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Analytical Methods

Development of a novel method for hot-pressure extraction of protein from chicken bone and the effect of enzymatic hydrolysis on the extracts

Xian-bing Dong [a], Xia Li [a], Chun-hui Zhang [a,*], Jin-zhi Wang [a], Chun-hong Tang [b], Hong-mei Sun [a], Wei Jia [a], Yin Li [a], Lin-li Chen [b]

[a] Institute of Agro-Food Science and Technology, Chinese Academy of Agricultural Sciences/Comprehensive Key Laboratory of Agro-Products Processing, Ministry of Agriculture, Beijing 100193, China
[b] College of Environmental and Biological Engineering, Natural and Health Food Research Institute, Chongqing Technology and Business University, Chongqing 400067, China

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ABSTRACT

To investigate the hot-pressure extraction of protein from chicken bone (CB), chicken bone extracts (CBE) was prepared from CB by heating at 130 ± 0.5 °C for 120 min, followed by filtration, standing, defatting, and concentration. Effects of enzymatic hydrolysis on the properties of hydrolysates were examined. Results showed CBE contained 25.59% of protein, and showed a desirable value of protein digestibility-corrected amino acid score for adult. The total amino acid (AA) content of CBE is 21.99%, among which 40.62% and 54.66% are essential and fresh AA, respectively. Forty kinds of volatile compounds were identified after 24 h of hydrolysis, with 2,3,5-trimethylpyrazine as the key flavor compound. After 8 h of hydrolysis of CBE, the content of small MW of peptides (400–1000 Da) increased by 74 times compared with that of 1 h. CBE and its hydrolysates demonstrate a new kind of potential suitable nutritional supplement in various foods.

1. Introduction

Meat is the major product of chickens, while their bones named as chicken bone (CB) or bone residue become a byproduct (Bhaskar, Modi, Govindaraju, Radha, & Lalitha, 2007). CB contains a notable amount of muscle, connective tissues and fat, and therefore represents a valuable source of proteins, containing ~51% moisture, 19% protein (collagen), 9% fat, and 15% ash (Fonkwe & Singh, 1994). Generally, depending on the deboning machine and on the kind of material used for deboning, the CB fraction yielded from 20% to 50% (Kijowski & Niewiarowicz, 1985). Approximately 28 million tons of chicken are consumed in China each year, and the total amount of raw chicken all over the world is 83 million tons in 2012 (USDA, 2012), producing 5.6–14 million tons of CB in China and 16.6–41.5 million tons of CB in the world, resulting a huge and valuable protein source.

Attempts to utilize CB as low value source of protein in animal feed have been reported (Kijowski & Niewiarowicz, 1985; McCurdy, Jelen, Fedec, & Wood, 1986). However, most of the CB left in the deboner is discarded due to microbial and aesthetic reasons, causing environmental pollutions and waste of valuable nutrients. Therefore, it is critical to develop an effective way to use the huge amount of CB as a protein source.

Several studies had been carried out to explore effective way of using animal bones. Extracting the protein from CB by acidic or alkaline hydrolysis has also been tried, but it is difficult to operate during the successive processing steps (Jelen, Earle, & Edwardson, 1979). Recently, enzymatic hydrolysis is employed to extract proteins and produce peptides (Morimura et al., 2002), which forms an effective way to recover proteins from the byproduct of animal processing industries. Unlike acidic or alkaline hydrolysis, enzymatic proteolysis is mild and controllable, which helps to improve the quality and functional properties of protein (Kristinsson & Rasco, 2000a). An enzymatic process using specific protease has been developed to produce flavorants from seafood byproduct (Baek & Cadwallader, 1995). However, due to the stable protein structure of bone, the enzymatic hydrolysis generally has lower DH, which is a means to determine the properties of protein hydrolysates. As a result, it is still difficult to extract proteins from the bone completely by enzymatic hydrolysis (Mahmoud, Malone, & Cordle, 1992).

Therefore, it is necessary to develop an effective way for better utilization of CB as a byproduct. The objective of present study is to develop a new method for the preparation of protein from chicken

* Corresponding author. Address: No. 2 Yuan Ming Yuan West Road, Haidian District, Beijing 100193, China. Tel.: +86 10 62819469; fax: +86 10 62815950.
E-mail address: dr_zch@163.com (C.-h. Zhang).

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bone, and to investigate the effect of enzyme hydrolysis on the resulting chicken bone extracts (CBE).

2. Materials and methods

2.1. Materials

After deboning, chicken bone, also named as bone residue, without head and leg was obtained from the Dayoo Group Company (Henan Province, China). The samples were cut into blocks at a size of ~3 × 5 cm and stored at −20 °C until use. The hydrolysis of CBE was performed using food-grade flavourzyme (500 LAPU/g) from Novozymes Co. LTD in China (Beijing, China). All other chemicals used were of analytical grade.

2.2. Preparation of CBE

CBE were prepared by hot-pressure extraction as shown in Fig. 1A. Frozen chicken bone was deboned by mechanical deboning. The chicken bone were then soaked in the water for 10 min to wash out the residual blood and then placed into the crane cage (Fig. 1B), hanging in hot-pressure extraction pot. They were then mixed with equal amount of distilled water (w/v) and extracted at 135 ± 0.5 °C for 120 min, respectively. The resulting soup was filtered through a 200-mesh sieve to remove the bone residues, and the filtrate was poured into a standing pot, incubated at 85 ± 1.0 °C for 120 min, and defatted by a tubular centrifuge at 16,000*g*. The supernatant (bone oil) was used as edible or condiment oil, while the aqueous layer was collected and concentrated by the vacuum condenser under the negative pressure of 0.08–0.1 MPa until the solid content reaching 30%. The concentrated CBE was stored at −20 °C until further use.

2.3. Preparation of protein hydrolysates

Defatted and concentrated CBE was used as substrate for hydrolysis. Flavourzyme at a ratio of 0.5% (w/w) was added to CBE in a water-bathed constant temperature vibrator (SHA-B, Ronghua Apparatus Co. LTD, Jiangsu, China) under 53 ± 1 C and pH 7.0 ± 0.2. Hydrolysates were sampled at time intervals of 0, 1, 3, 5, 8, 12, 17 and 24 h respectively. The hydrolysates were cooked at 95 °C for 10 min to inactive the enzyme and then centrifuged at 10,000*g*, 15 C for 15 min. The supernatant was collected for further studies.

2.4. Determination of the degree of hydrolysis (DH) and nitrogen recovery (NR)

Degree of hydrolysis (DH) was defined as the percentage of free amino groups cleaved from protein and calculated as the ratio between α-amino nitrogen (AN) and total nitrogen (TN) of CBE. The AN was determined using formol titration according to Nilsang, Lertsiri, Suphantharika, and Assavanig (2005) with modification. Briefly, 5.0 ml of hydrolysates were added with 50 ml distilled water. The mixture was adjusted to pH 8.2 using 0.05 M of NaOH. Ten ml of 38% (v/v) neutral formaldehyde solution was then added into the mixture, and titration was continued to the end point of pH 9.2. The consumed volume of NaOH after the addition of neutral formaldehyde solution was recorded as V_1. Meanwhile, 55 ml of distilled water was used as blank test, and the corresponding volume of consumed NaOH was recorded as V_0. AN was calculated using Eq. (1).

$$\mathrm{AN}(\%) = \frac{V_1 - V_0 \times 0.014 \times 0.05}{5.0} \times 100 \quad (1)$$

where 0.014 was the mass of nitrogen equivalent for 1.0 ml of 1 M of NaOH solution, and 0.05 was the concentration of standard NaOH solution, and 5.0 was the volume of hydrolysates of CBE. Nitrogen recovery (NR) was calculated as the ratio between TN in hydrolyzed supernatant and CBE according to Eq. (2).

$$\mathrm{NR}(\%) = \frac{\text{Total nitrogen in hydrolysed supernatant}}{\text{Total nigrogen in CBE}} \times 100 \quad (2)$$

2.5. Proximate analysis of CBE

Fat content was determined by 2050 Soxtec Auto Extraction Unit (Foss Tecator, Sweden). Moisture and ash contents were determined by the method of AOAC (1990). Total nitrogen (TN) was determined by Kjeldahl method using Kjeltec 2300 Analyzer (Foss Tecator, Sweden). The protein content was obtained by multiplying the TN value with a Kjeldahi factor of 6.25.

2.6. Determination of amino acid composition and amino acid score (AAS) of CBE

Total amino acid (TAA) composition was analyzed according to the method described by Spackman, Stein, and Moore (1958) with modifications. Briefly, 1.0 g sample and 10 ml of 6 M HCl were added into an empty tube, which was then sealed under vacuum and incubated at 110 ± 1 °C for 24 h. For determine the free amino acid (FAA) composition, 2 ml of hydrolysates at different time intervals of hydrolysis were mixed with equal amount (w/v) of 7% (v/v) 5-sulfosalicylic acid dehydrate respectively and incubated for 30 min to remove protein. The supernatant was then collected by centrifugation at 16,000*g* for 20 min and used to determine the FAA composition. The amino acid compositions for the samples were measured by L-8900 Amino Acids Automatic Analyzer (Hitachi LTD, Japan).

The *in vitro* protein digestibility (IVPD) was measured using the method described by Anyango, De Kock, and Taylor (2011). The AAS was determined according to the FAO/WHO/UNN-recommended pattern of amino acid requirements for pre-school (2–5 years), schoolchildren (10–12 years), and adults (FAO/WHO, 1990). AAS of the nine essential amino acids (EAAs, including threonine, valine, cystine + methionine, isoleucine, leucine, tyrosine + phenylalanine, lysine, histidine, and tryptophan) was calculated using Eq. (3), and the amino acid with lowest ratio was defined as the limiting amino acid (LAA). Protein digestibility-corrected amino acid score (PDCAAS) was then calculated using Eq. (4).

$$\mathrm{AAS} = \frac{\text{mg essentialamino acid in 1g of CBE}}{\text{essential amino acid required per g}} \quad (3)$$

$$\mathrm{PDCAAS}(\%) = \frac{\text{mg of limiting amino acidin 1 g of CBE}}{\text{mg of same amino acid in 1 g of reference protein}} \times \textit{in vitro}\ \text{digestibility} \quad (4)$$

2.7. Determination of volatile compounds in hydrolysates of CBE

The volatile compounds in hydrolysates were analyzed by headspace solid-phase microextraction-gas chromatography mass spectrometry (HS-SPME-GC–MS). One ml of hydrolysates was placed into a 15 ml glass vial. It was closed and shaken at 50 °C for 10 min. A SPME fiber (50/30 μm DVB/CAR on PDMS, Supelco, USA) was then exposed to the headspace above the opened vial at 50 °C for 30 min to collect sample. After sampling,