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Changes of moisture distribution and starch properties in fermented dough under subfreezing temperature storage

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Abstract

This paper took fermented dough as the research object. The difference between the subfreezing temperature and other freezing temperatures for long-term storage of fermented dough was discussed. The fermented dough was quick-frozen at -30 °C for 1 h and then stored at -6 °C, -12 °C and -18 °C for 5 d, 10 d and 15 d. The results indicated that with decreasing freezing temperature, the content of bound water in the dough increased gradually so that the water in the dough could be better preserved and the storage time of the dough could be prolonged. Although low temperatures could have a better storage effect, too low temperature and prolonged freezing time would destroy the structure of the starch in the dough. The structure of the starch stored at the subfreezing temperature (-12 °C) was better than that stored at other temperatures. Compared with the common freezing temperature (-18 °C), the subfreezing temperature not only achieves a better storage effect but also reduces energy consumption.

Keywords: fermented dough; subfreezing temperature; water distribution; starch; properties.

Practical Application: The fermented dough stored at subfreezing temperature (-12 °C) can not only guarantee the quality of dough, but also reduce the energy consumption and shorten the thawing time, which provides a new theory and technology for industrial production.

1 Introduction

With the development of science and the progress of the times, freezing technology is increasingly used in the food industry (Jiang et al., 2019). The frozen dough market has grown steadily in recent years due to consumer demand for convenient and high-quality baking products and a trend toward standardization of product quality (Kurek & Sokolova, 2020). Frozen dough technology can reduce production costs and promote chain enterprise standardization and product diversification (Gerardo-Rodríguez et al., 2017). However, the migration of water and the change in starch structure during the freezing process play vital roles in the quality of flour products (Tao et al., 2016). It has been found that recrystallization of ice crystals during freezing has many negative effects on frozen dough (Feng et al., 2020). For example, the gluten network structure is destroyed, and yeast fermentation activity is reduced. These factors will lead to changes in quality of frozen flour products, shortened shelf life, etc. (Mesa et al., 2019). Scholars have also conducted much research on these phenomena. Zhang et al. improved the moisture distribution and mechanical properties of dough by adding bamboo shoot dietary fiber (Zhang et al., 2017). Ke et al. added inulin to reduce the damage to the gluten protein network structure caused by frozen storage (Ke et al., 2020). Lu et al. used ε-poly-L-lysine-treated yeast to improve the freeze-thaw resistance of dough (Lu et al., 2020). All these methods increase the cost of production to varying degrees. Is it possible to improve the quality of frozen flour products without increasing production costs? No relevant reports have been seen yet.

The subfreezing temperature is the temperature between the freezing point and the common freezing temperature. Subfreezing storage not only preserves food for a long time but also minimizes the impact of freezing on food quality and reduces the cost of freezing (Meng et al., 2021). Qian et al. found that beef at subfreezing temperatures can significantly prolong the shelf life, and subfreezing temperature was used for the first time in the storage of beef (Qian et al., 2017). Meng et al. found that the subfreezing temperature of dough ranged from -9 °C to -12 °C (Meng et al., 2021). In this study, -12 °C was selected as the subfreezing temperature of fermented dough. The effects of subfreezing temperature storage on the water distribution and starch structure of fermented dough were investigated by comparing the moisture distribution of dough and the structural properties of starch under subfreezing temperature and other freezing temperatures. The purpose of this study is to enrich the theoretical research on the storage of fermented dough under subfreezing conditions and provide theoretical guidance and technical support for industrial production.

2 Materials and methods

2.1 Main materials and reagents

Wheat flour was purchased from Embryo Grain Health Food Co., Ltd., Xinxiang, China. Highly active dry yeast was provided by Angel Yeast Co., Ltd., Hubei, China. White granulated sugar was provided by Rongping Food Co., Ltd., Zhengzhou, China. Other chemical reagents in our experiments were of analytical grade.

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2.2 Prepare fermented dough

Two hundred grams of flour, 2 g of yeast, 2 g of sugar and 100 g of pure water were mixed using a dough mixer (DL-C03, Dongling Electric Co., Ltd., Guangdong, China) for 7 min to form dough with a smooth surface. The dough was cut into small pieces weighing 20 g. Then, the dough was wrapped with plastic wrap and fermented at 37 °C for 40 min in an intelligent biochemical incubator (LRH-150B, Huruiming Instrument Co., Ltd., Guangzhou, China). After this, the yeast dough was ready.

2.3 Freezing point of fermented dough

Insert the probe of the temperature sensor (L93-1, Hangzhou Luge Technology Co., Ltd., Hangzhou, China) into the fermented dough prepared in Section 2.2. Then the dough was quick-frozen at -30 °C for 1 h in a programmable cryogenic incubator (Hy-TH-80DH, Hongjin Testing Instrument Co., Ltd., Dongguan, China). Record the temperature every one minute during the freezing process. Measure the freezing point of the fermented dough.

2.4 Design of frozen storage of fermented dough

The dough prepared in Section 2.2 was quick-frozen at -30 °C for 1 h in a programmable cryogenic incubator (Hy-TH-80DH, Hongjin Testing Instrument Co., Ltd., Dongguan, China). Then they were frozen at -6 °C, -12 °C and -18 °C for 5, 10 and 15 days. Quick-frozen but unfrozen dough was used as control (ck).

2.5 Hydrogen proton density

Take the dough at room temperature, freezing point temperature, -6 °C, -12 °C, -18 °C for hydrogen proton density imaging measurement. The determination method was referred to Jiang et al., with some modifications(Jiang et al., 2021). Quick-frozen but unfrozen dough was used as control (ck).

2.6 Moisture distribution and migration of dough

Defrost the dough prepared in section 2.4 in the incubator at 30 °C for 1 h and determine the moisture distribution and migration. The determination method was referred to previous reports(Meng et al., 2021). The thaveled dough was placed in a nuclear magnetic test tube and placed in the center of the rf coil at the center of the permanent magnetic field. The FID test was used to adjust the resonance center frequency, and the CPMG pulse sequence measured the relaxation time of the sample (T_2). Then the CPMG pulse sequence was scanned. The CPMG pulse sequence parameters were set as follows: sampling frequency (SW), 100 kHz; repeated scanning time (NS), 4; half echo time (TE), 0.6 ms; and number of sampling points (TD), 400 014.

2.7 Freezable water content of dough

The dough prepared in Section 2.4 was defrosted in the incubator at 30 °C for 1 h. The content of freezable water (Fw) in the dough was determined by a Q200 differential scanning calorimeter (DSC) (TA Inc., USA). The determination method was performed according to a previous method with slight modifications (Zhang et al., 2020). After the dough was thawed, 20 mg of sample was taken from the center of the dough, sealed in a stainless-steel crucible (Shanghai Zhengji Scientific Instrument Co., Ltd., Shanghai, China) and placed in the instrument for measurement. An empty stainless-steel crucible was used as the reference. The initial temperature was 25 °C. The temperature was lowered to -40 °C at a rate of 5 °C/min, maintained for 1 min at -40 °C, and then heated to 30 °C at a rate of 5 °C/min. The nitrogen flow rate was 50 mL/min. TA Instruments analysis software (TA Instruments, New Castle, Delaware) was used to analyze the obtained curve by integrating the DSC results to scan the peak area of melting enthalpy (ΔH). To reduce the error, each integration was started at the melting temperature of ice, -10 °C, and ended at 20 °C. The proportion of freezable water (Fw) in frozen dough was calculated by Equation 1:

$$Fw = \Delta H / \left(\Delta H_0 \times W\right) \times 100\% \quad (1)$$

In the formula, ΔH is the melting enthalpy of the sample and ΔH_0 is the melting enthalpy of pure ice (335 J/g). W is water added to the dough sample (33%).

2.8 Starch isolation

The wheat dough prepared in Section 2.4 was defrosted in the incubator at 30 °C for 1 h. Starch in the dough was extracted by the Martin method (Tao et al., 2018). The gluten was washed out of the wheat dough with a sufficient amount of 0.1% NaOH solution. The starch suspension was then sifted through a 100-mesh sieve. Starch slurry was collected. The starch slurry was placed at 4 °C for 6 h and centrifuged at 3500 r/min for 20 min in a Multifuge X1R desktop high-speed centrifuge (Thermo company, Massachusetts, USA) to remove the upper pigment layer. The precipitate was washed with 300 mL distilled water and centrifuged. Washing with distilled water and centrifugation was repeated twice. The precipitate was resuspended in 400 mL distilled water, and the pH was adjusted to 7.0 with HCl. The centrifugation was repeated three times. The precipitate was collected after drying. The same method was used to separate and extract starch from the dough that was quick-frozen but not stored as the control (ck) starch samples.

2.9 Scanning Electron Microscopy (SEM)

The starch prepared in section 2.8 was observed for its microstructure. The starch sprayed with gold by ion sputtering. The microstructure of the sample was observed with a Quanta200 scanning electron microscope (SEM) (FEI Corporation, Oregon, USA). The scanning magnification was 800 ×.

2.10 Pasting properties

The pasting properties of starch samples prepared in section 2.8 were measured by RVA4500 rapid viscosity analyzer (TecMaster, Newport scientific instruments LTD., Australia). The procedure was slightly changed based on the previous procedure(Yang et al., 2021). Weigh 3.0g starch sample into the RVA aluminum cylinder, add 25 mL of water, stir evenly, put the aluminum cylinder into the instrument and start the measurement. The determination parameters were set as follows:

First, it was heated to 50 °C and kept for 1min, then to 95 °C in 3.7 min, and kept for 2.7 min at 95 °C. Finally, it was reduced from 95 °C to 50 °C in 3.8 min, and kept for 2 min at 50 °C. The viscosity curve of the sample during the treatment process was obtained. The pasting parameters of starch samples were obtained by using RVA software to record and analyze the data.

2.11 X-Ray Diffraction (XRD)

Take the starch samples prepared in section 2.8 to determine the crystal structure. The crystallization characteristics of the samples were measured by a Brooke X-ray diffractometer (Bruker D8 Advance A25, Germany). The test methods refer to the previously reported(Jiang et al., 2021).

2.12 Statistical analysis

SPSS 26.0 software (SPSS Inc., Chicago, USA) was used for statistical analysis and Origin 2017 software was used for plotting. Three parallels were measured for each sample, and the results were displayed as mean ± standard deviation. Significance level P is 0.05, when $P < 0.05$, data difference is very significant.

3 Results and discussion

3.1 Moisture distribution and migration of dough

Figure 1 a is the curve of the dough center temperature change measured by the temperature sensor in the process of quick-freezing. Figure 1 a shows that the dough enters the maximum

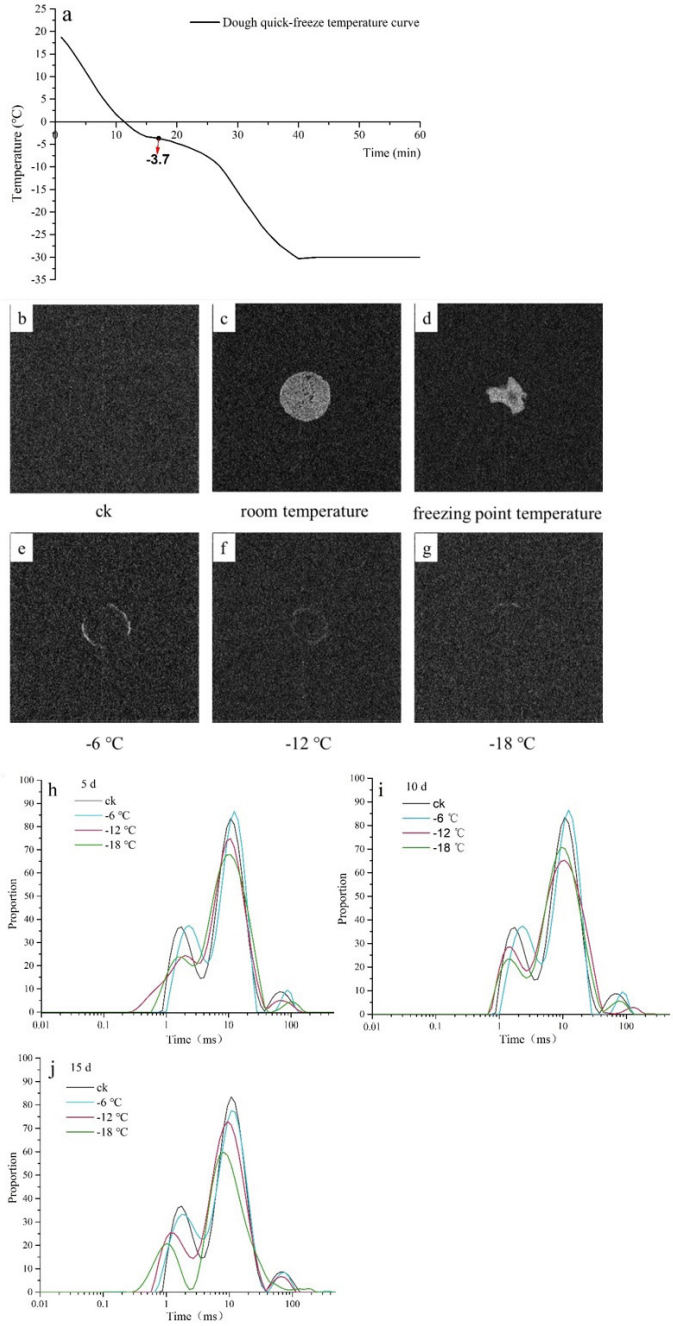

Figure 1. Moisture distribution and migration of dough (ck is the dough that has been quick-frozen but not stored).

ice crystal formation zone after 15 minutes of quick-freezing. The temperature during this process is called the freezing point of the dough (Xu et al., 2009). The temperature sensor measured the freezing point of the dough at -3.7 °C. Figure 1b-g show the hydrogen proton densities of frozen dough at ck, room temperature, freezing temperature, -6 °C, -12 °C and -18 °C, respectively. The brighter area of the image indicated that the signal strength was stronger, the hydrogen proton density was higher, and the water content available to microorganisms was higher (Jiang et al. 2020). The opposite was the same. Figure 1b shows the hydrogen proton density diagram of dough that has been quickly frozen but not stored. The brightness of this picture was the darkest, and the outline of the dough was almost invisible. After quick-freezing, the liquid water inside the dough was completely frozen, and there was almost no water available to microorganisms. Figure 1c shows the highest brightness. This result indicated that the liquid in the dough at room temperature had the highest fluidity, the highest free water content and the highest available water content for microorganisms. Figure 1d shows the hydrogen proton density of the dough at the freezing point (-3.7 °C). The outer contour of this picture was darker, and the center was brighter. This was because the dough was in the zone of maximum ice crystal formation, where the water on the outside has been frozen and the liquid water in the center has not been frozen. Figure 1e-g show hydrogen proton density maps of dough stored at -6 °C, -12 °C and -18 °C, respectively. There was a brighter small aperture in Figure 1e. This may be caused by the melting of a few ice crystals outside the dough when measured in the NMI chamber. The center part of the dough was still darker, indicating that the liquid water inside the dough frozen at -6 °C was completely frozen. Figure 1f, g had little difference in brightness. They were both darker. This meant that the water in the dough frozen at -12 °C and -18 °C was all frozen. The results showed that the subfreezing temperature had the same effect on the dough as common freezing temperatures and the water content available to microorganisms was lower; both could be stored for a long time.

Low-field NMR is a fast and nondestructive method to detect water distribution in food. The shorter the relaxation time, the more stable the water state. Figure 1h-j shows the measured moisture distribution curve of frozen dough. There were three peaks on the curve: T_{21} (0.01~3.00 ms), T_{22} (3.00~15 ms) and T_{23} (15~500 ms). Studies have shown that T_{21} represents water that is tightly bound to starch or protein, which can be called bound water. T_{22} reflects water bound to the frozen dough structure, which can be called weakly bound water. T_{23} corresponds to free water (Zhang et al., 2018). Figure 1h-j and Table 1 show that when the frozen storage temperature was -12 °C and -18 °C, the peak apex time of the bound water in the dough shifted to the left, while when frozen at -6 °C, the peak apex time of the bound water in the frozen dough shifted to the right. This result indicated that the water state of the dough was more stable when the dough was frozen at -12 °C and -18 °C. The peak time of weakly bound water had little difference at different freezing storage temperatures. By comparing the proportion of each peak area in Table 1, it can be seen that when frozen at -6 °C, with the increase in freezing time, the proportion of the peak area of the bound water gradually decreased, while that of the weakly bound water gradually increased. When frozen at -12 °C and -18 °C, with increasing freezing time, the proportion of the peak area of weakly bound water decreased, while that of bound water increased. By observing the relaxation time of bound water and weakly bound water in Table 1, it could be found that the peak-point time of both bound water and weakly bound water gradually increased with the increase in the freezing time of the dough stored at -6 °C, while the peak-point time of the dough stored at -12 °C and -18 °C gradually decreased. Comprehensive analysis showed that when the dough was frozen at -12 °C and -18 °C, the weakly bound water in the dough gradually bound closely with starch and protein, and part of the weakly bound water turned into bound water. This phenomenon corresponds to the change in the peak area ratio mentioned above. It also shows that the moisture distribution and migration of dough frozen at -12 °C are similar to those of dough frozen at -18 °C. The results show that freezing the dough at a subfreezing temperature (-12 °C) can achieve the effect of freezing at -18 °C.

3.2 Freezable water content of dough

The state of water plays an important role in the quality and stability of dough during freezing and storage of food

Table 1. Changes in the relaxation time (T_2) and water fraction (A_2) of fermented dough stored at different temperatures for different times.

Storage time (d)	Storage temperature (°C)	T_2(ms)			A_2(%)		
		T_{21}	T_{22}	T_{23}	A_{21}	A_{22}	A_{23}
5	ck*	1.748	10.723	65.793	25.385	69.479	5.136
	-6	1.98	9.326	86.975	20.144	76.812	3.044
	-12	1.748	10.723	65.793	18.652	78.944	2.404
	-18	1.322	10.723	100	18.271	79.921	1.808
10	ck	1.748	10.723	65.793	25.385	69.479	5.136
	-6	2.009	10.233	57.224	17.954	80.174	1.872
	-12	1.52	10.233	65.793	20.282	77.522	2.196
	-18	1.15	9.326	75.646	22.367	75.216	2.417
15	ck	1.748	10.723	65.793	25.385	69.479	5.136
	-6	2.31	12.328	49.77	15.786	82.157	2.057
	-12	1.52	9.326	65.793	24.064	73.688	2.248
	-18	1.15	8.111	74.753	25.144	73.126	1.73

*ck is the dough that has been quick-frozen but not storage.

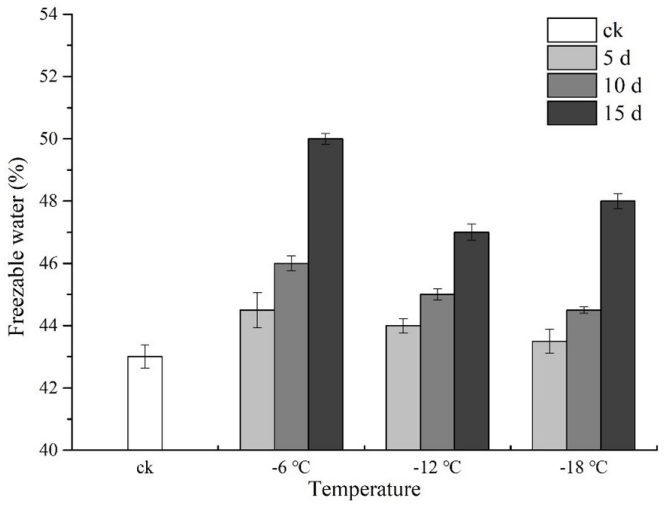

Figure 2. Freezable water content of dough (ck is the dough that has been quick-frozen but not stored).

(Gerçekaslan, 2021). Freezable water content is a key factor affecting the nucleation, size and distribution of ice crystals (Ding et al., 2015). Therefore, it is necessary to know the content of freezable water in the dough. Figure 2 shows the content of freezable water in the dough. The figure shows that the content of freezable water inside the dough increased with the extension of the freezing time regardless of whether the dough was frozen at -6 °C, -12 °C or -18 °C. When the freezing time was 5 d, the content of freezable water in the dough had little difference at different freezing temperatures. When the freezing time was extended to 15 d, the content of freezable water in the dough frozen at -12 °C was obviously lower than that in the dough frozen at other temperatures. This may be due to the migration of water inside the dough when it was frozen at -6 °C. Small ice crystals accumulated to form larger ones. The larger ice crystals broke down the gluten network inside the dough, releasing some of the water that had been inside the dough and leading to an increase in freezable water. This phenomenon could be alleviated when the dough was stored at a lower temperature. Thus, prolonged storage of dough at the subfreezing temperature (-12 °C) minimized the rise in freezable water content.

3.3 Microstructure of starch

Observation of the microstructure of wheat starch is helpful to understand the changes in the microstructure of dough during freezing (Peighambardoust et al., 2010). Scanning electron microscopy (SEM) is a method of looking at the microstructure of foods and visualizing the changes that occur in wheat flour and dough (Rosell et al., 2013). We performed scanning electron microscopy observations on the extracted starch sample, and the obtained starch microstructure is shown in Figure 3. Figure 3 shows that there were two particle sizes of large starch (type A starch) and small starch (type B starch) in both control starch (ck) and starch after frozen storage. Starch particles (ck) that were only quick-frozen but not put in frozen storage were round or oval, complete in shape and smooth on the surface. This is consistent with previous reports (Ao & Jane, 2007). However, after the starch was in frozen storage, the granules became uneven and the surface of the granules had dents and cracks. The longer the frozen storage time, the more serious the situation. More damaged starch particles were found in frozen storage at -18 °C. In comparison, the starch stored at -12 °C was damaged less, and the surface state of starch was smoother and more complete. This was consistent with the above results that the freezable water content of the dough was lower at -12 °C. During the freezing process, the freezable water in the dough was frozen into ice, and the size of the ice crystals gradually increased over time. The ice matrix occupied the interior or wall channel of the particle (Silvas-García et al., 2016). The swelling and pressure on the particles increased. Therefore, the higher the freezable water content is, the more serious the surface damage of starch particles. Therefore, the starch particles in the dough frozen at the subfreezing temperature (-12 °C) were in a relatively good state.

3.4 Pasting characteristics of starch

The pasting characteristics of starch are of great value in evaluating the process design and quality of frozen products (Ee et al., 2020). A rapid viscosity analyzer (RVA) is usually used to measure the pasting characteristics of starch (Wang et al., 2021). The viscosity and processing characteristics of starch can be measured by precisely controlled temperature and shear force to reflect the change in starch pasting properties (Kayode et al., 2021). Table 2 shows the effects of freezing time and temperature on the pasting characteristics of wheat starch in dough. Among them, ck was starch that had only been quick-frozen but not stored. Table 2 shows that the pasting characteristics of starch after frozen storage were higher than those of the control group. This result showed that the process of frozen storage did have varying degrees of influence on starch. This was consistent with previous reports (Kumar et al., 2018). The peak viscosity of starch stored at -18 °C was substantially higher than that of the control group. This may be due to the too low freezing temperature and too long freezing storage time, which made the damage to the starch surface more serious. This affected the integrity of starch granules, led to partial leaching of soluble substances, promoted binding with water, and thus increased the peak viscosity during gelatinization. This was consistent with the result observed in Section 3.3 that the starch surface was rougher at -18 °C. By comparing the breakdown viscosity of starch at different freezing temperatures under the same frozen storage time, we found that when the storage time was 5 d, there was no substantial difference in the breakdown viscosity of starch at different storage temperatures. When the storage time was 10 d, the breakdown viscosity of starch at -18 °C began to increase. When the storage time increased to 15 d, the breakdown viscosity of starch stored at the subfreezing temperature (-12 °C) was substantially different from that of starch stored at the other two temperatures. The starch stored at -12 °C had a lower breakdown viscosity and better thermal stability. This result indicated that the subfreezing temperature (-12 °C) is more advantageous in long-term frozen storage. Through the comparison of the setback viscosity of starch, we found that freezing would increase the setback viscosity of starch. The retrogradation of starch causes the aging of foods with high

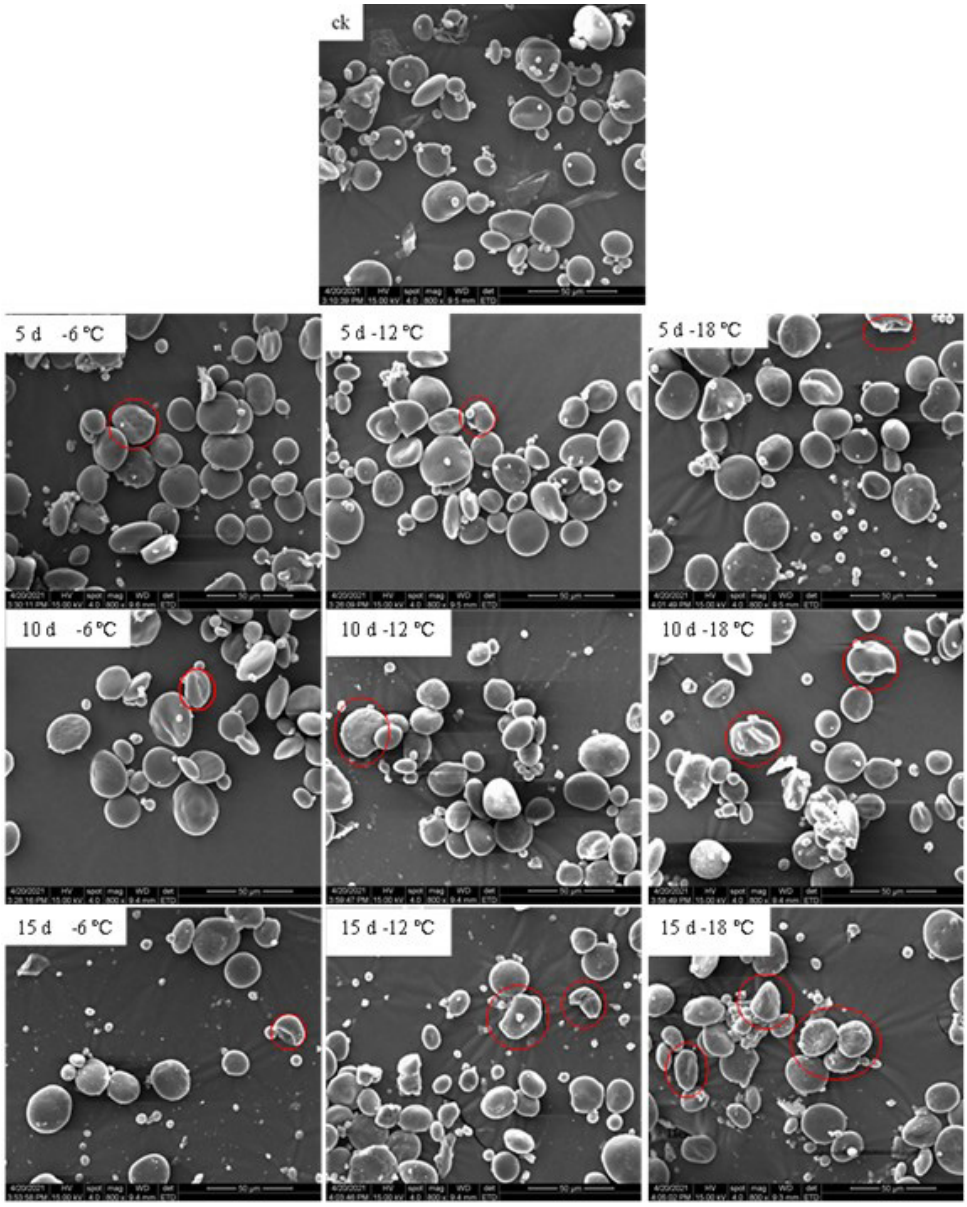

Figure 3. Microstructure of starch (ck is starch extracted from dough that has been quick-frozen but not stored).

Table 2. Pasting characteristics of starches treated by different storage time and temperature.

Storage time (d)	Temperature (°C)	PT/°C	PV/cp	BV/ cp	SV/ cp
5	ck	91.52±0.38^{a}	2056.67±24.91^{d}	336.67±58.59^{b}	835.67±56.39^{b}
	-6	85.30±0.48^{c}	3237.00±23.26^{a}	484.00±35.55^{a}	1264.67±99.05^{a}
	-12	87.68±0.46^{a}	2951.00±2.00^{b}	486.67±80.26^{a}	1165.67±11.55^{a}
	-18	91.18±0.03^{a}	2733.67±22.74^{c}	492.67±75.43^{a}	1215.67±35.50^{a}
10	ck	91.52±0.38^{b}	2056.67±24.91^{b}	336.67±58.59^{b}	835.67±56.39^{b}
	-6	93.63±0.80^{a}	1725.67±11.02^{c}	381.67±50.86^{b}	908.00±132.48^{b}
	-12	93.38±0.46^{a}	2067.00±9.00^{b}	359.00±5.57^{b}	902.33±10.07^{b}
	-18	85.55±0.01^{c}	3415.67±40.08^{a}	547.00±20.66^{a}	1339.67±76.53^{a}
15	ck	91.52±0.38^{a}	2056.67±24.91^{c}	336.67±58.59^{d}	835.67±56.39^{d}
	-6	90.10±0.48^{b}	2721.67±34.08^{b}	442.33±9.61^{b}	1176.67±25.17^{b}
	-12	86.93±0.42^{c}	3881.33±29.77^{a}	412.67±20.50^{c}	955.00±44.31^{c}
	-18	84.17±0.55^{d}	3871.33±41.40^{a}	638.00±53.23^{a}	1703.00±51.54^{a}

Values are means ± SD. Means values with the same letters in a column do not differ significantly ($p \geq 0.05$). PV, BV, FV, SV, ST are the peak viscosity, breakdown viscosity, final viscosity, setback viscosity, and pasting temperature, respectively. ck is starch extracted from dough that has been quick-frozen but not stored.

starch content and shortens the shelf life of foods. The sensory quality and storage quality of wheat starch food were also affected. However, under the same freezing storage time, the starch stored at -12 °C had a lower setback viscosity than that stored at other temperatures. The results showed that the retrogradation of starch could be inhibited to the maximum extent by storing dough at the subfreezing temperature. In conclusion, frozen storage did not change the overall shape of the starch pasting curve but substantially increased the peak viscosity, breakdown viscosity and setback viscosity and intensified the swelling of starch particles. However, the starch in the dough stored at the subfreezing temperature (-12 °C) had better thermal stability and could better inhibit retrogradation.

3.5 Starch crystalline structure

The regular arrangement, stacking and orientation of the starch double helix structure can be organized into a monoclinic crystal lattice or hexagonal crystal lattice. The ordered crystal structure is formed by the orientation, regular arrangement and tight packing of these crystal lattices. As shown in Figure 4, both the control wheat starch and the frozen wheat starch showed obvious diffraction characteristic peaks at 2θ of approximately 15°, 17°, 18° and 23°, which were typical A-type crystals, consistent with previous research results (Szymonska & Wodnicka, 2005). These results indicated that cryopreservation did not change the crystallization types of wheat starch. However, the diffraction peak intensity of frozen storage starch decreased, especially after 15 days of frozen storage, and the decreasing trend was more obvious. The relative crystallinity of starch that was only quickly frozen but not put in frozen storage was 33.4%. The relative crystallinity of starch decreased from 31.2% to 25.6% during freezing storage, and the lower the freezing storage temperature was, the lower the relative crystallinity was. This may be because during freezing storage, the growth of ice crystals destroys the hydrogen bonds within and between the molecules of starch, resulting in the unwinding of the double helix structure, hindering the orientation and tight arrangement between the double helix structures, and ultimately leading to a substantial decrease in the relative crystallinity of starch. The results showed that the low freezing temperature could destroy the ordered structure of starch molecules and their crystalline structure.

4 Conclusion

In this study, the effects of freezing storage temperatures of -6 °C, -12 °C and -18 °C on the structure and physicochemical properties of fermented dough were investigated. It was found that the water distribution of the fermented dough was similar at the subfreezing temperature of -12 °C and the common freezing

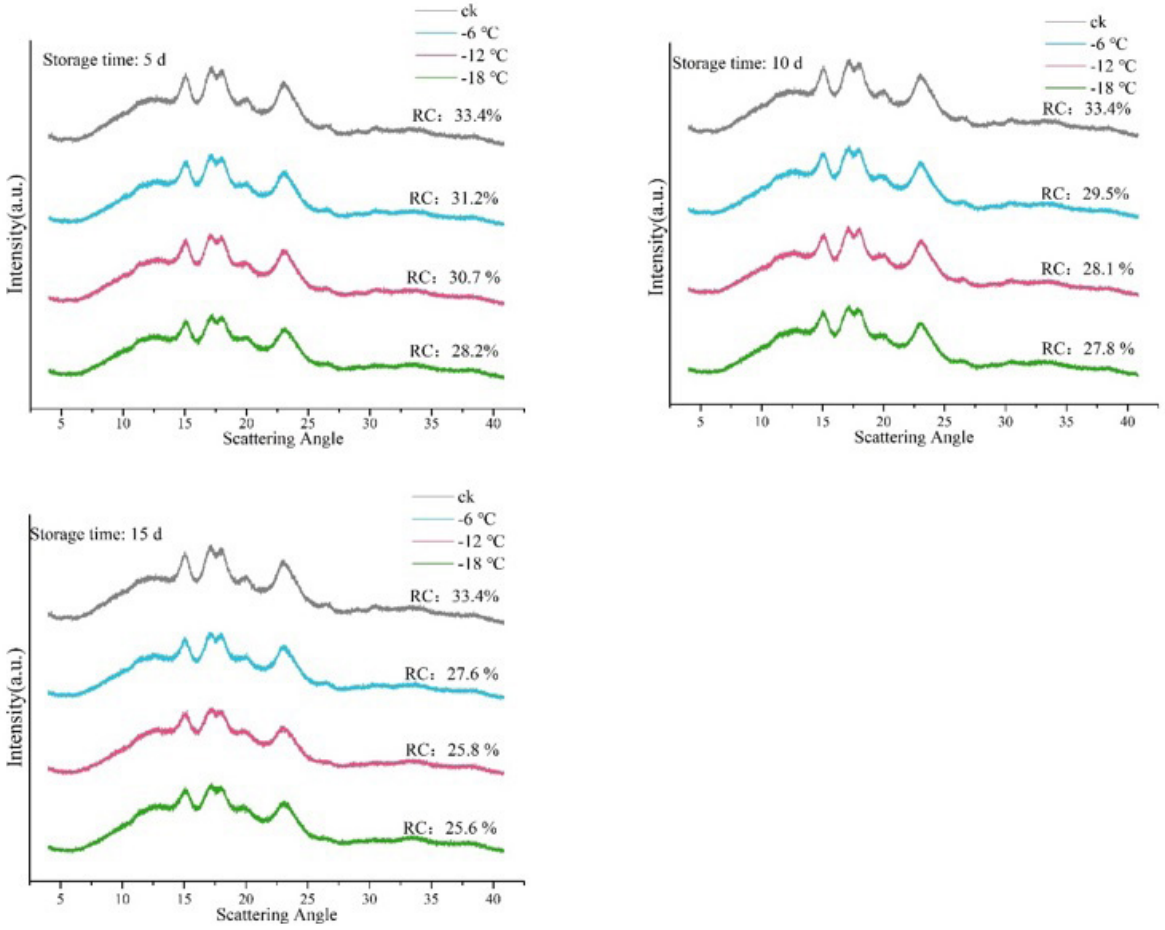

Figure 4. X-ray diffraction patterns of starch (RC, relative crystallinity, ck is starch extracted from dough that has been quick-frozen but not stored).

temperature of -18 °C, and the content of freezable water was lower at the subfreezing temperature of -12 °C. By observing the microstructure of starch, it was found that the starch particles frozen at -12 °C were in better condition and less damaged than those frozen at -18 °C. This conclusion is consistent with the conclusion drawn by the study on the crystal structure change of starch. The analysis of starch pasting showed that starch at subfreezing temperature had better thermal stability and could inhibit starch retrogradation to the greatest extent. The reduction of temperature results in better preservation of the moisture of the fermented dough starch and improves the storage time of the dough. However, too low freezing temperature will destroy the structure of starch, which will become more obvious with the extension of storage time. In conclusion, the freezing effect of fermented dough at subfreezing temperature (-12 °C) is better than that at -6 °C and -18 °C. Compared with the common freezing temperature -18 °C, the subfreezing storage temperature not only guaranteed the quality of dough, but also reduced the energy consumption in the freezing storage process and shortened the thawing time, which was beneficial to industrial production.

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