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Purification and identification of antioxidant peptides from peanut protein isolate hydrolysates using UHR-Q-TOF mass spectrometer

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ABSTRACT

Peanut protein isolate (PPI) was hydrolysed with alcalase to obtain antioxidant peptides. To purify these peptides, the peanut protein isolate hydrolysates (PPIH) were separated by ultrafiltration (MWCO = 3 kDa) and the obtained PPIH-II (Mw < 3 kDa) with higher antioxidant activity was further separated by gel filtration chromatography (Sephadex G-15). After filtration, both peptides, P1 and P4, with stronger antioxidant capacity were fractionated using preparative high performance liquid chromatography (P-HPLC). Three antioxidant peptides were finally purified from P1 and P4 using the UHR-Q-TOF mass spectrometer, and the amino acid sequences of the peptides were identified as Thr-Pro-Ala (286 kDa), Ile/Leu-Pro-Ser (315 kDa) and Ser-Pro (202 kDa), respectively.

1. Introduction

Free radicals are unavoidable metabolic byproducts that are the result of oxidative metabolism, which may attack macromolecules such as proteins and DNA, leading to serious diseases including hypertension, cancer, coronary heart disease and senile dementia (Halliwell & Gutteridge, 1999; Moskovitz, Yim, & Choke, 2002; Urso & Clarkson, 2003). There is a significant amount of evidence that antioxidants play an important role in human health, as they can protect the human body against damage from free radicals (Chen, Chi, & Xu, 2011). Food-derived protein hydrolysates or peptides are potential natural antioxidants without marked adverse effects, and commonly used in food such as weaning foods, dry mixes, baked foods, whipped toppings and salad dressings, and pharmaceutical applications (Guo, Kouzuma, & Yonekura, 2009; Wu, Wang, Ma, & Ren, 2009). Recently, many kinds of proteins have been hydrolysed to obtain peptides with an antioxidant capacity, such as chickpea albumin (Kou et al., 2013), barley glutelin (Xia, Bamdad, Gänzle, & Chen, 2012), egg white protein (Chen, Chi, Zhao, & Lv, 2012), tilapia frame protein (Fan, He, Zhuang, & Sun, 2012), chickpea protein (Zhang, Li, Miao, & Jiang, 2011) and cottonseed protein (Gao, Cao, & Li, 2010).

China is one of the largest producers of peanuts, accounting for 46.5% of the total peanut production worldwide (Jamdar et al., 2010). Peanut seeds contain 25–29% protein, 20–23% carbohydrate and 40–50% oil (Latifa, Pfannstielb, & Makkara, 2013), and peanut meal is a byproduct generated after extracting the oil from the peanuts, which contains 47–55% of the proteins but with poor functional properties (Yu, Aahmedna, & Goktepe, 2007). In recent years, fermented peanut meal has been used to prepare peanut peptides, and the antioxidant activity of peanut peptides has also been determined (Tang et al., 2012; Zhang et al., 2011). However, peanut protein isolate (PPI) has a higher purity of proteins and better functional properties than other peanut protein products, such as flour or concentrate (Wu et al., 2009). To the best of our knowledge, little research has been done on the hydrolysates from peanut protein isolate.

It is known that antioxidants usually contain 3–16 amino acid residues, and their composition, structure and correct positioning in the peptide sequence is related to their antioxidant activity (Sarmadia & Ismaila, 2010; Tang et al., 2010). In this work, PPI was hydrolysed with alcalase to obtain antioxidant peptides, while ultrafiltration, gel filtration chromatography and preparative high performance liquid chromatography (P-HPLC) were used to purify the antioxidant peptides. The objective of this study was to identify the antioxidant peptides using UHR-Q-TOF mass spectrometry in order to determine their potential value in the food industry.

2. Materials and methods

2.1. Materials

Defatted peanut flour was kindly provided by Qingdao Changshou Group Co. Ltd. (Shandong, China). The protein, fat,

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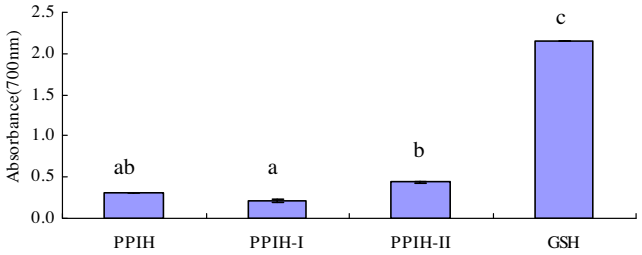

Fig. 1. The reducing power of PPIH after ultrafiltration. Different letters show significant differences ($p < 0.05$).

crude fibre and carbohydrate contents of the defatted peanut flour were 51.8%, 8.0%, 10.5% and 29.7%, respectively. Alcalase 2.4 L, reduced glutathione (GSH) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA), and all other reagents used in this experiment were analytical-grade.

2.2. Preparation of peanut protein isolate (PPI)

The alkaline extraction and acid precipitation method described by Kim, Kim, and Nam (1992) was used to prepare the PPI. The defatted peanut flour was mixed with distilled water in the ratio of 1:10, adjusting the pH to 9.0 with 1 N NaOH, and stirred at 40 °C in a water bath for 1 h with the stirring speed of 100 rpm. After the extraction process, the suspended material was filtered through a 100-mesh standard sieve. The sediment was centrifuged at 10,000 rpm for 30 min, and the resulting supernatant was adjusted to pH 4.5 with 1 N HCl and centrifuged at 4000 rpm for 15 min. The precipitates were then washed with distilled water and centrifuged several times at 4000 rpm for 15 min until a pH of 7.0 was reached. The PPI was dried at 40 °C for 48 h in an air oven, ground to pass through a 100-mesh standard sieve and stored at 4 °C, from where samples were drawn for analysis.

2.3. Preparation of peanut protein isolate hydrolysates (PPIH)

The PPIH was prepared using the method described by Li, Jiang, Zhang, Mu, and Liu (2008), with some modifications. The PPI (20 g) was suspended in distilled water (1000 ml) and hydrolysed with alcalase (400 μl) at 60 °C, after adjusting the pH of the mixture to 8.0 with 1.0 M NaOH. After maintaining the pH constant with 1.0 M NaOH during the hydrolysis time (3 h), the enzyme was inactivated at 95 °C for 10 min. Then, the hydrolysate solution was cooled down to room temperature, neutralised with 1 M HCl and centrifuged at 4000 rpm for 15 min. The supernatants were lyophilized and stored at −20 °C for further use.

2.4. Purification of PPIH

2.4.1. Ultrafiltration

The ultrafiltration of the PPIH was determined by using the method of Ren et al. (2008), with some modifications. The PPIH (9 g) was dissolved in 100 ml of distilled water and fractionated according to molecular size through an ultrafiltration (UF) membrane bioreactor system (Millipore Minitan system, Millipore, Bedford, MA), with a molecular weight cut off (MWCO) of 3 kDa. PPIH-I and PPIH-II represent the fractions with molecular weight (Mw) distributions of >3 kDa and <3 kDa, respectively. All PPIHs recovered were freeze-dried and the antioxidant activity of each fraction was determined.

2.4.2. Gel filtration chromatography (GFC)

The low molecular weight peptide fraction with the highest antioxidant activity after ultrafiltration was further separated using Sephadex G-15 and a gel filtration column (2 × 50 cm) eluted with distilled water, at a flow rate of 0.5 ml/min. Each fraction was measured at 220 nm. Fractions with active peaks were collected and lyophilized for an antioxidant activity assay.

2.4.3. Preparative high performance liquid chromatography (P-HPLC)

The samples of the antioxidant peptides, after separation by Sephadex G-15, were isolated with P-HPLC by using the method of Sun, Pan, Guo, and Li (2012), with some modifications. The desired peak after GFC purification was prepared by dissolving it in distilled water. The solution was applied onto a P-HPLC column (1.9 × 15 cm, Shiseido Fine Chemicals, Tokyo, Japan) with a linear gradient of acetonitrile (0–50% v/v, in 50 min) containing 0.05% (v/v) trifluoroacetic acid (TFA) with a flow rate of 5 mL/min. The

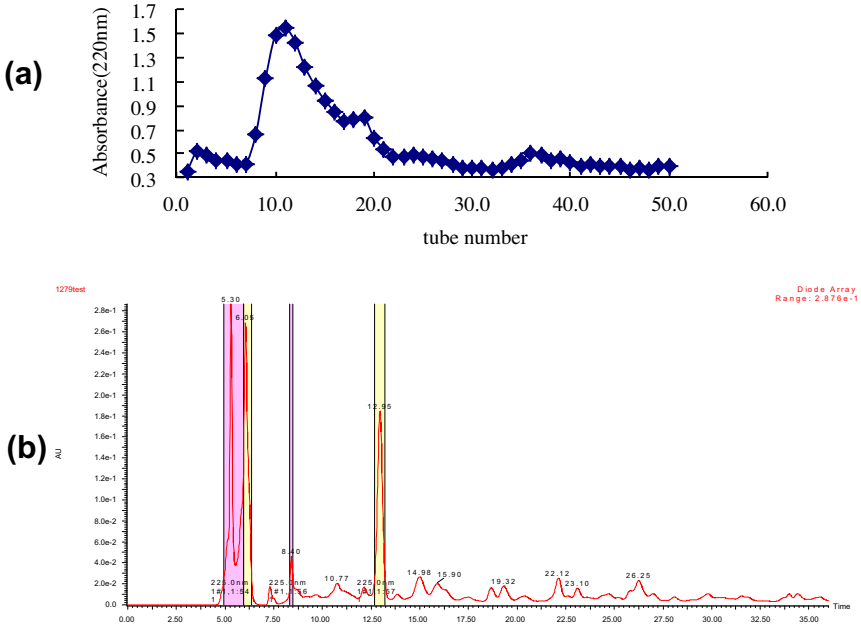

Fig. 2. Chromatogram of peanut peptides (Mw < 3 kDa) using Sephadex G-15 (a) and PPIH (3 kDa) separated after Sephadex G-15 (b).