

Comparative investigation of three types of surfactants on the co-stabilization behavior of myofibrillar protein emulsions under low-salt conditions

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Abstract: This study systematically investigated the effects of three small-molecule surfactants, Tween-20, sodium dodecyl sulphate (SDS), and soy lecithin (SL), on the structure and emulsifying properties of myofibrillar protein (MP). All three surfactants altered MP conformation and reduced surface hydrophobicity via non-covalent interactions. Tween-20 acted primarily through hydrophobic interactions, whereas SDS combined electrostatic and hydrophobic binding, modifying surface charge. SL functioned through hydrogen bonding and hydrophobic interactions with charge shielding effects. With increasing surfactant concentration, the Tween-20-treated emulsion exhibited gradual reductions in particle size to 522.43 nm and ζ -potential to -2.78 mV. The SDS-treated emulsion showed an initial decrease in particle size to 1 562 nm at 0.5%, followed by an increase to 4 315 nm at 1.0%, with ζ -potential decreasing to -26.0 mV. The SL-treated emulsion displayed decreasing trends in both particle size to 2 052.33 nm and ζ -potential to -4.85 mV. Both Tween-20 and SDS produced stable emulsions at 0.1%–0.5%; at 1.0%, Tween-20 caused slight phase separation, whilst SDS caused obvious phase separation. SL exhibited phase separation at all concentrations. Molecular docking confirmed distinct binding modes: Tween-20 bound through hydrophobic interactions, whereas SDS and SL formed hydrogen bonds with specific amino acid residues. These findings clarify surfactant stabilisation mechanisms in MP emulsions, guiding surfactant selection for meat processing.

Keywords: myofibril protein; small-molecule surfactants; oil-in-water emulsion

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1 Introduction

Oil-in-water (O/W) emulsions are dispersion systems composed of two immiscible liquids. They are widely used in the food industry, for instance in meat products, sauces, and fat substitutes. Their physicochemical properties directly determine the texture, mouthfeel, and stability of the final products^[1]. As such, maintaining emulsion stability is critical to product quality. However, emulsions are inherently prone to instability, exhibiting phase separation, flocculation, and coalescence over time. These phenomena significantly compromise emulsion stability and quality, thereby affecting the final products^[2]. In O/W emulsions, the interfacial film formed at the oil–water interface acts as a physical barrier against droplet coalescence, and its properties directly govern emulsion stability. Therefore, the key to improving emulsion stability is to construct a robust and functional interfacial film, the properties of which depend critically on the choice of interfacial material.

Proteins, owing to their amphiphilic nature and excellent film-forming ability, are particularly suitable for constructing interfacial films. Emulsified meat products such as frankfurters are essentially O/W emulsions stabilised by proteins. In such emulsified

meat systems, myofibrillar protein (MP), which accounts for 55%–65% of total muscle protein, acts as the key emulsifier by adsorbing at the oil–water interface^[3]. Nevertheless, emulsions stabilised solely by MP often exhibit limited stability under low ionic strength conditions, which restricts their practical application. Various strategies have been explored to improve MP functionality, including amino acid and polyphenol addition^[4–5], glycation modification^[6], small-molecule surfactant incorporation, and high-pressure processing^[7]. Among these, small-molecule surfactants are particularly effective at improving the stability of MP emulsions.

Surfactants are amphiphilic low molecules composed of hydrophilic or charged moieties and hydrophobic moieties^[8]. They can be classified into cationic, anionic, nonionic, and zwitterionic types. Small-molecule surfactants are the most widely applied in food processing owing to their wide variety, low toxicity, and low cost. Nevertheless, existing research on small-molecule surfactant–protein interactions has focused primarily on non-meat proteins. For example, Chen et al.^[9] investigated the effects of monoglycerides on the interfacial properties and stability of soy protein-stabilised emulsions; Ulaganathan et al.^[10] explored the

interactions between sodium dodecyl sulphate (SDS) and β -lactoglobulin; and Saha et al.^[11] studied the interactions between bovine serum albumin and ionic/nonionic surfactants. However, the interaction mechanisms between different types of small-molecule surfactants and MP, particularly under low ionic strength conditions, remain unclear.

Therefore, this study aimed to systematically compare the effects of three small-molecule surfactants, namely anionic (SDS), nonionic (Tween-20), and natural amphiphilic molecules (soy lecithin (SL)) at various concentrations (0.10%, 0.25%, 0.50%, and 1.00%) on MP-stabilised O/W emulsions under low ionic strength conditions (0.3 mol/L NaCl). The interaction mechanisms between surfactants and MP and their effects on emulsion stability were investigated by combining spectroscopic analysis, emulsifying property measurements, and molecular docking simulations. The findings provide a theoretical foundation for the development of emulsified meat products with enhanced quality and stability.

2 Materials and methods

2.1 Materials

Tween-20 was provided by Tianjin Zhiyuan Biochemical Reagent Co., Ltd. (Tianjin, China); SL was supplied by Hefei BASF Biotechnology Co., Ltd. (Hefei, China); and SDS was provided by Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Pork loin and soybean oil were purchased from a local Walmart supermarket (Chengdu, China). All other reagents used in the experiments were of analytical grade.

2.2 Extraction of MP

Pork *longissimus dorsi* muscle was used as the raw material. Visible fat and connective tissue were removed first. MP was extracted according to the method of Li et al.^[12], and protein concentration was determined by the biuret method. Briefly, 30 g of minced lean muscle was mixed with four volumes of extraction buffer (10 mmol/L phosphate, 0.1 mol/L NaCl, 2 mmol/L MgCl₂, 1 mmol/L ethylene glycol tetraacetic acid, pH 7.0). The mixture was homogenised at 6 500 r/min for 60 s and centrifuged at 4 000 r/min at 4 °C for 15 min. After discarding the supernatant, the extraction was repeated twice. The pellet was then washed with four volumes of 0.1 mol/L NaCl, homogenised for 60 s, and centrifuged under the same conditions. This washing procedure was repeated once. After removal of the supernatant, the pellet was resuspended in four volumes of 0.1 mol/L NaCl and homogenized for 60 s. The homogenate was filtered through four layers of gauze, and the pH was adjusted to 6.0 with 0.1 mol/L HCl. Finally, the suspension was centrifuged at 4 000 r/min at 4 °C for 15 min to obtain purified MP. The extracted MP was stored at 4 °C and used within 48 h.

2.3 Preparation of small-molecule surfactant-MP mixed solutions

Small-molecule surfactant-MP mixed solutions were prepared according to the method of Zhang et al.^[13] with minor modifications. First, individual small-molecule surfactant solutions at 0.2%, 0.5%, 1.0%, and 2.0% (m/V) were prepared in 20 mmol/L phosphate buffer (pH 6.5, NaCl-free). Each solution was sonicated and left overnight to ensure complete dissolution. Meanwhile, an MP stock solution (20 mg/mL) was prepared in 20 mmol/L phosphate buffer (pH 6.5, containing 0.6 mol/L NaCl). Each surfactant solution was then mixed with the MP stock solution at a

1:1 (V/V) ratio and homogenised at 8 000 r/min for 2 min. The final concentrations were: 10.0 mg/mL MP, 0.3 mol/L NaCl, and 0.10%, 0.25%, 0.50%, and 1.00% (m/V) surfactants, numbered as 0.10% X, 0.25% X, 0.50% X, 1.00% X, respectively, X stands for surfactant type. Two control groups were included: MP solution with 0.3 mol/L NaCl (no surfactant, numbered as 0 (control 1)) and MP solution with 0.6 mol/L NaCl (normal salt and no surfactant, numbered as 0 (control 2)).

2.4 Analysis of protein structure-related parameters

2.4.1 Determination of surface hydrophobicity

The surface hydrophobicity of MP was determined according to the method of Bai et al.^[14] with minor modifications. First, the protein solution was diluted to a concentration of 4 mg/mL using phosphate-buffered saline (PBS). An aliquot of 1 mL of the diluted protein solution was mixed with 200 μ L of bromophenol blue (BPB) solution (1 mg/mL in distilled water), and the same volume of PBS was mixed with 200 μ L of BPB solution as a control. Both mixtures were sonicated and then centrifuged at 4 000 r/min for 15 min at 4 °C. After centrifugation, the supernatant was collected, diluted tenfold with PBS, and the absorbance was measured at 595 nm. The surface hydrophobicity of MP was expressed as BPB binding amount and calculated using the following formula:

$$\text{BPB binding amount } (\mu\text{g}) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 200 \mu\text{g}$$

2.4.2 Determination of intrinsic fluorescence spectroscopy

The tertiary structure of MP was analysed by measuring intrinsic tryptophan fluorescence according to the method of Li et al.^[15] with minor modifications. The MP concentration was adjusted to 0.05 mg/mL, and fluorescence spectra were acquired using a fluorescence spectrophotometer. The measurement parameters were set as follows: emission wavelength range, 300–400 nm; excitation wavelength, 280 nm; slit width, 5 nm; and scan rate, 1 200 nm/min.

2.4.3 Determination of ultraviolet (UV) absorption

UV absorption spectroscopy of MP was performed according to the method of Zhang et al.^[16] with minor modifications. The protein solution was diluted to 0.5 mg/mL, and its UV absorption spectrum was measured using a UV-visible spectrophotometer over a wavelength range of 230–350 nm.

2.4.4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted to analyse the protein molecular weight distribution according to the method of Chen et al.^[17] with minor modifications. First, the protein solution was diluted to 2.0 mg/mL with phosphate buffer. Next, 100 μ L of the diluted solution was mixed with an equal volume of 5 $\times$ loading buffer (10 g/100 mL SDS, 0.5 g/100 mL bromophenol blue; 25% Tris-HCl (0.25 mol/L), 50% glycerol, and 25% deionised water (V/V)), and the mixture was boiled for 5 min. Approximately 10 μ L of each sample was loaded onto a gel consisting of a 5% stacking gel and a 12% resolving gel. Electrophoresis was performed at 80 V for 20 min, and then the voltage was increased to 120 V until completion. The gel was stained for 15 min with Coomassie brilliant blue staining solution (0.1 g/100 mL Coomassie brilliant blue R-250; 6.8% acetic acid, 50% methanol, and 43.2% deionised water (V/V)), and destained

overnight in destaining solution (7.5% acetic acid, 5.0% methanol, and 87.5% deionised water (V/V)).

2.5 Determination of emulsion physical stability parameters

2.5.1 Preparation of O/W emulsions

O/W emulsions were prepared according to the method of Zhu et al.^[18] with minor modifications. The oil-to-aqueous phase ratio was 1:4 (V/V). MP was diluted to 5.0 mg/mL in 20 mmol/L phosphate buffer, and 40 mL of this solution was mixed with 10 mL of soybean oil. The mixture was homogenised using a high-speed homogeniser at 13 000 r/min for 1 min, with a 20 s pause every 20 s to prevent excessive temperature increase.

2.5.2 Determination of creaming index (CI)

CI was determined following the method reported by Cui et al.^[19] with minor modifications. Emulsion samples were transferred to sealed weighing bottles and allowed to stand at room temperature for 24 h. The thickness of the oil layer and the clear layer was measured, and photographs of the bottles were taken, at 0 and 24 h to record changes in the emulsion and aqueous layers.

2.5.3 Determination of particle size

Emulsion particle size was measured according to the method of Zhang et al.^[20] with minor modifications. Emulsions were diluted to 0.1 mg/mL with phosphate buffer, and particle size distribution was analysed using a Zetasizer Nano (Malvern Panalytical, UK). The relative refractive index was set to 1.09, corresponding to the ratio of the refractive index of soybean oil to that of the aqueous phase. The surface-weighted mean diameter ($D_{3,2}$) and volume-weighted mean diameter ($D_{4,3}$) of the emulsions were recorded.

2.5.4 Determination of ζ -potential and polydispersity index (PDI)

The ζ -potential of the emulsion samples was determined according to the method of Li et al.^[21] with minor modifications. Emulsions were diluted to 0.1 mg/mL with phosphate buffer, and measurements were performed using a ζ -potential analyser (Malvern Instruments Ltd., Malvern, UK). The refractive index was set to 1.33, and the sample was equilibrated for 120 s before measurement. Each sample was measured in triplicate, and the average value was used as the final result. The PDI was simultaneously recorded from the same measurement.

2.6 Molecular docking

The crystal structure of the target protein was retrieved from the RCSB Protein Data Bank (<https://www.rcsb.org>). Molecular docking was performed using AutoDock 4.2 software. The structures of the small-molecule surfactants were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/compound>) and optimised using Chem3D 16.0 software (Revvity, Inc., USA). Global docking was carried out, and the conformation with the lowest binding energy was selected. The interactions between each ligand (small-molecule surfactant) and the protein were visualised and analysed using PyMOL software (Schrödinger, Inc., USA).

2.7 Data processing

All experiments were performed in triplicate, and the results are expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using SPSS 27 (IBM SPSS Statistics, Chicago, IL, USA). One-way analysis of variance was employed to evaluate the

effects of surfactant type and addition level on emulsion stability, with Duncan's multiple range test applied for post hoc analysis at a significance level of $P < 0.05$. All figures were plotted using Origin 2021 (OriginLab Corp., USA).

3 Results and discussion

3.1 Surface hydrophobicity

The binding of small-molecule surfactants to MP typically induces protein unfolding, exposing internal hydrophobic groups^[22]. As shown in Table 1, the BPB binding amount did not differ significantly between the two surfactant-free control groups (control 2 and control 1 groups) ($P > 0.05$). Notably, the SDS-treated groups exhibited a sharp reduction in BPB binding capacity compared with the two surfactant-free control groups. The Tween-20-treated groups showed a concentration-dependent decrease, with little change at 0.1% and a substantial reduction at higher concentrations. In contrast, the SL-treated groups displayed only a modest reduction across all concentrations. This observation is consistent with that of Chang et al.^[23], who demonstrated that the presence of surfactant micelles can encapsulate proteins within the micelles, thereby reducing the surface hydrophobicity value.

Table 1 Influence of surfactant type and concentration on surface hydrophobicity of MP emulsion.

Surfactant addition (%)	BPB binding amount (μg)		
	Tween-20	SDS	SL
0 (control 1)	71.82 $\pm$ 0.60 ^{Aa}	71.13 $\pm$ 0.58 ^{Aa}	71.62 $\pm$ 0.33 ^{Aa}
0 (control 2)	71.42 $\pm$ 0.43 ^{Aa}	71.06 $\pm$ 0.21 ^{Aa}	71.98 $\pm$ 0.47 ^{Aa}
0.10	66.72 $\pm$ 0.15 ^{Bb}	18.87 $\pm$ 0.44 ^{Cb}	69.94 $\pm$ 0.85 ^{Ab}
0.25	62.60 $\pm$ 0.31 ^{Bc}	11.79 $\pm$ 0.73 ^{Cc}	65.53 $\pm$ 0.45 ^{Ac}
0.50	46.91 $\pm$ 0.43 ^{Bd}	08.80 $\pm$ 0.09 ^{Cd}	64.44 $\pm$ 0.17 ^{Ac}
1.00	37.99 $\pm$ 0.48 ^{Be}	06.93 $\pm$ 0.20 ^{Ce}	69.59 $\pm$ 0.23 ^{Ab}

Note: Values are the mean of three independent replicates $\pm$ SD. Different uppercase letters indicate significant differences among surfactant types (including the control) at the same addition level; different lowercase letters indicate significant differences among addition levels within the same surfactant type ($P < 0.05$).

The distinct effects of the three surfactants on surface hydrophobicity can be attributed to their different ionic natures and binding modes. SDS, as an anionic surfactant, possesses a hydrophobic carbon chain and a hydrophilic sulphate head group. Upon binding to MP, the sulphate head groups adsorb to the MP surface, increasing hydrophilicity, whilst the hydrophobic chains insert into the MP interior^[24]. This configuration renders the MP surface in the emulsion highly hydrophilic whilst shielding hydrophobic residues in the interior, which ultimately alters the spatial structure of MP. In contrast, nonionic surfactants such as Tween-20 lack a charged head group and cannot form this ordered arrangement of hydrophilic and hydrophobic regions on the MP surface, and therefore have a less pronounced effect on the surface hydrophobicity of the emulsion.

Notably, a rebound in BPB binding amount (to 69.59 μg) was observed in the 1.00% SL-treated group. This may be attributed to the formation of SL micelles under low ionic strength conditions, which can expose additional hydrophobic regions on the MP surface^[25]. Nevertheless, the BPB binding amount at 1.00% remained lower than that of the two control groups, indicating that the overall effect of SL was still a net reduction in surface hydrophobicity.

3.2 Intrinsic tryptophan fluorescence

The intrinsic fluorescence of proteins arises from aromatic amino acid residues (tryptophan and tyrosine), which emit fluorescence upon excitation at 280 nm^[12]. As shown in Figure 1, the two surfactant-free control groups exhibited characteristic peaks at 334 nm (control 1) and 336 nm (control 2), with the control 1 group showing higher fluorescence intensity. For Tween-20 (Figure 1a), the fluorescence intensity increased to a maximum at 0.25% and then decreased. For SDS (Figure 1b), the intensity peaked at 0.50%. For SL (Figure 1c), the intensity peaked at 0.10% and then decreased.

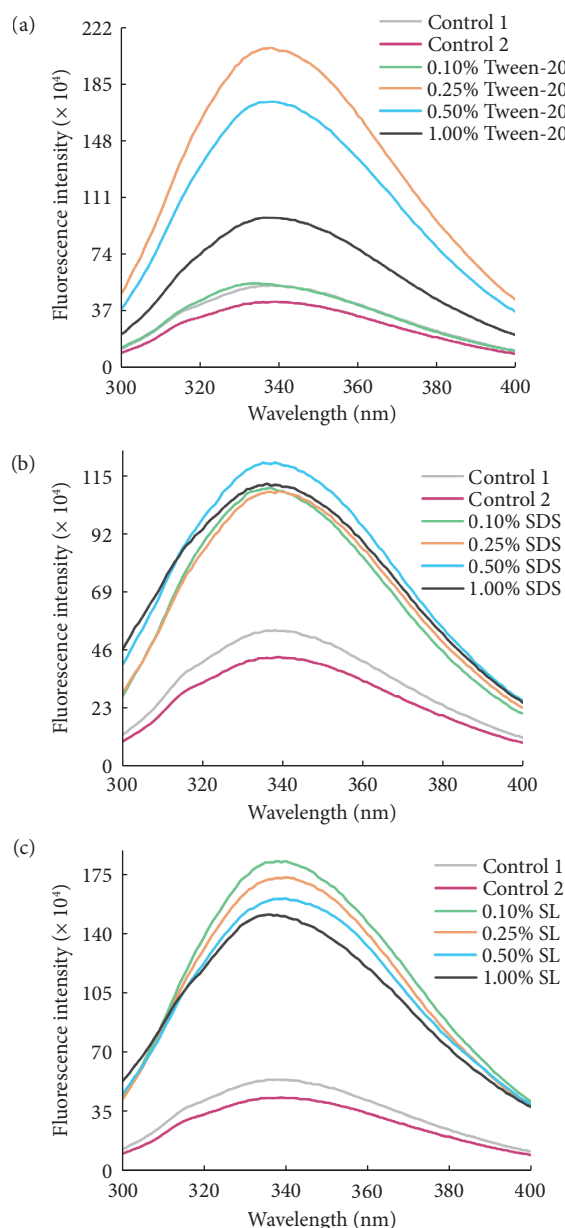


Figure 1 Fluorescence spectra of MP emulsions with (a) Tween-20, (b) SDS, and (c) SL at concentrations of 0%, 0.10%, 0.25%, 0.50%, and 1.00%.

The higher fluorescence intensity observed in the control 1 group compared with the control 2 group suggests a more compact MP conformation under lower ionic strength, in which tryptophan residues are less exposed to the aqueous environment^[26]. For the

surfactant-treated groups, the fluorescence intensity initially increased and then decreased with increasing concentration, with maximum intensities observed at 0.10% for SL, 0.25% for Tween-20, and 0.50% for SDS. This indicates that the three surfactants altered the tryptophan microenvironment to different extents. The subsequent decrease in intensity at higher concentrations suggests that excessive surfactant binding induces further conformational changes. $\lambda_{\max}$ also varied with surfactant type and concentration. Tween-20 and SL showed a slight red shift at low concentrations followed by a slight blue shift at higher concentrations, whereas SDS exhibited only a red shift across all concentrations. At low surfactant concentrations, weak non-covalent binding may induce partial unfolding, exposing tryptophan residues to a more polar environment and causing a red shift. At higher concentrations, strengthened interactions may promote the formation of compact protein-surfactant aggregates, resulting in a blue shift. SDS showed no blue shift, suggesting that its strong electrostatic binding maintains tryptophan residues in a more exposed state, even at high concentrations^[27].

BPB binding amount primarily reflects the accessibility of surface-exposed cationic residues and hydrophobic patches. Upon surfactant addition, surfactant molecules occupy or shield these sites, thereby reducing BPB accessibility and leading to an apparent decrease in surface hydrophobicity. This observation, however, does not necessarily indicate a loss of intrinsic hydrophobic character. In contrast, Trp fluorescence is highly sensitive to the local polarity around tryptophan residues. At low surfactant concentrations, surfactant binding-particularly via hydrophobic tail interactions-can displace water molecules from the vicinity of Trp residues, reducing the local dielectric constant and enhancing fluorescence intensity. As the surfactant concentration increases beyond a critical threshold, further binding may induce partial unfolding, exposing buried Trp residues to the aqueous polar environment, which leads to fluorescence quenching.

Notably, SDS-treated MP consistently exhibited elevated fluorescence across the tested concentration range, suggesting that within this concentration window, the local polarity reduction around Trp residues predominates over unfolding-induced quenching. For Tween-20 and SL, the progressive decline in fluorescence at higher concentrations likely reflects gradual conformational perturbation or enhanced dynamic quenching, which may be attributed to their different charge characteristics and hydrophobic chain lengths compared to SDS, leading to distinct binding modes and critical micelle behaviours. Thus, the observed trends are not contradictory but rather reflect sequential or competing events at different structural levels: surface site shielding (reduced BPB binding) versus local microenvironmental changes around Trp residues (altered fluorescence). This interpretation is consistent with previous reports on surfactant-protein interactions^[28].

3.3 UV absorption spectra

UV absorption spectroscopy in the near-UV region reflects changes in the microenvironment of amino acid side chains, which can be used to evaluate of protein conformational changes induced by small-molecule interactions^[29]. Figure 2 shows the UV absorption spectra of MP mixed with different small-molecule surfactants. All groups exhibited characteristic absorption peaks at 260–280 nm. As shown in Figure 2a, the peak wavelength of the Tween-20-treated

groups remained at approximately 272 nm, with no significant shift observed across the tested concentration range. However, the absorbance intensity initially increased with increasing Tween-20 concentration up to 0.50% and then decreased at 1.00%. In the SDS-treated groups (Figure 2b), the peak wavelength remained stable at 276 nm across all concentrations. For the SL-treated groups (Figure 2c), the peak wavelength shifted from 268 to 275 nm as the concentration increased from 0.10% to 0.50%, and then shifted slightly to 274–276 nm at 1.00%. Among the three surfactants, the SL-treated groups exhibited the highest UV absorbance, followed by the Tween-20-treated groups, whilst the SDS-treated groups showed the lowest.

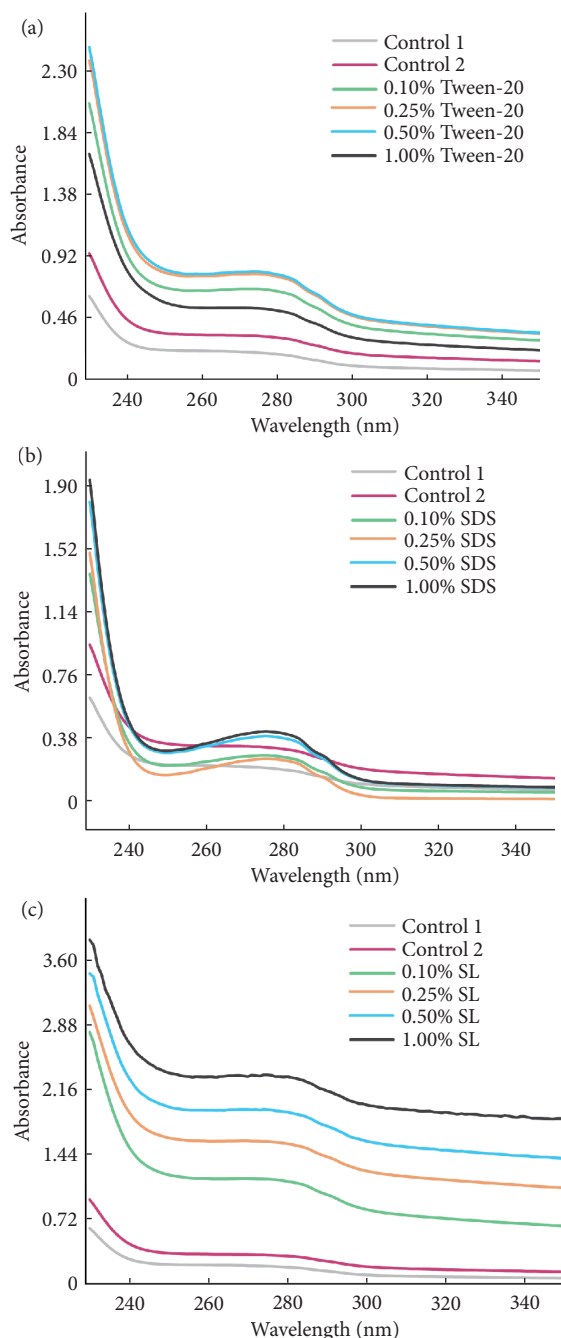


Figure 2 UV absorption spectra of MP emulsions with (a) Tween-20, (b) SDS, and (c) SL at concentrations of 0.10%, 0.25%, 0.50%, and 1.00%.

The reasons for the above phenomenon may be related to the influence of different surfactants on the MP microenvironment. In the Tween-20-treated groups, the absorption peak remained at approximately 272 nm with no significant shift across all tested concentrations, whereas the absorbance intensity first increased as the Tween-20 concentration rose to 0.50% and then decreased when the concentration was further raised to 1.00%. This trend likely reflects enhanced UV absorption due to non-covalent interactions between Tween-20 and MP at lower concentrations^[30], while the subsequent decrease at 1.00% may be associated with the presence of excess surfactant micelles. These micelles could alter the aggregation state of the protein-surfactant complexes, thereby affecting the microenvironment around the aromatic residues. In the SDS-treated groups, the characteristic peak remained stable at 276 nm across all concentrations. Compared with the control 1 group (272 nm), SDS shifted the peak to 276 nm, similar to the control 2 group, but no further concentration-dependent shift was observed. The continued increase in UV absorbance with rising SL concentration, in contrast to the decrease observed for Tween-20 at 1.00%, suggests that SL-MP complex formation may progressively enhance the effective local concentration or alter the microenvironments of chromophoric residues, and did not induce shielding effects at 1.00%. This is consistent with the ability of SL, as a natural phospholipid, to form stable complexes with MP, as also reported by Wu et al.^[31] for phospholipid-protein interactions.

3.4 SDS-PAGE analysis

SDS-PAGE separates proteins by molecular weight and visually reflects changes in protein subunits, such as aggregation, fragmentation, or degradation, through the size and staining intensity of protein bands^[32]. Figure 3 shows the SDS-PAGE patterns of MP treated with different small-molecule surfactants. High-molecular-weight aggregates appeared at the top of the gel in all MP samples. The two surfactant-free control groups exhibited relatively intact and clear protein bands that were consistent with the molecular weight markers. The three surfactants exerted different effects on MP. In the Tween-20-treated groups (0.10%–1.00%), no significant changes in band intensity were observed with increasing concentration. In the SDS-treated groups (0.10%–1.00%), however, the overall band intensity was markedly reduced, with particularly noticeable weakening of myosin heavy chain (MHC) and myosin light chain (MLC) bands at 0.25% and 1.00%. The SL-treated groups (0.10%–1.00%) also showed a significant decrease in band intensity, with the lowest intensity observed at 1.00%. This phenomenon may be attributed to the differential interactions between the three surfactants and MP. As a non-ionic surfactant, Tween-20 interacts weakly with MP and did not substantially alter the subunit structure at the concentrations tested, consistent with the unchanged band patterns. For SDS and SL, the reduced band intensity likely reflects surfactant-induced conformational changes and/or protein-surfactant complex formation in the original emulsion, rather than definitive subunit dissociation. However, the loading buffer used for SDS-PAGE contains a high concentration of SDS, which fully denatures and saturates proteins prior to electrophoresis. For samples pre-treated with SDS, the surfactant already bound to the protein may alter the final surfactant-to-protein stoichiometry during sample preparation, potentially affecting dye-binding capacity or the migration behaviour of the resulting complexes. Therefore, the reduced band intensity observed in the SDS-treated groups is more

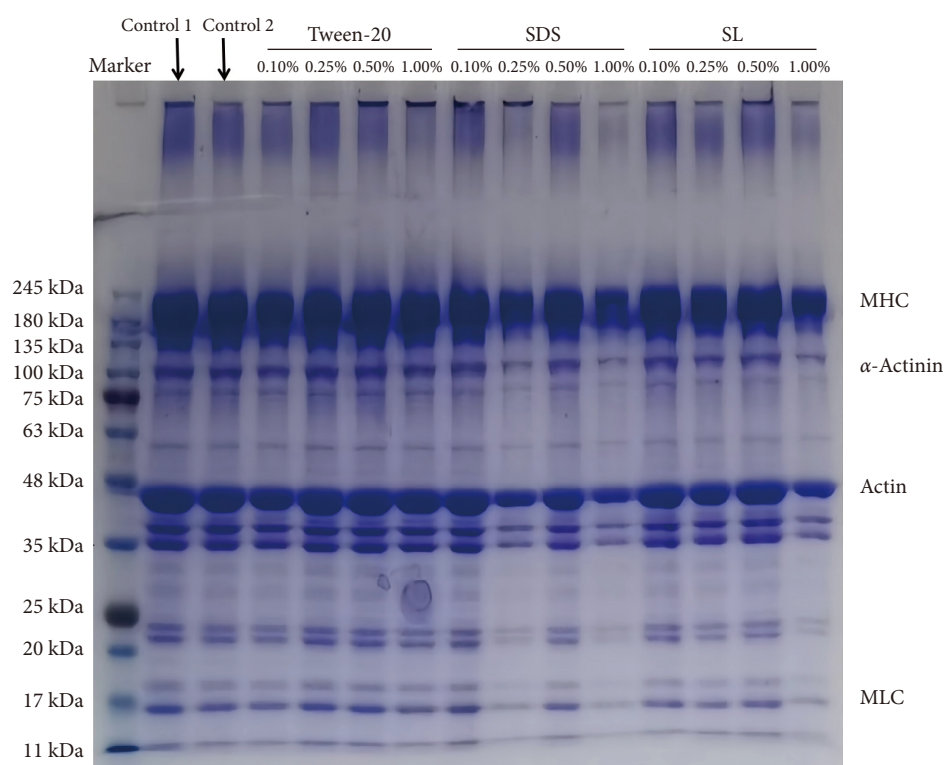


Figure 3 SDS-PAGE profiles of MP emulsions with different types and concentrations of small-molecule surfactants.

appropriately interpreted as evidence of extensive protein-surfactant interaction and conformational disruption, which may include partial dissociation and altered electrophoretic mobility, rather than complete subunit dissociation of MP in the original emulsion.

3.5 CI analysis

Emulsion stabilisation involves both steric stabilisation by the emulsifier adsorption layer and electrostatic repulsion between droplets, with steric stabilisation being the dominant mechanism. As shown in Figure 4, after standing for 24 h, no phase separation was observed in the control 2 group, whereas clear phase separation occurred in the control 1 group. In the Tween-20-treated groups, no phase separation was observed at 0.10%–0.50%, whilst slight phase separation was noted at 1.00%. In the SDS-treated groups, no phase separation was observed at 0.10%–0.50%, but obvious phase separation occurred at 1.00%. In contrast, all SL-treated groups exhibited phase separation across all tested SL concentrations.

This phenomenon may be attributed to the different mechanisms of action of the three surfactants. In the Tween-20-treated groups, at low concentrations (0.10%–0.50%), Tween-20 adsorbed onto the MP surface without disrupting the intermolecular forces between particles, thereby maintaining emulsion stability. At 1.00%, however, excess Tween-20 may have disrupted the adsorption equilibrium on the MP surface, resulting in slight phase separation^[3]. In the SDS-treated group, at low concentrations (0.10%–0.50%), SDS molecules adsorbed onto the MP surface, imparting negative charges that generated electrostatic repulsion and thereby maintained particle dispersion and emulsion stability. At 1.00%, however, SDS may have formed micelles that interacted with MP particles^[8], promoting aggregation and emulsion destabilisation, as evidenced by the obvious phase separation. As noted by Ravera et al.^[34], ionic surfactants at low concentrations can bind as monomers to specific sites on the native

state of proteins, thereby exerting a stabilising effect. In the SL-treated groups, phase separation occurred at all tested concentrations. This may be attributed to several factors. First, the negatively charged SL molecules formed a charge barrier on the MP particle surface, which may have enhanced intermolecular forces and promoted aggregation. Second, SL may have formed micelles that participated in emulsification, but its relatively weak surface activity and susceptibility to decomposition or condensation further compromised emulsion stability^[35].

3.6 ζ-Potential

The ζ-potential provides a measure of the electrostatic repulsion or attraction between particles and is therefore closely associated with the stability of colloidal systems. A higher absolute ζ-potential indicates stronger electrostatic repulsion, which favours particle dispersion and system stability; conversely, a lower absolute value suggests that attractive forces predominate, thereby promoting aggregation or coagulation^[36]. The results are presented in Figure 5a. All samples exhibited negative ζ-potentials. The control 2 group showed a higher absolute ζ-potential than the control 1 group. In the Tween-20-treated groups, the absolute ζ-potential decreased from 7.39 mV at 0.10% to 2.78 mV at 1.00%, showing a concentration-dependent reduction. In the SL-treated groups, the absolute value declined from 13.3 mV at 0.10% to 4.85 mV at 1.00%. By contrast, in the SDS-treated group, the absolute ζ-potential increased from 6.91 mV at 0.10% to 26.0 mV at 1.00%.

This observed variation may be attributed to differences in the interaction mechanisms between the various surfactants and the MP particles. In the control groups, the control 1 group exhibited a lower absolute ζ-potential, which was likely due to the lower ionic strength promoting self-aggregation among MP molecules, thereby reducing their solubility and dispersibility. Since Tween-20 is a

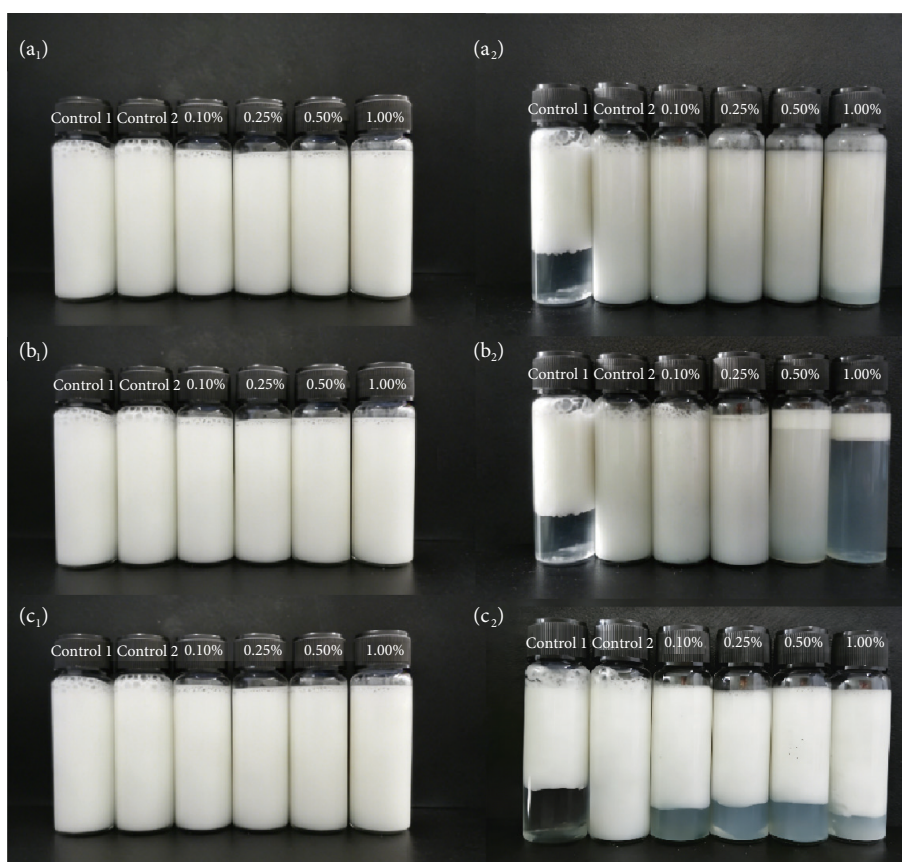


Figure 4 CI of MP emulsions with (a) Tween-20, (b) SDS, and (c) SL. Subscript 1, after 0 h of storage; subscript 2, after 24 h of storage. The visual appearance was used to determine the CI.

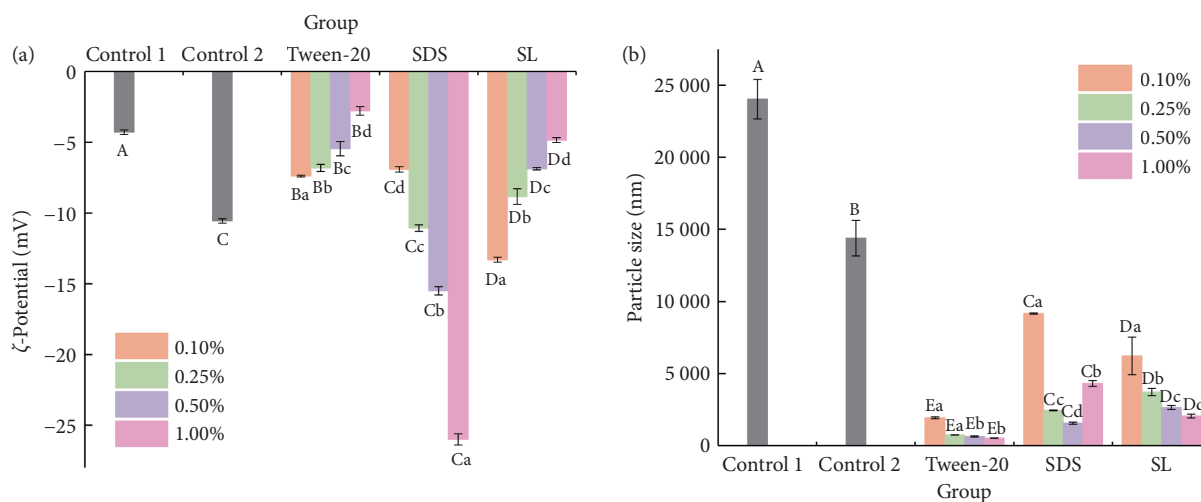


Figure 5 Effects of small-molecule surfactant types and concentration on the (a) ζ -potential and (b) particle size of emulsions. Different uppercase letters indicate significant differences among surfactant types (including the control) at the same addition level ($P < 0.05$); different lowercase letters indicate significant differences among addition levels within the same surfactant type ($P < 0.05$).

non-ionic surfactant, the observed decrease in ζ -potential may be ascribed to steric hindrance altering the shear plane, or to a dilution effect. As reported by Shen et al.^[37], Tween-20 adsorbs onto MP particle surfaces via hydrophobic interactions, leading to a reduction in the measured ζ -potential. The behaviour of SL was somewhat distinct: as a zwitterionic surfactant, its interaction with MPs likely involves both steric and charge-screening effects^[38]. In contrast, with increasing SDS concentration, the absolute ζ -potential rose from 6.91 (0.10%) to 26.0 mV (1.00%). This

increase is attributable to the anionic nature of SDS, which ionises and adsorbs onto MP particle surfaces; the extent of adsorption increases with concentration, thereby resulting in a higher absolute ζ -potential.

3.7 Particle size

Particle size can reflect the aggregation state of proteins and the size of their aggregates^[39]. The effects of different small-molecule surfactants on emulsion particle size are shown in Figure 5b. In the

two surfactant-free control groups, the emulsion particle sizes were relatively large: 24 036.67 nm for the control 1 group and 14 380 nm for the control 2 group. After the addition of small-molecule surfactants, the particle sizes of all emulsion groups decreased significantly ($P < 0.05$). With increasing Tween-20 concentration, the particle size of MP emulsions gradually decreased from 14 380 nm in the control 2 group to 522.43 nm under 1.00% Tween-20 conditions. For the SDS-treated groups, the particle size reached its minimum value (1 562 nm) at 0.50% concentration; when the SDS concentration further increased to 1.00%, the particle size rebounded to 4 315 nm. In the SL-treated groups, the particle size decreased progressively from 24 036.67 nm in the control 1 group to 2 052.33 nm under 1.00% SL conditions.

This may be attributed to the concentration-dependent adsorption behaviour of SDS on the MP particle surface. At low concentrations (0.10%–0.50%), SDS molecules primarily adsorbed onto the MP particle surface, imparting negative charges to the particles and enhancing electrostatic repulsion, thereby promoting dispersion and reducing particle size. At 1.00%, however, excess SDS molecules may have formed micelles in solution, which induced interparticle aggregation through depletion interactions or hydrophobic effects, causing the particle size to increase from 1 562 to 4 315 nm. For the SL-treated groups, as the SL concentration increased, more SL molecules adsorbed at the oil-water interface, and the resulting protein-surfactant complexes could more effectively cover the oil droplet surface and reduce interfacial tension, thereby continuously decreasing the emulsion particle size^[35].

3.8 PDI analysis

We also analysed changes in the PDI of the emulsions (Table 2). PDI reflects the width of the particle size distribution: a lower PDI value indicates a more uniform particle size distribution, while a higher PDI value indicates a broader distribution and poorer system homogeneity^[40]. As shown in Table 2, the control 2 group exhibited a significantly lower PDI than the control 1 group ($P < 0.05$), indicating a more uniform particle size distribution under high-salt conditions. Following the addition of small-molecule surfactants, the emulsion PDI generally increased. The PDI of the Tween-20-treated groups remained close to 1 with increasing concentration. In the SDS-treated and SL-treated groups, the PDI increased from 0.40 and 0.42 at 0.10% to 0.99 at 1.00%, respectively. Notably, the SL-treated groups exhibited decreasing particle size (from 24 036.67 to 2 052.33 nm) alongside decreasing absolute ζ -potential (from 13.3 to 4.85 mV), yet the PDI remained high.

The distinct PDI trends observed for the three surfactants can be explained by their different stabilisation mechanisms. For Tween-20, the high PDI may be attributed to a bimodal particle size distribution resulting from competitive displacement of interfacial

protein by the nonionic surfactant, producing low oil droplets alongside larger protein flocs^[41]. Despite the high PDI, the strong steric repulsion provided by the hydrophilic chains of Tween-20 prevented droplet coalescence, thereby maintaining emulsion stability. For SDS, the progressive increase in PDI corresponded to the initial decrease and subsequent rebound in droplet size, suggesting that excess SDS induced droplet aggregation at higher concentrations. For SL, the high PDI despite decreasing droplet size suggests that the system became more heterogeneous, possibly due to the formation of SL-MP complexes with varied compositions, which likely contributed to the phase separation observed at all SL concentrations.

3.9 Molecular docking simulation

Molecular docking was performed to predict the binding modes between the three surfactants and MP. As shown in Figure 6, the results revealed distinct binding mechanisms. Tween-20 was located within a binding cavity of myosin and bound to seven amino acid residues (Ile-578, Ala-585, Val-586, Cys-587, Tyr-462, Lys-564, and Leu-565) through hydrophobic interactions and van der Waals forces (Figures 6a–c). SDS bound to three amino acid residues (Met-675, Pro-309, and Tyr-100) via hydrophobic interactions and van der Waals forces, and additionally formed two hydrogen bonds with Asn-200 and Glu-159 (bond lengths: 3.3 and 1.6 Å, respectively) (Figures 6d–h). SL formed two hydrogen bonds with Lys-142 and Leu-141 (bond lengths: 2.6 and 3.5 Å), suggesting that hydrogen bonds may also contribute to stabilising the complex, and bound to five additional residues (Phe-451, Glu-216, Leu-143, Lys-142, Lys-657) through hydrophobic interactions and van der Waals forces (Figures 6i–l).

Overall, the binding between these surfactants and MP was mainly stabilised by hydrophobic interactions, van der Waals forces, and hydrogen bonds, with specific interactions varying by surfactant type. Tween-20 could occupy a binding cavity within myosin via hydrophobic anchorage involving seven amino acid residues, thereby stabilising the system predominantly through steric hindrance. In contrast, hydrogen bonds with Asn-200 (3.3 Å) and Glu-159 (1.6 Å, exceptionally short), which may suggest a high-affinity docking mode. This strong hydrogen bond may account for the pronounced unfolding effect of SDS on MP. Meanwhile, SL exhibited an intermediate binding behaviour, engaging both hydrogen bonding and hydrophobic contacts. Such mechanistic distinctions provide a molecular basis for understanding the differential effects of these surfactants on the emulsifying activity of MP. It should be noted that, in the absence of quantitative experimental validation, the molecular docking results should be regarded as supportive rather than conclusive evidence.

Table 2 PDI of MP emulsion with different types and concentrations of small-molecule surfactants.

Surfactants addition (%)	Tween-20	SDS	SL
0 (control 1)	0.97 ± 0.01 ^{Aa}	0.95 ± 0.08 ^{Aa}	0.96 ± 0.03 ^{Aa}
0 (control 2)	0.40 ± 0.04 ^{Ab}	0.41 ± 0.05 ^{Ac}	0.43 ± 0.06 ^{Ac}
0.10	1.01 ± 0.08 ^{Aa}	0.40 ± 0.08 ^{Bc}	0.42 ± 0.02 ^{Bc}
0.25	1.04 ± 0.07 ^{Aa}	0.45 ± 0.05 ^{Cc}	0.54 ± 0.06 ^{Bb}
0.50	1.09 ± 0.02 ^{Aa}	0.57 ± 0.02 ^{Bb}	1.00 ± 0.03 ^{Aa}
1.00	0.99 ± 0.02 ^{Aa}	0.99 ± 0.01 ^{Aa}	0.99 ± 0.01 ^{Aa}

Note: Values are means of three independent replicates ± SD. Different uppercase letters indicate significant differences among types at the same addition level; different lowercase letters indicate significant differences among addition levels within the same surfactant type ($P < 0.05$).

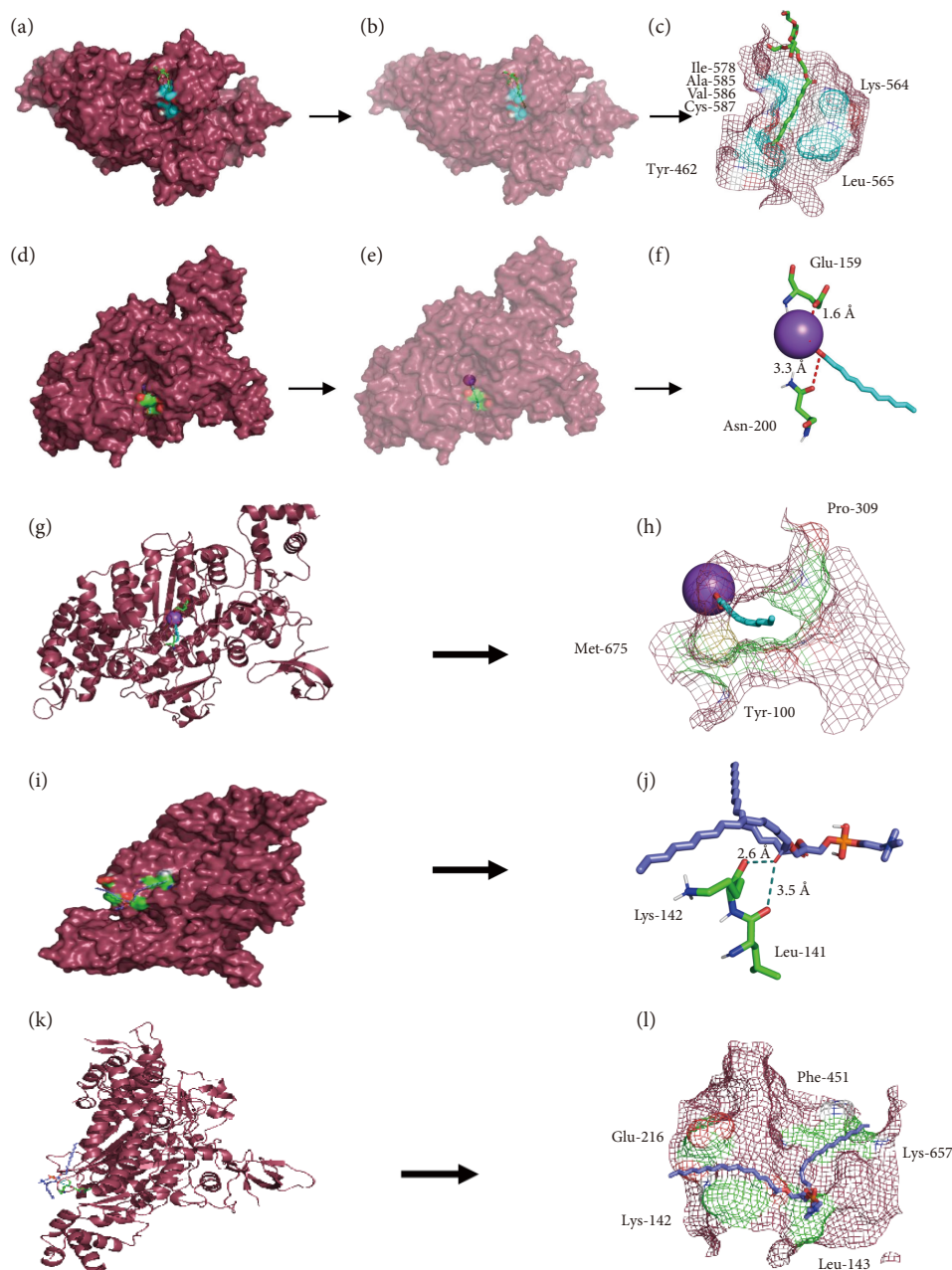


Figure 6 MD simulation of (a–f) Tween-20, (g–j) SDS, and (k–l) SL with myosin, respectively. (a, b, d, e, g, i, k) Overall surface binding modes; (c, f, h, j, l) detailed binding conformations and key interacting residues.

4 Conclusions

This study systematically elucidated the stabilisation mechanisms of three small-molecule surfactants, Tween-20, SDS, and SL, in MP-stabilised emulsions. Analyses of surface hydrophobicity, fluorescence spectroscopy, and UV absorption spectroscopy revealed that all three surfactants induced unfolding of MP structure and altered the microenvironment of internal aromatic amino acid residues, with SDS exerting the most pronounced effect on protein conformation. SDS-PAGE results further confirmed that SDS and SL markedly disrupted the structural integrity of myosin subunits, whereas the influence of Tween-20 was comparatively moderate. Molecular docking analysis indicated that Tween-20 associated with MP primarily via hydrophobic interactions, whereas both SDS and SL formed hydrogen bonds with specific amino acid

residues in addition to hydrophobic forces. In terms of emulsion stability, the Tween-20-treated group exhibited the optimal performance, followed by the SDS-treated group, whereas all SL-treated formulations displayed pronounced phase separation. This observation may be attributed to the fact that Tween-20, being non-ionic, engaged in weaker interactions with MP and likely formed a mixed adsorption layer at the oil-water interface through competitive displacement. In contrast, both SDS and SL were capable of forming protein-surfactant complexes with MP; moreover, at elevated concentrations, they could alter the surface charge characteristics of MP, thereby inducing protein aggregation and subsequent emulsion destabilisation. The surfactant type and concentration jointly influenced emulsion stability by synergistically modulating protein conformational changes and intermolecular interactions. Consequently, caution should be exercised when

employing ionic surfactants in the development of functional emulsion systems based on meat proteins. The present findings may offer useful guidance for the selection of appropriate surfactants in functional emulsion formulations intended for meat processing applications.

Conflict of interest

The authors declared that they have no conflicts of interest to this work.

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Author contributions

Huanhuan Xu: Writing-review & editing, conceptualization; Limei Mo: Validation; Mei Zhong: Data curation; Xiuwen Lin: Supervision; Linjing Gao: Investigation; Qisheng Zhang: Resources; Jinxing Liu: Resources; Jiaxin Chen: Funding acquisition, writing-review & editing; Wenwen Hou: Formal analysis, writing-review & editing.

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