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Effects of different storage temperatures on the structure and physicochemical properties of starch in frozen non-fermented dough

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Abstract

This study investigated the effects of different storage temperatures on the structure and physicochemical properties of starch in frozen nonfermented dough. The results showed that the water activity and water loss rate of dough samples decreased with decreasing storage temperature. The density of hydrogen protons in the dough sample decreased as well. The freezable water content in dough increased with decreasing freezing temperature. The results showed that with decreasing freezing temperature, the formation and recrystallization of ice crystals in the process of freezing storage resulted in an increase in depressions and holes in wheat starch grains, internal and external damage to starch, and a gradual decrease in the relative crystallinity. The porous surface of starch particles and their loose molecular order structure promote the infiltration of water molecules into the interior of the starch particles. The combination of starch chain and water molecules makes the peak viscosity of starch in the dough gradually rise, but diminishes its stability when heated. Compared with the control group, the starch recovery value increased, and the starch enthalpy value increased gradually. The structure and physicochemical properties of starch in nonfermented dough stored at -12 °C and -18 °C were similar.

Keywords: non-fermented dough; different temperature; storage; starch; physicochemical properties.

Practical Application: These results provide theoretical guidance for energy savings and emission reduction and optimization of storage temperature.

1 Introduction

Traditional flour products generally have two processing stages: on-site production and sales, but it is difficult to achieve a unified standard that balances intensive production management while maintaining the quality of finished products.

Centralizing production and standardizing processes, storage methods, and transportation in distributing to diverse sales regions has various impacts on food that result in the deterioration of products. After frozen flour products rise, the early processing processes, such as forming and fermenting, are separated from the late cold chain processing processes, which not only improves traditional production but also enables achieving standardization and large-scale production.

Frozen dough technology offers a strong advantage in helping to ensure maintenance of product quality while reducing production and labor costs. At present, freezing has been used in the food industry at home and abroad and developed rapidly worldwide. Frozen nonfermented dough is usually used in traditional Chinese flour products such as soup dumplings and steamed dumplings, which are exceedingly popular among consumers (Zhang et al., 2018). While there have been comprehensive studies on the quality change of fermented dough in frozen storage, there are few studies on changes in the quality of nonfermented dough at different frozen storage temperatures. Today, two main problems plague the frozen flour industry. First, dewatering and cracking of dough during freezing storage reduces the appearance quality and volume of products and causes cracks in the skin of raw dumplings, wontons, and spring rolls. Second, frozen storage leads to gluten protein denaturation, which is prone to skin embrittlement, loss of elasticity, coarsening of texture, loss of original puffiness, and degradation of flavor (Simmons et al., 2012; Meziani et al., 2012).

Multiple factors affect the quality of frozen dough and pasta products. During freezing, crystallization changes the water phase distribution, which changes the structure and function of biochemical components. The quality of dough deteriorates due to moisture migration after an extended period of freezing (Xin et al., 2018). Ice crystals weaken the gluten network and change the rheological properties of frozen dough. Wang et al. (2017). observed that the formation of ice crystals in nonfermented dough stored for 24 weeks resulted in disruption of the gluten matrix, a discontinuous network and more fractures, and increased separation of starch particles, resulting in less gas retention by the gluten matrix, reduced dough volume, and increased wake time of frozen dough. The formation of large ice crystals also destroys starch particles during recrystallization, further reducing the ability of gluten to retain gas during awakening. Therefore, ice crystallization reduces the gas retention of frozen dough.

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Starch is the main ingredient of flour, accounting for 75% of the weight of flour. The water absorption and expansion rate of starch controls the water holding capacity of flour, affects the softness of finished products and, ultimately the quality of finished products (Wang et al., 2015; Shevkani et al., 2017). Slow freezing can form large ice crystals, causing mechanical damage to starch grains, which results in the release of more fat, protein, and amylose when thawed. With the extension of freezing time, amylopectin content increases, and amylose content decreases. Freezing treatment can deform, break, or coalesce wheat starch grains. Changing the structure of starch disrupts the balance of the starch-gluten network (Zhao et al., 2018).

Earlier studies found that storage freezing at different temperatures influences the quality of dough, and that storage at -9 °C to -12 °C can effectively prevent damage to the gluten network structure of frozen dough by ice crystals and maintain the elasticity of gluten protein. SEM results showed that storage at -9 °C and -12 °C could diminish the separation of the gluten network from starch particles in dough (Meng et al., 2021). In this study, we studied the structure and physicochemical properties of nonfermented dough and its starch after storage at 6 °C, -12 °C and -18 °C for 15 days to provide reference for industrial production of frozen dough.

2 Materials and methods

2.1 Materials

Wheat flour was purchased from Xinxiang Embryo granule Food Co., LTD. (Henan, China). NaOH and HCl were purchased from Tianjin Guangfu Technology Development Co., LTD. (Tianjin, China).

2.2 Prepare non fermented dough

800 g wheat flour and 400 g distilled water were weighed, then mixed the raw materials evenly to prepare the dough by using a dough kneading machine (DL-CO3, dongling electric appliance Co., LTD., guangdong, China), and then stood for 30 min. The dough was divided into several small doughs of 5 g and 20 g. After this, the dough was ready.

2.3 Design of frozen storage of non 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).

2.4 Determination of the properties of dough stored at different temperatures

Freezing curve of dough

The sensor of the water temperature recorder (L93-1, Hangzhou Luge Technology Co., LTD., China) was inserted into the dough center, and the dough was placed in a cryogenic refrigerator (BD-106DT, Changhong Meiling Co. LTD., China) at -30 °C for quick freezing. The core temperature of dough was recorded every 1 min between reaching 4 °C and reaching -24 °C. The freezing curve was drawn with time as the abscissa and the dough core temperature as the ordinate (Jiang et al., 2021).

Determination of relaxation time and ^1H density in dough by low field nuclear magnetic resonance (LF-NMR)

The relaxation time of the water in dough frozen at different temperatures for different times was measured by a LF-NMR imaging analyzer (NMI20-040V-I, Suzhou Niumai Analytical Instrument Co., LTD., Jiangsu, China) according to the method of Wang et al. (2022) with some modifications. The dough sample was placed in a nuclear magnetic test tube 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. The spin relaxation time (T_2) of the sample was measured by CPMG pulse sequence, and the CPMG pulse sequence scanning test was carried out. CPMG test parameters: main frequency = 20 (MHz), offset frequency = 628049.19 (Hz), sampling number T_D = 40014, repeat scan number NS = 4, sampling interval T_W = 2000 ms, half echo time τ = 6.52 μs, temperature = 32 °C. The RELAXATION map and T_2 were obtained by inversion of CPMG relaxation attenuation curve using T_2 inversion fitting software (Miklos et al., 2015). Fresh dough was used as the control (CK).

^1H density: parameters of MRI test were set as follows. repetition time (TR) = 500 ms; Signal receiving bandwidth SW = 200 kHz; Sampling times NS = 4; According to T_2 values measured in CPMG sequence, echo time TE = 0.2 ms was selected for imaging. Fresh dough was used as a control (CK).

Determination of freezable water in dough

The freezable water in dough was determined according to the method of Jiang et al. (2020). with some modifications. Q200 DSC (TA Instruments Waters LLC, New Castle, DE, USA) was used to determine the content of freezable water (Fw) in frozen dough storage at different temperatures and different times. After the dough was thawed, 20 mg sample (wet base) from the center of the dough was weighed and sealed in an empty aluminum pan, then conducted the following determination. The sample was cooled from 25 °C to -40 °C at a cooling rate of 5 °C/min, kept at -40 °C for 1 min, and then heated to 30 °C at a heating rate of 5 °C/min. The enthalpy (ΔH) of each sample was calculated by TA software. Fresh dough was used as a control (CK). The proportion of freezable water (Fw) in the frozen dough is calculated by the following formula (Equation 1):

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

Where is ΔH_0 enthalpy of water (335 J/g), W is water content of dough sample.

Determination of dough texture

The texture of dough was determined according to the method of Qin et al. (2022) with some modifications. The texture characteristics of dough (radius of 3 cm) were measured by texture analyzer (Stable Microsystems, London, UK), and the hardness, springiness, cohesiveness, gumminess, and resilience of dough were obtained. The P36R probe (diameter of 10 mm) was used and the test parameters were as follows: pretest speed 2 mm/s, test speed is 1 mm/s, post-test speed 1 mm/s, compression ratio 70%, trigger force 5 g, and compression time interval 5 s.

2.5 Determination of properties of starch separated from frozen dough

Separation of starch from frozen dough

The frozen wheat dough storage at -6 °C, -12 °C and -18 °C was sampled on 5th day, 10th day and 15th day. The dough was thawed for 1 h at 30 °C in an incubator (LRH-150B, Huruoming Instrument Co., LTD., Guangzhou, China). The gluten was then washed away with 0.1% NaOH solution by using the Martin method until the washed water is not blue when added to the iodine solution, then collected the starch slurry. The starch slurry was washed with distilled water three times, and sifted with a 100-mesh sieve, then centrifuged at 3500 rpm for 20 min in a centrifuge (L550, Changsha High-tech Industrial Development Zone Xiangyi Centrifuge Instrument Co., LTD., Changsha, China), removed the upper pigment layer, then washed the precipitate with 300 mL distilled water and centrifuged again. Repeated washing and centrifugation twice. The precipitate was suspended again in 400 mL distilled water, and the pH was adjusted to 7.0 with HCl (0.01 mol/l) using a pH meter (PHS-3C, Shanghai Shengxi Instrument Co., LTD., Shanghai, China), then centrifuged. The precipitate was poured into large petri dishes, dried in an oven (DHG-9140-A Electric thermostatic air blowing drying oven, Shanghai Sanfa Scientific Instrument Co., LTD., Shanghai, China) and passed through a 150-mesh sieve (Yang et al., 2019). All starch samples are collected and packed in PE bags for the next analysis.

The starch, which was separated from the dough that only conducted quick-frozen process at -30 °C but not frozen storage, was used as a control (CK).

Microstructure observation of starch (SEM)

Microstructure of starch was observed according to Jiang et al. (2021). A small amount of starch samples was placed on an aluminum carrier platform with conductive tape. The carrier platform was placed in a gold-plated instrument, and the sample was sprayed with carbon and gold-plated for 90 s with ion sputtering coating instrument. A Quanta 200 scanning electron microscope (FEI Corporation, Oregon, USA) was used to observe the microstructure of starch samples. Starch extracted from dough without frozen storage was used as control (CK) (Zhang et al., 2015).

Determination of gelatinization properties of starch

3 g starch was put into RVA aluminum cylinder, and 25 mL water was added. After evenly stirring, gelatinization properties of starch were determined by using a rapid viscosity analyzer (RVA) (TecMaster, Newport Scientific Instruments LTD., Australia). The parameters are set as follows: the sample was kept at 50 °C for 1 min, heated to 95 °C in 3.7 min, kept at 95 °C for 2.70 min, then reduced from 95 °C to 50 °C in 3.8 min, and kept at 50 °C for 2 min. The viscosity curve was obtained and the data was analyzed automatically. Starch extracted from unfrozen dough was used as control (CK).

Determination of thermodynamic properties of starch

According to the experimental method of Koo et al. (2005)., a differential scanning calorimeter (DSC) was used to measure the thermodynamic properties of starch. Accurately weigh 5.0 mg starch and 10.0 mg water in a stainless steel crucible, then sealed and put in a fridge at 4 °C for 12 h. An empty crucible was used as a reference (to eliminate instrumental errors). The parameters were set as follows: scanning temperature ranges from 25 °C to 100 °C, and heating rate is 10 °C/min. The initial temperature (T_o), peak temperature (T_p) and terminal temperature (T_c) of gelatinization and the endothermic enthalpy (ΔH) of gelatinization were analyzed from DSC curves. Starch extracted from unfrozen dough was used as control (CK).

Crystal structure analysis of starch (X-ray diffraction)

Crystal characteristics of starch samples were determined by X-ray diffractometer (Bruker D8 Advance A25, Germany). The starch was placed in a sealed glass dryer containing ultra-pure water for water equilibrium for 24 h. The scanning range was from 4° to 40° (2θ), the scanning rate was 2°/min, and the X-ray diffraction pattern was obtained. Jade 6.0 software (Materials Data Inc., Livermore, CA, USA) was used to analyze the relative crystallinity (peak area divided by the total area of the diffraction pattern) (Thanatuksorn et al., 2009).

2.6 Statistical analysis

The experiments were repeated three times. All data was statistically analyzed using SPSS 17.0 software (SPSS Inc., Chicago, USA) and standard deviations were calculated. Data results were presented as mean ± standard deviation and plotted using Origin 2017(Origin Lab Co., Massachusetts, USA) software. Duncan test showed significant difference ($P < 0.05$).

3 Results and discussion

3.1 Properties of dough stored at different temperatures

Dough freezing curve

We placed a temperature probe in the center of dough to monitor its temperature during the quick-freezing process. Figure 1 shows that the freezing curve has a precooling stage. After 20 minutes, the dough entered a period of maximum ice crystal formation while the center temperature reached and remained at -4 °C remained for a period of time, and then continued to fall as the dough entered the deep-freezing stage. The stages of the temperature change offer a reference guide for the following determination of hydrogen proton density.

Relaxation time

Changes in the relaxation time of dough stored at different temperatures are shown in Figure 2. Two transverse relaxation times (T_2) characterize the different states of water in all dough samples, which include bound water in the range of 0.01~10 ms (T_{21}) and free water in the range of 10-1000 ms (T_{22}) (Shao et al., 2016; Cheng et al., 2018; Zang et al., 2017). A_{21} and A_{22} are the proportions of water in the two states of water, which are listed in Table 1. As seen from Figure 2 and Table 1, the values of A_{21} and A_{22} for the dough frozen for 5, 10 and 15 days at the three freezing temperatures showed no significant difference from that of the control group. While the total peak area of dough frozen at -18 °C was significantly different from that of the control group and -6 °C group at three different freezing days ($P < 0.05$), it was not significantly different from that of the -12 °C group. The total peak area of dough was the highest at -18 °C and reached 1042.04, 1063.16 and 1037.03 at 5, 10 and 15 days, respectively, showing that the water loss of dough was the least at -18 °C.

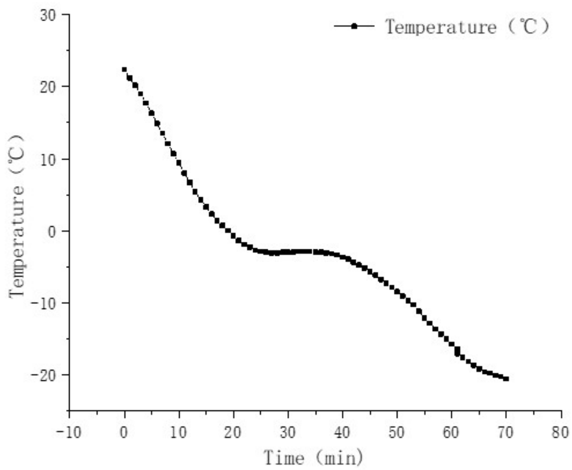
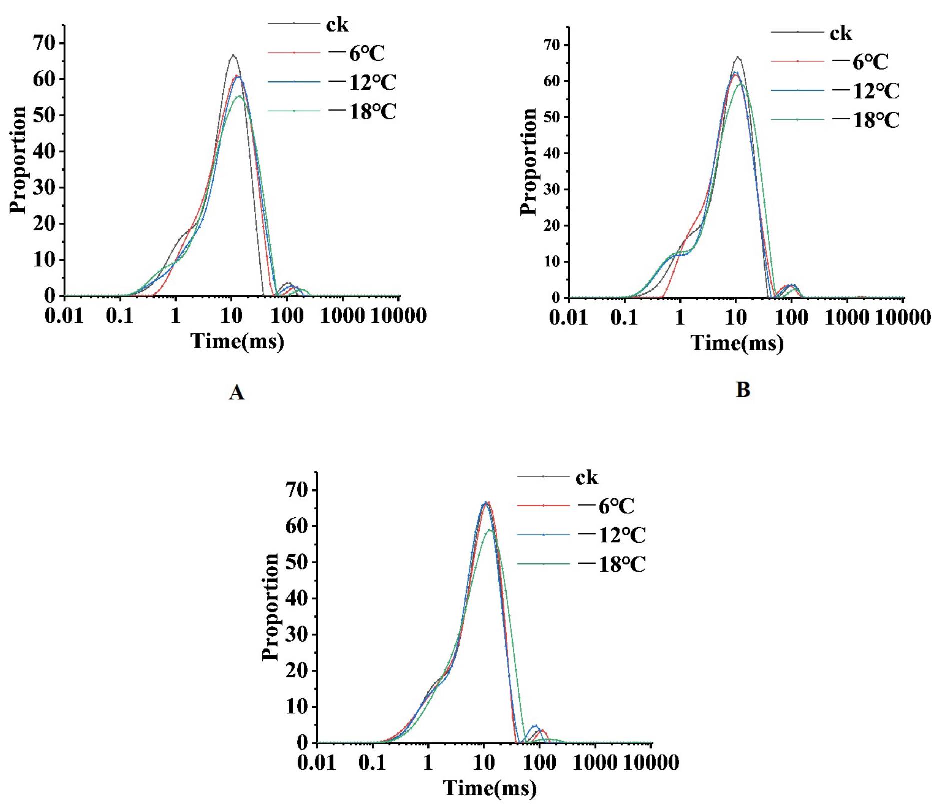


Figure 1. Freezing curve of dough center during refrigeration.

¹H density image

To quickly and efficiently analyze the distribution of water in the dough at different freezing temperatures, we used MRI to measure the hydrogen proton density image in the dough (Figure 3). As shown in Figure 3, the brighter area shows the strong hydrogen proton signal intensity in the dough, namely, the strong density; the darker area shows the weak hydrogen proton signal intensity, namely, the relatively low density (Borompichaichartkul et al., 2005). During freezing, the low density of hydrogen protons is less free water and a relatively low chemical rate that occurs in the dough. The results show that the fresh dough has strong



C

Figure 2. T_2 relaxation patterns of non-fermented dough frozen at different storage temperatures for different periods of time. A, 5 days; B, 10 days; C,15 days.

Table 1. The variation of moisture in different states of frozen dough during freezing storage.

Storage time (d)	Storage temperature (°C)	T_{21} (ms)	T_{22} (ms)	A_{21} (%)	A_{22} (%)	The total peak area
ck		10.72 ± 0.01[b]	100.00 ± 26.24[ab]	98.42 ± 0.32[a]	1.58 ± 0.32[a]	1006.81 ± 20.21[a]
5	-6	12.33 ± 1.07[a]	151.99 ± 11.43[a]	99.04 ± 0.07[a]	0.96 ± 0.07[a]	1024.85 ± 16.57[a]
	-12	12.33 ± 0.93[ab]	100.00 ± 8.65[b]	98.62 ± 0.07[a]	1.37 ± 0.07[a]	1031.46 ± 15.17[a]
	-18	12.33 ± 0.01[a]	151.99 ± 26.76[ab]	99.28 ± 11.67[a]	0.73 ± 0.43[a]	1042.04 ± 6.58[a]
ck		10.72 ± 0.01[ab]	100.00 ± 26.24[a]	98.42 ± 0.32[a]	1.58 ± 0.32[a]	1006.81 ± 20.21[b]
10	-6	9.33 ± 0.81[b]	86.98 ± 20.25[a]	98.18 ± 9.77[a]	1.74 ± 0.36[a]	1001.97 ± 23.48[ab]
	-12	9.33 ± 0.81[b]	100.00 ± 28.36[a]	98.24 ± 0.39[a]	1.73 ± 0.35[a]	1022.94 ± 17.60[ab]
	-18	12.33 ± 0.93[a]	114.98 ± 8.65[a]	99.15 ± 0.30[a]	0.85 ± 0.30[a]	1063.16 ± 11.69[a]
ck		10.72 ± 0.01[b]	100.00 ± 26.24[a]	98.42 ± 0.32[a]	1.58 ± 0.32[a]	1006.81 ± 20.21[b]
15	-6	12.33 ± 0.01[ab]	114.98 ± 12.17[a]	98.83 ± 0.02[a]	1.17 ± 0.02[a]	1009.16 ± 7.87[b]
	-12	10.72 ± 0.93[b]	86.98 ± 23.28[a]	98.02 ± 0.50[a]	1.98 ± 0.50[a]	1013.01 ± 8.95[ab]
	-18	12.33 ± 1.07[a]	132.19 ± 30.77[a]	99.23 ± 0.47[a]	0.73 ± 0.49[a]	1037.03 ± 10.79[a]

Note: Values are mean ± standard error, different letters in the same column mean significant difference ($P < 0.05$), [a] is the maximum. CK, quick-frozen but not refrigerated dough.

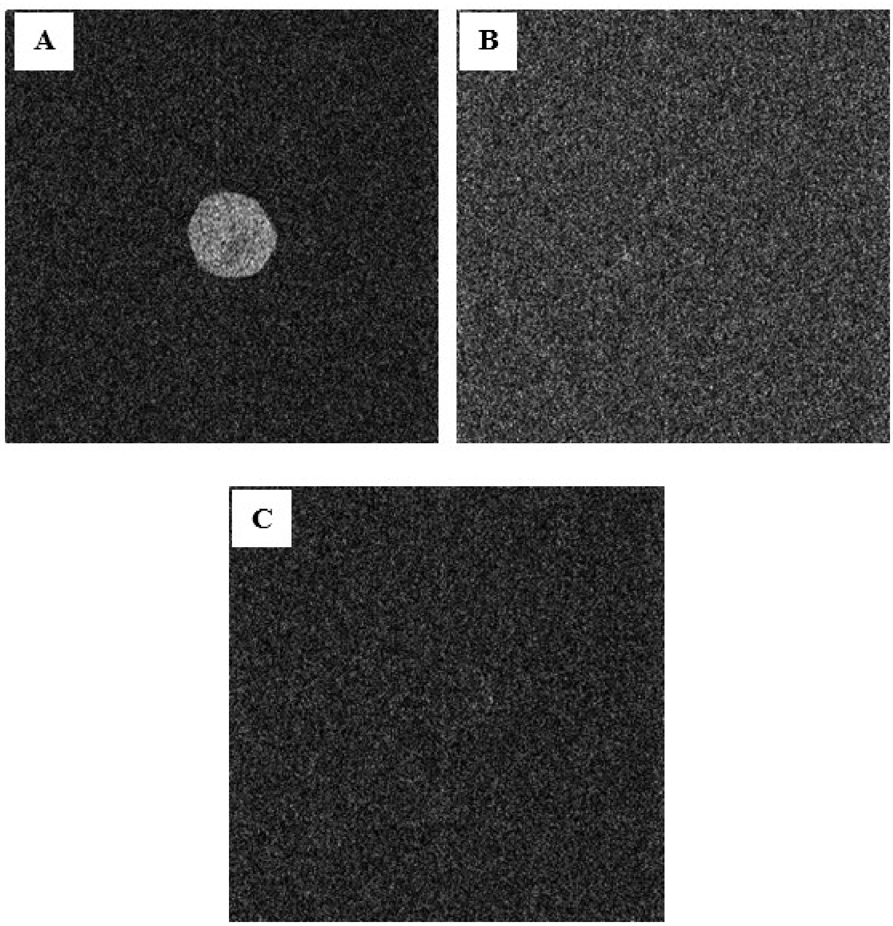


Figure 3. Density distribution diagram of fresh dough, dough quick-frozen to -4 °C and dough stored at -6 °C for 5 days. A, fresh dough; B, freeze to -4 °C dough;C, freeze the dough for 5 days at -6 °C.

unfrozen brightness, and the central part of the dough has been frozen at -4 °C. The brightness of the hydrogen proton density image of the dough samples frozen at -6 °C for 5 days is the same as that when the dough is frozen to -4 °C, which indicates that the free water content of the samples frozen at -6 °C for 5 days is very low, which is consistent with that when it is frozen to -4 °C, which is also consistent with the measurement results of T_2 relaxation time.

Freezable water

Table 2 shows the measurement results for F_W in dough stored for 5 days, 10 days and 15 days at different frozen storage temperatures. As seen from Table 2, the proportions of freezable water in the dough frozen at different temperatures for 5 days, 10 days and 15 days showed a significant difference compared with that in the control group, showing a gradual upward trend. Freezing storage increased the content of freezable water, which may be related to the changes in ice crystal nucleation dynamics during freezing storage; that is, during freezing storage, water migration, ice crystal generation and recrystallization resulted in water redistribution in the dough or in the gap of the gluten network; thus, the ice crystal volume increased, which increased the proportion of F_W (Wang et al., 2021; Kontogiorgos et al., 2008). However, with the extension of freezing storage time, there is no significant difference in F_W between -12 °C and -18 °C. These results showed that the storage effects at -12 °C and -18 °C were similar.

Table 2. The freezable water content (Fw) of frozen dough changes during freezing storage.

Storage time (d)	Storage temperature (°C)	Fw
ck	-6	0.22 ± 0.01^{c}
5		0.24 ± 0.01^{b}
	-12	0.30 ± 0.01^{a}
	-18	0.30 ± 0.01^{a}
ck		0.22 ± 0.01^{d}
10	-6	0.24 ± 0.01^{c}
	-12	0.26 ± 0.01^{b}
	-18	0.29 ± 0.01^{a}
ck		0.22 ± 0.01^{b}
15	-6	0.25 ± 0.01^{a}
	-12	0.25 ± 0.01^{a}
	-18	0.26 ± 0.01^{a}

The values were mean ± standard error (n=3), different letters in the same column indicated significant difference ($P < 0.05$), and ^{a} was the maximum. CK, quick-frozen but not refrigerated dough.

Texture

It can be seen from Table 3, compared with the control group (ck), the hardness of dough frozen storage for 5 d, 10 d and 15 d increased and the elasticity decreased with the decrease of temperature; the adhesion increased first and then decreased, and higher than those of the ck, while the cohesion and resilience of dough were not significantly different from that of the ck. This may be because the long-term freezing storage reduces the free water content in the dough, and the continuous recrystallization of water during the freezing storage leads to the redistribution of water in the dough, and finally leads to the change of dough texture characteristics (Gerardo-Rodríguez et al., 2016; Hernandez-Chavez et al., 2019; Zhang et al., 2022). With the extension of frozen storage time, the texture properties of dough changed to a certain extent, but the change range was small in the range of -12 °C to -18 °C, indicating that storage effects of -12 °C and -18 °C are similar.

3.2 Properties of starch separated from frozen dough

Microstructure

As shown in Figure 4, the size distribution of starch particles in the control group was different, with most being round or oval, smooth and complete. Compared with the control group, with the decrease in freezing storage temperature at -6 °C, -12 °C and -18 °C on Days 5, 10 and 15, the surface structure of particles was damaged to varying degrees, and the surface was rough and uneven, which might be due to the starch particles broken by ice crystals during freezing storage. The results showed that ice crystal nucleation caused mechanical damage in starch grains. This is consistent with earlier results (Yaqoob et al., 2019).

Table 3. The texture of frozen dough changes during freezing storage.

Storage time/d	Storage temperature /°C	Hardness/g	Springiness/g	Cohesiveness/g	Gumminess/g	Resilience/g
ck		1194.09 ± 104.03^{c}	0.29 ± 0.04^{a}	0.39 ± 0.02^{a}	472.06 ± 61.46^{a}	0.05 ± 0.01^{b}
5	-6	1265.67 ± 59.99^{bc}	0.23 ± 0.01^{a}	0.40 ± 0.01^{a}	482.90 ± 2.63^{a}	0.06 ± 0.01^{a}
	-12	1372.88 ± 21.89^{ab}	0.33 ± 0.22^{a}	0.41 ± 0.09^{a}	559.33 ± 130.65^{a}	0.05 ± 0.01^{ab}
	-18	1410.02 ± 8.03^{a}	0.20 ± 0.01^{a}	0.39 ± 0.01^{a}	490.48 ± 1.12^{a}	0.05 ± 0.01^{ab}
ck		1194.09 ± 104.03^{c}	0.29 ± 0.04^{a}	0.39 ± 0.02^{a}	472.06 ± 61.46^{a}	0.05 ± 0.01^{a}
10	-6	1316.14 ± 0.43^{b}	0.25 ± 0.03^{a}	0.40 ± 0.04^{a}	536.81 ± 49.94^{a}	0.05 ± 0.01^{a}
	-12	1426.87 ± 6.87^{a}	0.28 ± 0.11^{a}	0.42 ± 0.08^{a}	564.69 ± 54.74^{a}	0.05 ± 0.01^{a}
	-18	1440.83 ± 5.35^{a}	0.27 ± 0.14^{a}	0.35 ± 0.07^{a}	499.68 ± 108.37^{a}	0.05 ± 0.01^{a}
ck		1194.09 ± 104.03^{c}	0.29 ± 0.04^{a}	0.39 ± 0.02^{ab}	472.06 ± 61.46^{a}	0.05 ± 0.01^{b}
15	-6	1359.09 ± 10.48^{b}	0.32 ± 0.04^{a}	0.42 ± 0.04^{a}	572.65 ± 53.25^{a}	0.06 ± 0.01^{a}
	-12	1455.32 ± 8.13^{ab}	0.24 ± 0.04^{a}	0.35 ± 0.02^{b}	511.48 ± 29.25^{a}	0.05 ± 0.01^{ab}
	-18	1491.69 ± 22.74^{a}	0.25 ± 0.05^{a}	0.40 ± 0.02^{ab}	589.86 ± 15.08^{a}	0.05 ± 0.01^{a}

The values were mean ± standard error (n = 3), different letters in the same column indicated significant difference ($P < 0.05$), and ^{a} was the maximum. CK, quick-frozen but not refrigerated dough.

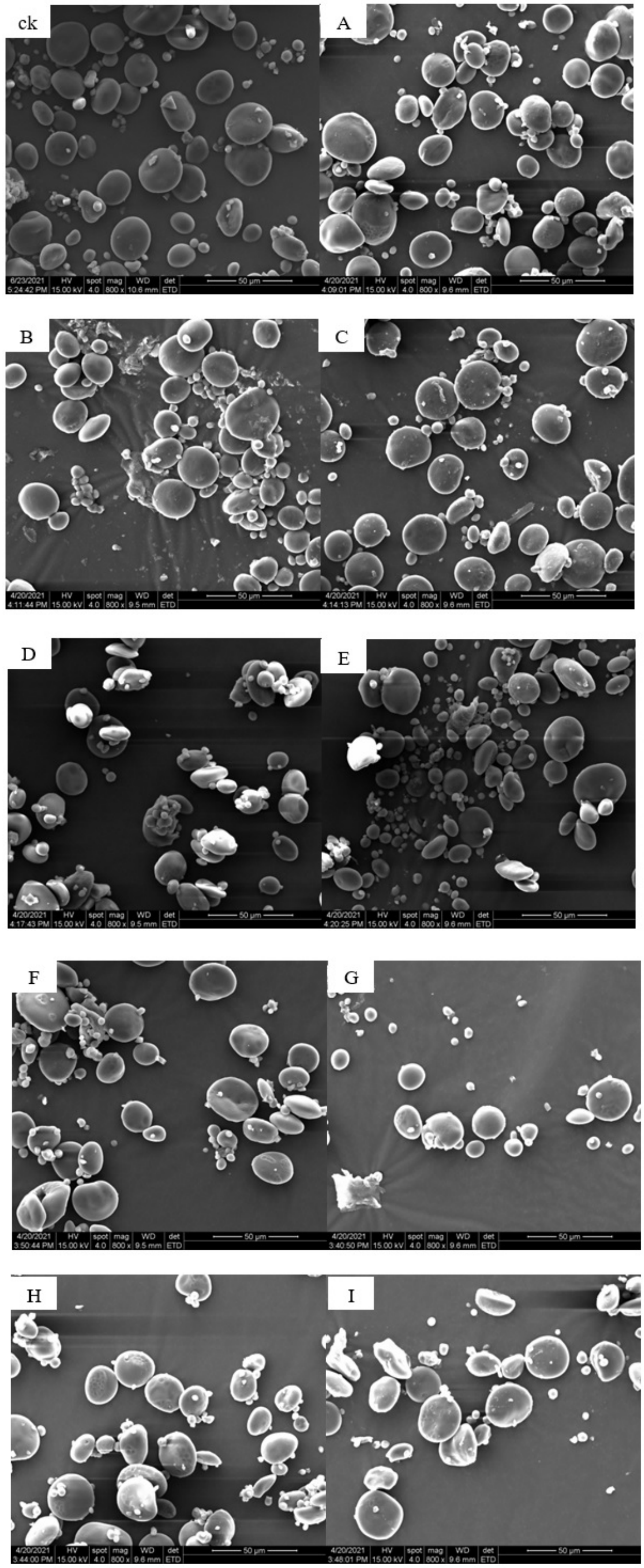


Figure 4. Microstructure of starch in non-fermented dough frozen at different temperature and for different time. CK, dough that has been frozen but not frozen; A, frozen at -6 °C for 5 days; B, frozen at -12 °C for 5 days; C, frozen at -18 °C for 5 days; D, frozen at -6 °C for 10 days; E, Frozen at -12 °C for 10 days; F, frozen at -18 °C for 10 days; G, frozen at -6 °C for 15 days; H, frozen at -12 °C for 15 days; I, frozen at -18 °C for 15 days.

Pasting properties

Peak viscosity refers to the maximum viscosity reached before heating makes the sample begin to gelatinize and cool. The attenuation value is the difference between the peak viscosity and the lowest viscosity. Our results showed that as attenuation value decreased the thermal stability increased. After swelling, starch particles have higher strength and are not easy to rupture. The regression value is the difference between the lowest viscosity and the final viscosity. The higher the regression value is, the easier the aging. Low gelatinization temperature is beneficial to reduce processing energy consumption time.

As shown in Table 4, the peak viscosity, lowest viscosity, final viscosity, and setback of the starch samples in the dough frozen for 5 days decreased first and then increased from -6 °C, -12 °C to -18 °C, reaching the maximum at -18 °C and was significantly higher than the starch samples in the control group. The three temperatures of frozen dough and the attenuation value and retrogradation of starch were higher than those in the control group and were significantly different from those in the control group, which may be due to the decrease in freezing storage temperature. The formation of ice crystals and recrystallization phenomenon caused by the mechanical damage structure of starch and starch granule surface holes make free water particles more likely to enter the internal space. Intramolecular and intermolecular hydrogen bonds are formed with stretched starch molecules, so the peak viscosity increases, and the integrity of starch particles is destroyed, leading to the reduction of thermal paste stability and easy regeneration. These results are consistent with earlier reports (Blazek & Copeland, 2008; Karakelle et al., 2020).

The peak viscosity, final viscosity and setback of the starch samples in the dough frozen for 10 days increased first and then decreased from -6 °C, -12 °C to -18 °C and were significantly higher than those in the control group. Moreover, the attenuation values of the starch in the dough frozen at three temperatures decreased gradually and were all higher than those in the control group. However, the peak viscosity, lowest viscosity, attenuation value, final viscosity, regression value, peak time and gelatinization temperature of starch did not change significantly from -12 °C to -18 °C. The results showed that the storage effect was similar for -12 °C and -18 °C.

The peak viscosity, the lowest viscosity, the decay value, the final viscosity and the recovery value of the starch samples in the dough after frozen storage for 15 days increased first and then decreased from -6 °C, -12 °C to -18 °C. The peak viscosity of starch samples in wheat dough stored at three freezing temperatures was higher than that in the control group. The α-amylase activity was inhibited but not destroyed, perhaps because the water migration was less during freezing storage at -18 °C. The α-amylase activity was not completely inhibited at -6 °C, but the α-amylase activity was not completely inhibited at -12 °C. The attenuation value of starch in the dough under freezing storage at -12 °C was higher than that in the control group, and there was a significant difference, which might be because during freezing storage at -12 °C, the water activity in the dough was relatively large, which caused mechanical damage to the starch and gluten network structure, leading to the decrease in heat stability. The starch recovery value of the frozen dough at three temperatures was higher than that of the control group, and there was a significant difference between the two groups. It may be that the formation and recrystallization of ice crystals caused mechanical damage to the starch structure during the frozen storage process, leading to starch rupture and easy recovery. In addition, the gelatinization temperature of frozen samples stored at -12 °C for 10 and 15 days was the lowest, which was beneficial to reduce the processing energy consumption time.

Thermal properties

Table 5 shows the effects of different freezing storage temperatures on the thermodynamic properties of starch in frozen dough on different days. As seen from Table 4, compared with the control group, the To, Tp and Tc of starch in dough frozen for 5, 10 and 15 days at three different freezing temperatures decreased, indicating that the crystalline structure of starch in the frozen dough was unstable after freezing storage treatment, which ultimately led to the decrease in the thermal stability of the frozen dough (Li et al., 2021).

Table 4. Gelatinization properties of starch in frozen dough during freezing storage.

Storage time/d	Storage temperature /°C	Peak /cp	Lowest/cp	Attenuation values /cp	Final /cp	Setback /cp	Peak time /min	Pasting temperature/°C
ck		2503.33 ± 22.03^{b}	2124.33 ± 56.36^{b}	379.00 ± 39.23^{c}	3073.33 ± 55.77^{b}	949.00 ± 15.62^{b}	6.91 ± 0.03^{a}	88.53 ± 0.51^{bc}
5	-6	2516.33 ± 41.31^{b}	2120.33 ± 79.58^{b}	396.00 ± 38.74^{c}	3122.00 ± 108.06^{b}	1001.67 ± 33.02^{b}	6.91 ± 0.10^{a}	89.37 ± 0.49^{ab}
	-12	2267.67 ± 75.75^{c}	1754.33 ± 36.50^{c}	513.33 ± 91.73^{b}	2712.67 ± 91.78^{c}	958.33 ± 112.22^{b}	7.00 ± 0.01^{a}	89.90 ± 0.48^{a}
	-18	3342.33 ± 56.01^{a}	2710.33 ± 72.84^{a}	632.00 ± 18.52^{a}	4427.33 ± 93.52^{a}	1717.00 ± 35.51^{a}	6.95 ± 0.04^{a}	87.65 ± 0.52^{c}
ck		2503.33 ± 22.03^{b}	2124.33 ± 56.36^{b}	379.00 ± 39.23^{b}	3073.33 ± 55.77^{b}	949.00 ± 15.62^{b}	6.91 ± 0.03^{a}	88.53 ± 0.51^{ab}
10	-6	2587.00 ± 23.64^{ab}	2142.00 ± 45.40^{b}	445.00 ± 30.12^{a}	3279.33 ± 111.64^{ab}	1137.33 ± 75.06^{ab}	6.87 ± 0.12^{a}	89.40 ± 0.48^{a}
	-12	2954.67 ± 383.28^{a}	2545.00 ± 354.29^{a}	409.67 ± 32.65^{ab}	3721.00 ± 526.57^{a}	1176.00 ± 187.80^{a}	6.84 ± 0.10^{a}	86.62 ± 1.62^{c}
	-18	2950.33 ± 23.80^{a}	2557.67 ± 22.12^{a}	392.67 ± 2.08^{ab}	3711.00 ± 19.97^{a}	1153.33 ± 14.64^{a}	6.89 ± 0.08^{a}	87.17 ± 0.03^{bc}
ck		2503.33 ± 22.03^{c}	2124.33 ± 56.36^{c}	379.00 ± 39.23^{b}	3073.33 ± 55.77^{d}	949.00 ± 15.62^{d}	6.91 ± 0.03^{ab}	88.53 ± 0.51^{b}
15	-6	2507.67 ± 25.01^{c}	2093.67 ± 24.83^{c}	414.00 ± 49.49^{b}	3297.00 ± 83.72^{c}	1203.33 ± 108.49^{c}	6.78 ± 0.10^{b}	90.15 ± 0.48^{a}
	-12	3660.00 ± 65.55^{a}	2966.00 ± 128.93^{a}	694.00 ± 140.55^{a}	4757.67 ± 143.96^{a}	1791.67 ± 151.58^{a}	6.96 ± 0.08^{a}	86.87 ± 0.45^{c}
	-18	3315.67 ± 23.80^{b}	2775.67 ± 80.70^{b}	540.00 ± 75.72^{ab}	4357.00 ± 48.51^{b}	1581.33 ± 84.71^{b}	6.91 ± 0.03^{ab}	87.68 ± 0.46^{bc}

Note: Values are mean ± standard error, different letters in the same column mean significant difference ($P < 0.05$), ^{a} is the maximum. CK, quick-frozen but not refrigerated dough.

ΔH can be used to stand for the energy needed to decompose intermolecular hydrogen bonds of starch particles, which is related to the degree of order of starch molecules in dough (Tao et al., 2015; Zeng et al., 2014). After frozen storage, ΔH changes to different degrees (Xu et al., 2019). Compared with the dough without freezing storage, the ΔH of dough shows a trend of gradually increasing, which is consistent with the research results of Lu & Grant (1999). which showed that during freezing storage, the micromechanical forces generated by the formation and growth of ice crystals promote the rearrangement of starch chains, leading to the formation of a more ordered molecular structure and resulting in an increase in the ΔH of dough. The ΔH of dough is mainly affected by the degree of order of starch molecules in the dough. The increase in ΔH indicates that the energy needed to change the chemical composition of wheat flour from ordered form to disordered form is increased. Therefore, freezing storage treatment increases the intermolecular hydrogen bonds of starch and increases the degree of ordering of wheat flour. The ΔH of dough frozen at -12 °C for 5 days, 10 days and 15 days is slightly lower than -18 °C, showing that the storage effect of -12 °C is like that of -18 °C.

X-ray diffraction

The crystallinity of starch can directly affect the comprehensive properties of starch molecular products. According to Figures 5A, 5B and 5C in Figure 5, starch samples in the frozen

Table 5. Thermodynamic properties of starch in frozen dough during freezing storage.

Storage time/d	Storage temperature /°C	T_0/°C	T_p/°C	T_c/°C	ΔH /(J/g)
ck		76.93 ± 3.22^a	85.68 ± 0.22^a	89.48 ± 0.42^a	0.15 ± 0.06^b
5	-6	60.54 ± 0.45^b	70.52 ± 0.01^b	87.55 ± 0.57^b	2.67 ± 0.09^a
	-12	60.72 ± 0.84^b	70.24 ± 0.02^{bc}	87.62 ± 0.41^b	2.55 ± 0.10^a
	-18	59.35 ± 2.37^b	70.17 ± 0.23^c	87.52 ± 0.32^b	2.62 ± 0.22^a
ck		76.93 ± 3.22^a	85.68 ± 0.22^a	89.48 ± 0.42^a	0.15 ± 0.06^c
10	-6	60.81 ± 1.53^b	70.29 ± 0.02^c	88.30 ± 0.68^b	2.90 ± 0.19^a
	-12	61.11 ± 0.26^b	70.61 ± 0.01^b	87.93 ± 0.10^b	2.41 ± 0.03^b
	-18	59.70 ± 0.40^b	69.00 ± 0.01^d	87.55 ± 0.19^b	2.91 ± 0.04^a
ck		76.93 ± 3.22^a	85.68 ± 0.22^a	89.48 ± 0.42^a	0.15 ± 0.06^c
15	-6	60.45 ± 1.35^b	69.27 ± 0.01^c	83.58 ± 0.30^d	1.52 ± 0.05^b
	-12	60.04 ± 0.70^b	69.75 ± 0.01^b	88.25 ± 0.29^b	2.85 ± 0.06^a
	-18	58.90 ± 0.91^b	69.84 ± 0.01^b	87.36 ± 0.11^c	2.85 ± 0.06^a

Note: Values are mean ± standard error, different letters in the same column mean significant difference ($P < 0.05$), ^{a} is the maximum. CK, quick-frozen but not refrigerated dough.

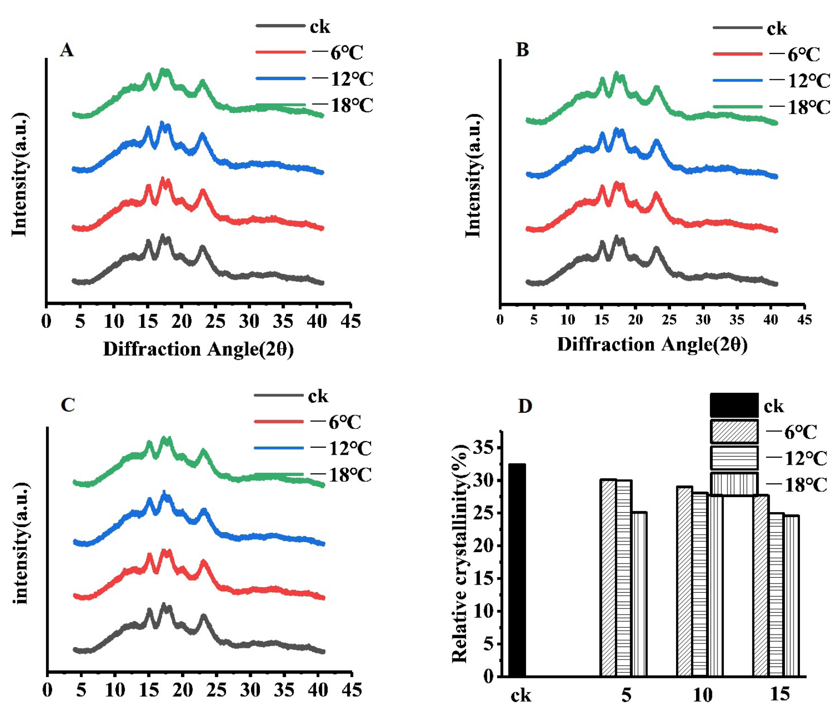


Figure 5. X-ray diffraction spectra of starch in non-fermented dough frozen at different temperatures for different periods of time.

wheat dough in the control group, at three temperatures and on three different days, all had obvious peak shapes at 15°, 17°, 18° and 23°, which were typical starch crystallization peaks and A-type crystals (Chen et al., 2015; Benavent-Gil & Rosell, 2017), indicating that freezing treatment had no significant effect on starch crystal type in the dough (Szymońska et al., 2000). This shows that freezing at low temperature does not dissolve the double helix crystal structure of starch.

The relative crystallinity of starch refers to the proportion of starch crystals, which is another important indicator to measure the characteristics of starch crystals. We saw a trend told simply as the greater the crystallinity of starch the higher the strength and hardness, and the better the stability but the lower the elasticity and fracture strength. The relative crystallinity of starch is affected by the content and length of the amylopectin chain, the orientation of the double helix in the crystallization region, and the degree of interaction between the double helixes.

As shown in Figure 5D, on Days 5, 10 and 15, the relative crystallinity of wheat flour showed an overall downward trend following the decrease in freezing storage temperature at -6 °C, -12 °C and -18 °C and was lower than that of the control group. Partial gelatinization, double helix movement in the freezing process and mechanical damage may lead to degradation of starch molecules, a decrease in the specific gravity of the crystallization zone and particle damage (Xiao et al., 2017; Hoover & Manuel, 1996; Tran et al., 2011). The results show that frozen storage can destroy the ordered structure of starch molecules as well as the crystal structure. When stored at -12 °C to -18 °C for 10 or 15 days, the relative crystallinity of starch samples did not decrease significantly, showing that with the extension of frozen storage time, the crystal structure of starch molecules was basically stable at both -12 ° and -18 °C.

4 Conclusion

By low field NMR analysis of relaxation time, ^{1}H density imaging and rapid determination of the freezable water content in dough, we concluded that the content of free water in dough decreases with the decrease of freezing temperature and the extension of freezing time, through which the hydrogen proton density in the dough decreased continuously and the frozen water content in the dough increased gradually, which effectively reduced the deterioration of dough quality.

Through analysis of the microstructure of starch, and the gelatinization properties (RVA), thermodynamic properties (DSC), and relative crystallinity of starch, our results showed that the interior and surface of starch particles suffer different degrees of mechanical damage with decreasing freezing temperature and the extension of freezing time; that the peak viscosity and retrogradation value of starch in dough showed an upward trend, indicating that the stability of hot paste became worse and that the starch was easier to retrograde; that frozen starch Δ H showed a gradually increasing trend; and that the crystalline structure of starch did not change, but the relative crystallinity showed a downward trend as a whole. The results showed that the characteristics of dough and starch would change after frozen storage, which s suggests ways to improve the quality of frozen flour products.

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