Identification of the proteins in broth of stewed traditional Chinese yellow-feathered chickens

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Abstract – The binding of flavor compounds to proteins affect the flavor perception, thus identification of the major proteins in meat-based broth will provide the basis for understanding protein adsorption of flavor compounds. The present study aimed to identify the major proteins in traditional Chinese chicken broth and to describe the structural changes of proteins during stewing (1, 2, or 3 h). As stewing time increased, protein content in the broth significantly increased. SDS-PAGE indicated that the macro-molecule proteins in the broth were mainly gelatin and actin and that the micro-molecule proteins fractions (<10 KDa) increased substantially. The gelatin had an ordered structure even after 3 h of stewing, as seen by circular dichroism (CD) spectroscopy. These findings suggested that gelatin was the structural protein in the broth system.

Key Words – chicken soup; yellow-feathered chickens; gelatin; flavor

I. INTRODUCTION

The yellow-feathered chicken breed, local to Asia, is highly preferred by Chinese consumers due to its unique flavor and textual characteristics and is mainly processed for use in pot-stewed products such as chicken broth and chicken soup. Traditional chicken soup recipes are popular in China, and often call for chicken meat, water, and spices such as (ginger, garlic and star anise). Additionally, salt is added in soup only before eating. Earlier research has reported that the aroma compounds in chicken broth mainly include methylpyrazine, 2-ethyl-4-methylthiazole, 3-(methylthio) propanal, and (*E*,*E*)-2,4-decadienal [1]. These volatile compounds tend to be bound by the proteins in the broth, since proteins may provide hydrophobic pockets, amino acid side chains and terminal ends for interaction [2]. As a consequence, changes in protein content and conformations may lead to different interactions between proteins and volatile compounds, affecting the flavor perception in protein-based food products. Although studies such as these on broth flavor have been performed, few have been conducted on the major proteins in broth. Zhang et al. has reported that the major proteins in crucian carp soup derive from myosin heavy chain and sarcoplasmic proteins. Given this result, it is possible that the thermal degradation products of myofibrillar and sarcoplasmic proteins would be the predominant proteins in chicken soup. This work aimed to illuminate whether these or other major proteins exist in chicken broth produced by long-term boiling of traditional Chinese yellow-feathered chicken meat.

II. MATERIALS AND METHODS

Live, domesticated, yellow-feathered female chickens that had been fed for 300 days were slaughtered and cleaned by a commercial chicken-processing company (Sungo, Henan, China). The bone-in chickens were evenly cut into two pieces and cooked in 4-L stainless steel pots (ST22J1, Supor, Hubei, China) with purified water (Yibao, Shenzheng, China) at an amount weighing twice the mass of the chicken meat. Three sets of 10 chickens were stewed for 1, 2, or 3 h. The concentration of protein in chicken broth was measured using the Biuret method. The original broth and adjusted broth (to protein concentration of 1.0 mg/mL) were prepared for SDS-PAGE. The CD spectrum was obtained using a Chirascan CD Spectrometer (Applied Photophysics Ltd, London, England).

III. RESULTS AND DISCUSSION

The electrophoretic patterns of proteins in chicken broth obtained at different stewing times are shown in Fig. 1. The gel loaded with the original concentrations of the chicken broth samples indicated that the staining density of the major protein bands and the protein size distributions were similar between the stewing times (Fig. 1a) for macromolecule protein fractions greater than 10 KDa. However, in the concentration-adjusted protein samples, the staining density of protein bands greater than >10 KDa decreased as stewing time increased (Fig. 2b). Additionally, Protein concentration in chicken broth increased remarkably with increased stewing time, from 1.25 mg/mL after 1 h to 3.82 mg/mL after 3 h. These results indicated that the proportion of large proteins in the chicken broth decreased as stewing time increased. The increase in protein concentration (to 3.82 mg/mL) can be mainly attributed to increases in the smaller protein fractions (<10 KDa) with prolonged stewing. The predominant protein bands in the gel were concentrated in the vicinities of 130 kDa and 40 kDa, which matched the α subunit of collagen (130 kDa for α_1 and α_2) and actin,

respectively. These identities were confirmed using liquid chromatography-mass spectrometry (data not shown). These results indicated that the predominant proteins in chicken broth were derived from collagen (gelatin) and myofibrillar proteins. Moreover, the α bands were representative for the unfolding polypeptide chains of the triple helix, and α bands generally disappeared in the severely denatured state, as seen in bands of less than 100 kDa [3]. Together these results suggested that an ordered gelatin structure still existed in chicken broth even after 3 h of stewing, since the α subunit was still present.

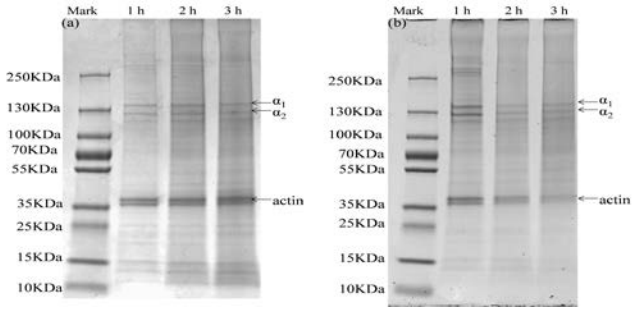

Figure 1 Electophoresis pattern of proteins of chicken broth obtained at different stewing times. (a) original protein samples. (b) adjusted protein samples.

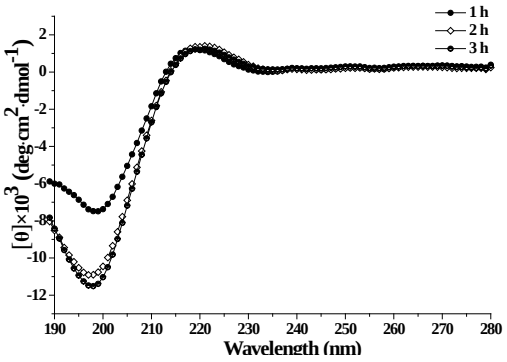

Figure 2 Circular dichroism (CD) spectroscopy of chicken broth obtained after different stewing times

UV-CD spectrum was performed to evaluate the secondary structure of proteins in chicken broth after different stewing times (Fig. 2). A positive peak at ~220 nm and a negative peak at ~199 nm were found in chicken broth, which are characteristic of the CD spectrum of collagen or gelatin. This result confirmed the presence of the α_1 and α_2 subunits of collagen protein as seen in the SDS-PAGE analysis. This CD result can eliminate the role of actin in the structural profiles of chicken broth, due to the present of two negative bands, near 208 and 222 nm of CD spectrum, that are characteristic of myofibrillar proteins. The positive peak at 220 nm commonly represents a triple helical conformation, and a negative peak at 197 nm suggests an aggregation of collagen or gelatin molecules [4]. In the chicken broth, there is no significant difference in the positive peak and a marked increase in the negative peak of the CD spectrum (Fig. 2). A decrease in the negative peak at 197 nm is likely due to promoted aggregation of gelatin molecules.

IV. CONCLUSION

In a broth made from boiled chicken, the remarkable increase in protein concentration with prolonged stewing could be mainly attributed to the increase in the small proteins fraction (<10 KDa). The large proteins in chicken broth mainly included gelatin and actin, which were derived from collagen and myofibrillar proteins, respectively. The gelatin retained an ordered structure even after 3 h of stewing, indicating that it is likely the major structural protein in the broth system.

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