoire de Génie Enzymatique et de Microbiologie, Université de Sfax, Ecole Nationale d'Ingénieurs de Sfax, PB 1173, 3038 Sfax, Tunisia
[f] Higher Institute of Biotechnology of Sfax, PB 1175, 3038 Sfax, Tunisia

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ABSTRACT

The composition, functional properties and *in vitro* antioxidative activity of the peptidic fraction of carotenoproteins from shrimp (*Parapenaeus longirostris*) by-products generated by enzymatic treatment with Alcalase® was evaluated. The peptidic fraction of carotenoproteins (PFCP) contained 80.8 ± 0.21% protein, 2.74 ± 0.3% lipid, 14.4 ± 0.14% ash, 1.13 ± 0.08% chitin and 1.08 ± 0.02 μg total carotenoid/g of sample. The amino acid profile of PFCP showed a high percentage of essential amino acids, such as arginine, lysine, histidine and leucine. Therefore, PFCP had a high nutritional value and could be used as a supplement to poorly balanced dietary proteins. PFCP showed an excellent solubility and possessed interfacial properties, which were governed by their concentrations. The antioxidant activities of PFCP at different concentrations were evaluated using various *in vitro* antioxidant assays, including the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical method, reducing power, chelating effects assay and β-carotene bleaching. The antioxidant activity of PFCP, based on their protection of supercoiled DNA strand from scission by peroxyl and hydroxyl radicals into the nicked circular form was also investigated.

Results from this study suggest that the peptidic fraction of carotenoproteins is a good source of natural antioxidants and peptides with interesting functionalities.

1. Introduction

A significant increase in the amount of waste resulting from the industrial processing of fish has become a problem, both for the environment and for the processing plants. About 45% of processed seafood consists of shrimp, the waste of which is composed of the exoskeleton and cephalothorax. This waste can represent 50–70% of the total weight of the raw material, and is a rich source of biomolecules, including chitin, proteins, carotenoid, flavours, nutritive components, and enzymes. Therefore, making use of such waste is of economical value and has drawn much interest from researchers in recent years.

Shrimp waste has been explored as a potential source of carotenoids, proteins, and chitin. In crustaceans, carotenoids occur as carotenoproteins, which are stable complexes of carotenoids bound to a high-density lipoprotein (Shahidi & Metusalach & Brown, 1998). Studies have been carried out on the recovery of carotenoids from shrimp waste in the form of carotenoprotein by different techniques (Sachindra & Mahendrakar, 2005; Sowmya & Sachindra, 2012). Attempts have also been made to isolate carotenoproteins from crustacean processing discards by using proteases (Cremades et al., 2003; Holanda & Netto, 2006; Klomklao, Benjakul, Visessanguan, Kishimura, & Simpson, 2009; Sila, Nasri, Jridi, et al., 2012).

Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), t-butylhydroquinone (TBHQ) and propyl gallate, have been widely used in food products to delay the deterioration caused by lipid oxidation. However, these antioxidants pose potential health hazards, and their use is restricted in some countries. Thus, it is essential to develop safe and natural antioxidants as alternatives to synthetic ones. Several peptides from protein ingredients have been found to possess antioxidant capacity (Korhonen & Pihlanto, 2003; Sarmadia & Amin, 2010). Bioactive peptides are regarded as specific protein fragments which

* Corresponding author. Address: Higher Institute of Biotechnology of Sfax, Route Sokra km 4, BP. 1175, 3038 Sfax, Tunisia. Tel.: +216 74 674 354; fax: 216 74 674 364.
E-mail address: ali.bougatef79@gmail.com (A. Bougatef).

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are inactive in the parent protein sequence. They can exert several physiological functions after they are released by enzymatic hydrolysis (Faithong, Benzakul, Phatcharat, & Binsan, 2010; He, Chen, Sun, Zhang, & Gao, 2006; Synowiecki & Al-Khateeb, 2000).

Functional properties can be defined as the overall physicochemical behaviour of performance of proteins in food systems during processing, storage and consumption. Protein hydrolysates have an excellent solubility at a high degree of hydrolysis (Gbogouri, Linder, Fanni, & Parmentier, 2004). High solubility of fish protein hydrolysate, over a wide range of pH is a substantially useful characteristic for many food applications. Furthermore, it influences the other functional properties, such as emulsifying and foaming (Kristinsson & Rasco, 2000a).

The deep-water rose shrimp (*P. longirostris*) is a large decapod crustacean. It has a pink-orange carapace with a reddish rostrum. It shows a wide geographic distribution, being found in the eastern Atlantic from northern Spain to the southern waters of Angola and the whole basin of the Mediterranean.

The objective of this investigation was to study some functional properties and the antioxidant activity and bioactive potential of the peptidic fraction of carotenoproteins extracted from deep-water pink shrimp by treatment with Alcalase®.

2. Materials and methods

2.1. Reagents

Chemicals, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), β-carotene and L-ascorbic acid, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals, namely potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, sodium hydroxide, $FeCl_3$, ferrozine and other solvents, were of analytical grade.

2.2. Materials

The deep-water pink shrimp (*P. longirostris*) waste, consisting of heads, cephalothorax, shells and appendix, were obtained fresh from a shrimp processing plant located in Sfax, Tunisia. Prior to use, the shrimp shells were washed thoroughly with distilled water and grounded. The shells were then stored at −20 °C until further analysis.

2.3. Preparation of the peptidic fraction of carotenoproteins (PFCP)

2.3.1. Extraction of carotenoprotein

Carotenoprotein from shrimp waste was recovered by the method of Sila, Nasri, and Bougatef (2012), with slight modification. Ground shrimp wastes were blended with three volumes of 0.5 M EDTA, pH 8.0 (optimum for Alcalase® activity) using a waring blender. Alcalase® was added to the mixture at a level of 1000 U/g samples and shaken at 200 rpm in a shaking incubator (DAIHAN Labtech CO., Ltd.) at 50 °C (optimum for Alcalase® activity) for 2 h. After incubation, the mixture was filtered through several layers of cheesecloth, and the filtrate was adjusted to pH 4.5 with 2 M HCl. The precipitate was recovered by centrifugation at 5000*g* for 30 min at 4 °C using a refrigerated centrifuge (MED-instrument MPW-350 R). The pellet obtained was dissolved in 20 ml of cold 5 mM sodium phosphate buffer (pH 7). Samples were lyophilised and the dry matter was referred to as "carotenoprotein".

2.3.2. Enzymatic hydrolysis and protein recovery

Dried carotenoprotein was resuspended (v/w) in Tris–HCl buffer (0.05 M), pH 8.0. The mixture was treated with Alcalase® at a level of 100 enzymatic U/g samples. Proteolysis was carried out at 50 °C for 30 min with continuous stirring. During the reaction, the pH of the mixture was maintained by continuous addition of 4 N NaOH. After the required digestion time, the reaction was stopped by heating the solutions for 15 min at 80 °C to inactivate enzymes.

The hydrolysed protein and pigment moieties in the reaction mixture were separated by ultrafiltration using a stirred ultrafiltration cell (Millipore 8400) with a 30.0 KDa MW cut-off membranes (PBGC membrane, Millipore). The rententate was lyophylised and referred as the peptidic fraction of carotenoproteins (PFCP).

2.4. Determination of the degree of hydrolysis

The degree of hydrolysis (DH), defined as the percent ratio of the number of peptide bonds cleaved (h) to the total number of peptide bonds in the substrate studied (h_{tot}), was calculated from the amount of base (NaOH) added to keep the pH constant during the hydrolysis according to the following equation:

$$\mathrm{DH}\ (\%) = \frac{h}{h_{tot}} \times 100 = \frac{B \times Nb}{MP} \times \frac{1}{\alpha} \times \frac{1}{h_{tot}} \times 100$$

where B is the amount of NaOH consumed (ml) to keep the pH constant during the proteolysis of the substrate. Nb is the normality of the base, MP is the mass (g) of the protein ($N \times 6.25$), and α represents the average degree of dissociation of the α-NH_2 groups in the protein substrate expressed as:

$$\alpha = \frac{10^{\mathrm{pH-pK}}}{1 + 10^{\mathrm{pH-pK}}}$$

where pH and pK are the values at which the proteolysis was conducted. The total number of peptide bonds (h_{tot}) in the protein substrate was assumed to be 9.22 meq g^{-1}.

2.5. Determination of chemical composition

The moisture and ash content were determined according to the AOAC standard methods 930.15 and 942.05, respectively (AOAC, 2000). The total nitrogen content of peptidic fraction of carotenoproteins (PFCP) was determined using the Dumas method. Samples were heated to 1050 °C following AOAC 992.15 (AOAC, 2000) in a LECO model FP-2000 protein/nitrogen analyser calibrated with EDTA). The crude protein was estimated by multiplying the total nitrogen content by a factor of 6.25. Crude fat was determined gravimetrically after Soxhlet extraction of dried samples with hexane. All measurements were performed in triplicate.

2.6. Total carotenoids

The total carotenoid content was determined according to the method of Saito and Regier (1971), with slight modification. One gram of carotenoproteins, previously prepared, was homogenised in 25 ml of cold acetone (−20 °C) for 2 min and the homogenate was filtered through a Whatman No. 1 filter paper under vacuum. The filtrate was placed in a separatory funnel and was partitioned with 25 ml of petroleum ether. The separatory funnel and contents were shaken gently and were left to stand at room temperature (25 °C) for 10 min. The lower layer was drawn off. The top layer was washed twice with 25 ml of distilled water. The petroleum ether layer obtained was dried by occasional shaking with 15 g of anhydrous sodium sulphate for 30 min. The dried material was filtered through a coarse sintered glass funnel. The residual sodium sulphate was then washed with small volumes of petroleum ether several times to remove all the pigments. The washings were pooled with the filtrate and then evaporated under vacuum at 50 °C using a rotary evaporator (Rotary evaporator, Stuark, UK).