EVALUATION OF GOOSE LIVER ISOLATE AS A FOOD INGREDIENT: A NEW APPROACH FOR GOOSE BY-PRODUCT UTILIZATION

Xue Zhao, Xin Li, Xing-lian Xu*, Su Zhuang, Tong Xing

College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, P.R. China

*Corresponding author: Xinglian Xu Ph.D E-mail address: xlxus@njau.edu.cn

I. INTRODUCTION

Nowadays, food industries pursue a more efficient processing stream with a view to enhancing or recovering value of poultry products [1]. Goose liver protein is a byproduct of broilers contained plenty of nutrients and had high digestibility (~97%) [2]. However, due to its undesirable odor and color, as well as liability to oxidation, the economic value of goose livers is therefore lowered. During isoelectric solubilization/precipitation (ISP) processing, protein is subjected to extreme pH conditions followed by a recovery step at an isoelectric pH. The ISP procedure changes protein conformation, thus modified protein gelation and emulsification properties [3]. For now, the effects of ISP process on poultry protein are centered on meat paste system, of which functionalities decided by mainly myofibrillar conformation. However, goose liver is a complicated system contained mostly water-soluble profile such as myoglobin and enzyme. Hence, the empirical conclusion drawn from meat muscle is not practical in liver protein recovery and modification. The objectives of this study were to evaluate the composition, conformation and emulsion properties of ISP isolated goose liver protein for better understanding its potential as a functional agent.

II. MATERIAL AND METHODS

Fresh goose liver was purchased from a local facility. Livers were minced into paste with a Waring blender (GM200, Retsch, Germany). Protein was isolated from goose liver through ISP process as previously described [4]. The liver batter was homogenized with ice-cold deionized water at a ratio of 1:6 (W/V). The pH of resulting liver slurry was adjusted to reach final pH of 2.0, 2.5, 3.0, 11.0, 11.5 and 12.0 using 2 M NaOH or HCl. The slurry was then centrifuged at 10000 g (Avanti J-E, Beckman Coulter, CA) for 10 min and the supernatant was collected. Afterwards the pH of the supernatant adjusted to 5.5. Then the protein was separated through centrifuging at 10000 g for 10 min and treated as ISP isolated liver protein. Non-ISP treated goose liver paste was labeled as control (CON).

Proximate composition of recovered fractions (crude protein, total lipid and ash content) was determined according to standard methods. The Kjeldahl method was used to determine crude protein content. To assess total lipid content, the Soxhlet extraction with petroleum ether was performed. Ash content was measured by placing 5 g of samples in a crucible and incineration in a muffle furnace at 550 °C for 24 h. The total pigment content was determined according to the method described before. Protein samples (10 g) was mixed with 40 ml acetone, 1 ml deionized water and 1 ml HCl. The mixture was stirred and the absorbance was read at 640 nm. The total pigment was calculated as the absorbance value multiplied by a coefficient of 17.18.

The hydrophobicity of the protein sample was determined using the BPB method [5]. The ISP-extracted protein and meat paste were suspended in a phosphate buffer (50 mM sodium phosphate, 0.6 M NaCl, and pH 6.5), and the protein concentration was adjusted to 5 mg/ml. Subsequently, 200 µl of 1 mg/ml BPB (bromophenol blue) sodium salt (in distilled water) was added to 1 ml of protein suspension and mixed well. The samples and control were centrifuged for 15 min at 2000 g. The absorbance of the supernatant at 595 nm was measured. The amount of BPB bound (µg) = 200 µg × $(A_{control} - A_{sample}) / A_{control}$

Raman spectra were recorded in a HR800 spectrometer (Horiba Jobi Yvon S.A.S., Longjumeau, France). The Raman spectra were acquired in the range 400cm^{-1} to 3600 cm^{-1}, for each sample, 3 scans with 30 s exposure time, 2 cm^{-1} resolution and 120 cm^{-1}/min sampling speed were conducted.

Both EAI and ESI were determined according to the method described by Chan et al [6]. The protein content was adjusted to 6.13 g protein/100 g emulsion by adding pre-cold deionized water and 35 g soybean oil. Mixtures were homogenized for 30 s at 10000 rmp, 3 times to obtain liver protein/soybean oil emulsion. Immediately after homogenization, aliquots of 50 µl of emulsions were diluted to 5 ml with 0.1% SDS solution. The absorbance of the emulsion was measured at 500 nm at 0 min and 10 min, respectively, and treated as A_0 and A_{10}. The EAI and ESI were calculated as follows equation: EAI= 2.33 × A_0, ESI= 10 × $[A_0/(A_0 - A_{10})]$

All data were submitted to analysis of variance (ANOVA) using the general linear model procedure of Statistical Analysis System (SAS 8.2. SAS Inst. Inc., NC, USA, 2000). Differences between least squares means were determined using Duncan's multiple range comparison, and were reported as significant at the 0.05 level.

III. RESULTS AND DISCUSSION

Proximate compositions of the ISP-isolated liver protein and CON are presented in Table 1. The lipid reduction in the ISP recovered protein fraction is crucial assessment to evaluate the economical feasibility of ISP as a protein isolation technology. Higher fat content could lead protein sample to more oxidative sensibility and get rancidity faster. According to the results, the alkali-aided proteins had significant higher protein content and lower ash and fat content, which implicated an efficient impurity reduction rate of ISP process.

The total pigment contents of control liver paste and ISP isolated protein were presented in Fig. 1A. It is shown all alkali-aided samples have similar ($p > 0.05$) remained pigment content, and significantly lower ($p < 0.05$) than acid-

aided samples. Besides, all ISP isolated protein lost partial pigment compared to the CON, which proves that ISP process could reduce the pigment content of goose livers successfully.

Table1 Proximate analysis (g/100g, dry basis) and secondary structure of ISP-isolated goose liver proteins

pH	Proximate analysis			Secondary structure			
	Protein (%)	Ash (%)	Fat (%)	α-helix	β-sheet	β-turn	Random coil
2.0	89.1±0.5^{c}	2.9±0.1^{c}	8.0±0.1^{d}	66.8±1.0^{b}	6.2±0.3^{d}	9.5±0.5^{a}	16.9±0.1^{a}
2.5	87.8±1.0^{d}	3.3±0.3^{b}	8.9±0.0^{c}	66.7±1.3^{b}	7.0±0.2^{d}	9.4±0.7^{a}	16.5±0.6^{a}
3.0	87.4±0.9^{d}	3.5±0.3^{b}	9.1±0.1^{b}	67.0±1.8^{b}	8.6±0.5^{c}	10.3±0.6^{a}	13.5±0.8^{b}
11.0	90.7±0.3^{b}	2.4±0.2^{d}	6.9±0.1^{e}	66.5±1.6^{b}	13.2±0.4^{a}	10.0±0.5^{a}	9.9±0.3^{c}
11.5	90.5±1.0^{b}	2.6±0.3^{d}	6.8±0.2^{e}	66.4±1.1^{b}	12.7±0.2^{ab}	10.1±0.9^{a}	10.0±0.7^{c}
12.0	92.1±1.1^{a}	2.3±0.3^{d}	5.6±0.5^{f}	67.5±1.5^{b}	11.6±0.4^{b}	9.9±1.7^{a}	9.67±0.8^{c}
CON	83.2±0.4^{e}	5.1±0.2^{a}	11.7±0.1^{a}	71.1±1.6^{a}	9.14±0.9^{c}	9.6±1.2^{a}	10.1±0.8^{c}

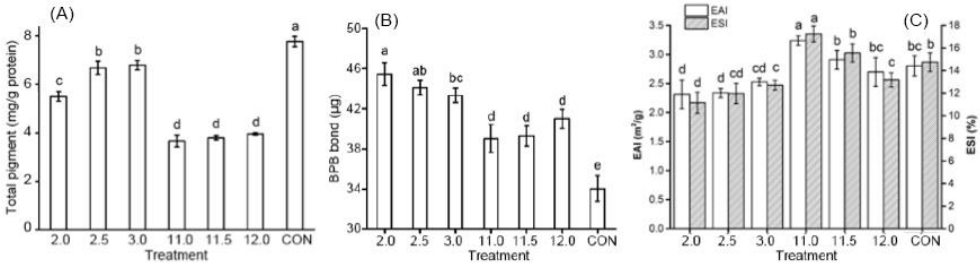

Fig.1 The content of total pigment (mg/g protein) (A), hydrophobic group content (B), emulsifying activity index and emulsifying stability index (C) of protein extracted from goose livers at different pHs. Note: a-d Different letters indicate significant differences ($P < 0.05$), n=4.

Changes in surface hydrophobicity were associated with the emulsion properties of muscle proteins. In this study, surface hydrophobicity of goose liver protein increased significantly ($P < 0.05$) after ISP treatment (Fig. 1B). For acid-isolated protein, the hydrophobicity was improved more than alkali-aided samples.

Based on the calculated portion of secondary structure, by derivation of Amide I spectra (Table 1), solubilizing under extreme pH conditions led to a significant change of secondary structure. Compared to CON, the total α-helix of all ISP-isolated samples was significantly decreased. Simultaneously, the proportions of disordered secondary structures, including β-turn and random coil, increased ($P < 0.05$) in alkaline solubilized samples.

The emulsion activity index (EAI) and emulsion stability index (ESI) of isolated goose liver protein were shown in Fig.1C. The isolated proteins solubilized with pH 11.0 presented highest EAI and ESI. Compared to acid isolated proteins, alkali-aided samples showed better emulsion ability. Although alkaline treatment increased surface hydrophobicity of protein, which could enhance protein to absorb to surface of oil droplet and further improve emulsion ability, however, pH 12.0 and acid treatment may induce sever protein denaturation of proteins, thus decrease their emulsify capacity.

IV. CONCLUSION

The ISP treatment could effectively decrease the fat and impurity content in goose liver, thus raise the ratio of protein in isolated protein. Additionally, total pigments, which played an important role as a pro-oxidation component, were also decreased significantly, indicating a possibility for better oxidative stability. Both secondary and tertiary structure of goose liver was changed during ISP treatment, reflected by higher surface hydrophobicity and lower α-helix. The EAI and ESI were both improved when liver protein was subjected to pH 11.0 solubilization. Overall, ISP treatment could isolate and modify goose liver protein and enhance its value.

ACKNOWLEDGEMENTS

This research was funded by China Agricultural Research System (Beijing, China, CARS-41); Overseas Expertise Introduction Center for Discipline Innovation ("111 Center") On Quality & Safety Control and Nutrition of Muscle Food（B14023).

REFERENCE

1. Mullen, A. M., Álvarez C, Zeugolis, D. I., Henchion, M., O'Neill, E., & Drummond, L. (2017). Alternative uses for co-products: harnessing the potential of valuable compounds from meat processing chains. Meat Science, 206(1), 15-33.
2. Li, X., Xue, S., Zhao, X., Zhuang, X., Han, M., Xu, X. L., & Zhou G. H. (2018). Gelation properties of goose liver protein recovered by isoelectric solubilisation/precipitation process. International Journal of Food Science & Technology, 53, 356-364
3. Nolsøe, H., & Undeland, I. (2009). The Acid and Alkaline Solubilization Process for the Isolation of Muscle Proteins: State of the Art. Food and Bioprocess Technology*, 2*(1), 1-27.
4. Zhao, X., Xing, T., Chen, X., Han, M. Y., Deng, S. L., & Xu, X. L., & Zhou, G. H. (2017). Changes of molecular forces during thermo-gelling of protein isolated from PSE-like chicken breast by various isoelectric solubilization/precipitation extraction strategies. Food and Bioprocess Technology, 10(7), 1240-1247.
5. Chelh, I., Gatellier, P., & Santé-Lhoutellier, V. (2006). Technical note: A simplified procedure for myofibril hydrophobicity determination. Meat Science, 74(4), 681-683.
6. Chan, J. T. Y., Omana, D. A., & Betti, M. (2011). Functional and rheological properties of proteins in frozen turkey breast meat with different ultimate ph. Poultry Science, 90(5), 1112-1123.