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Research Article

Mechanism study on egg yolk addition improving the gel properties of low-salt chicken breast paste

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Abstract: This study investigated the improvement of gel properties in low-salt chicken breast mince and its underlying mechanism by adding different proportions of egg yolk (EY). Results showed that adding 1.0% EY significantly improved the textural properties of the low-salt chicken breast mince, the hardness increased from 900 to 1 059 g, and the springiness rose from 0.88 to 0.95. Meanwhile, the cooking loss decreased from 12.3% to 9.02%, and the water-holding capacity (WHC) increased from 60.67% to 82.67%. The relaxation peaks of immobilized water (T_{22}) and free water (T_{23}) both shifted toward lower relaxation time, which resulted in a dense microstructure and enhanced WHC, comparable to the positive control group. Further extraction of myofibrillar protein (MP) revealed that 1.0% EY addition significantly increased particle size and surface hydrophobicity while reducing free sulfhydryl content, the particle size increased from 44.29 to 96.63 μm, the surface hydrophobicity rose from 151.9 to 164.20 μg, and the free sulfhydryl content decreased from 18.99 to 15.14 μmol/g, this was accompanied by a structural shift from α-helix to β-sheet and enhanced fluorescence intensity. Additionally, increased disulfide bond and hydrophobic interactions enhanced cross-linking between EY and MP. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and laser confocal analysis confirmed protein aggregation, forming a compact gel network structure. This study elucidates the improvement of EY on the gel properties of low-salt minced meat and its mechanism of action, providing a theoretical basis for its application in low-salt minced meat products.

Keywords: gel properties; egg yolk; myofibrillar protein; gel network structure

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1 Introduction

Minced meat products generally refer to a category of meat-based foods produced by mechanically grinding and chopping raw materials such as poultry, livestock, or aquatic products, followed by the addition of seasonings and other ingredients, and subsequent processing through methods such as cooking and aging[1]. The primary protein component in muscle tissue is myofibrillar protein (MP), which accounts for approximately 55% to 60% of total muscle protein. This protein fraction includes key structural proteins such as myosin and actin[2] and plays a critical role in determining the gelation properties and overall quality of meat products. MP contributes to desirable texture and sensory characteristics in processed meats and provides essential functional properties, including water-holding capacity (WHC) and gel-forming ability[3]. Chicken muscle tissue comprises over 90% crude protein and essential amino acids, establishing it as a high-quality protein source. In particular, chicken breast is recognized as the leanest and most nutrient-dense portion, widely incorporated into health-oriented diets due to its characteristic "one high and three lows" profile (high protein, low fat, low cholesterol, and low calories). It serves as a raw material for various processed products, including chicken breast meatballs, sausages, and jerky. Salt is an indispensable ingredient in meat processing, contributing not only to product quality but also to microbial inhibition, flavor enhancement, and preservation. Typically, meat products contain 2% to 4% salt. A content of 2% to 3% promotes the solubilization of myofibrillar proteins, facilitating the formation of a dense and uniform gel network that improves WHC[4]. However, excessive salt intake has been identified as a dietary risk factor for various diseases. Epidemiological evidence suggests that long-term high-sodium diets are strongly associated with an increased risk of chronic conditions, such as hypertension and cardiovascular disease[5–6]. The Chinese Dietary Guidelines (2022) recommend that the daily salt intake for adults should be no more than 5 g per person. As public health awareness increases, low-salt diets have gained widespread popularity.

A drastic reduction in sodium content significantly inhibits the solubilization and sufficient dissociation of MP, which in turn prevent these proteins from unfolding and cross-linking effectively. This phenomenon readily leads to the formation of a gel network with a loose structure and heterogeneous pores, ultimately, resulting in the overall quality deterioration of meat products[7]. Direct reduction sodium chloride addition weakens the intermolecular electrostatic shielding effect, thereby impeding the ordered aggregation of MPs and the establishment of a three-dimensional (3D) gel scaffold. Consequently, the reduced sodium level in meat batter gels induces quality deterioration, which is characterized by

decreased WHC, reduced gel strength, and diminished juiciness[8]. According to Bower et al.[9], reducing salt concentrations in beef and turkey meat adversely affected their textural properties and water retention. Therefore, indiscriminate salt reduction may prove counterproductive. Current research on low-salt meat products primarily focuses on three strategies: incorporation of functional ingredients, optimization of processing methods, and utilization of exogenous additives as alternatives. The addition of functional components such as dietary fiber and plant proteins can serve as effective salt substitutes. Zhao et al.[10] demonstrated that incorporating quinoa protein into porcine MP effectively enhanced the structural stability and functionality of low-salt MP. Similarly, previous research has reported that the addition of soybean protein isolate (SPI) to emulsified casings can improve both emulsion stability and eating quality of sausages[11]. Despite ongoing progress, several challenges remain in low-salt meat product research. These include limitations in the applicability of current technological processes, which cannot be universally extended to all types of minced meat products, as well as the relatively high cost of alternative ingredients and their associated modification procedures. Consequently, further investigation is still required to develop meat products with reduced sodium content. EY, a widely available food raw material with low cost and high nutritional value, accounts for approximately 28%–29% of the total mass of a whole egg. It is rich in bioactive functional components and possesses outstanding emulsifying, gelling and interfacial adsorption properties. These superior functional attributes endow EY with considerable potential to ameliorate the quality of meat batter gels. However, few studies have systematically investigated the underlying mechanisms by which EY may improve the gel properties of low-salt chicken breast mince, warranting further exploration in this area.

In this study, low-salt and high-salt control groups were established to investigate the effects of incorporating varying concentrations of EY on the gel properties of low-salt chicken breast mince. The quality attributes of the meat batter gels were evaluated by measuring color differences, textural properties, WHC, and microstructural characteristics. Furthermore, in a MP model system, the study examined how EY modulates the physicochemical properties of MP and its influence on the organization of protein network structures. By elucidating the enhancing effect of EY on the eating quality of low-salt chicken breast mince and its underlying mechanism of action, this research provides a theoretical basis for the application of EY in low-salt minced meat products.

2 Materials and methods

2.1 Materials

Chicken breast and eggs were purchased from Jingdong Supermarket (Rizhao, China). Sodium chloride (salt) was provided by Guizhou Salt Industry Group Co., Ltd. (Guiyang, China). Hydrochloric acid, sodium chloride, sodium dihydrogen phosphate, and potassium sodium tartrate were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bromophenol blue, tris(hydroxymethyl)aminomethane, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) kit, and Nile blue staining solution were obtained from Beijing Solarbio Technology Co., Ltd. (Beijing, China).

2.2 Preparation of minced chicken breast

Frozen chicken breasts were thawed at 4 °C, and any visible fat and connective tissue were removed. The prepared meat was then minced using a meat grinder (MJ-MCE1803, Midea Group Co., Ltd., Guangzhou, China) for 30 s. Subsequently, the minced meat was transferred to a mixer, where cold water and salt were added according to the formulation. For the experimental groups, EY was also incorporated at this stage, and the mixture was homogenized for 1 min. To allow for adequate MP dissolution, the batter was allowed to stand for 2 min. During the entire chopping and mixing process, the temperature of the meat batter was maintained below 10 °C. Finally, the remaining ice water was added, and the batter was chopped for an additional 1 min. The resulting batter was divided into two portions. One portion was reserved for rheological property analysis and stored at 4 °C. The other portion was stuffed into collagen or natural casings and cooked in a 70 °C water bath for 20 min. After cooking, the samples were immediately cooled under running tap water and then stored overnight at 4 °C prior to further analysis.

The formulations for the different treatment groups were as follows: positive control (PC) group: 100 g chicken breast, 20 g ice water, and 2.4 g salt (total mass 122.4 g), resulting in a final salt content of 2% (*m*/*m*). Control (CK) group: 100 g chicken breast, 20 g ice water, and 1.2 g salt (total mass 121.2 g), resulting in a final salt content of 1% (*m*/*m*). Experimental groups: 100 g chicken breast, 20 g ice water, 1.2 g salt (total mass 121.2 g, 1.0% salt content), supplemented with varying levels of EY at 0.6% (0.73 g), 0.8% (0.98 g), and 1.0% (1.22 g) (*m*/*m*, based on total mass), respectively, and the samples were coded as 0.6% EY, 0.8% EY, 1.0% EY, respectively.

2.3 Extraction of MP

MP was extracted from fresh chicken breast muscle following the protocol established by Park et al.[12]. The protein concentration was adjusted to 40 mg/mL with 20 mmol/L phosphate-buffered saline (PBS, pH 7.0) supplemented with different concentrations of NaCl. Two control groups were formulated: the high-salt control contained 0.6 mol/L NaCl (PC), while the low-salt control contained 0.3 mol/L NaCl (CK). On the basis of CK, EY was incorporated at graded levels to yield final concentrations of 0.6%, 0.8% and 1.0% (*m*/*m*, relative to the total mass of the mixture), and the samples were coded as 0.6% EY, 0.8% EY, 1.0% EY, respectively. All sample mixtures were subjected to water bath heating at 70 °C to induce gel formation, followed by cooling to ambient temperature prior to subsequent characterization.

2.4 Measurement of meat batter gel properties

2.4.1 Color measurement

The color of chicken gel was measured using a colorimeter (3NH, Shenzhen Sanenshi Technology Co., Ltd., Shenzhen, China) following the method of Gao et al.[13]. The lightness (L^*), redness (a^*), and yellowness (b^*) values were recorded.

2.4.2 Texture profile analysis (TPA)

Following the method described by Pietrasik et al.[14], cylindrical chicken breast batter gels with a height of 2 cm were prepared. A texture analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) equipped with a P/36R probe was used to measure their hardness, elasticity, and chewiness. Compression ratio 30%; pre-test

speed 5.0 mm/s; test speed 2.0 mm/s; return speed 2.0 mm/s; trigger force auto-5.0 g; interval time 5 s; data acquisition rate 200 pps. Triplicate measurements were performed for each sample, and the results were presented as mean values.

2.4.3 Cooking loss measurement

Cooking loss was measured according to the method of Li et al.[15] with minor modifications. Approximately 30 g of raw meat batter (m_1) was placed into a 50 mL centrifuge tube and centrifuged briefly to remove air bubbles. The tube was then heated in a 70 °C water bath for 30 min. After heating, the tube was removed, cooled, and the surface moisture was wiped dry. The sample was weighed again (m_2). Cooking loss was calculated using the following equation (1):

$$\text{Cooking loss}\ (\%) = \frac{m_1 - m_2}{m_1} \times 100 \quad (1)$$

2.4.4 Determination of WHC

WHC was determined following the method of Geng et al.[16]. Cooked samples were cut into thin slices (5 mm thickness), weighed (m_1), wrapped in three layers of filter paper, and placed into 50 mL centrifuge tubes. The tubes were centrifuged at 4 °C and 5 000 × g for 10 min. After centrifugation, the samples were removed and weighed again (m_2). WHC was calculated using the following equation (2):

$$\text{WHC}\ (\%) = \frac{m_2}{m_1} \times 100 \quad (2)$$

2.4.5 Low-field nuclear magnetic resonance (LF-NMR) measurement

Water distribution and mobility were measured using a LF-NMR analyzer (NMI20-025V-1, Suzhou Niumag Analytical Instrument Corp., Suzhou, China) according to Zhang et al.[17] with minor modifications. Cooked gel samples were cut into uniform sizes and placed into 25 mm diameter NMR tubes. The transverse relaxation time (T_2) was measured using the Carr-Purcell-Meiboom-Gill (CPMG) sequence.

2.4.6 Dynamic rheological measurement

The dynamic rheological properties of raw meat batters were determined using a rheometer (MARS Iq Air, Thermo Fisher Scientific, Beijing, China) according to the method of Zhao et al.[18]. A small amount of sample was evenly spread on the platform of a 50 mm parallel plate. The test parameters were set as follows: frequency 0.1 Hz, strain 2%, gap 1 mm, initial temperature 20 °C, heating rate 2 °C/min, and final temperature 80 °C. Silicone oil was applied to the exposed sample edges to prevent moisture evaporation during heating.

2.5 Microscopic morphological observation

2.5.1 Field emission scanning electron microscopy (FE-SEM)

The microstructure of the meat batter gels was examined using FE-SEM (Thermo Scientific Apreo 2C, Thermo Fisher Scientific, Shanghai, China) according to the method described by Yang et al.[19]. Samples were frozen at −80 °C for 6 h and then subjected to vacuum freeze-drying. The freeze-dried specimens were mounted onto aluminum stubs and sputter-coated with gold.

2.5.2 Confocal laser scanning microscopy (CLSM)

The microstructure of meat batter gels was observed using CLSM (FV3000, Olympus Corp., Tokyo, Japan) according to the method described by Xiao et al.[20]. The MP concentration was adjusted to 2 mg/mL. The MP sample (1 mL) was mixed with Nile blue staining solution (40 μL) and vortexed thoroughly. After 30 min of reaction in the dark, 30 μL of the stained mixture was dispensed onto a glass slide, covered with a coverslip, and excess liquid was gently removed. Protein distribution was observed and imaged under an excitation wavelength of 633 nm.

2.6 Physicochemical properties measurement of MP

2.6.1 Particle size distribution determination

The particle size distribution of MP samples was determined according to the method described by Yan et al.[21] with minor modifications. The MP concentration was adjusted to 1.0 mg/mL. Particle size measurements were performed using a laser diffraction particle size analyzer (SALD-2300, Shimadzu Corp., Suzhou, China). The instrument parameters were set as follows: scattering angle 90°, equilibration time 30 s, and deionized water as the dispersant. The refractive indices (RI) were set to 1.330 for water and 1.450 for protein. The viscosity of the dispersant (water) was set to 0.887 2 mPa·s (corresponding to the viscosity of water at 25 °C).

2.6.2 Surface hydrophobicity determination

Surface hydrophobicity was determined using the bromophenol blue (BPB) binding method according to Wang et al.[22] with minor modifications. Briefly, 1.0 mL of MP sample solution (1.0 mg/mL) was transferred into a centrifuge tube, and 200 μL of BPB (1.0 mg/mL) was added. The mixture was vortexed for 10 min and then centrifuged at 8 000 × g for 10 min. An aliquot of 0.4 mL of the supernatant was collected and mixed with 3.6 mL of PBS. The absorbance was measured at 595 nm and recorded as A. For the blank, PBS was used instead of the protein sample, and the absorbance was recorded as A_0. The surface hydrophobicity, expressed by the binding amount of BPB, was calculated using the following equation (3):

$$\text{Surface hydrophobicity}\ (\mu\text{g}) = \frac{A_0 - A}{A_0} \times 200 \quad (3)$$

2.6.3 Free thiol content determination

The free thiol content was determined according to the method of Wei et al.[23] with minor modifications. Briefly, 1.0 mL of MP sample (4.0 mg/mL) was mixed with 5.0 mL of buffer solution (0.086 mol/L Tris, 0.09 mol/L glycine, 4 mmol/L ethylene diamine tetraacetic acid (EDTA), pH 8.0). Then, 30 μL of DTNB solution (4 mg/mL) was added. The mixture was vortexed thoroughly and allowed to react at room temperature for 30 min. Absorbance was measured at 412 nm against a blank prepared without DTNB. The free thiol content was calculated using the molar extinction coefficient of 13 600 L/(mol·cm) according to the following equation (4):

$$\text{Free thiol content}\ (\mu\text{mol/L}) = \frac{73.53 \times A_{412\ \text{nm}} \times D}{C} \quad (4)$$

Where 73.53 is the molar extinction coefficient, $A_{412\ \text{nm}}$ is the absorbance at 412 nm, D is the dilution factor, and C is the protein concentration (4.0 mg/mL).

2.7 MP structure determination

2.7.1 SDS-PAGE

SDS-PAGE was performed according to the method of Wang et al.[24] with slight modifications. MP samples (2 mg/mL) were mixed with loading buffer at a 3:1 ratio (*V*/*V*) and heated in boiling water for 10 min, followed by centrifugation at 10 000 × *g* for 10 min. Electrophoresis was carried out using a 12% separating gel and a 5% stacking gel. After the stacking gel had solidified, 10 μL of each sample and marker were loaded into the wells. Electrophoresis was initially run at a constant voltage of 90 V. When the dye front migrated into the separating gel, the voltage was increased to 120 V. Upon completion, the gel was stained with Coomassie brilliant blue R-250 solution (0.1 g/100 mL R-250, 45% methanol, 10% acetic acid) for 1 h. Subsequently, the gel was destained with a solution containing 10% methanol and 10% acetic acid until the protein bands became clearly visible against a transparent background. The destaining solution was replaced multiple times during this process. Gel images were captured using a gel documentation system (Model Tanon 4600SF, Tanon Science & Technology Co., Ltd., Shanghai, China).

2.7.2 Fourier transform infrared (FTIR) spectroscopy determination

FTIR spectra were obtained according to the method described by Wang et al.[25]. Meat batter gels were mixed with dried KBr, ground into a fine powder, and pressed into transparent pellets. The pellets were then analyzed using a FTIR spectrometer (Nicolet iS 5, Thermo Fisher Scientific, New York, USA). Spectra were recorded in the wavenumber range of 4 000–400 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans per sample. Spectral data processing was performed using OMNIC software. The amide I band (1 700–1 600 cm^{-1}) was selected for second derivative calculation and Fourier self-deconvolution, followed by Gaussian and Lorentzian curve fitting. The relative content of each protein secondary structure in the samples was calculated based on the peak area of the fitted curves.

2.7.3 Fluorescence spectroscopy determination

The fluorescence spectrum of the MP sample solution was measured according to the method of Yuan et al.[26] with minor modifications. The MP concentration was adjusted to 1.0 mg/mL. Fluorescence measurements were performed using a fluorescence spectrophotometer (Qubit Flex, Thermo Fisher Scientific, Beijing, China). The excitation wavelength was set at 280 nm, and emission spectra were recorded from 300 to 450 nm. Both excitation and emission slit widths were set to 20 nm.

2.8 Statistical analysis

All measurements were performed in triplicate. Statistical significance analysis was conducted using IBM SPSS Statistics 22 software (IBM, USA). Experimental results were expressed as mean ± standard deviation (SD). Data visualization was performed using Origin 2024 graphing software (OriginLab Co., USA).

3 Results and discussion

3.1 Meat batter gel characteristics

3.1.1 Color analysis

Color is one of the most important sensory attributes influencing consumer acceptance of meat products. As shown in Table 1, compared with the CK and PC groups, the L^* of low-salt chicken breast batters gels significantly decreased with increasing EY addition ($P < 0.05$), reaching the minimum value of 77.94 ± 0.47 in the 1.0% EY group. This decrease may be attributed to the high protein and lipid content in EY, which promotes protein-lipid interactions and Maillard reactions during thermal processing, thereby accelerating the formation of pigmented compounds[27]. The a^* values were significantly lower in both the 1.0% EY and PC groups ($P < 0.05$). In contrast, the b^* values increased progressively with increasing EY concentration ($P < 0.05$), reaching the highest level in the 1.0% EY group. This increase can be explained by the presence of natural fat-soluble yellow pigments in EY, primarily composed of xanthophylls[28]. These pigments were uniformly dispersed within the meat batter system, leading to a significant enhancement in product yellowness.

Table 1 Effect of different EY addition levels on color difference of chicken breast meat batters gels.

Group	L^*	a^*	b^*
CK	86.81 ± 0.19^{a}	3.30 ± 0.04^{b}	11.01 ± 0.03^{d}
0.6% EY	84.95 ± 0.78^{b}	3.67 ± 0.02^{a}	11.70 ± 0.01^{c}
0.8% EY	78.40 ± 0.30^{c}	3.46 ± 0.29^{ab}	11.80 ± 0.00^{b}
1.0% EY	77.94 ± 0.47^{cd}	3.05 ± 0.01^{c}	13.83 ± 0.02^{a}
PC	85.59 ± 0.11^{b}	2.68 ± 0.01^{d}	10.59 ± 0.02^{e}

Note: Different lowercase letters in the same column indicate significant differences ($P < 0.05$).

3.1.2 TPA

Hardness, springiness, and chewiness are critical indicators for evaluating the textural properties of meat batter gels, directly reflecting the compactness of the gel network structure and the sensory quality of the final product[29]. As shown in Table 2, compared with the CK group, all low-salt experimental groups exhibited varying degrees of improvement in hardness, springiness, and chewiness. The hardness reached its maximum at the 1% EY addition level, which was significantly higher than that of the other low-salt groups ($P < 0.05$). Meanwhile, springiness also increased progressively with EY concentration ($P < 0.05$), peaking at 0.957 in the 1.0% EY group. Notably, this value was not significantly different from that of the PC group ($P > 0.05$), indicating that 1.0% EY supplementation could effectively compensate for the loss of springiness caused by salt reduction. These findings are consistent with the report by Lü et al.[30], demonstrating that EY addition effectively improves the textural quality of low-salt meat batter gels.

Table 2 Effects of different EY addition levels on the texture of chicken breast meat batter gels.

Group	Hardness (g)	Springiness	Chewiness (g)
CK	900.79 ± 8.57^{cd}	0.886 ± 0.015^{d}	641.09 ± 16.16^{c}
0.6% EY	957.34 ± 44.00^{c}	0.919 ± 0.025^{c}	737.60 ± 74.26^{c}
0.8% EY	822.95 ± 118.50^{d}	0.946 ± 0.003^{b}	675.86 ± 94.41^{c}
1.0% EY	1 059.12 ± 13.81^{b}	0.957 ± 0.008^{ab}	922.86 ± 32.77^{b}
PC	1 257.32 ± 34.95^{a}	0.975 ± 0.012^{a}	1 147.75 ± 38.27^{a}

Note: Different lowercase letters in the same column indicate significant differences ($P < 0.05$).

The chewiness followed a similar trend to that of hardness and springiness, reaching its maximum in the 1.0% EY group. This is consistent with its physical meaning, as chewiness is essentially a

comprehensive oral texture parameter determined by both hardness and springiness. EY is rich in high-quality proteins and lipid bioactive components, which participate in the conformational rearrangement and intermolecular cross-linking of MP, effectively occupy the internal pore structures of the gel network, and gel network and restrict the disordered migration and subsequent loss of water, concurrently, it induces protein molecules to form a more uniformly distributed and compact 3D network structure[31], this densified and more stable network structure not only enhances gel elasticity but also further augments its mechanical strength, which ultimately translates into simultaneous improvements in hardness, elasticity, and chewiness at the macroscopic level.

3.1.3 Water retention properties analysis

Cooking loss can intuitively reflect the effect of different EY additions on the WHC of meat batter. As shown in Figure 1A, with the addition of EY, the cooking loss gradually decreased. When the EY addition reached 1.0%, the cooking loss significantly decreased to 9.02% ($P < 0.05$), which was the closest to that of the PC group. This may be because EY is rich in protein and lipid components. When EY is added to low-salt meat batter, its fat droplets fill the gaps in the protein network structure, thereby effectively preventing water loss[32]. Concurrently, EY proteins interact intermolecularly with meat batter MPs under heating conditions, promote protein cross-linking and rearrangement, and form a more compact 3D gel network during the cooking process. This enhances the binding capacity of the system for free water, ultimately reducing the cooking loss rate.

WHC is an important indicator for evaluating the quality of meat products. As shown in Figure 1B, with the addition of EY, the WHC increased significantly and reached a peak at the 1.0% EY addition level, with its over all value comparable to that of the PC group. This indicates that EY can effectively bind water in the meat batter and system and maintain the water content in the batter, which is consistent with the findings of Leng et al.[33]. The incorporation of EY simultaneously enhances both protein–water and protein–protein interactions, thereby facilitating the formation of a compact and dense gel network. This significantly improves the binding and entrapment capacity for free water, reduces water loss, and ultimately leads to a marked improvement in the WHC of meat batter gels.

LF-NMR can detect the water composition and distribution in meat batter gels, primarily reflected by the T_2. As shown in Figure 1C, three peaks appeared in the T_2 spectrum within the T_2 distribution of 0.01–10 000 ms, where T_{21} (0.1–10 ms) represents bound water, T_{22} (20–200 ms) represents immobilized water, and T_{23} (300–3 000 ms) represents free water. Immobilized water is associated with the MP structure[34]. Higher proportions of immobilized water and bound water indicate a more stable network structure of the meat batter gel. Prolonged T_2 suggests difficulty in retaining water, leading to water loss[35]. As shown in Table 3, with the addition of EY, the relaxation peak of T_{21} first shifted toward a longer T_2, and then shifted to a shorter T_2 at 1.0% EY addition. This may be attributed to that the addition of EY enhanced the polarity of protein molecular chains, promoted the formation of weak water-binding sites, and thus increased the water migration activity[36]. Furthermore, with the addition of 1% EY, the relaxation peaks of T_{21}, T_{22} and T_{23} all shifted to significantly shorter T_2 ($P < 0.05$), and T_{22} reached its minimum value at 1.0% EY addition level. This change indicates that an appropriate amount of EY can promote the tight binding between water molecules and protein molecules, reduce the mobility and freedom of water molecules, facilitate the construction of a more compact and stable gel network structure, and enhance the water binding and entrapment capacity of the system. These findings are in good agreement with the aforementioned results of WHC and cooking loss rate.

3.1.4 Dynamic rheological properties

The storage modulus (G') is an important parameter for characterizing the textural properties of food. As shown in Figure 2A, with increasing temperature, the proteins in chicken

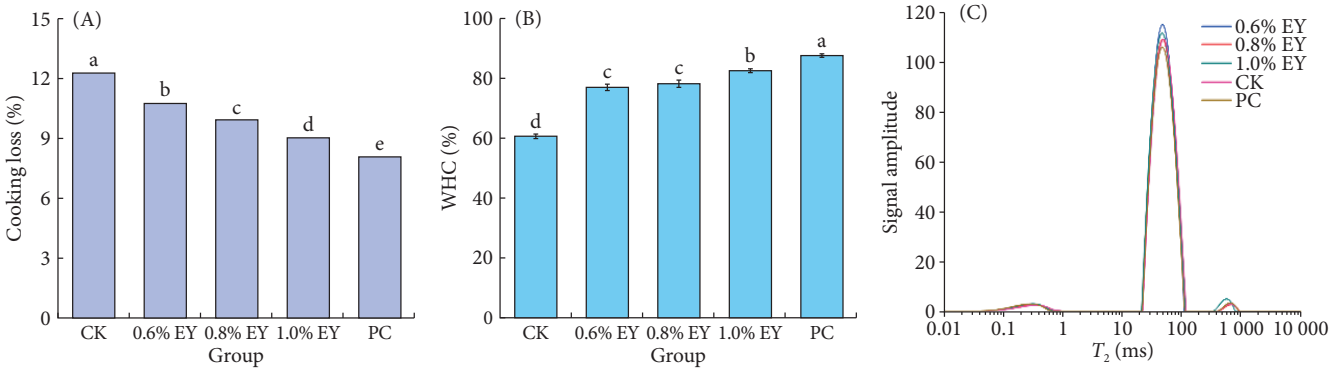

Figure 1 Effect of different EY addition levels on water retention properties of meat batter gels. (A) Cooking loss; (B) WHC; (C) T_2. Different lowercase letters indicate significant differences ($P < 0.05$).

Table 3 Effect of different EY addition on LF-NMR T_2 (ms) of low-salt chicken breast minced meat gels.

Group	T_{21}	T_{22}	T_{23}
CK	0.309 ± 0.007^{d}	53.481 ± 0.610^{a}	939.617 ± 28.500^{c}
0.6% EY	0.339 ± 0.010^{c}	53.481 ± 0.610^{a}	1 581.619 ± 65.630^{a}
0.8% EY	0.375 ± 0.010^{b}	53.481 ± 0.610^{a}	1 093.016 ± 0.000^{b}
1.0% EY	0.331 ± 0.010^{cd}	48.129 ± 0.000^{c}	741.890 ± 44.350^{d}
PC	0.419 ± 0.020^{a}	51.409 ± 0.590^{b}	658.160 ± 7.530^{e}

Note: Different lowercase letters in the same column indicate significant differences ($P < 0.05$).

breast batter underwent thermal denaturation, leading to gel formation. A higher G' value indicates greater gel strength[37]. In the temperature range of 20–40 °C, the G' value remained relatively stable with increasing temperature. During this stage, thermal expansion of MPs and cross-linking of myosin heads resulted in a relatively weak gel network structure[38]. In the range of 40–60 °C, G' showed a significant upward trend. This increase may be attributed to further temperature elevation inducing denaturation of myosin tails, altering protein conformation and enhancing intermolecular interactions, which disrupted the initially formed weak network and markedly promoted the gelation process. Notably, during this stage, the sample with 1.0% EY addition exhibited a faster increase rate of G' and consistently maintained higher values than the PC group, indicating that appropriate EY addition facilitated protein cross-linking and network strengthening. Finally, in the range of 60–80 °C, the G' value continued to increase with rising temperature, reaching the highest level in the 1.0% EY group. This dynamic change revealed that EY addition significantly enhanced the elasticity of meat batter during heating, with 1.0% EY addition being more favorable for increasing G', which corroborated the elasticity results. As shown in Figure 2B, The loss modulus (G'') curve exhibited a similar trend to that of G'. Throughout the heat-induced gelation process, G'' values remained lower than G', indicating that the protein gel exhibited elastic properties.

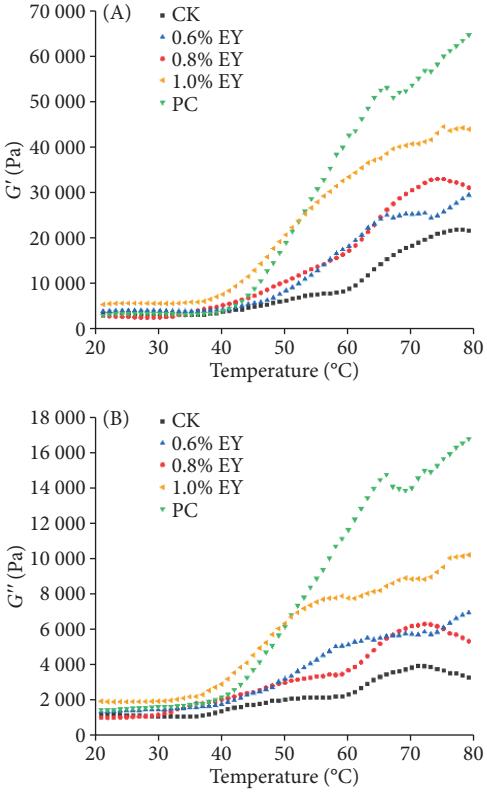

Figure 2 Effect of different EY addition on the dynamic rheological behavior of chicken breast mince meat batter. (A) G'; (B) G''.

3.2 Microscopic morphology

As shown in Figure 3A, the CK group exhibited numerous cavities and an incompact structure. This may be attributed to the low ionic strength of the system under low-salt conditions, which resulted in insufficient electrostatic shielding effect of sodium ions on MP. The inadequate sodium ion concentration reduced the number of ionic cross-linking sites, compromised the integrity of the gel network, and restricted protein solubilization and intermolecular cross-linking. Consequently, the system failed to form a continuous and intact 3D network, ultimately leading to the formation of a large number of pores[39]. After the addition of EY, the number of internal cavities in the meat batter gels decreased significantly, and the gel network structure became dense and uniformly distributed. When the EY addition level reached 1%, the compactness and structural regularity of the gels had approached that of the PC group. The proteins and bioactive components in EY can undergo intermolecular association and interaction with MP, guide the ordered aggregation and cross-linking of proteins, effectively fill the network gaps and reduce the pore size, thereby constructing a more continuous and compact gel scaffold[40]. Consistent with the microstructural evolution observed in this study, Zhao et al.[10] previously showed that supplementation with exogenous proteins promotes the assembly of MPs into a highly ordered and compact gel network. This well-developed network effectively immobilizes free water and occludes the interstitial spaces, thereby improving the overall gel integrity. This finding is in good agreement with the results of texture analysis, WHC and cooking loss analysis.

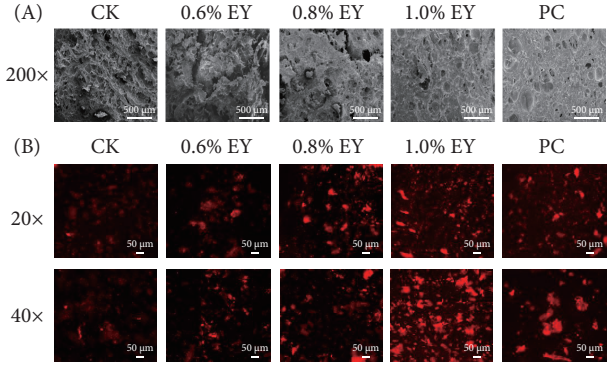

Figure 3 Effects of different EY additions on the microstructure of chicken breast meat batter gels. Images of (A) FE-SEM and (B) CLSM.

When MP binds with Nile blue, it appears red under specific excitation wavelengths, while dark regions correspond to protein-free aqueous areas[41]. As shown in Figure 3B, with the addition of EY, the area of the red regions increased, indicating a significant elevation in the proportion and content of protein-enriched regions. The addition of 1.0% EY significantly promoted the ordered aggregation of proteins and resulted in their more uniform distribution in the gel system, and this trend was more pronounced at 40× magnification. This may be attributed to the significant intermolecular interactions between EY proteins and MP, which can induce the spatial conformational unfolding of MP and expose more active sites capable of binding to Nile blue fluorescent dye, thereby leading to a marked increase in fluorescent response regions. Concurrently, the interactions between EY components and MP drive the formation of more ordered protein structures with more homogeneous distribution. Previous studies have demonstrated that gel structures with smaller pore sizes and more uniform network arrangement generally possess superior water binding capacity[42]. The present fluorescence observation results further corroborate from the perspectives of protein distribution and microscopic pores that an appropriate amount of EY can optimize protein aggregation morphology and spatial distribution, and reduce gel pore defects.

3.3 Physicochemical properties of MP

Particle size reflects the dimensions of particles in a solution system. As shown in Figure 4A, with the addition of EY, the particle size gradually increased. This may be attributed to the presence of components such as lecithin and cholesterol in EY, whose hydrophilic groups can serve as hydroxyl donors, facilitating the aggregation and cross-linking of MP, leading to larger protein aggregates and consequently increased particle size[43]. The particle size of the PC group was significantly higher than that of the other experimental groups ($P < 0.05$). This may be explained by the fact that changes in the average particle size of proteins are primarily caused by intramolecular cross-linking and aggregation. High salt concentration increases the ionic strength of the solution, shielding the surface charges of MP molecules and allowing them to approach each other more readily, resulting in aggregation and thus larger particle sizes[44].

Surface hydrophobicity reflects the hydrophobicity of proteins and the extent to which hydrophobic amino acid residues are exposed on the protein surface. Hydrophobic interactions are the main force maintaining the tertiary structure of proteins. As shown in Figure 4B, with the addition of EY, surface hydrophobicity significantly increased, reaching 164.2 μg at 1.0% EY addition ($P < 0.05$), which was close to that of the PC group. EY is abundant in lipid components, which can bind to the hydrophobic regions on the surface of MPs via hydrophobic affinity interactions, disrupt the hydrophobic regions on the surface of MPs via hydrophobic affinity interactions, disrupt the inherent hydrophilic–hydrophobic balance of protein molecules, and induce the unfolding of protein tertiary structures. A large number of hydrophobic amino acid residues originally buried within the molecular interior are extensively exposed, ultimately resulting in a significant increase in the surface hydrophobicity of the system[45]. Furthermore, the increased surface hydrophobicity further strengthens intermolecular hydrophobic association, promotes the mutual aggregation of MPs, and forms protein complexes or aggregates with more stable structures, which is consistent with the increase in particle size. Concurrently, the exposure of hydrophobic groups in turn leads to a more stable aqueous dispersion system, thereby enhancing the overall WHC[46].

Free thiol content can be used to evaluate the exposure of sulfhydryl groups on the protein surface. As shown in Figure 4C, after EY addition, the free thiol content significantly decreased ($P < 0.05$), which is consistent with the findings of Xu et al.[47] which reported similar results when adding mealworm powder to shrimp MP. This decrease may be attributed to interactions between EY proteins and MP, which alter protein conformation and expose sulfhydryl groups originally buried within the molecular interior. These exposed free thiol groups are susceptible to oxidation, forming disulfide bonds, thereby reducing the free thiol content and modifying the internal interactions within the protein system, ultimately. This alters the intramolecular interactions of proteins and thereby affects the dynamic properties of the mixed protein system[48]. Moreover, under heating conditions, protein cross-linking and aggregation are promoted, facilitating the formation of a more compact protein gel network[49]. The PC group exhibited the highest free thiol content, which may be because NaCl increases the exposure of reactive thiol sites, leading to an increase in free thiol content[50].

3.4 MP structure

The SDS-PAGE patterns of MP with different additions of EY are shown in Figure 5A. MP is primarily composed of myosin heavy chain (MHC, 220 kDa), paramyosin (100 kDa), actin (42 kDa), tropomyosin (35 kDa), and myosin light chain (MLC, 15–25 kDa)[51]. Among these components, the reduction in MHC is highly correlated with enhanced gel strength and texture properties[52]. Compared with the control groups, the experimental groups with EY addition exhibited narrower MHC bands. This phenomenon indicates that certain components in EY, such as lipids and proteins, have formed strong intermolecular cross-links with MPs, possibly via mechanisms including disulfide bonds and hydrophobic interactions, which facilitate the formation and strengthening of the protein network structure. Meanwhile, in the region below approximately 35 kDa molecular weight, the band distribution and intensity showed no significant changes. This indicates that under these conditions, low-molecular-weight proteins or peptides did not substantially participate in the cross-linking reaction, or that their content and reactivity in the system were relatively low. These further reveals that the addition of EY mainly affects the interactions of high-molecular-weight components in MPs, thereby specifically improving the textural properties of the gels.

FTIR spectroscopy can reflect changes in the secondary structure of proteins. As shown in Figure 5B, compared with the CK group, the EY-supplemented groups exhibited more pronounced characteristic absorption peaks near 3 274 cm^{-1}, corresponding to the amide A band associated with N–H stretching vibrations. This observation suggests that EY addition may enhance intermolecular or intramolecular hydrogen bonding interactions among proteins in the meat batter. Additionally, characteristic absorption peaks were detected at 2 946 cm^{-1}, which are attributed to aliphatic C–H

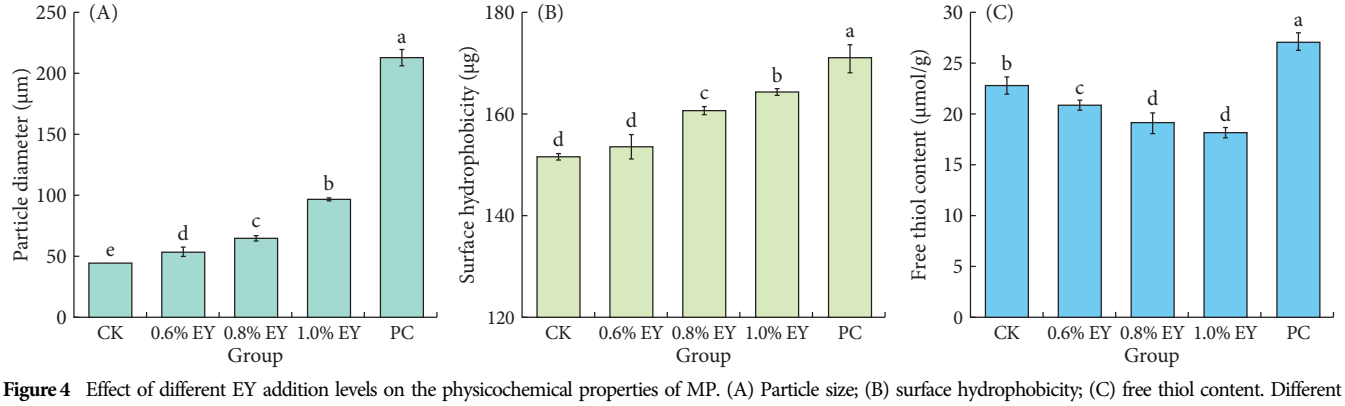

Figure 4 Effect of different EY addition levels on the physicochemical properties of MP. (A) Particle size; (B) surface hydrophobicity; (C) free thiol content. Different lowercase letters indicate significant differences ($P < 0.05$).

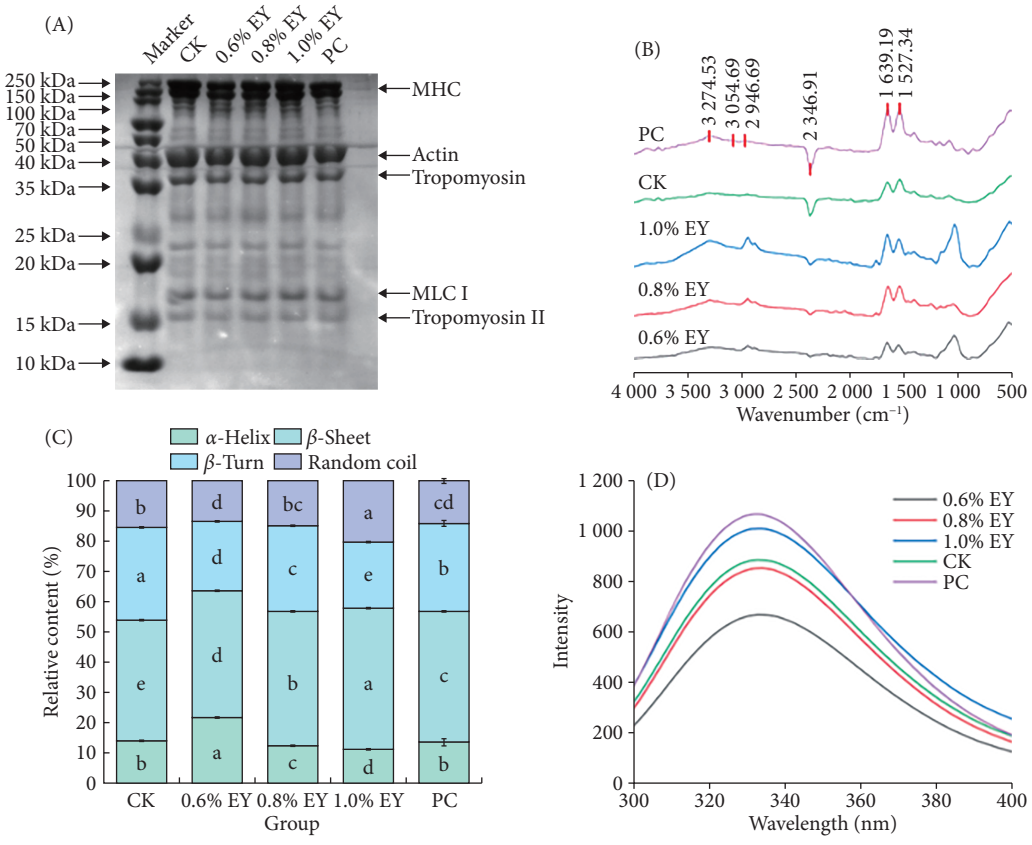

Figure 5 Effect of different EY addition levels on MP structure. (A) SDS-PAGE pattern; (B) FTIR spectra; (C) relative content of secondary structure; (D) fluorescence spectrum. Different lowercase letters indicate significant differences ($P < 0.05$).

stretching vibrations. Within the wavenumber range of 1 700–1 600 cm^{-1}, corresponding to the amide I band, characteristic absorption peaks were observed. After fitting analysis and quantitative calculation of the amide I band, the relative contents of protein secondary structures were obtained. As shown in Figure 5C, analysis and calculation of the relative contents of secondary structures revealed that the addition of EY significantly decreased the relative content of α-helix in MP while increasing that of β-sheet ($P < 0.05$). The α-helix relative content decreased from 22.1% to 11.4%, whereas the β-sheet relative content increased from 41.6% to 46.8%, indicating a transition of more α-helix to β-sheet in the secondary structure. Notably, the β-sheet relative content in the 1% EY group was significantly higher than that in the PC group ($P < 0.05$). This conformational change is considered more conducive to the formation of an ordered and tightly packed gel structure, thereby enhancing gel strength[53].

Intrinsic fluorescence spectroscopy is commonly used to characterize changes in the tertiary structure of proteins and can provide insights into conformational alterations of proteins. As shown in Figure 5D, in the low-salt system, the maximum fluorescence emission wavelength of MP was near 333 nm. With the increasing of EY addition, the maximum fluorescence emission wavelength shifted to around 334 nm, with a red shift of 1 nm, indicating the conformational change of proteins. This phenomenon may be attributed to the disruption of the original hydrophobic interaction balance of the protein in the low-salt system upon EY addition, accompanied by increased hydrophobicity, which induced moderate unfolding of the hydrophobic structure of the proteins. This conformational change gradually exposed fluorescent groups such as tryptophan residues[54]. Meanwhile, hydrophobic interactions between EY components and proteins further optimized the hydrophobic microenvironment surrounding the fluorescent groups, reducing the fluorescence quenching effect caused by polar solvent molecules, ultimately leading to a significant enhancement in the fluorescence intensity of the system[55].

4 Conclusion

This study investigated the effect of EY addition on the quality improvement of low-salt chicken breast meat batter and its underlying mechanism. The results demonstrated that EY addition effectively improved the gel quality of chicken breast meat batter, promoted the tight binding between water and proteins within the gel, and resulted in a more compact and uniform gel network structure. Furthermore, EY addition facilitated the unfolding of MP structure, leading to the exposure of hydrophobic groups and promoting the aggregation of complex protein molecules. This was accompanied by a decrease in free sulfhydryl content and an increase in disulfide bond formation, thereby enhancing cross-linking between protein molecules, which was substantiated by SDS-PAGE showing dense cross-linking patterns. FTIR spectra revealed a transition from α-helices to β-sheets in the protein secondary structure, while CLSM demonstrated protein aggregation, collectively contributing to the formation of a more compact protein network structure. This study elucidates the mechanism by which EY improves the gel quality of chicken breast meat batter and its effects on myofibrillar proteins, providing a theoretical basis for the application of EY in the development of low-salt chicken breast meat products. Nevertheless, several

limitations still exist in the present study. Specifically, the synergistic effects of complex excipients and the flavor profiles of the final product have not been systematically investigated. Accordingly, future research efforts will focus on optimizing the compounding formulations with other excipients and systematically characterizing the flavor properties of the product, so as to further facilitate its industrial transformation and practical application.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Duo Shang: Conceptualization, methodology, investigation, formal analysis, data curation, writing-original draft, writing-review & editing. Chao Ai and Hui Teng: Methodology, validation, writing-review & editing. Lei Chen: Methodology, validation, supervision, writing-review & editing. Yingmei Wu and Xin Li: Formal analysis, writing-review & editing. Qun Huang: Conceptualization, methodology, writing-review & editing, supervision, project administration, funding acquisition.

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