Food Chemistry 168 (2015) 662–667

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Short communication

Purification and characterization of three antioxidant peptides from protein hydrolyzate of croceine croaker (*Pseudosciaena crocea*) muscle

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ARTICLE INFO

Article history:
Received 18 May 2014
Received in revised form 24 July 2014
Accepted 24 July 2014
Available online 5 August 2014

Keywords:
Croceine croaker (*Pseudosciaena crocea*)
Protein hydrolysate
Peptide
Antioxidant activity

ABSTRACT

Three antioxidant peptides were purified from protein hydrolysate of croceine croaker (*Pseudosciaena crocea*) muscle prepared using pepsin and alcalase, and identified as Tyr-Leu-Met-Ser-Arg (PC-1), Val-Leu-Tyr-Glu-Glu (PC-2), and Met-Ile-Leu-Met-Arg (PC-3) with molecular weights of 651.77, 668.82, and 662.92 Da, respectively. PC-1 exhibited the highest scavenging activities on DPPH (EC_{50} 1.35 mg/ml), superoxide (EC_{50} 0.450 mg/ml), and ABTS (EC_{50} 0.312 mg/ml) radicals, but PC-2 exhibited the strongest hydroxyl radical scavenging activity (EC_{50} 0.353 mg/ml) among the three peptides. PC-1 also showed effective inhibition on lipid peroxidation in the model system. The good activities of isolated peptides might be benefit from the smaller size and hydrophobic and/or aromatic amino acids within their sequences.

1. Introduction

Oxidation is an important reason of food spoilage, which can also lead to the changes of flavour, colour, texture, and loss of nutritive value. In addition, it was confirmed that superfluous radical generation during oxidative stress was closely related to the progress of many diseases (Ji, Sun, Zhao, Xiong, & Sun, 2014). At present, synthetic chemicals, such as BHT and BHA, are used as antioxidants for preventing radical formation or eliminating them from the chain reaction in food and biosystems. Nevertheless, their applications are limited in some countries because of their underlying damages (Puchalska, Marina, & García, 2014). So, there is a strong demand for natural antioxidants from natural sources.

Bioactive peptides showed a variety of biological activities, such as antihypertensive, enhancing immunity, and antioxidant activities. At present, peptides with high antioxidant activity have been prepared from protein hydrolysates of seafood or seafood by-products, such as flounder fish muscle (Ko, Lee, Samarakoon, Kim, & Jeon, 2013), horse mackerel viscera (Sampath Kumar, Nazeer, & Jaiganesh, 2011), *Sphyrna lewini* muscle (Wang, Li, Chi, Zhang, & Luo, 2012), horse mackerel and *Otolithes ruber* skin (Sampath Kumar, Nazeer, & Jaiganesh, 2012), *Mytilus coruscus* (Kim et al., 2013), oysters (Wang et al., 2014), and blue mussel (Wang et al., 2013). These antioxidants are thought to be safer than the synthetic ones.

Croceine croaker (*Pseudosciaena crocea*) is a kind of important economic fish in China, and its population collapsed in the 1970s due to overfishing. At present, the production of croceine croaker has exceeded the demand of people through large-scale artificial breeding, and the enzymatic hydrolysis would be one of the most efficient methods to resolve the excess croceine croakers. In the study, three novel antioxidant peptides were isolated from protein hydrolysate of croceine croaker muscle, and their antioxidant activities were evaluated.

2. Materials and methods

2.1. Materials

Croceine croaker (*P. crocea*) was provided by Zhejiang Dahaiyang Sci-Tech Co., Ltd. (Zhejiang, China). DPPH, DEAE-52 cellulose, trifluoroacetic acid (TFA), Sephadex G-15, papain, and alcalase were bought from Sigma–Aldrich (Shanghai) Trading Co., Ltd.

2.2. Preparation of protein hydrolysate

The muscle of croceine croaker was pounded to homogenate, and the homogenate was defatted using isopropanol on the method of Wang et al. (2013). Defatted homogenate was dispersed in distilled water to obtain 10% (w/v) protein slurry, and hydrolyzed using papain (enzyme/substrate ratio 1%, 50 °C, pH 7.0 and 4 h) and

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http://dx.doi.org/10.1016/j.foodchem.2014.07.117
0308-8146/

alcalase (enzyme/substrate ratio 2%, 50 °C, pH 9.5 and 4 h), respectivley. Half of hydrolysates prepared using papain for 4 h was taken out and its pH was adjusted to 9.5 with 1 N NaOH, and alcalase (enzyme/substrate ratio 2%) was added for an additional 4 h of hydrolysis. After proteolysis, the hydrolysates were heated to 90 °C for 15 min and centrifuged at 5000×*g* for 15 min, and the supernatant with the highest activity, referred to as CCH-S, was lyophilized.

2.3. Isolation of antioxidant peptides from CCH-S

CCH-S solution was fractionated by ultrafiltration membranes (MWCO 3 and 10 kDa), and three fractions termed CCH-S-I (MW > 10 kDa), CCH-S-II (MW 3–10 kDa), and CCH-S-III (MW < 3 kDa) were collected and lyophilized.

CCH-S-III solution (5 ml, 40.0 mg/ml) was added to a pre-equilibrated DEAE-52 cellulose column (1.6 × 60 cm) and stepwise eluted with ultrapure water, 0.1, 0.5, and 1.0 M NaCl solution at a flow rate of 1.0 ml/min, respectively. Fractions (5 ml) were collected and detected at 280 nm. Three fractions (Fr.A, Fr.C, and Fr.D) were pooled and lyophilized.

Fr.C solution (2 ml, 25.0 mg/ml) was fractionated by Sephadex G-15 column (2.6 × 80 cm) and eluted with ultrapure water at a flow rate of 1.0 ml/min. Each eluate (3 ml) was collected and monitored at 280 nm, and two subfractions (Fr.C-1 and Fr.C-2) were collected and lyophilized.

Fr.C-1 was finally separated by RP-HPLC on a Zorbax, SB C-18 column (4.6 × 250 mm) using a linear gradient of acetonitrile (0.05% TFA). The eluate was determined at 280 nm, and three peptides (PC-1, PC-2, and PC-3) were isolated and lyophilized.

2.4. Determination of degree of hydrolysis (DH) and antioxidant activity

DH was analysed on the previously method (Chi et al., 2014) (Supplementary material 1). Lipid peroxidation inhibition assay and DPPH/hydroxyl/superoxide/ABTS radical scavenging assays were determined on the method of Wang et al. (2012) (Supplementary material 2). EC_{50} was defined as the concentration at which a sample caused a 50% decrease of the initial racical concentrations.

2.5. Identification of peptide sequences and molecular mass

The peptide sequences were determined on an Applied Biosystems 494 protein sequencer. Molecular masses were determined using a Q-TOF mass spectrometer coupled with an electrospray ionization source (ESI). Ionization was carried out in positive mode with a capillary voltage of 3500 V. Nitrogen was maintained at 40 psi for nebulization and 9 l/min at 350 °C for evaporation temperature.

Table 1
DH and DPPH/hydroxyl radical scavenging activities of hydrolysates and membrane fractions (*c* = 10 mg/ml).

	DH (%)	DPPH radical scavenging activity (%)	Hydroxyl radical scavenging activity (%)
CCH-P	10.24 ± 0.76^{a}	11.86 ± 0.75^{a}	13.11 ± 1.07^{a}
CCH-A	16.32 ± 0.65^{b}	15.48 ± 1.22^{b}	16.32 ± 1.47^{b}
CCH-S	21.09 ± 0.92^{c}	18.96 ± 1.42^{c}	22.79 ± 1.63^{c}
CCH-S-I	–	8.38 ± 1.97^{A}	13.78 ± 1.84^{A}
CCH-S-II	–	17.38 ± 1.97^{B}	20.42 ± 1.56^{B}
CCH-S-III	–	24.73 ± 1.69^{C}	29.97 ± 2.15^{C}

–, no detected.
CCH-P, prepared using papain; CCH-A, prepared using alcalase; CCH-S, prepared using papain then alcalase. $^{a-c}$ or $^{A-C}$ Values with different letters in the same column indicated significant difference for the same composition determination ($p < 0.05$).

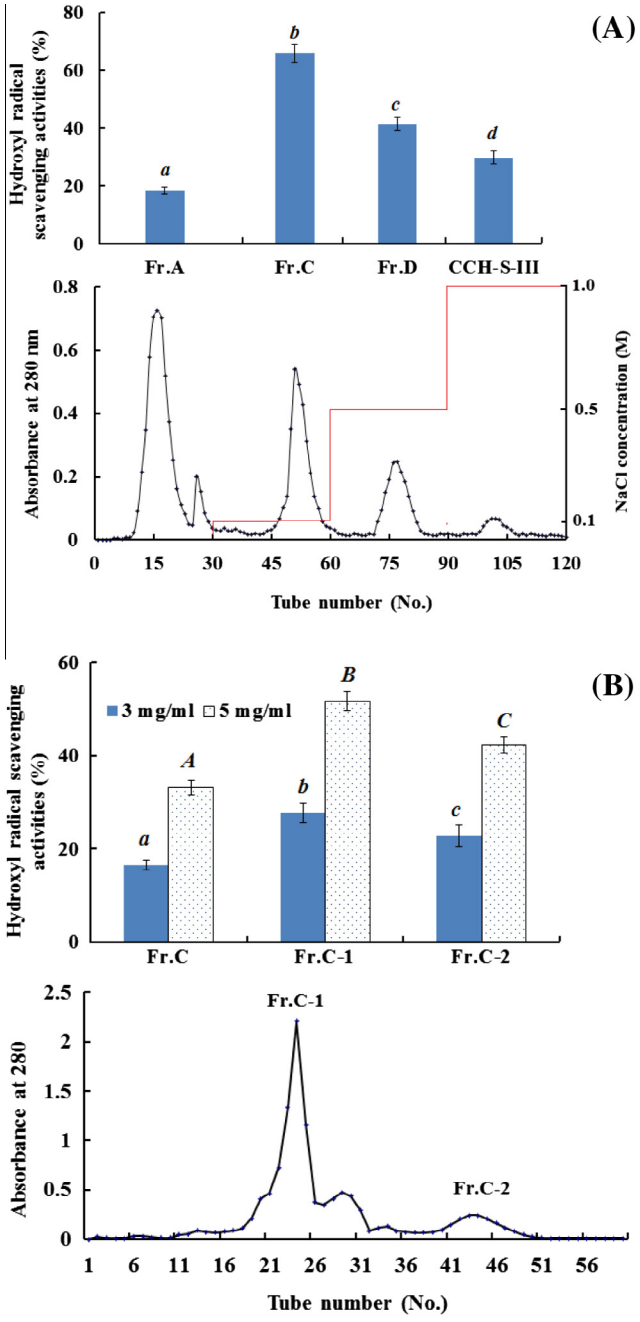

Fig. 1. Chromatographic purification and hydroxyl radical scavenging activity of fraction CCH-S-III and its subfractions. (A) DEAE-52 cellulose chromatography. $^{a-d}$ Column wise values with same superscripts indicated no significant difference ($p > 0.05$). (B) Sephadex G-15 gel chromatography. $^{a-c}$ or $^{A-C}$ Column wise values with same superscripts of this type indicated no significant difference ($p > 0.05$).

2.6. Statistical analysis

Data were presented as means ± standard deviation (SD) ($n = 3$). ANOVA test (SPSS 19.0 software) was applied to compare the mean value of each treatment. Significant differences were determined by using Duncan's multiple range Test ($p < 0.05$).

3. Results and discussion

3.1. Preparation of protein hydrolysates

Bioactive peptides are often functionally inactive in native proteins and must be discharged by proteolysis (*in vivo* digestion,