Process Biochemistry 51 (2016) 431–443

Contents lists available at ScienceDirect

Process Biochemistry

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

Physicochemical, functional properties and antioxidant activities of porcine cerebral hydrolysate peptides produced by ultrasound processing

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ARTICLE INFO

Article history:
Received 22 September 2015
Received in revised form
15 December 2015
Accepted 16 December 2015
Available online 21 December 2015

Keywords:
Bioactive peptides
Porcine cerebral protein
Hydrolysis
Molecular characterization
Functional properties
Antioxidant activity

ABSTRACT

There is a great demand for developing efficient extraction methods to reduce the extraction time and increase the yields and activities of functional antioxidants. The yields, physicochemical, functional properties and antioxidant activities of ultrasound-pretreated porcine cerebral hydrolysate peptides (UPCHPs) were studied and compared with traditional enzymolysis (PCHPs). The results showed that UPCHPs had higher peptides concentration, smaller molecular weight, and higher concentration of hydrophilic and late-eluting hydrophobic peptides than PCHPs at hydrolysis time of 20 min. However, the contents of antioxidant amino acids (Gly, Ala, Arg, Pro, His, Val, Leu, Lys and Phe) in both hydrolysate peptides were above 60%. UPCHPs with an excellent solubility had high reducing power activity and scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (72%), 2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radicals (73%) and hydroxyl radicals (56%) at 2 mg/mL. Moreover, UPCHPs showed the moderate iron chelating activity (60%). In general, UPCHPs with good functional properties could serve as a potential antioxidant food ingredients.

1. Introduction

Bioactivity peptides are found in milk, egg, meat and fish as well as many plants [1–4], beyond their nutritional value, that are participating in many physiological events and playing crucial roles in the regulation of metabolism, circulation, behavior and so on [5–7]. Based on the amino acid composition and sequences, these peptides can exhibit diverse activities, such as opiate-like [8], mineral binding [9], immunomodulatory [10], antimicrobial [11], antioxidant [12], antithrombotic [13], hypocholesterolemic [14] and antihypertensive functions [15].

The isolation of peptides is mainly based on controlled enzymolysis process [16]. In the process of enzymatic hydrolysis, length of hydrolysis period can alter the degree of hydrolysis by increasing the number of free amino acids or reducing the number of peptide bonds. Previous studies suggested extensive hydrolysis could adversely affect functional properties of peptides [17] and short hydrolysis preparation had shown greater biological activities than long hydrolysis preparation [18]. However, traditional enzymolysis has many disadvantages that arise mainly from the low contact frequency and the decreased enzyme activity. Ultrasound technology, as a novel physical processing technology, has attracted much attention in assisted extraction of bioactive components [19–21]. Mechanical and thermal effects enacted by cavitation are regarded as the fundamental process responsible for the initiation of most of the sonochemical reactions in liquids, which can result in enhanced mass transfer, increased contact frequency between substrate and enzyme and so on [22–24].

Porcine brain (*Sus scrofa domestica brisson*) has the highest level of cholesterol (1352–2195 mg/100 g) and phospholipids in comparison with other meat by-products. For this reason, the United States Department of Health and Human Services recommends limited the amounts of them to be eaten [25]. However, there is a growing interest in the utilization and disposal of hydrolysates of porcine cerebral protein (PCP) which is used for the treatment of neural system diseases and preventing the age-dependent dementia [26], characterizing by progressive loss of cognitive capability [27]

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http://dx.doi.org/10.1016/j.procbio.2015.12.011
1359-5113/

and pathological changes in brain [28]. Cerebrolysin (Ebewe Pharmaceutical, Austria) from cerebral protein hydrolysates has been found widespread clinical application for more than 40 years. It is known to be a mixture of 75% free amino acids and 25% short-chain peptides, probably peptides are physiologically active ingredient [29].

Ultrasound processing can enhance the productivity and favor retention of product quality. Accordingly, the objectives of this research were to study the effects of ultrasound-assisted extraction from PCP and compare with traditional enzymolysis on the peptides yield, physicochemical, functional properties and antioxidant activities.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), L-leucine, 2,4,6-trinitrobenzenesulphonic acid (TNBS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade. Sodium dodecyl sulphate (SDS) was from Bio-Rad Laboratories (Hercules, CA, USA). All solutions were freshly prepared in distilled water.

2.2. Preparation of protein substrate from porcine brain

Protein substrate from porcine brain was prepared by the method reported in our previous study [30]. Porcine brains were minced and added portion-wise to 5 volumes (v/w) of boiling water. They were boiled for 10 min after addition of the final portion. PCP was extracted thoroughly by ethanol treatment with a mince:ethanol ratio of 1:2 at 70 °C for 30 min to obtain degreased powder. The powder was then mixed with water to form slurry. The deposited solid was separated from the liquid by centrifuging at 4000 × *g* at room temperature for 15 min. It was then dried and stored in a desiccator for further analysis.

2.3. Production of porcine cerebral hydrolysate peptides

The ultrasonic pretreatment of the PCP and enzyme hydrolysis was also prepared reported in our previous study [30]. A probe ultrasonic reactor (SC-II, Chengdu Jiuzhou Ultrasonic Technology Co., Ltd.) working with a single frequency of 20 kHz and a maximum power of 80 W was used in the ultrasonication experiments for 5 min. The pretreated sample solution passed through the probe by countercurrent method which two peristaltic pumps were used to keep the material solutions in a counter-current flow state. Ultrasonic porcine cerebral protein (UPCP) solution obtained from the above section reacted with alcalase (E/S ratio, 2000 U/g). The traditional enzymolysis was prepared from PCP with alcalase using the same procedure of UPCP enzymolysis. The pH of the reaction solution was adjusted to 8.5 and then the solution was incubated in a water bath at 50 °C. During the whole period of hydrolysis, the pH was maintained at 8.5 by continuous addition of 0.5 mol/L NaOH. At the end of the incubation period, adjusting pH to 7.0 with 1 mol/L HCl, the enzymatic hydrolysis was terminated by boiling the mixture for 10 min. Then the mixture was centrifuged at 4000 × *g* for 15 min. The supernatants of hydrolysates were collected and stored at 4 °C. The hydrolysate peptides from UPCP were abbreviated to UPCHPs. The other hydrolysate peptides from PCP were abbreviated to PCHPs.

2.3.1. Degree of hydrolysis

The α-amino acid content was determined according to the method of Benjakul and Morrissey [31]. To properly diluted hydrolysate samples (125 μL), 2.0 mL of 0.2 mol/L phosphate buffer (pH 8.2) and 1.0 mL of 0.01% TNBS solution were added. The solution was mixed thoroughly and placed in a temperature controlled water bath (Model W350, Memmert, Schwabach, Germany) at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 mL of 0.1 M sodium sulphite. The mixtures were cooled at room temperature for 15 min. The absorbance was read at 420 nm on a spectrophotometer (TU-1810, Puxi General Instrument Co., Ltd., China) and α-amino acid was expressed in terms of L-leucine. Degree of hydrolysis (DH) was calculated as follows:

$$\mathrm{DH} = \left[\frac{(L_t - L_0)}{(L_{\max} - L_0)}\right] \times 100 \quad (1)$$

where L_t is the amount of α-amino acid released at time t. L_0 is the amount of α-amino acid in the original acid-solubilised protein substrate. $L_{\max}$ is total α-amino acid in the original acid-solubilised protein substrate obtained after acid hydrolysis (6 mol/L HCl at 100 °C for 24 h).

2.3.2. Determination of polypeptide concentration

The polypeptide concentration (μg/mL) was determined using a reported Folin-phenol colorimetric method as explained by Qu et al. [32]. A 4 mL Folin-phenol reagent A was mixed with 0.5 mL sample and incubated for 10 min at room temperature, after which, a 0.5 mL Folin-phenol reagent B was added, and the absorbance was read at 500 nm on a spectrophotometer (TU-1810, Puxi General Instrument Co., Ltd., China) after 30 min incubation at room temperature. Standards of 0–50 μg/mL BSA were assayed simultaneously with triplicate assays to calculate the polypeptide concentrations of samples.

2.4. Physicochemical properties of the peptides fractions

2.4.1. Determination of the molecular weight distribution

The molecular weight distributions (MWD) of the samples were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF-MS, ultraflex-tof-tof, BRUKER, Germany). Linear model of flight distance was 1.22 m. Resolution was ACTH18-39 (m/z = 2465.2). Sensitivity was 1fmol ACTH18-39 (m/z = 2465.2) as signal to noise ratio was greater than 10 to 1.

2.4.2. Proximate composition

Proximate composition (protein, ash and protein recovery) was estimated as per Association of Official Analytical Chemists [33] methods.

2.4.3. Determination of mineral analysis

The mineral elements including potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe) and copper (Cu) were determined after the wet-digestion according to the method described by Do et al. [34]. with slight modification. A 0.2000 g sample was placed into polyfluoroethylene (PTFE) tube, to which a 10 mL mixture of HNO_3 and $HClO_4$ (v/v, 4:1) were added. The tube was closed and heated at 130 °C for 60 min in an aluminum block. After cooling to room temperature, 2 mL of H_2O_2 were added, and the solution was heated again for 10 min at 115 °C. Then, 0.1 mL of 40% HF was added, followed by a heating at 115 °C for 10 min and a second addition of H_2O_2 (2 mL) until the solution was clear. After digestion, the solution was allowed to cool to room temperature. A blank digest was carried out in the same way. The solution was finally transferred to a 25 mL calibrated volumetric flask with ultrapure water. The samples were then determined by