Food Chemistry 179 (2015) 290–295

Contents lists available at ScienceDirect

Food Chemistry

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

Isolation, identification and synthesis of four novel antioxidant peptides from rice residue protein hydrolyzed by multiple proteases

Qiao-Juan Yan [a,*], Lin-Hua Huang [a], Qian Sun [b], Zheng-Qiang Jiang [b,*], Xia Wu [b]

[a] *College of Engineering, China Agricultural University, Beijing 100083, China*
[b] *College of Food Science and Nutritional Engineering, Beijing Key Laboratory of Functional Food from Plant Resources, China Agricultural University, Beijing 100083, China*

ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form 18 December 2014
Accepted 31 January 2015
Available online 7 February 2015

Keywords:
Rice residue protein
Multiple proteases
Antioxidant activity
Synthesis
Peptides

ABSTRACT

Multiple proteases were optimized to hydrolyze the rice residue protein (RRP) to produce novel antioxidant peptides. An antioxidant peptide fraction (RRPB3) with IC_{50} of 0.25 mg/ml was purified from the RRP hydrolysate using membrane ultrafiltration followed by size exclusion chromatography and reversed-phase FPLC. RRPB3 was found to include four peptides (RRPB3 I–IV) and their amino acid sequences were RPNYTDA (835.9 Da), TSQLLSDQ (891.0 Da), TRTGDPFF (940.0 Da) and NFHPQ (641.7 Da), respectively. Furthermore, four peptides were chemically synthesized and their antioxidant activities were assessed by DPPH radical scavenging, ABTS radical scavenging assay and FRAP-Fe^{3+} reducing assay, respectively. Both RRPB3 I and III showed synergistic antioxidant activity compared to each of them used alone. All four synthetic peptides showed excellent stability against simulated gastrointestinal proteases. Therefore, the peptides isolated from RRP may be used as potential antioxidants in the food and drug industries.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Oxidation as an important process in aerobic organisms leads to the formation of free radicals which plays a critical role in the development of some serious health disorders such as cancer, cardiovascular diseases (Afonso, Champy, Mitrovic, Collin, & Lomri, 2007; Devasagayam et al., 2004). Additionally, oxidation has direct harmful effects on food flavor and texture. Thus, antioxidant additives, which significantly delay or inhibit oxidation, are important in the food industry (Teng, Fang, Song, & Gao, 2011). However, chemical synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ), are limited due to their toxic effects on human's enzyme systems (Williams, Iatropoulos, & Whysner, 1999). Natural antioxidants such as peptides have attracted more attention owing to their safety and wide distribution properties in recent years (Lee et al., 2013). Antioxidant peptides derived from various food proteins have been considered as ideal natural antioxidants in the food industry or medical treatment (Sarmadi & Ismail, 2010; Singh, Vij, & Hati, 2014). They are found to be safe and potentially exert a beneficial health effect due to their low toxicity, high activity and easy absorption (Piu et al., 2014). Usually, the peptides with the antioxidant activity rely on their specific amino acid sequences, which can be possibly obtained from the proteins via enzymatic hydrolysis (Bougatef et al., 2010). So far, numerous antioxidant peptides have been isolated and identified from different protein hydrolysates such as fishes (Najafian & Babji, 2012), duck skin (Lee et al., 2013), Spanish dry-cured ham (Escudero, Mora, Fraser, Aristoy, & Toldra, 2013), chickpea (Zhang, Li, Miao, & Jiang, 2011), rice bran (Zhang et al., 2010) and soybean-based infant formulas (Puchalska, Marina, & Garcia, 2014).

Rice proteins are known as one of the most valuable plant proteins due to their excellent nutritional and hypoallergenic properties in comparison with other cereals and legume proteins (Zhao et al., 2012). Until now, there are some reports on the enzymatic hydrolysis of rice proteins to isolate and identify functional peptides with antioxidant, oral tolerogenic or flavor enhancer (Takagi, Hiroi, Hirose, Yang, & Takaiwa, 2010; Zhang et al., 2010;

Abbreviations: ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; BHT, butylated hydroxytoluene; BLAST, basic local alignment search tool; DPPH, 1,1-diphenyl-2-picrylhydrazyl; HPLC, high-pressure liquid chromatography; FPLC, fast protein liquid chromatography; FRAP, ferric reducing ability of plasma; OPA, O-phthalaldehyde; RRP, rice residue protein; RRPB3, the third peak of peptide B fraction produced from RRP; TPTZ, 2,4,6-tripyridyl-s-triazine.

* Corresponding authors at: PO Box 294, China Agricultural University, No. 17 Qinghua Donglu, Haidian District, Beijing 100083, China. Tel.: +86 10 62737689; fax: +86 10 82388508.

E-mail addresses: yanqj@cau.edu.cn (Q.-J. Yan), zhqjiang@cau.edu.cn (Z.-Q. Jiang).

http://dx.doi.org/10.1016/j.foodchem.2015.01.137
0308-8146/© 2015 Elsevier Ltd. All rights reserved.

Zhao et al., 2012). However, the low yield and activity of these functional peptides from rice proteins made them unpractical for commercial application. Rice residue protein (RRP), which was extracted from the by-products in rice processing, is of high nutritional value but largely underutilized as an animal feed at present. In this study, a multiple-protease hydrolysis process was investigated to produce the RRP hydrolysates with high yield and antioxidant activity. Antioxidant peptides were further isolated, sequenced, synthesized and characterized.

2. Materials and methods

2.1. Materials

Rice residue protein (RRP) of food grade (consisting of more than 70.0% protein, less than 3.0% fat, 5.0% crude fiber, 6.0% ash and 16.0% other substances on dry basis, and 8.0% moisture) was supplied by Hetian Food Company (Liaoning province, China). Alcalase (2.4 AU-A/g), Flavourzyme (500 LAPU/g) and Protamex (1.5 AU-NH/g) were obtained from Novoenzymes, Denmark. Pepesin (250 U/g), Trypsin (250 U/g) and Papain (2000 U/g) were purchased from Amresco (Solon, USA). Gly-Leu and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, MO, USA). The ABTS radical scavenging and FRAP-Fe^{3+} kits were purchased from Beyotime Institute of Biotechnology (Beijing, China). All other chemicals used were of analytical grade.

2.2. Antioxidant capacity assays

Three methods viz. DPPH, ABTS and FRAP assays were used to determine the antioxidant capacity according to the reported protocols (Gimenez, Moreno, Lopez-Caballero, Montero, & Gomez-Guillen, 2013; Wang et al., 2014). Solvent blanks were measured for each assay. Assays for each sample were performed in triplicates. The results were presented as means ± standards errors.

DPPH radicals scavenging capacity assay was done according to the method of Fukumoto and Mazza (2000) with slight modifications. In brief, an aliquot of 4 ml of each peptide solution was mixed with 1 ml of methanol containing 1 mM DPPH radicals. The mixture was allowed to stand for 30 min in the dark, and absorbance was monitored at 517 nm. The reduction of absorbance at 517 nm between the blank and a sample was used to calculate the scavenging activity. The IC_{50} value was defined as the concentration of peptide required to scavenge 50% of DPPH radicals. BHT was used as positive controls.

ABTS radical-scavenging capacity assay was performed using the method of Wiriyaphan, Chitsomboon, and Yongsawadigul (2012) with minor modifications. The stock solutions included 7.4 mM ABTS radical solution and 2.6 mM potassium persulfate. The working solution was then prepared by mixing both stock solutions in equal quantity and allowed them to react for 12 h at room temperature in darkness. The solution was then diluted by mixing 1 ml ABTS radical solution with 60 ml methanol to obtain an absorbance of 1.17 ± 0.02 unit at 734 nm. Fresh ABTS radical solution was prepared for each assay. Samples (150 μl) were reacted with 2850 μl of the ABTS radical solution for 2 h in darkness, the absorbance was then measured at 734 nm. The linearity of the standard curve was within the range from 25 to 600 mM Trolox. Results were expressed in mM Trolox equivalent (TE)/g fresh mass.

The FRAP-Fe^{3+} reducing capacity assay was carried out as described by Escudero et al. (2013) with slight modifications. Briefly, the stock solutions included 300 mM acetate buffer pH 3.6 (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $FeCl_3 \cdot 6H_2O$ solution and maintained at 37 °C before use. Samples (150 μl) were incubated with 2850 μl of the FRAP solution for 30 min in darkness. Absorbance of the color product (ferrous tripyridyltriazine complex) was then measured at 593 nm. The standard curve was linear between 25 and 800 mM Trolox. Results were expressed in mM TE/g fresh mass.

2.3. Peptide concentration determination

The peptide concentration in the extracts was determined by OPA assay (Wang et al., 2008). The procedure involved mixing 2.5 μl of sample with 100 μl of OPA mixture (2.5 ml of sodium tetraborate, 1 ml of 5% (w/v) SDS, 100 μl of 40 mg/ml OPA in methanol, 10 μl 2-mercaptoethanol, and 1.39 ml water). The mixture was held for 8 min at room temperature and the absorbance was then measured at 340 nm. The peptide content was calculated by interpolation using a Gly-Leu (a dipeptide), standard calibration curve in the range from 0 to 5 mg/ml.

2.4. Preparation of antioxidant RRP hydrolysates

The hydrolysis conditions such as different proteases, substrate concentration, initial pH, hydrolysis temperature and incubation time were optimized to produce antioxidant peptides. The initial hydrolysis conditions were as follows: amount of protease, 2.5%; concentration of substrate (RRP), 5%; hydrolysis time, 4 h; temperature and pH were set up according to the optimal conditions of each enzyme [Alcalase (pH 8.5 at 55 °C), Flavourzyme (pH 6.0 at 50 °C), Protamex (pH 7.0 at 50 °C), Pepsin (pH 3.0 at 37 °C), Papain (pH 6.0 at 55 °C), Trypsin (pH 8.0 at 37 °C)]. Enzymatic hydrolysis was carried out in a temperature-control shaker at a speed of 200 rpm. Then the proteases were terminated after boiling for 10 min. The hydrolysate supernatant (RRP hydrolysate) was separated by centrifugation at 5000 rpm for 15 min and then concentrated in a rotary evaporator for further use.

2.5. Purification of antioxidant peptides from the RRP hydrolysate

The RRP hydrolysate was filtrated sequentially using 10,000 Da and 3000 Da membranes (Millipore, USA). Subsequently, the sample containing peptides below 3000 Da were properly diluted in 50 mM phosphate buffer (pH 7.0) and loaded onto a Sephadex G-15 gel filtration column (2.5 × 90 cm) pre-equilibrated with the same buffer. The column was then eluted with the buffer, and the fractions exhibiting antioxidant activity measured by the DPPH radical scavenging activity assay were pooled and concentrated in a rotary evaporator. The antioxidant fractions were further separated on a reversed-phase SOURCE 15RPC column (2.5 × 10 cm) (GE healthcare, USA) by Fast Protein Liquid Chromatography (FPLC) system (GE healthcare, USA). The antioxidant peptides were eluted with a liner gradient of aqueous acetonitrile solution (0–40%) containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 1.0 ml/min. The elution peaks were monitored at 214 nm, and their antioxidant activities were assessed by the DPPH radical scavenging activity assay.

2.6. Identification of amino acid sequences

The fractionated peptides with highly antioxidant activities were identified by their amino acid sequences using Nano-UPLC/MS/MS mass spectrometry (Waters, MA, USA). According to the manufacturer's recommendation, the peptide fractions were firstly washed and concentrated with a Symmetry C_{18} column (Waters, MA, USA) and subjected to the nanoACQUITY UPLC with a BEH C_{18} column coupled to a SYNAPT G2-S electronic spray ion

Download English Version:

https://daneshyari.com/en/article/7592633

Download Persian Version:

https://daneshyari.com/article/7592633

Daneshyari.com