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Antioxidant properties of papain hydrolysates of wheat gluten in different oxidation systems

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Abstract

Enzymatic hydrolysis was used for preparing hydrolysates from wheat gluten which is by-product during production of wheat starch. The enzyme used for the hydrolysis was papain. The hydrolysate was separated based on the molecular weight of the peptides by membrane ultrafiltration (UF) with a molecular weight cut-off of 5 kDa into permeate (P) and retentate (5-K) fractions. The antioxidative activities of the hydrolysate and its UF fractions were investigated by using the TBA method and scavenging effect of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The three fractions showed strong antioxidative activities in the linoleic acid oxidation system, and exhibited DPPH radical scavenging activity. The antioxidative activity of the P fraction was almost the same as that of vitamin E at pH 7.0. The molecular weight distribution of the P fraction was concentrated in 4.2 kDa (86.5%) after gel permeation chromatography fractionation using an HPLC system.The P and 5-K fractions had higher surface hydrophobicities (H_0) at pH7.0 compared with the hydrolysate. The resulting UF fractions were superior to the hydrolysate in terms of antioxidative activities.

Keywords: Wheat gluten hydrolysate; Antioxidative activity; 2-Thiobarbituric acid; 1,1-Diphenyl-2-picrylhydrazyl radical scavenging; Molecular weight distribution

1. Introduction

Lipid or fatty acid oxidation will result in quality deterioration and shorten the shelf-life of food products. The main cause is that oxidation can generate some free radicals that lead to fatty acid decomposition and development of undesirable rancid odors and flavors (Akoh & Min, 2002; Nawar, 1996). Numerous lines of evidence have indicated that free radicals play a critical role in a variety of pathological conditions including the processes of aging, cancer, multiple sclerosis, inflammation, coronary heart and cardiovascular diseases, senile dementia, arthritis and atherosclerosis (Blake & Winyard, 1995; Halliwell & Gutteridge, 1990; Halliwell & Gutteridge, 1999). Following the growing realization that a wide range of herbal medicines and foodstuffs may be credited for preventive effects on chronic diseases due to their radical scavenging or antioxidant properties, although the overall function in vivo has yet to be clarified, increasing attention has been directed to the development of safe and effective functional foods and the extraction of novel potential antioxidants from medicinal plants (Gordon, 1996; Potterat, 1997). Synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, may be used to prevent food products from deterioration during storage, and to extend the shelf-life of the food products. However, the demand for natural antioxidants has recently increased because of questions about the long-term safety and negative consumer perception of synthetic antioxidants (Yu et al., 2002).

Recently, some protein hydrolysates have been reported to exhibit antioxidant activity. The protein hydrolysates

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have effective antioxidative activities against the peroxidation of lipids and/or fatty acids (Amarowicz & Shahidi, 1997; Diaz & Decker, 2004; Pena-ramos & Xiong, 2002; Sakanaka, Tachibana, Ishihara, & Juncja, 2004).

Wheat gluten and wheat starch are economically important coproducts produced during wet processing of wheat flour. Wheat gluten includes two main components, glutenins and gliadins. They are highly polymorphic polypeptides, consisting of more than 60 different molecular weight species ranging in M_r from 30,000 to 90,000 kDa (Payne, Nightingale, Krattiger, & Holt, 1987; Shewry, Halford, & Tatham, 1992). In the food industry, wheat gluten is traditionally used as an additive to improve the baking quality of flour. It is readily available in large quantities and at low prices. The use of wheat gluten in food and non-food applications is gaining much interest. However, little information about antioxidants derived from wheat gluten proteins can be found.

In this present study, the antioxidant activities of wheat gluten hydrolysate and its UF fractions obtained by papain hydrolysis and membrane ultrafiltration were investigated in comparison with that of commercial antioxidant VE in two different oxidation systems. Meanwhile, amino acid composition and surface hydrophobicity of the hydrolysate and its UF fractions derived from wheat gluten were evaluated.

2. Materials and methods

2.1. Raw materials

Wheat gluten, produced by the Martin process, was obtained from Lianhua Co., China. Gluten contained 71.5% (m/m, dry basis) protein, 6.8% moisture. Papain (600,000 U/g) was purchased from Guangzhou Enzyme Co. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was of analytical grade from Nacalai Tesque (Kyoto, Japan). Linoleic acid (about 99%) and 2-thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals were of analytical grade.

2.2. Preparation of wheat gluten hydrolysate and its UF fractions

Eight percent of an aqueous dispersion of wheat gluten was heated in a water bath at 90 °C for 30 min prior to enzymatic hydrolysis.The enzymatic hydrolysis was carried out at 50 °C, constant pH 6.5 with an enzyme to substrate ratio[E/S] of 6000 U/g for 6 h. The enzyme was inactivated by heating at 100 °C for 10 min.The resulting hydrolysate was then rapidly cooled to ambient temperature in the ice bath, and was passed through a 5 kDa molecular weight cut-off (A/G Technology Co., model UFP-5-C, Needham, MA, USA) membrane. The hydrolysate and its UF fractions (Permeate, P fractions; retentate, 5-K fraction) were freeze-dried and stored at −20 °C until use.

2.3. Measurement of antioxidant activity

2.3.1. Scavenging effect of DPPH radical

The free radical scavenging activity was measured using the method of Chen, Muramoto, Yamauchi, Fujimoto, and Nokihara (1998). The dried hydrolysate and its UF fractions were dissolved in 4 ml distilled water at protein concentration with 0%, 0.04%, 0.08%, 0.12%, 0.16%, 0.2% and 0.24% (m/v,). A 1 ml of 40 μM DPPH radical in ethanol was added to the sample solution. The absorbance at 517 nm was measured after 30 min of incubation at 25 °C. The residual radicals in the samples were calculated according to the following equation:

$$\text{Residual DPPH radicals } (\%) = 100 - [(\text{DPPH blank} + \text{control sample}) - \text{DPPHsample}/(\text{DPPH blank})] \times 100$$

where DPPH blank is the value for 4 ml of water/1 ml of ethanol including 40 μM DPPH, the DPPH sample is the value of 4 ml of sample solution/1 ml of ethanol including 40 μM DPPH, and the control sample is the value of 4 ml of sample solution/1 ml of ethanol.

2.3.2. TBA method

The hydrolysate and its UF fractions at 250 mg of protein dissolved in 4.87 ml of distilled water, 0.13 ml of linoleic acid, 10 ml of ethanol, and 10 ml of 50 mM phosphate buffer (pH 7.0) were mixed in glass flasks. The flasks were sealed tightly with silicone rubber caps and kept at 40 °C in the dark. At regular intervals, aliquots of the reaction mixtures were withdrawn with a microsyringe for measurements of the oxidation using the thiobarbituric acid (TBA) method with minor modification (Ohkawa, Ohishi, & Yagi, 1979). The reaction mixture (50 μl) was added to a mixture of 0.8 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 0.8% TBA solution in water. The mixture was cooled in ice bath, it was centrifuged (5000 rpm) for 10 min. The absorbance of the supernatant was determined at 532 nm and antioxidative activity was expressed as malondialdehyde (MDA) concentration.

2.4. Molecular size distribution analysis

Molecular size distribution profile of the hydrolysate and its UF fractions was determined by HPLC (Water's 1525, USA) on a GPC column. GPC column (7.8 mm × 30 mm, Protein-Pak60) with exclusion limits of 1–20 kDa was connected. The elution buffer was 0.05 M Tris–HCl (pH 7.2), flow rate 0.7 ml/min, and monitored at 280 nm.

2.5. Amino acid analysis

The PICO TAG method, with modification, was used for measuring the amino acid profile of the hydrolysate and its UF fractions (Bildlngmeyer, Cohen, Tarvin, & Frost, 1987). The dry sample(weight equivalent to 4%