# Mitigating effect of fucoidan versus sodium alginate on quality degradation of frozen dough and final steamed bread

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ABSTRACT

The impact of fucoidan (FD) and sodium alginate (SA) addition (0.3, 0.6, and 0.9 g/100 g wheat flour, dry basis) and freezing time on the rheology, water, structural characteristics of dough, and the quality of end steamed bread was explored in this study. The results showed FD was more effective in improving the textural characteristics of frozen dough compared with SA. Meanwhile, the freezable and free water content of SA dough were lower than those of FD dough, with the most pronounced effect observed at 0.9%. Adding SA increased the storage modulus, loss modulus, and disulfide bond content of the dough. The addition of FD induced a denser gluten protein network with fewer pores. Furthermore, the addition of FD reduced the hardness and chewiness of steamed bread and increased its specific volume and lightness. Overall, FD could alleviate the quality deterioration of frozen dough and the corresponding steamed bread.

## 1. Introduction

Steamed bread, a traditional Chinese staple food, is renowned for its rich nutritional value and energy content (Zhang et al., 2022b). As the pace of modern life accelerates and the demand for nutritious and convenient foods grows, the accessibility of high-quality steamed bread has become a priority. In response to this demand, the emergence of frozen dough technology has significantly transformed the steamed bread industry. This technology has revolutionized traditional production methods, providing consumers with a convenient and efficient alternative to conventional baking techniques (Omedi, Huang, Zhang, Li, & Zheng, 2019; Zhu, 2021). Nowadays, the adoption of frozen dough technology has gained considerable momentum in the food industry. Freezing reduces production costs and waste, improves operational efficiency, facilitates centralized production, and encourages scalability (Xuan et al., 2017). However, there are many problems associated with frozen dough technology, such as the accelerated formation of ice crystals, the denaturation of starch, and the damage to yeast cells (Wang, Yang, Gu, Xu, & Jin, 2017). Now, numerous studies have been dedicated to the exploration of novel freezing techniques (Li, Zhang, Liu, Wang and Zhang, 2019b), the use of food additives (Lin et al., 2021), and the implementation of cryoprotective measures to counteract the detrimental alterations in frozen dough quality. Among the above methods, the addition of food polysaccharides stands out as the most economical, simple, and effective way to improve dough characteristics and the quality of frozen dough products.

Food polysaccharides have been used as dough quality improvers to inhibit water loss, slow starch aging, and maintain yeast activity. Several studies have shown that polysaccharides play an essential role in the dough system by binding tightly to water molecules and gluten proteins. The incorporation of polysaccharides can change the shape of ice crystals, effectively inhibit ice recrystallization, and maintain yeast activity (Zhao et al., 2022). Additionally, the addition of polysaccharides can stabilize the three-dimensional structure of gluten network (Cheng et al., 2024), inhibit the short-term degradation of starch (Qiu et al., 2017), enhance the rheological properties of dough (Wang et al., 2023c), and improve the quality of wheat products.

Sodium alginate (SA) is a natural polysaccharide extracted from brown algae, composed of β-D-mannuronic acid and α-L-guluronic acid linked by (1 → 4) bonds, forming a hydrogel through ionic cross-linking with divalent cations (Hong, Zhang, Xu, Wu, & Xu, 2021). SA exhibits high viscosity, providing the stability, viscosity, and safety required for food applications. The addition of SA to frozen dough can stabilize the gluten network structure, making the dough more resistant to deformation (Feng, Mu, Zhang, & Ma, 2020). However, considering the diverse needs for product improvement, further exploration and development of other food polysaccharides are warranted.

FD is a natural functional polysaccharide (Hadjkacem et al., 2023). It

belongs to the class of sulfated polysaccharides and consists of an α-L-fucoidan backbone linked by α (1 → 3) and α (1 → 4) bonds (Koh, Lim, Lu, & Zhou, 2020). Studies have indicated that sulfated polysaccharides isolated from FD can reduce starch digestibility and glycemic potential (Koh, Chong, Lu, & Zhou, 2022). Additionally, the incorporation of FD has been shown to improve specific volume and softness in dough, while also imparting biological properties such as anticancer and antioxidant effects (Koh et al., 2020). Based on its positive impact on dough quality, it is hypothesized that adding FD to frozen dough could hinder water migration, minimize ice crystal formation, mitigate gluten network damage, and consequently alleviate the alterations in dough and steamed bread quality induced by freezing.

Currently, studies have primarily focused on investigating the effects of FD on starch hydrolysis capacity, yeast fermentation capacity, and bread quality. However, the impact of FD on dough and steamed bread quality remains unstudied, and there is a lack of literature on its utilization in frozen products. Furthermore, the potential mechanism of influence of FD on the frozen dough and steamed bread remains to be clarified. This study investigated the effect of the addition of FD and SA and freezing time on the texture, rheology, water distribution, and structural properties of dough, along with the quality of steamed bread. The mechanism of the effect of FD on frozen dough properties and the corresponding steamed bread quality was elucidated. This study provides a theoretical foundation for the application of FD in frozen dough-based products, further enhancing the value of FD in food processing.

## 2. Material and methods

### 2.1. Material

Both food-grade FD (95% purity, 4.37 μm median particle size) and food-grade SA (98% purity, 44.83 μm median particle size) were purchased from Qingdao Mingyue Seaweed Group Co., Ltd. (Shandong, China). The plain wheat flour used in this study was sourced from Xiangnian Nature Flour Co., Ltd. (Nanyang, Henan, China). The flour composition, determined according to the AOAC (2000) method, consists of 12.15% protein, 66.48% starch, 13.04% moisture, 1.63% fat, and 0.31% ash (dry basis, w/w). Dry yeast was procured at Yihai Kerry Foodstuffs Marketing Co., Ltd. (Shanghai, China). Glycine, urea, and mercaptoethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All chemicals were of analytical grade unless specified.

### 2.2. Farinograph test

Farinograph properties of FD- and SA-wheat flour blends were analyzed using a model JFZD electronic farinograph (Oriental Fude Technology Development Co., Ltd., Beijing, China) according to the method in the AACC (2010) guidelines. Based on preliminary experiments, the amounts of FD and SA were 0.3, 0.6, and 0.9 g/100 g of wheat flour (dry basis). In each test, FD- or SA-wheat flour blend was placed in the kneader. Water was added within 20 s. The mixture was blended until the dough reached a maximum consistency of 500 ± 20 FU. The dough was then kneaded for an additional 12 min before the test was stopped. The water absorption, dough development time, stability time, degree of softening, and farinograph quality number were obtained. Each test was repeated three times.

### 2.3. Extensograph test

The extensograph properties of FD and SA dough were carried out using a model JMLD150 dough extensograph (Oriental Fude Technology Development Co., Ltd., Beijing, China) following the AACC (2010) guidelines. FD- or SA-wheat flour blend was weighed and placed in a kneading bowl with 6 g of NaCl. An appropriate amount of distilled water, as determined by farinograph quality, was added to achieve a

dough consistency of 480 to 520 FU. The dough was divided into masses of 150 ± 0.5 g using scissors. These dough masses were then shaped and allowed to rise in a dough box for 45, 90, and 135 min. After the rising period, the corresponding dough mass was placed on the dough extensograph for testing to obtain the extensograph curve. The maximum resistance to extension, extensibility, area under the curve, and the ratio of maximum resistance to extensibility of the dough were obtained. Each measurement was repeated three times.

### 2.4. Preparation of dough and steamed bread

To prepare the dough, FD or SA was evenly mixed with 1000 g of wheat flour using a model HM740 dough mixing machine (Hauswirt, Qingdao, Shandong, China). The amount of FD or SA was 0.3, 0.6, and 0.9 g/100 g wheat flour (dry basis), according to the preliminary tests. Following the mixing of dry ingredients, 0.8 g of dry yeast and an appropriate amount of ice water, as determined by farinograph quality, were added. The mixture was initially stirred at 25 °C at 60 rpm for 3 min and then stirred at 120 rpm for 4 min to form the dough. After 5 min of maturing at 25 °C, the dough was divided into several 50 g portions and kneaded by hand until smooth and shaped. One portion of the dough was used directly for testing, while the others were immediately placed into polyethylene bags and frozen in a model BW-86 L486 ultra-low-temperature refrigerator (Haier, Qingdao, Shandong, China) at −70 °C until the central temperature reached −20 °C. The frozen dough was then transferred to a refrigerator at −18 °C for freezing for 14, 28, and 56 days, respectively. For further testing, the frozen dough was thawed at a model OMJ-32 fermenting box (Hebei Ouxinuo Food Machinery Co., Ltd., Hengshui, Hebei, China) at 30 °C with 75% humidity for 40 min.

For the preparation of steamed bread, the thawed dough was steamed in a 30 cm composite steel steamer (Qingzhan Stainless Steel Co., Ltd., Jieyang, Guangdong, China) at 100 °C and 0.101 MPa for 15 min. After steaming, the bread was left to stew for an additional 3 min, then removed and cooled to 25 °C for subsequent measurements. Dough and steamed bread without FD and SA served as the controls.

### 2.5. Total water content of dough

Based on a slightly modified method 925.10 (AOAC, 2000), the total water content of the dough was determined with a model 101-3ES oven (Ever Bright Medical Treatment Instrment Co., Ltd., Beijing, China). About 3 g of the sample was spread evenly on the vessel and dried in a model DHG-9040 drying oven (Guangzhou Kenton Instrument Co., Ltd., Guangzhou, Guangdong, China) at 105 °C for 6 h. Afterward, the doughs were sealed and allowed to cool in a desiccator at 25 °C, then weighed. All doughs were carried out in three technical replicates. The total water content (%, g/g) was calculated by using Eq. (1):

$$Total\ water\ content = (W_1 - W_2)/W_1 \times 100 \tag{1}$$

where, $W_1$ is the mass (g) of the dough before drying and $W_2$ is the mass (g) of the dough after drying.

### 2.6. Rheological properties of dough

The rheological properties of dough with different amounts of FD or SA were determined using a model DHR-2 discovery hybrid rheometer (TA Instruments, New Castle, DE, US) according to the slightly modified method (Guo et al., 2022). During the whole test, the parallel plates were at a distance of 2,000 μm. The environmental temperature was 25 °C, the strain was 0.5%, the strain frequency was 0.1 to 100 Hz, the strain range was 0.01% -100%, and the rheometer was scanned over this range to obtain a linear viscoelastic region. About 4 g dough was removed from the dough core and placed on the plate. When the plate was decreased to 2,000 μm, the spilled dough was scraped off, and the surface was coated with mineral oil to prevent dough variation. The

storage modulus, loss modulus, and loss tangent of the dough were obtained. All the doughs were tested three times.

## 2.7. State of water within dough

The freezable water content of dough was measured using a model DSC-1 differential scanning calorimeter (Mettler-Toledo Instrument Co., Ltd., Schwerzenbach, Zurich, Switzerland) according to the method with minor modification (Li et al., 2021). Approximately 20 mg of dough was enclosed within the 40 μL flat-bottomed aluminum crucible. An empty aluminum box was used as a control. The dough was initially equilibrated at 25 °C for 5 min, cooled from 25 to −30 °C at a speed of 10 °C/min, held for 1 min, and then heated to 30 °C at a speed of 10 °C/min. The melting peak enthalpy was recorded during heating. Three technical replicates were performed on all doughs. The freezable water (*FW*) and non-freezable water (*NFW*) content (%, g/g) of the dough was measured using Eqs. (2) and (3):

$$FW = \Delta H/(\Delta H_0 \times W_t) \times 100 \tag{2}$$

$$NFW = (1 - FW) \times 100 \tag{3}$$

where, $\Delta H$ is the melting peak enthalpy (J/g) of the heat absorption curve; $\Delta H_0$ is the melting peak enthalpy (334 J/g) of pure water; $W_t$ is the total water content (%) of the dough.

## 2.8. Water distribution of dough

The water distribution of dough was obtained using a model NMI20–015 V-1 low-field nuclear magnetic resonance analyzer (Niumag Electronic Technology Co., Ltd., Shanghai, China) with a 1 Hz resonant frequency (He et al., 2020). About 1.5 g of dough was weighed from the core of the dough and filled into the nuclear magnetic resonance (NMR) vial, ensuring that the dough covered the bottom of the vial. Before testing, the NMR permanent field was maintained at 25 °C. The sampling frequency was 200 kHz, the interval time was 3,500 ms, the number of echoes was 2,000, the echo time was 0.2 ms, and the accumulation number was 16. The minimum value of relaxation time was 0.01 ms, the maximum value was 10,000 ms, the number of relaxation time points was 100, and the number of iterations was 100,000. The spin-spin relaxation time $T_2$ of the dough was measured using the Carr-Purcell-Meiboom-Gill pulse sequence, and the inversion spectra of the corresponding dough were generated according to the inversion procedure of $T_2$. The peak area percentage of the $^1H$ NMR spectra was finally determined. Three technical repeats were performed for all doughs.

## 2.9. Free sulfhydryl and disulfide bond content of dough

The free sulfhydryl and disulfide bond content of dough was recorded as previously described (Lu, Guo, Fan, Wang, & Yan, 2023). The dough was cut into 1 cm × 1 cm × 1 cm and placed in a freeze-dryer, lyophilized at −70 °C for 1 day. The freeze-dried samples were ground in a mortar, then sieved through a 100-mesh sieve. Approximately 0.075 g of lyophilized powder was added to 1 mL of buffer (pH 8.0, consisting of 10.4 g Tris, 6.9 g glycine, and 1.2 g EDTA). 4.7 g of guanidine hydrochloride was added and vortexed for 1 min. The mixture was then fixed to 10 mL with buffer.

Determination of free sulfhydryl content. The sample solution (1 mL) was mixed with 4 mL of uroguanidine hydrochloride solution and 0.05 mL of Ellman's reagent (DTNB, 4 mg/mL). Next, the absorption value ($A_{412}$) of the resulting solution was measured using a model UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan) at 412 nm. Three technical replications were carried out. The content (μmol/g) of free sulfhydryl ($C_{SH}$) was calculated using Eq. (4):

$$C_{SH} = 73.53 \times A_{412} \times D/C \tag{4}$$

where, 73.53 is the value of the extinction coefficient; $A_{412}$ is the absorbance of the sample at 412 nm; $D$ is the dilution factor; $C$ is the crude protein concentration (mg/mL).

Determination of disulfide bond content. Sample solution (1 mL) was mixed with 0.05 mL of mercaptoethanol and 4 mL of ureidoguanidine hydrochloride solution on a model ZD-85 gas bath thermostatic oscillator (Guoyu Instrument Manufacturing Co., Ltd., Changzhou, Jiangsu, China) and shaken at 25 °C for 1 h. Next, 10 mL of 12% trichloroacetic acid was added, and the solution was left at 25 °C for 1 h. The mixture was then centrifuged at 5,000 × g in a model H2050R centrifuge (Xiangyi Laboratory Instrument Development Co., Ltd., Changsha, Hunan, China) at 25 °C for 10 min. After removing the supernatant, the above steps were repeated twice. The precipitate was dissolved in 10 mL of 8 M urea, shaken until completely dissolved, and then 0.04 mL of Ellman's reagent (DTNB, 4 mg/mL) was added. The absorbance of the samples at 412 nm was recorded. Three technical replications were carried out. The disulfide bond content ($C_{SS}$, μmoL/g) was calculated using Eq. (5):

$$C_{SS} = (C_{SH'} - C_{SH})/2 \tag{5}$$

where, $C_{SH'}$ is the total sulfhydryl content (μmol/g), including disulfide bond derivatives and free sulfhydryl groups in solution; $C_{SH}$ is the free sulfhydryl content (μmol/g) in solution.

## 2.10. Microstructure of dough

The microstructure of dough was visualized using a model TM3000 scanning electron microscopy (Hitachi High-Tech Co., Ltd., Shanghai, China) according to the method of Wang et al. (2023b). The dough was freeze-dried in a vacuum freeze-dryer at −70 °C for 24 h and then cut into 1 cm × 1 cm × 1 cm pieces. Suitable-sized samples were sprayed with gold. Subsequently, the samples were scanned on a carrier table under an accelerating voltage of 20 kV. Sample images were viewed at magnifications of 800 × and 2,000 × .

## 2.11. Textural properties of dough and steamed bread

The textural properties of the sample were tested as previously described using a model SMS TA.XT Express Texture Analyzer (Stable Micro Systems Co., Ltd., Surrey, England, UK) fitted with an aluminum cylindrical probe (P/36R) (Liu et al., 2022). Approximately 2 g of dough and 2 cm × 2 cm × 2 cm of steamed bread were respectively cut from the core of the sample and placed on the operating table of the mass spectrometer. The weight and height of the instrument were calibrated via Exponent Lite Express with a correction weight of 1 kg and a specified drop height of 20 mm. The test speed was 1 mm/s, the trigger force was 5 g, the compression degree was 60%, and the compression interval was 2 s. The hardness, springiness, cohesiveness, and chewiness of the dough were obtained. Additionally, the hardness, springiness, and chewiness of the steamed bread were recorded. Each measurement was conducted in triplicate for all samples.

## 2.12. Specific volume of steamed bread

The specific volume of steamed bread was determined as previously described with minor modifications (Zhang et al., 2022b). The mass (g) of the steamed bread was weighed using a weighing balance. The volume (mL) of steamed bread was determined using the millet displacement method. Each treatment was repeated three times. The specific volume (mL/g) of the steamed bread was calculated using Eq. (6):

$$Specific\ volume = Volume/Mass \tag{6}$$

### 2.13. Colorimetry of steamed bread

The color of steamed bread was determined using a model Minolta CR-400 colorimeter (X-rite Pantone Co., Ltd., Michigan, Grand Rapids, US) by the method (Xie et al., 2023). Calibration was carried out using standard tiles prior to measurement. The core of steamed bread was cut into 2 cm × 2 cm × 1 cm cubes for measurement. L* was the lightness (0, black; 100, white). a* was the red-green axis index (+a*, red; −a*, green). b* was the yellow-blue axis index (+b*, yellow; −b*, blue). Each sample was tested three times.

### 2.14. Statistical analysis

The data obtained from three technical replicates were statistically analyzed using SPSS software (version 26.0, IBM Corp., Armonk, NY, US) and presented as mean ± standard deviation. Pearson's correlation coefficient was used for correlation analysis. Image plotting of the post-statistical data was performed using Origin Software 2021 (Origin Lab Corp., Northampton, MA, US). A significance level of $P < 0.05$ was considered statistically significant.

## 3. Results and discussion

### 3.1. Farinograph properties

The effect of different amounts of FD or SA on the faringraph of wheat flour is shown in Table S1. Adding FD or SA significantly ($P < 0.05$) increased the water absorption of the dough. The maximum water absorption of the dough was observed when FD or SA was added at 0.9%, which significantly ($P < 0.05$) increased by 12.34% and 2.59%, respectively, compared with the control. It is because the two food polysaccharides have inherently high water absorption, resulting in enhanced water absorption during the dough formation process. The FD dough apparently had a higher ($P < 0.05$) water absorption compared with SA dough at the same amount, indicating that FD has a stronger hydrogen bonding interaction with the water in the dough system. Dough development time reflects the formation and stability of the gluten network. Similarly, dough development time showed an increasing trend with the increase in the additive amount of FD or SA. This suggests that the formation time of the gluten structure is closely related to the water absorption of polysaccharides (Li, Zhu, Yadav and Li, 2019a). The dough development time and the degree of softening of dough with FD addition were higher than those of dough with SA addition at the same addition amount, indicating that SA better enhances the rigidity of the dough.

Here, the stability time of the dough decreased ($P < 0.05$) with increasing amounts of FD. The stability time of FD dough reached its lowest value at 0.9%, which was 30.10% lower compared with the control. This is because food polysaccharide has more molecular force binding sites, which in turn weakens the stability of the gluten protein network structure (Ma et al., 2016). Conversely, the stability time of dough increased ($P < 0.05$) with increasing amounts of SA. This implies that SA is more effective in enhancing the stability of the dough compared with FD. The trend of the farinograph quality number for dough with the addition of FD and SA was consistent with the trend of the dough's stability time. This finding indicates that SA is more effective in strengthening the gluten protein structure.

### 3.2. Extensograph properties

The extensograph properties of wheat dough after 135 min of fermentation are shown in Table S2. The good extensibility makes wheat dough easier to stretch and less likely to break (Zhu, Tao, Wang, & Xu, 2023). The extensibility values of FD dough gradually increased ($P < 0.05$) with the increase in additive amount. Conversely, SA dough exhibited the opposite trend. This finding indicates that 0.9% FD

maximally weaken the gluten force, and make the dough easier to rheology, while 0.9% SA maximally enhance the stability of the cross-linked mesh structure between polysaccharides and gluten proteins.

The area under the curve reflects the elasticity and toughness of the dough (Yu et al., 2019). The area under the curve for the dough with 0.9% FD was the minimum ($P < 0.05$), being 16.60% lower compared with the control. In contrast, the area under the curve for the dough with 0.9% SA was the maximum ($P < 0.05$), being 54.25% higher than the control. This finding indicates that the addition of 0.9% SA significantly ($P < 0.05$) enhances the tensile strength of the dough. This is because of the formation of more viscoelastic colloid after water absorption by SA.

Here, the maximum resistance to extension is the maximum resistance generated when the dough is stretched (Xie, Yuan, Fu, An and Deng, 2022b). The maximum resistance to extension of the FD dough significantly ($P < 0.05$) decreased, while the maximum resistance to extension of the SA dough significantly ($P < 0.05$) increased. This indicates that SA can bind more tightly with gluten proteins to enhance the strength of the network structure compared with FD. The ratio of maximum resistance to extension to extensibility reflects the relationship between these two parameters, with a larger ratio indicating a more glutenous dough. It can be seen that the trend is the same as the maximum resistance to extension.

### 3.3. Dynamic rheological properties of dough

The storage modulus (G′), loss modulus (G″), and loss tangent (tan δ) of dough are demonstrated in Fig. 1. It can be seen that G′ values were higher than G″ values. With the extension of freezing time, the G′ and G″ values significantly ($P < 0.05$) decreased for all samples, with the most significant changes observed at 56 days. This is because of the damage caused by ice crystal formation on the protein structure and glutenin aggregates. Another reason is that mechanical interactions among ice crystals destroy yeast cells, causing them to release some compounds, such as glutathione, as a way to reduce the resistance of the dough (Wang et al., 2022). The G′ and G″ values of the FD dough were lower than those of the control at various freezing time, with the lowest at 0.9% addition (Figs. 1a-d). This is likely due to the competitive nature of FD with the dough for water, ultimately hindering gluten protein formation. However, all SA dough had higher ($P < 0.05$) G′ and G″ values at various freezing time compared with FD dough at the same amount, with the highest at 0.9% addition. This is because SA limits the molecular interactions and migration in the dough, thus making the dough elasticity remain better under stress (Guarda, Rosell, Benedito, & Galotto, 2004). Another reason is that SA has thickening properties, which make it better adhere to the starch-gluten matrix, preventing depolymerization of gluten proteins and leaching of starch granules, thereby maintaining the best elastic behavior of the dough to the maximum extent.

At the same freezing time, the tan δ value of the dough exhibited an increasing trend in the presence of FD compared with the control. In contrast, the increase in tan δ was inhibited by the addition of SA. Notably, after 56 days of freezing, the dough with 0.9% SA demonstrated the lowest ($P < 0.05$) tan δ value among all samples, indicating that a high addition (0.9%) of SA could maintain the best viscoelasticity of the dough.

### 3.4. Texture properties of dough

Hardness is the force required to compress the dough, reflecting the most direct indicator of wheat flour product quality (Awoyale et al., 2022). The hardness, springiness, cohesiveness, and chewiness of dough are shown in Fig. 2. The freezing, FD, or SA significantly ($P < 0.05$) affected the hardness, cohesiveness, and chewiness of the dough. The hardness of all samples progressively ($P < 0.05$) increased during the extension of freezing time to 56 days, while the control showed the most prominent rate of growth. After 56 days of freezing, the hardness of the
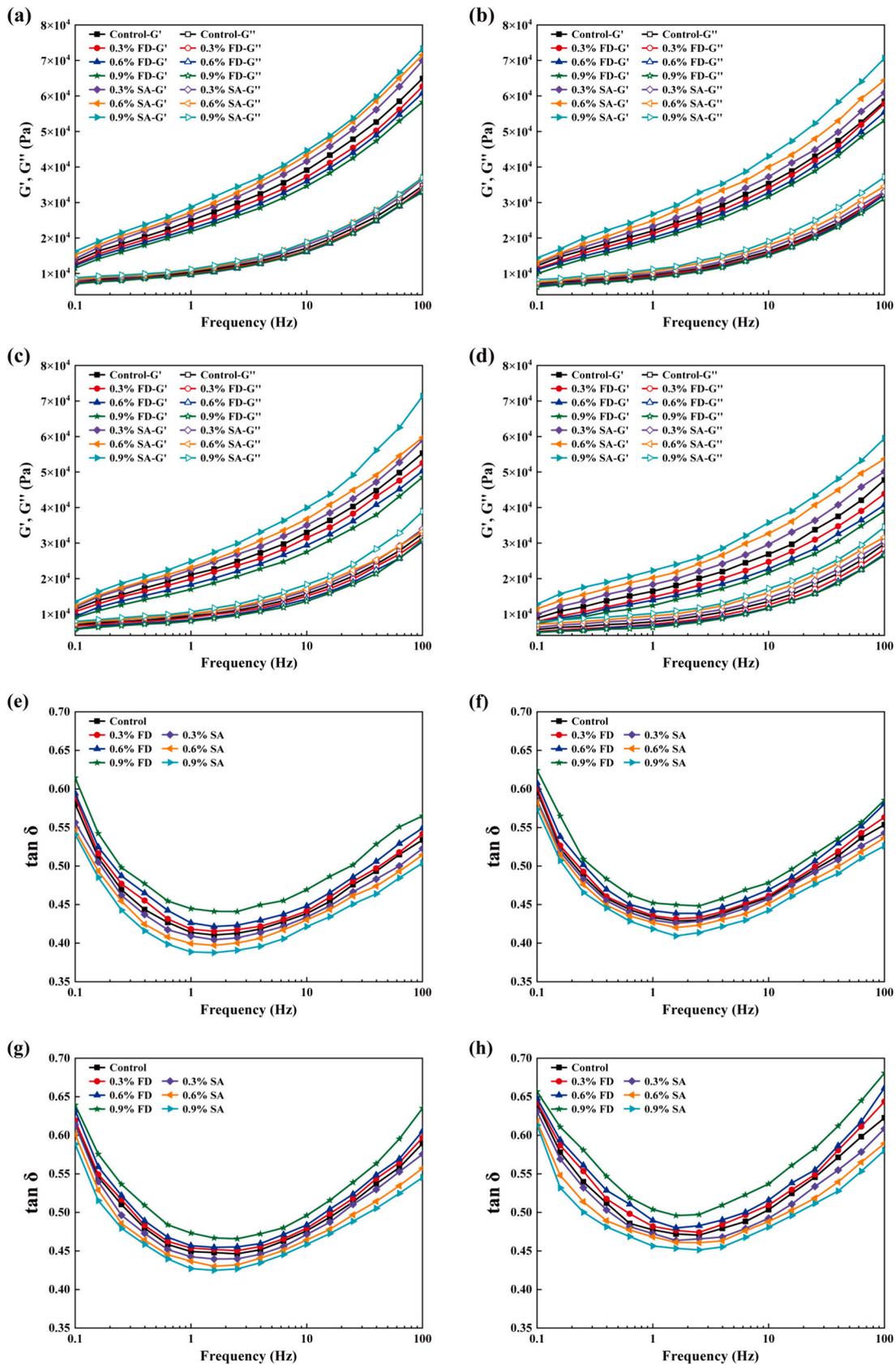
**Fig. 1. Rheological properties of dough with different amounts of fucoidan (FD) or sodium alginate (SA) versus control dough without FD and SA addition.** (a-d) storage moduli (G′) and loss moduli (G″) of dough frozen for 0, 14, 28, and 56 days, respectively. (e-h) loss tangent (tan δ) of dough frozen for 0, 14, 28, and 56 days, respectively.
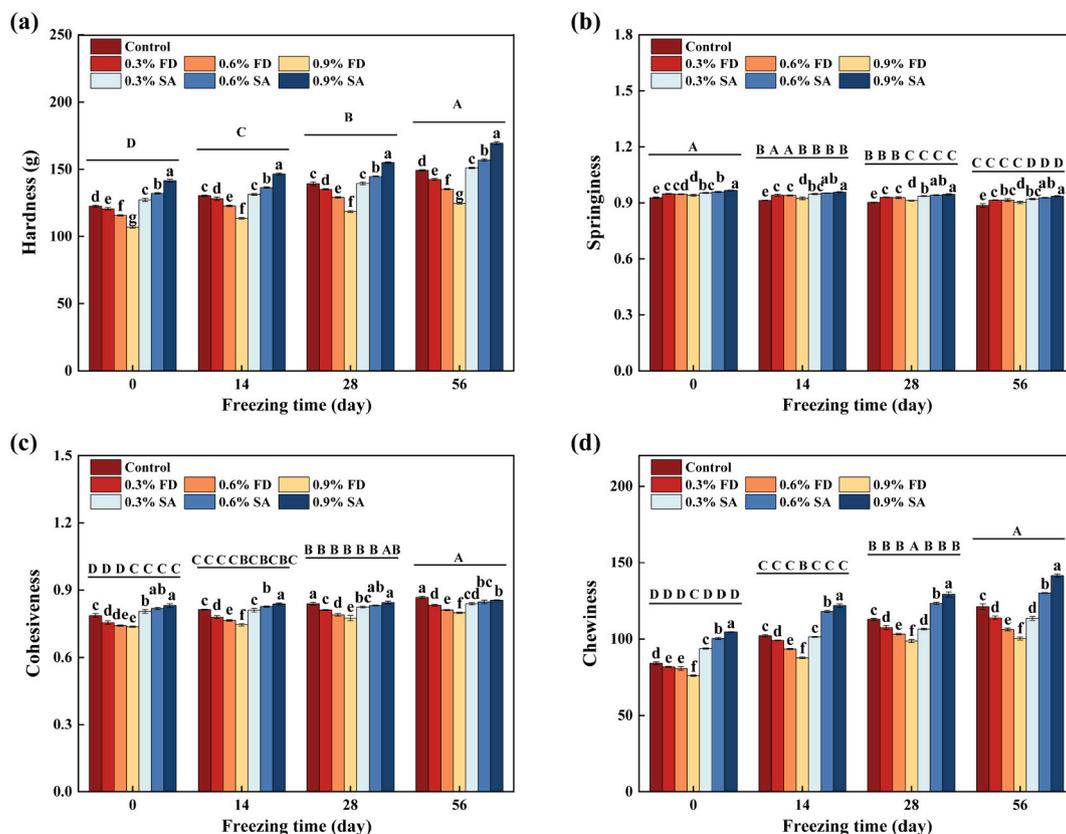
**Fig. 2.** **Textural properties of dough with different amounts of fucoidan (FD) or sodium alginate (SA) versus control dough without FD and SA addition.**
Error bars indicate the mean standard deviation of the three determinations. Different lower case letters indicate a significant ($P < 0.05$) difference among different amounts of polysaccharides at the same freezing time, and different upper case letters are significantly ($P < 0.05$) different among different freezing time at the same amount of polysaccharide.

control significantly ($P < 0.05$) increased to 21.76%. On the one hand, the formation and growth of ice crystals during freezing may destroy the dough system. On the other hand, freezing makes the internal water of dough migrate faster to its surface, resulting in a reduction in the dough's water content and a rapid cross-linking reaction between starch and protein molecules, thereby increasing the hardness (Zhang et al., 2022a). Dough hardness gradually decreased with the increase in FD amount, reaching the lowest ($P < 0.05$) value at 0.9%. After 56 days of freezing, the hardness was 124.74 g, representing a 16.43% reduction compared with the control. This result is attributed to the interactions between FD and water within dough systems, which inhibited the integrity of the gluten network and the swelling of starch granules, leading to alterations in the starch-gluten network and a reduction in hardness. Conversely, dough hardness showed a rising trend with the increase in SA amount. The maximum hardness of dough was observed when SA was added at 0.9%, which increased by 13.41% compared with the control. This is because SA strengthens the protein network and inhibits water migration in dough systems, thereby increasing the dough's hardness (Liu et al., 2020). Adding FD is more favorable than SA for improving dough hardness, especially at 0.9% FD.

The springiness of all the dough showed a decreasing trend as the freezing time increased (Fig. 2b). The effect of different amounts of food polysaccharides on dough springiness was not considerable. However, it can be seen that the springiness of FD dough decreased with the increase in the amounts, suggesting that the simultaneous water absorption of FD and wheat flour hinders the formation process of gluten. Conversely, the addition of SA increased the springiness of the dough, which is consistent with the rheological results. The trends in dough cohesiveness with hardness were generally consistent.

The addition of FD reduced chewiness and mitigated the negative

effects of freezing on dough texture. Chewiness was lowest ($P < 0.05$) with 0.9% FD, showing a 17.29% reduction compared with the control after 56 days of freezing, indicating that a higher amount of FD enhances dough textural properties. Conversely, dough chewiness gradually increased with the increase in SA amount. Interestingly, the chewiness of 0.3% SA dough was lower than that of the control after 14 days of freezing. This suggests that SA has a moderate impact on the quality of frozen dough. In conclusion, 0.9% FD most effectively improves dough textural properties.

### 3.5. State of water within dough

The water within frozen dough is mainly composed of freezable water and non-freezable water. During the freezing process, freezable water transforms into ice crystals, which is a key cause of deterioration in dough quality, and its content directly affects the size and distribution of ice crystals in the dough system. The enthalpy is obtained by area integration of the thermogram, and reflects the thermal changes of the sample during the melting process. The enthalpy, freezable water, and non-freezable water content of dough are displayed in Fig. 3. The enthalpy of all the samples was raised as an extension of freezing time (Fig. 3a). Likewise, the trends in the freezable water content of all samples aligned with the corresponding enthalpy from 0 to 56 days of freezing. The freezable water content of the control after 56 days of freezing demonstrated a significant ($P < 0.05$) increase of 22.01% compared with the unfrozen sample (Fig. 3b). These could be attributed to the exposure of gluten's hydrophobic groups during freezing, resulting in the liberation of water from the network structure of gluten proteins and their conversion to freezable water (Wang et al., 2023a). However, with the addition of FD, a significant ($P < 0.05$) drop in
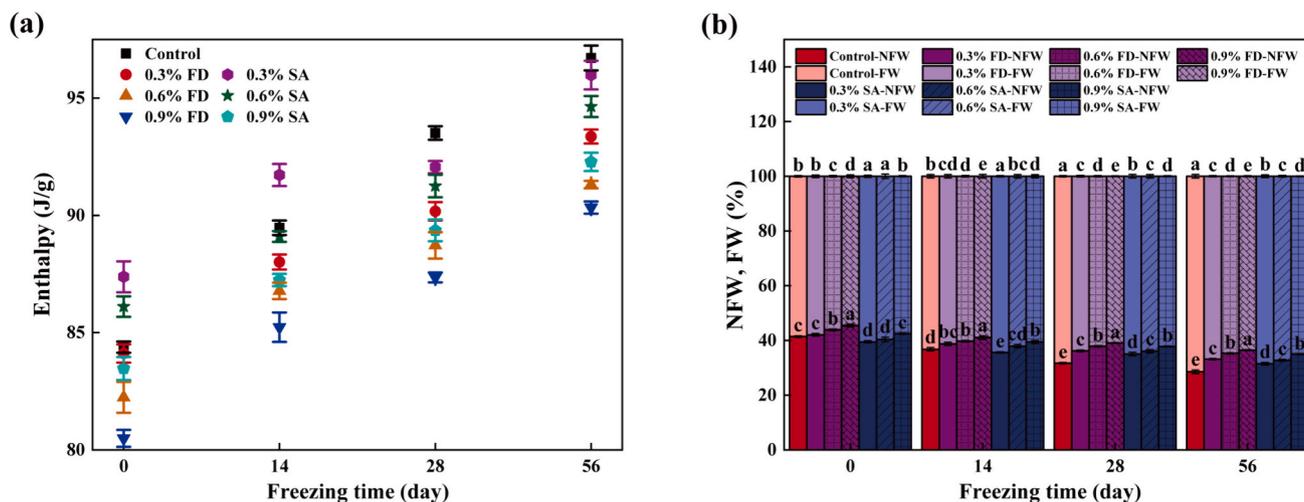
**Fig. 3.** Enthalpy, freezable water (FW) content, and non-freezable water (NFW) content of dough with different amounts of fucoidan (FD) or sodium alginate (SA) versus control dough without FD and SA addition. Error bars indicate the mean standard deviation of the three determinations. Different lower case letters indicate significant ($P < 0.05$) differences among different amounts of polysaccharides at the same freezing time, and different upper case letters are significantly ($P < 0.05$) different among different freezing time at the same amount of polysaccharide.

freezable water of all dough could be observed at different freezing time. The lowest freezable water content was reached when the FD was 0.9%, demonstrating an 11.08% lower value compared with the control after 56 days of freezing. This result suggests that FD further reacts with free water and affects the distribution of water in the gluten network, minimizing water loss from the dough system during freezing.

Here, as the freezing period extended to 28 days, the freezable water content of the dough containing 0.9% SA showed a significant ($P < 0.05$) reduction of 8.93% compared with the control. The reason is probably that as a viscous polysaccharide, SA effectively binds to the gluten protein-starch system, thereby minimizing water loss and impeding the rise in freezable water content. This suggests that high-viscosity food polysaccharides can inhibit the rise of freezable water content in frozen dough (Ke et al., 2020). The freezable water content of FD dough was significantly ($P < 0.05$) lower compared with SA dough when the additive amount was the same. In summary, FD effectively reduces the freezable water content compared with SA, with the most pronounced effect observed at the additive amount of 0.9%.

### 3.6. Water distribution of dough

The $T_2$ relaxation time reflects the state of the hydrogen proton resonance over time. There are three proton groups in the dough system. $T_{21}$ represents strongly bound water, primarily tightly bound to protein molecules or starch granules. $T_{22}$ represents weakly bound water, indirectly bound to the starch-gluten network, whereas $T_{23}$ denotes free water, primarily attached to gluten proteins and starch surfaces. The peak area proportions of $T_{21}$, $T_{22}$, and $T_{23}$ are denoted as $A_{21}$, $A_{22}$, and $A_{23}$, which are used to indicate the amount of strongly bound water, weakly bound water, and free water, respectively.

The water changes within the dough are displayed in Fig. 4. As the freezing time increased from 0 to 56 days, the $T_2$ relaxation time was constantly prolonged, suggesting that freezing enhances the mobility of water. With the addition of FD, the $A_{23}$ content of the dough was significantly ($P < 0.05$) reduced. Notably, at a concentration of 0.9%, the $A_{23}$ content reached its lowest value, showing a reduction of 28.40% compared with the control after 56 days of freezing. This is consistent with previous findings (Xie et al., 2022a), which suggest that polysaccharides are able to form hydrogen bonds with water in the dough system, resulting in a more secure adsorption of water into the starch-gluten network structure, which reduces the amount of free water in the frozen dough and inhibits the loss of water out of the dough. When

the freezing time was 56 days, the $A_{23}$ value of SA dough was lowest when the amount of SA was 0.9%, which was 23.55% lower than that of the control. This is because of the high viscosity content of SA, leading to a strong gluten-starch bond within the dough. This enhances the bond stabilized water molecules, restricts the water flow, and minimizes the damage caused by freezing. The $A_{23}$ value of FD dough was lower than that of SA dough with the same amount. This suggests that FD, as a hydrophilic hydrocolloid, exhibits a greater ability to hinder water migration within the dough system, with optimal results observed at an additive amount of 0.9%.

### 3.7. Free sulfhydryl and disulfide bonds of dough

Disulfide bonds, formed by free sulfhydryl oxidation, are important chemical bonds for maintaining the stability of the three-dimensional gluten network (Zhang et al., 2020). The amounts of free sulfhydryl and disulfide bonds in the gluten structure of FD and SA dough at different freezing time are presented in Fig. S1. At 56 days, the disulfide bond content of frozen control significantly ($P < 0.05$) decreased by 16.32% compared with that of the unfrozen control, suggesting that the formation of ice crystals and recrystallization during freezing cause the fragmentation of the disulfide bonds. FD had a comparatively lesser impact on the gluten network (Figs. S1a-b). This is because FD competes with the gluten structure for water, hindering the polymerization of the gluten network and affecting the structural stability of gluten. Interestingly, as the freezing time increased, the disulfide bond content of FD was gradually higher than that of the control. After 56 days of freezing, dough with 0.3% FD addition had the greatest disulfide bond content, which was 14.11% higher than the control, suggesting that the presence of FD can inhibit freezing-induced disulfide bond breaking. This is because the high water-holding capacity of FD inhibits the transformation of water into ice crystals, thus alleviating the freezing-induced free sulfhydryl breakage. However, the free sulfhydryl of SA dough was significantly ($P < 0.05$) lower at all freezing time compared with the FD dough at the same amount. After 56 days of freezing, the dough with 0.9% SA had the lowest free sulfhydryl content and the highest disulfide bond content, with a significant ($P < 0.05$) decrease of 2.01 μmol/g in free sulfhydryl content and an increase of 1.61 μmol/g in disulfide bond content compared with the dough with 0.9% FD. This finding indicates that 0.9% SA has a strong reducing effect and is able to provide the best protection for gluten. Concurrently, SA forms a strong bond with gluten proteins, reinforcing the stability of the three-
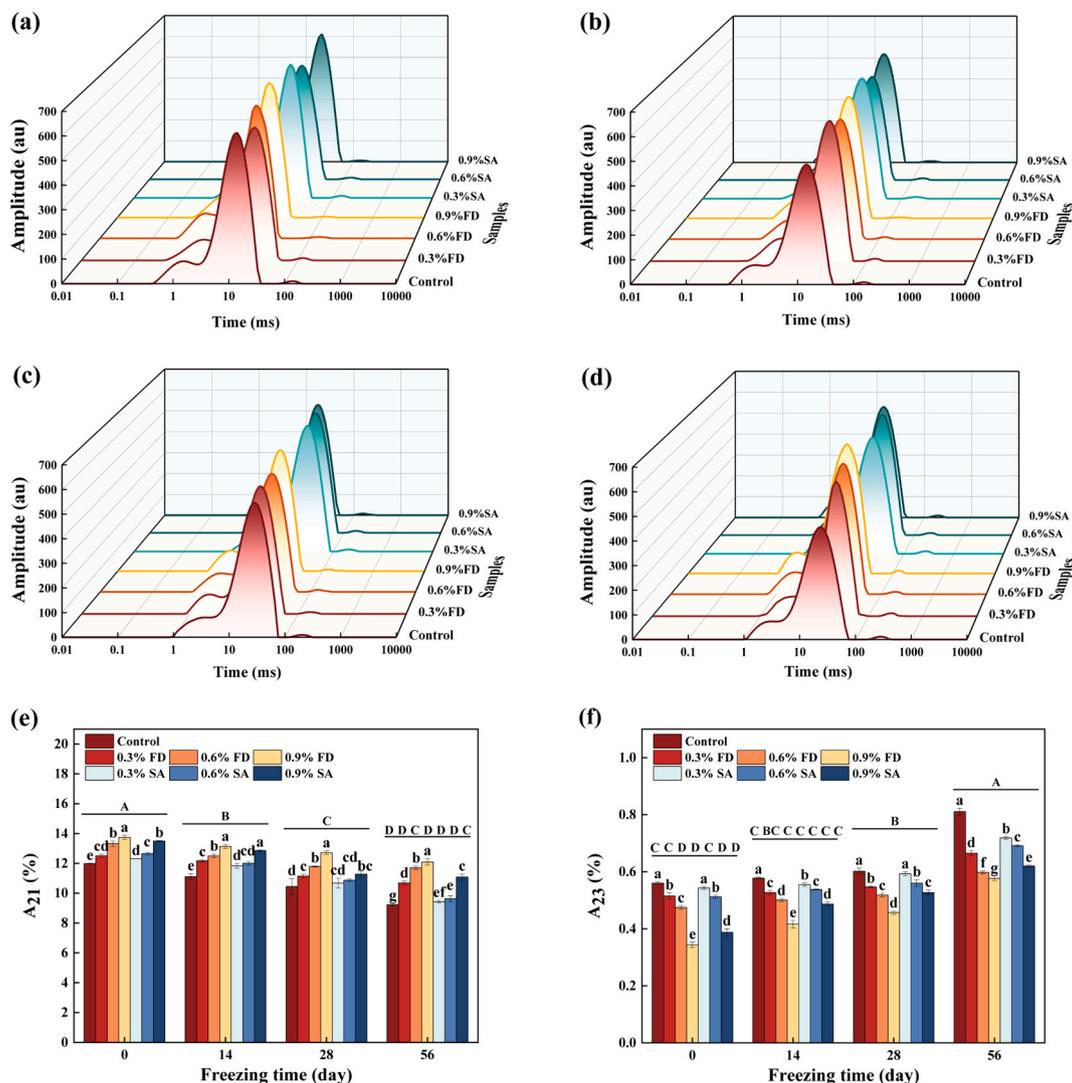
**Fig. 4. T$_2$ distribution of relaxation times, A$_{21}$, and A$_{23}$ of dough with different amounts of fucoidan (FD) or sodium alginate (SA) versus control dough without FD and SA addition.** (a-d) T$_2$ relaxation times of dough for freezing from 0 to 14, 28, and 56 days, respectively. (e-f) A$_{21}$ and A$_{23}$ of dough frozen for 0, 14, 28, and 56 days, respectively. Error bars indicate the mean standard deviation of the three determinations. Different lower case letters indicate significant ($P < 0.05$) differences among different amounts of polysaccharides at the same freezing time, and different upper case letters are significantly ($P < 0.05$) different among different freezing time at the same amount of polysaccharide.

dimensional network. Furthermore, SA effectively curbed water migration within the dough system, consequently minimizing ice crystal-induced disruption of the gluten protein matrix and restraining the decline in disulfide bond content, in line with the findings from LF-NMR analysis.

### 3.8. Microstructure of dough

The typical microstructures of the dough are shown in Fig. 5. During freezing, the depolymerization of gluten macromolecules and the disorder of starch are unavoidable. This is because of the uneven arrangement of the polar and non-polar residues of the protein, as well as the clusters of ice crystals. When ice crystals are combined with hydrophilic residues of proteins, the starch becomes irregularly segregated (Silvas-García et al., 2016). However, the incorporation of FD or SA, particularly at levels of 0.3% FD or 0.9% SA, noticeably ameliorated this condition. Dough containing 0.3% FD exhibited a more compact gluten network compared with the control (Figs. 5b and h). This is attributed to the strong binding of FD to water, which restrains the formation of ice crystals and recrystallization. Consequently, this hinders the mechanical

harm to the gluten network during the freezing process, leading to a more uniform gluten network. This is consistent with the study of Wu, Liu, Hu, Wang, and Zhao (2022), who reported that the food polysaccharide effectively bound water and slowed down the growth of ice crystals, thus reducing the disruption of the organizational bonding system in gluten. After 56 days of freezing, the addition of 0.9% SA resulted in a more pronounced improvement of starch granule indentations, with their surfaces appearing smoother compared with the control (Figs. 5c and i). Simultaneously, the starch granules were distributed more evenly within the gluten network. The reason may be that the high viscosity of SA enhances the structure of the starch-gluten network and resists mechanical damage caused by ice crystal formation. In one study, the high-consistency hydrocolloid can ameliorate the structural damage caused by freezing to the starch-gluten network (Wang et al., 2023b). The results indicate that FD and SA effectively mitigate the negative effects of freezing on the dough's microstructure.

### 3.9. Textural properties of steamed bread

Steamed bread hardness is a key sensory attribute in predicting
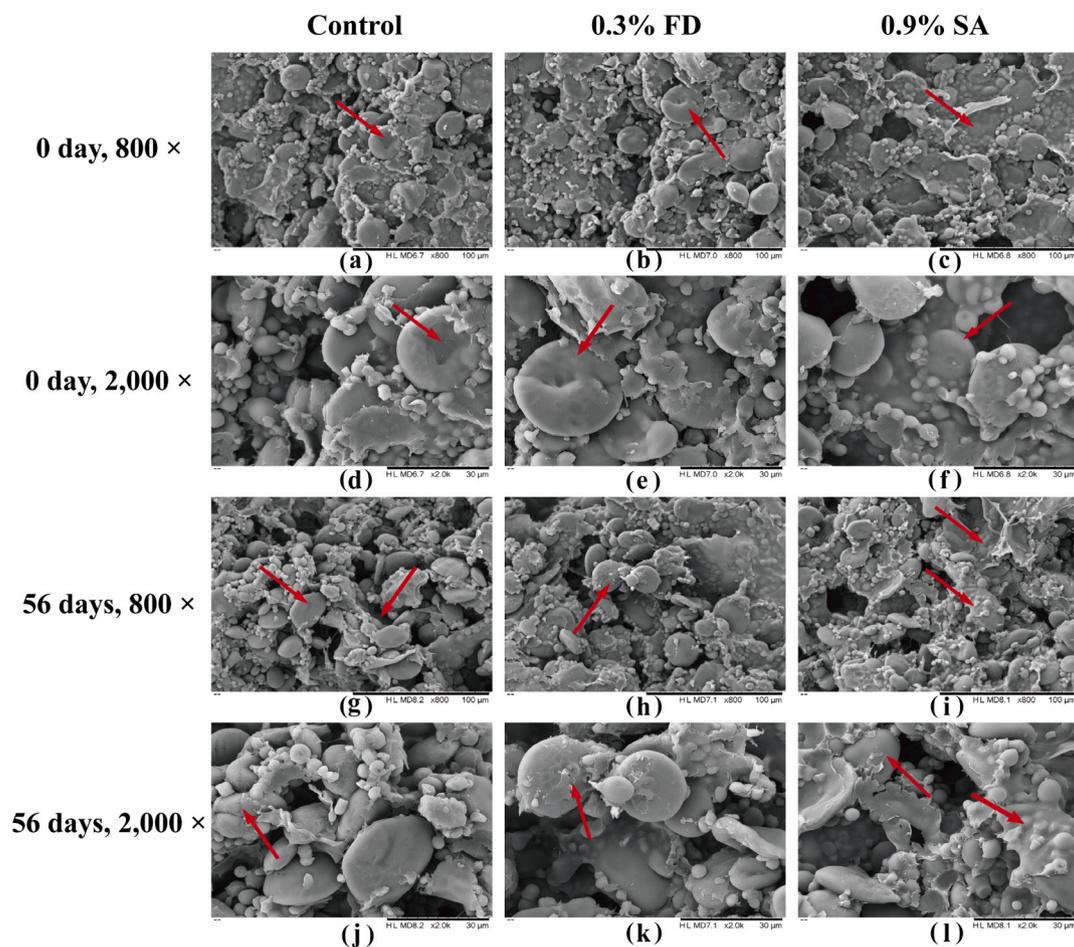
**Fig. 5. SEM images of dough after freezing for 0 day (a-f) or 56 days (g-l)**. (a-c, g-i) Control dough, dough with 0.3% FD, and dough with 0.9% SA at a magnification of 800 ×, respectively. (d-f, j-l) Control dough, dough with 0.3% FD, and dough with 0.9% SA at a magnification of 2,000 ×, respectively.

steamed bread texture (Fu et al., 2021). The effects of FD or SA on the textural properties of the steamed bread are shown in Table S3. With the extension of freezing time from 0 to 56 days, all frozen dough steamed bread showed a significant ($P < 0.05$) increase in hardness and chewiness, concurrently with a progressive decline in springiness, indicating freezing causes a degradation in the quality of frozen dough steamed bread (Yang et al., 2023). The hardness of FD steamed bread significantly ($P < 0.05$) diminished with rising FD amounts, reaching a minimum of 0.9%, resulting in a significant ($P < 0.05$) reduction of 33.48% compared with the control after 56 days of freezing. This is because the combination of FD with hydrogen bonds in the dough inhibits water migration and loss, thereby reducing dough hardness. Moreover, FD inhibits gluten formation and starch swelling. This is consistent with the rheological properties of the dough. After 56 days of freezing, the hardness of steamed bread containing 0.3% SA exhibited a noteworthy ($P < 0.05$) decrease of 4.90% compared with the control. This finding suggests that a lower amount (0.3%) of SA yields a more favorable impact on enhancing the finished products' hardness (Kang, Reddy, Park, Choi, & Lim, 2018). The change in springiness of SA steamed bread was similar to hardness. After 56 days of freezing, steamed bread containing 0.9% FD had the lowest chewiness, which was significantly ($P < 0.05$) reduced by 25.27% compared with SA steamed bread. It indicates that FD contributes to the fluffiness of the steamed bread. In short, the addition of FD and SA can alleviate the damage of yeast and gluten protein structure. Consequently, this enhances the dough's air-holding capacity during the steaming process.

### 3.10. Specific volume of steamed bread

The specific volume of steamed bread is shown in Table S3. The specific volume of frozen steamed bread was significantly ($P < 0.05$) lower than that of unfrozen steamed bread. This is because the formed ice crystals destroy the internal network structure of the dough and weaken the air-holding capacity of the steamed bread during the freezing process (Liu, Guo, & Zhu, 2019). The specific volume of steamed bread exhibited a positive correlation with the addition of FD during the same freezing period. After 56 days of freezing, the specific volumes of FD (0.3%–0.9%) steamed bread were 0.33, 0.45, and 0.56 mL/g higher than those of the control, highlighting the capacity of FD to enhance the fermentation performance of frozen dough. In contrast, the specific volume decreased with increasing levels of SA addition, suggesting that high levels of SA dough were too reinforcing for dough fermentation. With the extension of freezing time, specific volumes of SA dough gradually increased, suggesting that SA enhances the air retention capacity of steamed bread. Overall, adding 0.9% FD has a more pronounced effect on increasing the specific volume of the steamed bread compared with SA.

### 3.11. Color of steamed bread

The values of lightness, red-green axis index and yellow-blue axis index for different FD and SA steamed bread are shown in Table S4. The lightness values of the steamed bread all increased with the extension of freezing time, indicating that freezing damages the starch-gluten three-dimensional structure, resulting in a large amount of starch exposure,

which ultimately leads to an increase in the lightness of the steamed bread. The addition of FD significantly ($P < 0.05$) increased the lightness compared with the control. This is because FD can inhibit damage to steamed bread caused by water loss and ice crystal formation, making the distribution of air holes more uniform and the lightness of the steamed bread higher. Conversely, SA gradually reduced the lightness of steamed bread, reaching its lowest value with 0.9% SA, an 8.60% reduction compared with 0.9% FD after 56 days of freezing. This indicates that 0.9% FD is more beneficial than SA for improving the lightness of steamed bread. In addition, the red-green axis index of SA steamed bread was lower. This is because SA has strong adsorption properties and can be tightly bound to the gluten structure to inhibit the fermentation of the yeast cells, so that the dough could not be fully fermented and the color was greenish. The yellow-blue axis index of steamed bread was elevated by the addition of FD and SA, which was mainly attributed to the addition of polysaccharides to promote the occurrence of melodic reactions in the dough during the heating process (Xie et al., 2023).

*3.12. Pearson's correlation analysis*

To comprehensively investigate the mechanism of the influence of FD and SA on the properties of frozen dough, correlation analyses were performed to characterize the texture, rheology, water state, and structure of frozen dough (56 days) with FD and SA (Fig. 6). Freezable water was positively correlated with hardness, cohesiveness, chewiness and free water, and negatively correlated with springiness. These findings indicate that dough hardness, cohesiveness, chewiness, and free water increased with increasing freezable water content. Throughout the freezing process, it was confirmed that the strongly bound water in the dough was more readily converted to free water, leading to a steady rise in the free water content. Simultaneously, a continual transfer of water occurred from the interior of the dough to its surface, ultimately contributing to the deterioration of the dough's textural properties. Interestingly, freezable water had a highly significant ($r = -0.90$, $P \leq 0.01$) negative correlation with strongly bound water, indicating that the effective inhibition of the reduction of strongly bound water in a frozen dough matrix can significantly lessen freezable water content. Freezable water was positively correlated with free sulfhydryl and negatively correlated with disulfide bonds, indicating that the increase in freezable water content of frozen dough causes the appearance of

mechanical damage to the gluten protein network. Strongly bound water was negatively correlated with hardness, cohesiveness, and chewiness and positively correlated with springiness, indicating that as the strongly bound water in the frozen dough increases, there is a downward trend in hardness, cohesiveness, and chewiness, accompanied by a rise in the springiness of the dough. It means that during freezing, FD and SA enhance the stabilization of strongly bound water, thus leading to better textural properties. The disulfide bond content in the dough showed a highly significant ($P \leq 0.001$) negative correlation with free sulfhydryl and a significant ($P \leq 0.05$) positive correlation with G′ and G″, suggesting that the viscoelastic properties of dough deteriorate due to the increase in free sulfhydryl content and disulfide bond breakage by freezing.

*3.13. Mechanism*

Based on the obtained results, the mechanisms of the effects of the addition of FD and SA on the quality degradation of frozen dough and final steamed bread were proposed. Both FD and SA are hydrophilic polysaccharides with polyhydroxyl structures, which enhance hydrogen proton signaling strength. This structural feature allows water molecules to more easily bind and form hydrogen bonds with the polysaccharides. By binding water molecules, FD and SA impede the migration of water during freezing, which is a common cause of quality degradation in frozen dough. This reduced water migration results in lower freezable water and free water content (Figs. 3 and 4). Furthermore, FD specifically inhibits starch swelling and water loss during freeze-thaw cycles, contributing to the improved textural properties of the dough. This results in a dough that maintains its structure and moisture content better during freezing, leading to better final product quality.

FD and SA interact with gluten proteins through disulfide bonds. This interaction affects the rheological and structural properties of the gluten network. High-viscosity SA binds tightly to gluten proteins, forming a gluten protein-SA complex. This complex inhibits ice crystal formation and prevents disulfide bond breakage during freezing (Fig. S1), enhancing the elasticity and integrity of the gluten network (Fig. 1). SA enhances the orderliness of the gluten structure, resulting in a more continuous and cohesive gluten network. FD retains water and inhibits the enlargement of ice crystals within the dough. This improvement leads to more intact starch granules and a stronger, more elastic dough structure (Fig. 5).

## 4. Conclusions

In this study, the effects of adding FD and SA and freezing time on the texture, rheology, water distribution, and structural properties of dough, as well as the quality of steamed bread, were examined. These impact mechanisms and Pearson's correlation analysis were elucidated. The findings revealed that FD improved the quality of frozen dough and steamed bread. It increased the water absorption, development time, and extensibility values of the dough compared with SA. After 56 days of freezing, both FD and SA made the viscoelasticity, disulfide bond content, and integrity of the gluten-starch structure much better than the control. However, FD's effect was slightly weaker than SA's. Additionally, FD significantly reduced the hardness and chewiness of both the dough and steamed bread while enhancing the specific volume and lightness of the steamed bread, surpassing the effects of SA. In summary, FD effectively inhibits ice crystal formation, reduces freezable water content, and minimizes structural damage to gluten proteins in frozen dough, thereby improving the quality of steamed bread. The optimal effect was observed at an additive amount of 0.9%.

## Ethics statement

The authors declare no applicable ethical considerations with regards to the information contained in the article.



**Fig. 6.** Correlation analysis of texture, rheology, structural properties, and water distribution of frozen dough (56 days) with FD or SA. HA, hardness; SP, springiness; CO, cohesiveness; CH, chewiness; G′, storage moduli; G″, loss moduli; ΔH, the enthalpy of melting; FW, freezable water; $A_{21}$, strongly bound water ratio; $A_{22}$, weakly bound water ratio; $A_{23}$, free water ratio; SH, free sulfhydryl; SS, disulfide bond.

## CRediT authorship contribution statement

**Xue Yang:** Writing – original draft, Methodology, Investigation, Conceptualization. **Jinying Guo:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Mengli Niu:** Validation, Software, Methodology, Data curation, Conceptualization. **Can Lu:** Visualization, Validation, Data curation. **Ping Wang:** Resources, Methodology, Conceptualization. **Denglin Luo:** Resources, Methodology, Formal analysis, Conceptualization.

## Declaration of competing interest

All authors declare no conflict of interest.

## Data availability

Data will be made available on request.

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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.2024.101608.

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