INFLUENCE OF pH ON CHEMICAL AND GELATION PROPERTIES OF PROTEIN EXTRACTED FROM PSE-LIKE CHICKEN BREAST BY ALKALI-AIDED PROCESSING

Xue Zhao, Tong Xing, Xing-lian Xu*

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

Abstract - The chemical and gel properties of meat paste prepared by pH adjustment method and protein isolated using isoelectric/precipitation (ISP) process from PSE-like chicken breast muscle were studied. The ultimate pH of protein gels were adjusted to 6.2 and 7.0. Increases in reactive sulfhydryl content and surface hydrophobicity were found when precipitation at pH 6.2 compared to 5.5. However, various ultimate pH showed no significant impact on surface hydrophobicity. Hardness of gels as measured by textural profile analysis was improved using 6.2 as precipitation pH compared to 5.5. In addition, the results indicated that protein precipitated at pH 6.2 formed a harder gel than precipitation at pH 5.5, possibly due to the higher surface hydrophobicity and S-S bridge formation. Overall, network characteristics of ISP-treated protein gels were strongly dependent on precipitation pH and ultimate pH.

Key words - surface hydrophobicity, sulfhydryl content, gel hardness

I. INTRODUCTION

With the increasing demand of poultry meat, broilers have been intensively selected for rapid growth and breast muscle mass and conformation. However, this has been accompanied by severe meat quality defects, among which pale, soft, exudative (PSE) -like meat is the most serious [1]. PSE-like meat has been characterized by undesired sensory qualities, poor water holding capacity and impaired functional properties, which causes huge loses to modern meat industry [2]. Thus, different technology has been widely studied to improve the functionality of PSE-like meat muscle protein [3,4].

Isoelectric solubilization/precipitation (ISP) process has shown great potential as a new method in improving gel properties of protein recovered from poultry muscle [5,6]. For now, much work has been concentrated on soluble pH effects on gel properties [5-7]. Noticeably, Marmon et al. [8] proved, for protein extracted from tilapia, precipitation at pH 6.5 induced harder gel strength and finer microstructure. Hence, it is of importance to understand the specific mechanism exists with various precipitation pH values during processing to optimize the functionality of the proteins.

Recently, we have found that protein isolated from PSE-like chicken can form well-developed gel when soluble pH was 11 and protein was collected at pH 5.5. However, how ultimate pH and recovered pH influenced protein chemical and gelation properties need further illumination. Thus, the present study was carried out to compare the role of precipitation pH and ultimate pH on gelation properties of PSE-like chicken meat paste and protein extracted by ISP methods.

II. MATERIALS AND METHODS

PSE-like chicken breasts were obtained from a local supplier (Yike, Suqian, Jiangsu，China). The pH_{24h} and L^*_{24h} were measured after slaughter 24 h. According to Li et al. [4], the PSE-like chicken breasts were chosen by pH_{24h} and L^*_{24h} value ($L^*_{24h} > 53$, $pH_{24h} < 5.7$). All the raw material was further conducted without freezing to prevent unwanted protein denaturation.

The experiment was carried out in two sets, pH-adjustment (PA) set and ISP set, respectively. In

PA set, the pH of meat paste was adjusted to 6.2 (normal pH of chicken breast meat), 7.0 (neutral pH) directly by adding 2 M NaOH drop wise. In ISP set, the samples were handled according to Hrynets et al. [5], the soluble pH was 11.0 and the precipitation pH was 5.5 and 6.2 respectively. At last, the pH of recovered sediments was adjusted to 6.2 and 7.0. The protein contents of the samples were adjusted to 100 mg/ml. All samples (including PA set) were mixed with 400 mM (~2%) NaCl. During the mixing process, the protein was kept in ice to prevent the temperature of the protein from exceeding 10 °C. Experimental treatments and treatment codes are presented in Table 1.

Table 1. Description of experiment treatments and treatment codes. Gels were formulated to contain 90% moisture, 2% NaCl. Treatment of protein, recovered pH and ultimate pH were experimental variables.

Treatment Code	1	2	3	4	5	6	7
Set	Control	PA	PA	ISP	ISP	ISP	ISP
Soluble pH	NA	NA	NA	11	11	11	11
Recovered pH	NA	NA	NA	6.2	6.2	5.5	5.5
Ultimate pH	5.57 (Initial pH)	6.2	7	6.2	7	6.2	7

(NA: not application; PA: pH adjustment; ISP: isoelectric solubilization/precipitation)

Hydrophobicity of non-solubilized protein was determined using the BPB method described by Chelh et al. [9]. The total and reactive sulfhydryl groups were estimated using protocols as detailed by Cao et al. [10] and Hrynets et al. [5] with a little modification.

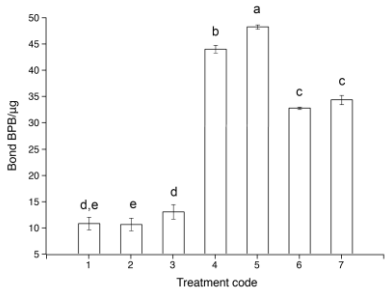
For gelation preparation, tubes filled with protein samples were heated at water bath from 20 °C to 80 °C with the heating rate kept at 1 °C/min and held at 80 °C for 20 min. Before analysis the samples were stored overnight at 4 °C.

The hardness of the gel was measured using texture profile analysis (TPA) method [11]. The test speed used for the test was 5 mm/s among the trigger force of 5 g and double compression cycle test was set up to 50% compression of the original height. During the test, the elapsed time was 1 s.

Reported results represent an average of each experiment assay. 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). Difference 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 DISSCUSSION

Figure. 1. Surface hydrophobicity of proteins. Data are given as mean ± SEM (n = 4). Different letters on the top of data bars indicate significant differences ($P < 0.05$).



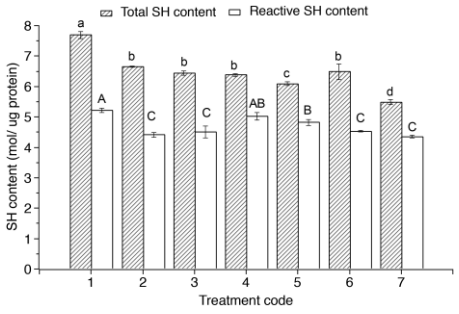
Changes in surface hydrophobicity were associated with the gelation of muscle proteins. In this study, pH adjustment alone showed a slight effect ($P > 0.05$) on the surface hydrophobicity of PSE-like chicken meat protein, whereas surface hydrophobicity increased significantly ($P < 0.05$) after ISP treatment (Fig. 1). For ISP-isolated protein, the hydrophobicity was improved slightly when ultimate pH increased from 6.2 to 7.0. This may be induced by the expansion of peptide chains, since the enhanced electrostatic repulsion force may lead to protein expansion.

The protein isolate recovered at pH 6.2 had a higher content of hydrophobic groups ($P < 0.05$) than that recovered at pH 5.5. This identical response to increased surface hydrophobicity suggested that the protein was only partially refolded compared to its isoelectric point state while raising the precipitated pH (from 5.5 to 6.2). Hydrophobicity of the myosin was observed increased upon refolding after ISP

process. Due to increased exposure of hydrophobicity groups, the protein aggregation promoted through non-covalent forces. This was in agreement with our TPA results.

Total and reactive sulfhydryl content (T-SH and R-SH, respectively) significantly influenced the gelation of muscle protein. SH content of muscle protein varied significantly with pH changes (Fig. 2). For meat paste counterparts, the T-SH content was highest as initial state and sharply decreased ($P < 0.05$) when pH was shifted upper to 6.2 (7.69 and 6.65 mol/ μ L, respectively). After that, the total SH content changed slightly even the pH was raised to 7.0 ($P > 0.05$). Compare to the control, protein subjected to ISP treatment showed significantly lower ($P > 0.05$) content of SH. It is known that the protein suffered from alkaline condition was more likely to oxidize SH to S-S bonds, because pKa of SH group of free cysteine is around 8.3, SH groups thus were deprotonated at pH 11.0 [12]. In addition, an oxidation reaction occurred because two cysteine residues came closer during protein folding-unfolding process, which was also associated with the reduction of SH with improvement of S-S [13]. A study by Hrynets et al. [5] performed on mechanically separated turkey meat showed that alkaline isolated protein had lower content of SH compared to raw sample. This was also seen with the chicken dark meat muscle proteins [13]. The data with different poultry meat indicated that the difference of SH-groups could explain the different gelation properties of the proteins.

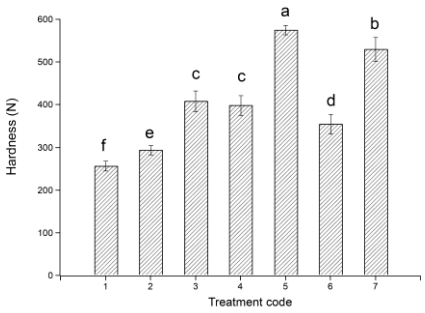
Figure. 2. Total sulfhydryl content and reactive sulfhydryl content of proteins. Data are given as mean ± SEM (n = 4). Different letters on the top of data bars indicate significant differences (P < 0.05).



Hardness of the protein-based gels was presented in Fig 3. Our exploration suggested that the hydrophobic group located in protein surface was exposed dramatically (Fig 1) during the extraction process. In this circumstance, in consideration of hydrophobic interactions play a major role in forming heat-induced gel, it was certain that ISP-treated protein showed stronger gel network. Furthermore, the increased sulfhydryl content (Fig 2) was attributed to higher hardness.

The pH of protein is believed to affect gelation abilities obviously. Higher pH gives the protein substantial negative charges and then efficiently strengthens the electrostatic repulsion. The repulsion force behind the protein formation contributes to more uniformly protein dispersion and evenly distributes the gel structure [14]. It was stated there was a direct connection between the dispersion of a protein gel network and its elasticity [15]. Accordingly, it could explain that the increase in gel pH, no matter whether the protein was conducted to ISP process, led to significantly increase in gel strength. Simultaneously, it was interesting to note that the different extent of improvement between the two types of protein (with or without ISP treatment) while pH was changed. It seems that the ISP-extracted proteins were more sensitive to pH changes. These distinctions were probably attributed to the unstable condition of the denaturing muscle protein. Overall, the results indicated that both procedures could significantly upgrade the gel qualities of protein from PSE-like chicken breast meat.

Figure 3. Hardness (Texture Profile analysis) of protein gels. Data are given as mean ± SEM (n = 3). Different letters on the top of data bars indicate significant differences (P < 0.05).



IV. CONCLUSION

Gel qualities of the muscle protein subjected to pH adjustment and ISP process were determined and compared. TPA results showed that gels precipitated at pH 6.2 were stronger than pH 5.5 and gels without ISP treatment, which may be attributed to surface hydrophobic group exposure and sulfhydryl oxidation during alkaline treatment. In conclusion, this study demonstrated that recovered pH and final pH were both crucial for gelation quality of ISP-isolated chicken breast muscle protein.

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