Effects of ethanol treatment on rheological and gel properties of chicken myofibrillar protein

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ABSTRACT
Culinary wine is often used to marinate meat products before cooking to remove unfavorable flavors. However, the effects of ethanol as the main component in culinary wine on the main proteins in meat products are not clear. Therefore, we studied the effects of ethanol treatment on the microstructure, rheological, and gelation properties of chicken myofibrillar protein (CMP) and the reasons for these changes. The viscosity coefficient of CMP solutions firstly decreased; then increased with increasing ethanol and reached the minimum at 0.9% ethanol. The gel strength, water holding capacity, and storage modulus of CMP gels reached the maximum at 0.9% ethanol treatment, and the scanning electron micrographs showed a more compact structure. The presence of ethanol decreased the dielectric constant of solution, resulting the changes of surface hydrophobicity and sulfhydryl groups of CMP solutions and gels. These changes contributed the evolution of the properties we mentioned above.

1. Introduction

Chinese rice wine is a traditional alcoholic beverage and widely used as culinary wine in South China (Lu et al., 2015; Yongmei et al., 2007). The traditional Chinese rice wine is made by glutinous rice and basically involves the following steps: the soaked rice is smelted and fermented after steamed, then sterilized, and finally stored and aged (Jin, Zhou, & Xu, 2017; Li, Jin, & Xu, 2013; Xu et al., 2016). Chinese rice wine is low-alcoholic that contains about 17% ethanol and a lot of amino acids, organic acids, and flavor substance (Lv et al., 2018; Xu et al., 2014; Yang, Xia, Wang, Yu, & Ai, 2017). Culinary wine made from Chinese rice wine has a wide range of applications in meat products. This was due to the unfavorable favors of the aldehydes, ketones, thioethers, and trimethylamines contained in the original meat. The addition of culinary wine allows these substances to be dissolved in ethanol and then evaporates with ethanol when the cooking temperature increased. The other substances in culinary wine also enhance the flavors of cooked meat products.

The impact of red wine as a culinary wine on meat products has been reported (Blackhurst, Pietersen, Neill, & Marais, 2011; Park et al., 2011), but the impact of Chinese culinary wine on the quality of meat products was rarely reported. Chinese culinary wine contains a large amount of organic acids, and the addition of a suitable amount of organic acid reduced the TBARs values and increased the brightness during the meat storage (Kang, Jang, Lee, Min, & Lee, 2002). The effects of ethanol, as the main ingredient of culinary wine and accounts for about 15% of the total composition, on the quality of meat products were not clear. Previous studies have shown that ethanol exhibited strong influence on the functional properties of protein, including

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altering the protein interactions, changing the secondary structures of protein, making the protein denaturation, and even reducing the protein denaturation temperature (Lin, Wei, Li, & Wang, 2004; Lin, Wu, & Liang, 1995; Nikolaids & Moschakis, 2018; van Koningsveld et al., 2002).

Chicken meat is the main meat product in the food market because of its low fat, high protein content, affordability, variety, and diversity. From the survey of National Chicken Council, Washington, DC, it was found that consumers buying chicken meal or snacks from markets and foodservice establishments reached 87% and 72% in 2 weeks, respectively (Caspermeyer, 2017); they expected the meat consumption of per capita will reach to the peak at 2018. Myofibrillar protein is a major protein (40–60%) in chicken meat, which determines the quality of meat products to a large extent (Xiong, 2014). There were many studies that proved the effects of different treatment conditions on the properties of myofibrillar proteins and myofibrillar protein gels to represent the impact of different treatments on the quality of meat products, such as high pressure (Zhang, Yang, Zhou, Zhang, & Wang, 2017), ultrasound (Wang, Yang, Tang, Ni, & Zhou, 2017), irradiation (Choia et al., 2015), and insoluble dietary fiber (Zhuang et al., 2017).

Raman spectroscopy was widely used to study the changes in the chemical forces of protein solutions and protein gels (Feng et al., 2018; Liu, Zhao, Xie, & Xiong, 2011; Wang et al., 2017; Zhang et al., 2017). The intensity ratio of the peaks at 850 and 830 cm\(^{-1}\) (I\(_{850}/I_{830}\)) in the Raman spectrum reflects that the hydroxyl groups (–OH) on the tyrosine (Tyr) benzene ring combined with the solubility of the protein to form hydrogen bonds (exposed) or the protein to form hydrogen bonds (buried) (Hildebrandt et al., 1988; Li-Chan, Nakai, & Hirotusuka, 1994). The amide I band in Raman spectrum of myofibrillar protein is around 1600–16,700 cm\(^{-1}\), and the amide I band is closely related to the secondary structure of protein (Alix, Pedanou, & Berjot, 1988; Zhang et al., 2017).

In this study, we used rheometers, texture analyzers, and Raman spectroscopy to study the effect of different volume fractions of 15% ethanol on the structures and properties of chicken myofibrillar protein (CMP); this research will provide a theoretical supplement for the impact of the main ingredients of culinary wine on meat products.

2. Materials and methods

2.1. Materials

Six-week old Arbor Acres (AA) chickens, 15 cocks and hens, were purchased from Qinglongshan Chicken Farm, Nanjing (China). After the slaughter, the chicken breasts were stored at −18°C and used within 1 month. Bovine serum albumin (BSA) and ethanol (chromatographic purity, 99.9%) were obtained from Sinophrarm Chemical Reagent Co., Ltd (Shanghai, China). All other chemicals were of analytical grade.

2.2. Extraction of CMP and preparation of CMP gels

The extraction and purification of CMP were performed according to the method of Zhang, Yang, Tang, Chen, and You (2015). The protein concentration of CMP was determined by the Biuret method using BSA as standard curve and used in 24 h.

Appropriate CMP was mixed with different volume of 15% ethanol, then dissolved them with phosphate buffer (0.6 mol/L KCl, 0.01 mol/L KH\(_{2}PO_{4}\), pH 6.0), and made the total volume of solution to 10 mL. The final CMP concentrations were 60, 30, 1, 0.5, 0.25, and 0.125 mg/mL, and the final ethanol volume fractions were 0.0%, 0.3%, 0.6%, 0.9%, 1.2%, and 1.5%. To simulate the process of treating meat products by using Chinese culinary wine before cooking, we placed the mixed solutions at room temperature for 30 min. The preparation of CMP gels is as follows: 5 mL CMP solutions (30 mg/mL) were added into 7-mL capped plastic tubes and heated in water bath from 20°C to 65°C at the rate of 1°C/min and kept at 65°C for 20 min. Since the optimal gel formation condition for proteins is 60–70°C, higher temperature is detrimental to gel formation, so we chose 65°C as the gel forming condition. Following the heating procedure, the tubes were cooled at room temperature for 1 h and then kept for 8 h at 4°C for morphological structure and textural measurement. The CMP with concentration of 60 mg/mL was used for Raman spectrum testing, 30 mg/mL for gel preparation and rheological measurement, 1 mg/mL for sulfhydryl groups content determination, 1, 0.5, 0.25, and 0.125 mg/mL for surface hydrophobicity measurement.

2.3. Surface hydrophobicity

The surface hydrophobicity (S\(_{0}\)-ANS) of CMP solutions and CMP gels was measured by ANS fluorescence probe and performed according to the method of Zhou, Yang, Wang, Wei, and Li (2019) with a fluorescence photometer (F-7000, Hitachi Corp., Tokyo, Japan). The peak values were recorded, then protein concentrations were plotted as abscissa, fluorescence intensity was plotted as ordinate, and the initial slope of the straight line was marked as S\(_{0}\)-ANS.

2.4. Sulphydryl groups (SH)

Total SH and free SH group contents of CMP solutions and CMP gels were measured by the method of Liu et al. (2011) with a UV-vis spectrophotometer (U-3900, Hitachi Corp., Japan). SH contents were calculated by the method described elsewhere (Zhao et al., 2016).

2.5. Raman spectroscopy

The secondary structures and hydrogen bonds of CMP solutions and CMP gels were measured by Raman spectroscopy using a Jobin Yvon Labram HR800 spectrometer (Horiba/Jobin, Yvon, Longjumeau, France). Samples were placed on a glass slide during measurement, and test conditions were monitored by the method of Zhang et al. (2017). Protein secondary structures were determined by the method of Alix et al. (1988), and the results were expressed as the percentage of α-helix, β-sheet, β-turn, and random coil.

2.6. Static rheological measurement

Static rheological measurement of CMP solutions (30 mg/mL) was measured by a rotational rheometer (MCR302, AntonPaar, Graz, Austria) with 50-mm parallel plate (PP 50) followed by the method of Hu et al. (2013). The following parameters were monitored: strain, 2%; constant frequency, 0.1 Hz; gap, 0.5 mm; shear rate, 100–1000 s\(^{-1}\). The shear
stresses were recorded, and the value of flow index \((n)\) and viscosity coefficients \((k)\) were calculated by power law equation (Eq.1):

\[ \tau = k \delta^n \]  

(1)

In which, \(\tau\) represents shear stress (Pa), \(\delta\) represents shear rate \((s^{-1})\), \(n\) represents flow index and \(k\) represents consistency index \((Pa\cdot s^n)\).

2.7. Dynamic rheological measurement

The gelation property of CMP solutions (30 mg/mL) during heating stage was determined by rotational rheometer (MCR302, AntonPaar, Graz, Austria) with 50 mm parallel plate (PP 50). The following parameters were monitored: strain, 2%; constant frequency, 0.1 Hz; gap, 0.5 mm. The temperature was increased from 20°C to 80°C at the rate of 2°C/min, storage modulus \((G'\) were recorded automatically.

2.8. Morphological structure measurement

Following fixation with 2.5% glutaraldehyde, CMP gels were diced and washed with phosphate buffer solution (0.1 mol/L, pH 7.0), and then rinsed with ethanol at different gradients to remove water. Then CMP gels were freeze-dried by a Freeze dryer (Free Zone 4.5 Plus, Labconco, Kansas City, MO). The morphological structural observations were studied using scanning electron microscopy (SEM) (TM 3000, Hitachi Corp., Tokyo, Japan) with the conditions of accelerating voltage 15 kV and 1500 magnification.

2.9. Gel strength

CMP gels’ strength was assessed using a texture analyzer (TA. XT. Plus, Stable Micro Systems, Surrey, UK) attached to cylinder stainless steel probe (P6, 6 mm in diameter). The following parameters were monitored: pre-test speed, 5 mm/s; test speed, 1 mm/s; post-test speed, 5.0 mm/s; trigger type, 5.0 g auto fore; and 5 mm of the distance before the test. Tests were repeated four times at room temperature.

2.10. Water holding capacity (WHC)

The WHC of CMP gels was determined by centrifugal method. Briefly, CMP gels were centrifuged at 4°C, 10,000 \(\times g\) for 10 min; WHC was calculated using the following equation (Eq.2):

\[ \text{WHC}\% = \frac{(m_2 - m)}{(m_1 - m)} \times 100\% \]  

(2)

In which, \(m\) represents empty tube weight, \(m_1\) is the total weight of gel + tube and \(m_2\) is the total weight of tube + CMP gel following centrifuge.

2.11. Statistical analysis

SPSS software (SPSS Inc., Ver.19, Chicago, IL) was used for data analysis, and all the values were presented as mean \pm standard deviation (mean \pm SD). The variance analysis was used to determine the significance between the data; significant differences were measured using the Duncan multiple test at \(p < 0.05\) level.

3. Results

3.1. Surface hydrophobicity

Figure 1 shows the surface hydrophobicity of CMP solutions and CMP gels with different volume fractions ethanol treatment. Compared with control, the \(S_0\)-ANS values of ethanol-treated CMP solutions were significantly \((p < 0.05)\) increased as ethanol concentration increased and then kept no
significant \((p > 0.05)\) changes when the ethanol concentration exceeds 0.9%. Compared with control, the \(S_0\)-ANS of CMP gels decreased with the increasing ethanol volume fractions, and reached minimum when the ethanol concentration was 0.9%, and then significantly increased \((p < 0.05)\) as the ethanol content further increased. The \(S_0\)-ANS of CMP gels was obviously higher than the \(S_0\)-ANS of CMP solutions.

### 3.2. Sulfhydryl groups

Figure 2a and b shows the total and free SH groups of CMP solutions and CMP gels. The total SH groups increased significantly \((p < 0.05)\) with the increasing ethanol concentration, and then decreased significantly \((p < 0.05)\) when the free SH groups increased \((p < 0.05)\) along with ethanol concentration. The total SH groups of CMP gels \((10.66 \, \mu \text{mol/g})\) were lower than total SH groups of CMP solutions \((13.67 \, \mu \text{mol/g})\) for the untreated sample. Compared with untreated sample (gels), the total SH groups significantly \((p < 0.05)\) decreased and showed no significant \((p > 0.05)\) change when ethanol concentration exceeded 0.9%, while the free SH groups significantly \((p < 0.05)\) increased and exhibited no significant \((p > 0.05)\) change when ethanol concentration exceeded 0.9%. Besides, with 0.9% ethanol treatment, the degree of total SH groups decreased \((2.0956 \, \mu \text{mol/g})\) was higher than that of free SH groups increased \((1.7463 \, \mu \text{mol/g})\).

### 3.3. Hydrogen bonds

Figure 3 shows the normalized intensity of \(I_{850}/I_{830}\) conjugate bimodal ration. The values of solutions and gels \(I_{850}/I_{830}\) gradually increased with the increasing ethanol.

![Figure 2](image1.png)

**Figure 2.** Sulfhydryl (SH) contents of CMP solutions (a) and CMP gels (b) with different fractions ethanol treatment (mean ± SD, \(n = 3\)).

![Figure 3](image2.png)

**Figure 3.** The normalized intensity in \(I_{850}/I_{830}\) of CMP gels and CMP solutions (mean ± SD, \(n = 3\)).
The values of CMP solutions were among $\beta$ for random coil, and $1670$ cm$^{-1}$ for $\alpha$-helix, $1660$–$1670$ cm$^{-1}$ for random coil, and $1670$–$1680$ cm$^{-1}$ for $\beta$-sheet (Wang et al., 2017). Table 1 shows the proportion of the secondary structure of CMP solutions and CMP gels. With $0.9\%$ ethanol treatment, the proportions of $\alpha$-helix of CMP solutions gradually decreased from $44.33\%$ to $31.46\%$ and $\beta$-sheet reduced from $26.70\%$ to $23.55\%$, $\beta$-turn increased from $24.49\%$ to $37.44\%$ and no significant changes of random coil were observed. The trends of $\alpha$-helix and $\beta$-sheet increasing and $\beta$-turn decreasing were found when the ethanol fractions exceeded $0.9\%$. The $\alpha$-helix was the major secondary structure of CMP solutions ($44.33\%$), and $\beta$-sheet was the major secondary structure of CMP gels ($33.50\%$). The $\alpha$-helix and $\beta$-turn of CMP gels showed a decline trend first and then showed an increase trend when ethanol contents are more than $0.9\%$, while the opposite trend was observed in $\beta$-sheet.

### 3.4. Secondary structure

In this paper, the amide band focused on $1640$–$1645$ cm$^{-1}$ and $1680$–$1690$ cm$^{-1}$ for $\beta$-turn, $1645$–$1660$ cm$^{-1}$ for $\alpha$-helix, $1660$–$1670$ cm$^{-1}$ for random coil, and $1670$–$1680$ cm$^{-1}$ for $\beta$-sheet (Wang et al., 2017). Table 1 shows the proportion of the secondary structure of CMP solutions and CMP gels. With $0.9\%$ ethanol treatment, the proportions of $\alpha$-helix of CMP solutions gradually decreased from $44.33\%$ to $31.46\%$ and $\beta$-sheet reduced from $26.70\%$ to $23.55\%$, $\beta$-turn increased from $24.49\%$ to $37.44\%$ and no significant changes of random coil were observed. The trends of $\alpha$-helix and $\beta$-sheet increasing and $\beta$-turn decreasing were found when the ethanol fractions exceeded $0.9\%$. The $\alpha$-helix was the major secondary structure of CMP solutions ($44.33\%$), and $\beta$-sheet was the major secondary structure of CMP gels ($33.50\%$). The $\alpha$-helix and $\beta$-turn of CMP gels showed a decline trend first and then showed an increase trend when ethanol contents are more than $0.9\%$, while the opposite trend was observed in $\beta$-sheet.

### 3.5. Static rheological

Static rheological behavior is an important property for protein solutions resisting against the external force. Figure 4a shows the effect of ethanol addition on the flow curves of CMP solutions. Shear stress decreased firstly and then increased with the increasing ethanol concentration and reached minimum at $0.9\%$ ethanol volume fractions. Shear stress followed by shear rate change was fitted by power law equation, the rheology curves of all samples matched well with the power law equation (Table 2) as illustrated by high regression coefficients ($R^2 > 0.95$). The flow index ($n$) of all samples showed no significant changes. Viscosity coefficient ($K$) firstly decreased and then increased with the increasing ethanol concentration and reached the minimum at $0.9\%$ ethanol.

| Ethanol addition | $N_{\text{exposed}}$ | $N_{\text{buried}}$ | $\alpha$-helix | Random coil | $\beta$-sheet | $\beta$-turn |
|------------------|---------------------|---------------------|---------------|-------------|--------------|-------------|
| CMP solutions    | 0.0%                | 0.6492 ± 0.0081$^d$ | 0.3508 ± 0.0081$^a$ | 44.33 ± 0.38$^b$ | 7.48 ± 0.30$^c$ | 26.70 ± 0.32$^d$ | 24.49 ± 0.16$^e$ |
|                  | 0.3%                | 0.6622 ± 0.0061$^c$ | 0.3378 ± 0.0061$^a$ | 39.77 ± 0.47$^c$ | 7.49 ± 0.17$^c$ | 25.41 ± 0.44$^d$ | 27.33 ± 0.45$^e$ |
|                  | 0.6%                | 0.6807 ± 0.0100$^b$ | 0.3193 ± 0.0100$^a$ | 37.58 ± 0.36$^d$ | 7.32 ± 0.24$^c$ | 24.57 ± 0.14$^c$ | 30.53 ± 0.28$^e$ |
|                  | 0.9%                | 0.6965 ± 0.0047$^c$ | 0.3035 ± 0.0047$^a$ | 31.46 ± 0.75$^d$ | 7.55 ± 0.39$^c$ | 23.55 ± 0.34$^d$ | 37.44 ± 0.74$^e$ |
|                  | 1.2%                | 0.6987 ± 0.0037$^d$ | 0.3013 ± 0.0037$^a$ | 44.42 ± 0.50$^c$ | 7.50 ± 0.08$^c$ | 24.53 ± 0.27$^c$ | 23.55 ± 0.74$^d$ |
|                  | 1.5%                | 0.7089 ± 0.0091$^a$ | 0.2911 ± 0.0091$^a$ | 45.56 ± 0.48$^c$ | 7.37 ± 0.19$^c$ | 25.45 ± 0.38$^d$ | 26.12 ± 0.58$^e$ |
|                  | 0.0%                | 0.6679 ± 0.0042$^b$ | 0.3211 ± 0.0042$^b$ | 27.10 ± 0.25$^d$ | 7.31 ± 0.27$^c$ | 33.50 ± 0.43$^d$ | 32.09 ± 0.69$^b$ |
|                  | 0.3%                | 0.6819 ± 0.0025$^c$ | 0.3181 ± 0.0025$^b$ | 26.21 ± 0.21$^b$ | 7.15 ± 0.26$^c$ | 36.81 ± 0.23$^d$ | 29.83 ± 0.21$^e$ |
|                  | 0.6%                | 0.6815 ± 0.0014$^d$ | 0.3149 ± 0.0014$^d$ | 23.33 ± 0.30$^d$ | 6.98 ± 0.15$^c$ | 40.13 ± 0.26$^c$ | 29.56 ± 0.49$^b$ |
|                  | 0.9%                | 0.7058 ± 0.0055$^b$ | 0.2942 ± 0.0055$^b$ | 20.85 ± 0.24$^a$ | 7.21 ± 0.25$^b$ | 43.80 ± 0.20$^a$ | 28.24 ± 0.22$^c$ |
|                  | 1.2%                | 0.7123 ± 0.0023$^a$ | 0.2877 ± 0.0023$^a$ | 22.55 ± 0.35$^b$ | 7.21 ± 0.13$^b$ | 40.54 ± 0.32$^a$ | 29.70 ± 0.63$^b$ |
|                  | 1.5%                | 0.7154 ± 0.0012$^a$ | 0.2846 ± 0.0012$^a$ | 23.64 ± 0.33$^c$ | 7.21 ± 0.21$^b$ | 39.98 ± 0.27$^c$ | 29.26 ± 0.62$^c$ |

Different letters (a–e) indicate significant difference ($p < 0.05$) among the same kind of samples in treated with different ethanol volume fractions.

Las letras diferentes (a-e) indican una diferencia significativa ($p < 0.05$) entre el mismo tipo de muestras con el tratamiento con diferentes fracciones en volumen de etanol.
3.6. Dynamic rheological

Figure 4b shows the $G'$ values change by temperature increase. The $G'$ curves of treated samples were higher than untreated sample (nearly doubled). At the temperature between 20°C to 45°C, $G'$ values increased with increasing ethanol content then decreased when ethanol volume fractions more than 0.9%. At the temperature between 45°C and 55°C, where is the peak of $G'$ curves, the peak values of treated samples are almost twice as much as untreated group. When the temperature exceeded 55°C, the development of $G'$ values was similar with the $G'$ values between 20°C and 45°C.

3.7. SEM

Figure 5 shows the microstructure of all samples at 1500 magnifications. Compared with untreated sample (Figure 5a), the surface of ethanol treated samples showed typical honeycombs and more compact and dense structure when the ethanol concentration was low. However, large amount of agglomeration occurred when the amount of ethanol exceeded 0.9% (Figure 5e, f).

3.8. Gel strength and WHC

The gel strength and WHC of CMP gel were widely used to evaluate the quality of meat product (Rosenvold & Andersen, 2003; Zhuang et al., 2018). Gel strength and WHC values have a significant change ($p < 0.05$) with the addition of ethanol as illustrated in Figure 6. Gel strength increased firstly and then decreased with the increasing volume fractions of ethanol, and a maximum (1093.019 g*mm) appeared at the ethanol concentration of 0.9%. The similar trend was also found in WHC.

4. Discussion

4.1. Change in chemical forces of CMP solutions and CMP gels

The addition of ethanol reduces the dielectric constant of solution and increases the electrostatic interaction between (or inside) the protein molecules (Fennema & Owen, 1996). The increase in electrostatic repulsion inside the molecule causes the protein molecules to unfold and thereby exposes more hydrophobic groups. The exposed hydrophobic groups increased the $S_0$-ANS of CMP solutions (Figure 1). More exposed proteins led to the hydrogen bonds between protein molecules gradually transform into hydrogen bonds between proteins and solutions, thereby increased the values of $N_{\text{exposed}}$. Most free SH groups buried in the interior zone of molecules, making it difficult to combine with Ellman’s reagent. The addition of ethanol made buried SH groups exposed to the solution, and detected by Ellman’s reagent, thereby increased the free SH groups. The total SH groups showed a downward trend after the ethanol content exceeded 0.9%, this might be due to the condition that high loading of ethanol promotes the accumulation of protein molecules (Nikolaidis & Moschakis, 2018).

During heat-induced protein gel formation, the high-order protein structures are destroyed and hydrophobic groups are adequately exposed (Xiong, Blanchard, Ooizumi, & Ma, 2010), and leading to an obvious increase in $S_0$-ANS of proteins (Figure 1) and a higher $I_{850}/I_{830}$ values in CMP gels than CMP solutions. Heating caused a decrease in the total SH groups of CMP, and indicating the formation of disulfide bonds between proteins (Monahan, German, & Kinsella, 1995). Liu et al. (2011) studied the evolution of disulfide bonds in the heat-induced gelation of pork and fish meat, and revealed that more disulfide bonds formed after heating. The changes in the total SH and free SH groups of CMP gels indicated that the addition of ethanol facilitated the formation of disulfide bonds of CMP gel. These disulfide bonds can be used to the protein cross-linking and support
the gel structure between protein molecules, and thus enhance the protein gel strength (Broersen et al., 2006; Zhao et al., 2016).

The addition of ethanol increased the exposure of protein molecules (Table 1), and heating process caused serious aggregation of exposed proteins, and disulfide bonds formed between the exposed proteins (Figure 2b), finally resulting in the decreased in $S_0$-ANS of CMP gels. The increase in $S_0$-ANS when the ethanol fractions were more than 0.9% might be due to more proteins exposed (Table 1), and no more disulfide bonds generated (Figure 2b).

### 4.2. Secondary structure

It is well known that $\alpha$-helix is stabilized by hydrogen bonds in peptide chains and $\beta$-sheet stabilized by hydrogen bonds between peptide chains (Zhang et al., 2017). In this work, the exposed proteins (CMP solutions) were increased with ethanol treatment (Table 1), and the reduced hydrogen bonds in the peptide chains, resulting in the transformation of $\alpha$-helix into the $\beta$-turn. But some papers pointed that ethanol treatment increased $\alpha$-helix and decreased $\beta$-structure ($\beta$-turn and $\beta$-sheet) of protein (Lin et al., 2004; van Koningsveld et al., 2002). These differences might be due to that they used high concentrations of ethanol, which caused the denaturation of protein. The same trends, namely the $\alpha$-helix and $\beta$-sheet increasing and the $\beta$-turn decreasing, were found when the ethanol fractions exceeded 0.9%. These changes might be due to the dehydration and aggregation of protein molecules (Nikolaidis & Moschakis, 2018).

The changes in secondary structure of CMP gels and CMP solutions indicated that $\alpha$-helix gradually converted into $\beta$-sheet during CMP gel formation. The same trend was found in heating egg white protein (Seguchi et al., 2004). Hu, Xu, Gong, and Kuang (2005) also found an apparent convert of photosystem I in which the $\alpha$-helix is converted to $\beta$-sheet during 60°C to 80°C. The decrease of $\alpha$-helix represented an increase in unfolding protein molecules and a decrease in intermolecular hydrogen bonds in peptide chains. The increase in the $\beta$-sheet indicated an increase of aggregation between the protein molecules. The $\alpha$-helix converted into $\beta$-sheet means that protein molecules aggregated during heating. The changes in the secondary structure of CMP gels demonstrated that 0.9% ethanol addition was beneficial to the aggregation of CMP gel. When the ethanol concentration exceeded 0.9%, the aggregation of protein molecules was gradually destroyed.

### 4.3. Rheological properties

The exposure of hydrophobic groups and the increase of total SH groups were the main reasons that result in the decrease in $K$ values (Xia, Kong, Xiong, & Ren, 2010). The decrease in the apparent viscosity of CMP (0.0–0.9%) might be due to the decrease of ordered structure ($\alpha$-helix and $\beta$-sheet) mainly and the increase of total SH groups. When the ethanol volume fractions exceeded 0.9%, the increase of $K$ values was due to the increase of ordered structure and the decrease of total SH groups of CMP solutions. The lower apparent viscosity makes protein have better potential technological functionality (Krešić, Lelas, Jambrak, Herceg, & Brnčić, 2008) and the higher viscosity indicates the protein is difficult to swallow. The evolution of $K$ values suggested that the proper addition of ethanol amount (0.9%) reduced the apparent viscosity of CMP solutions.

All samples showed a slightly downward trend and then a sharply increased trend in the range of 45–50°C (Figure 4b). During this temperature, degeneration and aggregation of myosin heavy chains and actin leading to the increase of $G'$ and the preliminary protein network structure was formed (Youling L. Xiong & Blanchard, 1994). $G'$ sharply decreased with the increase of temperature (50–55°C), which was attributed to the light meromyosin denaturation leading to the increase in the fluidity of filament. When the temperature

![Figure 6. Gel strength and water holding capacity (WHC) of untreated and treated CMP gels (mean ± SD, n = 4). Figure 6. Fuerza del gel y capacidad de retención de agua (WHC) de geles CMP sin tratar y tratados (media ± DE, n = 4).](390_L.ZHOU ET AL.)
exceeded 60°C, the denaturation of myosin filament became irreversible and CMP heat-induced gel gradually formed (Egelandsdal & Samejima, 1986).

At the temperature between 20°C and 45°C, the addition of ethanol increased the exposure of amino acid residues (Table 1), and the exposed amino acid residues (myosin heavy chains and actin) gradually aggregated with the temperature increase, resulting in an increase of G* values. At the temperature between 45°C and 55°C, it might be due to the ethanol addition allowed the myosin heavy chains and actin fully exposed and CMP gel elasticity increased as the temperature increased. When the temperature exceeded 55°C, the change of G* values might be due to the change of gel surface hydrophobicity (Figure 1) and SH groups (Figure 2b). The change in the G* curve indicated that 0.9% ethanol treatment favors gel formation of CMP.

4.4. Gel properties

The microstructure of CMP gels was uniform and smooth when the ethanol concentration was lower than 0.9%, while the uneven and larger lumps structure was appeared when the ethanol concentration was higher than 0.9% (Figure 5). The gel strength and WHC of CMP gel reached a maximum at the 0.9% ethanol treatment. These changes indicated that 0.9% ethanol treatment favors the formation of a CMP network, resulting in increased gel strength and WHC. Zhang et al. (2017) studied the effect of high pressure on CMP, and found a dense and homogeneous gel network structure was conducive to the improvement of the gel properties. The same trend was also found by Tornberg (2005). As the above results show that, 0.9% ethanol added to MP solution allowed proper exposure of protein molecules, thereby increased MP gel strength and WHC.

5. Conclusions

We used different volume fractions of ethanol to treat CMP solutions for 30 min, and found viscosity coefficient values of CMP solutions treated with 0.9% ethanol reached a minimum, and flow index values showed no significant change. The decrease in the ordered structure of proteins and increase in total SH groups were the reasons for the decrease in viscosity coefficient values of CMP solutions. The gel strength and WHC reached a maximum at 0.9% ethanol treatment, and G* displayed the same trend. SEM of CMP gels with 0.6% and 0.9% ethanol treatment showed a dense and homogeneous gel network. According to experimental results, we speculated that the evolution of electrostatic repulsion inside the molecule caused by the addition of ethanol was the main reason for the change of surface hydrophobicity, SH, and hydrogen bonds of the CMP solutions and CMP gels, which further caused the changes in the physical properties of CMP solutions and CMP gels.

Disclosure statement

No potential conflict of interest was reported by the authors.

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