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Effect of frozen storage temperature on the protein properties of steamed bread

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Abstract

In this paper, frozen steamed bread was used as the object to study the effect of frozen storage on the protein characteristics in a steamed bread system by measuring the changes in water holding capacity, oil holding capacity, emulsifying capacity, soluble protein content, surface hydrophobicity, secondary structure and molecular weights of gluten proteins in frozen steamed bread. The results showed that with an extension of frozen storage time, the disulfide bond (S-S) and total content of sulfydryl (-SH) decreased as a whole, and the molecular weights of steamed bread proteins were mainly distributed between 22 and 31 kDa, which were composed of a mixture of gliadin and low-molecular-weight glutenin subunits. With a decrease in frozen storage temperature, the soluble protein contents and protein water holding capacities of steamed bread first increased and then decreased, and the trend of the surface hydrophobicity was opposite. The frozen storage temperature had a significant effect on the protein properties of the steamed bread system. The changes in protein emulsification, oil holding capacity and soluble protein content were stable at -24 °C, which was suitable for long-term frozen storage.

Keywords: steamed bread; frozen storage; protein properties; structure; quality.

Practical Application: Freezing temperature regulate the characteristics of gluten protein in steamed bread.

1 Introduction

Fast-frozen dough products have become one of the important foods consumed by Chinese residents in daily life (Zhang et al., 2022; Zeng et al., 2022). Fast-frozen steamed bread is a relatively new staple product that is prepared by pre-steaming steamed bread, cooling, frozen storage and reheating processes and has drawn great attention among consumers. Research on frozen dough and frozen cooked noodles showed that destruction of the gluten protein network structure during frozen storage was the main reason for the deterioration in quality of frozen flour products (Wang et al., 2017). Changes in the biochemical components of frozen flour products during freezing were mainly due to decreases in yeast activity and air holding capacity and destruction of the gluten network and starch granule structure (Shi et al., 2013; Yang et al., 2019; Yu et al., 2020). The partially baked bread could undergo physicochemical changes during freezing, frozen storage and thawing that are related to ice crystals and water redistribution in the complete system (Gerardo-Rodríguez et al., 2021), the molecular weight of gluten protein decreases, and the structure of its fiber network weakens (Zhao et al., 2013). Wang et al. (2014b) found that γ-gliadin protein was easier to freeze by studying the characteristics of gliadin during frozen storage, which was the main reason for the decrease in protein foaming during frozen storage. In addition, it was also found that glutenin macropolymer (GMP) was depolymerized due to the breaking of interchain disulfide bonds (S-S) during frozen storage (Wang et al., 2014a). However, according to a study of a frozen noodle product system after heat treatment, frozen storage led to an increase in cooking loss, decrease in water absorption and increase in frozen water content (Liu et al., 2019). Moisture loss and starch aging occurred in frozen steamed bread, and the sensory quality of steamed bread deteriorated, but the changes in starch contents, ratios of amylopectin to amylose contents, protein contents and molecular weights were not obvious. Refrigerated bread can significantly reduce the digestion of starch (Xie et al., 2022). By exploring the aggregation and structural characteristics of heat-denatured gluten during frozen storage, Qian et al. (2021) found that the proteins in a steamed bread system were easier to depolymerize than those in a gluten system. The gluten quantities are the most important for dough formation (Alfaris et al., 2022). The gluten cross-linking behaviors of dough and steamed bread were different, and the gluten deterioration mechanism of frozen dough was also different from that of heat-denatured gluten proteins during frozen storage. In dough, gluten interacts with gliadin through noncovalent interactions such as hydrogen-bonded ion interactions and hydrophobicity to form a viscoelastic gluten network that gives the dough strength and ductility (Johansson et al., 2013). The degradation behavior of heat-denatured gluten proteins may be related to the heat-induced crosslinking of glutenin-gliadin disulfide bonds. Glutenin is more sensitive to heating than gliadin, so during cooking, glutenin preferentially polymerizes by oxidizing free-SH to S-S, followed by gliadin cross-linking to glutenin networks through S-S (Wang et al., 2016).

Research on the quality deterioration of frozen noodle products has mainly been conducted on frozen dough systems.

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Although quick-frozen cooked noodle systems after heat treatment have been studied, this research is limited to changes in quality (Qian et al., 2021). The purpose of this paper is to study the effects of different temperatures on the water holding capacity, oil retention, emulsification, emulsifying stability, soluble protein content, surface hydrophobicity, free sulfhydryl content, protein subunits and secondary structure of steamed bread during frozen storage to further provide a theoretical basis to optimize the freezing process of frozen steamed bread.

2 Materials and methods

2.1 Materials

Commercial steamed bread flour was purchased from One Plus One Natural Flour Co., Ltd. (Henan, China). Active dry yeast was obtained from Angel Yeast Industry Co., Ltd. (Hubei, China). All chemical reagents used in the experiments were of analytical grade.

2.2 Preparation of steamed bread and extraction of gluten proteins

The dough recipe used was based on the method described by Qin et al. (2022): 600 g of flour (10.87% moisture content, 11.30% protein content, 1.2% fat content and 32.43% wet gluten content); 4 g of baking powder; 30 g of white sugar; 4.2 g of active dry yeast; and 284 g of deionized water (75% farinograph water absorption, the optimum quantity of water established in Mixolab assays), and a kneader (DL-C03 dough mixer, Dongling Electric Co., Ltd., Guangdong, China) was used to prepare the dough. After the dough was awakened at 38 °C under 85% relative humidity for 30 min, the fermented dough was divided into 30-g pieces, rounded, molded and continuously fermented for 10 min. The small, fermented dough samples were steamed in a steamer for 15 min, which provided the steamed bread samples.

After cooling at room temperature for 1 h, the steamed bread samples were sealed in polypropylene food bags. After being frozen at -32 °C for 30 min, the steamed bread was frozen in a programmable constant temperature and humidity chamber (HYC-TH-800H Hongjin Testing Instrument Co., Ltd., Dongguan, China) at -6, -12, -18, -24 and -30 °C for 1, 2, 3 and 4 weeks, respectively.

Gluten proteins were extracted from frozen steamed bread by using the Martin method. The freeze-dried powders and deionized water of steamed bread with different treatments were mixed evenly at a ratio of 1:3, homogenized for 1 min, and then centrifuged with a Multifuge XIR Centrifuge desktop high-speed centrifuge (Thermo Fisher Scientific, Massachusetts, USA) at 3500 r/min for 15 min. The supernatant was poured out, and the light yellow part of the upper layer was scraped off. This operation was repeated once, and the remaining precipitate consisted of wet gluten and was then freeze-dried with a vacuum freeze dryer (Aiphal-2LDPlus, CHRIST Lyophilizer Co., Ltd., Lower Saxony, Germany) for 48 h.

2.3 Determination of water holding capacity and oil retention of protein

A 0.1-g sample was placed in a 10-mL centrifuge tube, and 2 g of deionized water was added and stirred evenly for 30 min, and centrifuging was then conducted with a Multifuge XIR Centrifuge desktop high-speed centrifuge (Thermo Fisher Scientific, Massachusetts, USA) at 3000 r/min for 10 min. The supernatant was removed, and the sediment weight was recorded. The ratio of weights after and before centrifugation (e.g., W/W0) was used to characterize the water holding capacity of the protein (Zhao et al., 2009).

According to the method of Vioque et al. (2000), to determine the oil retention, a 0.5-g sample was mixed evenly with 5 mL of soybean oil for 30 min and then centrifuged at 2200 r/min for 25 min. The supernatant was removed, and the sediment weight was recorded. The weight ratios after and before centrifugation (M/M0) were used to characterize the oil retention in protein.

2.4 Determination of emulsifying capacity and emulsion stability

A 20-mL 0.2% sample solution was mixed with 20 mL of soybean oil and stirred at 10000 r/min for 1 min in a high-speed shear dispersion emulsifier. A portion of the sample was separated and centrifuged at 3000 r/min for 10 min, and the total liquid volume and emulsion layer volume were recorded. The other part of the sample was placed in a constant-temperature water bath (50 °C) for 30 min. These samples were maintained at room temperature for 20 min and then centrifuged at 3000 r/min for 10 min, and these samples were used to determine the total liquid volumes and emulsion layer volumes (Wang et al., 2021).

2.5 Determination of thiol and disulfide bond content

Twenty-milligram protein samples were suspended in 5.0 mL of reaction buffer containing 10.4 g/L Tris, 6.9 g/L glycine, 1.2 g/L EDTA, and 8 mol/L urea, pH 8.0 and were allowed to react for 30 min at room temperature to determine the total sulfhydryl and free sulfhydryl contents according to Wang et al. (2018). The absorbances of the samples were measured at 412 nm against reaction buffer as a blank.

2.6 Determination of soluble protein content and surface hydrophobicity

The soluble protein contents were determined by referring to the Coomassie brilliant blue method. A 1-g sample was dissolved with 10 mL of phosphate buffer (0.01 M, pH 7.0) and stirred evenly for 1 h, and the solution was centrifuged at 4 °C in a closed high-speed refrigerated centrifuge (Thermo Fisher Scientific, Massachusetts, USA) at 8000 r/min for 15 min. A 0.2-mL volume of supernatant was mixed with 0.8 mL of distilled water and 5 mL of coomassie bright blue G-250 was added and left to stand for 15 min to measure the absorbance values. The soluble protein contents were calculated from the following formula (Equation 1):

$$soluble\ protein\ content = \frac{C \times V_T}{V_2 - F_W} \quad (1)$$

where C is the protein mass obtained from the standard curve, V_T is the total volume of sample protein dispersion, V_1 is the sample volume added during the determination and F_w is the total sample mass. At the same time, the standard curve was drawn (Figure 1). Bovine serum albumin solutions with different concentration gradients and their corresponding absorbance values were used as the horizontal and vertical coordinates, respectively.

The samples were prepared to provide 8 mg/mL solutions with phosphate buffer (0.02 mol/L, pH 7.0), and phosphate buffer was used as a control group. The hydrophobicity of the protein surface was calculated by the binding amount of bromophenol blue based on the method described by Wang et al. (2018).

2.7 SDS–PAGE

Proteins were separated in 1-mm thick preparation gel, which consisted of 5% concentrated gel and 12% separation gel. The 50-mg freeze-dried samples were dissolved in 1 mL of Tris-HCl (1 mol/L, pH = 6.8), which contained 5% (v/w) β-mercaptoethanol, 2% (w/w) SDS, 0.1% (w/w) bromophenol blue and 10% (v/w) glycerol. After heating in boiling water for 5 min, the samples were centrifuged at 10000 × g for 10 min, and 10 μL of supernatant was added to each channel for SDS–PAGE analysis. The samples were run at 100 V until the bromophenol blue indicator moved to the bottom of the gel. The gel was carefully removed and washed in distilled water for 3 min to remove SDS from the surface. Then, the gel was stained with 0.25% (W/V) coomassie bright blue R-250 for 1 h and decolorized with 10% (V/V) acetic acid for 2-3 h. A gel imaging system was used for photography and analysis (Han et al., 2019).

2.8 Statistical analysis

All experiments were conducted in parallel with three groups. The experimental data were analyzed by Origin 9.0 (Origin Lab Co., Massachusetts, USA) and SPSS 17.0 software (SPSS Inc., Chicago, USA), and the data are expressed as the averages ± standard deviations and were analyzed by single factor ANOVA. The Duncan test showed a significant difference ($P < 0.05$).

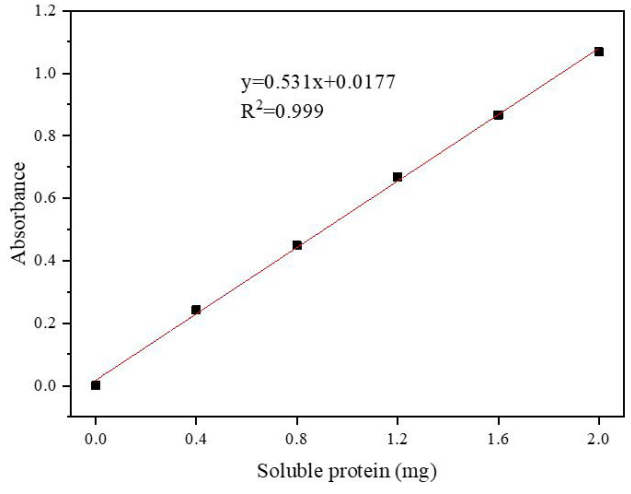

Figure 1. The standard curve of soluble protein.

3 Results and discussion

3.1 Water holding capacity

The protein water retention level is related to the degree of protein denaturation. It was found that the compact and continuous protein network structure in the gluten protein microstructure was more likely to intercept free water, which was conducive to improving protein water retention (Hu et al., 2013). The water-holding capacity of gluten proteins is mainly formed by covalent disulfide bonds through cysteine groups and hydrogen bonds formed by glutamine residues closely combined with water. The study shown in Figure 2A showed that the water-holding capacity of protein decreased with an extension of frozen storage time, and there were no significant differences in the water-holding capacities of protein at different temperatures after 4 weeks of storage, which might be related to ice recrystallization during cryopreservation. Small ice crystals aggregate to form large ice crystals that destroy the wheat gluten network. The larger the ice crystals are, the weaker the gluten network, the lower the gluten strength and the lower the water holding capacity (Xu et al., 2009). Within 2 weeks of storage, the protein water holding capacities of steamed bread at different temperatures first increased and then decreased. The higher the temperature, the smaller the protein water-holding capacity, which might be due to damage to the hydrophilic structures of various proteins and the exposure of more hydrophobic groups, leading to a decrease in protein binding power to water and decreased protein water holding capacity. The highest water holding capacity of steamed bread stored at -24 °C might be due to the lower hydrophobic amino acid content of steamed bread stored at -24 °C but also to the higher protein solubility and polar amino acid content (Samard & Ryu, 2019).

3.2 Oil retention of protein

Protein oil retention depends on the protein composition, protein denaturation and interactions with water and oil. Figure 2B shows that the oil-holding capacity of gluten increases with an extension of frozen storage time. The oil-holding capacity of protein in steamed bread frozen at -24 °C and -30 °C changed little. During frozen storage at -24 °C, the oil-holding capacity increased from 1.76 g/g to 1.96 g/g. The increased oil-holding capacity may result from the denaturation of gluten during frozen storage, and increasing numbers of hydrophobic groups are exposed. Hydrophobic groups or hydrophobic bonds affect the oil-holding capacity of protein. The more hydrophobic groups there are, the more oil gluten is able to contain to some extent (Ghribi et al., 2015). There were no significant differences in the oil-holding capacities of protein stored at different temperatures. The effect of different temperatures on the oil-holding capacity of protein was the same within 2 weeks, and the oil-holding capacity of protein was lowest at -18 °C. The oil-holding capacity of protein was smallest at -30 °C, and the highest capacity was 2.27 g/g at -6 °C for 4 weeks. The high oil-holding capacity may

be due to the exposure of hydrophobic groups that is caused by the destruction of the intermolecular structure of gluten proteins and the increased contents of hydrophobic amino acids, which might combine hydrophobic amino acids with the hydrocarbon side chains of oil, thereby improving the oil-holding capacity (Osen et al., 2014; Shevkani et al., 2015). The changes in the oil holding capacity of protein were stable at -24 °C and -30 °C during frozen storage.

3.3 Emulsifying capacity and emulsion stability

Emulsification is the capability of protein to diffuse at the oil-water interface, which is related to the properties of the adsorption layer. Proteins are linked with water and hydrophilic amino acids as well as to oil and hydrophobic amino acids. Hydrophilic and hydrophobic amino acids contribute to the mixing of water and oil in food systems. Increases in surface hydrophobicity can improve the emulsifying properties of proteins because hydrophobic and hydrophilic amino acids maintain a certain balance. The exposure of more hydrophobic groups at the water-oil interface can increase the emulsifying properties and emulsifying stability. Decreases in protein solubility also affect the migration and adsorption of proteins at the water-oil interface and thereby reduce the emulsifying activity of proteins (Lagrain et al., 2012).

Figure 3A shows that the emulsification of protein increased with decreasing temperature when frozen at different temperatures,

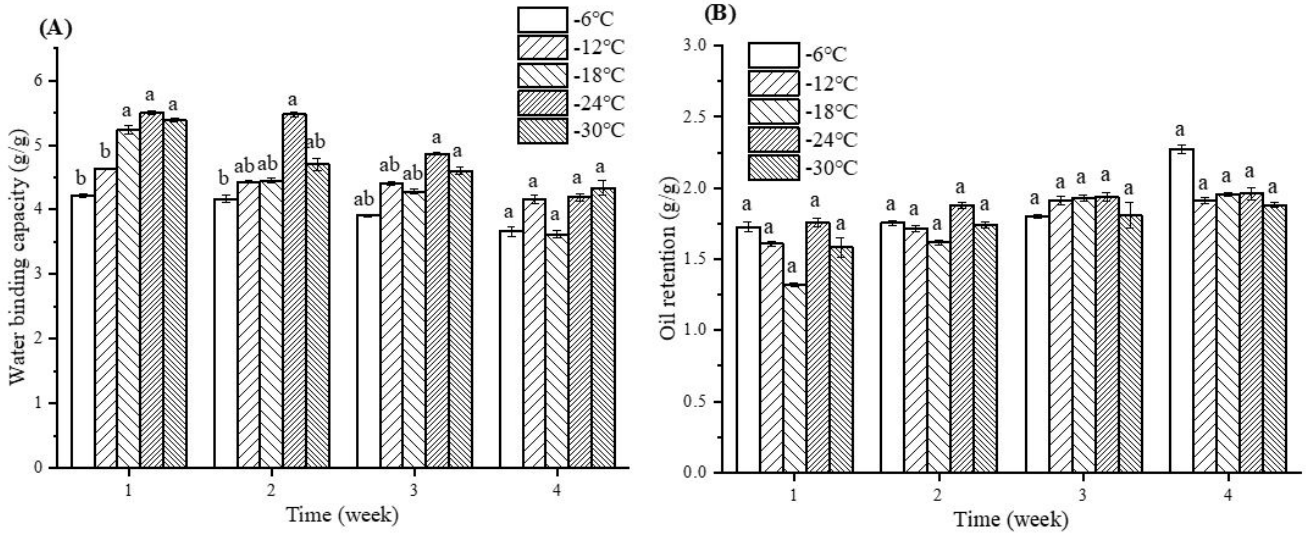

Figure 2. Changes of protein water holding capacity (A) and oil retention (B) under different frozen storage. [Note: Different lowercase letters "a~b" in the same week indicate significant difference at different frozen temperatures ($p < 0.05$)].

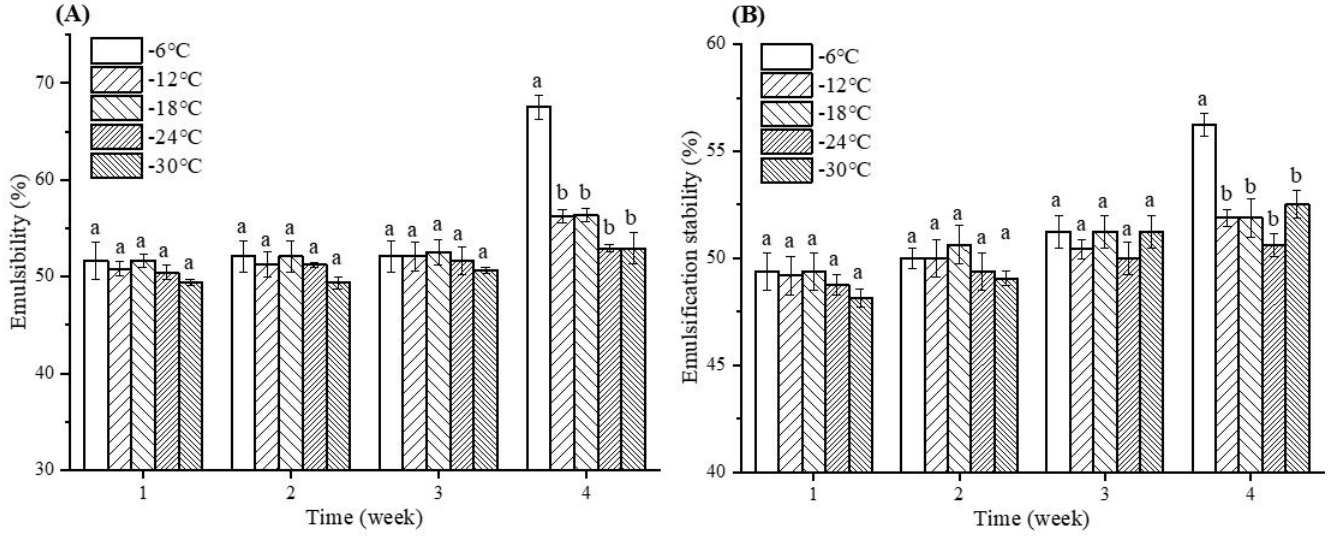

Figure 3. Changes of protein emulsifying capacity (A) and emulsion stability (B) under different frozen storage. [Different lowercase letters "a~b" in the same week indicate significant difference at different frozen temperatures ($p < 0.05$)].

and there was a significant difference in emulsification between -6 °C and other temperatures after frozen storage for four weeks ($p < 0.05$). With an extension of frozen storage time, the protein emulsification of frozen steamed bread increased at different temperatures, which might be due to the high hydrophobic amino acid contents. The surface hydrophobicity affected the emulsifying ability of proteins. The dissociation and partial unfolding of globular proteins led to the exposure of hydrophobic amino acid residues, resulting in increased surface activity and adsorption of larger amounts of emulsifying proteins at the oil-water interface (Agyare et al., 2009). Figure 3B showed with an extension of freezing time, the emulsifying stability of gluten at different temperatures was improved. The higher the freezing temperature before two weeks of frozen storage, the higher the emulsifying stability, and the lowest value occurred at -30 °C, while the highest occurred at -24 °C after more than two weeks of storage. The emulsifying stability of protein may be related to the increased surface hydrophobicity after the protein structure was destroyed and to the exposure of hydrophobic groups after freezing storage. An increase in surface hydrophobicity can improve the emulsifying stability of protein, which may be due to the balance of hydrophilic and hydrophobic groups at the interface, resulting in a good interaction between oil and wheat gluten, which results in a thicker and more stable emulsion (Matemu et al., 2011).

3.4 Thiol and disulfide bond content

The amount of free sulfhydryl groups reflects the changes in disulfide bonds, and there is a close relationship between disulfide bond amounts and gluten protein structures, which determines protein aggregation and maintains the stability of the protein matrix (Wang et al., 2014a). Yan et al. (2020) found that during the fermentation and cooking process of steamed bread, heating led to formation of disulfide bonds, and heat-induced protein denaturation exposed buried SH, resulting in in-SH being more easily oxidized and exchanged between SH and S-S, thus forming S-S between protein molecules and contributing to the structure of steamed bread. The free SH contents increased with decreasing pH. Table 1 shows that the S-S contents in steamed bread decreased significantly with an extension of frozen storage time, and the overall trend for total SH decreased with increasing frozen storage time. When the frozen storage temperatures were higher, the S-S contents decreased, and the free SH contents increased gradually as a whole, while the overall trend for total SH was the opposite. The overall trend for the disulfide bonds was opposite to that of free thiol, which was mainly due to the transition between Smurs and free-SH. The S-S levels decreased from 0.20 μmol/g to 0.13 μmol/g during storage at -24 °C, and there were no significant changes in the S-S levels at 2-3 weeks after storage. The increased free SH content was mainly due to the destruction of heat-induced disulfide bond crosslinking between gliadin and glutenin polymers. Zhao et al. (2012) found that during freezing storage, water redistribution and ice recrystallization may cause disulfide bond rupture and gluten polymer depolymerization, resulting in increased free-SH contents in gluten.

3.5 Soluble protein content and surface hydrophobicity

During the process of steamed bread cooking, wheat gluten proteins are crosslinked by disulfide bonds through the intermolecular oxidation of free SH and the conversion reaction between -SH and S-S to form protein polymers, and the covalent crosslinked structure of glutenin and gliadin is formed by heat induction (Qin et al., 2016). Protein depolymerization led to decreased relative molecular weights, which in turn led to increased soluble protein contents. Figure 4A shows that during the first two weeks of frozen storage, the protein solubility first increased and then decreased with decreasing quick-freezing temperatures. The soluble protein contents of steamed bread frozen at -12 °C were the highest and increased to 2.03 mg/g

Table 1. Contents of accessible thiol (SH_{free}), total thiol equivalent (SH_{eq}), and disulfide bonds (SS) of protein components of gluten.

Index	Temperature (°C)	Frozen store time (weeks)			
		1	2	3	4
SH_{eq} (μmol/g)	-6	0.99 ± 0.22aA	0.92 ± 0.25aAB	0.90 ± 0.23aAB	0.73 ± 0.07aB
	-12	1.01 ± 0.21aA	0.92 ± 0.27aA	0.79 ± 0.06aB	0.73 ± 0.08aB
	-18	1.01 ± 0.06aA	0.97 ± 0.13aA	0.96 ± 0.14aA	0.94 ± 0.23aA
	-24	1.02 ± 0.17aA	0.94 ± 0.08aB	0.96 ± 0.20aAB	0.86 ± 0.13aB
	-30	1.10 ± 0.35aA	1.00 ± 0.31aA	0.97 ± 0.22aA	0.92 ± 0.13aA
SH_{free} (μmol/g)	-6	0.69 ± 0.02aA	0.69 ± 0.02aA	0.69 ± 0.19aA	0.68 ± 0.10abA
	-12	0.65 ± 0.09abA	0.62 ± 0.09abA	0.56 ± 0.13abA	0.57 ± 0.10cA
	-18	0.63 ± 0.07abB	0.66 ± 0.15aB	0.69 ± 0.12aB	0.75 ± 0.12aA
	-24	0.63 ± 0.02abAB	0.56 ± 0.05bB	0.57 ± 0.06abB	0.75 ± 0.12aA
	-30	0.54 ± 0.04bB	0.53 ± 0.06bB	0.52 ± 0.04bB	0.61 ± 0.08abcA
SS (μmol/g)	-6	0.15 ± 0.10bA	0.12 ± 0.08bA	0.11 ± 0.02bA	0.03 ± 0.01cB
	-12	0.18 ± 0.06abA	0.15 ± 0.09abA	0.12 ± 0.04bA	0.07 ± 0.01bcB
	-18	0.19 ± 0.01abA	0.16 ± 0.01abA	0.14 ± 0.01abA	0.09 ± 0.05bcB
	-24	0.20 ± 0.07abA	0.19 ± 0.02abA	0.19 ± 0.07aA	0.13 ± 0.02abAB
	-30	0.28 ± 0.15aA	0.24 ± 0.12aA	0.23 ± 0.09aA	0.18 ± 0.02aA

Note: The results are expressed as mean ± SD (standard deviation); a~c: different letters in the same column indicate significant difference ($p < 0.05$); A~B: different letters in the same row indicate significant difference ($p < 0.05$).

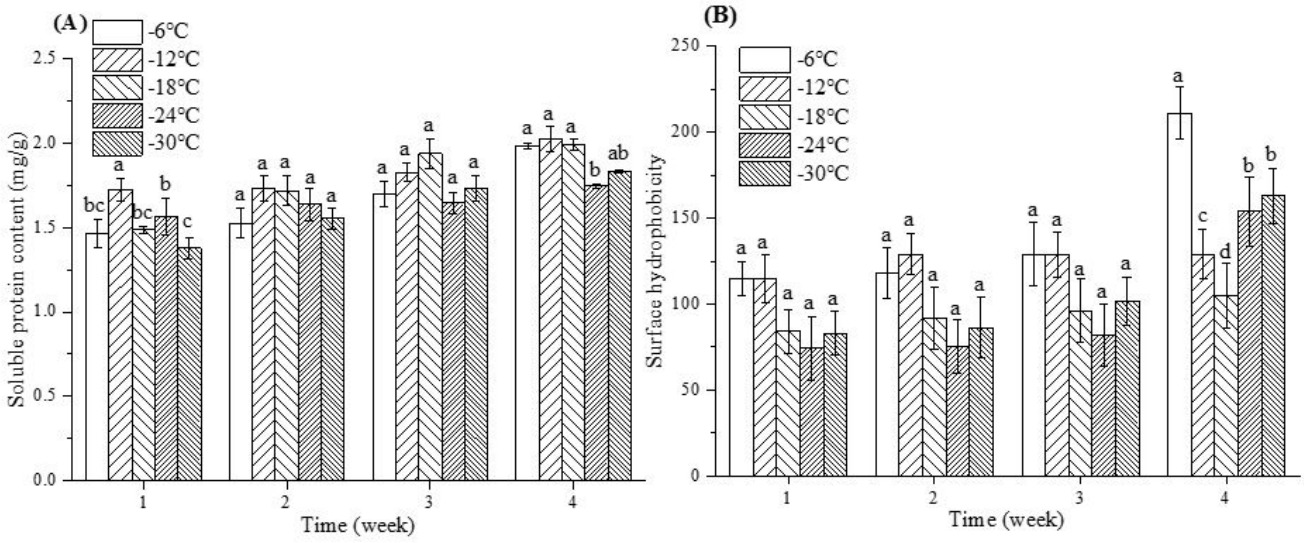

Figure 4. Changes of soluble protein content (A) and surface hydrophobicity (B). [Different lowercase letters "a~c" in the same week means significant difference at different frozen temperatures ($p < 0.05$)].

after 4 weeks of frozen storage. The soluble protein contents of steamed bread frozen for 3 and 4 weeks were lowest when frozen at -24 °C. During the whole freezing process, the soluble protein contents increased from 1.57 mg/g to 1.75 mg/g at -24 °C, and the solubility of protein was relatively stable. Water redistribution and ice recrystallization destroy the internal structure of gluten proteins, which further leads to depolymerization and shedding of gluten and an increase in protein solubility. The aggregation behavior and solubility of proteins are closely related to product quality. The soluble protein contents of steamed bread increased with an extension of storage time for different freezing storage temperatures. Protein depolymerization caused by freezing storage mainly destroys the thermally induced cross-linking of disulfide compounds between glutenin and gliadin (Qian et al., 2021).

Surface hydrophobicity plays an important role in the stability, conformation and functional properties of proteins that are in contact with polar water environments. This property can be used to analyze the number of hydrophobic residues on the surface of proteins (Hu et al., 2013). As shown in Figure 4B, After 3 weeks of storage above -30 °C, the smallest surface hydrophobicity was observed at -24 °C. After 4 weeks of frozen storage, it was found that the surface hydrophobicity first decreased and then increased with decreasing temperature, and the surface hydrophobicity was smallest at -18 °C. The lower the freezing temperature is, the lower the surface hydrophobicity, the smaller the degree of damage by ice crystals to the protein structure, and protein aggregation protects the hydrophobic groups of proteins. In the first three weeks of frozen storage, the surface hydrophobicity at -6 °C was equal to or even lower than that at -12 °C. This may be due to the partial denaturation of proteins at higher temperatures, and the exposure of hydrophobic groups may lead to production of more extensive protein-protein polymers, which are conducive to the aggregation of gluten proteins to form a solid and well-aggregated microstructure, which thereby enhances the binding of proteins to water and reduces the surface hydrophobicity (Wang et al., 2019). When frozen for 4 weeks, the surface hydrophobicity levels under different temperatures were significantly higher than those during the previous 3 weeks, which may be related to the high protein solubility, negligible aggregation behavior and exposure of hydrophobic components embedded in the protein structure due to denaturation. The increased surface hydrophobicity with an extension of frozen storage time may be due to the expansion of protein molecules to a certain extent, exposing some hydrophobic groups and regions between the interior parts of gluten molecules and the surfaces around polarity (Zhao et al., 2012).

3.6 SDS–PAGE

To evaluate the effect of frozen storage at different temperatures on the aggregation characteristics and primary structures of gluten proteins in steamed bread, the proteins were separated by SDS-PAGE using the molecular weights of the proteins. The electrophoresis map is shown in Figure 5. Bands above 43 kDa correspond to high-molecular-weight glutenin subunits, bands between 22-43 kDa correspond to a mixture of gliadin and low-molecular-weight glutenin subunits, and bands below 22 kDa are associated with small amounts of albumin and globulin (Tang et al., 2019). Zhao et al. (2012) reported that gliadin was crosslinked with glutenin through noncovalent forces and heat-induced disulfide bond exchange reactions during cooking. However, the cross-linking reaction was most sensitive and easily destroyed during frozen storage, which led to the depolymerization of proteins and increases in SDS soluble protein and gliadin band strength.

The gel atlas showed that SDS mainly extracted glutenin molecules of 22-31 kDa, while high-molecular-weight glutenin subunits might form large polymeric molecules, which had low solubility in nonreducing SDS-PAGE sample buffer after heating. These larger polymeric molecules could not enter the separation gel. Therefore, few bands were observed in the region above 70 kDa. The deeper color of the 22-31 kDa region suggested

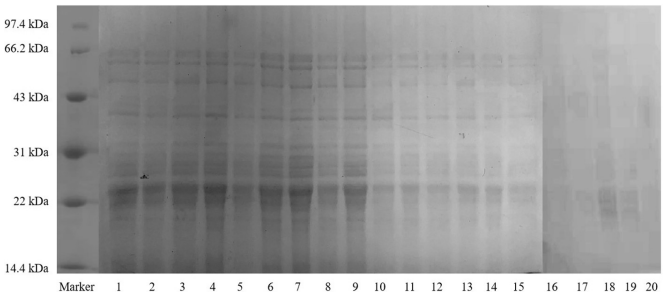

Figure 5. Changes of molecular weight distribution of protein in steamed bread under different frozen storage. (Note: Spectral bands from 1-5 are samples frozen under different frozen temperatures for 1 week; 6-10 are samples frozen under different frozen temperatures for 2 weeks; 11-15 are samples frozen under different frozen temperatures for 3 weeks; 16-20 are samples frozen under different frozen temperatures for 4 weeks , respectively).

this. Since this region mainly consists of a mixture of gliadin and low-molecular-weight glutenin subunits, this difference indicated that the aggregation of gliadin or low-molecular-weight glutenin was hindered by disulfide bonds during storage. Considering the results of disulfide bond conformation, it may preferentially destroy the disulfide bond crosslinking between glutenin molecules to hinder the aggregation of low-molecular-weight glutenin, making it easier to extract. In the early storage stage, protein band migration was more obvious at -24 °C, but in the late stage of freezing storage, no obvious band migrations were observed at different temperatures under the nonreducing mode. In addition, the protein bands did not show any significant changes, indicating that different temperatures had no effect on protein subunits. With increasing storage times, the optical densities of the bands on the separation gel decreased significantly, indicating that the soluble glutenin contents decreased. However, the nonreduced SDS-PAGE pattern showed that only the noncovalent bonds in the protein were cleaved, and the S-S bonds were not destroyed (Barbiroli et al., 2013). Glutenin has been proven to be the main component of insoluble GMP, which is formed by intermolecular S-S bonds (Wang et al., 2016).

For the 22 kDa range in the middle of the separation gel, the intensity of the band corresponding to gliadin changed. This may be due to the lack of intermolecular SH groups in gliadins, which makes S-S bonds more difficult to polymerize than other proteins (Sun et al., 2021). The slight decrease in gliadin band intensities may be caused by noncovalent interactions and SH-SS exchange (Hu et al., 2017).

4 Conclusion

In the first 3 weeks of frozen storage, the exposure of hydrophobic groups may lead to protein aggregation. There were no significant differences in the soluble protein contents among different temperatures in the middle of storage. The emulsifying stability and soluble protein content of frozen storage at -24 °C were the lowest, and the changes in Smurs contents were not significant. When stored for 4 weeks, there were no significant differences in the protein water holding capacities in frozen steamed bread at different temperatures, but there was a significant difference in emulsification among -6 °C and other temperatures. There were no significant differences in the water holding capacities of protein in frozen steamed bread at different temperatures, but there was a significant difference in emulsification among -6 °C and other temperatures. During the whole frozen storage process, the changes in emulsification, oil holding capacity and soluble protein content of protein at -24 °C were relatively weak, and the change in protein was the smallest. The optical densities of the bands on the separated gel decreased significantly, indicating that the soluble glutenin content decreased, which was more suitable for long-term frozen storage.

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