PREPARATION OF GELATIN FROM CHICKEN BONE RESIDUE WITH HOT-PRESSURE EXTRACTION

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Abstract – Hot-pressure extraction (HPE) was utilized to prepare chicken bone protein (CBP) at 130℃, and the effect of extraction time on the gelatin properties was investigated in this study. Results showed that HPE time had significant effect on the yield of CBP (P<0.05), leading to the degradation of protein and consequently affected the properties of CBP gelatin. Content of total soluble solids, crude protein and hydroxiproline increased significantly during extraction (0~120 min, P<0.05). Result of MW distribution suggested that protein experienced dramatic degradation from 40 to 120 min (P<0.05). The proportion of MW of 10~30 KDa decreased from 59.82% (40 min) to 13.99% (120 min), while ratio of MW <10 KDa increased from 35.46% (40 min) to 86.01% (120 min). Gel strength of 20, 40 and 60 min was better than that of 90 and 120 min, while CBP of 0 min could not gelation. This study illustrated a promising procedure to prepare gelatin from chichen bone byproducts without addition of acid and/or alkali, which provided a natural way to prepare gelatin from CBP.

Key Words –Chicken bone, Gelatin, Gel strength

I. INTRODUCTION

Gelatin, one of the most popular biopolymers, is widely used as emulsifier, gelling agent, stabilizer in food industry [1-2]. And thus the global demand for gelatin has been increasing over the years. Therefore, exploring different material sources and optimizing the extracting procedures of gelatin has received interest of researchers in the last decade [3]. The most common raw materials for gelatin extraction are pig skin (46%), bovine hide (29.4%), pork and cattle bones (23.1%) [3]. However, industrial applications call for more gelatin species, and thus raw materials from fish have received considerable attention in recent years [3]. For instance, amount of gelatin prepared by fish skin had been doubled from 2002 to 2007, but few study about chicken bone gelatin and its manufacturing method are reported.

Approximately 87 million tons chicken are produced all over the world in 2015 [4]. Consequently, due to the difference of machine and material used for deboning, about 17.4~43.5 million tons CB were produced [5]. CB contains about 19% protein, and collagen accounts for 35% to 40% of the total protein [5], representing a huge amount of collagen. Therefore, CB is a potential resources to prepare gelatin.

The protein source, age of the animal, and type of collagen, are considered closely related to gelatin properties [1]. Besides, the physicochemical properties including molecular weight (MW) distribution, content of total soluble solids (TSS), crude protein and hydroxiproline (Hyp) of gelatin are factors that influence its gelatin properties, which vary with materials and extraction process [2]. Therefore, lot of researchers focused on the relationship between the properties mentioned above and that of gelatin. For example, de Moraes et al. [2] studied the effect of temperature and pH on functional properties of collagen hydrolysates, and obtained highest content of soluble protein from bovine hide at highest temperature.

Besides the traditional time-consuming chemical method, innovative processing without addition of acid and/or alkali are attracting researcher's attention nowadays. For example, Wang et al. [5] reported that yield of collagen from CB with HPE can be as high as 94%, and obtained high content of protein at 130℃ for 90 min. Hence, we hypothesized that CBP prepared with HPE should have good gelatin properties. However, extraction time will affect the degradation of protein and thus influence functional properties of gelatin [2]. An optimization of extraction conditions and better knowledge of the gelatin

properties prepared by CBP could be beneficial to the application of CB. Therefore, effect of extraction time on gelatin properties from chicken bone protein was investigated in the present study, aiming to provide useful technology on preparation of edible gelatin using chicken bone byproducts with HPE.

II. MATERIALS AND METHODS

Materials

Frozen CB without leg or head was purchased from Protil Biotechnology Company (Henan Province, China). The CB was cut into small patches about 5×3×5 cm and stored at -20℃. All chemical reagents used were of HPLC or analytical grade.

Preparation of chicken bone protein

CBP was prepared by HPE according to Wang et al. [5] with slight modification. Briefly, after being removed blood by soaked in water (1:1, w/w) for 10 min, 20 kg of CB blocks of approximately 5×3×5 cm were placed into the crane cage and hang in HPE pot to extract CBP at 130±0.5℃ for 120 min, which was sampled at 0, 20, 40, 60, 90 and 120 min, respectively. The ratio of bone: water was 1:1.5 (w/w). All CBP samples were filtered through a 200-mesh sieve to remove the bone residues and defatted at 16000 × *g*, and then stored at -80℃.

Yield of TSS, crude ptotein and hydroxyproline

TSS content of CBP was determined by a MASTER-53M hand-held refractometer (ATAGO, Japan). Content of crude protein was determined by Kjeldahl method using Kjeltec 2300 Analyzer (Foss Tecator, Sweden) [5]. Hyp content was determined according to a previous published method [5].

Molecular weight distribution of Protein

MW distribution of CBP was performed with Agilent liquid chromatograph 1200 using UV detector (Agilent, CA, USA). The column used was TSK gel filtration column, 2000 SWXL 300 mm ×7.8 mm (Tosoh Co., Tokyo, Japan), whilst the mobile phase consisting of acetonitrile, water and trifluoroacetic acid (45:55:0.1, v/v/v) at a flow rate of 0.6 mL/min. The column temperature was 30℃ and the loading account was 10 μL.

Gel strength

CBP solutions of 6.67% (crude protein equivalent) were matured at 4℃ for 17±1 h to form gelatin. Gel strength was determined using the TA. XT2i Texture Analyzer (Stable Micro System, UK). The detailed test settings were as follows: test speed: 1.0 mm/s; ratio of compression: 30%; and trigger force: 5 g. Gel strength is defined as the initial force required to disrupt the gelatin.

Statistical analysis

All of the experiment were conducted in triplicates and expressed as mean ± SD. Statistical calculation was investigated using the statistical package SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Mean separation was performed using Duncan's multiple range tests in case of significant difference (P<0.05).

III. RESULTS AND DISCUSSION

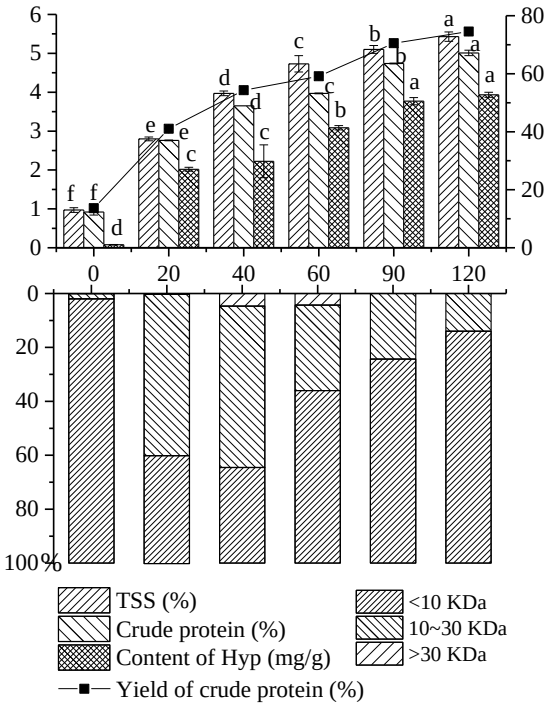
Content of TSS, crude protein, Hyp and yield of crude protein

Protein in CB are consist of collagen, sarcoplasmic and miofibrill, and collagen is the most abundant one [5]. Bone collagen is a heterotrimer composed of three α chains in a triple-helix structure, which is mainly stabilized by intra- and inter-chain hydrogen bonding. Collagen is the product of an almost continuous repeating of the Gly-X-Y- sequence, where X is mostly proline (Pro) and Y is mostly Hyp [6]. Several collagen molecules in cross-section constitute the basic unit of collagen fibrils by covalent bonds, causing difficult degradation or hydrolysis of collagen. One of the fundamental properties of collagen is thermal denaturation and shrinkage when it is heated above a specific temperature, and thus Hyp is one remarking

feature of the amino acid composition of animal bone.

It can be seen that TSS, crude protein, Hyp and yield of crude protein increased significantly during HPE (Figure 1). TSS content increased significantly from 0.97% to 5.43%, and crude protein content increased from 0.92% to 5.01% during extraction (P<0.05). The yield of crude protein before 90 min was higher than that of 90~120 min, indicating that most of the protein has been extracted out before 90 min. Similar to the variable content of crude protein, Hyp content increased rapidly from 0.08 mg/g to 3.77 mg/g before 90 min with HPE (P<0.05), whereby there was no significant difference between that of 90 and 120 min, indicating that hydrogen bonds and other interactions that stabilized the structure of collagen was destroyed by HPE treatment before 90 min, causing helix-to-coil transition and the formation of polypeptides with different MW, which will inevitably influencing the gelatin properties [2].

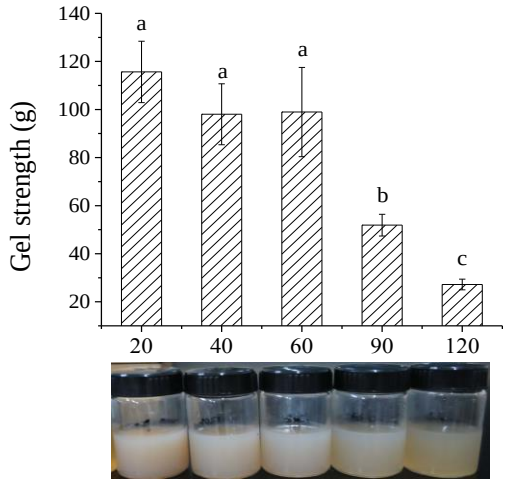
Figure 1. Physicochemical properties of chicken bone protein at different extraction time



MW distribution of CBP

The change of MW distribution (Figure 1) indicated that collagen and other proteins in CB degraded vigorously during HPE. During extraction (40~120 min), the proportion of MW of 10~30 KDa decreased from 59.82% to 13.99%. As expected, the proportion of MW <10 KDa increased from 35.46% to 86.01% (40~120 min). This could be due to the degradation and aggregation of protein during HPE process, which was also observed in collagen treated with pressure and heat [6]. The change of MW will partly affected the gelatin properties because gelatin with a higher content of low MW fragments may require more crosslinks per unit volume to form a gelatin compared to that with higher content of high MW fragments.

Figure 2. Gel strength of chicken bone protein at different extraction time



Gel strength of CBP gelatin

Gel strength is one of the most important functional properties of gelatin and is significantly related to the MW distribution of sample [6]. As shown in Figure 2, gel strength of CBE extracted at 20, 40 and 60 min was nearly 100 g, which was similar to that of cold fish gelatin [3]. However, CBP at 0 min was incapable of forming gelatin, and gel strength of gelatin of 90 and 120 min was much lower than

that of 20~60 min, suggesting that extraction time was significantly influence gel strength (P<0.05). This can be partly attribute to the difference of MW distribution of CBP at different extraction time, because proportion of high MW of CBP at 20~60 min was higher than that of 90 and 120 min. Similarly, Chen et al. [6] also proposed that gel strength of gelatin was positively correlated with the presence of high MW components and negatively correlated with the presence of low MW components.

IV. CONCLUSION

In conclusion, extraction time had significant effect on the physicochemical composition and gelatin properties of CBP with HPE. Given both the yield of protein and gel strength of gelatin, CBP of 40 and 60 min extracted at 130℃ is desirable to form gelatin. Results of this study illustrated a promising procedure to produce gelatin for factories from CB without utilization of acid and/or alkali liquor, which could be helpful for enriching the source of gelatin, and thus provide a natural way to prepare value-added gelatin from chicken bone residue.

ACKNOWLEDGEMENTS

This work was financially supported by National Natural Science Foundation of China (No. 31401623) and China-Agentina Food Science Technology Center of Chinese Ministry of Science (No. KY201401005).

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