

Effect of bacterial cellulose on freeze-thaw stability of rice starch gels

Hongshuai Zhu¹, Xinhua Xie^{1,*}, Chao Xu¹, Xiaohong Chang¹, Xiuyuan Wu¹, Yue Shen¹
¹College of Food Science and Technology, Henan Agricultural University, 63 Nongye Rd.
Zhengzhou, 450002, China
Email: xiexinhua9922@yeah.net

Abstract: The effect of bacterial cellulose (BC) on the freeze-thaw stability of rice starch gels during 5 freeze-thaw cycles were investigated, using scanning electron microscopy (SEM), rapid visco analyzer (RVA), X-ray diffractometry (XRD), differential scanning calorimetry (DSC) and textural analyzer (TA). SEM micrographs of freeze-thaw gels showed that smaller pore size and a thicker surrounding matrix corresponded with increasing bacterial cellulose concentration. RVA results showed that supplementation with bacterial cellulose could significantly decrease the breakdown and setback values, which meant that the short-term retrogradation of rice starch was inhibited. The freeze-thawed starch gels showed a typical B-type crystal structure in the XRD, significantly different from native starch (A-type crystal structure). The recrystallinity of rice starch was reduced from 7.22% to 5.61% with increasing bacterial cellulose concentration after five freeze-thaw cycles. DSC results showed that bacterial cellulose could significantly reduce the retrogradation enthalpy of rice starch, which was consistent with the XRD results. The textural properties of the rice starch gels during freeze-thaw cycles were improved with increasing bacterial cellulose concentration. This study showed that bacterial cellulose was an effective agent in preserving the quality of freeze-thawed rice starch based products.

Keywords: Rice starch, Bacterial cellulose, Freeze-thaw stability, Microstructure, Thermal properties.

1.Introduction

Innovative and new frozen ready-to eat food products are continually being launched into world markets as a result of lifestyle changes by consumers. With proper frozen storage, these products can be kept for up to one year. However, freezing of foods causes water transforming into ice, which could result in physical stress to the food matrix. When frozen food is thawed for consumption, the moisture is readily separated from the matrix and this causes texture softening, drip loss, and then leads to deterioration of overall product quality [1]. In the case of starch-based foods, freezing and thawing changes their textural properties drastically due to amylose and amylopectin crystallization and retrogradation [2], and subsequently make such foods unacceptable to consumers. Therefore, freeze-thaw stability is an important property, which can be used to evaluate the ability of starch to withstand the undesirable physical changes occurring during freezing and thawing.

Controlling the freeze-thaw stability of starch pastes and gels by the addition of hydrocolloids has been widely investigated [3]. Addition of hydrocolloids can alter both structural and rheological characteristics of starch such as gelatinization temperature and pasting properties [4] [5]. Furthermore, hydrocolloids can reduce starch retrogradation and improve the stability of frozen starch gel systems [6]. Ferrero et al [7] reported that adding xanthan gum to corn starch pastes minimizes amylose retrogradation, syneresis, and rheological changes after freezing. In addition, guar gum and locust bean gum were found to reduce syneresis in freeze-thawed corn starch and waxy *Amaranthus paniculatus* starch [8]. In both of these studies, the authors based their conclusions on the reduction to a slowing of retrogradation brought about by an interaction between the hydrocolloid and the starch [9]. But these hydrocolloids are increasingly important ingredients in the modern health-conscious food industry. Dietary fiber (DF) is among the most commonly used as natural food hydrocolloids in real food systems [10], and it has several physico-chemical functions, such as water binding and alteration of viscosity, which in turn can offer a range of health benefits and assist to reduce the risk of chronic diseases such as diabetes, obesity, cardiovascular disease, and diverticulitis [11]. Santos [12] reported that with more fiber content, the retrogradation of wheat flour was slower, crystallinity was smaller, and the recrystallization of starch could be delayed.

Bacterial cellulose (BC) is a unique type of DF. Compared with other DF, BC has several advantages. Firstly, BC produced by microorganism, is a highly pure form of cellulose and does not require harsh chemical treatments to isolate and purify, which is different from cellulose derived from plant sources. Secondly, BC fibers are within the Nano-scale with a fine 3D network structure [13]. Finally, BC fibers have a high aspect ratio with a diameter of 20-100 nm. As a result, BC has a very high surface area per unit mass. The quality,

combined with its highly hydrophilic nature, results in a very high water absorption and water holding capacity [14], which enables BC to be used as novel DF in food manufacturing processes. Although many efforts have been made to investigate the applications of DF on starch [4] [12] [15], only a few studies on using BC to improve the freeze-thaw stability of rice starch have been conducted.

The objective of this study was to determine the effect of BC on freeze-thaw stability of rice starch. Pastes and gels were characterized by scanning electron microscopy (SEM), rapid visco-analyzer (RVA), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and textural analyzer (TA). This research could make a contribution toward improving in the quality of frozen rice starch-based products.

2. Materials and methods

2.1 Materials

Rice starch was supplied by Hao Yi Kuai Co., Ltd. (An Hui, China). The contents of moisture, ash, protein, and amylose in the rice starch were 12.29%, 0.1%, 0.41%, and 22.04% w/w. Bacterial cellulose was purchased from Hainan Guangyu Biotechnology Co., Ltd (Hainan, China) with 10.16% moisture content.

2.2 Starch gel preparation

Rice starch slurry containing bacterial cellulose (0%, 5%, 10%, 15%, 20%, w/w, 6.0% total solids) was gelatinized by heating in a boiling water bath at 95 °C with continuous stirring for 30 min. The starch paste was sonicated for 20 min to remove air bubbles and then cooled in water bath at 30 °C for 120 min. After cooling, separate sample containers were filled with the rice starch gel samples for experiment.

2.3 Freezing and thawing

Rice starch gel samples were frozen at -18 °C for 22 h and then thawed at room temperature (25 °C) for 2 h. This freeze-thaw cycle was repeated for up to 5 cycles. The freezing experiments were carried out in two separate trials.

2.4 Scanning electron microscope

The freeze-thaw samples were freeze dehydration. Subsequently, the samples were stuck on a specimen holder and coated with gold palladium using a sputter coater. The microstructure of samples was observed at 250× resolution, respectively, with a scanning electron microscope (Quanta-250, FEI, Ltd., American) operating at an accelerating voltage of 3.00 kV.

2.5 Pasting profile

The pasting properties of the rice starch suspension (12%, w/w) with 0-20% bacterial cellulose (based on RS weight) were determined using a RVA-4500 rapid visco-analyzer (Perten, Australia), respectively. The slurry was held at 50 °C for 1 min, heated to 95 °C at a constant rate of 12 °C/min and then held at 95 °C for 2.5 min. It was subsequently cooled to 50 °C at the same rate and then maintained at 50 °C for 2 min. Paddle speed was 960 rpm for the beginning 10 s to disperse the sample, and then set at 160 rpm during the measurement. The viscosity was expressed in Cp units and the data were reported as the average of triplicate measurements.

2.6 X-ray diffraction analysis

The freeze-thaw samples were prepared as described in Section 2.2. After five freeze-thaw cycles, the freeze-dried samples were milled to pass through a 150-mesh sieve. The recrystallization analysis was carried out using a X'Pert Pro X-ray diffractometer (PANalytical, Nederland) equipped with a copper tube operating at 40 kV and 30 mA Cu-K α radiations. Diffractograms were obtained by scanning from 4° (2 θ) to 40° (2 θ) at a rate of 2°/min. MDI Jade 5.0 was used to analyze the diffractograms.

2.7 Thermal properties

The thermal properties were determined using a differential scanning calorimeter (Netzsch Co. Ltd., Germany). The weight ratio of the solid content of RS/BC mixtures to water was maintained at 1:3. The addition of BC was 0%, 5%, 10%, 15%, 20% (w/w, based on rice starch). The bacterial cellulose solutions were fully dispersed by being magnetically stirred for at least 2 h at room temperature (25 °C). Then RS was added while stirring in another 1 h. About 8 mg dispersions were weighted and transferred into an aluminum DSC pan. The calorimeter was calibrated with an indium standard. RS/BC were measured after being equilibrated for 24 h at 4 °C. The gelatinization procedure was measured from 25 °C to 100°C at the rate of 10 °C/min, followed by cooling to 25 °C at the same rate. Heating and cooling were performed in an atmosphere of nitrogen gas. The onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) were determined from the curves. The gelatinized enthalpy (ΔH) was calculated based on the area of the endothermic peak. Subsequently, the

gelatinized samples were stored at $-18\text{ }^{\circ}\text{C}$ for 22 h and thawed at room temperature ($25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) for 2 h. The samples were heated in the DSC at a rate of $10\text{ }^{\circ}\text{C}/\text{min}$ from $25\text{ }^{\circ}\text{C}$ to $100\text{ }^{\circ}\text{C}$. The enthalpy of melting the amylose-lipid complex were calculated. Analyses were performed in triplicate.

2.8 Texture measurement

The freeze-thawed samples were prepared as described in Section 2.2. At the end of each freeze-thaw cycle, the gel texture was determined at room temperature ($25 \pm 2\text{ }^{\circ}\text{C}$) using the texture profile analysis method (five replicates per treatment) with a Texture Analyzer (TA-XT plus, Stable Micro System, Surrey, UK). These specimens were subjected to deformation levels of 40 % of the original sample height for single compression and the gels were compressed twice. Hemispherical probe (P/0.5 HS) was punched into the gel with a constant crosshead velocity of 1 mm/s. Hardness was expressed as the maximum force exerted during the first compression cycle.

2.9 Statistical analysis

Results were reported as mean \pm standard deviation (SD) of triplicate analyses for each sample unless otherwise stated. A one-way analysis of variance and Tukey's test were used to establish the significance of differences among the mean values at the 0.05 significance level. The statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, USA) for windows program.

3. Results and Discussion

3.1 Scanning electron microscopy

To elucidate the relationship between freeze-thaw stability and the addition of BC to rice starch gels, the microstructure of freeze-thaw gels was examined using SEM. Images of treated specimens were shown in Fig. 1. For rice starch gels with different concentrations of BC, clear differences were observed in the microstructure of rice starch gels after one and five freeze-thaw cycles. All freeze-thaw starch gels developed a spongy structure which could be attributed to ice crystal formation and amylose retrogradation [9], a thick fibrillar network of rice starch gels were formed in the spongy structure during the repeated freeze-thaw cycles, similar findings were reported by Thunyaboon [16]. In rice starch gels without BC, the microstructure after one freeze-thaw cycle was characterized by large pores in the gel (Fig. 1 a₁). After five freeze-thaw cycles, the rice starch gels had bigger pores, the matrix surrounding the pores was stronger and the pores were very clearly defined (Fig. 1 a₂). These structural findings correlated well with the changes in the hardness values after 1-5 freeze-thaw cycles of rice starch gel with no BC added. After one freeze-thaw cycle, the rice starch gels containing 5% BC appeared to have smaller and less well-defined pores embedded in a weak matrix (Fig. 1 b₁). After five freeze-thaw cycles, the pore size in these gels were increased but the pores were still less well-defined than in starch gels without BC. Rice starch gels with 10% (Fig. 1 c₁,c₂), and 15% (Fig. 1 d₁,d₂) showed the similar results to those with 5% BC (Fig. 1 b₁,b₂). In 20% BC systems after one freeze-thaw cycle, the matrix surrounded the pores was thickest and the pores were smallest (Fig. 1 e₁). However, after five freeze-thaw cycles, the matrix surrounding pores of the rice starch gels became thinner and more compact and the pores became larger (Fig. 1 e₂). The specimen images showed that BC effectively stabilized the microstructure of rice starch gels because BC could maintain the matrix surrounding pores of rice starch gels. It could be speculated that the amylose retrogradation of rice starch gels may be retarded with the addition of BC by slowing amylose-amylose re-association [16].

3.2 Pasting properties

The effect of BC on the pasting profiles of RS was shown in Fig. 2. Statistical analysis of pasting parameters was also performed and summarized in Tab. 1. Compared with the control of RS alone, addition of BC at the concentration tested resulted in a significantly ($P < 0.05$) decrease in the peak and final values, whereas the pasting temperatures were significantly increased ($P < 0.05$). This might be ascribed to the inhibition effect of BC on the swelling and gelatinization of starch granules [17]. And BC induced a reduction of breakdown and setback values. This might be ascribed to the ultra-fine Nano-structure of BC, a large number of hydrophilic groups contained in its molecule, and many internal channels for retentioning water, so BC had strong water absorption and water holding capacity [18]. And it led to the water absorption of starch granules decreasing significantly, weakened the hydrogen bonding between starch molecules and water molecules [19]. The reduction of breakdown values elucidated that the starch granules would be more compact with the addition of BC and this could result in the decrease of leached amylose during heating [20]. Consequently, the setback values decreased with the addition of BC. Ordinarily, high setback values were associated with hardness of starch gels during freeze-thaw cycles and was used to indicate the extent of short-term retrogradation [21]. These results suggested that short-term retrogradation of rice starch could be depressed by BC. Accordingly, as a unique DF, BC might possess the potential capacity to anti-retrogradation of starch.

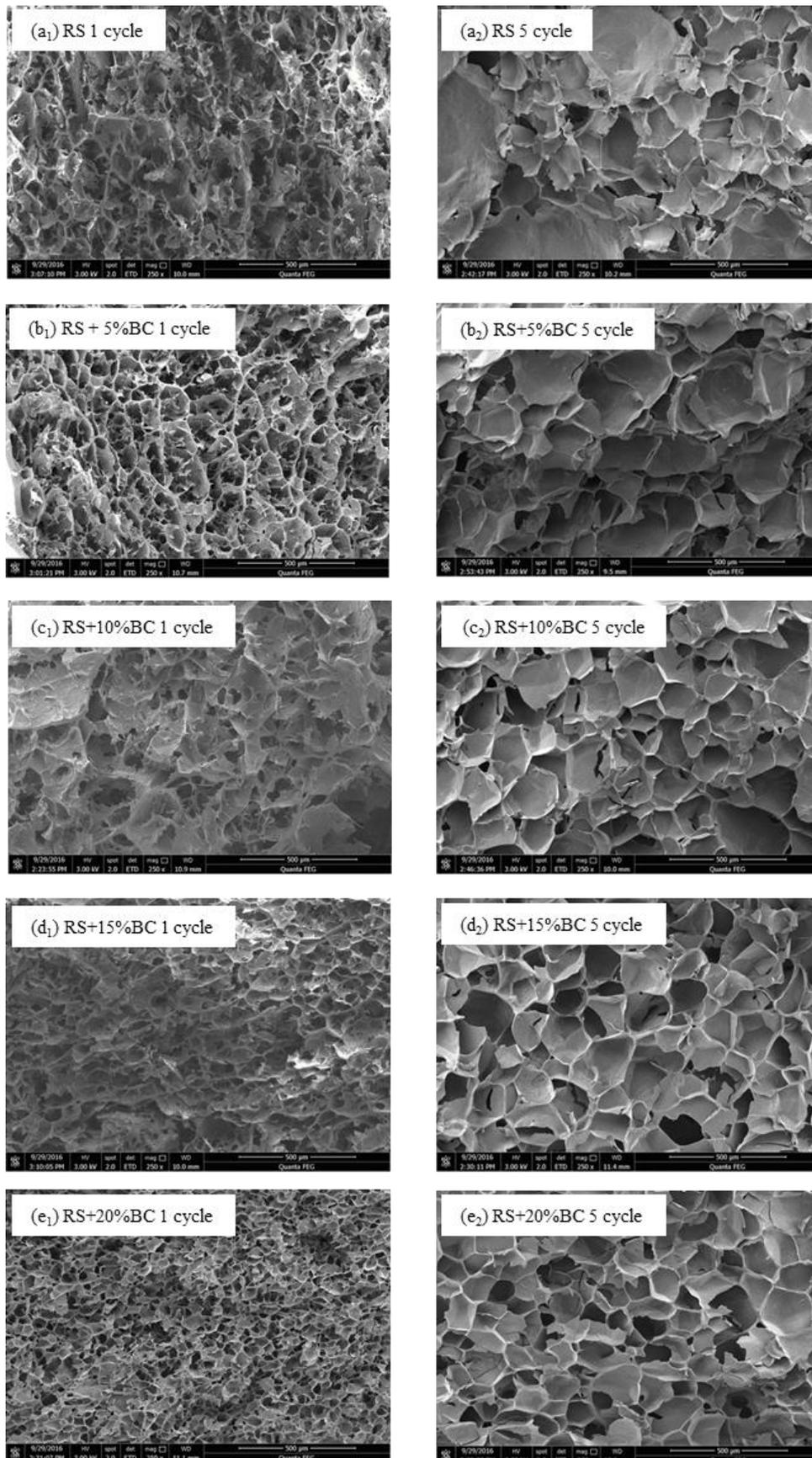


Fig. 1. SEM images of rice starch gels (6% w/w) containing BC (0%, 5%, 10%, 15%, and 20%) after one and five freeze-thaw cycle (250 \times , Bar = 500 μ m)

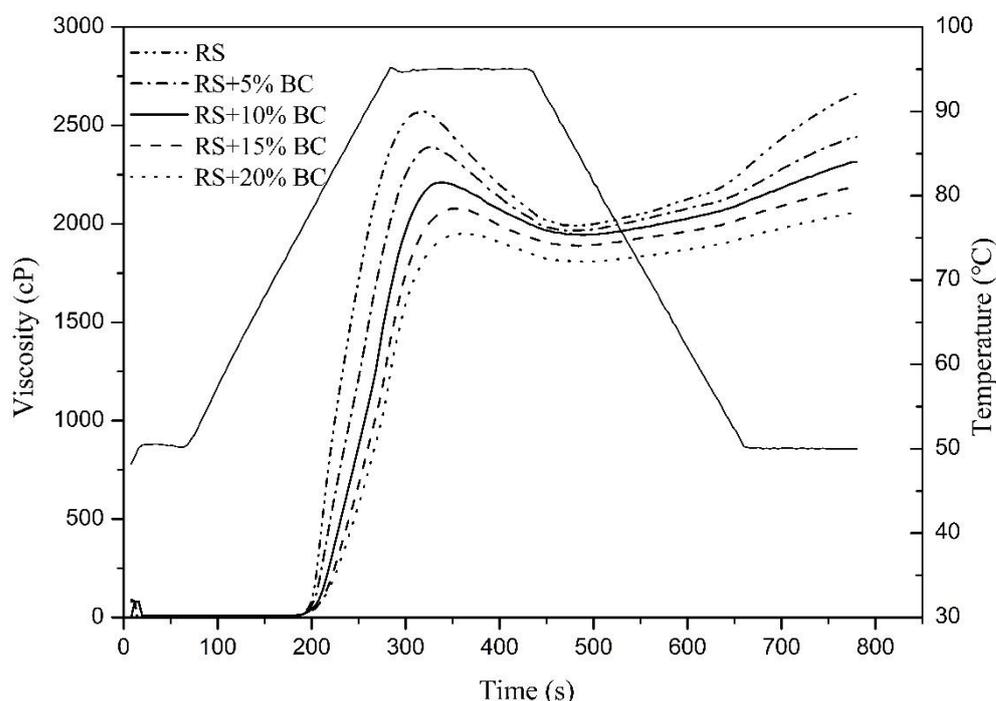


Fig. 2. Typical RVA pasting profiles of RS with different concentrations of BC

3.3 X-ray diffraction pattern

X-ray diffraction patterns of native starch and the freeze-dried starch after five freeze-thaw cycles were shown in Fig. 3. Native rice starch showed a typical A-type XRD pattern with strong peaks at 2θ close to 15.21° , 17.36° , 18.17° , and 23.14° , which was in good agreement with previous studies [22, 23]. The freeze-thawed starch showed a B-type diffraction pattern, and a well-defined peak at 2θ close to 17.02° was observed in all samples. The transformations of crystallinity from A-type to B-type after five freeze-thaw cycles were the result of the crystallization of amorphous starch melting, mainly because the amylopectin fraction increased during five freeze-thaw cycles [24, 25]. All the diffractograms in Fig. 2 showed an obvious peak at approximately 20.09° . And the peak was attributed to a well-formed V-type XRD pattern, which could be indicative of the amylose-lipid complex formation [19]. Hyo-Young and Seung-Taik [26] found that amylo maize starch treated by repeated freeze-thaw cycles showed combined B- and V-type crystalline patterns.

Apparently, it was noted that the relative crystallinity decreased from 7.22% to 5.61% with the concentration of BC increased from 0% to 20%, indicating that there was indeed a retarding effect of BC on the recrystallization of rice starch, which was consistent with the conclusion attained from the RVA experiment above.

3.4 Thermal properties

This experiment was carried out with an attempt to use differential scanning calorimetry (DSC) to assess the effects of BC on pasting and freeze-thaw stability of rice starch pastes. For the DSC measurements, total polysaccharides concentration of 25% w/w was used, which was higher than those in the other experiments (6% w/w) because the retrogradation process could not be observed with such a low concentration with the DSC used. The transition temperatures and enthalpies of rice starch with different concentrations of BC and corresponding freeze-thaw (five cycles) pastes are summarized in Table 2. During pasting, the onset (T_o), peak (T_p), and conclusion (T_c) temperatures of rice starch with BC addition significantly ($P < 0.05$) shifted to higher temperatures (from 68.17°C to 71.57°C , 71.70°C to 75.03°C , and 75.57°C to 79.16°C , respectively), which may originate from the interactions between RS and BC. However, the enthalpy of the pastes significantly decreased with increasing BC concentration. It might be due to the fact that the hydrophilic characteristic of BC could result in the lower heat transfer rates and the decreased mobility of water in the system [27]. As a result, the coupling forces between the crystallites and the amorphous matrix were changed, thus starch was more prone to being hydrated and lower thermal energy was needed [28].

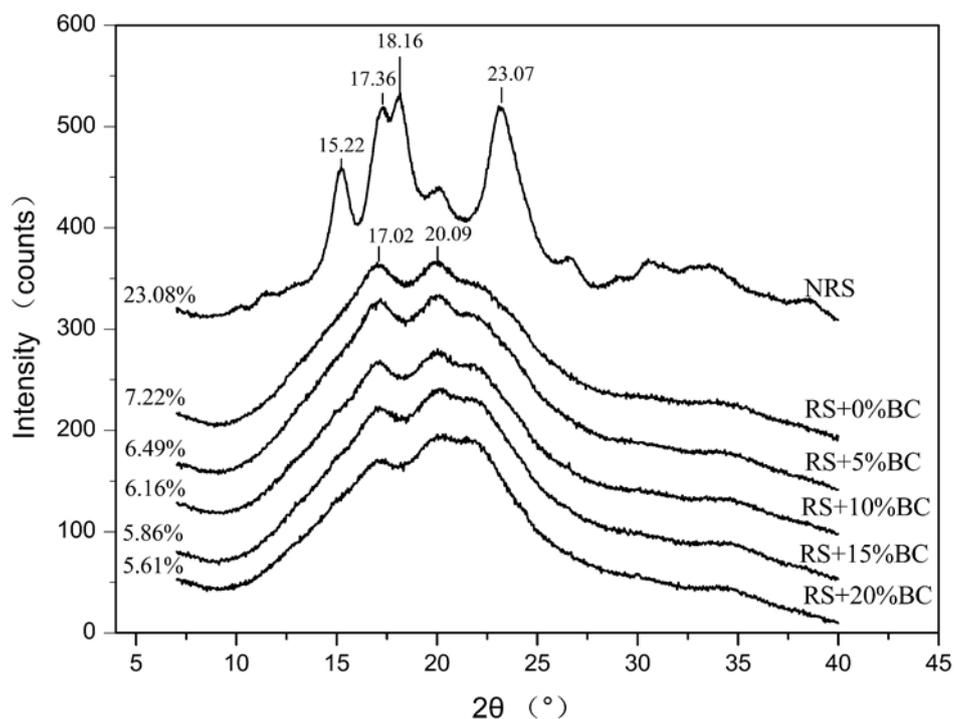


Fig. 3. X-ray diffraction patterns of native RS and freeze-dried RS gels with different concentrations of BC treated by five freeze-thaw cycles

DSC results of the freeze-thawed pastes demonstrated much lower transition temperatures and enthalpies than those obtained during pasting. However, neither the transition temperatures nor enthalpies appeared to be affected by BC addition applied in this study. The recrystallization of rice starch was retarded during repeated freeze-thaw cycles with the addition of BC. In the absence of BC, the endothermic peak for rice starch retrogradation ranged from 48.47 °C to 63.40 °C with a ΔH 4.05 J/g. With the concentration of BC increasing to 20% w/w, the melting enthalpy was significantly decreased to 2.06 J/g, approximately half of the value (4.05 J/g) for the rice starch alone. The endothermic peak was ranged from 51.77 °C to 66.67 °C, which was also significantly ($P < 0.05$) shifted to higher temperatures compared with the rice starch alone. The results above might be attributed to the interactions between RS and BC. When RS was heated in BC solution, the swelling and gelatinization of RS granules were suppressed at relative high BC concentration due to the competition of BC against starch granules for available water [29, 30]. This could lead to insufficient gelatinization of RS. And the partial gelatinized starch granules had higher proportion of ordered and denser structure [30], which might decrease the mobility of starch macromolecular chains and therefore inhibit the crystallization progress of rice starch molecules. And the swelling of rice starch granules was suppressed by BC, which also reduced the quantity of leached amylose of rice starch. Russell [31] reported that the amylose leached out from granules during gelatinization had been suggested to have synergetic effects on the retrogradation of amylopectin, so the reduction of leached amylose could also expect the inhibition of amylopectin retrogradation. These results suggested that the BC could significantly inhibit the recrystallization of rice starch during freeze-thaw cycles.

3.5 Textural properties

Textural changes occurred during freezing and thawing of starch gels with and without BC. The hardness changes were presented in Fig. 4. The results showed that the hardness values of all samples increased after the repeated freeze-thaw cycles. But the control sample showed the most pronounced increasing in hardness when the number of freeze-thaw cycles increased, which led to starch gel hardening and consequently unacceptable texture. The gel firmness was mainly caused by retrogradation of starch gels, which associated with the syneresis of water gels and crystallization of amylopectin, leading to harder gels [32]. However, a higher BC concentration to rice starch gel appeared to result in a smaller change in textural properties during freeze-thaw

treatments. The SEM images of the freeze-thaw gels (Fig. 1), which specimen images showed that BC effectively stabilized the microstructure of rice starch gels because BC could maintain the matrix surrounding pores of rice starch gels. This could explain the harder texture noted in freeze-thawed gels without BC. Incorporation of BC concentration up to a level of 20% into rice starch is thus considered to be advantageous to maintain desirable textural properties during freeze-thaw treatments.

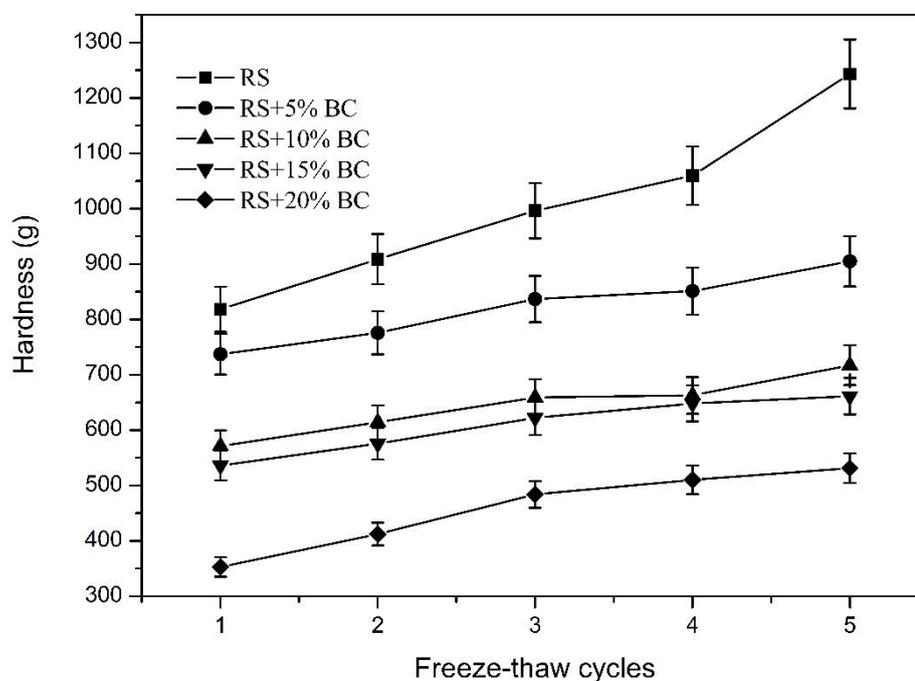


Fig. 4. Effect of number of freeze-thaw cycles on hardness of RS gels with different concentrations of BC. Error bars represent standard deviations

4. Conclusions

In summary, the study verified the effects of BC on freeze-thaw stability of rice starch gels that depended on the concentrations of BC by a variety of methods. BC was shown to be an effective agent for the reduction of recrystallinity, the spongy structure formation, and for limiting the increase in gel hardness of rice starch gels after repeated freeze-thaw cycles. Moreover, the pasting properties (peak viscosity, breakdown, final viscosity, setback) of rice starch decreased with the addition of BC, but the pasting temperature increased, BC could effectively retard the retrogradation of rice starch gels. This study showed that BC could be a useful additive for preserving quality in frozen rice starch-based food products. However, the application amount could not be very high considering the price of BC. Then the reasonable addition concentration of BC should be selected with comprehensive of both the effect of BC on the freeze-thaw stability and the cost of the products. Furthermore, the deep-seated mechanism on the molecular level involving the molecular characteristics of both BC and RS should be further investigated to uncover the essence behind the phenomenon.

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Tab. 1. Pasting characteristics of RS with different concentrations of BC

BC conc (%)	Peak viscosity (cP)	Trough (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Pasting temperature (°C)
0	2559.67 ± 10.59a	2004.33 ± 14.50a	555.33 ± 22.50a	2651.67 ± 11.85a	647.33 ± 18.14a	76.95 ± 0.39c
5	2385.66 ± 8.74b	1966.67 ± 1.53ab	417.00 ± 9.00b	2441.00 ± 1.00b	474.67 ± 1.53b	77.51 ± 0.08b
10	2216.67 ± 6.51c	1944.67 ± 1.53b	272.33 ± 5.03c	2321.33 ± 7.02c	376.33 ± 5.51c	78.37 ± 0.03b
15	2081.00 ± 17.69d	1893.67 ± 18.90bc	187.33 ± 3.21d	2193.33 ± 20.98d	299.67 ± 2.08d	79.17 ± 0.03a
20	1953.33 ± 10.21e	1819.33 ± 12.01d	137.67 ± 4.04e	2062.67 ± 9.29e	244.67 ± 3.06e	79.18 ± 0.02a

Note: Tests were performed in triplicate and the data were shown in mean ± standard deviation. Different letters in the same column indicated significant differences ($P < 0.05$) by Tukey's test.

Tab. 2. Thermal properties of native and freeze-thawed (five cycles) RS with different concentrations of BC

BC conc (%)	Native				Freeze-thawed			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
0	68.17 ± 0.35d	71.70 ± 0.10b	75.57 ± 0.15b	12.94 ± 0.14a	48.47 ± 0.21c	54.70 ± 0.05c	63.40 ± 0.17d	4.05 ± 0.05a
5	69.03 ± 0.21c	72.23 ± 0.12b	76.10 ± 0.30b	12.13 ± 0.12b	49.23 ± 0.35b	55.59 ± 0.10c	64.40 ± 0.36c	3.41 ± 0.14b
10	71.06 ± 0.29b	73.13 ± 1.45b	77.03 ± 1.62b	10.87 ± 0.15c	51.27 ± 0.35a	57.10 ± 0.76b	65.33 ± 0.12b	2.90 ± 0.02c
15	71.16 ± 0.23a	73.87 ± 1.62a	77.30 ± 0.17a	9.37 ± 0.28d	51.37 ± 0.34a	58.20 ± 0.53ab	66.30 ± 0.36a	2.38 ± 0.03d
20	71.57 ± 0.15a	75.03 ± 0.12a	79.16 ± 0.31a	8.50 ± 0.33e	51.77 ± 0.13a	59.20 ± 0.27a	66.67 ± 0.21a	2.06 ± 0.10e

Note: T_o : onset temperature; T_p : peak temperature; T_c : conclusion temperature; ΔH : enthalpy. Values are means ± SD (n=3). Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

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