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Research Article

Enhancing beef mince gel strength and water-holding capacity: the concentration effect of sarcoplasmic protein

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Abstract: Sarcoplasmic protein (SP), a water-soluble fraction naturally lost during post-ripening and industrial thawing. This study revealed the concentration-effect mechanism of SP in regulating the gel properties of beef mince by controlling the addition amount of SP (0%–0.5%, *m*/*m*). Compared with the control group, the incorporation of 0.3% SP maximized gel strength and water-holding capacity (95.33%) while increasing cooking yield. Dynamic rheology confirmed a higher storage modulus, indicating an elastic-dominant network. Raman spectroscopy revealed a shift from α-helix to β-sheet, while scanning electron microscope observation showed a denser, more uniform microstructure with reduced pore size. The results of the chemical force analysis revealed a significant enhancement of hydrophobic interactions and disulfide bond formation, which collectively drove the elevation of β-sheet relative content in the protein structure. Excess SP (> 0.4%) formed loose aggregates that impeded myosin cross-linking and weakened the gel. Therefore, controlled SP re-addition can improve the beef minced gel property. This study provides a theoretical basis for the intensive processing and utilization of SP and the quality improvement of beef gel products.

Keywords: sarcoplasmic protein; concentration effect; gel property; beef mince; intermolecular interactions

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1 Introduction

Beef is widely consumed owing to its nutritional characteristics, such as high protein, low fat, and low cholesterol. It is rich in high-quality proteins and essential amino acids, making it suitable for developing gel-type meat products[1]. The commercial value of beef mince products is largely dependent on their gel quality characteristics, including texture, gel strength, water-holding capacity (WHC), etc. The heat-induced gel characteristics of myofibrillar protein (MP), a major functional component of beef, have been widely studied. Mince products prepared based on MP, such as hamburger patties, luncheon meat, and meatballs, are highly prevalent in the market. However, in the industrial production of gel products, centrifugation or rinsing is applied to remove sarcoplasmic protein (SP)[2]. As the second-largest component accounting for 30%–35% of muscle protein, SP is highly soluble at low pH (6.0–7.5)[3]. However, the effect of SP on gels has long been overlooked. In addition, during the maturation of beef, anaerobic glycolysis of muscle glycogen produces lactic acid, causing a rapid pH drop that leads to the dissolution of SP, which also reduces the edible quality of gel products[4].

The relationship between SP and gel performance of minced meat has been increasingly studied in recent years. For instance, Xia et al.[5] confirmed that the addition of 20% SP (*m*/*m*) minimized the cooking loss of gel by 8.5%. However, Paker et al.[2] reported that SP competitively inhibited MP crosslinking, reducing the gel strength by 12%–15% at the same concentration. SP mainly consists of myoglobin and various glycolysis-related enzymes, with a significant number of thermostable enzymes[6]. These enzymes are involved in the maintenance of the color of minced meat and the regulation of the pH of the system. They can also influence the stability of the protein three-dimensional network structure, which further affects the quality characteristics of the gel. The processing of minced meat gel products involves steps such as chopping, heating, and shaping, which require prior dissolution of SP and the subsequent disintegration and dissolution of MP. The thermostable proteins in SP may alter the subsequent formation and gel performance of minced meat gels. As a water-soluble complex protein system, the composition and stability of SP can influence the gel performance of minced meat. Currently, most studies have focused on the interaction between SP and MP, limited studies have explored the regulation of the formation and characteristics of minced meat gels by recycled SP. Moreover, the dose-effect of SP dissolved during the processing is poorly understood. SP, as the protein that is first dissolved during the processing, serves as a precursor that influences the gel performance of minced meat. Therefore, the interaction between the dissolved SP and the minced meat gel itself deserves research attention.

This study analyzed the impact of gradient addition of SP (0%–0.5%, *m*/*m*) on the texture properties, rheological properties, molecular structure, interaction forces, and microstructure of gel, and the regulatory mechanism of different concentrations of SP on the gel network. Through systematic investigation of the effects of SP concentration on various physical and structural properties of

beef mince gels, this study provides a comprehensive understanding of SP's role in gel formation and stabilization. This is crucial for advancing fundamental science in meat gelation and protein interactions. This study will provide a theoretical support for developing beef mince products with high WHC and promoting high-value utilization of meat processing by-products.

2 Materials and methods

2.1 Materials

Fresh bovine hind leg meat (*semitendinosus* muscle, within 24 h post-slaughter, pH 5.8–6.2) was purchased from Hua Hong Jia Run Supermarket of South China Agricultural University (Guangzhou, China). The reagents used in the study included: sodium chloride purchased from Guangdong Salt Industry Group Co., Ltd. (Guangzhou, China); 8-anilino-1-naphthalene sulfonic acid (ANS) purchased from Sigma-Aldrich (Saint Louis, MO, USA); sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate dodecahydrate purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China); glutaraldehyde purchased from Tianjin Comio Chemical Reagent Co., Ltd. (Tianjin, China); acetic acid purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemical reagents were of analytical grade.

2.2 Extraction of SP and preparation of beef mince gel

2.2.1 Extraction of SP

The extraction of SP was determined following the method described by Xia et al.[5] with slight modifications. Fresh meat was trimmed of visible fat and fascia and then cut into small pieces, which were dispersed in a 10-fold volume of phosphate-buffered solution (PBS; 0.05 mol/L NaH_2PO_4/Na_2HPO_4, 0.1 mmol/L NaCl, pH 7.0) and homogenized at 10 000 r/min for 60 s. It was centrifuged at 5 000 × *g* for 15 min at 4 °C. The supernatant containing SP was filtered quickly through four layers of cheesecloth to remove the insoluble substance, and the filtrate was collected, followed by the addition of solid ammonia sulfate until saturation to salt out SPs under continuous stirring. The precipitated proteins were collected by centrifugation at 10 000 × *g* and 4 °C for 15 min. The whole extraction process was performed in an ice bath. The extract was freeze-dried and stored at −20 °C.

2.2.2 Preparation of beef mince gel

The minced meat was prepared at 4 °C. Briefly, fresh bovine hind leg meat was trimmed to remove visible fat and fascia and then chopped in a meat grinder (22, Suonso Machinery Co., Ltd., Wenzhou, China). Each portion was 50 g, and 6 g of ice-water, 1.5 g of salt, and a fully swollen SP solution were added. Six levels (0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, *m*/*m*) were prepared, and the mixture was chopped at 4 °C for 30 s. The chopped minced meat was heated in a constant-temperature water bath at 80 °C for 30 min. Following the formation of a gel, it was cooled to room temperature and stored at 4 °C.

2.3 Determination of WHC

The WHC was tested following the method described by Li et al.[7] with slight modifications. The gel was cut into thin slices with a thickness of about 1 mm. A sample of about 3 g was weighed, and the sample mass was recorded as m_0. It was placed in a 50 mL centrifuge tube and centrifuged at 4 °C and 1 000 r/min for 20 min. Subsequently, the surface water was blotted dry using a filter paper, and the mass of the thin slice was accurately weighed and recorded as m_1. WHC is calculated according to formula (1):

$$\text{WHC}\ (\%) = \frac{m_1}{m_0} \times 100 \quad (1)$$

2.4 Determination of cooking yield (CY)

CY was determined following the method described by Zhang et al.[8] with slight modifications. Briefly, 50 g of the minced meat was collected in a pre-weighed 100 mL centrifuge tube and treated in a water bath at 80 °C for 30 min, until the central temperature reached 75 °C. The water was blotted using a filter paper and then weighed. Each treatment group was measured in parallel 5 times. CY is calculated according to formula (2):

$$\text{CY}\ (\%) = \frac{m_2 - m_3}{m_4} \times 100 \quad (2)$$

Where m_2 and m_3 represent the weights of minced meat and centrifuge tubes before and after cooking (g), respectively, and m_4 is the weight of raw minced meat (g).

2.5 Determination of color difference

The beef mince gel was warmed to room temperature for 2 h, and then absorbent paper was used to wipe away moisture from the surface of the beef mince gel. The brightness value (L^*), redness value (a^*), and yellowness value (b^*) of samples from each group were measured using a colorimeter (SP62, Shenzhen 3nh Technology Co., Ltd., Shenzhen, China). Five positions of each sample were used for the determination of color changes, and each position was measured three times.

2.6 Determination of mechanical properties

2.6.1 Uniaxial compression test

The gel strength was tested as described by Wang et al.[9]. The beef mince gel was made into a cube (1.5 cm). The P/0.5 probe was used for testing. The testing parameters were set to a compression rate of 2.0 mm/s, a constant test speed of 1 mm/s, and a return speed of 2 mm/s. The applied force was 5.0 g, with a maximum penetration depth of 10 mm.

2.6.2 Texture profile analysis (TPA)

Using a texture analyzer (TA.XT Plus, Stable Micro System, Godalming, Surrey, UK) to evaluate the mechanical properties of the gel. The gel was made into a cube (1.5 cm). The TPA mode and P/36R probe were selected for testing, with a compression ratio set at 50%. The pre-test, test, and post-test speeds were uniformly maintained at 2 mm/s, the interval between two compression cycles was set to 5 s, and the trigger force was set to 5 g. Each sample group was measured in eight parallel replicates. Key parameters of the formed gel, including hardness, springiness, chewiness, and resilience, were recorded for subsequent analysis.

2.7 Determination of dynamic rheological

2.7.1 Temperature scan

The temperature scan was tested as described by Tang et al.[10] with slight modifications. The rheological properties of beef mince were tested using a rheometer (MCR301, Anton Paar GmbH, Graz, Austria). The PP50 probe was selected. The sample was evenly

smeared in the center of the sample stage, and the circumference of the fixture was sealed with paraffin. The gap between the upper and lower plates was 1.0 mm, the strain was 1%. The temperature was increased from 20 to 80 °C at a heating rate of 2 °C/min, then held at 80 °C for 30 min, and finally decreased from 80 to 20 °C at a cooling rate of 5 °C/min.

2.7.2 Frequency scan

The beef mince gel was placed in the center of the lower plate, and the upper plate was slowly lowered to the preset gap. The excess sample overflowing from the edge was scraped off. The frequency range was 0.1–100 rad/s and the strain amplitude was 0.1%. A frequency-scan experiment was carried out, and the corresponding storage modulus (G') and loss modulus (G'') were measured. The frequency-scan results were fitted using the power-law equation:

$$G' = K'\omega^{n'} \quad (3)$$

Where K' represents the rigidity of the gel (Pa·s), ω represents angular frequency (rad/s), and n' represents the frequency dependence of the gel.

2.8 Determination of low-field nuclear magnetic resonance (LF-NMR)

The meat pieces were placed in a cylindrical glass tube. The LF-NMR (MQ20, Bruker, Germany) of the gel was measured as described by Lin et al.[11]. The spin-relaxation time (T_2) was determined using the Carr-Purcell-Meiboom-Gill sequence, with an echo time of 0.5 ms, a repetition time of 2 500 ms, and 8 scans.

2.9 Determination of intermolecular forces

Intermolecular forces were measured as described by Zhang et al.[12] with slight modifications. Briefly, 0.2 g of minced beef gel sample was mixed with 10 mL of SA (0.05 mol/L NaCl), SB (0.6 mol/L NaCl), SC (0.6 mol/L NaCl + 1.5 mol/L urea), SD (0.6 mol/L NaCl + 8 mol/L urea), and SE (0.6 mol/L NaCl + 8 mol/L urea + 1.5 mol/L β-mercaptothion), respectively. The beef mince gel was homogenized at 10 000 r/min for 2 min, left to stand for 2 h, and then centrifuged at 5 000 r/min for 20 min at 4 °C. Changes in protein content between treatments (SB *vs.* SA, SC *vs.* SB, SD *vs.* SC, and SE *vs.* SD) were used to assess the effects of ionic bonds, hydrogen bonds, hydrophobic interactions, and disulfide bonds on protein solubility. The results are expressed as the amount of protein dissolved per mL of solution.

2.10 Determination of protein conformation in beef mince gel

2.10.1 Surface hydrophobicity

This test was performed following a method described by Liu et al.[13] with minor modifications. ANS was used as a fluorescent probe to determine the surface hydrophobicity. Briefly, 2 g of minced meat gel sample was dissolved in 20 mL of 0.02 mol/L PBS (pH 7.0) and homogenized at 10 000 r/min for 2 min. The supernatant was taken, and the protein concentration was determined by the biuret method. The protein concentration was adjusted to 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL. Then, 20 μL of 8 mmol/L ANS solution dissolved in PBS was added to 4 mL of the sample. After reacting in the dark for 15 min, the fluorescence intensity was measured. The wavelength was set at 390 nm (excitation) and 470 nm (emission), and the slit width was set to 5 mm. The surface hydrophobicity of the protein was the initial slope (S_0) of the fluorescence intensity relative to the protein concentration.

2.10.2 Raman spectroscopy

Assay using Raman spectroscopy (DXR 2xi Micro, Thermo Fisher Scientific, Waltham, MA, USA). The gel was cut into thin slices of 0.2 mm and wrapped with tin-foil paper. Each sample was scanned more than 3 times at an excitation wavelength of 785 nm, emission power of 300 mW, and a scanning range of 400–3 600 cm^{-1}. The characteristic peak of phenylalanine (1 004 cm^{-1}) was used as an internal standard to normalize the spectral intensity. The data were fitted and processed using PeakFit 4.2 software (Beijing Uone Technology Co., Ltd., Beijing, China).

2.10.3 Intrinsic fluorescence spectroscopy

Fluorescence spectra were measured using a fluorescence spectrophotometer (RF-5301PC, Shimadzu, Japan). The method reported by Chen et al.[14] was adopted with slight modifications. Briefly, samples from each group (1.0 g) were added to 15 mL of 0.02 mol/L PBS (pH 7.0) and homogenized at 10 000 r/min for 30 s at 4 °C. The concentration of the beef protein solution in each group was adjusted to 0.5 mg/mL. The excitation wavelength was 280 nm, the emission wavelength was 300–400 nm, and the slit width was 2.5 mm.

2.11 Appearance photography and micro-morphology observation of beef mince gel

2.11.1 Appearance photography

The beef mince gel was cut into square blocks. A digital camera (ILCE-7 M2, Sony, Japan) was used to record images of the prepared minced meat gel under a unified background. ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used for network void analysis.

2.11.2 Scanning electron microscope (SEM) observation

For SEM observation, the gel was cut into pieces of the same dimensions (1 cm × 1 cm × 1 cm). The gel sample was fixed with 2.5% glutaraldehyde solution for 30 min, then soaked in PBS for 30 min, washed twice, frozen for 24 h, and freeze-dried in a freeze-dryer (DGJ-10, Bodeng Biological Science and Technology Co., Ltd., Shanghai, China). It was fixed to the sample stage with conductive double-sided tape, sputter-coated with gold, and observed under a SEM (SU70, Hitachi, Japan). The micro-structure at a magnification of 300× was obtained under an acceleration voltage of 25 kV.

2.12 Statistical analysis

The data were presented as the mean ± standard deviation. The experimental data were analyzed for significance using one-way analysis of variance and post-hoc multiple comparison Duncan in SPSS Statistics 25.0 statistical software (International Business Machines Co., Armonk, NY, USA). The significance level was $P < 0.05$.

3 Results and discussion

3.1 Effects of SP on WHC and CY of beef mince gel

The minced beef gel is a continuous protein-water cross-linked matrix, and WHC is often used as an indicator of the degree of

interaction between water and protein[15]. CY is considered the core parameter used for investigating the processing loss in meat products. As shown in Figure 1A, both WHC and CY initially increased and then declined, with SP significantly enhancing the gel's WHC and CY ($P < 0.05$). Specifically, WHC reached a maximum of 95.33% at 0.3% SP addition, while CY peaked at 96.3% when SP content was 0.4%. However, WHC and CY decreased significantly ($P < 0.05$) once SP addition exceeded 0.4%. Myoglobin in SP contains numerous hydrophilic groups, such as $-COOH^-$ and $-NH_2$, which form a stable hydrogen-bond network with water molecules to improve gel WHC[16]. Under thermal induction, SP, due to its relatively low molecular weight, first undergoes denaturation and aggregation to form a weak gel-like network, enhancing the system's water retention capacity. Additionally, SP's excellent emulsifying property effectively reduces the interfacial tension of the meat emulsion, preventing oil-water phase separation and thereby significantly increasing CY[17]. The reduction in WHC and CY caused by excessive SP may be influenced by several factors. Firstly, the denatured SP forms a protein network that interacts with the structural proteins between the myofilaments, generating osmotic pressure between myofibrils or across the sarcolemma. This causes a transverse contraction of the lattice between the myofilaments, decreasing WHC. Secondly, calpain in SP can disintegrate the Z-line, releasing intact α-actin that is hydrolyzed by various cathepsins, to weaken the gel network[18]. Thirdly, excessive SP competes with myosin for water-solubility sites. Its denatured amorphous structure may either embed within the gel network or adsorb onto the surface of MP, forming a physical barrier that impedes myosin head cross-linking and hinders the formation of an ordered gel network structure.

3.2 Effects of SP on the color of beef mince gel

Color is an important sensory attribute influencing the consumer acceptance of meat products. Figure 1B depicts the effects of varying concentrations of SP on the color of beef mince gel. With increasing SP addition, the L^* initially increased and then decreased; the change of b^* is not significant ($P > 0.05$). Notably, the 0.3% SP group exhibited the highest WHC; the retained water in the gel enhanced light reflection, thereby increasing the L^*[19]. The addition of 0.2% SP increased the a^*, which can be attributed to the oxygen-binding property of myoglobin present in SP. Myoglobin contains globin and heme, the latter is a porphyrin ring that attaches to a central iron atom. The Fe^{2+} in myoglobin may be affected by oxygen and free radicals[20]. When heated to 60 °C, the porphyrin ring is disrupted, allowing the release of Fe^{2+}, which is then oxidized, changing the color of the gel from red to brown. Under low-concentration conditions, the peroxiredoxin in SP can eliminate the peroxides and free radicals generated by the auto-oxidation of myoglobin[8], thus retarding the deterioration of the gel color. However, when the SP concentration increased to 0.4%, the L^* and a^* of the gel decreased as a consequence of aggravated protein oxidation[21].

3.3 Effects of SP on the gel properties of beef mince gel

Gel strength is closely associated with the sensory texture of meat products, reflecting the stability of the gel network and serving as a vital indicator of gel quality[22]. Figure 1C shows the alterations in the gel properties of beef mince. Compared to the control group, adding 0.1%–0.4% SP significantly enhanced the gel strength ($P < 0.05$), with a trend of initial increase followed by a decrease as SP content rose. Specifically, gel strength peaked at 524 g when SP addition was 0.3%, which was consistent with the trend of WHC (Figure 1A). Additionally, when the gel strength was relatively high, water entrapped within its network structure was less likely to be extruded by external forces[19]. Transglutaminase in SP can catalyze the glutamine-lysine residue reaction, and the resultant peptide bonds could confer resistance against protein hydrolysis, augmenting the strength of the minced-meat gel. Meanwhile, protease inhibitors in SP have been crucially linked to the suppression of myosin heavy chain degradation, thus maintaining the stability of the gel network structure[23]. Nonetheless, a significant decrease in gel strength was observed at SP concentrations > 0.4% ($P < 0.05$), attributed to excessive SP aggregation that forms a loose network and hinders myosin head cross-linking, ultimately weakening the gel structure.

Hardness, defined as the maximum peak pressure detected by the instrument probe upon the initial gel compression, besides being a key parameter that predominantly reflects a gel network's resistance to deformation, is also directly intertwined with a product's taste and texture[24]. As shown in Table 1, hardness exhibited a distinct pattern, initially decreasing and then increasing. Specifically, SP inhibited the cross-linking of MP at low concentrations (0.1%–0.2%), leading to a notable decline in hardness from 742.53 to 681.11 g ($P < 0.05$). Conversely, the gel hardness peaked at 1 031.11 g at 0.3% SP, signifying a 38.86% increase relative to the control group ($P < 0.05$). This is because introducing SP effectively increases the pH of the beef mince system, promoting MP solubility and the dissociation of actomyosin into actin and myosin. Enhanced protein-protein interactions (PPIs) further facilitate MP aggregation, forming a dense gel network and increasing hardness. However, when SP concentration reached 0.4%, excessive SP denaturation formed a physical barrier that impeded MP cross-linking, resulting in a significant decrease in hardness ($P < 0.05$) and degraded texture

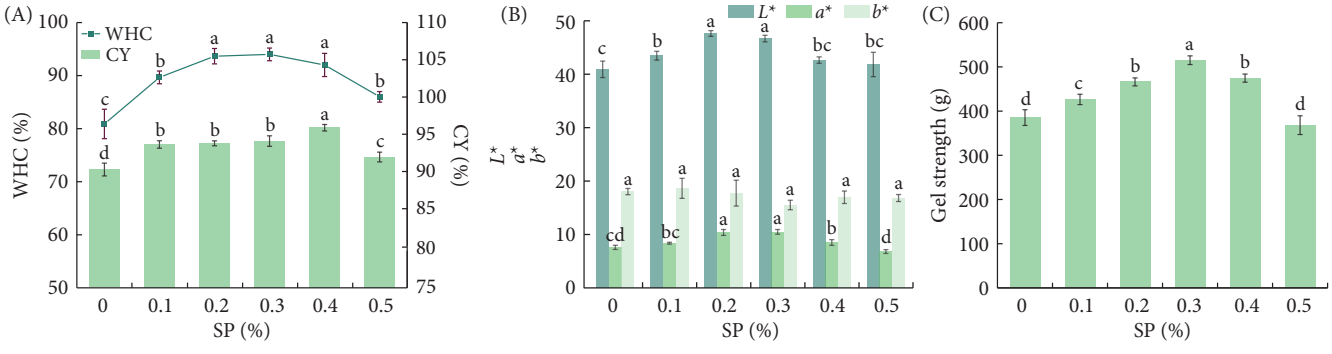

Figure 1 Effects of SP treatments with different concentrations on the (A) WHC and CY, (B) color difference, and (C) gel strength of beef mince gel. Values are shown as mean ± standard deviation ($n = 3$). Different lowercase letters denote significant differences ($P < 0.05$) by Duncan's multiple comparison test.

Table 1 Effect of different concentrations of SP treatments on the textural properties of beef mince gel.

SP (%)	Hardness (g)	Springiness (mm)	Chewiness (g·mm)	Resilience
0	742.53 ± 10.03^{d}	0.90 ± 0.01^{a}	524.86 ± 12.71^{b}	0.37 ± 0.02^{b}
0.1	681.11 ± 16.68^{b}	0.93 ± 0.01^{a}	504.50 ± 16.86^{ab}	0.40 ± 0.02^{ab}
0.2	693.80 ± 37.03^{b}	0.88 ± 0.04^{a}	466.57 ± 40.90^{a}	0.37 ± 0.04^{b}
0.3	1 031.12 ± 10.68^{a}	0.92 ± 0.02^{a}	742.59 ± 37.84^{c}	0.38 ± 0.02^{b}
0.4	997.46 ± 48.36^{c}	0.94 ± 0.01^{a}	726.76 ± 25.84^{c}	0.38 ± 0.01^{b}
0.5	959.79 ± 26.55^{c}	0.94 ± 0.04^{a}	623.39 ± 39.78^{d}	0.40 ± 0.02^{ab}

Note: Values are shown as mean ± standard deviation (n = 3). Different lowercase letters in a column denote significant differences (P < 0.05) by Duncan's multiple comparison test.

properties. Chewiness, correlated with gel juiciness and WHC, also peaked at 742.59 g·mm when SP was added at 0.3%. Notably, no significant differences in springiness and resilience were observed among different SP addition groups (P > 0.05).

3.4 Effects of SP on the rheological properties of beef mince gel

3.4.1 Temperature scan curve analysis

G' and G'' represent the elastic and viscous aspects of the minced meat gel structure, respectively. Figure 2 shows the results of the temperature scan. G' of the gels with 0%–0.3% SP exhibited similar change trends, with three distinct stages: in the initial stage (20–52 °C), G' increased significantly, peaking at 50 °C. Conversely, the rapid decrease in G' was primarily attributed to the denaturation of the myosin tails. This denaturation weakened the protein network, which in turn enhanced system fluidity and ultimately disrupted the weak low-temperature gel (52–62 °C). In the third stage (62–80 °C), myosin heavy chain denaturation and unfolding promoted protein aggregation, and G' increased rapidly, driving a rapid G' increase as the system formed an elastic gel network. During the isothermal stage, G' and G'' remained stable. In the cooling stage, protein molecules formed new cross-links via hydrophobic interactions and hydrogen bonds, further increasing gel elasticity. Notably, G' continued to rise at this stage. At SP addition amounts > 0.3%, G' increased rapidly in the first stage, followed by a gel-weakening stage. Nonetheless, the initial temperature of the third stage changed from 52 to 62 °C as the denaturation temperature of SP was lower than that of MP. Notably, SP was denatured first at 40 °C, resulting in loose aggregates. Meanwhile, the gel's G' increased rapidly, and the aggregates embedded some myosin, increasing the temperature at which the protein was denatured. At 62 °C, myosin head denaturation began, and the gel's G' increased rapidly. At the end of cooling, the 0.5% SP group exhibited the highest G', indicating this concentration maximizes gel elasticity. In contrast, 0.4%–0.5% SP resulted in significantly lower G' in the initial stage; G' and G'' increased exponentially at 30 °C but showed no elevation during cooling. This suggests that excessive SP forms a physical barrier, hindering hydrogen-bond networks and hydrophobic interactions of meat proteins, consistent with previous trends in WHC and gel strength.

3.4.2 Frequency scan curve analysis

Figure 3A shows the frequency-scan curves. The 0.3% SP group had the highest G' and G'', and $G' > G''$ across all groups, indicating that the gel system was mainly elastic[25]. With increasing SP content beyond 0.3%, G' and G'' decreased. Notably, higher G' correlates with denser cross-links, stronger deformation resistance, and greater hardness, consistent with TPA results. Additionally, G' and G'' showed low frequency dependence, confirming that the beef mince gel forms a stable cross-linked network. The gel's G' across different groups was fitted using the power-law equation (R^2 > 0.98), and Figure 3B shows the results. The smaller the value of n', the greater the influence of covalent bonds on gel formation[26]. Compared to the control group, SP addition significantly increased n' (P < 0.05), suggesting weakened covalent bond contributions to gel formation, with no further significant change in n' when SP reached 0.5% (P > 0.05). A higher K' indicates a more viscous, well-structured gel network[27]. SP addition significantly increased K' (P < 0.05), with a trend of initial increase followed by a decrease (P < 0.05). This indicates that appropriate SP promotes gel rigidity and strength via enhanced non-covalent interactions (offsetting weakened covalent bonds), while SP content > 0.4% impairs other

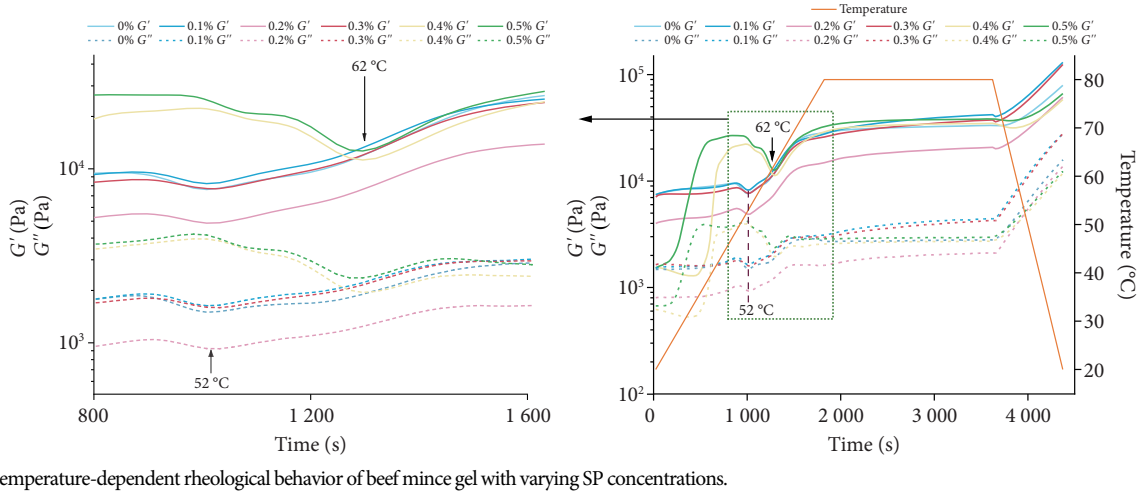

Figure 2 Temperature-dependent rheological behavior of beef mince gel with varying SP concentrations.

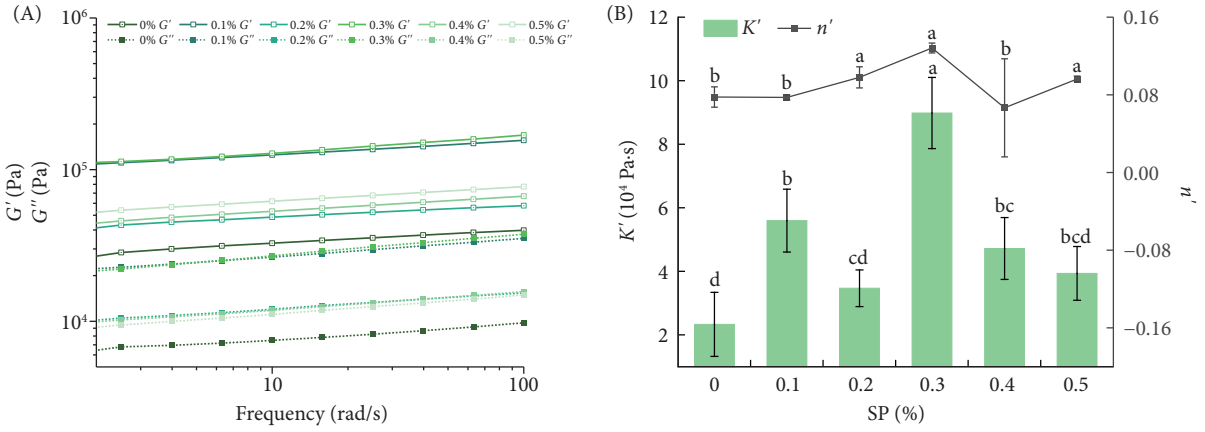

Figure 3 (A) Frequency-dependent viscoelasticity of SP-modified beef mince gels and (B) K' and n' fitted by the power-law equation. Values are shown as mean ± standard deviation ($n = 3$). Different lowercase letters denote significant differences ($P < 0.05$) by Duncan's multiple comparison test.

meat protein interactions, reducing gel rigidity, consistent with gel strength changes. Higher rigidity correlates with stronger deformation resistance, hardness, and chewiness. Thus, 0.3% SP effectively produces a gel with good palatability, strong chewiness, and brittle texture.

3.5 Effects of SP on LF-NMR of beef mince gel

LF-NMR measures the T_2 to characterize the mobility and distribution of water molecules[28]. Notably, T_{21} (0.1–10 ms), T_{22} (10–100 ms), and T_{23} (100–1 000 ms) represent bound, immobilized, and free waters, respectively. Figure 4A shows the changes in T_2 curve. After adding SP, T_{23} shortened, indicating that free water mobility decreased. A shorter T_2 indicates a stronger protein-water binding ability[29]. Furthermore, myoglobin oxidation could change the MP aggregation degree and effectively promote protein conformation transformation, thus enhancing the gel network's water-capturing ability. Figure 4B shows the proportions of the three types of water in the gel. The control group had the highest free water relative content (38%), which explains its lowest WHC, as free water is prone to loss under external stimuli. With increasing SP concentration, free water relative content decreased progressively, while bound and immobilized water relative content increased. At 0.3% SP, the relative content of bound water increased from 8% to 19%, and that of the immobilized water increased to 59%, indicating that part of the retained free water was converted into immobilized and bound waters, which was attributable to the self-aggregation of SP to form a loose gel network, thus enhancing the free water retention ability. The polar groups (e.g., –COOH, $–NH_2$) on the surface of SP could also bind to water molecules through hydrogen bonds, reducing water mobility. When SP concentration exceeded 0.3% (*m*/*m*), the bound water relative content began to decline. Excessive SP competes with MP for water-binding sites, leading to the loss of immobilized water in the MP matrix. Meanwhile, water molecules are adsorbed onto the surface of SP aggregates, resulting in uneven water distribution within the gel[30].

3.6 Effects of SP on the intermolecular forces of beef mince gel

Ionic bonds, hydrogen bonds, hydrophobic interactions, and disulfide bonds are the major chemical forces maintaining the gel network structure. Figure 5A shows the changes in the main intermolecular forces in the minced beef gel. Compared to the control group, adding SP resulted in significantly fewer ionic bonds between the gel molecules ($P < 0.05$), while the hydrogen bonds, hydrophobic interactions, and disulfide bonds exhibited an initial increase followed by a decrease. Ionic bonds represent electrostatic attractions between groups with opposite charges. Notably, SP's excellent solubility leads to its surface negative charges competing with myosin for cations, disrupting the original ionic bond balance, explaining the reduced ionic bond proportion. The increase in

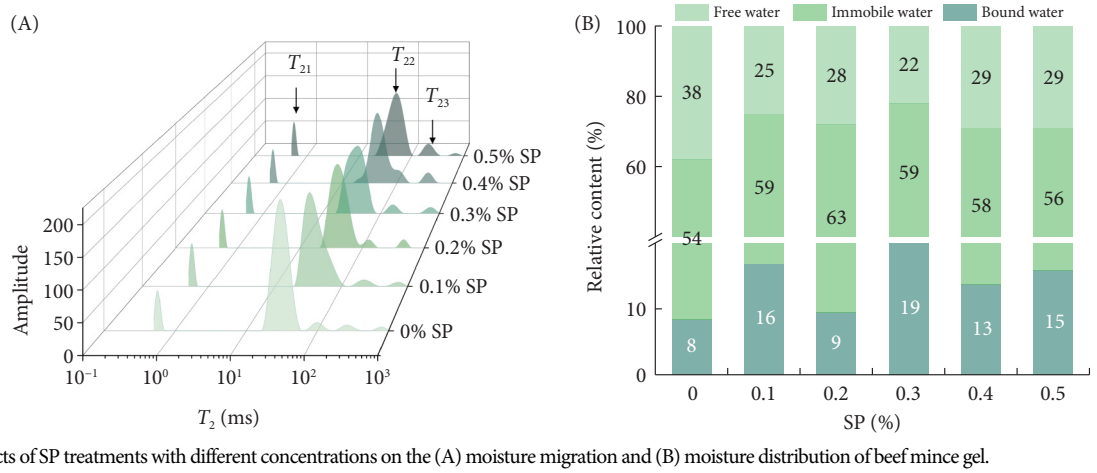

Figure 4 Effects of SP treatments with different concentrations on the (A) moisture migration and (B) moisture distribution of beef mince gel.

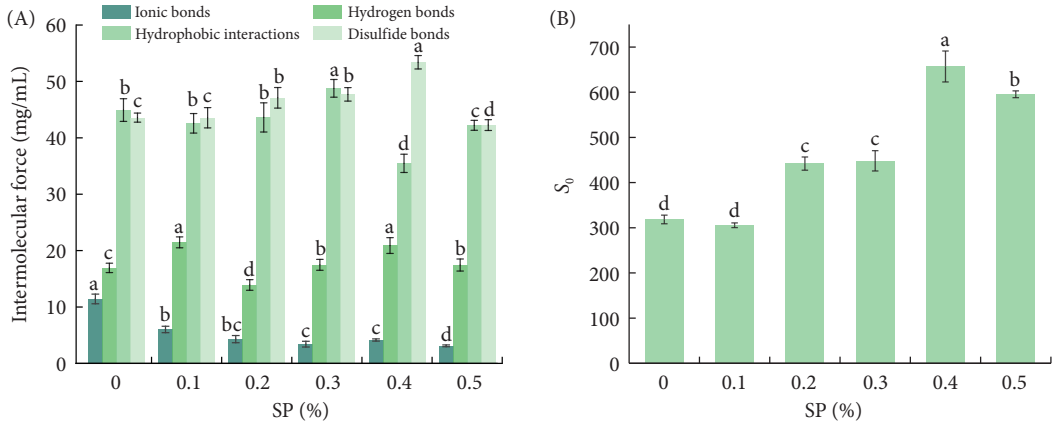

Figure 5 Effects of SP treatments with different concentrations on (A) intermolecular interactions and (B) surface hydrophobicity of beef mince gel. Values are shown as mean ± standard deviation ($n = 3$). Different lowercase letters denote significant differences ($P < 0.05$) by Duncan's multiple comparison test.

hydrogen bonds could be attributed to the interaction between the denatured SP-exposed polar groups and water molecules. However, excessive SP could decrease hydrogen bond interactions in the gel network. The hydrophobic interaction was greatest in the gel with 0.3% SP, and decreased significantly at greater concentrations, indicating that the SP concentration effect influenced the hydrophobic interaction, which was unconducive to gel network formation, ultimately decreasing the gel strength. The proportion of disulfide bonds was greatest at 0.4% SP. Disulfide bonds result from the oxidation of cysteine's sulfhydryl groups. High temperatures induce the unfolding of the cysteine structure in SP, exposing the sulfhydryl groups, which are then oxidized to form disulfide bonds. The proportion of disulfide bonds decreased with increasing SP content ($P < 0.05$) mainly because excessive SP content accelerates protein aggregation, affecting the cross-linking of myosin heads into a gel network. As the dominant forces stabilizing the gel network, hydrophobic interactions showed a trend consistent with WHC and gel strength, indicating a close correlation between hydrophobic interactions and gel functional properties.

3.7 Effects of SP on the surface hydrophobicity of beef mince gel

Surface hydrophobicity is a key indicator reflecting the distribution and exposure degree of hydrophobic groups on protein surfaces, and it significantly influences protein functional properties[31]. Figure 5B shows the changes in surface hydrophobicity. Compared to the control group, adding 0.2% SP resulted in a significantly higher surface hydrophobicity of the protein in the minced meat gel ($P < 0.05$). With gradually increasing SP concentration, surface hydrophobicity first rose and then declined, peaking at 0.4% SP. The proportion and distribution of hydrophobic amino acids are core factors determining protein surface hydrophobicity. After SP integrates into the system, it shifts the pH away from the protein's isoelectric point, increasing the net charge of protein molecules and enhancing intermolecular electrostatic repulsion. This accelerates protein unfolding, exposing internal hydrophobic groups that bind to ANS, thereby increasing surface hydrophobicity. Additionally, SP itself has relatively high surface hydrophobicity, which further strengthens hydrophobic interactions between proteins[32]. The system's surface hydrophobicity significantly decreased at 0.5% SP ($P < 0.05$). This is due to the rapid denaturation and aggregation of SP, which encapsulates myosin. Consistent with previous research results on the gel's chemical forces, our findings suggested that the contents of ionic and hydrogen bonds in the system were low, hindering the exposure of the hydrophobic regions within the protein. Moreover, enhanced myosin-myosin interactions and hydrophobic interactions drive the formation of polymer aggregates containing non-polar amino acids, leading to re-embedding and shielding of partial hydrophobic groups, ultimately reducing surface hydrophobicity[33].

3.8 Effects of SP on the micro-structural environment of beef mince gel

Unique Raman spectral characteristics of specific functional groups provide insights into protein microenvironments and spatial structure. As shown in Figure 6A, an S=S stretching vibration was observed at 510–540 cm^{-1}, with intensity peaking at 0.4% SP before decreasing at 0.5% SP. This trend aligns with intermolecular force results (Figure 5A), as SP-induced protein unfolding exposes –SH that form S–S cross-links, while excessive SP aggregation at high concentrations hinders –SH collision and cross-linking. The peak at 760 cm^{-1} was assigned to Trp residues, which are sensitive to the polarity of their local environment. With SP addition, Trp was exposed, leading to a decrease in the spectral intensity at 760 cm^{-1} and confirming partial protein unfolding. The peak near 1 450 cm^{-1} was attributable to the C–H bond of aliphatic amino acids[34], with increased intensity at 0.2% SP reflecting side-chain rearrangement that facilitates hydrophobic packing and stabilizes the gel network (Figure 6B). The amide I band (1 600–1 700 cm^{-1}), which is highly sensitive to the protein backbone conformation, showed a notable decrease and subsequent increase in intensity with SP addition, indicating structural reorganization of the polypeptide backbone.

The secondary structure composition was quantitatively derived from deconvoluting the amide I band in Figure 6C, including α-helix (1 650–1 658 cm^{-1}), β-sheet (1 665–1 680 cm^{-1}), β-turn (1 680–1 690 cm^{-1}), and random coil (1 660–1 665 cm^{-1}). With SP addition, the α-helix relative content decreased significantly ($P < 0.05$), while the β-sheet relative content increased, reaching a maximum at 0.2% SP. This α-helix to β-sheet transition is a critical molecular-level event. The α-helix decrease is attributed to SP-induced pH shifts that enhance electrostatic repulsion and break intramolecular hydrogen bonds, stabilizing the helical structure, while the extended β-sheet structure forms intermolecular hydrogen bonds to construct a rigid, continuous gel network, directly contributing to enhanced gel strength and WHC.

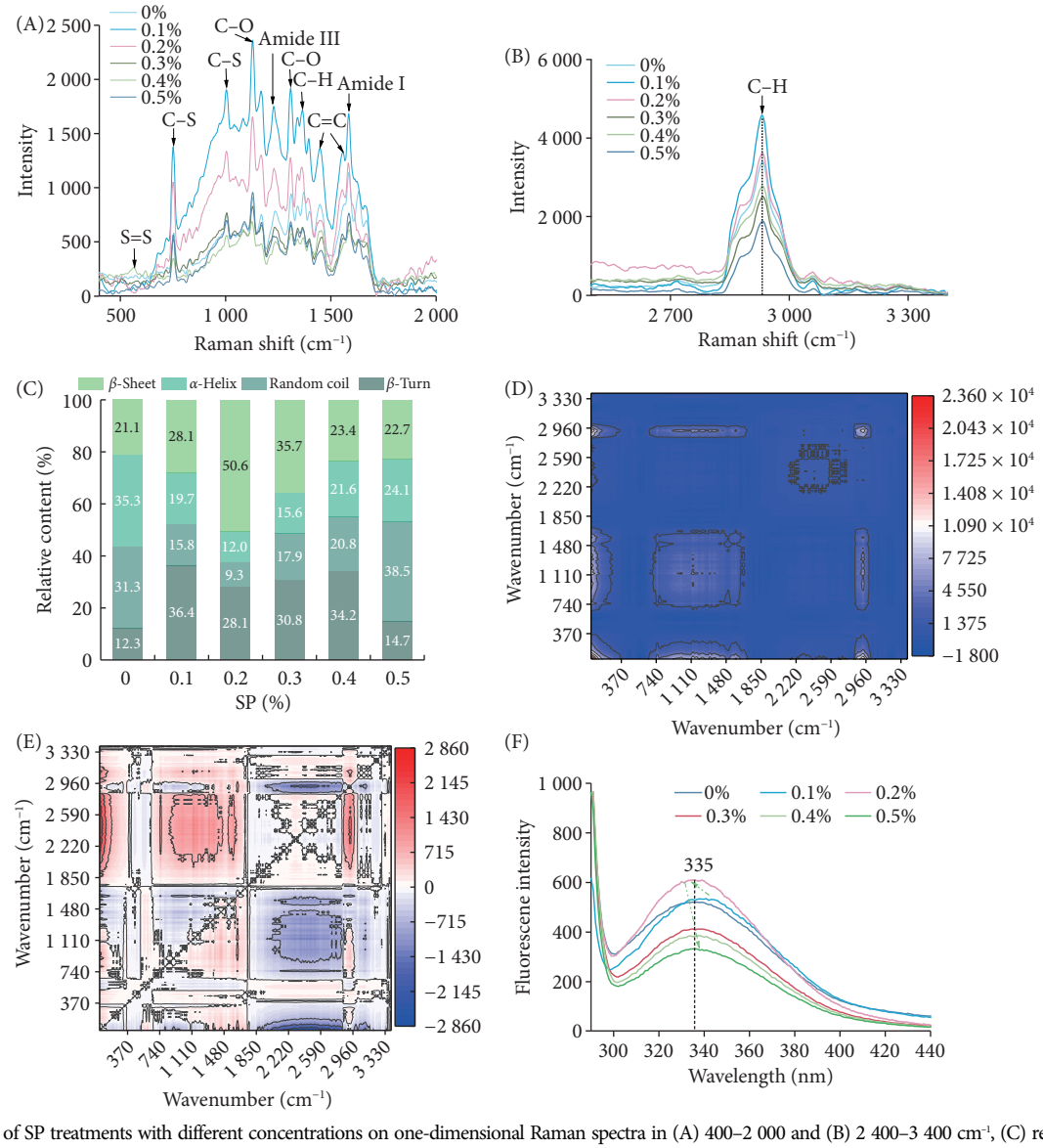

Figure 6 Effects of SP treatments with different concentrations on one-dimensional Raman spectra in (A) 400–2 000 and (B) 2 400–3 400 cm^{-1}, (C) relative content of secondary structure, two-dimensional Raman correlation (D) synchronous and (E) asynchronous spectra, and (F) intrinsic fluorescence of beef mince gel.

Thereafter, with increasing SP content, the β-sheet relative content gradually decreased, while the random-coil relative content increased. The unfolding of the protein could break the hydrogen bonds, decreasing the α-helix relative content. Specifically, the transformation of the protein secondary structure exposes the intramolecular sulfhydryl and hydrophobic groups, increasing the system's hydrophobic interactions, consistent with the changes in intermolecular forces (Figure 5A). At 0.5% SP, the random coil relative content reached 38.5%. The excessive conversion to disordered random coils disrupts the ordered gel matrix, leading to a looser network and the observed deterioration of textural properties and WHC[35].

Two-dimensional correlation spectroscopy is often employed to analyze the influence of physical and chemical factor-induced external disturbances on the protein unfolding process in a reaction system[36–37]. Figures 6D and E show the synchronous and asynchronous spectra of beef mince gels with adding different concentrations of SP. In the synchronous spectrum, strong peaks at 1 480 (amide II, C–N/N–H) and 2 960 cm^{-1} (C–H stretching) indicated these groups underwent the most significant changes in response to SP. The corresponding asynchronous spectrum showed a cross-peak between these two wavenumbers, revealing a sequential response: the change at 1 480 cm^{-1} (protein backbone) preceded that at 2 960 cm^{-1} (hydrophobic side chains). This sequence provides direct spectroscopic evidence that SP first disrupts the hydrogen-bonding network of the protein backbone, leading to α-helix unfolding, which then triggers the rearrangement of hydrophobic side chains. The subsequent exposure and interaction of these hydrophobic groups are a key driver for the formation of a strong gel network. This molecular sequence aligns well with the WHC trend: WHC increased up to 0.3% SP as the backbone rearrangement and hydrophobic association created a dense, elastic matrix, but declined at higher SP concentrations as excessive disruption prevented effective network assembly.

3.9 Effects of SP on the intrinsic fluorescence of gel

Intrinsic fluorescence spectroscopy is a sensitive and specific tool for probing the spatial conformation of proteins, with Trp residues serving as the primary intrinsic fluorophore[38]. Figure 6F shows the changes in intrinsic fluorescence. With increasing SP concentration

(from 0% to 0.5%), the fluorescence emission peak (λ_{max}) shifted from 335 to 338 nm, accompanied by a continuous increase in fluorescence intensity that peaked at 610.04 (17.2% higher than the control) at 0.2% SP. SP first competes with MP for bound water molecules, disrupting the hydration layer on the protein surface. This hydration perturbation reduces the intramolecular hydrogen-bonding stability of MP, inducing partial unfolding of the polypeptide chain. Consequently, Trp residues migrate from the hydrophobic core to the relatively polar hydrophilic interface of the protein molecule, while the moderate polarity increase does not trigger significant quenching, the reduced steric hindrance around Trp residues and decreased self-quenching between adjacent Trp molecules jointly enhance fluorescence intensity. When the SP concentration is below 0.3%, both fluorescence intensity and WHC significantly increase compared to the control group ($P < 0.05$). Partial unfolding of MP exposes more hydrophilic groups to increase water-binding sites, while also revealing hydrophobic groups and –SH groups that promote intermolecular interactions. The enhanced hydrophobic interactions and formation of disulfide bonds between MP molecules construct a dense, continuous, and elastic gel network. At 0.5% SP, the maximum fluorescence intensity decreased to 332.30, and λ_{max} stabilized at 336 nm. At higher SP concentrations, excessive hydration competition and charge perturbation accelerate protein denaturation and unfolding, leading to uncontrolled PPIs[39]. These enhanced interactions drive the formation of large, dense protein aggregates, where Trp residues are re-embedded into hydrophobic micro domains within aggregates or interact with quenching groups on adjacent molecules. The increased steric hindrance and static quenching effect jointly reduce fluorescence intensity, while λ_{max} stability suggests no further change in Trp microenvironment polarity, only a shift in the dominant factor governing fluorescence intensity from solvent accessibility to aggregation-induced quenching.

3.10 Effects of SP on the appearance and microstructure of beef mince gel

Figure 7 shows the appearance characteristics of the beef mince gel. As shown in Figures 1A and B, compared with the control group, SP-incorporated gels exhibited a subtle brown tint, with L^* increasing first and then decreasing as SP concentration increased. A uniformly distributed small-hole structure also emerged within the gel. At 0.4% SP content, the number of holes on the gel surface increased, making the surface relatively rough. The voids on the gel's surface were further examined using ImageJ software, revealing that the control group gel had more disordered hole structures and aggregates, with a relatively rough surface. Adding an appropriate quantity of SP reduced both the number and diameter of the holes within the gel. Exposure of hydrophobic groups enhanced intermolecular interactions, forming a uniform network structure via disulfide bonds and hydrophobic interactions. However, at 0.4% SP, the dense gel network structure was disrupted, featuring irregular pores and a substantial number of cavity structures.

SEM facilitates a more intuitive comparison of the differences in the three-dimensional (3D) network structures of minced meat gels[40]. The microstructure of the gel is directly related to its WHC, texture, and gel strength. As shown in Figure 1C, proteins undergo heat-induced cross-linking to form distinct 3D gel networks, the

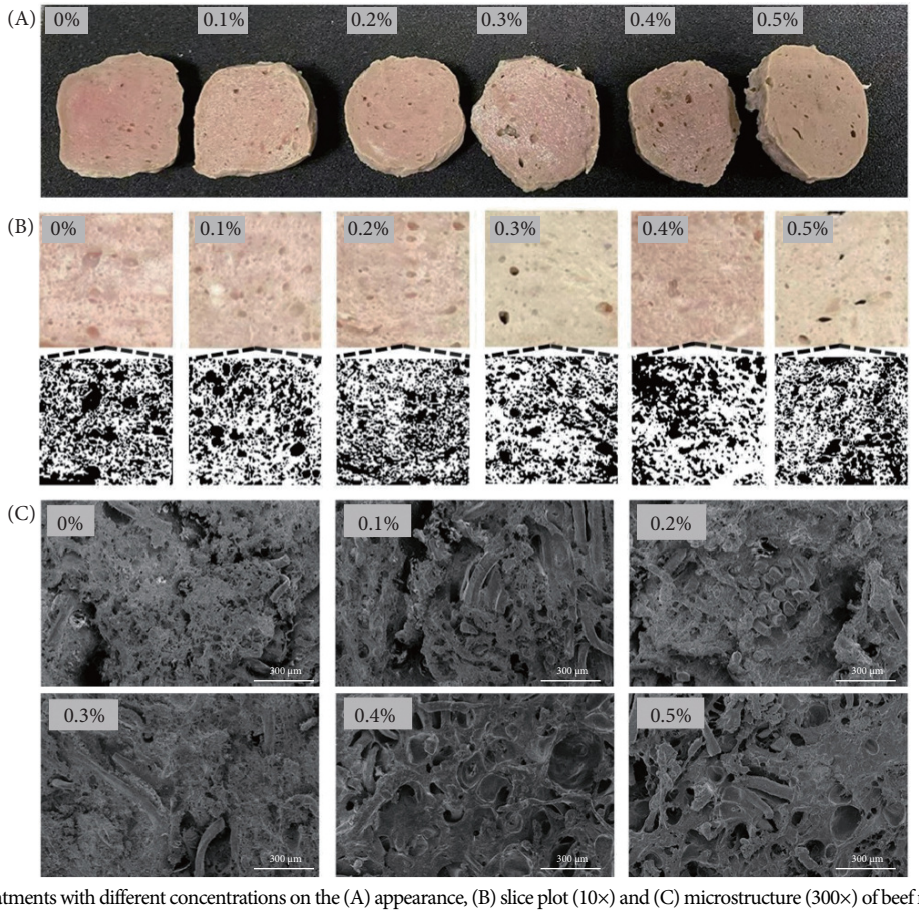

Figure 7 Effects of SP treatments with different concentrations on the (A) appearance, (B) slice plot (10×) and (C) microstructure (300×) of beef mince gel.

control group exhibited a loose network with numerous irregular cavities distributed chaotically. This is attributed to insufficient cross-linking of MP in the absence of SP, limited MP unfolding leads to minimal exposure of hydrophobic groups and –SH groups, preventing effective cross-linking. Conversely, upon adding 0.1% SP, irregular aggregates in the network decreased, and cavity diameter reduced. The 0.3% SP group formed the most compact and uniform network, with evenly distributed cavities interconnected into a honeycomb-like structure. This optimization arises from the sequential synergy between SP and MP, due to its small particle size, SP undergoes thermal denaturation at 30–40 ℃, preceding MP. The resulting micro aggregates act as cross-linking nuclei, providing anchor points for subsequent MP unfolding and β-sheet assembly. When SP-induced aggregation rate matches MP unfolding rate, the two components form continuous cross-links via PPIs, ultimately constructing a dense network. After denaturation, SP creates a porous structure between the myofilaments, forming a gel matrix conducive to water retention. The SP-generated micelles upon heating facilitate PPIs, and the enhanced degree of protein cross-linking results in a more compact network structure[41]. Owing to its smaller particle size and more rapid denaturation and aggregation rates upon heating, SP can serve as a filler during gel formation. At 0.4% SP, the network contained numerous disordered massive aggregates, and cavity diameter increased with uneven distribution. Excessive SP rapidly aggregates into rigid particles, hindering the ordered arrangement of MP molecules. These aggregates not only encapsulate partially uncross-linked MP but also disrupt continuous β-sheet assembly, creating fracture zones in the network. This structural disruption directly contributes to deteriorated macroscopic properties[42]. Overall, low SP concentrations follow a moderate unfolding to ordered cross-linking to dense network pathway, simultaneously enhancing gel properties. In contrast, high SP concentrations induce excessive aggregation to network fracture to enlarged cavities, resulting in loose structures with abundant but inefficient water-retention space and reduced pressure resistance.

4 Conclusion

This study systematically investigated the effects of varying SP concentrations on the physical properties, molecular conformation, and microstructure of beef mince gels. Results indicate that 0.3% SP was the optimal concentration, which significantly improved the gel's WHC and CY to 95.33% and 96.3%, respectively, while increasing gel hardness by 38.86%, demonstrating enhanced rigidity and elasticity. Moderate SP induced partial unfolding of MP, exposing hydrophobic groups and sulfhydryl groups to promote disulfide bond cross-linking and hydrophobic interactions, facilitating the assembly of a uniform network with evenly distributed pores. However, when SP concentration exceeded 0.4%, the gel network was disrupted. Excessive SP aggregated rapidly and inhibited ordered cross-linking, leading to irregular pores and reduced intermolecular forces, thereby decreasing gel properties. This study provides a research basis for clarifying the mechanism by which SP regulates the gel properties of beef mince and their subsequent industrial applications.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors contribution

Baosheng Lu: Writing-original draft, validation, methodology, formal analysis, data curation. Ziyuan Wang: Methodology, conceptualization. Shanguang Guo: Investigation, software. Nan Xiao: Investigation, resources, validation. Minmin Ai: Supervision, methodology, resources, writing-review & editing.

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