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Evaluation of the antifreeze effects and its related mechanism of sericin peptides on the frozen dough of steamed potato bread

Shengxiang Gong[1,2] | Danlu Yang[2] | Qiaoyu Wu[2] | Shaoyun Wang[3] | Zhong Fang[1] | Yue Li[1] | Feifei Xu[1] | Zhengwu Wang[2] | Jinhong Wu[1,2]

[1]The Key Laboratory of Synthetic and Biological Colloids, Ministry of Education, Jiangnan University, Wuxi, China

[2]Department of Food Science and Engineering, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China

[3]Institute of Food and Marine Bioresources, College of Biological Science and Technology, Fuzhou University, Fuzhou, China

Correspondence
Jinhong Wu, Department of Food Science and Engineering, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China.
Email: wujinhong@sjtu.edu.cn

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Abstract

This study investigated the effects of sericin peptides (SPs) on frozen potato dough with trehalose as a reference. The results found that SPs decreased freezable water content in frozen dough and increased yeast survival ratio significantly after 4-week frozen storage. Additionally, compared with control group and frozen dough containing 5% of trehalose, frozen dough with 5% of SPs showed less starch granule separation and more complete gluten membrane during 8-week storage. Specific volume of the frozen dough with 5% of SPs decreased by 6.3%, significantly lower than the control and reference groups. Furthermore, compared with trehalose, SPs effectively decreased the hardness and gumminess of steamed potato breads, as well as enhanced their resilience. The quality improvement of frozen potato dough with SPs could be attributed to ideal structural matching, hydrogen bonds, and non-bond interactions between sericin peptides and ice surface, as well as the inhibition of the formation and recrystallization of ice crystals.

Practical applications

Frozen dough method is widely used in baked flour products, providing fresh, safe, consistent quality products and simplifies the manufacturing process. However, the overall quality of frozen dough decreased gradually during frozen storage. Traditional antifreezes (trehalose and sorbitol) have high calories, which are not conducive to diabetics and obese patients. Therefore, it is essentially important to find a new and efficient antifreeze to replace traditional antifreezes. SPs, the hydrolysate of sericin, exhibited a strong cryoprotection and antifreeze activity according to this study. We can expect a potential application of SPs in cell protection and food preservation.

1 | INTRODUCTION

Sericin is a globular protein derived from the outer sticky layer of natural silkworm cocoon. Sericin is water-soluble and exhibits high hydrophilic amino acids content, which attribute to its excellent water retaining characteristics (Zhang, 2003). Sericin peptide, the hydrolysate of sericin, is biologically active and demonstrates anti-oxidative, cosmetic, hypoglycemic, and anticancer effects on the human body (Fan, Wu, Chen, Mao, & Ren, 2010). In addition, sericin peptide has been found to possess high cryoprotective activity. Tsujimoto, Takagi, Takahashi, Yamada, and Nakamori (2001) overpressed dimers of sericin peptides in E. coli, and found that the transformants exhibited a prominent increase in cell viability and inhibition of the denaturation of lactate dehydrogenase after

Shengxiang Gong and Danlu Yang contributed equal to this paper.

freeze. Wu, Wang, Wu, and Wang (2012) had also suggested that sericin enzymatic peptides could protect grass carp surimi against freeze-induced denaturation. Recently, Wu et al. (2015) isolated and characterized a novel sericin antifreeze peptide which was demonstrated to prevent cells or tissues from freeze denaturation, and its thermal hysteresis activity reached as high as 0.94°C.

Potato is the third largest food crop in the world. Fresh potatoes are rich in nutrients and possess various medicinal effect. Known as the "second bread" in Europe, potatoes have always been a staple food and have been widely consumed throughout history. Consequently, highly processed potato products might have important impact on development. Steamed bread is a traditional fermented flour product in China that has high nutritional value. Steamed bread is produced by steaming instead of baking, therefore, formation of acrylamide is avoided due to the lack of Maillard reaction involved (Ma et al., 2016). However, steamed bread has relatively short shelf life and cannot be produced in large-scale. Therefore, novel production process to improve shelf life of potato steamed bread needs to be investigated.

Frozen dough method is widely adopted for baked flour products since it ensures the production of fresh, safe, and quality consistent products following a simple manufacturing process (Jia et al., 2016). However, the overall quality of frozen dough gradually decreases during frozen storage (Phimolsiripol, Siripatrawan, Tulyathan, & Cleland, 2008). Wang et al. found that the process of steamed bread dough deterioration during frozen storage is a remarkable and notorious phenomenon, leading to poor loaf volume and significant degradation of textural properties (Wang, Jin, & Xu, 2015; Wang, Tao, Jin, & Xu, 2015). Two factors have been identified as possible reasons for the worsening of bread quality: firstly, a decrease in gassing power owing to a decline in both yeast viability and activity; and secondly the gradual loss of dough strength (Ribotta, León, & Añón, 2001). In order to suppress the deterioration of frozen dough during storage, researchers proposed adding antifreeze into the frozen dough, which can significantly improve its antifreeze ability, as well as reduce its freezing point (Zhang, Zhang, Shen, & Deng, 2018). Ding et al. demonstrated that antifreeze protein (BaAFP-1) was highly effective in preserving frozen doughs by improving their thermal properties and restricting water mobility and distribution (Ding et al., 2015). Bai et al. (2018) also showed that trehalose exhibited significant inhibitory effect on the thawing of shrimp during frozen storage.

Trehalose, a ubiquitous stress metabolite in organisms, is widely used as antifreeze in frozen dough. However, trehalose is a carbohydrate-based antifreeze, which increases the sweetness of the dough, rendering it unsuitable for diabetics (Cheung, Liceaga, & Li-Chan, 2010). Therefore, this study investigates sericin peptide, a protein-based antifreeze, while using trehalose as a reference. To evaluate the effects of sericin peptide on frozen dough of steamed potato bread at freezing and throughout frozen storage, sericin peptide was added to the formula of dough. The aim of this study is to clarify the antifreeze effects and the related mechanism of sericin peptides on the frozen dough of steamed potato bread.

2 | MATERIALS AND METHODS

2.1 | Materials

Wheat flour (11% of protein, 1.5% of lipid, and 73.5% of carbohydrate) was purchased from Yihai Kerry (Yanzhou) Oils & Grans Industries Co., Ltd (Yanzhou, China). Potato flour (8.95% of protein, 1.85% of lipid, and 68.12% of carbohydrate) was obtained from Xisen Potato Powder Co., Ltd (Inner Mongolia, China). Sericin peptides (SPs) were purchased from Huzhou New Tenth biotechnology Co., Ltd (Huzhou, China). Trehalose and dried yeast (Angel brand, Hubei, China) were purchased from a local supermarket (Shanghai, China). All other chemicals were of analytical grade or higher.

2.2 | Cryoprotective effect of SPs on the survival of frozen yeast

One gram of dry yeast was suspended in 50 ml of sterilized saline water. Trehalose (2% and 5%, wt/vol) and SPs (2% and 5%, wt/vol) were added to the yeast suspensions, respectively. Suspension without antifreeze was used as the control. Suspension was mixed for 10 min, and then transferred to a quick freezing cabinet at −36°C for 2 hr. Subsequently, the suspension were transferred to a refrigerator at −18°C and stored for 0, 1, 2, 3, and 4 weeks. Frozen suspension was thawed at 38°C and 65% rh in a fermentation cabinet for 40 min. According to the method (Shibata, Obase, Itoh, & Amemiya, 2018), yeast survival ratio was calculated. Using a 10-fold serial dilution method, the thawed suspension was diluted with sterilized saline water to a concentration of 10^{-5}–10^{-7}. Hundred microliter of each sample was spread on potato dextrose agar (PDA) plates, and incubated at 28°C for 3 days. Plates with colonies at 10–150 colony-forming units (CFUs) were selected and counted.

2.3 | Preparation of frozen-thawed potato dough

The dough was prepared with 800 g wheat flour, 200 g of potato flour, 20 g of yeast, 20 g of sucrose, and 600 g of water. Doughs with trehalose contained 20 g or 50 g of trehalose (2% and 5% of flour basis), while doughs with SPs contained 20 g or 50 g of SPs (2% and 5% of flour basis). Dough without antifreeze was used as the control. Then, the dough was mixed in a vertical mixer until the gluten was fully stretched. The fresh dough was immediately divided into 100 g pieces. The divided dough was shaped and transferred to a quick freezing cabinet to be frozen at −36°C for 2 hr, and then stored at −18°C for 0, 1, 2, 3, and 4 weeks. After different freezing periods, the potato dough was thawed in a fermentation cabinet at 38°C and 65% rh for 1 hr.

2.4 | Determination of freezable water content

Using the method described by Bot (2007), the freezable water content of the dough samples was assessed using a NETZSCH 204 F1 differential scanning calorimetry (DSC) equipped with a refrigerated

cooling system (NETZSCH, GmbH, Selb, Germany). The instrument was operated under a dry nitrogen atmosphere, and an empty pan was used as the reference. Approximately 10 mg of the thawed dough was weighed and immediately sealed in an aluminum pan. The temperature profile was as follows: equilibration at 20°C for 5 min, cooling to −30°C at a rate of 5°C/min, equilibration at −30°C for 5 min, then heating to 30°C at a rate of 5°C/min. The melting enthalpies (ΔH_m) of potato dough samples with a series of moisture contents were determined. Freezable water content was calculated directly from the enthalpy (ΔH) of dough divided by that of pure water.

2.5 | Microstructure observation

Fragments of 5 mm × 5 mm × 5 mm were dissected from the center part of freeze-thawed potato doughs. Firstly, the fragments were fixed using 2.5% of glutaraldehyde at 4°C for 12 hr. Then, they were washed three times with 0.1 M of phosphate buffer (pH 7.4) for 15 min each time. Next, they were eluted with a gradient concentrations of ethanol (30%, 50%, 70%, 80%, 95%, and 100%) for 15 min in turn. The samples were then dried in an automated critical point dryer (Leica EM CPD300, Leica Microsystems, Germany), and sprayed with gold in a vacuum sprayer (Leica EM SCD050, Leica Microsystems, Germany). Dough fragments were further observed using a scanning electron microscope (Hitachi S-3400N II, Hitachi Science Systems, Ltd., Japan). Micrographs at 1,200 × magnification were captured.

2.6 | Baking performance of frozen potato dough

Thawed potato dough pieces were hand-molded, fermented, and then steamed for 20 min. Steamed breads were cooled to room temperature. The weight of steamed bread was measured, and their specific volume was calculated according to the AACC method 10-05.01 (AACC International, 2010). Uniform slices of 25 mm in thickness were taken from the center of steamed breads. Subsequently, their textural characteristics were measured by a universal TA texture analyzer (Shanghai Teng Ba Instrument Technology Co., Ltd., Shanghai, China) equipped with a P/36R cylindrical probe. The test program was set to the texture profile analysis (TPA) mode with pre-test speed of 2 mm/s, test speed of 1 mm/s, and posttest speed of 2 mm/s. The compression level was 50% of the original height. Results were recorded for the following parameters: hardness, cohesiveness, and chewiness. The potato dough without antifreeze was used as control.

2.7 | Statistical analysis

All experiments were performed in triplicates, and the data were shown as mean ± standard deviation. Analysis of variance (ANOVA) was conducted using the SPSS 16.0 soft (SPSS Inc., USA). The data were analyzed using Duncan's multiple range tests at a significance level of $p < 0.05$.

3 | RESULTS AND DISCUSSION

3.1 | Cryoprotective effect of SPs

It has been widely reported that the quality of frozen dough is closely related to yeast survival ratio (Nguyen et al., 2018). During the frozen storage of dough, the formation of ice crystals could cause yeast cell death and decrease their viability (Acker & Mcgann, 2003). Therefore, the cryoprotective effect of SPs could be examined by investigating the yeast survival ratio after different durations of frozen storage. As shown in Figure 1, the yeast survival ratio decreased during the 4 weeks of frozen storage, with the most severe deaths of yeast observed in the first 2 weeks. The main reason could be that during the quick freeze procedure and frozen storage, the freezable water in the system crystallizes and recrystallizes (Yadav, Patki, Sharma, & Bawa, 2009), which could lead to the formation of large ice crystals in the system that damage the yeast cell membrane (Ribotta et al., 2003). In the early stage of frozen storage, yeasts were seriously damaged by ice crystals and mortality increased rapidly. After that, the decrease in yeast survival ratio slowed down, potentially attributing to activation of the yeast's own anti-reverse regulation, such as the production of trehalose, to protect itself from harmful environment.

As shown in Figure 1, after the same duration of frozen storage, the yeast survival ratio of the control group was significantly lower than those of the experimental groups supplemented with trehalose and SPs. An obvious trend was demonstrated in Figure 1, indicating that the yeast survival ratio significantly increased with an increase in trehalose and SPs concentrations. This observation was consistent with previous reports (Zhang, Zhang, & Wang, 2007). A maximum yeast survival ratio of 49.22% was observed after 4 weeks of frozen storage at 5% of SPs concentration in frozen dough. This indicated that, compared to trehalose, the cryoprotective effect of SPs is significantly ($p < 0.05$) more prominent. This phenomenon

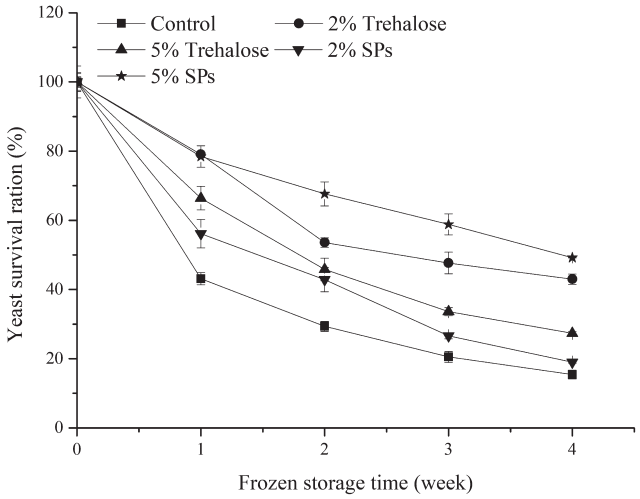

FIGURE 1 The cryoprotective effect of SPs and trehalose of different concentrations at various durations of frozen storage. Bars represent standard deviations

could potentially be attributed to the inhibitory effect of SPs on ice formation.

3.2 | Effect of SPs on freezable water content

The moisture content of food commonly consists of two portions, namely bound water and bulk water. Bulk water flows freely and freezes at 0°C, therefore, it is called freezable water. While bound water does not freeze even at −40°C, therefore, it is referred to as unfreezable water (Ross, 2010). During the frozen storage and transport of dough, the crystallization and recrystallization of freezable water could damage the gluten network structure of dough and dampen yeast viability. (Yadav et al., 2009).

As shown in Figure 2, freezable water content varies depending on storage time and levels of antifreeze content. For fresh potato doughs, the freezable water content decreased following trehalose and SPs addition, indicating that the antifreeze prevented the formation of ice crystals in the doughs. However, the freezable water content of frozen potato doughs increased significantly with increase in storage time. After 4 weeks of frozen storage, the freezable water content of the control group increased by 20.52%, which was significantly ($p < 0.05$) higher than those of frozen potato doughs containing 5% of trehalose and 5% of SPs (14.44% and 10.53%). In addition, as shown in Figure 2, compared with trehalose, SPs are more effective in reducing the freezable water content and inhibiting the formation of ice crystals. The decrease in freezable water content after addition of SPs could be attributed to the interactions between water molecules and the side chains of SPs. As theorized by Wu et al. (2015), water molecules could interact with SPs via hydrogen bonds, hydrophobic interaction, and non-bond interaction, which could prevent water migration and the formation of freezable water.

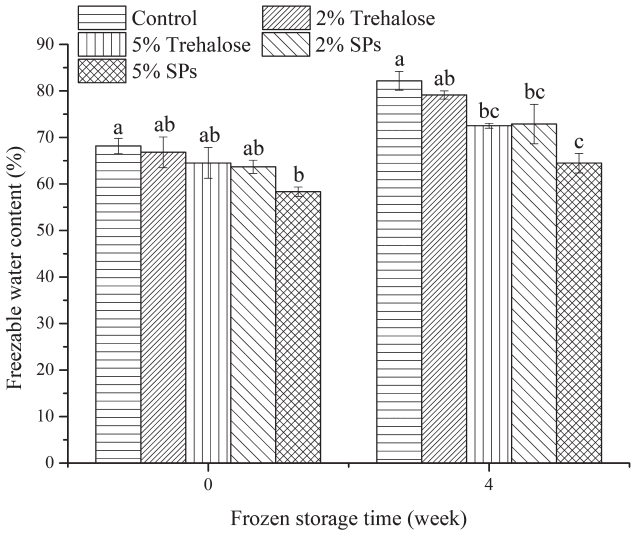

FIGURE 2 Variation of freezable water content in doughs containing different concentrations of SPs and trehalose at various durations of frozen storage. Means indicated by the same letter for the same storage time do not differ significantly ($p < 0.05$)

3.3 | Microstructure of frozen potato dough

The frozen potato doughs with addition of 5% of antifreeze were chosen as *SEM* experimental samples, because high content of antifreeze is associated with more obvious protection against gluten network structure. The *SEM* images of frozen potato dough after 0, 4, and 8 weeks of storage at a magnification of 1,200 × are shown in Figure 3.

The many oblate granules of different sizes were representative of starch granules, while the homogenous membrane indicated the network structure of wheat gluten. For fresh potato dough with no antifreeze (Figure 3a), dough with 5% of trehalose (Figure 3b), and dough with 5% of SPs (Figure 3c), the starch granules were firmly embedded in the relatively complete gluten membrane. However, as shown in Figure 3d,g, after 4 and 8 weeks of storage, the gluten membrane of the control group was significant thinner, large caverns were found in the microstructure, and starch granules were exposed, indicating the destruction of the gluten network structure. The gluten membrane of frozen potato dough with 5% of trehalose was also thinner and impaired with holes (Figure 3d,h). However, the ice crystals in dough with 5% of trehalose caused less damage to gluten structure than the control group. Variations in the frozen potato dough with 5% of SPs after 4 and 8 weeks of storage were shown in Figure 3f,i. The starch granules were still evenly embedded on the gluten membrane, and the membrane was relatively intact with only a few holes. Kontogiorgos, Regand, Yada, and Goff (2007) reported that frozen dough with 0.1% of ice structuring proteins showed fewer holes compared with control dough, however the difference was not significant, potentially due to the low content of ice structuring proteins in the frozen dough studied.

During frozen storage, ice crystals formed by crystallization or recrystallization of water in dough could cause irreversible damage to the gluten network structure. Holes could be found on the gluten membrane of dough after these ice crystals had melted (Zounis, Quail, & Wootton, 2002). In addition, substances with reducing property released by dead and injured yeasts could result in depolymerization of the gluten network (Nguyen et al., 2018). SPs can inhibit recrystallization and affect the growth state of ice crystals, thereby, protecting the structural network of gluten. Indeed, our results showed that frozen potato dough with 5% of SPs possessed a complete gluten membrane even after 8 weeks of storage, indicating that SPs can effectively protect the integrity of the gluten network structure and improve the quality of frozen potato dough.

3.4 | Effect of SPs on specific volume of steamed bread

Specific volume is one of the important parameters for analyzing the quality of fermented flour products and correlated to the rheology and fermentation properties of dough (Banu, Stoenescu, Ionescu, & Aprodu, 2011; Huang, Kim, Li, & Rayas-Duarte, 2008; Kahraman et al., 2008; Mudgil, Barak, & Khatka, 2016). Many researchers have reported that specific volume of frozen dough

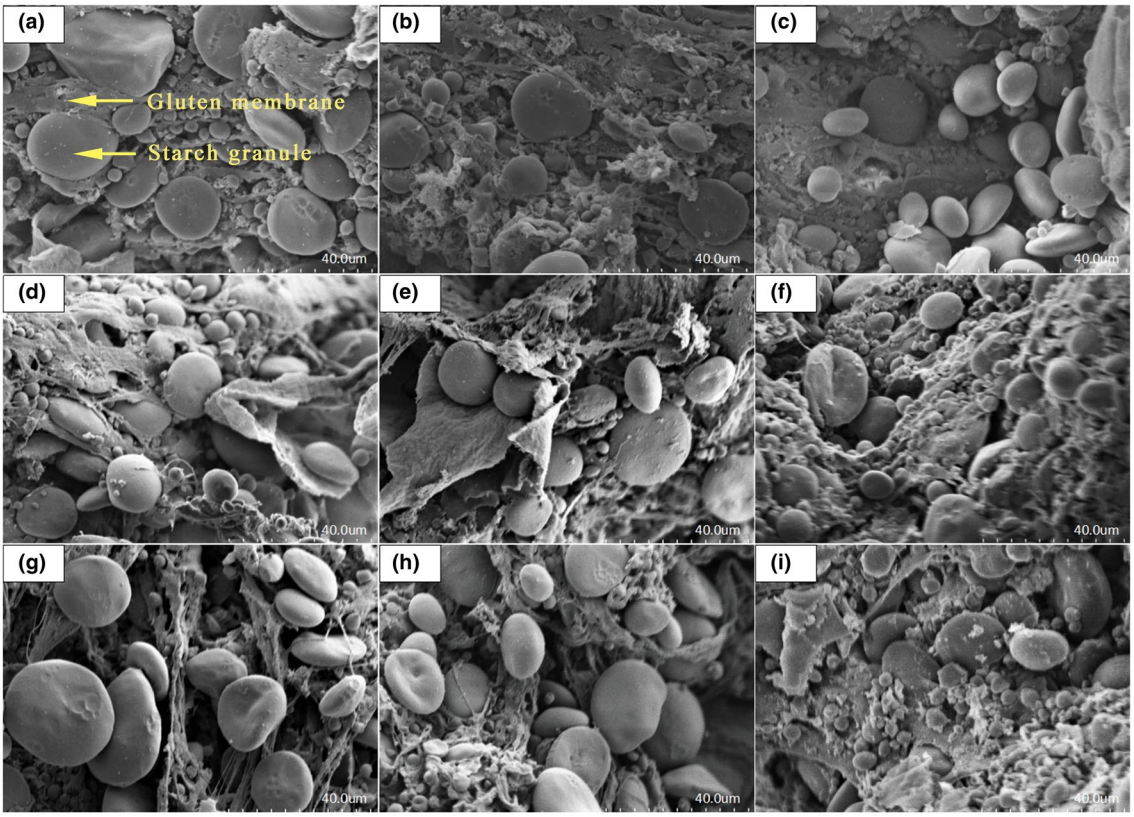

FIGURE 3 Scanning electron microscopic analysis of microstructure of frozen potato dough at 1,200× ((a–c) represent doughs stored at −18°C at 0 weeks with no antifreeze, 5% of trehalose, and 5% of SPs, respectively; (d–f) represent doughs stored at −18°C at 4 weeks with no antifreeze, 5% of trehalose, and 5% of SPs, respectively; (g–i) represent doughs stored at −18°C at 8 weeks with no antifreeze, 5% of trehalose, and 5% of SPs, respectively)

could be weakened as frozen storage period increases (Yi & Kerr, 2009). As shown in Table 1, the specific volume for all steamed potato breads decreased with increase in duration of frozen storage. The decrease in volume of steamed bread could attribute to the damage of gluten network and the decrease in yeast activity. The specific volumes of the experimental groups with the addition of 2% of trehalose and SPs were slightly higher than that of the control group. However, the addition of 5% of trehalose and SPs showed the most significantly improvement in the specific volume of steamed potato breads with increasing time in frozen storage. After 4 weeks of frozen storage, the specific volume of the control group decreased from 1.98 to 1.44 cm^3/g (27.3%), while the specific volume of experimental group with 5% of trehalose decreased from 1.85 to 1.72 cm^3/g (7.1%). In addition, the specific volume of experimental group with 5% of SPs also decreased from 2.06 to 1.93 cm^3/g (6.3%). These results indicated that the addition of trehalose and SPs could effectively inhibit the decrease in specific volume of steamed breads with increasing time in frozen storage. Compared with trehalose, the inhibitory effect of SPs was significantly ($p < 0.05$) higher. Our results demonstrated that SPs could effectively reduce freezable water content of frozen potato dough, inhibit ice crystal formation and recrystallization, and protect the gluten network and yeast cells of dough. Consequently, SPs addition could improve the gas production ability of yeast and gas holding capacity of gluten. These improvement led to an increase in the specific volume of steamed potato breads.

TABLE 1 Effect of SPs on the specific volume (cm^3/g) of steamed bread

Time	Control	2% Trehalose	5% Trehalose	2% SPs	5% SPs
0 week	1.98 ± 0.08^a	1.98 ± 0.07^a	1.85 ± 0.19^a	2.09 ± 0.07^a	2.06 ± 0.11^a
1 week	1.73 ± 0.06^a	1.88 ± 0.08^a	1.82 ± 0.29^a	1.98 ± 0.13^a	2.06 ± 0.26^a
2 weeks	1.70 ± 0.10^a	1.78 ± 0.05^a	1.77 ± 0.07^a	1.98 ± 0.10^a	2.03 ± 0.13^a
3 weeks	1.47 ± 0.04^a	1.68 ± 0.06^a	1.77 ± 0.06^a	1.85 ± 0.20^b	2.02 ± 0.08^b
4 weeks	1.44 ± 0.02^a	1.65 ± 0.02^b	1.72 ± 0.05^c	1.82 ± 0.11^d	1.93 ± 0.08^d

Notes: Values are expressed as mean ± *SD* of triplicate samples. Means with the same letters in a row do not differ significantly ($p < 0.05$).

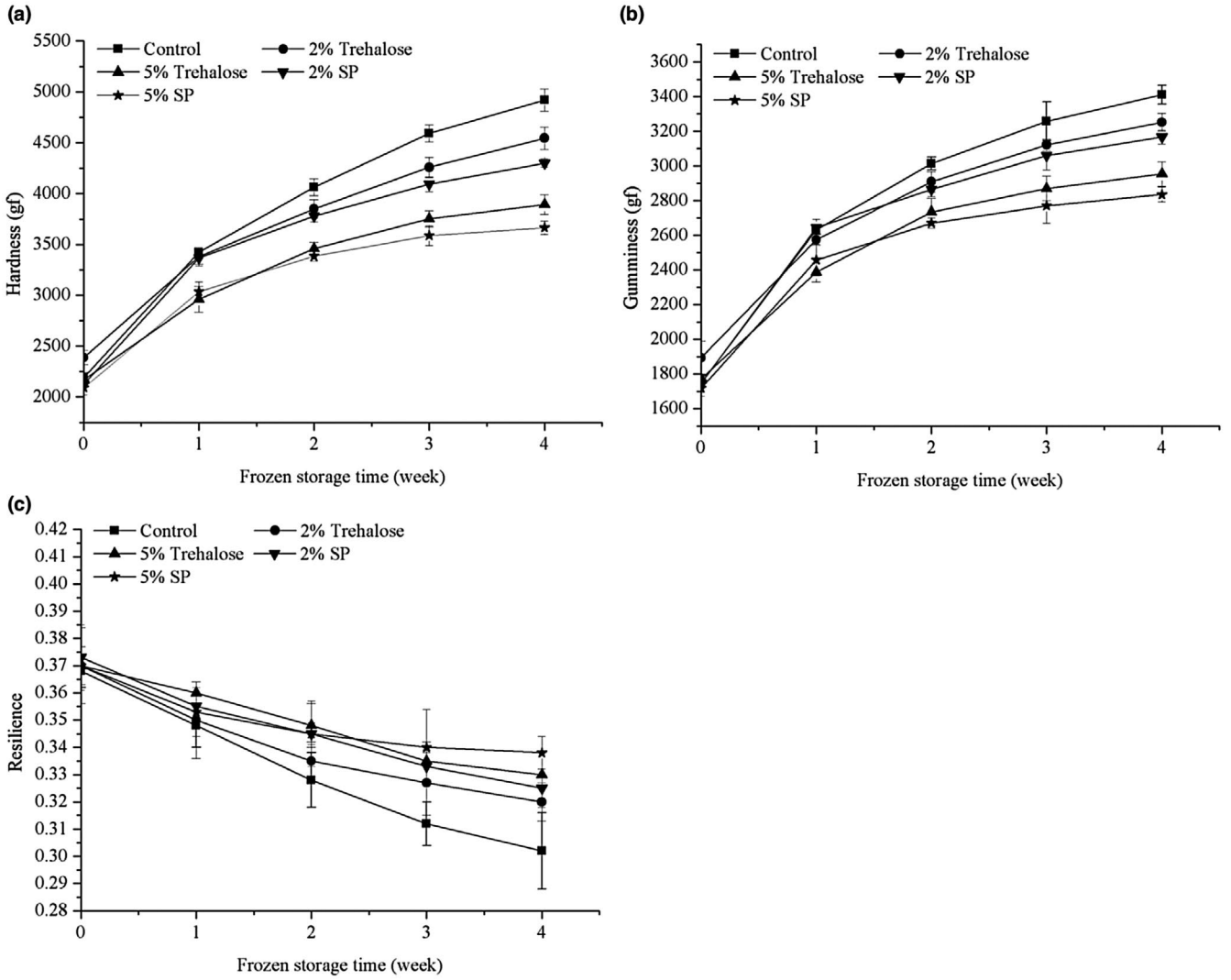

FIGURE 4 Effect of SPs on hardness (a), gumminess (b), and resilience (c) of steamed potato breads

3.5 | Effect of SPs on the texture of steamed bread

Results of TPA test for all steamed potato breads were illustrated in Figure 4. In this study, the hardness, resilience, and gumminess of bread were used to evaluate the quality of steamed potato bread. Many studies have suggested that, hardness and gumminess are negatively correlated with the quality of steamed bread, while resilience is positively correlated. As shown in Figure 4a–c, the hardness and gumminess of steamed potato breads increased with frozen storage time, while their resilience gradually decreased. Compared with the control group, the hardness and gumminess of steamed bread with 5% of trehalose decreased by approximately 21% and 13%, respectively, while elasticity increased by approximately 9% after 4 weeks of frozen storage. In addition, in steamed bread with 5% of SPs, the hardness and gumminess decreased by approximately 26% and 17%, respectively, while elasticity increased by approximately 12% after 4 weeks of frozen storage. Therefore, SPs were more effective than trehalose in improving the quality of frozen potato dough.

It was widely accepted that deterioration of frozen dough quality is mainly due to the formation and growth of ice crystals. During frozen storage, the formation of intracellular ice crystals could kill the yeast cells and decrease their viability (Nguyen et al., 2018). Furthermore, substances with reducing properties released by dead and injured yeasts, along with ice recrystallization during prolonged storage, could damage the network structure of gluten, and result in the separation of starch granules and reduction in gas holding capacity of gluten (Wang, Tao, et al., 2015). In this study, SPs significantly improved textural and baking characteristics of frozen potato dough. SP addition was also closely correlated with increase in yeast survival ratio and reduction in frozen water content. These results could be attributed to the perfect structural match, hydrogen bonds, and non-bond interactions between SPs and surface of ice. Cheng et al. (2002) had demonstrated that the hydrogen bonds formed between ice surface and antifreeze protein could distort ice surface layers and prevent ice recrystallization. Furthermore, non-bond interaction, such as Coulomb and van der Waals, could also play an important role in the prevention of ice crystal growth (Dalal & Sonnichsen, 2000).

4 | CONCLUSION

This study investigated the application of SPs in frozen potato dough. SPs were added in frozen potato doughs and the quality of dough throughout the storage process and subsequent baking performance were investigated. At concentration of 5%, SPs effectively reduced freezable water content, preserved the gluten network, and protected yeast cells of frozen potato dough. The special volume and textural properties of steamed potato breads with SPs were superior to those of steamed potato breads with trehalose. Furthermore, SPs could inhibit ice crystal formation and recrystallization and improve the gas production ability of yeast and gas holding capacity of gluten. Overall, compared with trehalose, SPs demonstrated greater cryoprotective effect and could be used as antifreeze for improving the quality of frozen dough.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

ORCID

Danlu Yang https://orcid.org/0000-0002-7872-2340

Zhengwu Wang https://orcid.org/0000-0002-3946-0974

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