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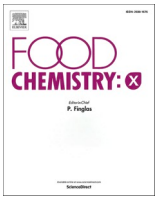

# Insight into curdlan alleviating quality deterioration of frozen dough during storage: Fermentation properties, water state and gluten structure

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ABSTRACT

Curdlan was effective in alleviating quality deterioration of frozen dough during storage. This research explored the mechanisms from perspectives of fermentation properties, water state and gluten structure of frozen dough during storage, and the performance of corresponding steamed bread. Results showed that curdlan addition improved the gas-releasing capability and gas-holding capability of frozen dough, meanwhile enhanced the specific volume and textural properties of corresponding steamed bread. The melting enthalpy and NMR results demonstrated that curdlan restricted the conversation of bound water into freezable water, and inhibited the moisture migration in frozen dough. Frozen dough with 0.5% curdlan had significantly lower gluten macropolymers (GMP) depolymerization degree and free sulfhydryl (SH) content than the control, indicating that curdlan alleviated the depolymerization of GMP. Microstructure results proved that the deterioration of the structure was retarded by curdlan. This study contributes to understanding the theories for curdlan alleviating the deterioration of frozen dough during storage.

## 1. Introduction

Frozen dough could be manufactured industrially and transported under cold chain to restaurants or retail stores for thawing, fermentation and cooking into final pastry. Frozen dough technology helped retain the dough freshness, standardize the product quality and save the cost (Zhu, 2021).

However, the deterioration of frozen dough during storage remains a challenging issue (Lu and Zhu, 2023). The ice recrystallization brought about water migration, as well as damage to gluten network structure and reduction of yeast viability (Luo et al., 2018, Wang et al., 2014). The depolymerization of gluten macropolymers (GMP) occurred due to the breakage of interchain disulfide (SS) bonds (Wang et al., 2016a). Furthermore, the polymerization ability of gluten and gliadin during heating was lowered by frozen storage, resulting in reduced specific volume, deteriorated internal texture and weakened sensory properties of final products (Wang et al., 2018). How to maintain the performance and alleviate the deterioration of frozen dough became a critical task for both enterprises and researchers (Lu and Zhu, 2023).

In order to improve the freezing tolerance of frozen dough and enhance the final product quality, additives including antifreeze proteins, hydrocolloids, emulsifiers and enzymes were commonly used (Song et al., 2019, He et al., 2020, Guan et al., 2023). With good water holding capacity, hydrocolloids such as carboxymethyl cellulose sodium, konjac glucomannan, and xanthan gum could inhibit the moisture migration and restrain the recrystallization of ice crystals, thus lowing the damage to the gluten network and the yeast (Wu et al., 2022, Lu et al., 2023). Water extractable arabinoxylan also induced partial agglomeration of GMP in frozen dough, resulting in higher GMP content (Wang et al., 2016b). Curdlan is a linear water-insoluble polysaccharide with good hydrophilicity, which consists of glucose units via $\beta$-(1,3)-glycosidic bonds with a polymerization degree of 400–500 (Yuan et al., 2021). Curdlan has been employed to enhance the freeze–thaw stability of frozen cooked noodles (Liu et al., 2022b). Curdlan delayed the water migration, and strengthened the gluten network structure, thus maintaining the integrity of gluten network in frozen cooked noodles (Liang et al., 2022, Liang et al., 2021). Although the mechanisms of curdlan improving the frozen tolerance of the frozen cooked noodles that had gone through heat treatment have been revealed, the research about curdlan alleviating quality deterioration of frozen dough is limited. The gluten network of raw dough is formed by covalent SS bonds, hydrophobic interaction, ionic bonds and hydrogen bonds (Ribotta et al.,

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2001). Nevertheless, owing to the crosslinking of glutenin and gliadin via disulfide bonds, as well as interchange reactions between SH and SS during heat treatment, the gluten network of cooked noodles is quite different from that of the raw dough (Obadi et al., 2021).

Therefore, this study was to explore the impact of curdlan on the fermentation properties, moisture state, gluten aggregation and microstructure of frozen dough during storage, as well as the quality of the corresponding steamed bread. In particular, changes in freezable water content, water distribution state, molecular weight of gluten and GMP depolymerization degree of frozen dough during storage were analyzed. The specific volume, textural and sensory properties of corresponding steamed bread were systematically evaluated. This research provides scientific support for promoting the application of curdlan in improving the freezing tolerance of frozen dough.

## 2. Materials and method

### 2.1. Materials

Curdlan was supplied by Haios Biology Co., Ltd (Shandong, China). Wheat flour, provided by Jinyuan Co., Ltd (Henan, China), contains 73.60% starch, 12.38% moisture, 13.44% protein and 0.49% ash. Instant dry yeast (*Saccharomyces cerevisiae*) was provided by Angel Yeast Co., Ltd (Hubei, China) and stored at 4 °C. Distilled water was made in laboratory.

### 2.2. Preparation of frozen dough and steamed bread

The basic ingredients of dough were wheat flour (100 g), curdlan (0%, 0.1%, 0.3%, 0.5%, 0.7% and 0.9%), yeast (1 g) and water (50 g). Curdlan and yeast were dispersed in distilled water, respectively. All materials were poured into dough mixer and stirred for 8 min. The obtained dough was divided into 100 ± 0.5 g samples, molded, packed with polyethylene bags, frozen at −30 °C for 2 h, and stored at −18 °C for 0, 2, 4, 6, 8, 10 and 12 weeks, respectively. Subsequently, the dough was thawed in an incubator at 30 °C and 80% humidity for 1 h to be determined.

For steamed bread making, frozen dough was thawed at 4 °C for 12 h, and fermented at 35 °C and 85% humidity for 50 min. The dough was molded, fermented again under same condition for 15 min, steamed in a steamer for 20 min and then taken out to cool down naturally.

### 2.3. Quality evaluation of steamed bread

The specific volume was accessed by millet replacement method (Zhao et al., 2021). The textural properties were determined using a TA-XT2i Texture Analyzer equipped with a P/36R probe (Stable Microsystems, UK). Slices of 15 mm thickness in the center of the steamed bread were taken to be evaluated. Sensory evaluation was conducted by 10 trained panelists (5 males and 5 females) using the literature method (Zhao et al., 2021). The sensory evaluation criteria were presented in Table S1.

### 2.4. Fermentation properties of frozen dough

The fermentation properties were tested by a rheofermentometer (F3, Chopin, Villeneuve-La-Garenne, France) with cylindrical weight of 2000 g. The test was conducted over 3 h at 35 °C. Fermentation properties including maximum fermentation height ($H_m$), gas retention volume ($V_r$), total gas releasing volume ($V_t$) and gas retention coefficient (R) were obtained.

### 2.5. Freezable water content of frozen dough

The enthalpy ($\Delta H$) was tested by a differential scanning calorimeter (DSC-8000, PerkinElmer, USA). The sealed DSC pan was cooled 40 °C to −20 °C at 10 °C/min, kept at −20 °C for 5 min, and then heated at 1℃/min to 10 °C (Liu et al., 2022b). The freezable water content (*FW*) was calculated using the formula:$FW(\%) = \frac{\Delta H}{\Delta H_0 \times W_t} \bullet 100\%$, where $\Delta H$ (J/g) was the enthalpy of the melting peak of the heat absorption curve; $\Delta H_0$ (334 J/g) was the enthalpy of the melting peak of moisture;$W_t$ (%) was the moisture content.

### 2.6. Water distribution state of frozen dough

The water distribution was analyzed by low field nuclear magnetic resonance (LF-NMR) (MicroMR-CL-I, Niumag Corporation, China) using the reported method (Liang et al., 2021). The transverse relaxation time ($T_2$) was measured using the Carr-Purcell-Meiboom-Gill (CPMG) sequence with parameters echo time 0.1 ms, echoes number of 2500, and 4 scans.

### 2.7. Molecular weight of gluten

The gluten in frozen dough was extracted using the literature method (Qian et al., 2021). Freeze-dried frozen dough powder was extracted with sodium phosphate buffer (PBS, 0.05 M, pH 6.8) containing 2% SDS under non-reducing condition. The powder was also extracted with the above PBS with 0.1% dithiothreitol (DTT) under reducing condition.

The molecular weight analysis was carried out on Agilent 1206 HPLC equipped with diode array detector and Zenxin sec-300 column (7.8 × 300 mm, 3 μm, Sepax Technologies, China). HPLC conditions: sample amount, 100 μL; elution solvent, PBS with 2% SDS; flow rate, 0.7 mL/min; column temperature, 30 °C; and detector wavelength, 214 nm. SDS-soluble polymers (SP), SDS-soluble monomers (SM), GMP and depolymerization degree were calculated from following formulas:

$$\mathrm{SP}\,(\%) = \frac{\mathrm{A_P}}{\mathrm{A_r}} \times 100\%$$

$$\mathrm{SM}\,(\%) = \frac{\mathrm{A_M}}{\mathrm{A_r}} \times 100$$

$$\mathrm{GMP}\,(\%) = 100\% - \mathrm{SP} - \mathrm{SM}$$

$$\text{Depolymerization degree}\,(\%) = \left(\frac{\mathrm{GMP_0} - \mathrm{GMP_d}}{\mathrm{GMP_0}}\right) \times 100\%$$

where $A_P$ was the peak area of SP, $A_M$ was the peak area of SM, and $A_r$ was the total peak area of proteins under reducing condition; $GMP_0$ and $GMP_d$ were the GMP proportions of gluten without storage and after storage, respectively.

### 2.8. Free sulfhydryl (SH) content

Free SH content of gluten was determined by Ellman's method using Tris-Glycine and 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) (Wang et al., 2018). The absorbance was measured at 412 nm against the blank. The linear regression equation was calculated from the standard curve of L-cysteine as $Y$=0.098$x$-0.0004, $R^2 = 0.9997$. Free SH content (μmol/g) was calculated from the absorbance values using the calibration curve.

### 2.9. Microstructure of frozen dough

The microstructure of dough was characterized by using scanning electron microscopy (SEM) (Quanta FEG-250, FEI, Netherlands). Small samples (8 mm × 8 mm × 8 mm) were taken from the center of freeze-dried dough, and observed by using SEM with 3.0 KV accelerating voltage (Li et al., 2021).

### 2.10. Statistical analysis

Each date was measured in triplicate. Experimental dates analysis was performed using the SPSS package (IBM Inc., New York, USA). Statistical differences were determined by independent sample T-test analysis and Duncan multiple comparation ($p < 0.05$).

## 3. Results and discussion

### 3.1. Quality evaluation of steamed bread

Specific volume, textural and sensory properties were measured to evaluate the quality of steamed bread made from frozen dough. As Fig. 1 showed, enhanced specific volume of steamed bread with the addition of curdlan was observed ($p < 0.05$), especially the maximum specific volume of 2.17 $cm^3 \cdot g^{-1}$ was obtained with 0.5% curdlan. After frozen storage for 10 weeks, a decrease in specific volume from 1.92 $cm^3 \cdot g^{-1}$ to 1.51 $cm^3 \cdot g^{-1}$ with reduction of 21.3% was detected in the control, as well as a reduction from 2.17 $cm^3 \cdot g^{-1}$ to 1.80 $cm^3 \cdot g^{-1}$ with reduction of 17.1% for steamed bread containing 0.5% curdlan. It was mainly attributed to the deterioration of frozen dough, which involved the destruction of gluten network structure and the reduced survival of yeast during frozen storage (Yadav et al., 2009). The positive effect of curdlan on the specific volume was probably related with the inhibition effect of curdlan on the deterioration of frozen dough during storage, which was further confirmed in this study.

As Fig. 2(a) presented, curdlan addition induced a decline in the hardness of steamed bread from fresh dough ($p < 0.05$), which reached the lowest of 2943 g with curdlan addition of 0.5%. Frozen storage of 10 weeks witnessed an increase in the hardness of the control from 2536 g to 4346 g ($p < 0.05$), while that of sample with 0.5% curdlan rose from 2205 g to 3520 g ($p < 0.05$). It was indicated that the increment in hardness of sample with 0.5% curdlan (59.6%) was lower than that of the control (71.4%) with same storage time. As is indicated in Fig. 2(b) and Fig. 2(c), the decrease of springiness and resilience with the extension of storage time was mainly due to the worsened air holding capacity of frozen dough. Cohesiveness presented a decreasing tendency during frozen storage time of 10 weeks, but 0.5% curdlan significantly inhibited the descend of cohesiveness ($p < 0.05$), as Fig. 2(d) showed. Meanwhile, the chewiness and adhesiveness gradually increased with prolonged storage time, and 0.5% curdlan effectively restricted the increments ($p < 0.05$), as Fig. 2(e) and Fig. 2(f) showed. Overall, the quality of steamed bread made from frozen dough was improved by 0.5% curdlan, indicating the enhanced freezing tolerance of frozen dough.

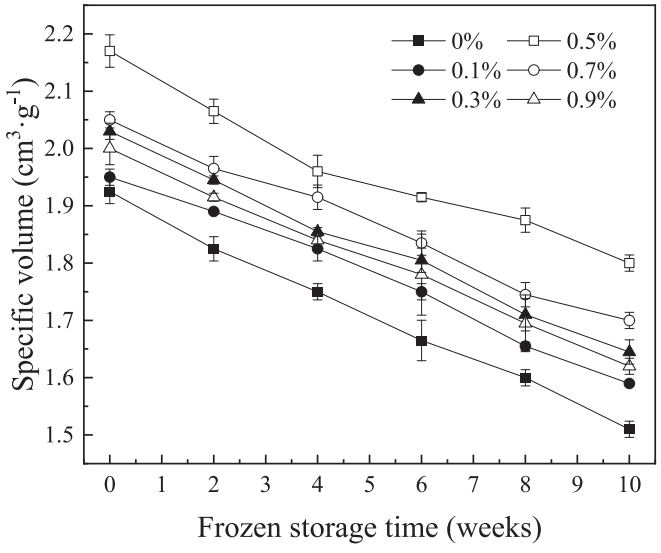


**Fig. 1.** Effect of curdlan on the specific volume of steamed bread made from frozen dough with different storage time.

Sensory evaluation results in Table 1 showed that 0.5% curdlan increased the sensory score from 82.7 to 88.9 ($p < 0.05$). Steamed bread with 0.5% curdlan presented better appearance, internal textural, mouse-feel and flavor than other samples. Further increase of curdlan content to 0.9% reduced the sensory score to 80.6. The sensory score of all samples presented downward trends during frozen storage process, resulting from the reduced consumers acceptability of steamed bread. After 10 weeks of storage, the sensory score of steamed bread with 0.5% curdlan decreased to 63.9, significantly higher than that of the control (58.6) ($p < 0.05$).

### 3.2. Rheofermentation properties of frozen dough

Rheofermentation properties provide the gas production and retention performance of frozen dough. As Fig. 3 showed, $H_m$ and R of fresh dough first increased and then decreased with curdlan content increasing from 0 to 0.9% ($p < 0.05$). Frozen dough with 0.5% curdlan reached the maximum $H_m$ and R of 33.6 mm and 98.0%, respectively. Meanwhile, no significant difference was observed in $V_t$ between all samples before storage ($p > 0.05$). It was concluded that 0.5% curdlan improved the gas-holding capability of frozen dough, which might be ascribed to the strengthened gluten network structure by curdlan (Wang et al., 2014). $H_m$, $V_t$ and R of all frozen doughs drastically declined during storage ($p < 0.05$), suggesting the reduced gas-releasing and gas-holding capability of frozen doughs. The reduced activity of yeast gave rise to decreased gas-releasing capability (Lu et al., 2021a). The reduced gas-holding capability and dough inflation properties was attributed to the gluten depolymerization (Wang et al., 2020). Addition of curdlan significantly increased $H_m$, $V_t$ and R of frozen dough with same storage time, especially for 0.5% curdlan ($p < 0.05$), which was in accordance with the steamed bread characteristics in Section 3.1. It was speculated that addition of 0.5% curdlan alleviated the depolymerization of the glutenin macropolymer (GMP) during frozen storage.

### 3.3. Freezable water content of frozen dough

Moisture in frozen dough was composed of freezable water and non-freezable water. The freezable water content in frozen dough was accessed by testing melting of enthalpy (ΔH). As Table 2 showed, curdlan addition significantly reduced the ΔH of frozen dough without storage ($p < 0.05$), implying that curdlan promoted the conversion of freezable water into non-freezable water. For instance, 0.9% curdlan decreased the freezable water content from 30.24% to 21.18% ($p < 0.05$), resulting from the strong water-holding ability of curdlan. Similar downward trend was observed in previous study in which curdlan addition reduced freezable water content in frozen cooked noodles (Liang et al., 2021).

The moisture content of all samples presented declining trend during storage ($p < 0.05$), which was mainly ascribed to the continuous evaporation of surface water and the migration of inner water to the surface throughout frozen storage process (Selomulyo and Zhou, 2007). ΔH and freezable water content of all samples had upward tendency during storage. After 10-week storage, 8.80 J/g, 7.23 J/g and 6.44 J/g increment were observed for 0%, 0.5% and 0.9% curdlan dough, respectively. Freezable water content of the control increased from 30.24% to 39.07% ($p < 0.05$), while that of frozen dough with 0.5% curdlan rose from 22.71% to 29.61% ($p < 0.05$) after 10 weeks storage. The connection between water molecules and amino acids in protein was damaged by the formation and recrystallization of ice crystals during storage, leading to releasing of bound water from gluten (Lu et al., 2021b). Although curdlan addition could not prevent the separation of water molecules, it restrained the changes in some extent. Therefore, less non-freezable water was available for ice crystals

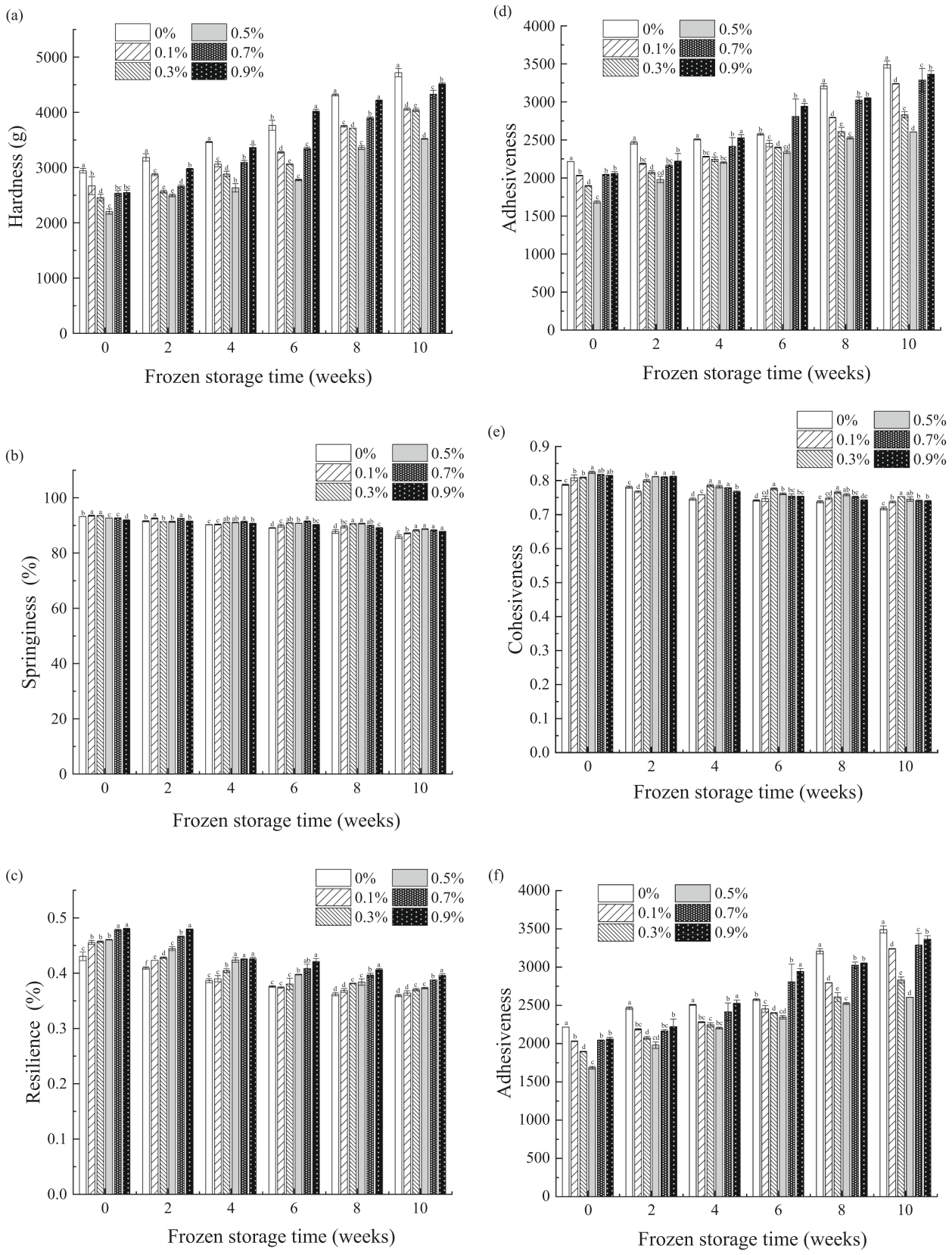


**Fig. 2.** Effect of curdlan on the textural properties of steamed bread made from frozen dough with different storage time: (a) hardness, (b) springiness, (c) chewiness, (d) adhesiveness, (e) cohesiveness, (f) resilience.

**Table 1**
The effect of curdlan on sensory score of steamed bread made from frozen dough with different storage time.

| Curdlan | Frozen storage time (weeks) | Specific volume | Shape | Smoothness | Color | Visual internal texture | Firmness | Stickiness | Texture in mouth | Flavor | Total scores |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0% | 0 | 14.4 ± 0.2$^{a}$ | 8.5 ± 0.2$^{a}$ | 4.5 ± 0.0$^{a}$ | 9.2 ± 0.0$^{a}$ | 12.2 ± 0.3$^{a}$ | 8.4 ± 0.0$^{a}$ | 8.0 ± 0.1$^{a}$ | 9.2 ± 0.2$^{a}$ | 8.3 ± 0.1$^{a}$ | 82.7 ± 0.5$^{a}$ |
| | 2 | 13.4 ± 0.3$^{b}$ | 7.9 ± 0.3$^{b}$ | 4.3 ± 0.1$^{ab}$ | 8.8 ± 0.2$^{ab}$ | 11.5 ± 0.2$^{b}$ | 8.1 ± 0.1$^{b}$ | 7.4 ± 0.2$^{b}$ | 8.7 ± 0.2$^{ab}$ | 7.9 ± 0.2$^{ab}$ | 78.0 ± 0.7$^{b}$ |
| | 4 | 12.6 ± 0.1$^{c}$ | 7.2 ± 0.2$^{c}$ | 4.1 ± 0.1$^{b}$ | 8.2 ± 0.4$^{b}$ | 10.8 ± 0.2$^{c}$ | 7.8 ± 0.2$^{b}$ | 6.8 ± 0.2$^{c}$ | 8.2 ± 0.2$^{b}$ | 7.4 ± 0.1$^{b}$ | 73.1 ± 1.1$^{c}$ |
| | 6 | 11.9 ± 0.4$^{d}$ | 6.6 ± 0.2$^{d}$ | 3.9 ± 0.2$^{bc}$ | 7.7 ± 0.3$^{bc}$ | 10.1 ± 0.3$^{d}$ | 7.5 ± 0.3$^{bc}$ | 6.2 ± 0.1$^{d}$ | 7.6 ± 0.2$^{c}$ | 6.9 ± 0.3$^{bc}$ | 68.4 ± 1.3$^{d}$ |
| | 8 | 10.9 ± 0.3$^{e}$ | 6.1 ± 0.3$^{de}$ | 3.7 ± 0.2$^{c}$ | 7.1 ± 0.2$^{c}$ | 9.3 ± 0.2$^{e}$ | 7.3 ± 0.2$^{bc}$ | 5.8 ± 0.2$^{d}$ | 7.0 ± 0.1$^{d}$ | 6.5 ± 0.3$^{c}$ | 63.7 ± 0.9$^{e}$ |
| | 10 | 10.0 ± 0.5$^{e}$ | 5.5 ± 0.4$^{e}$ | 3.5 ± 0.1$^{c}$ | 6.5 ± 0.2$^{d}$ | 8.5 ± 0.5$^{e}$ | 7.0 ± 0.3$^{c}$ | 5.0 ± 0.4$^{e}$ | 6.5 ± 0.4$^{d}$ | 6.1 ± 0.3$^{c}$ | 58.6 ± 1.6$^{f}$ |
| 0.5% | 0 | 16.9 ± 0.1$^{a}$ | 9.0 ± 0.0$^{a}$ | 4.5 ± 0.0$^{a}$ | 8.5 ± 0.0$^{a}$ | 14.5 ± 0.2$^{a}$ | 7.5 ± 0.1$^{a}$ | 9.5 ± 0.0$^{a}$ | 9.5 ± 0.0$^{a}$ | 9.0 ± 0.1$^{a}$ | 88.9 ± 0.2$^{a}$ |
| | 2 | 15.5 ± 0.3$^{b}$ | 8.4 ± 0.4$^{ab}$ | 4.3 ± 0.1$^{ab}$ | 8.2 ± 0.2$^{ab}$ | 13.5 ± 0.3$^{b}$ | 7.1 ± 0.2$^{ab}$ | 8.9 ± 0.0$^{b}$ | 9.1 ± 0.1$^{b}$ | 8.6 ± 0.0$^{b}$ | 83.6 ± 0.6$^{b}$ |
| | 4 | 14.4 ± 0.4$^{c}$ | 7.7 ± 0.3$^{b}$ | 4.1 ± 0.1$^{b}$ | 7.9 ± 0.3$^{b}$ | 12.5 ± 0.2$^{c}$ | 6.7 ± 0.3$^{b}$ | 8.3 ± 0.2$^{c}$ | 8.6 ± 0.2$^{c}$ | 8.2 ± 0.2$^{c}$ | 78.4 ± 1.2$^{c}$ |
| | 6 | 14.1 ± 0.3$^{c}$ | 7.0 ± 0.3$^{b}$ | 4.0 ± 0.2$^{b}$ | 7.5 ± 0.2$^{bc}$ | 11.5 ± 0.2$^{d}$ | 6.3 ± 0.3$^{bc}$ | 7.6 ± 0.2$^{d}$ | 8.0 ± 0.2$^{d}$ | 7.7 ± 0.3$^{c}$ | 73.7 ± 1.4$^{d}$ |
| | 8 | 13.9 ± 0.2$^{c}$ | 6.3 ± 0.2$^{c}$ | 3.9 ± 0.1$^{b}$ | 7.1 ± 0.2$^{c}$ | 10.5 ± 0.3$^{e}$ | 5.9 ± 0.2$^{c}$ | 6.9 ± 0.2$^{e}$ | 7.4 ± 0.2$^{e}$ | 7.1 ± 0.2 $^{cd}$ | 69.0 ± 2.1$^{e}$ |
| | 10 | 13.1 ± 0.4$^{d}$ | 5.8 ± 0.5$^{c}$ | 3.8 ± 0.2$^{b}$ | 6.7 ± 0.3$^{c}$ | 9.5 ± 0.5$^{f}$ | 5.5 ± 0.4$^{c}$ | 6.0 ± 0.3$^{f}$ | 7.0 ± 0.4$^{e}$ | 6.5 ± 0.4$^{c}$ | 63.9 ± 2.3$^{f}$ |
| 0.9% | 0 | 15.0 ± 0.0$^{a}$ | 8.5 ± 0.0a | 4.0 ± 0.0$^{a}$ | 8.5 ± 0.1$^{a}$ | 11.5 ± 0.2$^{a}$ | 8.2 ± 0.0$^{a}$ | 9.0 ± 0.1$^{a}$ | 8.5 ± 0.0$^{a}$ | 7.5 ± 0.0$^{a}$ | 80.7 ± 0.4$^{a}$ |
| | 2 | 14.2 ± 0.2$^{b}$ | 8.1 ± 0.1$^{b}$ | 3.9 ± 0.1$^{a}$ | 8.2 ± 0.1$^{ab}$ | 11.0 ± 0.3$^{ab}$ | 8.0 ± 0.1$^{a}$ | 8.4 ± 0.2$^{b}$ | 8.1 ± 0.2$^{ab}$ | 7.3 ± 0.1$^{ab}$ | 77.2 ± 1.3$^{b}$ |
| | 4 | 13.4 ± 0.3$^{c}$ | 7.5 ± 0.2$^{c}$ | 3.8 ± 0.1$^{ab}$ | 7.9 ± 0.2$^{b}$ | 10.4 ± 0.2$^{b}$ | 7.8 ± 0.3$^{ab}$ | 7.8 ± 0.1$^{c}$ | 7.7 ± 0.2$^{b}$ | 7.1 ± 0.2$^{b}$ | 73.4 ± 1.2$^{c}$ |
| | 6 | 13.3 ± 0.3$^{c}$ | 6.8 ± 0.2$^{d}$ | 3.8 ± 0.2$^{ab}$ | 7.5 ± 0.2$^{bc}$ | 9.8 ± 0.2$^{bc}$ | 7.5 ± 0.3$^{b}$ | 7.2 ± 0.3$^{d}$ | 7.4 ± 0.3$^{b}$ | 6.8 ± 0.2$^{bc}$ | 70.1 ± 2.1$^{d}$ |
| | 8 | 12.3 ± 0.4$^{d}$ | 6.2 ± 0.5$^{de}$ | 3.7 ± 0.1$^{b}$ | 7.1 ± 0.0$^{c}$ | 9.3 ± 0.3$^{c}$ | 7.5 ± 0.3$^{b}$ | 6.5 ± 0.3$^{de}$ | 7.0 ± 0.2$^{bc}$ | 6.5 ± 0.3$^{c}$ | 66.1 ± 2.4$^{e}$ |
| | 10 | 11.3 ± 0.3$^{e}$ | 5.7 ± 0.4$^{e}$ | 3.7 ± 0.2$^{b}$ | 6.7 ± 0.3$^{c}$ | 8.8 ± 0.5$^{c}$ | 7.2 ± 0.2$^{b}$ | 5.8 ± 0.4$^{e}$ | 6.5 ± 0.4$^{c}$ | 6.4 ± 0.5$^{c}$ | 62.1 ± 2.5$^{f}$ |

Data are expressed as mean ± standard deviation (n = 3). $^{a\text{-}f}$ Values labelled with different letter are significantly different ($p < 0.05$).

formation during frozen storage. The inhibiting effect of curdlan on the rise of freezable water content was mainly due to the retarded moisture mobility and strengthened gluten network by curdlan, which was proved in Section 3.4 and 3.5.

### 3.4. Water distribution of frozen dough

The moisture distribution behavior is extremely essential to the quality of frozen dough. Fig. 4 showed the $T_2$ relaxation distribution curves of frozen dough, in which $T_{21}$, $T_{22}$ and $T_{23}$ were bound water, immobilized water and free water, respectively. $T_{21}$, $T_{22}$ and $T_{23}$ value as well as the related peak area ratio ($A_{21}$, $A_{22}$ and $A_{23}$) were showed in Table S2.

As displayed in Fig. 4, T2 shifted to the left in the frozen dough with added curdlan before storage, implying that curdlan addition lowered the moisture mobility due to enhanced interaction strength between moisture and solids (Liu et al., 2022b). Addition of 0.9% curdlan increased $A_{21}$ from 13.38% to 14.71%, which indicated that curdlan increased the bound water content ($p < 0.05$). It was consistent with the result in Section 3.2, which found that curdlan addition reduced the freezable water content in frozen dough.

After storage for 10 weeks, $A_{21}$ decreased from 13.38% to 11.76% ($p < 0.05$), whereas $A_{23}$ increased from 0.54% to 1.13% for the control sample ($p < 0.05$). The result was mainly because that frozen storage treatment damaged the gluten network, leading to the releasing of bound water from the dough (Wang et al., 2017). $A_{21}$ decreased from 13.44% to 13.35% ($p < 0.05$), and $A_{23}$ increased from 0.68% to 0.95% for the sample with 0.5% curdlan ($p < 0.05$). Therefore, $A_{21}$ and $A_{23}$ of frozen dough with 0.5% curdlan was relatively more stable during storage, indicating that curdlan restricted the moisture migration in frozen dough during storage process. It was because of the strong hydrophilicity of curdlan and the enhanced crosslinking of gluten network in frozen dough caused by curdlan, which led to a stronger interaction with moisture in frozen dough (Liang et al., 2021). Moreover, peaks of $T_{22}$ and $T_{23}$ shifted to the left significantly for all samples after 10 weeks storage ($p < 0.05$), but $T_{22}$ and $T_{23}$ in frozen dough with curdlan shifted less than that of the control dough ($p < 0.05$). It was demonstrated that addition of curdlan (0.1%-0.5%) positively retarded the moisture migration of frozen dough during storage.

### 3.5. Molecule weight and free SH content of gluten proteins

SE-HPLC chromatograms of SDS-soluble gluten protein extracted under non-reducing condition were shown in Fig. 5. The chromatograms consist of three fractions: large polymeric glutenin (F1, Mw≈91 ~ 698 kDa), monomeric gliadin and glutenin (F2, Mw≈16 ~ 91 kDa), peptide chain (F3, Mw < 16 kDa) (Wang et al., 2015). F1 was recorded as SDS-soluble polymeric protein (SP), while F2 and F3 were denoted as SDS-soluble monomeric protein (SM). Total gluten proteins including GMP were dissolved under reducing condition, due to the breakage of the SS bonds by using of DTT. GMP was insoluble in SDS under non-reducing condition, but soluble in DDT under reducing condition. Fractions of SP, SM and GMP, and GMP depolymerization degree were summarized in Table 3.

Incorporation of curdlan resulted in relatively higher level of monomers fraction in the fresh dough. Frozen dough induced reduction of GMP, as well as increase of SP and SM in all samples, suggesting the occurrence of gluten depolymerization. After 10 weeks storage, the

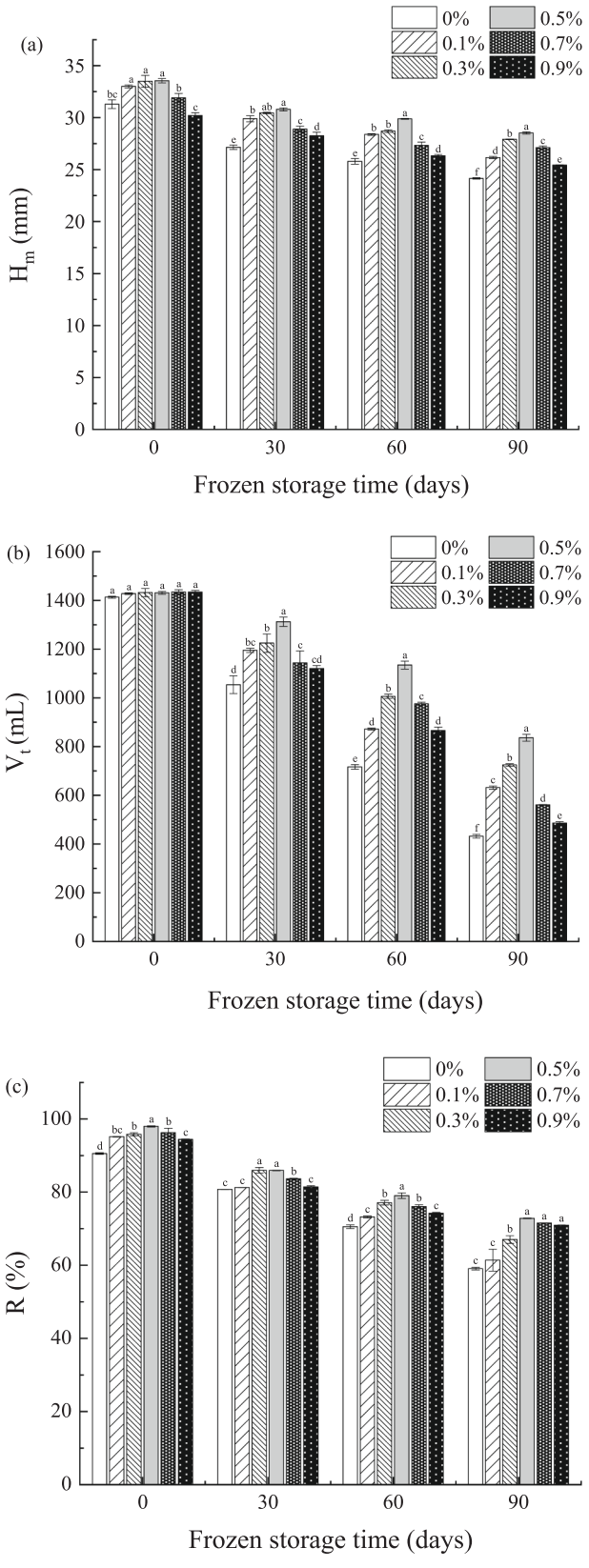


**Fig. 3.** Effect of curdlan on the fermentation properties of frozen dough during frozen storage. $H_m$, $V_t$ and R refer to maximum dough fermentation height, total release gas volume produced by the yeast cells and gas retention coefficient, respectively.

**Table 2**
Effect of curdlan on freezable water content of frozen dough during frozen storage.

| Curdlan (%) | Frozen storage time (weeks) | ΔH(J/g) | Moisture content (%) | Freezable water (%) |
|---|---|---|---|---|
| 0 | 0 | 40.40 ± 0.78$^{f}$ | 40.00 ± 0.00$^{a}$ | 30.24 ± 0.38$^{f}$ |
| | 2 | 42.23 ± 0.42$^{d}$ | 39.84 ± 0.02$^{b}$ | 31.80 ± 0.22$^{e}$ |
| | 4 | 44.17 ± 0.39$^{d}$ | 39.73 ± 0.03$^{c}$ | 33.36 ± 0.20$^{d}$ |
| | 6 | 45.96 ± 0.67$^{c}$ | 39.48 ± 0.11$^{d}$ | 35.11 ± 0.34$^{c}$ |
| | 8 | 47.38 ± 0.55$^{b}$ | 38.52 ± 0.25$^{e}$ | 36.90 ± 0.26$^{b}$ |
| | 10 | 49.20 ± 0.87$^{a}$ | 37.78 ± 0.33$^{f}$ | 39.07 ± 0.45$^{a}$ |
| 0.5 | 0 | 30.33 ± 0.30$^{e}$ | 40.07 ± 0.02$^{a}$ | 22.71 ± 0.22$^{f}$ |
| | 2 | 31.85 ± 0.27$^{d}$ | 39.95 ± 0.04$^{b}$ | 23.92 ± 0.14$^{e}$ |
| | 4 | 33.27 ± 0.34$^{c}$ | 39.77 ± 0.05$^{c}$ | 25.10 ± 0.17$^{d}$ |
| | 6 | 34.90 ± 0.51$^{b}$ | 39.36 ± 0.12$^{d}$ | 26.60 ± 0.26$^{c}$ |
| | 8 | 36.14 ± 0.63$^{ab}$ | 38.85 ± 0.09$^{e}$ | 27.91 ± 0.35$^{b}$ |
| | 10 | 37.56 ± 0.72$^{a}$ | 38.06 ± 0.23$^{f}$ | 29.61 ± 0.37$^{a}$ |
| 0.9 | 0 | 28.30 ± 0.20$^{e}$ | 40.07 ± 0.00$^{a}$ | 21.18 ± 0.16$^{f}$ |
| | 2 | 29.62 ± 0.35$^{d}$ | 39.75 ± 0.04$^{b}$ | 22.36 ± 0.19$^{e}$ |
| | 4 | 30.89 ± 0.28$^{c}$ | 39.63 ± 0.02$^{c}$ | 23.39 ± 0.16$^{d}$ |
| | 6 | 32.15 ± 0.46$^{b}$ | 39.49 ± 0.09$^{d}$ | 24.43 ± 0.25$^{c}$ |
| | 8 | 33.48 ± 0.57$^{ab}$ | 39.05 ± 0.17$^{e}$ | 25.72 ± 0.30$^{b}$ |
| | 10 | 34.74 ± 0.61$^{a}$ | 38.29 ± 0.33$^{f}$ | 27.22 ± 0.32$^{a}$ |

Data are expressed as mean ± standard deviation (n = 3). $^{a\text{-}f}$ Values labelled with different letter are significantly different ($p < 0.05$).
$\Delta H$ refers to the melting enthalpy.

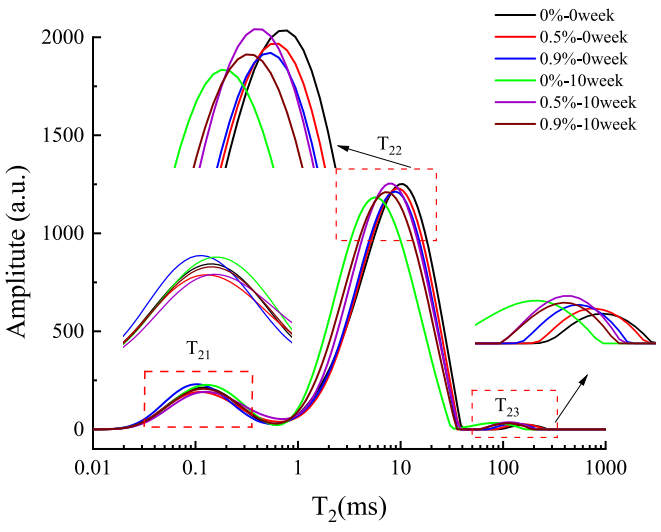


**Fig. 4.** Effect of curdlan on the $T_2$ relaxation distribution curves of frozen dough during frozen storage. $T_{21}$, bound water; $T_{22}$, immobilized water; $T_{23}$, free water.

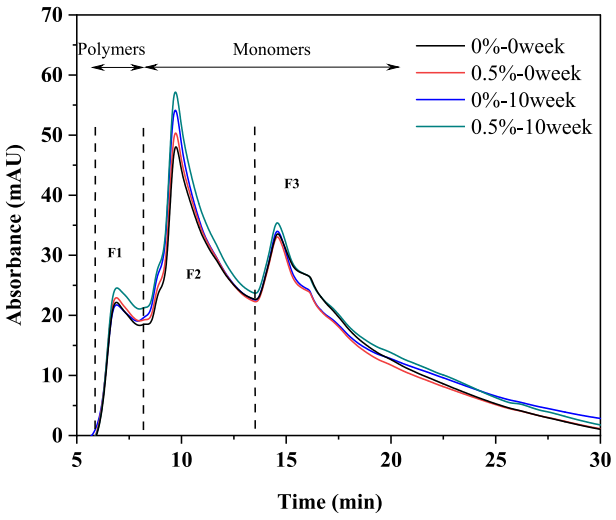


**Fig. 5.** SE-HPLC chromatograms of SDS-soluble gluten protein. F1, large polymeric glutenin; F2, monomeric gliadin and glutenin; F3, peptide chain.

increment of SM level (8.79%) for the control was remarkably higher than that of SP (2.07%) ($p < 0.05$), implying that GMP preferred to depolymerize into monomers rather than polymers. However, addition of curdlan suppressed the increment of both polymers and monomers during 10 weeks storage. Higher proportion of GMP (22.76%) was detected in dough with 0.5% curdlan than that in the control (21.32%) after 10 weeks of storage. The GMP depolymerization of the dough with 0.5% curdlan (22.82%) was remarkably lower than that of the control (33.73%) ($p < 0.05$), implying that 0.5% curdlan alleviated the depolymerization of GMP during frozen storage. It was reported that curdlan interacted with gluten via hydrophobic and steric-hindrance interactions, which enhanced the gluten network structure (Liu et al., 2022a).

The depolymerization of GMP was induced by the breakage of SS bonds. Therefore, free SH content was tested to investigate the effect of curdlan on the gluten network structure. After frozen storage for 10 weeks, free SH content of the control increased from 6.72 μmol/g to 8.84 μmol/g ($p < 0.05$), while that of the dough with 0.5% curdlan increased from 6.57 μmol/g to 8.61 μmol/g ($p < 0.05$), resulting from the breakage of SS bonds. The breakage of SS bonds was mainly caused by ice crystallization. Furthermore, protein denaturation caused by low-temperature freezing was also a contributor (Wang et al., 2016b). Moreover, frozen dough with 0.5% curdlan had significantly lower free SH content than the control with same storage time ($p < 0.05$), further proving the alleviation effect of curdlan on the depolymerization of GMP. This effect was directly related with the retardation of moisture migration, which had been proved in Section 3.4. Furthermore, this effect was probably connected with the enhancement of gluten network structure by interaction with curdlan, which should be further studied.

### 3.6. Microstructure of frozen dough

SEM images of frozen dough before and after frozen storage were shown in Fig. 6. For the control sample before frozen storage, the surface was smooth and regular with starch granules embedded in gluten network. Frozen dough with 0.5% curdlan also showed uniform surface. However, gluten protein for sample with 0.9% curdlan was not continuous, and starch granules were not be completely embedded in gluten network. This suggested that excessive curdlan was not benefit for the formation of gluten network. After 10 weeks of storage, the starch granules were seriously damaged for the control sample, and some grooves and cracks appeared for the control sample. Additionally, the gluten network structure collapsed, which resulted in uncomplete embedment of starch granules. The destruction of the structure of frozen dough was primarily caused by water migration and ice recrystallization. Frozen dough with 0.5% curdlan showed less fragmentation, which proved that curdlan protected the frozen dough structure during storage.

## 4. Conclusion

Fermentation properties showed that curdlan retained the gas production and retention capability of frozen dough during storage. Textural results suggested that curdlan alleviated the increment of the hardness, chewiness, adhesiveness and cohesiveness, as well as the reduction of springiness and resilience of steamed bread. The melting enthalpy and NMR results demonstrated that curdlan restricted the conversation of bound water into freezable water, and inhibited the moisture migration in frozen dough during storage. Besides, 0.5% curdlan caused lower GMP depolymerization degree and free SH content of frozen dough. Therefore, curdlan had alleviation effect on the depolymerization of GMP, which was not only related with the retarded migration of moisture, but also probably connected with the strengthening effect of curdlan on the gluten network structure. Microstructure results proved that the deterioration of the structure during frozen storage was retarded by curdlan. Therefore, curdlan enhanced the freezing tolerance and alleviated the quality deterioration of frozen dough during storage. Furthermore, it is necessary to explore the interaction between curdlan and gluten in frozen dough.

## CRediT authorship contribution statement

**Beibei Zhao:** Conceptualization, Investigation, Data curation, Formal analysis, Writing – original draft, Project administration, Writing – review & editing, Funding acquisition. **Liuyu Hou:** Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Ting Liu:** Methodology, Data curation, Formal analysis. **Xinru Liu:** Methodology, Data curation, Formal analysis. **Shijian Fu:** Methodology, Data curation, Formal analysis. **Hua Li:** Conceptualization, Data curation, Project administration, Writing – review & editing, Funding acquisition.

## 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 work reported in this paper.

**Table 3**
Proportions of polymers, monomers and GMP, GMP depolymerization and free SH of frozen dough.

| Curdlan (%) | Frozen storage time (weeks) | Polymers (%) | Monomers (%) | GMP (%) | GMP depolymerization (%) | Free SH content (μmol/g protein) |
|---|---|---|---|---|---|---|
| 0 | 0 | $17.75 \pm 0.12^{b}$ | $50.07 \pm 0.08^{b}$ | $32.17 \pm 0.12^{a}$ | – | $6.72 \pm 0.05^{b}$ |
| | 10 | $19.82 \pm 0.15^{a}$ | $58.86 \pm 0.18^{a}$ | $21.32 \pm 0.22^{b}$ | $33.73 \pm 0.11^{a}$ | $8.84 \pm 0.06^{a}$ |
| 0.5 | 0 | $17.89 \pm 0.18^{b}$ | $52.63 \pm 0.12^{b}$ | $29.49 \pm 0.14^{a}$ | – | $6.57 \pm 0.04^{b}$ |
| | 10 | $19.32 \pm 0.14^{a}$ | $57.92 \pm 0.09^{a}$ | $22.76 \pm 0.15^{b}$ | $22.82 \pm 0.09^{b}$ | $8.61 \pm 0.07^{a}$ |

Data are expressed as mean ± standard deviation (n = 3). $^{a-e}$ Values labelled with different letter are significantly different ($p < 0.05$).
GMP, gluten macropolymers; free SH, free sulfhydryl groups.

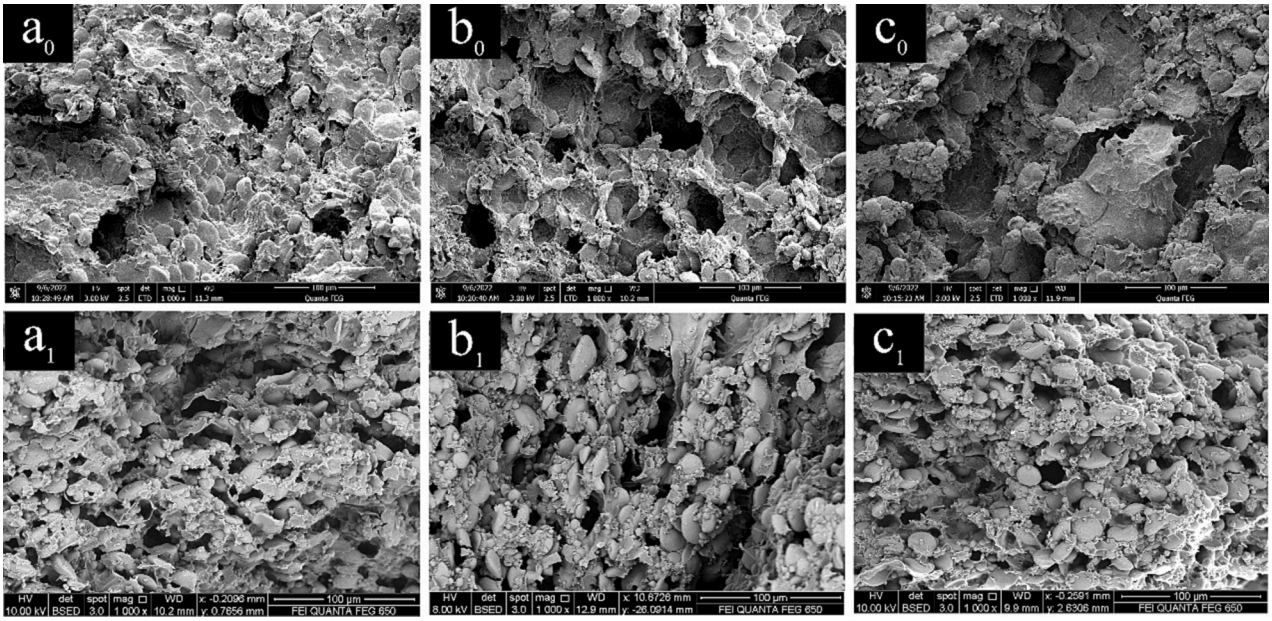


**Fig. 6.** Scanning electron microscope of dough×1000 magnification. a, b and c represent the control, dough with 0.5% curdlan and dough with 0.9% curdlan, respectively. 0 and 1 represent the dough without frozen storage and the frozen dough after 10 weeks of storage.

## Data availability

Data will be made available on request.

## Acknowledgments

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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.2023.100832.

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