Food Chemistry 118 (2010) 403–410

Contents lists available at ScienceDirect

Food Chemistry

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

Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis

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ARTICLE INFO

Article history:
Received 22 November 2008
Received in revised form 18 April 2009
Accepted 5 May 2009

Keywords:
Functional properties
Purification
Antioxidant peptide
Porcine plasma protein hydrolysate

ABSTRACT

Antioxidant activity and functional properties of porcine blood plasma protein hydrolysates (PPH) prepared with Alcalase at 6.2%, 12.7% and 17.6% of degree of hydrolysis (DH) were investigated. The PPH showed stronger radical-scavenging ability and possessed stronger Cu^{2+}-chelation ability and a reducing power compared to non-hydrolysed plasma protein ($P < 0.05$). The antioxidant activity of PPH, indicated by thiobarbituric acid-reactive substance (TBARS) values in a liposome-oxidising system, increased with increasing DH ($P < 0.05$). The Alcalase hydrolysis increased protein solubility from its original 68.46–81.79% (non-hydrolysed) to 82.95–94.94% (hydrolysed) over a broad pH range (3.0–8.0). However, hydrolysis decreased surface hydrophobicity and suppressed emulsifying and foaming capacity of the plasma protein. To identify antioxidant peptide, PPH was subjected to ultrafiltration, ion-exchange chromatography and reverse-phase high performance liquid chromatography (RP-HPLC), and the amino acid sequences of isolated peptides were determined by liquid chromatography/tendem mass spectrometry (LC–MS/MS). The peptide with the strongest antioxidant activity had the amino acid sequence of His-Asn-Gly-Asn. The results indicated that PPH could be used as a novel antioxidant but may be of limited utility as an emulsifying or foaming agent.

1. Introduction

Functional properties of proteins can be modified by physical, chemical and enzymatic treatments through changing the protein structure. Enzymatic treatment is a particularly attractive technique to modify proteins due to the milder process conditions required, the relative ease to control the reaction, and minimal formation of by-products (Mannheim & Cheryan, 1992). Selective enzymatic hydrolysis under controlled conditions has been used to improve the solubility and enhance the emulsifying and foaming properties of wheat gluten (Agyare, Xiong, & Addo, 2008) and fish protein (Shahidi, Han, & Synowiecki, 1995).

The cleavage of peptide bonds leads to an increase in the concentration of free amino and carboxyl groups, which increases solubility. Hydrolysis also disrupts the protein tertiary structure and reduces the molecular weight of the protein and, consequently, alters the functional properties of proteins (Adler-Nissen, 1986; Kristinsson & Rasco, 2000). However, extensive hydrolysis could have a negative impact on the functional properties (Kristinsson & Rasco, 2000).

In addition to their functionalities, protein hydrolysates, such as hydrolysed wheat protein (Liyana-Pathiranaa & Shahidi, 2007), zein protein (Kong & Xiong, 2006), and porcine haemoglobin (Chang, Wu, & Chiang, 2007), have been shown to possess antioxidant activity. A typical enzymatic protein hydrolysate is a mixture of proteoses, peptones, peptides, and free amino acids (Chang et al., 2007). The levels and compositions of free amino acids and peptides were reported to determine the antioxidant activities of protein hydrolysates (Wu, Chen, & Shiau, 2003). The type of proteases used and the degree of hydrolysis also affect the antioxidant activity (Kong & Xiong, 2006).

Many antioxidative peptides also have been identified from a variety of food proteins, such as royal jelly protein (Guo, Kouzuma, & Yonekura, 2009) and egg yolk (Park, Jung, Nam, Shahidi, & Kim, 2001). Marcuse (1962) reported that several amino acids, such as Tyr, Met, His, Lys, Gly and Trp, are generally accepted as antioxidants despite their prooxidative effects in some cases. Kawashima, Itoh, Miyoshi, and Chibata (1979) noted that some di- and tri-peptides containing aromatic amino acid residues, as well as peptides containing Tyr, Pro and His, showed strong antioxidant activity.

Animal blood, collected from packing plants, is a valuable protein source from which bioactive peptides can be produced. Presently, the utilisation of porcine serum protein is limited. The production of protein hydrolysates with antioxidant activity and improved functional properties would be of economical interest as well as processing significance. Unlike bovine plasma protein and its hydrolysates, which are restricted or banned from food uses due to the concern with potential transfer of animal diseases,

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doi:10.1016/j.foodchem.2009.05.013

plasma protein from wholesome swine is considered safe for human consumption.

In this study, the antioxidant activity of porcine plasma protein hydrolysates (PPH) prepared by Alcalase-hydrolysis was evaluated. The surface hydrophobicity, solubility, and emulsifying and foaming properties in relation to the degree of hydrolysis were examined. Meanwhile, the antioxidative peptides were isolated from the hydrolysate, and their amino acid sequences were determined.

2. Materials and methods

2.1. Materials and chemicals

Porcine plasma protein was obtained from Beidahuang Meat Corporation (Harbin, Heilongjiang, China). The dry porcine plasma protein powder contained 70% protein as determined by the Kjeldahl method of determination (AOAC, 2000). Alcalase 2.4 L (6×10^4 u/g) was obtained from Novozymes (Bagsvaerd, Denmark). Testing chemicals, including 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Soybean L-α-phosphatidylcholine, and 1-anilino-8-naphthalene-sulphonate (ANS) 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) was heat pretreated (90 °C, 5 min) and then hydrolysed with Alcalase at 55 °C. 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 Alcalase (pH 8.0) before hydrolysis was initiated, and it was readjusted to the optimal value every 15 min during hydrolysis with 1 M NaOH. The hydrolysates were produced by varying the hydrolysed time to 0.5, 2 and 5 h, which yielded 6.2%, 12.7%, and 17.6% of degrees of hydrolysis (DH), respectively.

After hydrolysis, the pH of the solution was brought to 7.0, and the solution was then heated at 95 °C for 5 min to inactivate the enzyme. The hydrolysates were freeze-dried (LGJ-1 Freeze-Dryer, Shanghai, China), pulverised, placed in sealed bags, and stored at 4 °C until use. DH of hydrolysed protein was determined using a pH-stat method (Adler-Nissen, 1986).

2.3. Determination of antioxidant activities

2.3.1. Thiobarbituric acid-reactive substances (TBARS)

The antioxidant activity of PPH was initially assessed by means of TBARS analysis in an oxidising liposome system. Liposomes were prepared from soybean phosphatidylcholine according to the method of Decker and Hultin (1990). A series of mixed solutions of 5 mL of liposome with 1 mL of PPH (40 mg protein/mL) at 6.2%, 12.7%, and 17.6% of DH were prepared. The control solution was prepared by mixing 1 mL of water instead of 1 mL of protein solution (40 mg protein/mL) with 5 mL of liposome. Oxidation was initiated by adding 0.1 mL of 50 mM $FeCl_3$ and 0.1 mL of 10 mM sodium ascorbate into the liposome/protein solution and continued for 60 min in a 37 °C water bath. The concentrations of TBARS (secondary products from lipid oxidation), with or without the presence of PPH, were determined according to the method which outlined by Kong and Xiong (2006).

2.3.2. 2,2-Diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was determined according to the method of Yen and Hsieh (1995) with slight modification. Aliquots (4.0 mL) of the DPPH radical solution (1×10^{-4} M, in methanol) were each incubated with 1.0 mL of PPH (40 mg protein/mL in distilled water) at room temperature for 30 min in the dark. The absorbance of reacted solutions was measured at 517 nm.

2.3.3. Reducing power

The reducing power of PPH was measured using the ferric reducing/antioxidant power (FRAP) assay (Benzie & Strain, 1996). Absorbance (593 nm) of samples was taken at 30 s intervals for up to 8 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).

2.3.4. Metal chelating activity

The metal chelating effect was determined as previously described (Kong & Xiong, 2006).

2.4. Surface hydrophobicity

The determination of superficial hydrophobicity followed the method described by Akita and Nakai (1990) using 1-anilino-8-naphthalene-sulphonate (ANS) as the fluorescence probe. Fluorescence intensity (FI) was measured with a fluorometer (F-4500 model, Hicathi, Tokyo, Japan) at an excitation wavelength of 374 nm and an emission wavelength of 485 nm. After drawing a graph of the fluorescence as a function of protein concentration, the inclination was calculated and considered as the hydrophobicity.

2.5. Determination of functional properties

2.5.1. Solubility

To determine protein solubility, 200 mg of PPH samples were dispersed in a buffer solution (0.02 M sodium phosphate and 0.01 M citric acid), in pH 3.0–8.0. The mixtures were stirred at room temperature for 30 min and centrifuged at 7500g for 15 min. Protein contents in the supernatants were determined using the Biuret method (Gornall, Bardawill, & David, 1949). Total protein content in PPH samples was determined after solubilisation of the samples in 0.5 M NaOH. Protein solubility was calculated as follows:

$$\text{Solubility } (\%) = (A/B) \times 100$$

where A is protein content in supernatant and B is total protein content in sample.

2.5.2. Emulsifying properties

The emulsifying properties were determined according to the method of Pearce and Kinsella (1978) with a slight modification. Vegetable oil (10 mL) and 30 mL of 0.2% PPH solution were mixed in a buffer solution (0.02 M sodium phosphate and 0.01 M citric acid, pH 3.0–8.0) and homogenised at 20,000 rpm for 1 min using a homogenizer (IKA T18 basic, IKA-Werke GmbH & Co., Staufen, Germany). An aliquot of the emulsion (50 μL) was pipetted from the bottom of the container at 0 and 10 min after homogenisation and mixed with 5 mL of 0.1% sodium dodecyl sulphate (SDS) solution. Absorbance at 500 nm was measured using a spectrophotometer (UT-1800, Pgeneral, Beijing, China), using 0.1% SDS solution as the blank. The emulsifying activity index (EAI) and the emulsion stability index (ESI) was calculated as follows:

$$\text{EAI}(\text{m}^2/\text{g}) = (2 \times 2.303 \times A_{500})/[0.25 \times \text{Protein weight}(\text{g})]$$

where A_{500} represents the absorbance at 500 nm.