LWT - Food Science and Technology 42 (2009) 956–962

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Free radical scavenging activity of porcine plasma protein hydrolysates determined by electron spin resonance spectrometer

Qian Liu [a], Baohua Kong [a,*], Lianzhou Jiang [a], Xuhai Cui [b], Jing Liu [a]

[a] College of Food Science, Northeast Agricultural University, Harbin, Heilongjiang 150030, China
[b] Department of Life Science, Zaozhuang University, Zaozhuang, Shandong, 277160, China

ARTICLE INFO

Article history:
Received 22 March 2008
Received in revised form
10 December 2008
Accepted 17 December 2008

Keywords:
Free radical scavenging activity
Plasma protein
Enzymatic hydrolysis
Electron spin resonance

ABSTRACT

A study was conducted to investigate the antioxidant capacity of porcine plasma protein before and after enzymatic hydrolysis. Porcine plasma protein was hydrolyzed by using Alcalase with degree of hydrolysis (DH) ranged from 0 to 17.8%. The free radical scavenging effects of porcine plasma protein hydrolysates (PPH) were evaluated by electron spin resonance (ESR) spectrometer. The reducing power of PPH increased with increasing of DH ($P < 0.05$). The 5-h PPH exhibited the strongest inhibition of lipid oxidation, as indicated by lowest thiobarbituric acid-reactive substance values in a liposome-oxidizing system, and the strongest free radical scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH$^{\bullet}$), hydroxyl ($^{\bullet}$OH) and superoxide ($O_2^{\bullet-}$) radicals. The increase of protein concentration enhanced ($P < 0.05$) free radical scavenging effect of PPH. Although non-hydrolyzed plasma protein displayed an antioxidative effect, it was far less potent than PPH. The results indicated that the antioxidant capacity of porcine plasma protein could be enhanced by enzymatic hydrolysis of Alcalase.

1. Introduction

It is known that many human diseases can be caused by free radicals and natural antioxidants can act as free radical scavengers (Chang, Wu, & Chiang, 2007; Gill & Cross, 2000; Meisel, 1997). Free radical-mediated lipid peroxidation oxidative stress and antioxidants are widely discussed in many current research areas. Formation of free radicals such as hydroxyl radical ($^{\bullet}$OH) or superoxide radical ($O_2^{\bullet-}$) is an unavoidable consequence in the respiration process of cells in aerobic organisms (Je, Qian, Byun, & Kim, 2007). Since free radicals are very unstable, they are immediately neutralized by antioxidant in the cell once they are generated in normal metabolism pathway. The free radical can cause damage of tissue by reacting with other chemicals in the body. Lipid peroxidation is one example of oxidative stress caused by free radical reacting with lipid. Numerous studies revealed that lipid peroxidation is involved in the occurrence of many chronic diseases (Butterfield et al., 2002). Specially, the food lipid peroxidation can greatly affect the nutritional value of the food. Consumption of such kind of food can potentially cause disease conditions of human being due to the toxic reaction products formed in lipid peroxidation process. Therefore, looking for functional ingredients that possess antioxidant activity in the food has become hot research subject in the food science (Lindmark-Mansson & Akesson, 2000).

Protein hydrolysate is obtained through hydrolysis of protein with some kinds of enzyme, and is a mixture of proteoses, peptones, peptides, and free amino acids (Chang et al., 2007). Some protein hydrolysates have been found to have specific functional activities, such as inhibitory activity on angiotensin I converting enzyme (ACE) (Hyun & Shin, 2000; Pan, Luo, & Tanokura, 2005), immunological regulatory activity (Cross & Gill, 2000; Mills, Alcocer, & Morgan, 1992), and antioxidant activity (Amarowicz & Shahidi, 1997; Klompong, Benjakul, Kantachote, & Shahidi, 2007; Kong & Xiong, 2006; Peña-Ramos, Xiong, & Arteaga 2004; Peng, Xiong, & Kong, 2009; Sakanaka & Tachibana, 2006; Shahidi, Han, & Synowiecki, 1995; Yamaguchi, Naito, Yokoo, & Fujimaki, 1980). Production of protein hydrolysates with antioxidant activity and improved functional properties would be of economical interest as well as processing significance. Porcine blood is an important by-product of the meat industry and is considered as a potential source of nutritional and functional protein. Some studies have showed that porcine plasma protein could be used as functional ingredients (Wismer-Pedersen, 1979), proteinase inhibitor (Benjakul, Visessanguan, & Srivilai, 2001), and filler in meat or surimi products (Satterlee, Free, & Levin, 1973).

Electron spin resonance (ESR) trapping technique is based on the measurement of transitions of unpaired electrons in a magnetic field, which can detect and identify molecules that have unpaired

* Corresponding author. Tel.: +86 451 55191794; fax: +86 451 55190577.
E-mail address: kongbh63@hotmail.com (B. Kong).

0023-6438/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2008.12.007

electrons, such as free radicals, transition metal ions and defects in material (Cox & Symons, 1986). The technique also provides a sensitive, direct and accurate means of monitoring reactive species at room temperature, and the antioxidant activity also can be effectively determined by ESR spectrometer (Antolovich, Prenzier, Patsalides, McDonald, & Robards, 2002).

The aims of this study were to investigate the antioxidant properties of porcine plasma protein before and after enzymatic hydrolysis by using the ESR technique on the basis of their abilities to scavenge DPPH radical, hydroxyl radical and superoxide radical, and even further, to understand the mode of action of porcine plasma protein hydrolysates (PPH) as antioxidant.

2. Materials and methods

2.1. Materials

Porcine plasma protein was obtained from Beidahuang Meat Corporation (Heilongjiang, China). The dry porcine plasma protein power contained about 70% protein, 15% ash, 13% moisture and 1.5% lipid as determined by the method of AOAC (2000). Alcalase 2.4L (6×10^4 U/g) was obtained from Novo Nordisk (Bagsvaerd, Denmark). The testing chemicals including Ferrous sulfate ($FeSO_4 \cdot 7H_2O$), Disodium ethylenediaminetetraacetate (EDTA), BHA (butylated hydroxyanisole), L-ascorbic acid, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ), 1,1-diphenyl-2-pycrylhydrazyl (DPPH), ribofavin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of porcine plasma protein hydrolysates (PPH)

Porcine plasma protein solution (40 mg protein/ml) with heat pretreatment (90 °C, 5 min) was hydrolyzed with Alcalase at 55 °C for 0.5, 1, 2, 3, 4, 5, 6 and 7 h. The reason for heat pretreatment of porcine plasma protein solution is because heat pretreatment can change the accessibility of the susceptible bonds by the protease and increase the DH and antioxidative activity of hydrolysates (Peña-Ramos & Xiong, 2002). The enzyme to substrate ratio [E/S] was 2:100 (g/g). The pH of the porcine plasma protein solution was adjusted to the optimal values for the Alcalase (pH 8.0) before hydrolysis was initiated, and was readjusted to the optimal value every 15 min during hydrolysis with 1 M NaOH. After hydrolysis, the pH of the solution was brought to 7.0 by using 1 M HCl, and the solution was then heated at 95 °C for 5 min to inactivate the enzymes. The hydrolysates were then freeze-dried (LGJ-1 Freeze-Dryer, Shanghai, China), the lyophilized hydrolysates were stored at 4 °C until use.

2.3. Degree of hydrolysis (DH)

Degree of hydrolysis (DH) of PPH was determined using a pH-stat method (Adler-Nissen, 1986). The DH of PPH was calculated based on the following equations:

$$\mathrm{DH}(\%) = \frac{h}{h_{tot}} \times 100$$

$$h = B \times N_b \times 1/\alpha \times 1/MP$$

where B = base consumption (ml), N_b = concentration of base (1 M NaOH), $1/\alpha$ = calibration factors for pH-stat ($1/\alpha = 1.105$), MP = mass of protein (g), h = hydrolysis equivalents, and for porcine plasma protein, h_{tot} = 8.8 mmol/g protein (Adler-Nissen, 1986).

2.4. Reducing power

The reducing power of PPH was measured by using the ferric reducing/antioxidant power (FRAP) assay (Benzie & Strain, 1996). Aliquots of 3.0 ml of FRAP reagent, prepared freshly and incubated at 37 °C, were mixed with 0.3 ml of distilled water and 0.1 ml of sample, or distilled water (for the reagent blank). The FRAP reagent contained 2.5 ml of 10 mM TPTZ solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 ml of 0.3 M acetate buffer, pH 3.6. Absorbance (593 nm) of samples as well as blank solutions was taken at 4 min. Sample FRAP values were calculated based on a $FeSO_4$ standard curve (prepared with 100–1000 μM $FeSO_4 \cdot 7H_2O$), and were expressed as $FeSO_4$ equivalent (μM).

2.5. Lipid oxidation

Liposomes were prepared from soybean phosphatidylcholine according to the method of Decker and Hultin (1990) and Kong and Xiong (2006). To measure the antioxidant activity of PPHs, a series of mixed solutions of 5 ml of liposome and 1 ml of hydrolysates (40 mg protein/ml) were prepared. The control solution was prepared by mixing 1 ml of water instead of 1 ml of protein solution with 5 ml of liposome. Lipid oxidation was initiated by iron redox cycling by adding 0.1 ml of 50 mM $FeCl_3$ and 0.1 ml of 10 mM sodium ascorbate into the liposome/protein solution (6 ml). Samples were incubated in a 37 °C water bath for 1 h, and lipid oxidation was immediately determined as described below.

2.6. Thiobarbituric acid-reactive substances (TBARS)

The concentration of TBARS (secondary products from lipid oxidation) was determined according to the method of Sinnhuber and Yu (1958) with slight modifications as described by Kong and Xiong (2006).

2.7. Electron spin resonance (ESR) measurement

Experimental conditions of ESR measurement were as follows: magnetic field, 3385.0 Gs; power, 20.00 mW; modulation frequency, 100.0 kHz; amplitude, 1.0 GS; sweep time, 300 s for DPPH and hydroxyl radicals, and 400 s for superoxide radicals.

2.7.1. Scavenging effect on DPPH radical

DPPH radical scavenging activity was measured by using the method described by Nanjo et al. (1996). A 60 μl sample (or ethanol itself as control) was added to 60 μl of DPPH (60 μM) in ethanol solution. After mixing vigorously for 10 s, the solution was then transferred into a 100 μl quartz capillary tube, and the spin adduct was measured on an ER 200D-SRC ESR spectrometer (Bruker, Germany) exactly 2 min later. DPPH radical scavenging activity (RSA) was calculated based on the following equation, in which H and H_0 were the height of the third resonance peak for samples with and without protein, respectively.

$$\mathrm{RSA} = (H_0 - H)/H_0 \times 100\%$$

2.7.2. Scavenging effect on hydroxyl radical

Hydroxyl radicals (•OH) were generated by the Fenton reaction in the system that contain 200 μl 0.3 M DMPO, 200 μl 10 mM $FeSO_4$ and 200 μl 10 mM H_2O_2 in a phosphate buffer solution (Rosen & Rauckman, 1984). Aliquots of 20 μl Samples (the control was pH 7.4 phosphate buffered solution (PBS)) were mixed with 60 μl Fenton reaction solution, and then transferred into a 100 μl quartz capillary tube. After 2.5 min, the ESR spectrum was recorded using an ESR spectrometer. The RSA was calculated as above with H and H_0