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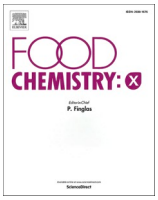

Synergistic cryoprotective effects of mannan oligosaccharides and curdlan on the grass carp surimi

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ABSTRACT

The cryoprotective effects of mannan oligosaccharides (MOS) and curdlan (CU) on the quality of grass carp surimi after freeze-thaw cycles (FTCs) were assessed using the response surface methodology. The optimal contents of MOS (6.79 %, *w*/w) and CU (0.45 %, w/w) produced minimum thawing losses and the highest gel strength of surimi after five times FTCs. MOS, CU, and their mixture demonstrated cryoprotective effects on grass carp surimi. Compared to MOS or CU alone, MOS-CU displayed synergistic cryoprotective effects, as evidenced by the better prevention of thawing losses of surimi, the superior retardation of the aggregation and denaturation of MP, the amelioration of the gel strength and WHC of surimi gel. Moreover, the MOS-CU mixture demonstrated cryoprotective effects equivalent to those of commercial cryoprotectant on grass carp surimi from zero to five times FTCs and even outperformed CC after seven times FTCs.

1. Introduction

Surimi, characterized by its high-protein and low-fat content, can be processed into various products with unique textures and flavors, including fish sausage, fish balls, and crab sticks. These characteristics have led to a gradual increase in the demand for surimi and surimi-based products in recent years. Surimi, which contains high levels of moisture and protein, is prone to microbial growth, resulting in a limited shelf life at room temperature. To address this challenge, freezing and cold chain logistics have been widely employed to inhibit microbial proliferation and slow down biochemical reactions, such as protein and lipid oxidation, thereby prolonging the quality guarantee period of surimi (L. Cai et al., 2020). Despite the benefits of freezing and cold chain logistics, temperature fluctuation during frozen storage and transportation can lead to repeated freeze-thaw cycles. These cycles can cause ice crystal formation and recrystallization, which in turn can induce protein denaturation and oxidation, lipid oxidation, and water migration in surimi, ultimately resulting in its quality deterioration (Sun et al., 2022). Therefore, there is a need to develop effective strategies to mitigate these issues and enhance the stability and quality of surimi products during frozen storage and transportation.

Commercial cryoprotectants, such as the mixture of 4 % sucrose and 4 % sorbitol (CC), are commonly utilized to mitigate the degradation of surimi quality during freezing-thawing. However, CC is characterized by high sugar and high calories, which imparts saccharine sweetness to surimi products and pose potential health risks to consumers. Given these drawbacks, there is a growing interest in developing healthier cryoprotectants to address these issues. Recent studies have extensively investigated the effects of low-sweetness and low-calorie saccharides, including Konjac glucomannan (Walayat et al., 2022) and chitosan (Dey & Dora, 2011) on the freezing and thawing stability of surimi. The main antifreeze mechanisms of polysaccharides and oligosaccharides can be summarized as follows: (a) The hydroxyl groups in the polysaccharides and oligosaccharides can bind with water molecules, inhibiting water mobility and reducing the proportion of freezable water of surimi, thereby controlling the ice crystal formation and preventing protein denaturation (Cao et al., 2022). (b) The hydroxyl groups on the polysaccharides and oligosaccharides chain form numerous hydrogen bonds with the ice interface, providing the necessary energy to inhibit ice crystal growth and recrystallization (W. Zhang et al., 2022). (c) Polysaccharides and oligosaccharides could interact with protein molecules through hydrogen bonding, hydrophobic interaction, and CH–π interaction, which is essential for stabilizing proteins and avoiding aggregation and destabilization during freezing and thawing (Sun et al.,

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2022). (d) Certain polysaccharides and oligosaccharides enhance the gelation properties of surimi, even during freezing and thawing, thereby exhibiting antifreeze effect (Sun et al., 2024). The effectiveness of novel cryoprotectants has been acknowledged, nevertheless, there is still a gap in their industrial production and application. Therefore, unflagging efforts are essential to explore more novel and effective cryoprotectants, particularly those are easily available and healthy.

Mannan oligosaccharide (MOS) is a low-grade polymeric sugar composed of 2–10 monosaccharide molecules. These molecules are linked by β-1,4-D-mannopyranosidic bond or α-1,2 or α-1,3 or α-1,6 glycosidic bond, forming linear or branched structures (Singh et al., 2018). MOS possess physiologically active functions, including improving intestinal flora structure and enhancing cognitive function (Liu et al., 2021). The high content of free hydroxyl groups in MOS facilitates the conversion of free water into bound water (Moreira & Filho, 2008), which is beneficial for stabilizing frozen food matrices. MOS also exhibits antioxidative abilities, which could enhance the stability of proteins and lipids in surimi during frozen storage (Xue et al., 2022). Given these properties, we hypothesize that MOS could serve as a potential cryoprotectant for surimi. However, compared with other oligosaccharides such as xylooligosaccharides (Walayat, Xiong, Xiong, Moreno, Li, et al., 2020), Konjac oligo-glucomannan (Walayat et al., 2022), trehalose and alginate oligosaccharides (B. Zhang et al., 2017), the cryoprotective effects of MOS in food system remain relatively unexplored.

Curdlan (CU) is a linear, non-branched bioactive polysaccharide colloid composed of β-1,3-glycosidic bonds (Wu et al., 2015). CU has been shown to significantly enhance the qualities of surimi gel by increasing hardness, chewiness, and water-holding capacity. These improvements are attributed to its ability to promote a denser and more ordered gel structure through enhanced hydrogen bonding and protein stability (Cai & Zhang, 2017; Wu et al., 2015; Zhang et al., 2024). Additionally, CU also shows significant potential as a cryoprotectant. It maintains the structural integrity and improves the physicochemical properties of frozen gluten (Liu et al., 2024; Zhao et al., 2023) and myofibrillar proteins in shrimp surimi (M. Lu et al., 2024). When combined with magnetic field-assisted freezing, CU facilitates the formation of smaller ice crystals. This process subsequently reduces the solubility, turbidity, and mean particle size of myofibrillar proteins in shrimp surimi. Moreover, this combination can also slow down the oxidation of myofibrillar proteins, maintaining high sulfhydryl content and stabilizing secondary and tertiary structures (Lu et al., 2024). Despite these promising findings, limited information is available regarding the influence of CU on the quality attributes of surimi during freeze-thaw cycles (FTCs).

The synergistic antifreeze effects of complex cryoprotectants have been reported in previous studies, such as a mixture of sorbitol and sodium tripolyphosphate (Yoo, 2014), egg white proteins and β-cyclodextrin (Walayat, Xiong, Xiong, Moreno, Niaz, et al., 2020), egg white proteins and xylooligosaccharides (Walayat, Xiong, Xiong, Moreno, Li, et al., 2020), whey protein isolate hydrolysates and CC (sucrose and sorbitol) (Li et al., 2013), salted egg white and CC (Yu et al., 2022), etc. Despite these efforts, no prior studies have specifically focused on the synergistic cryoprotective effects of mannan oligosaccharide (MOS) and curdlan (CU).

Therefore, this study aimed to investigate the cryoprotective effects of the combination MOS and CU on the qualities of grass carp surimi during FTCs, and optimize the formulation of complex cryoprotectants (MOS and CU) using the response surface methodology (RSM). Additionally, the optimized composite cryoprotectants on the changes of thawing losses, water mobility, gel properties, and protein structure of MP in grass carp surimi were evaluated during FTCs. This research can provide valuable data for the combined utilization of MOS and CU as a novel cryoprotectant in the surimi processing industry, potentially improving product quality.

2. Material and methods

2.1. Materials and reagents

Fresh grass carp was purchased from a nearby RT supermarket (Changsha, China). Mannan oligosaccharides, sucrose, and sorbitol were obtained from Shanghai Yuanye Biotechnology Co., Ltd., Sinopharm Chemical Reagent Co., Ltd., and Beijing Solarbio Science & Technology Co., Ltd., respectively. Curdlan was sourced from Jiangsu Yiming Biological Technology Co., Ltd. All other chemicals utilized were of analytical grade from Sinopharm Chemical Reagent Co., Ltd.

2.2. Preparation of surimi sample

Surimi was prepared according to method (Mo et al., 2024). Fresh fish heads and tails were removed, and only the white meat was used. The white meat was mixed with five times its volume of deionized water and subjected to centrifugation (5810R; Eppendorf AG, Hamburg, Germany) for 15 min (11,000 g, 4 °C). This procedure was repeated twice. Afterward, the precipitation was resuspended in a 0.5 % NaCl solution and centrifuged again under the same condition for another 15 min. Cryoprotectant including MOS (6.79 %, *w*/w), CU (0.45 %, w/w), MOS-CU (6.79 %–0.45 %, w/w), and commercial cryoprotectants (CC, 4 % sucrose and 4 % sorbitol, w/w) were added to surimi. The moisture content of the surimi was adjusted to 76.5 %. A control group was established using surimi without any added cryoprotectant.

2.3. Freeze-thaw cycles (FTCs) of surimi sample

FTCs of the surimi sample were in the following procedures. The samples were frozen at −18 °C (7 days) and then thawed at 4 °C (12*h*). This process was a freeze-thaw cycle (FTC). The FTCs were repeated 1, 2, 3, 5, and 7 times, with the corresponding surimi samples labeled as FTC1, FTC2, FTC3, FTC5, and FTC7, respectively. Surimi samples prepared as described in Section 2.2 but not subjected to freezing were designated as FTC0.

2.4. Response surface experimental design

Response surface methodology (RSM) was utilized to study the cryoprotective effects of the independent variables (mannan oligosaccharide, curdlan) on the evaluated response variables (gel strength and thawing losses of surimi after FCT5). The amount of antifreeze agent was optimized using a central composite design (CCD) with five levels for each variable, coded as −1.41, −1.00, 0.00, 1.00, and 1.414, respectively (Table S1). The experimental design comprised 13 points, including five replications at the center point. Two response variables were evaluated: gel strength (Y_1) and thawing losses (Y_2). Design Expert Version 10.0.7 was used to obtain design experiments based on CCD. The RSM methodology was practiced to achieve multi-objective optimization including 1) minimizing the thawing losses of surimi and 2) maximizing the gel strength of surimi after FCT5.

2.5. Moisture mobility measurement

The moisture mobility of surimi was measured using an LF-NMR analyzer (NMI20, Niumag Electric Co., China) (Yu et al., 2022). The parameters were set: TW, 2500; NECH, 4000; NS, 8. The inversion method is a simultaneous iterative reconstruction technique (SIRT).

2.6. Thawing losses

The samples were weighed prior to freezing, and their mass was recorded as M_1. The thawed samples were wiped with filter paper and weighed again; the mass of the thawed samples was then recorded as M_2(Cao et al., 2022):

$$Thawing\ loss(\%) = \frac{M_1 - M_2}{M_1} \times 100 \quad (1)$$

2.7. Myofibrillar proteins (MP) extraction and the determination of protein content

MP was extracted from surimi after each FTC (Mo et al., 2024). The thawed surimi was homogenized in tenfold volume of Tris-maleate buffer (50 mmol/L NaCl-20 mmol/L Tris-maleate, pH 7.0), followed by centrifugation for 15 min (10,000, 4 °C), and the supernatant was poured out. The above operation was repeated twice. The resulting precipitate was added in 3 times volume of Tris-maleate buffer (0.6 mmol/L NaCl-20 mmol/L Tris-maleate pH 7). The mixture was then incubated at 4 °C for 1 h and subsequently centrifuged for 15 min (10,000, 4 °C). The obtained supernatant was MP solution. The content of MP was determined by the Biuret method.

$$\text{MP content}\left(\frac{mg}{g}\right) = \frac{CxV}{g} \quad (2)$$

C: MP concentration, V: the volume of the MP, and g: the weight of surimi used to extract MP.

2.8. Total and active sulfhydryl content of MP

The determination of total and active sulfhydryl groups using Ellman's reagent with slight modifications (Zhou et al., 2006). The diluted sample (4 mg/mL, pH 8) was mixed with 4 mL Ellman reagent and 0.5 mL Tris-HCl buffer, mixed thoroughly, and reacted at 40 °C for 15 min. The absorbance was determined at 412 nm with 0.6 mol/L KCl solution as blank. For active sulfhydryl content was taken as 2.25 mL of diluted MP solution and mixed with 15 μL of Ellman's reagent, incubated at 4 ± 1 °C for 1 h, and the absorbance was measured at 412 nm using a UV-2660 spectrophotometer (Shimadzu, Kyoto, Japan).

$$sulfhydryl\ content = \frac{A}{C} \times D \quad (3)$$

where A is the absorption value, C is the extinction coefficient (13,600 M^{-1} cm^{-1}), D is the dilution number, and the sulfhydryl content is expressed as micromoles per 10^5 g of protein.

2.9. Surface hydrophobicity of MP

The surface hydrophobicity of the MP was assessed using an ANS probe (Yin et al., 2019). Four different protein concentrations (0.5, 0.25, 0.125, and 0.0625 mg/mL) were prepared for each MP sample. Subsequently, 4 mL of each MP solution was mixed with 20 μL of ANS solution (8.0 Mm ANS, 0.01 M Tris-HCl, pH 8.0). Fluorescence intensity was measured using a fluorescence spectrophotometer (G9800A; Agilent Technologies, Sanata Clara, CA, USA). The parameter was follows: excitation wavelength: 374 nm; emission wavelength: 485 nm. Finally, the initial slope of the fluorescence intensity against MP concentration curve represents surface hydrophobicity.

2.10. Endogenous fluorescence intensity of MP

The tertiary structure changes of MP were determined as described by (Yu et al., 2022).Adjust the concentration of MP solution to 0.05 mg/mL using 0.6 mol/L KCl. Tryptophan residues test conditions: excitation wavelength, 295 nm; excitation and emission slit width, 5 nm; emission wavelength, 305–400 nm; scanning speed, 12,000 nm/min. Tyrosine residues test conditions: excitation wavelength at 280 nm and emission wavelength range from 290 to 420 nm.

2.11. Preparation of surimi gel

The method of surimi gel preparation was according to the procedure (Mo et al., 2024). At each FTC, place the thawed surimi into a blender (model 3205; De'Longhi, Treviso, Italy) and blend for 2 min. Then, add 2.5 % NaCl and continue chopping for 3 min. Transfer the prepared surimi paste into a casing with a diameter of 20 mm. Next, prepare the surimi gel by following this procedure: heat it for 60 min at 40 °C, then immediately at 90 °C for another 30 min. After heating, cool the mixture in cold water and refrigerate it at 4 °C.

2.12. Puncture properties of surimi gel

The puncture properties of surimi gel were assessed by a texture analyzer TA-XT plus (Stable Micro Systems, Godalming, UK) with slight modifications (Xie et al., 2024). The measurement was performed with a P/0.5 s spherical probe at a test speed of 1 mm s^{-1} and a puncturing distance of 15 mm. Breaking force and deformation distance were recorded. Gel strength (g*mm) was calculated by breaking force (g) and deformation distance (mm).

2.13. Water holding capacity (WHC)

Surimi gel was cut into thin slices 0.5 cm thick (W_1) and subsequently layered between two sheets of filter paper. The prepared sample was centrifuged at 3000 rpm for 15 min at 4 °C and weighed again (W_2) after removing the wrapped filter paper. The WHC was then determined using the following calculation (Walayat et al., 2022):

$$WHC(\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (4)$$

2.14. Statistical analysis

All indicators were measured at least three times in parallel and the results were expressed as mean ± standard deviation. Plots were made using Origin 2018 and SPSS Statistics 22 was used for data significance analysis ($p < 0.05$).

3. Results and discussion

3.1. Optimization of the composite cryoprotectants and experimental validation

Gel strength is a critical parameter for assessing the edible qualities of surimi products(C. Zhang et al., 2024), and thawing losses is a crucial factor influencing the product production yield(M. Lu et al., 2024). During freezing and thawing, the gel strength of surimi always descends accompanied by the accelerating thawing losses. The gel strength (Y_1) and thawing losses (Y_2) of surimi after FTC5, as affected by the independent variables (MOS (A), CU (B), are presented in Table S2. The gel strength of surimi after FTC5 ranged from 1467.99 to 2167.85 g•mm, while the thawing losses ranged from 0.90 % to 2.75 %. RSM-CCD was employed to obtain the optimal mixture formulation of the composite cryoprotectants. The second-order polynomial equation was applied to describe the correlation between the dependent and independent variables, as shown in Eq.5 and Eq.6.

Y_1=2073.24+213.33*A-40.10*B-6.44*AB-104.69*A^2–251.33*B^2 (5).

Y_2=0.97–0.36*A+0.28*B-0.26*AB+0.28*A^2+0.62*B^2 (6).

Where Y_1 and Y_2 are the desirability values of gel strength and thawing losses after 5 FTCs. A and B are the coded values of the MOS, CU.

The coefficients of determination (R^2) for the gel strength and the thawing losses obtained by regression modeling were 0.9821 and 0.9873, respectively. This suggests that the quadratic polynomial model

fits the experimental data well. The regression parameters obtained for ANOVA are shown in Table S3. The model p (<0.0001) along with the p (>0.05) for lack of fit for both models presented implied that the quadratic model achieved an adequate fit for both responses.

Regarding gel strength, the linear terms (A, B) and quadratic terms (A^2, B^2) were significant (p<0.05), whereas the interaction term (AB) was insignificant (p>0.05). Fig. 1(A) and 1(B) show the effects of MOS and CU on the gel strength of surimi after FTC5. The gel strength initially increased with rising MOS content (2.010 % to 6.874 %, w/w) and CU content (0.201 % to 0.454 %, w/w), but subsequently decreased as the concentrations of MOS and CU continued to increase. MOS or CU absorbs water and expands during heating, filling the surimi gel network (Wu et al., 2015). Nevertheless, excessive MOS or CU will form a large number of gel clumps during heating, which may hinder the contact between hydrogen bonds or disulfide bonds, thus destroying the gel network (H. Liu et al., 2024). Several studies have shown that CU can markedly improve the textural qualities (such as the gel strength, resilience, and springiness) of surimi gels, along with the water-holding capacity (Hu et al., 2015; M. Lu et al., 2024).

Regarding thawing losses, the linear terms (A, B), interaction terms (AB) and quadratic terms (A^2, B^2) were all significant (p<0.05). As shown in Fig. 1(C) and Fig. 1(D). The thawing losses of surimi decreased with the increase of MOS content (2.010 % to 6.268 %, w/w) and CU content (0.201 % to 0.485 %, w/w) and then increased with a further raising of MOS and CU content. Since MOS and CU possess a large amount of hydroxyl group, they could form more hydrophobic interactions and hydrogen bonds with protein and water during FTCs. In a similar study, Xie et al. (Xie et al., 2024)also found that tamarind seed polysaccharide (TSP) could prevent the texture deterioration in surimi gel during FTCs, since a regular gel network was formed between TSP and protein through hydrogen bonding. However, excessive amounts of MOS and CU increased the thawing losses of surimi. This observation is consistent with findings by Lu et al., (M. Lu et al., 2024), who speculated that the excessive CU interfered with protein interactions in surimi, disrupting the ordered network structures, hence the thawing losses of surimi containing 0.8 % CU was much higher than that of surimi containing 0.6 % CU.

The optimum mixture formulation of the composite cryoprotectants for obtaining maximum gel strength and minimum thawing losses of surimi using the RMS-CCD model was 6.79 % MOS and 0.45 % CU. For each 100 g of surimi, MOS-CU was incorporated to decrease CC by 131.81 KJ. The experimental gel strength and thawing losses at the optimum mixture formulation were 2191.31 ± 42.04 g•mm and 0.85 %, respectively, comparable with the predicted gel strength of 2175.36

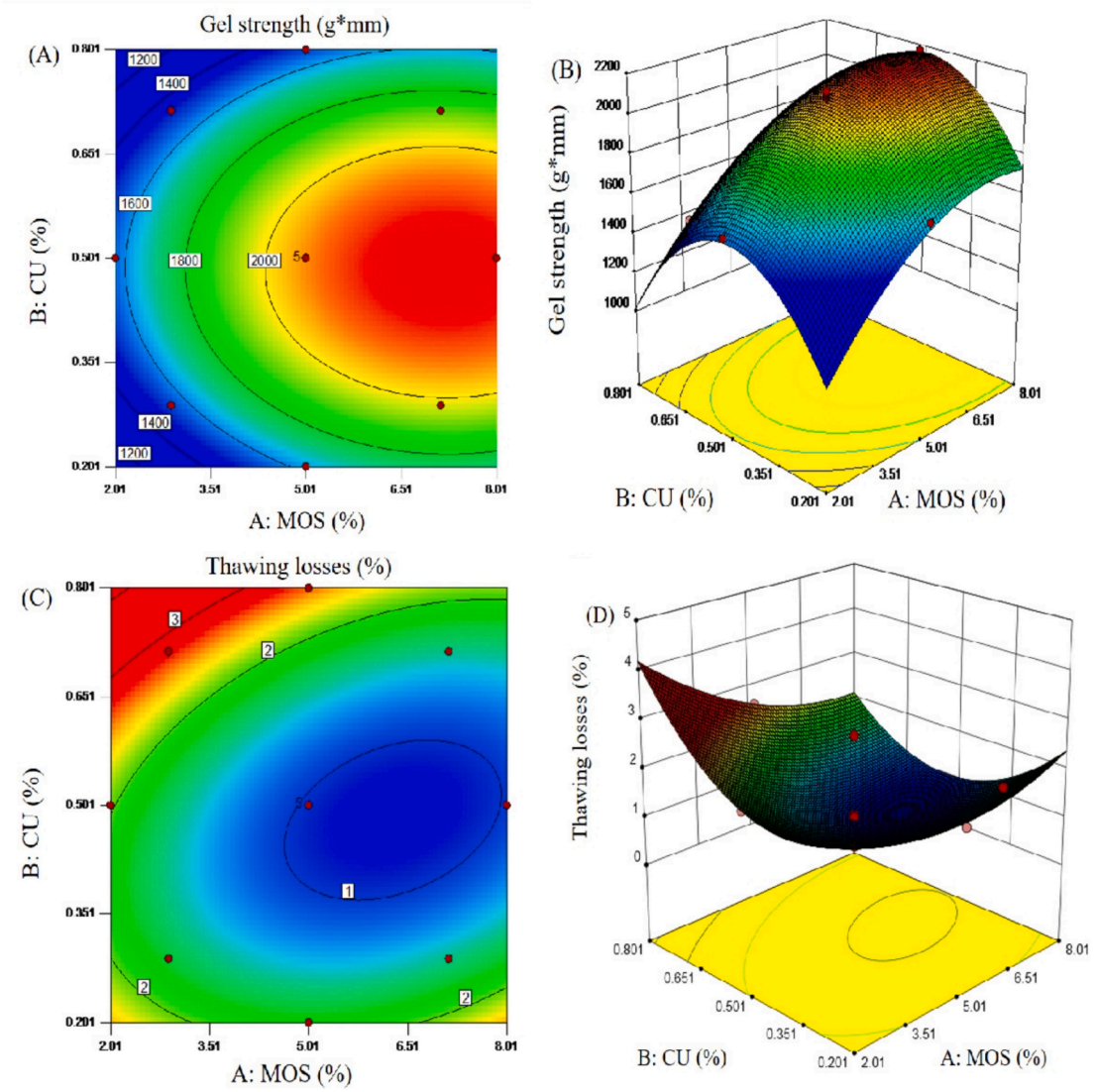

Fig. 1. Contour plots (A) and 3D surface plots (B) of gel strength and contour plots (C) and 3D surface plots (D) of thawing losses of surimi after 5 freeze-thaw cycles. Note: MOS, mannan oligosaccharide; CU, curdlan.

g•mm and thawing losses of 0.89 %. These findings confirmed that Eq.5 and Eq.6 were well-suited for predicting surimi gel strength and thawing losses after FTCs.

3.2. Freeze-thaw stability of surimi with the optimized cryoprotectants of MOS and CU

3.2.1. Thawing losses of surimi

The thawing losses of surimi increased with prolonged FTCs ($p<0.05$), as illustrated in Fig. 2(A). Upon thawing, the ice crystals in surimi melt and proteins undergo denaturation. Consequently, partial bound water of surimi converted into free water, which cannot be reassociated with protein, contributing to the elevated thawing losses (Xie et al., 2024). In the FTC1, the thawing losses were significantly lower for the MOS (0.42 %), CU (0.69 %), MOS-CU (0.33 %), and CC (0.38 %) groups compared with the control group (1.28 %), indicating that cryoprotectants can effectively inhibit the thawing losses, and the MOS-CU exhibited the lowest thawing losses ($P<0.05$). The thawing losses of MOS, and CU groups exhibited insignificant differences during FTC5 ($P>0.05$). However, after FTC7, the thawing losses (CC group) reached 5.43 %, markedly surpassing the losses observed in the MOS, CU, and MOS-CU ($P<0.05$). These findings revealed that MOS and CU could effectively prevent the increase of thawing losses of surimi during FTCs, and the performance of the optimized composite cryoprotectants (6.79 % MOS-0.45 % CU) surpassed that of commercial cryoprotectants. According to Zhao et al., (Zhao et al., 2023), curdlan could restrict the conversation of bound water into freezable water in frozen dough due to its superior water-retention ability. Analogous findings were reported by Zhang et al., (B. Zhang et al., 2017; B. Zhang, Fang, et al., 2018; B. Zhang, Hao, et al., 2018; B. Zhang, Zhang, et al., 2018) who provided that Xylo oligosaccharides, trehalose, alginate oligosaccharides, carrageenan oligosaccharides, and Kappa-carrageenan oligosaccharides could substitute water molecules surrounding myosin by forming hydrogen bonds with the amino acids' polar residues of, ultimately enhancing the water holding ability of myosin and reducing the thawing losses of frozen shrimp. Therefore, it is reasonable to speculate that the presence of hydroxyl groups in MOS and CU could be intact with water and protein by hydrogen bonds and reduce the content of freezable water in surimi, thereby inhibiting ice recrystallization and retarding the thawing losses of surimi during FTCs. Furthermore, CU exhibits high viscosity, which may alter the hydration behavior of protein in surimi and restrict the growth of ice crystals.

3.2.2. Moisture mobility of surimi

Low-field NMR measured the relaxation time (T2), which is typically associated with the degree of freedom in hydrogen protons (M. Lu et al., 2024). As shown in Fig. 2(B), as FTCs increased, T_{21} and T_{23} of surimi increased, while T_{22} decreased. This phenomenon suggests that moisture migration occurs in surimi. The MOS, CU, MOS-CU, and CC groups exhibit a gradual decline in T_{22} compared to the control group, indicating an improvement in the water-holding capacity of surimi. Additionally, repeated FTCs accelerate recrystallization, contributing to heightened protein denaturation and thawing losses. As shown in Table 1, A_{22} of surimi decreased with the prolonged FTCs, while A_{21} and A_{23} raised. After 7 FTCs, compared with the control group, the MOS, CU, MOS-CU, and CC groups significantly delayed the decrease of A_{22} ($p<0.05$). These results showed that MOS and CU inhibited the loss of partially immobilized water in surimi, and the performance of the optimized composite cryoprotectants (6.79 % MOS-0.45 % CU) surpassed that of CC. During repeated FTCs, partially immobilized water converts into free water, which accelerates the recrystallization and may destroy protein structure, thereby giving rise to the quality deterioration of surimi. However, the combination of MOS and CU hindered the mobility of water within the surimi, inhibited ice crystal recrystallization, and minimized the thawing losses of surimi during FTCs. This finding was consistent with the results of thawing losses of surimi (Fig. 2 (A)). It may be because MOS has abundant hydroxyl groups and CU has a high viscosity, which can firmly bind water molecules through ionic and hydrogen bonds. Consequently, this binding optimizes the distribution of water molecules within the entire structure of surimi proteins, thus enhancing the stability during FTCs.

3.2.3. MP content of surimi

MP, which accounts for 50 % to 60 % of the total protein in surimi, is vital for the gel-forming ability of surimi (S. Li et al., 2022). AS FTCs increased, the MP content in each group showed a downward trend

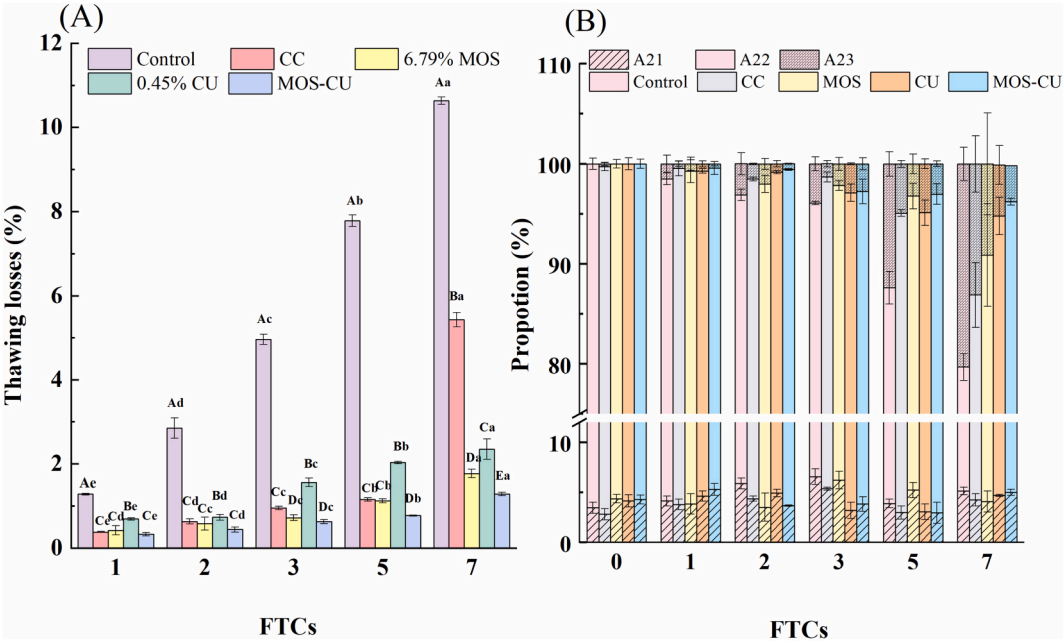

Fig. 2. Effect of MOS, CU, MOS-CU or CC on the thawing losses (A) and moisture mobility (B) of surimi during the freeze-thaw cycles (0, 1, 2, 3, 5 and 7). Different uppercase letters indicate significant differences between different antifreeze agents for the same number of freeze-thaw cycles ($p < 0.05$), and different lowercase letters indicate significant differences between different freeze-thaw cycles for the same antifreeze agent ($p < 0.05$).

Table 1
Proportion of water peak area during FTCs of surimi.

	FTCs	Control	CC	MOS	CU	MOS-CU
A_{21}(%)	0	3.46 ±0.56^{c}	2.82 ±0.56^{d}	4.36 ±0.42^{b}	4.15 ±0.60^{ab}	4.29 ±0.45^{ab}
	1	4.14 ±0.50^{c}	3.79 ±0.52^{bc}	3.85 ±1.03^{b}	4.63 ±0.50^{a}	5.29 ±0.59^{a}
	2	5.87 ±0.54^{ab}	4.37 ±0.25^{b}	3.52 ±1.40^{b}	4.92 ±0.37^{a}	3.69 ±0.07^{bc}
	3	6.56 ±0.77^{a}	5.36 ±0.16^{a}	6.23 ±0.88^{a}	3.20 ±0.81^{bc}	3.83 ±0.72^{bc}
	5	3.89 ±0.43^{c}	2.98 ±0.65^{cd}	5.23 ±0.77^{ab}	3.07 ±0.78^{c}	2.96 ±1.06^{c}
	7	5.14 ±0.37^{b}	4.26 ±0.61^{b}	4.08 ±1.05^{b}	4.69 ±0.10^{a}	4.99 ±0.31^{a}
A_{22}(%)	0	96.54 ±0.56^{a}	96.91 ±0.41^{a}	95.64 ±0.42^{a}	95.85 ±0.60^{a}	95.71 ±0.45^{a}
	1	94.37 ±0.59^{b}	95.75 ±0.75^{ab}	95.41 ±1.13^{a}	94.66 ±0.21^{a}	94.29 ±0.64^{b}
	2	91.02 ±0.58^{c}	94.11 ±0.18^{bc}	94.46 ±0.85^{a}	94.26 ±0.17^{a}	95.74 ±0.10^{a}
	3	89.52 ±0.17^{c}	93.33 ±0.49^{bc}	91.61 ±0.50^{a}	93.92 ±0.87^{a}	93.42 ±1.22^{b}
	5	83.73 ±1.63^{d}	92.10 ±0.31^{c}	91.56 ±1.27^{a}	92.05 ±1.26^{b}	94.02 ±1.04^{b}
	7	74.55 ±1.34^{e}	82.65 ±3.24^{d}	86.82 ±5.13^{b}	90.10 ±1.88^{c}	91.23 ±0.34^{c}
A_{23}(%)	0	/	0.27 ±0.17^{c}	/	/	/
	1	1.49 ±0.87^{e}	0.46 ±0.25^{c}	0.74 ±0.67^{b}	0.71 ±0.31^{c}	0.42 ±0.23^{c}
	2	3.12 ±1.11^{cd}	1.52 ±0.07^{c}	2.02 ±0.55^{b}	0.82 ±0.33^{c}	0.58 ±0.06^{c}
	3	3.92 ±0.69^{c}	1.32 ±0.33^{c}	2.15 ±0.65^{b}	2.88 ±0.09^{b}	2.75 ±0.60^{b}
	5	12.37 ±1.21^{b}	4.92 ±0.34^{b}	3.21 ±0.98^{b}	4.88 ±0.49^{a}	3.02 ±0.29^{ab}
	7	20.31 ±1.67^{a}	13.09 ±2.82^{a}	9.09 ±5.10^{a}	5.11 ±1.94^{a}	3.59 ±0.02^{a}

Different lowercase letters in the same column represent data that are significantly different ($p < 0.05$).

(Fig. 3(A)), indicating that the surimi underwent denaturation during the FTCs. Meanwhile, after FTC5, the decrease rates of CC, MOS, CU, and MOS-CU groups were 57.06 %, 58.65 %, 61.98 %, and 52.64 %, respectively, all significantly lower than the control group of 81.36 % ($p<0.05$). These results indicate that MOS, CU, MOS-CU, and CC can effectively inhibit the structural changes of MP. This inhibition may be attributed to a significant reduction in the size of ice crystals formed during the freezing process following the addition of cryoprotectants, thereby preventing the irreversible destruction of MP (Walayat et al., 2021). After FTC7, there is no significant difference in MP content between CC and MOS-CC. Hence, regarding MP, the cryoprotective effectiveness of MOS-CU is equivalent to that of CC, which protects surimi protein from denaturation and aggregation during FTCs to some extent. Zhang et.al (T. Zhang et al., 2023) also found that there was no significant difference in salt-soluble protein content of grass carp surimi, added with konjac glucomannan or CC ($p>0.05$). They attributed this result to the destruction of MP structure during FTCs, which exposes hydrophobic amino acids and accelerates MP aggregation and denaturation through hydrophobic interactions.

3.2.4. Conformation and structure of myofibrillar proteins (MP) in surimi

Total and active sulfhydryl groups.

The concentration of total sulfhydryl (SH_T) and active sulfhydryl (SH_A) groups of MP are depicted in Fig. 3(B) and (C), respectively. SH_T and SH_A contents of MP in surimi dropped drastically, especially within the first three FTCs. This decline can be attributed to the oxidation of sulfhydryl group into disulfide bond during the FTCs, which induces cross-linking between MP and decreases sulfhydryl group content(Yin et al., 2019). Within 3 FTCs, SH_T and SH_A contents of MP in the control group declined rapidly from 10.23 mol/10^5 g to 2.84 mol/10^5 g and from 4.39 mol/10^5 g to 1.30 mol/10^5 g, with descent rate of 72.24 % and 70.39 %, Whereas the decrease rates of the CC, MOS, CU, and MOS-CU groups were 55.34 % and 55.10 %, 60.21 % and 49.17 %, 46.78 % and 48.07 %, and 52.34 % and 38.78 %, respectively. During FTCs, the MP structure is damaged by ice crystals, exposing its internal reactive sulfhydryl groups which are oxidized to form disulfide bonds, thereby reducing the levels of SH_T and SH_A. This finding aligns with Liu et al.,(J. Liu et al., 2019), which found that after 50 days of freezing, 0.5 % konjac oligo-glucomannan can inhibit MP denaturation in surimi and slow the reduction of sulfhydryl content. After FTC7, the highest amount of SH_T and SH_A of MP was observed in the MOS-CU group, followed by CU, CC, MOS and control groups. From the perspective of the SH groups, MOS-CU was more effective in hindering the SH oxidation of MP than CC. According to Lu et al.,(Z.-Y. Lu et al., 2020), MOS supplementation reduces oxidative damage by decreasing ROS and displayed its powerful antioxidant activity, hence the addition of MOS could reduce MP oxidation. CU forms an irreversible triple helix structure when heated, which hinders the oxidative crosslinking of SH_A during FTCs through steric hindrance(H. Liu et al., 2024). As a result, MOS-CU can serve as a potential substitute for traditional cryoprotectants and inhibit MP denaturation by providing a protective barrier against oxidative changes in MP.

Surface hydrophobicity.

The level of hydrophobicity on the surface of a protein reflects its tendency to aggregate, and is determined by the number of hydrophobic residues present (J. Liu et al., 2019). Surface hydrophobicity values are negatively related to the structural integrity of surimi MP (Chen et al., 2022). As presented in Fig. 3(D), the surface hydrophobicity of MP in surimi increased with FTCs. This is because the repeated FTCs induced the unfolding of MP, and subsequently, aromatic amino acids buried in the intramolecular exposed to the surface, thus contributing to the increase in surface hydrophobicity of MP (Q. Liu et al., 2014). The control group exhibited the highest increase in surface hydrophobicity values after FTC7, indicating that the addition of cryoprotectants protects surimi MP from conformational changes. Additionally, the surface hydrophobicity of surimi MP in the MOS and CU groups exhibited was significantly higher than that of the CC group throughout the first three FTCs ($p<0.05$). However, the MOS group exhibited inferior surface hydrophobicity compared to the CU group, suggesting that MOS could further effectively mitigate the aggregation and denaturation of MPs. The molecular weight and steric hindrance of MOS are much smaller than that of CU, which makes it easier to maintain the protein structure of MP (Xie et al., 2024). This speculation could be evidenced by higher MP content and SH content and lower surface hydrophobicity of MP in the MOS group than those in the CU group, as illustrated in Fig. 3(A)(B). The MOS-CU group performed the best antifreeze activity, as evidenced by its lowest surface hydrophobicity during FTCs. Zhou et al.(Zhou et al., 2006) concluded that delaying the exposure of hydrophobic groups during cryopreservation at −18 °C with 8 % (*w*/w) concentration of alginate and sodium lactate effectively inhibited the increase in surface hydrophobicity.

Endogenous Fluorescence Intensity.

The fluorescence intensity was utilized to analyze alterations in the microenvironment surrounding tryptophan and tyrosine residues within amino acids, aiming to provide further elucidation on changes occurring in the tertiary structure of MP during FTCs (Wan et al., 2023). As shown in Fig. 4 and Fig. 5, the fluorescence intensity of MP exhibited a significantly decrease in all groups with the increase FTCs, ($p<0.05$). After FTC7, the fluorescence intensity of the Trp residue in the control group decreased by 30.84 %, a more significant decline than observed in the CC group (23.21 %), MOS group (24.57 %), CU group (27.27 %), and MOS-CU group (23.83 %). It is shown that denaturation of tryptophan residues and protein unfolding enhance hydrophobic interactions and exposure to natural proteins (Wan et al., 2023). This exposure may promote the protein-protein interaction linked to the adverse alterations

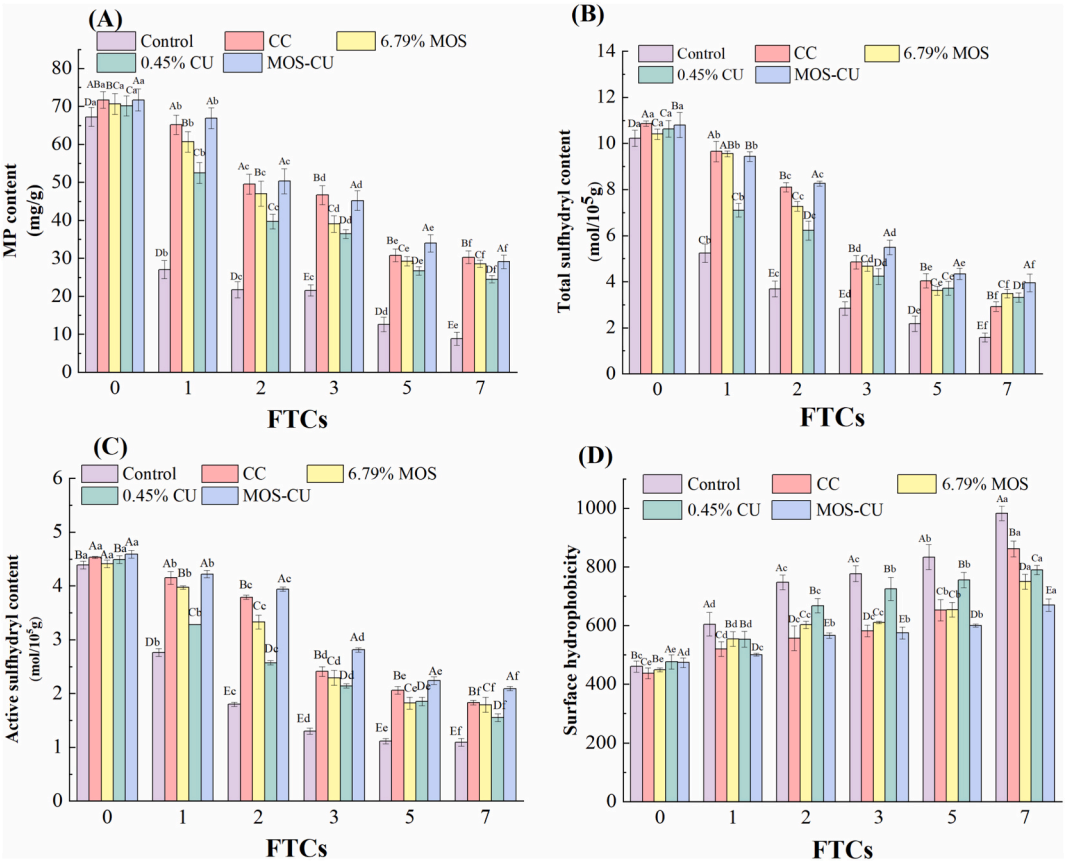

Fig. 3. Effect of MOS, CU, MOS-CU or CC on the MP content (A), total sulfhydryl content (B), active sulfhydryl content (C) and surface hydrophobicity (D) of surimi MP during the freeze-thaw cycles (0, 1, 2, 3, 5 and 7). Note: CC, commercial cryoprotectant (4 % sucrose and 4 % sorbitol); 6.79 % MOS, the addition of mannan oligosaccharides was 6.79 %; 0.45 % CU, the addition of curdlan was 0.45 %; MOS-CU, synergistic cryoprotectant (6.79 % mannan oligosaccharides-0.45 % curdlan). Different uppercase letters indicate significant differences between different antifreeze agents for the same number of freeze-thaw cycles ($p < 0.05$), and different lowercase letters indicate significant differences between different freeze-thaw cycles for the same antifreeze agent ($p < 0.05$).

that occurred during FTCs (Walayat et al., 2022). No significant difference was observed in the tryptophan fluorescence intensity in the MOS, CU, and CC groups ($p>0.05$). This suggests that adding cryoprotectants effectively delayed the reduction of tryptophan exposure in proteins and that the optimized cryoprotectants delayed the effect better. This is because MOS, CU can effectively inhibit the MP oxidative denaturation, thereby slowing down the degree of exposure of tryptophan and aggregation of protein molecules. The final result is to delay the decline of endogenous fluorescence intensity. Maximum fluorescence emission wavelength (λ_{max}) reflects the tertiary structure of MP, however, the blue or redshift of the MP λmax during FTCs did not occur.

3.2.5. *Gel properties evaluation of surimi*

Gel properties, including gel strength, water holding capacity (WHC), hardness, elasticity, etc. are mainly associated with the structure and conformation of MPs in surimi (Sun et al., 2024). Puncture properties, such as breaking force, deformation distance and gel strength, were employed to evaluate the gel-formation ability of surimi during FTCs (Fig. 6). Adding CC, MOS, and CU in fresh surimi (FTC0) resulted in an enhanced deformation distance and gel strength, but no significant effect on the breaking force, indicating that upgrading the elasticity of surimi gel. Additionally, after FTC7, the gel strength and deformation distance of the MOS and CU groups were lower than the MOS-CU groups, indicating that complex cryoprotectants could enhance the quality of surimi gel. Except for the CC group, the surimi gel properties decreased gradually with the increase of FTCs, suggesting a decline in surimi the gel-forming ability. The present observation is reasonable, possibly due to the irreversible damage to MP caused by ice crystals formed by water molecules during the FTCs and the oxidative denaturing of MP, which reduces the solubility of salt-soluble proteins and decreases gel strength (Chen et al., 2022). This is consistent with findings reported by Lin et al.,(Lin et al., 2019), who pointed out that konjac oligo-glucomannan (KOG) effectively delays MP denaturation during surimi cryostorage thereby preserving the gel properties of surimi. Interestingly, after FTC7, the CC group exhibited higher gel strength than that observed after FTC5. This finding could be attributed to the higher thawing losses of the CC group after FTC7, which resulted in the protein concentration, thereby increasing the gel strength and declining the yield of surimi-based products.

After FTC5 and FTC7, the gel strength of control, CC, MOS, CU and MOS-CU decreased by 39.48 % and 44.30 %, 28.55 % and 26.35 %, 35.58 % and 44.31 %, 31.23 % and 36.56 %, 24.56 % and 28.92 %, respectively. After 7 FTCs, it is commendable that there was no significant difference between the MOS-CU group and the CC group ($p>0.05$). It may be that MOS and CU can interact with surimi protein, prevent protein freezing denaturation, and enhance its gel strength, and the hydrophilic amino acids in MOS and CU can stabilize the hydration layer around surimi protein and may also have a positive effect on the network structure of surimi gel.

As seen in Fig. 6(D), the WHC of surimi gels showed a decreasing trend during FTCs. MP denaturation may lead to alterations in the gel network structure, which reduces the WHC (Chen et al., 2022). The addition of MOS, CU, MOS-CU, and CC slowed the rate of decline in WHC of surimi during the FTCs compared to the control. After 5 FTCs, no significant difference among the CC, MOS, CU, and MOS-CU groups ($p>0.05$), indicating that cryoprotectants could enhance the quality of

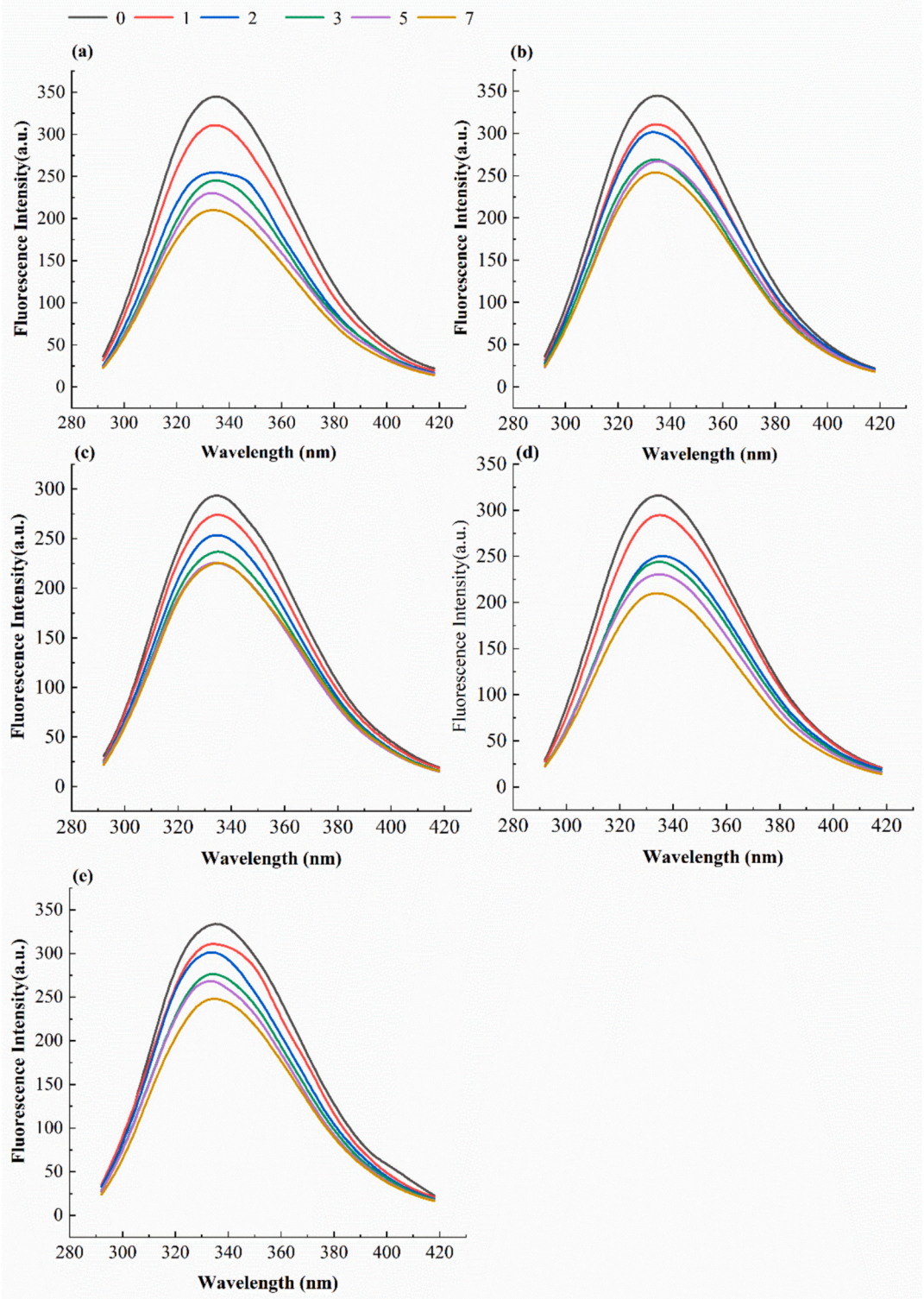

Fig. 4. Effect of MOS, CU, MOS-CU or CC on tryptophan endogenous fluorescence intensity of MP during the freeze-thaw cycles (0, 1, 2, 3, 5 and 7). (a) control, (b) CC, (c) 6.79 % MOS, (d) 0.45 % CU, (e) MOS-CU.

surimi gel. After 7 FTCs, WHC of MOS and CU groups were significantly higher than that of the CC group ($p<0.05$), while the MOS-CU group exhibited superior performance of WHC. It is speculated that MOS and CU altered the interaction between the gel matrix and water molecules, by enhancing the three-dimensional network structure within the gel, and reducing the water loss of surimi. The inclusion of fistular onion stalk polysaccharide (Sun et al., 2024) and tamarind seed polysaccharide (Xie et al., 2024) can help slow down the decrease in the WHC of surimi during FTCs or frozen storage. This is because it enhances the binding between water molecules and MP, thereby immobilizing more free water within the gel network. When comparing the cryoprotective effects of MOS, CU, and MOS-CU, surimi with MOS-CU exhibited the most remarkable freeze-thaw stability as evidenced by the highest gel strength and WHC of the MOS-CU group from FTC1 to FTC7, as

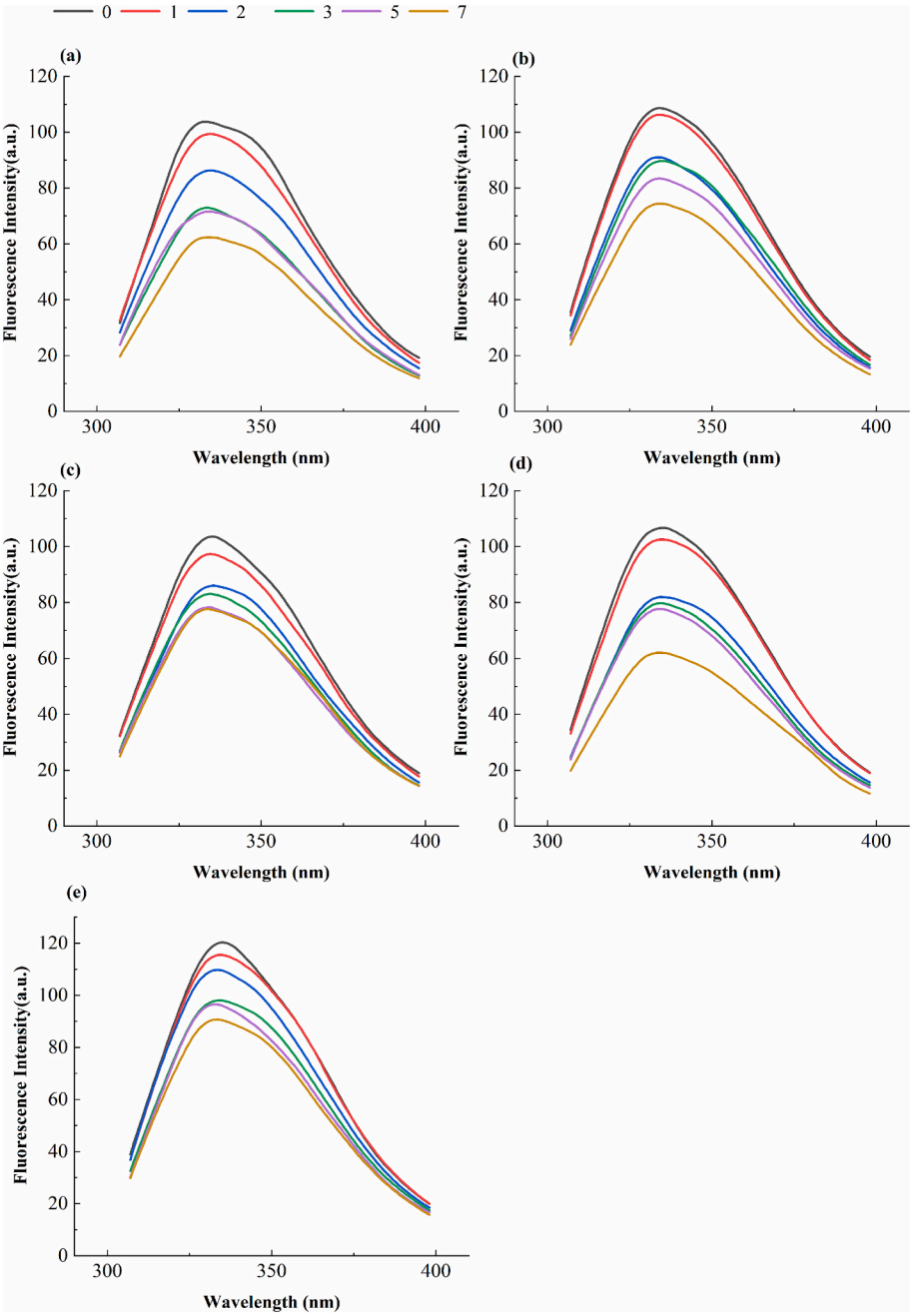

Fig. 5. Effect of MOS, CU, MOS-CU or CC on tyrosine endogenous fluorescence intensity in MP during the freeze-thaw cycles (0, 1, 2, 3, 5 and 7). (a) control, (b) CC, (c) 6.79 % MOS, (d) 0.45 % CU, (e) MOS-CU.

displayed in Fig. 6 (CD).

4. Conclusion

In this study, mannan oligosaccharides (MOS) and curdlan (CU) were explored as potential cryoprotectants for surimi using response surface methodology. The results demonstrated that the optimal supplementation levels of MOS (6.79 %) and CU (0.45 %) significantly reduced thawing losses and enhanced gel strength in surimi after five freeze-thaw cycles (FTC5). Furthermore, the cryoprotective effects of the optimized cryoprotectant (MOS-CU, 6.79 % MOS and 0.45 % CU) on surimi were evaluated over 7 times FTCs. Compared to MOS or CU alone, the MOS-CU mixture exhibited superior performance in reducing the thawing losses of surimi, inhibiting the transition of partially immobilized water to free water, delaying the deterioration of myofibrillar proteins (MP), and slowing down the decrease in gel strength and WHC of surimi gel, especially after seven freeze-thaw cycles (FTC7). This synergistic effect indicated that MOS-CU can effectively protect surimi quality during freezing and thawing processes. Importantly, MOS-CU could achieve a cryoprotectant effects on the surimi comparable to those of the commercial cryoprotectant (CC) during the FTC0 to FTC5, and its performance was even superior to that of CC after FTC7. This finding highlights the potential of MOS-CU as a novel cryoprotectant that not only enhances surimi quality but also offers a healthier alternative with lower sweetness and calories. The results of this study provide valuable insights into the feasibility and practically of using MOS and CU in the surimi industry.

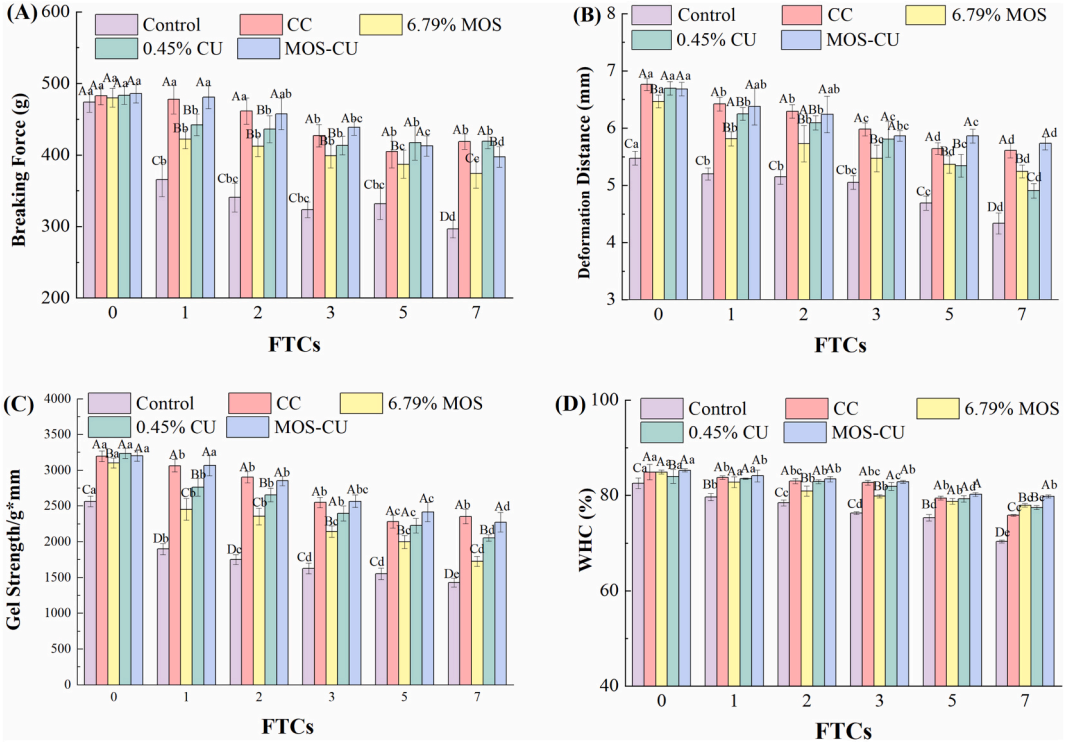

Fig. 6. Effect of MOS, CU, MOS-CU or CC on the breaking force (A), deformation distance(B), gel strength (C) and WHC(D) of surimi gel during the freeze-thaw cycles (0, 1, 2, 3, 5 and 7) of surimi gel. Note: CC, commercial cryoprotectant (4 % sucrose and 4 % sorbitol); 6.79 % MOS, the addition of mannan oligosaccharides was 6.79 %; 0.45 % CU, the addition of curdlan was 0.45 %; MOS-CU, synergistic cryoprotectant (6.79 % mannan oligosaccharides-0.45 % curdlan). Different uppercase letters indicate significant differences between different antifreeze agents for the same number of freeze-thaw cycles ($p < 0.05$), and different lowercase letters indicate significant differences between different freeze-thaw cycles for the same antifreeze agent ($p < 0.05$).

CRediT authorship contribution statement

Yanxin Lin: Writing – original draft, Investigation, Data curation. **Lingzhi Zhang:** Writing – review & editing, Investigation. **Wanting Tang:** Writing – review & editing, Investigation. **Jing Ren:** Writing – review & editing. **Yijie Mo:** Writing – review & editing. **Xiao Guo:** Writing – review & editing. **Lizhong Lin:** Writing – review & editing. **Yuqin Ding:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102250.

Data availability

Data will be made available on request.

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