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Purification and identification of antioxidant peptides from corn gluten meal

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ABSTRACT

Corn gluten meal was hydrolyzed by alkaline protease and Flavourzyme to obtain the antioxidant peptides. The antioxidant activities of the hydrolysates or peptides were evaluated by free radical scavenging capacity (1,1-diphenyl-2-picrylhydrazyl/2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt/hydroxyl radical/superoxide radical anion), metal ion (Fe^{2+}/Cu^{2+}) chelating activity and lipid peroxidation inhibitory capacity. The hydrolysates were separated by ultrafiltration, and those with molecular weight <10 kDa exhibited highest antioxidant activity in all relevant assays. The hydrolysates were subsequently purified by gel filtration chromatography, and fraction F3 showed the highest antioxidant activity. Three peptides were identified from fraction F3 using LC–ESI–Q–TOF MS/MS as Leu-Pro-Phe (375.46 Da), Leu-Leu-Pro-Phe (488.64 Da) and Phe-Leu-Pro-Phe (522.64 Da). These peptides exhibited good free radical scavenging activity and lipid peroxidation inhibitory effect. Thus, corn gluten meal may be used as a potential source of antioxidant peptides for food and nutraceutical applications.

1. Introduction

Oxidation is an essential process for life to perform biological functions such as catabolism of proteins, fats and carbohydrates (Gulcin, 2009). During the oxidation, free radicals such as superoxide anion radicals ($O_2^{\cdot -}$) and hydroxyl radicals ($^{\cdot}OH$) are generated in the living body (Evans, Goldfine, Maddux, & Grodsky, 2003). These free radicals, which are physiologically produced, play important roles in biological systems that exert diverse functions like signaling roles and providing defense against infections (Johansen, Harris, Rychly, & Ergul, 2005). Nevertheless, when these radicals are produced in excess, they can accumulate in cells and cause harm over time. This can result in protein damage, DNA mutation, oxidation of membrane phospholipids (Lee, Koo, & Min, 2004) and modification in low density lipoproteins, which in turn can initiate several diseases, including diabetes, atherosclerosis, arthritis, coronary heart diseases and cancer (Chandrasekara & Shahidi, 2011). In addition, free radical-induced lipid oxidation is a major concern in the food industry, because the oxidation of fats and oils during processing and storage of food products has a negative effect on the quality and nutritive value of lipids (Rajapakse, Mendis, Jung, Je, & Kim, 2005). Therefore, it is important to inhibit the formation of excessive free radicals occurring in the living body and food stuffs (Zhong, Ma, Lin, & Luo, 2011). Antioxidants can act at different levels in an oxidative sequence, protecting the human body from free radicals and retarding the progress of many chronic diseases which are influenced by oxidative reactions (Pihlanto, 2006). However, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are widely used in the food industry have potential

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risks on human health (Pihlanto, 2006; Shahidi & Zhong, 2008). Thus, more attention has been focused on the development safe and effective functional foods and antioxidative agents from natural sources.

In recent years, hydrolyzed proteins from many animal and plant sources such as peanut kernels (Hwang, Shyu, Wang, & Hsu, 2010), rice bran (Revilla et al., 2009), sun flower protein (Megías et al., 2008), frog skin (Qian, Jung, & Kim, 2008), milk casein (Blanca, Ana, Lourdes, & Isidra, 2007), egg yolk protein (Sakanaka & Tachibana, 2006) and canola (Cumby, Zhong, Naczk, & Shahidi, 2008) have been found to possess antioxidant activity. Corn gluten meal is a major by-product of corn wet milling, containing 60% (w/w) protein. However, its low water solubility and severely imbalanced amino acid composition makes it difficult to be used as a food additive. In China, Over 840,000 tonnes of corn gluten meal are produced every year (Lin et al., 2011). At present, corn gluten meal is mainly used as feedstuff or discarded. Producing hydrolysates with antioxidant properties from corn gluten could effectively increase its value in the marketplace (Li, Han, & Chen, 2008a). In the present study, corn gluten meal was hydrolyzed by alkaline protease and Flavourzyme to produce hydrolysates with antioxidant activities. The antioxidant activities of hydrolysates with different molecular weight distribution were compared. Moreover, the hydrolysates that exhibited strong antioxidant activities were fractionated by gel filtration chromatography. Antioxidant activities of the fractions were determined and peptides that showed highest antioxidant activity were identified by LC–MS/MS. Furthermore, the peptides were synthesized according to the obtained amino acid sequences to confirm their antioxidant activities.

2. Materials and methods

2.1. Materials

Corn gluten meal was obtained from Dacheng Ltd. (Changchun, Jilin, China). Alkaline protease and Flavourzyme were purchased from Pangbo Biological Engineering Co. Ltd. (Nanning, Guangxi, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), ferrozine, linoleic acid and Sephadex G-25 were purchased from Sigma to Aldrich (Shanghai, China). All other chemicals used were of analytical grade.

2.2. Preparation of hydrolysates with different molecular weight distribution

The method of preparing the corn gluten meal hydrolysates was previously developed in our laboratory (Zhuang, Tang, Dong, Sun, & Liu, 2013). Corn gluten meal was mixed with deionized water (1:25, w/v). The obtained mixture was adjusted to pH 9.5 by adding 0.1 M NaOH and heated in a water bath at 55 °C. Then the alkaline protease (8%, w/v) was added to hydrolyze the corn gluten meal over a 75 min period. After being heated in a boiling water bath for 10 min, the pH and temperature were adjusted to 7, 50 °C, respectively. Then Flavourzyme (4.2%, w/v) was added to hydrolyze the corn gluten meal for another 66 min. At the end of the hydrolysis period, the mixture was heated in boiling water for 10 min to inactivate the proteases. The hydrolysates were then centrifuged at 10,000*g* for 10 min at 4 °C. The obtained supernatant was ultra-filtered through a membrane with a cut off molecular weight of 30 kDa. This process yielded two fractions: retentate (fraction 1, represented hydrolysates >30 kDa) and permeate (M_W < 30 kDa). The permeate was further ultra-filtered through a membrane with a cut off molecular weight of 10 kDa to obtain the second retentate (fraction 2, represented hydrolysates between 30 and 10 kDa) and permeate (fraction 3, represented hydrolysates <10 kDa). The obtained hydrolysates with different molecular weight distribution were lyophilized and stored at −20 °C until use (no longer than 90 d).

2.3. Determination of antioxidant activities

2.3.1. DPPH radical scavenging activity assay

The DPPH radical scavenging activity of hydrolysates or peptides was measured using a modified method of Zhang, Li, Miao, and Jiang (2011). DPPH radical (2 ml 0.1 mM) dissolved in 95% ethanol was added to 2 ml of sample solution. The mixture was shaken and left for 30 min at room temperature. Absorbance of the resulting solution was measured at 517 nm. For the blank, 2 ml of distilled water were used instead of the sample. The radical scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity}(\%) = [(\text{Blank absorbance} - \text{Sample absorbance})/\text{Blank absorbance}] \times 100.$$

2.3.2. ABTS radical scavenging activity assay

The ABTS radical scavenging activity of hydrolysates or peptides was determined according to the method of Tironi and Añón (2010) with some modifications. The ABTS radical cation was generated by mixing ABTS stock solution (7 mM) with potassium persulphate (2.45 mM) and allowing the resultant mixture in the dark at room temperature for 16 h before use. The ABTS radical solution was diluted in 0.2 M phosphate buffered saline (pH 7.4), to an absorbance of 0.70 ± 0.02 at 734 nm. The diluted ABTS radical solution (2 ml) was mixed with 200 μl of sample. After 10 min, the absorbance was read at 734 nm. For the blank, 200 μl of distilled water were used instead of the sample. The ABTS scavenging activity of samples was calculated as follows:

$$\text{ABTS scavenging activity}(\%) = [(\text{Blank absorbance} - \text{Sample absorbance})/\text{Blank absorbance}] \times 100$$

2.3.3. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of hydrolysates or peptides was determined according to the method of Wang, Wang, Dang, Zheng, and Zhang (2013) with some modifications. Briefly, 1 ml of 6 mM $FeSO_4$ solution was mixed with 1 ml of sample solution and 1 ml of 6 mM H_2O_2 solution. The mixture was shaken and left for 10 min at room temperature. Then 1 ml of 6 mM salicylic acid was added to the mixture, after 30 min, the absorbance was determined