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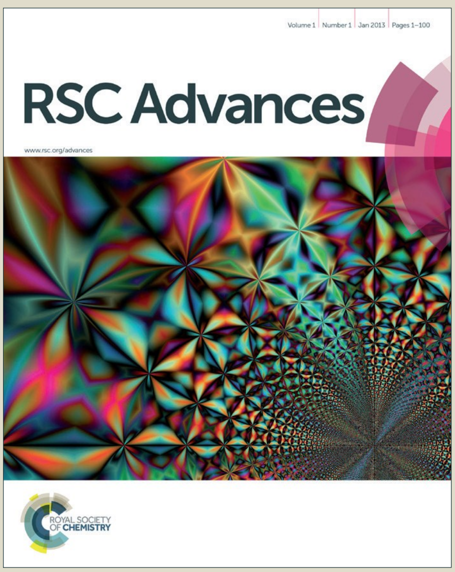

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Effect of freezing rate on rheological, thermal and structural properties of frozen wheat starch

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Abstract

Native wheat starch were frozen at different rates (0.18, 0.37 and 1.54 ^{o}C/min) and then were evaluated for dough making. Result showed that damaged starch content was higher in the starch frozen at a slow rate than that frozen at a fast rate. It seemed that a slow freezing rate allowed the formation of ice macrocrystals and affected the integrity of starch granules. The slow freezing rate resulted in a more crystalline and stable structure of double helix for starch, which increased gelatinization temperatures and enthalpy. The pasting behavior was also influenced by the slow freezing rate as indicative of a significant increase (p < 0.05) in breakdown and setback values. Farinograph test was performed in reconstituted dough by using wheat starch-gluten mixtures as model systems. Water absorption, dough development and stability were affected in samples with starch frozen at a slow rate. The dough frozen at slow rate displayed the lowest bread and the hardest crumb among samples. Rapid freezing resulted in a less structural and functional changes in starch compared with slow freezing.

Key words: Wheat starch; Freezing rate; Reconstituted dough; Bread quality.

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1 Introduction

The frozen dough market has grown steadily in recent years due to consumer demand for convenience and high quality baked products. Frozen dough was obtained from highly mechanized processes in big companies, which reduced production costs and manufacturers can supply a product of uniform quality at any time.[1] However, bread made from frozen dough present greater staling and other quality changes due to prolonged storage and freeze-thaw cycles.[2] The ice crystals formed during frozen storage reportedly caused physical damage to the food structure, resulted in loss of gas retention, poor loaf volume and strong alteration textural properties.[3]

Wheat starch was found to be important for frozen dough quality, where freezing exerted some stress on the starch granules that cause deterioration in granule integrity. Wolt and D'appolonia (1984) investigated differences in starch obtained from breads baked from frozen doughs. Highly significant positive correlations were found between amylose-amylpectin ratio, proof time, and loaf volume. Amylose-amylopectin ratios were also negatively correlated with frozen dough storage time. Lu and Grant (1999) compared the thermal properties of wheat starches isolated from original wheat flour and frozen doughs after subjecting to 16 weeks of frozen storage. The T_o, T_p, and ΔH of the frozen dough starch was found to be increased with frozen storage time. The scanning electron microscopy and atomic force microscope analysis of the starch upon freezing and thawing shows a coarse surface and broad granular channel.[6-8] Those alterations in the frozen starch caused a lower specific volume and firmer crumb in the bread, increasing the dough retrogradation.[9, 10] The cause of these alterations was associated to formation of ice crystal in starch matrix, involving in the amount and size of ice crystal which was dependent on freezing rate.[11] Slow freezing usually lead to the process of ice crystal growth. On the other hand, rapid freezing resulted in less ice formation (since more water is in glassy state) and showed low glass transition temperature (T_g).[12] While several researchers have studied the effects of storage time or temperature on yeast viability and dough structure,[1, 3, 5] less has been done on the influence of freezing rate. Especially in frozen dough preparations, where freezing intervened between dough formation and bread baking, the role of starch still have not been fully investigated. Starch is the main composition in bread, which determined the rheological properties of dough and baking quality.[13] It means that this is an area that needs to be researched more since freezing rate may play an important role in the forms of ice crystals and the integrity of starch in frozen dough.

In the present study, we examined the changes in the rheological, thermal and structural properties

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of starch frozen at different freezing rates. A reconstitution dough system, with fractions of the same flour such as starch and gluten, has been used to understand the starch transformations from dough to bread.

2 Materials and Methods

2.1 Materials

Wheat starch was purchased from Puluoxing Starch Co., Ltd. (Hangzhou, China). Commercial wheat gluten [protein content (N × 5.7) 62.1 %] was obtained from Weijing Co., Ltd. (Shanghai, China). Active dry yeast (Angel brand, Hubei,China), sugar, and salt were purchased from a local supermarket. All other chemicals and reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Suzhou, China) and were of analytical grade unless otherwise stated.

2.2 Preparation of Freezing-Treated Starch

The wheat starch was dispersed in deionized water by stirring at 20 ^{o}C for 3 hours to produce starch suspensions with a final concentration of 40% (w/w). Then the samples were immersed into a freezer, until the core temperature reached -20 ^{o}C, and then stored at -30 ^{o}C, -40 ^{o}C and -70 ^{o}C, respectively. The freezing rate (Fr) expressed in ^{o}C/min was calculated by Eq. (1), based on the definition the International Institute of Refrigeration (1986) and the reported by Meziani, et al. (2011):

$$\mathrm{Fr} = \frac{(T2 - T1)}{(t2 - t1)}$$

where T1 is the initial freezing temperature, T2 is the final freezing point (-20^{o}C), and (t2 - t1) is the time elapsed between the beginning and the end of freezing.

In present work, the freezing rates are estimated at: 0.18 ^{o}C/min, 0.37 ^{o}C/min and 1.54 ^{o}C/ min, respectively, for -30 ^{o}C, -40 ^{o}C and -70 ^{o}C. After the storage of 8 weeks, samples were thawed in a water bath at 25 ^{o}C for 2 hours. The supernatants was collected by centrifugation at 2200 × g for 20 min before starch residues were dried at 40 °C for 2 days. Control samples were stirred at 20 ^{o}C for 3h and then directly centrifuged at the same conditions.

2.3 Chemical Composition Contents

The apparent amylose content was determined by the iodine binding colorimetric method.[14] Damaged starch (%) values were obtained according to AACC 76-30A method [15] following analysis through fungal enzyme *Aspergillusoryzae* (10065, Sigma Chemical Co., St. Louis, MO, USA).

2.4 Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra were recorded using a Thermo Nicolet iS10 FT-IR Spectrometer (Thermo Electron

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Corp., Madison, WI, USA). Spectra at 1045 and 1022 cm^{-1} was recorded in triplicate for each sample to analyze the crystalline structures of wheat starch.[16]

2.5 Differential Scanning Calorimetry (DSC)

A SIINT instrument (X- DSC 7000 model, Japan) was used to study the thermal transitions of wheat starch modified by different freezing rates. For comparison, a spectrum of native cassava starch is also included. thermal transitions of samples. The prepared starch samples (3 mg) were accurately weighed in triplicate in aluminum pans. Deionized water was added in a ratio of 1:2 (w/w, sample dry basis: water). For comparison, native wheat starch was included. Pans were kept 1 h at 25 ^{o}C and then were heated from 30 ^{o}C to 90 °C at 10°C/min (together with an empty reference pan). From thermograms gelatinization enthalpy (ΔH) and onset (T_o), peak (T_p) and conclusion temperatures (T_c) were obtained.

2.6 Pasting Properties

The pasting profiles were analysed using a rapid visco-analyzer (Model RVA-4C, Newport Scientific Pty. Ltd., Warrriewood, Australia). The starch concentration used in the present study was 8 % (Dry weight, 28 g total weight). The suspension was stirred manually using the plastic paddle before the RVA run. Test profile was programmed according to the general pasting method (Standard 2). The slurries were first held at 50 ^{o}C for 1 min, heated at a rate of 6.0 ^{o}C/min to 95 ^{o}C, maintained at that teperature for 5 min, cooled to 50 ^{o}C at a rate of 6.0 ^{o}C/min and held at 50 ^{o}C for 2 min. Constant paddle rotating speed (160 rpm) was used throughout the entire analysis except for a speed of 960 rpm for the first 10 s to disperse the samples. Breakdown value representing the differences between peak viscosity and trough viscosity, and setback value representing the differences between trough viscosity and final viscosity were calculated from the RVA curves. The viscosity was expressed in cP units.

2.7 Rheological Properties of Dough

Water absorption, development and stability times of dough prepared from reconstituted flours with were determined in a farinograph with 300 g stainless bowl (Brabender, OHG, Duisberg, Germany) according to the approved method 54–21.[15] The reconstituted flours consisted of wheat starch and gluten in a ratio of 86/14 calculated on dry basis content. For comparison, wheat starch frozen at different rates were included in this study.

2.8 Baking Properties

The reconstituted dough were transformed into bread according to a modified description by Tao,

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et al. (2015). The reconstituted flour was made from 300 g of starch-gluten blends (in a ratio of 86/14), 4.5 g of fresh yeast, 10.5 g of sugar and 4.5 g of salt and then hydrated to 60% (dry matter base) using deionized water. The dough was mixed for 6.5 min and then molded into 60 g. The doughs were proofed at 37 °C with 80 % relative humidity for 90 min before baking (15min, 210 °C).

Specific volume of breads was determined by the rapeseed displacement. Firmness of bread containing native and treated starch was analyzed by TA.XT2i (Perten Instruments, Hägersten, Sweden) using a 25 mm cylindrical acrylic probe. The 10-mm-thick bread slice was compressed at a pre-test speed 3 mm/s, test speed 1 mm/s, and post-test speed 5 mm/s. The firmness of bread crumb was recorded as the force at 50 % strain and performed at least in triplicate.

2.9 Statistical Analysis

All data were expressed as mean ± standard deviation (SD) of three replicates. Data were analyzed using one-way analysis of variance and means were compared by Duncan's multiple range tests using an SPSS package (version 13.0 for Windows, SPSS Inc., Chicago, IL). The probability value of $p < 0.05$ was considered significant.

3 Results and Discussion

3.1 Composition Analyses

Table 1 showed the effect of freezing rate on chemical components of starch granules. Damaged starch content (DS) was higher for the starch granules frozen at a slow rate (0.37 ^{o}C/min) than that frozen at a fast rate (1.54 ^{o}C/min). The DS content increased further when starch was frozen at 0.18 ^{o}C/min. However, amylose content decreased from 28.9 % for native starch to 26.2 % for starch frozen at 0.18 ^{o}C/min. According to Morrison*, et al.* (1994), increasing damaged starch could decrease double-helix content and crystallinity decreased with an increase in amylose content. An explanation for the differences in the present study might be caused by mechanical force varieties. Freezing water exerted pressure on the granules due to phase transformation, the internal or external ice crystals of granules occupied more space than an equal amount of water.[6] In this manner, a broad granular channel could be observed with leaching materials when thawing. It has been reported that the location of amylose in starch granules was proposed to be mainly in the central amorphous core, with some amylose molecules also dispersed among the amylopectin clusters arranged around the core.[18] The leaching amylose from starch granules indicated a slight increase in the relative crystallinity, as quantified by FTIR spectroscopy (Table 1). The intensity ratio of 1045/1022 cm^{-1}, as an indication of

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the starch order,[16] was significantly increased from 0.623 for native starch to 0.791, 0.682 and 0.645 for starch frozen at 0.18 °C/min, 0.37 °C/min and 1.54 °C/min, respectively. This finding was in agreement with the research of Meziani*, et al.* (2011), who reported that slow freezing treatment resulted in an increase of crystallinity degree of dough. Freezing treatment induced the reorganization of starch molecules by decreasing the amorphous material with an increase in crystallinity. The loss of amylose from the inside granules possibly lead to the adjustment of the internal structures.[19]

3.2 Thermal Transition

Table 2 showed gelatinization properties of native and frozen starches. There were no significant differences in onset, peak and conclusion temperatures between native starch and starch frozen with a fast rate of 1.54 °C/min ($p > 0.05$). However, the counterpart temperatures of starch frozen with a slow rate (0.18 and 0.37 °C/min) were higher than those of native starch. Similar changes were observed on the enthalpy values that the starch frozen at a slow rate had slightly higher enthalpy values than that at a fast rate. Incorporation of these changes, a slow freezing rate showed a more crystalline and stable structure of double helix for starch, which made the granules more resistant to be gelatinized.[8, 10] More energy was required to disrupt the organized helical complex structure formed by interaction between amylose-amylose and amylose-amylopectin chains.[20, 21]

3.3 Pasting properties

The pasting profiles and important parameters including breakdown and setback values of starches were shown in Fig.1. Freezing treatment did not change the overall shape of pasting profiles, however, caused a marked increase in peak viscosity. Freezing at -40 °C (0.37 °C/min) increased peak viscosity of wheat starch more greatly than the treatment at -30 °C (0.18 °C/min), whereas frozen starch at a fast rate (1.54 °C/min) showed a slightly higher than the native starch. This fact is because a slow freezing rate allowed the formation of ice macrocrystals, which affected the integrity of starch granules.[9] During heating, some soluble materials leached from the coarse surface and allowed more free swelling of amylopectin molecules.

The freezing rate was also a factor that affected the breakdown and setback values (Fig. 1b). The breakdown values were significantly different between the starches processed with freezing rates of 0.18 and 1.54 °C/min ($p < 0.05$). It seemed that freezing treatment facilitated a disruption of the granular structure. Such a structure was sensitive to the shearing forces freezing step and then leached amylose. This pattern also affected the setback values which was indicative of the stability of the paste

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and the tendency to reorganize during the cooling step.[22] Setback values of starch frozen at a slow rate (0.18 ^{o}C/min) were slightly higher than that at a fast rate (1.54 ^{o}C/min). Increases in the setback values could be ascribed to the reorganization of starch chains and a formation of amylose junction zones [21]. Thus, the slow freezing accelerated the retrogradation of starch paste, which agreed well with the previous studies.[9, 23, 24]

3.4 The Reconstituted Breads Qualities

A farinograph study showed that the additions of freezing treated starch to reconstituted wheat flour increased the water absorption, whereas the addition of fast freezing treatment (1.54 ^{o}C/min) has little effect (Fig.2). Higher water absorption values for -30 ^{o}C (0.18 ^{o}C/min) than -40 ^{o}C (0.37 ^{o}C/min) flour mixtures might be expected since -30 ^{o}C had a higher amount of damage starch than -40 ^{o}C did (Table 1). Dough development time was only slightly affected by fast freezing rate but was significantly increased by the slow freezing treatment. Freezing decreased the dough stability, producing the weakest dough, but fast freezing rate made little changes. This observation could be ascribed to the leaching of amylose from the starch granules due to slow freezing treatment. The importance of amylose was illustrated by Dexter*, et al.* (1979). Starches of different botanical origin were mixed with durum semolina gluten. With decreasing amylose content in the reconstituted samples, cooking quality deteriorated. Amylose was supposed to bind to a protein fraction and, in this way, contribute to the formation of a protein network.[26]

As might be expected from the farinograph characteristics (Fig.2), the freezing treated starches decreased the baking performance of reconstituted wheat flour (Fig.3). The specific volume (SV) of control reconstituted bread was 3.01 g/cm^3. There was significant difference between the slow freezing (0.18 ^{o}C/min) and rapid freezing (1.54^{o}C/min) (p < 0.05). These values confirmed the influence of freezing rate on the baking quality of starches: the SV of bread containing fast freezing-treated starches (1.54 ^{o}C/min) did not differ from the initial value (native starch); the SV of the samples made from slow freezing rate-treated starches (0.18 and 0.37^{o}C/min) was significantly lower than their initial values. The volume loss was probably due to leaching of soluble materials, such as amylose. Amylose was a good indicator of bread loaf which banded to a protein fraction and, in this way, contributed to the formation of a protein network that avoided amylose-leaching during cooking.[27, 28] Another possible explanation for this was that the slow freezing resulted in more damage on starch granules while rapid freezing produced less. Therefore, it was recommended for breadmaking from frozen dough that the

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flour used could not have more than 7% damaged starch,[23] since excessively high levels of damaged starch increase the water absorption capacity of flours, creating problems during dough handling and fermentation.[29]

Concerning crumb hardness, slow freezing-treated starch (0.18°C/min) breads crumb was almost twice harder than control bread crumb (Fig. 3). Similar observations were reported by our previous studies.[8] Damaged starch due to ice recrystallization, released amylose to form a network of amylose, leading to an increase in the crumb hardness.[10, 30] Meanwhile, the enhanced firmness of breads could be also interpreted as the shrinkness of the bread loaf. Firmness was inversely correlated with specific volume, thus a lower bread specific volume results in greater firmness due to denser crumb and more compact.[31] These findings caused by various freezing rates pointed to an important role of starch granules in determining the bread texture.

4 Conclusions

Reconstituted dough, with fractions of native and modified wheat starch, has been used to evaluate the influence of freezing rate on the starch transformations from dough to bread. The overall results confirmed that the slow freezing rate had a significant effect on the integrity of starch granules with leaching some amylose molecules. The various structure characteristics of starch granule were further corroborated by significant variations in thermal properties and pasting behavior. In starch-gluten reconstituted dough, starch frozen at a slow rate was able to absorb more water, which affected the dough development and stability. Regarding the specific volume of bread, the reconstituted dough with starch frozen at slower rate, was significantly lower than that of the native starch, which was assigned to the limitation of water distribution. This change resulted in greater firmness due to denser crumb and more compact. The results of this study to understand the mechanisms of freezing on the wheat dough starch properties, it will be interesting to provide a noble insight into the role of starch granules for the baking quality of frozen dough.

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Tables

Table 1 Amylose, damaged starch (DS) and Short-Range Order (FTIR) of Native and Freezing-Treated Starches

Samples	Amylose (%)	DS (%)	1045/1022 ratio
Not Frozen	28.9±0.3a	20.15 ± 0.15	0.623 cd
-30 ºC	26.2±0.1b	22.10 ± 0.10b	0.791 a
-40 ºC	27.0±0.2c	21.30 ± 0.10a	0.682 b
-70 ºC	27.5±0.4c	19.85 ± 0.15a	0.645 c

Values are expressed as mean ± SD of triplicate samples. Means with the same letters in a column do not differ significantly ($p > 0.05$).

Table 2 DSC Parameters of Native and Freezing-Treated Starches

Samples	T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH (J/g)
Not Frozen	56.5 ± 0.3 b	61.6 ± 0.1b	66.3 ± 0.2 a	9.5 ± 0.5 c
-30 ºC	58.1 ± 0.1 a	62.6 ± 0.2 a	65.6 ± 0.2 c	11.8 ± 0.2 a
-40 ºC	57.8 ± 0.2 a	62.3 ± 0.1 a	66.0 ± 0.1 ab	10.4 ± 0.2 b
-70 ºC	56.3 ± 0.5 b	61.3 ± 0.3 b	65.9 ± 0.2 bc	9.3 ± 0.4 c

Values are means ± SD. Means with the same letters in a column do not differ significantly ($p > 0.05$). T_o, T_p and T_c are the temperatures of the onset, peak and conclusion of gelatinization, respectively. ΔH is the gelatinization enthalpy.

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Figure captions

Fig.1 Pasting curves (a) and variations of setback and breakdown values (b) of native starch and starch frozen at 3 different rates. Error bars indicate the standard error for three independent experiments. The letters a and b indicate significant differences ($p < 0.05$).

Fig.2 Farinograph characteristics of mixtures of wheat gluten and wheat starches frozen at 3 different rates. Error bars indicate the standard error for three independent experiments. The letters a and b indicate significant differences ($p < 0.05$).

Fig.3 Changes in the specific volume and firmness values of reconstituted bread with native and frozen starches after baking. Error bars indicate the standard error for three independent experiments. The letters a and b indicate significant differences ($p < 0.05$).

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Fig.1

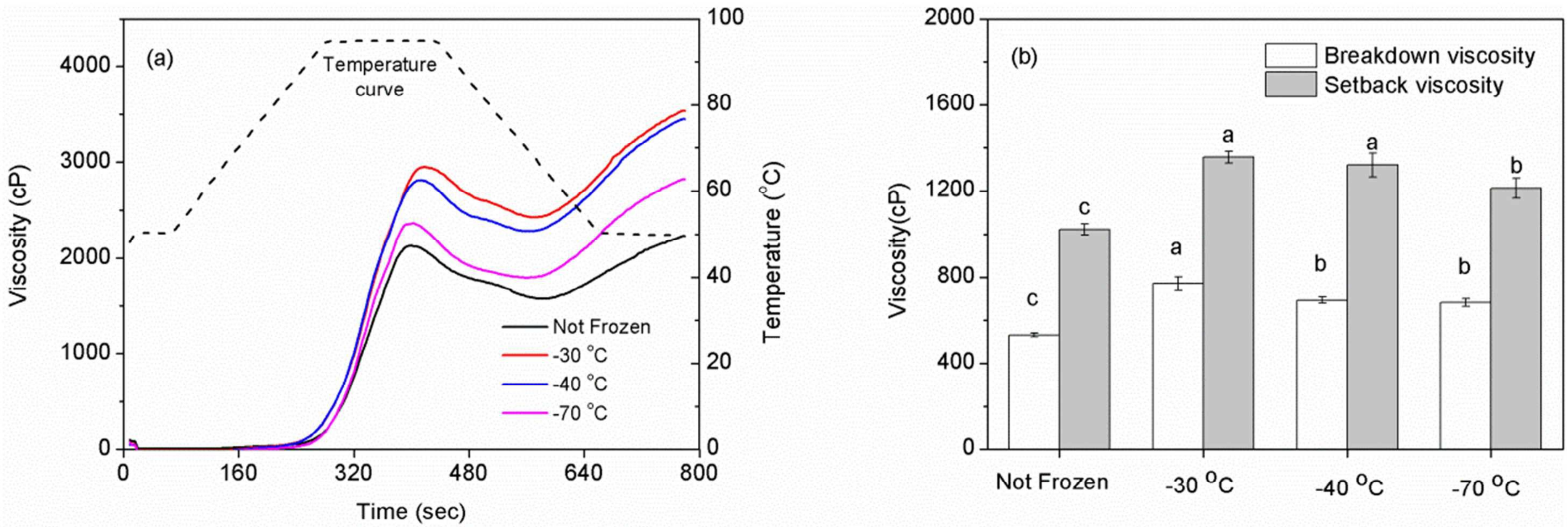

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Fig.2

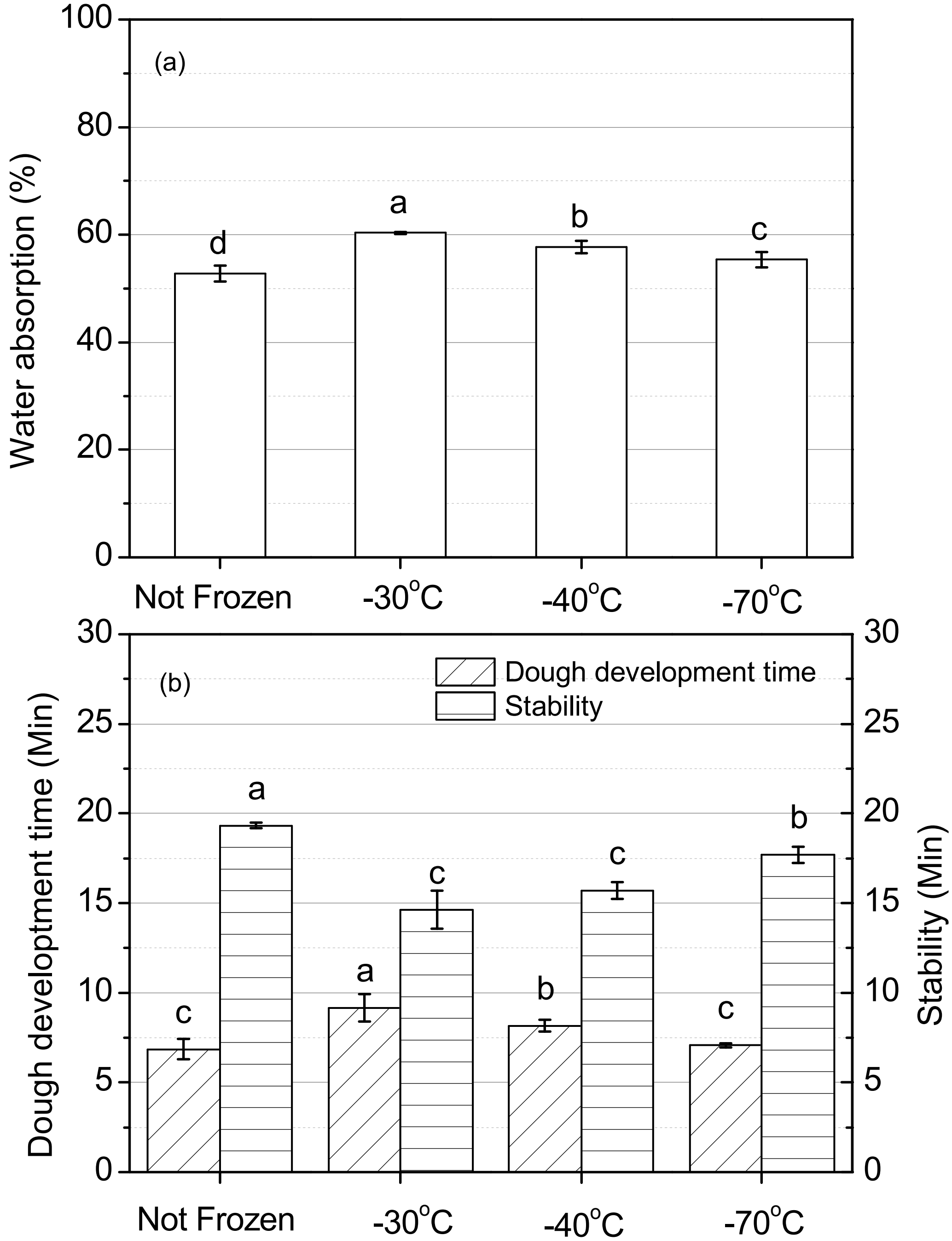

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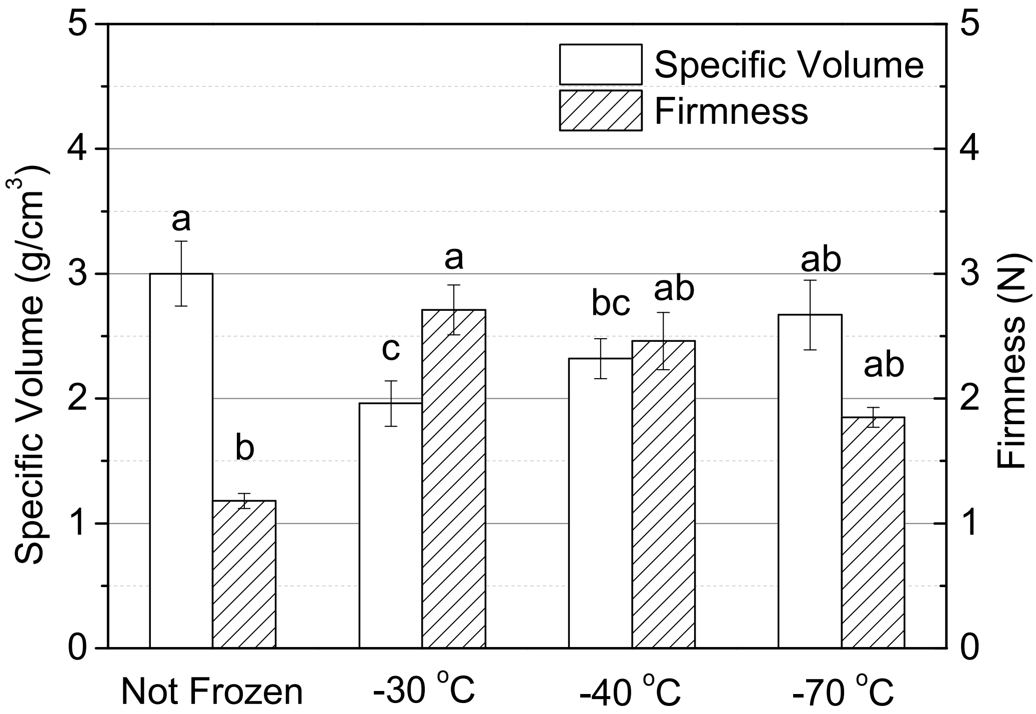
Fig.3



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