Effects of L-lysine on thermal gelation properties of chicken breast actomyosin

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Abstract The effects of L-lysine (L-Lys) on the water holding capacity (WHC) and texture of actomyosin (AM) gel and the possible mechanisms were investigated. L-Lys increased the WHC and hardness of the AM gel. These effects may be related to the even and continuous microstructure of the gel according to the scanning electron microscopy analysis. Furthermore, L-Lys increased the surface hydrophobic residues and the reactive sulfhydryl groups. L-Lys decreased the storage modulus at the first transition temperature but increased it at the second transition temperature and the third transition enthalpy. These results suggested that L-Lys varied the thermal behaviors and the microstructure of the AM gel by increasing the surface hydrophobicity and reactive sulfhydryl groups, ultimately contributing to the increased WHC and hardness. The changes in pH did not fully explain the results from the present study. The results were useful for understanding previous findings and may serve as a reference for the preparation of reduced-sodium and phosphate-free meat products.

Keywords L-Lysine (L-Lys) · Actomyosin (AM) · Thermal gelation · Water holding capacity (WHC) · Hardness

Introduction

Water holding capacity (WHC) and texture are the two important parameters when evaluating the quality of meat products [1, 2]. The current methods to improve the WHC and texture of meat products usually involve the utilization of NaCl and food-grade phosphates [1, 3, 4]. However, excessive intake of NaCl and phosphorus has been reported to pose health problems [4, 5]. A reduction in NaCl and phosphates can cause a distinct decline in the quality properties of meat products [1, 2]. Therefore, it is important to find an appropriate ingredient that can remedy the negative effects caused by reducing the NaCl/phosphate amount.

The use of L-Lysine (L-Lys) has attracted considerable interest in the meat industry [6–8]. Recently, L-Lys was demonstrated to improve the cooking yield and texture of pork sausage [6] and frozen white shrimp [8]. However, the mechanisms regarding the effects of L-Lys on WHC and texture of meat products is not clear.

WHC and texture are substantially dependent on the gelation of myofibrillar proteins [9, 10]. Actomyosin (AM), a complex of myosin and actin, is one of the major myofibrillar proteins. AM largely contributes to the WHC and textural properties of meat products [11, 12]. Different from myosin and other myofibrillar proteins, AM exhibits distinctive thermal stability and rheological and gelling properties, which depend on pH, ionic strength, heating rate, and other factors [12, 13].

Some studies have shown that L-Lys increases the solubility [14, 15], surface hydrophobicity and reactive sulfhydryl content and decreases the α -helical content of porcine myosin [14]. The solubility, conformation, and intermolecular bonds of myofibrillar proteins affect the texture, WHC, and other characteristics of meat products

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[13, 15]. From these previous reports, it is possible to suggest that L-Lys may be effective in improving the WHC and texture of myofibrillar protein gels. However, to our knowledge, there is no literature about the effects of L-Lys on the WHC and texture of AM. The objective of this study is to investigate the effects of L-Lys on the WHC and hardness of heat-induced AM gel. Additionally, we propose that the mechanisms for thermal gelation of L-Lys and the AM system can be determined from changes at the molecular level.

Materials and methods

Materials

L-Lys (99%), was purchased from Shanghai Juyuan Biotechnology Co., Ltd. (Shanghai, China). The pH of a 0.3% L-Lys solution was 9.45 ± 0.03 at room temperature.

Three batches of boneless and skinless chicken breasts (10 kg), obtained from birds slaughtered at approximately 40 days of age, were purchased from the Carrefour Group (a local supermarket). After visible fat and connective tissue were trimmed, the chicken breasts were minced twice using an MGB-120 meat chopper with a 5-mm (inner diameter)-hole plate (YEEKAI, Guangdong, China). All the procedures were carried out at 4 °C. The minced meat was packaged and stored at −18 °C until use.

Extraction of AM

The extraction of AM was carried out according to previous methods with some modifications [11]. The minced meat (200 g) was homogenized for 10 min in 500 mL of cold 20 mM potassium phosphate buffer (pH 7.0) containing 50 mM KCl with an FK-A grinder (Jincheng Guosheng experimental instrument factory, Jiangsu, China) and then centrifuged with a GL-21M centrifuge (Xiangyi Laboratory Instrument Development Co., Ltd., Changsha, China) at $8000 \times g$ for 10 min. The homogenization and centrifugation procedures were repeated three times. The precipitate was stirred for 30 min with 2.5 volumes of 0.6 M KCl in 20 mM potassium phosphate buffer (pH 7.0) using a JJ-1 agitator (Jincheng Guosheng experimental instrument factory, Jiangsu, China). Subsequently, the mixture was centrifuged at $10,000 \times g$ for 10 min, and the supernatant was filtered through three layers of gauze. The resulting filtrate was diluted with three volumes of cold distilled water and centrifuged at $10,000 \times g$ for 10 min. The collected precipitate (AM) was dissolved in 0.6 M KCl buffer (20 mM potassium phosphate, pH 7.0) and

adjusted to a concentration of 25 mg/mL. The protein concentrations were determined using the Biuret method [9]. The protein composition of the extracted AM was validated using SDS-PAGE with a gel imaging system (Universal Hood II, Bio-rad Co., USA). All procedures were carried out at 4 °C in a cold chamber.

Preparation of L-Lys-AM solutions

The AM solution mentioned above (10 mL) was treated as the follows: Sample 1, without L-Lys; Sample 3, 0.1% L-Lys; Sample 4, 0.2% L-Lys; and Sample 5, 0.3% L-Lys. The pH values of the AM solutions were immediately determined [6]: Sample 1, pH 6.43; Sample 3, pH 7.12; Sample 4, pH 7.88; and Sample 5, pH 8.41. Because pH is related to the gelling properties of myofibrillar proteins [8, 11], the pH of the AM solution without L-Lys was adjusted to approximately 7.12 using a dilute KOH solution (Sample 2). The AM solutions were stored for approximately 12 h at 4 °C for their next use.

Preparation of L-Lys-AM gels

The AM solution mentioned above (10 mL) was transferred to a 10-mL beaker (inner diameter: 25 mm, height: 35 mm) and then heated. The temperature was increased from 20 to 80 °C for 30 min and then maintained at 80 ± 2 °C for 30 min in a water bath. The sample was immediately cooled using flowing tap water and stored for approximately 12 h at 4 °C for subsequent analysis.

Determination of WHC

The WHC of the gel mentioned above was determined according to a previously described procedure [9]. Specifically, approximately 10 g of the gel was centrifuged at $1000 \times g$ for 10 min at 4 °C. The WHC was calculated as the percentage of the residual gel's weight after centrifugation relative its initial weight. Each sample was measured in quadruplicate ($n = 4$).

Determination of gel hardness

The gel hardness were measured using a TA-XT plus Texture Analyzer (Stable Micro System Co., Surrey, UK) at approximately 20 °C [11]. The gel was subjected to a compression test using a cylindrical probe (P/0.5 in., aluminum) at a trigger type button with a 1.5 mm/s pre-test speed, a 1.0 mm/s test speed, a 4.0 mm distance and a 5 g trigger force. The maximum sustained compression force was determined to be the gel hardness. Each sample measurement was repeated five times ($n = 5$).

Scanning electron microscopy (SEM)

According to a previous protocol [6], the gel sample ($5 \times 5 \times 1$ mm) was dried for nearly 20 h using an FD-1A-50 lyophilizer (Beijing, Beijing Boyikang Experimental Instrument Co. LTD., China). Subsequently, the SEM observations were carried out using a scanning electron microscope (Jeol, JSM 6490LV, Tokyo, Japan). At least two fields from each sample were examined, and one of the fields was presented.

Determination of surface hydrophobic residues

Surface hydrophobic residues were determined using 8-anilino-1-naphthalene sulfonic acid (ANS) according to a previously described procedure with some modifications [14]. The AM solutions were diluted to 1 mg/mL AM with 0.6 M phosphate buffer (pH 6.5). To 4 mL of the diluted AM solution, 20 mL of 15 mM ANS solution (dissolved in 0.6 M phosphate buffer, pH 7) was added. Subsequently, the AM solutions were fully stirred in the dark for 20 min at 25 °C, and the fluorescence of the 0.2 mL ANS-AM conjugate was measured with a fluorophotometer (930A, Shanghai Sanco Instrument Co., Ltd., China) using 380 and 470 nm as excitation and emission wavelengths, respectively. Each sample was measured in quintuplicate, and the results were expressed as the mean ($n = 5$).

Determination of the reactive sulfhydryl groups

Reactive sulfhydryl groups were measured according to a previously described protocol with some modifications [14]. The AM solution was diluted to 1 mg/mL AM with 0.6 M phosphate buffer (pH 8). To 4 mL of the diluted AM solution, 50 μ L of 5,5'-dithiobis-(2-nitrobenzoic acid) solution (10 mM in 0.6 M phosphate buffer, pH 8) was added. Subsequently, the AM solution was incubated for 20 min. The absorbance of the solution was determined at 412 nm using a spectrophotometer (ThermoFisher FC, Bio-Tek, USA). The sulfhydryl content was obtained by dividing the absorbance by the molar extinction coefficient ($EM = 13,600$). The results were expressed as micromoles of sulfhydryl per 100 mg of AM. Each sample was measured in quintuplicate, and the results were expressed as the mean ($n = 5$).

Dynamic rheology

After the samples were treated with L-Lys, the dynamic oscillatory measurements were performed immediately with a rheometer (Discovery HR-3, TA instrument Co., USA) in oscillatory mode, according to a previously

described method with slight modifications [11]. A parallel steel plate geometry (60 mm) with a 550 μ m gap was used. The samples were heated from 20 to 80 °C at 2 °C/min followed by cooling from 80 to 20 °C at 4 °C/min. A continuous, approximately 0.8 Hz oscillation and a 2% strain were applied to monitor the storage modulus (G_0). Each sample was measured in triplicate ($n = 3$).

Differential scanning calorimeter (DSC)

DSC measurements were carried out using a Micro DSC VII calorimeter (Setaram, Caluire, France) according to a previously described procedure with some modifications [9]. First, 10–15 mg of the AM solution was accurately weighed in a DSC vessel, equilibrated for 10 min at 20 °C, and then heated over a 20–85 °C temperature range at a 5 °C/min rate. An empty vessel was used as the reference. Nitrogen gas was used to eliminate water condensation in and under the DSC vessel. The thermal transition temperature (T_M) was described as the maximum transition temperature of the main endothermic peak, while the apparent enthalpy (ΔH , J/g of AM) was described as the global area under the DSC curve. Each sample was measured in triplicate, and the results were expressed as the mean ($n = 3$).

Statistical analysis

The analyses of variances, means and standard errors were determined using Excel 2003 (Microsoft Office Excel 2003 for Windows). A $p < 0.05$ significance level was used to determine the differences between the samples.

Results and discussion

WHC and hardness

WHC and hardness are two important indexes to measure the properties of a gel. As illustrated in Fig. 1(A), the application of L-Lys led to significant increases in WHC in all cases compared to the control group ($p < 0.05$). Moreover, the effects of L-Lys on WHC depended on the levels of L-Lys. WHC reached the highest values at 0.2% ($p < 0.05$). The present results suggest that the application of L-Lys may favor the increase in the yield of meat products. L-Lys reportedly increased the solubility of porcine myosin [14, 15], which may contribute to the increased WHC. This effect may be related to the changes in pH induced by L-Lys. To further clarify this, the WHC was also measured when the pH of AM solution was adjusted to 7.12 (Sample 2). The results showed that Sample 2 had slightly lower WHC than the control samples ($p > 0.05$), indicating that the increase in pH was not

Fig. 1 Effects of L-Lys on the WHC (A) and hardness (B) of actomyosin gels. L-Lys/pH of five samples: Sample 1 0%/6.43, Sample 2 0%/7.12, Sample 3 0.1%/7.12, Sample 4 0.2%/7.88, and Sample 5 0.3%/8.41. The different letters (a–d) are significantly different ($p < 0.05$)

responsible for the increased WHC under the present conditions.

As shown in Fig. 1(B), the samples treated with L-Lys had higher gel hardness than the control samples in all cases ($p < 0.05$). Furthermore, this effect on gel hardness depended on the L-Lys levels: gel hardness increased from 15.7 to 172.1 g when L-Lys concentration increased from 0 to 0.2%, but decreased from 172.1 to 135.8 g when L-Lys increased from 0.2 to 0.3%. It was reported that L-Lys increased the pH of chopped meat mixture [6], and pH is one of the important factors that affect the hardness of myofibrillar proteins gel [16]. Therefore, the hardness of Sample 2 was also investigated in order to clarify if L-Lys affected gel hardness by changing the pH of the AM solution. However, Sample 2 had slightly lower hardness than the control group ($p > 0.05$), indicating that the changes in pH could be ruled out. The present results also indicate that the WHC and hardness of AM gel may be improved by adding an appropriate amount of L-Lys. This

may be an interesting finding for the manufacture of low-sodium and phosphate-free meat products.

Microstructure (SEM)

The microstructure of protein gels is an important determinant of WHC and hardness [9, 17]. Therefore, the microstructure of the protein gels were investigated to reveal the relationship between WHC, texture and microstructure of AM gel. As shown in Fig. 2, the different sizes of caves and aggregates were distributed in a coarse gel in the absence of L-Lys. A similar microstructure was also found in chicken salt-soluble proteins gel [9, 18]. The caves and aggregates disappeared and tended to form a continuous and even gel at 0.1–0.3%. This effect may contribute to the increased WHC and hardness. Similarly, L-arginine reportedly induced chicken salt-soluble proteins to form a similar microstructural gel [9]. pH is one of the

Fig. 2 Effects L-Lys on the microstructure of actomyosin gels (A1–E1 500, A2–E2 2000). (A1, A2) Sample 1, (B1, B2) Sample 2, (C1, C2) Sample 3, (D1, D2) Sample 4, and (E1, E2) Sample 5. L-Lys/pH of five samples: Sample 1, 0%/6.43; Sample 2, 0%/7.12; Sample 3, 0.1%/7.12; Sample 4, 0.2%/7.88; and Sample 5, 0.3%/8.41

important factors that affects the microstructure of protein gels [16]. Therefore, Sample 2 was also examined in order to determine the effects of pH on the microstructure of the protein gel. The results showed a coarse gel with different cluster sizes. A possible reason could be that the optimum pH for gel formation is approximately 6.0 using purified myosin, AM, or myofibrillar proteins [13]. The results suggested that the changes in pH were not a crucial factor that affected the microstructure of AM gel in the presence of L-Lys. On the other hand, both surface hydrophobicity and reactive sulfhydryl groups usually participate in thermal gelation of meat proteins [11]. L-Lys reportedly increased the surface hydrophobicity and the reactive sulfhydryl content of porcine myosin [14], which may be responsible for the continuous and uniform gel. The addition of 0.3% L-Lys also formed a compact and dense AM gel. However, the samples treated with 0.3% L-Lys exhibited a more concavo-convex microstructure with larger caves and bigger aggregations, compared with the ones treated with 0.2% L-Lys. This may explain why the samples treated with 0.3% L-Lys had lower WHC and hardness than the ones treated with 0.2% L-Lys. The present results indicate that the structure of AM gel may be changed by adding an appropriate amount of L-Lys and result in increased WHC and hardness.

Surface hydrophobic residues and reactive sulfhydryl groups

The surface hydrophobic residues and reactive sulfhydryl groups participate in the formation of gel and may have important effects on the WHC and texture of gel [11]. The effects of L-Lys on the surface hydrophobic residues and reactive sulfhydryl groups of AM were further investigated. The fluorescence intensity decreased at 0.1% L-Lys but increased at 0.2–0.3% L-Lys [Fig. 3(A)], whereas the reactive sulfhydryl groups increased in all cases compared to the control group ($p < 0.05$) [Fig. 3(B)]. L-Lys was also reported to increase the surface hydrophobicity and reactive sulfhydryl groups of porcine myosin at pH 6.5 [14]. Sample 2 was examined in order to clarify the effects of pH on surface hydrophobicity and sulfhydryl group. It was found that Sample 2 had lower fluorescence intensity than the control group ($p < 0.05$). Previous literature also showed that fluorescence intensity decreased with increasing pH from 6.0 to 8.0 in scallop *Patinopecten yessoensis* actomyosin [19]. Sample 2 had more reactive sulfhydryls than the control group, but lower reactive sulfhydryls than the samples treated with L-Lys ($p < 0.05$). The present results preclude the possibility that pH changes were responsible for the increased fluorescence intensity and reactive sulfhydryls. On the other hand, Guo et al. [14]

Fig. 3 Effects of L-Lys on the surface hydrophobicity (A) and reactive sulfhydryl groups (B) of actomyosin. L-Lys/pH of five samples: Sample 1 0%/6.43, Sample 2 0%/7.12, Sample 3 0.1%/7.12, Sample 4 0.2%/7.88%, and Sample 5 0.3%/8.41. The different letters (a–d) are significantly different ($p < 0.05$)

observed that L-Lys caused the secondary structural changes of porcine myosin at pH 6.5. Therefore, the increases in surface hydrophobic residues and reactive sulfhydryl groups may be related to secondary structural changes of AM. It is well known that surface hydrophobicity and sulfhydryl groups are usually involved in the thermal gelation of myofibrillar proteins [11, 13]. Therefore, the increased surface hydrophobicity and sulfhydryl group due to the addition of L-Lys may contribute to the increased WHC and hardness of AM gel.

Dynamic rheological properties

The storage modulus (G_0), associated with the hardness of the gel [9, 11], reflects the gelation process of AM during heating. The G_0 values for the control group distinctly increased at approximately 42 °C, reached the first peak at approximately 50 °C, drastically declined, and steadily increased again at nearly 60 °C [Fig. 4(A)]. This was in accordance with previous findings [12]. The increase in G_0

Fig. 4 Effects of L-Lys on the storage modulus (G_0) during heating (A, B) of actomyosin solutions. L-Lys/pH of five samples: *Sample 1* 0%/6.43, *Sample 2* 0%/7.12, *Sample 3* 0.1%/7.12, *Sample 4* 0.2%/7.88, *Sample 5* 0.3%/8.41

values at 42 °C was due to the cross-linking of myosin filament, causing the transformation from a viscous solution to an elastic network [11]. The decrease in G_0 values after 50 °C was due to either the dissociation of actin–myosin complex [20] or the helix-to-coil transformation of myosin, resulting in enhanced molecular “fluidity” [21]. This temperature range was also suggested to be the stage at which the rate of bond disruption because of thermal aggregation at this point surpassed that of bond formation due to thermal denaturation [18]. This led to the reorganization and realignment of the protein molecules. The second increase in G_0 values after 60 °C was attributed to the increasing crosslink among proteins to intensify the gel matrix and form a firm and irreversible gel [9, 11].

The treatment with L-Lys had no obvious influence on the curve shapes of G_0 values of AM during heating and cooling [Fig. 4(A), (B)]. This effect suggested that L-Lys did not change the relative importance of the various chemical bonds that formed the AM gel network [22]. Similarly, the dynamic rheological curve shapes did not obviously change in salt-soluble meat proteins in the presence of either L-arginine [9] or peanut protein isolate

Fig. 5 Effects of L-Lys on the CD spectra of actomyosin. L-Lys/pH of five samples: *Sample 1* 0%/6.43, *Sample 2* 0%/7.12, *Sample 3* 0.1%/7.12, *Sample 4* 0.2%/7.88, and *Sample 5* 0.3%/8.41

[18]. The G_0 values of the samples treated with L-Lys were significantly lower than those of the control group at the second (42–50 °C) and third (50–60 °C) temperature ranges ($p < 0.05$), which suggested that L-Lys slowed the cross-linking rate during the second temperature range and increased the dissociation of actin–myosin complex and/or the transformation of myosin from helix to coil structure during the third temperature range. Additionally, L-arginine reportedly caused a decrease in G_0 values of chicken salt-soluble proteins during the second and third temperature ranges [9]. The aggregation of proteins prior to heating inhibits myofibrillar protein gel formation, and the myosin tail participates in the thermal gelation of myofibrillar proteins [13]. Therefore, the present results may explain why the addition of L-Lys caused the disappearance of clusters and the formation of a uniform and continuous gel. On the other hand, the dissociation of actin–myosin complex contributed to enhanced WHC [23]. This may be a plausible explanation for how L-Lys increased the WHC of AM gel. Compared to the control group, L-Lys-treatment had lower G_0 values of AM during the fourth (>60 °C) temperature range. These results seem to be in conflict with the description that the samples treated with L-Lys had higher hardness than the control samples. However, L-arginine reportedly increased the G_0 values of chicken salt-soluble proteins during the corresponding temperature range [9]. The differing results may be due to the different protein and/or amino acid species. To investigate the effects of pH on the G_0 values, *Sample 2* was tested. Compared to the control, *Sample 2* had lower G_0 values with a similar curve shape during heating and cooling. The decrease in G_0 values was also observed when pH was elevated from 5.5 to 8.0 [16]. A possible reason could be that the pH of AM solution shifted the isoelectric points of myofibrillar proteins, which increased

Table 1 Effects of L-Lys on T_M and ΔH of chicken actomyosin

Sample	1	2	3	4	5
T_{M1} (°C)	55.84 ± 0.38 ^c	55.08 ± 0.63 ^b	53.94 ± 0.39 ^b	53.90 ± 0.55 ^b	53.55 ± 0.11 ^a
ΔH_1 (J/g)	0.266 ± 0.031 ^a	0.229 ± 0.031 ^a	0.326 ± 0.055 ^{ab}	0.467 ± 0.010 ^b	0.279 ± 0.022 ^a
T_{M2} (°C)	63.76 ± 0.23 ^a	63.81 ± 0.71 ^a	64.60 ± 0.62 ^b	64.40 ± 0.83 ^b	64.72 ± 0.27 ^b
ΔH_2 (J/g)	0.041 ± 0.008 ^a	0.071 ± 0.043 ^a	0.038 ± 0.020 ^a	0.057 ± 0.026 ^a	0.072 ± 0.040 ^a
T_{M3} (°C)	71.18 ± 0.73 ^b	71.45 ± 0.37 ^b	70.72 ± 0.41 ^a	71.59 ± 0.23 ^b	71.45 ± 0.38 ^b
ΔH_3 (J/g)	0.080 ± 0.075 ^a	0.313 ± 0.037 ^b	0.277 ± 0.069 ^b	0.430 ± 0.091 ^c	0.299 ± 0.047 ^b

L-Lys/pH of five samples: Sample 1, 0%/6.43; Sample 2, 0%/7.12; Sample 3, 0.1%/7.12; Sample 4, 0.2%/7.88; and Sample 5, 0.3%/8.41. The different letters (a–c) in the same row are significantly different ($p < 0.05$)

electrostatic repulsion, leading to the decreased G_0 values. Compared to the groups treated with 0.1% L-Lys, Sample 2 had lower G_0 values during the second temperature range but higher G_0 values during the third and fourth temperature ranges and during cooling. Since the two samples had the same pH, the decreases in the G_0 values were not completely attributed to the changes in pH in the presence of L-Lys.

The G_0 values drastically increased during cooling in all cases [Fig. 5(B)], which may be important for the final hardness of the AM gel. Some chemical bonds such as hydrogen bonds may form and/or reinforce, which may contribute to the increase in the G_0 values during cooling. The present results may explain the above contradictions between G_0 values of AM during the fourth temperature range and the final strength of AM gel.

DSC

DSC can disclose the changes in the native structure damage during heating, which reflects the interactions between meat proteins and additives [9, 11]. Therefore, the DSC of AM was also investigated to further explore the effects of L-Lys on the gelation process of AM during heating (Fig. 5). Three major characteristic peaks at 55.84 °C (T_{M1}), 63.76 °C (T_{M2}), and 71.18 °C (T_{M3}) were found in the control group samples. These were in accordance with the previous findings [9, 11]. The slight differences in endothermic transitions between our results and earlier findings may be partially due to different sample conditions. Temperatures for these endothermic peaks tend to vary depending on the muscle type, pH, and heating conditions [24]. The 55.84 °C transition may be attributed to the myosin head, the 63.76 °C transition can be ascribed to the myosin tail [18], and the 71.18 °C transition can be assigned to actin [11].

L-Lys had an obvious influence on the DSC curve of AM. First, the addition of L-Lys caused a decrease in T_{M1} in all cases ($p < 0.05$) but an increase in ΔH_1 at 0.2% L-Lys ($p < 0.05$) (Table 1). This suggested that the denaturation

of a myosin head occurred at a lower temperature but at a higher energy in the presence of L-Lys. Second, T_{M2} increased ($p < 0.05$), while ΔH_2 had no obvious change in all cases ($p > 0.05$), indicating that the denaturation of myosin tail occurred at a higher temperature in the presence of L-Lys. Partial denaturation followed by irreversible aggregation of myosin heads and helix-coil transitions of myosin tails is usually involved when myosin participates in thermal gelation of muscle proteins [25]. Therefore, it was possible that the addition of L-Lys changed the relative speed between aggregation and gelation of AM, which caused an even three-dimensional network, finally contributing to the increased WHC. On the other hand, the thermal stability of myosin tails was positively correlated to WHC [9]; theoretically, the addition of L-Lys may be helpful to increase the WHC of AM gel. Finally, L-Lys had no obvious influence on T_{M3} ($p > 0.05$) but increased ΔH_3 significantly ($p < 0.05$). This showed that the denaturation of actin occurred at a higher energy in the presence of L-Lys. However, the disappearance of the third thermal transition peak was observed in chicken salt-soluble proteins [9]. The differences in endothermic transitions between the present results and previous ones may be partially due to different conditions [24]. Sample 2 had lower T_{M1} but higher ΔH_3 ($p < 0.05$) than the control. The results suggested that the effects of L-Lys on DSC curve could not be ascribed to the shift in pH.

In conclusion, L-Lys increased the WHC and hardness of the AM gel. SEM showed that the treatment with L-Lys resulted in an even and continuous gel. Additionally, L-Lys increased the surface hydrophobicity and reactive sulfhydryl groups. DSC disclosed that L-Lys decreased T_{M1} and G_0 values but increased T_{M2} and ΔH_3 . These results suggested that the addition of L-Lys caused the increases in the surface hydrophobicity and reactive sulfhydryl groups, which may vary the thermal behaviors of AM and the microstructure of AM gel, ultimately contributing to the increased WHC and hardness. The changes in pH induced by L-Lys did not completely explain the present results. The results were also interesting for applications in the

manufacture of reduced-sodium and phosphate-free meat products.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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