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Effect of sucrose on the freeze–thaw stability of rice starch gels: Correlation with microstructure and freezable water

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ABSTRACT

Rice starch gels subjected to repeated freezing and thawing tend to decrease in quality. This study investigated how the addition of sucrose to rice starch gels affects factors commonly used to measure quality. Rice starch gels containing 0–20% sucrose were treated to 5 freeze–thaw cycles. The result showed that sucrose effectively reduced the % syneresis. Scanning electron micrographs of freeze–thaw gels showed that smaller pore size and a thicker surrounding matrix corresponded with increasing sucrose concentration. Furthermore, the amount of freezable water in starch systems decreased with increasing sucrose concentrations, which also corresponded with gel microstructure. These results suggest that re-association of starch chains (retrogradation) induced by freeze–thaw treatment is retarded by sucrose. This study showed that sucrose is an effective agent for preserving the quality of freeze–thawed rice starch gels.

1. Introduction

New and innovative frozen food products are continually being launched into world markets as a result of lifestyle changes by consumers. Upon freezing, however, water in these foods transforms into ice, often resulting in physical stress to the food matrix. When a frozen food is thawed for consumption, the moisture is readily separated from the matrix and it causes softening of the texture, drip loss, and often lead to deterioration of overall product quality (Rahman, 1999).

In the freezing process, when starch pastes or gels are frozen, phase separation occurs upon formation of ice crystals. Upon thawing, a phenomenon known as syneresis occurs with starch pastes and gels because the water can be easily expressed from the dense network (Karim, Norziah, & Seow, 2000). Repeating the cycle of freezing and thawing enforces the phase separation and ice growth (Eliasson & Kim, 1992). As the ice crystals become larger, the syneresis and sponge formation occurs more readily. Syneresis in freeze–thawed gel is due to the increase of molecular association between starch chains, in particular retrogradation of amylose (Morris, 1990), expelling water from gel structure (Saartratra, Puttanlekb, Rungsardthong, & Uttapap, 2005). Thus the amount of syneresis is a useful indicator for the tendency of starch to retrograde (Karim et al., 2000).

Freeze–thaw stability is an important property that is used to evaluate the ability of starch to withstand the undesirable physical changes that may occur during freezing and thawing. This property may be simply evaluated by gravimetric measurement of the water of syneresis that separates from starch pastes or gels (Schoch, 1968; Wu & Seib, 1990). Multiple freeze–thaw cycles that involve subjecting samples to repeated freezing and intermittent thawing to room temperature over a period of 2–4 h are known to drastically accelerate retrogradation and syneresis (Radley, 1976; Yuan & Thompson, 1998).

The effect of starch modification on freeze–thaw stability of different starch gels has been investigated in numerous studies (Hung & Morita, 2005; Kaur, Singh, & Singh, 2004; Pal, Singhal, & Kulkarni, 2002; Reddy & Seib, 2000). However, few studies have investigated the role of food ingredients on freeze–thaw stability in starch gels. Sucrose is one of the major food ingredients and about 10^8 tonnes are produced annually (Izydorezyk, 2005). It is a common ingredient in baked and processed foods. Research on the effects of sucrose upon the retrogradation of starch gels has been conflicting and inclusive. Sugars have been shown to retard retrogradation. I'Anson et al. (1990) and Chang and Liu (1991) found that sugars reduced crystallinity in retrogradated wheat starch gels. Kohyama and Nishinari (1991) found that sugars decreased retrogradation in sweet potato starch pastes. Katsuta, Nishimura, and Miura (1992) reported that sugars inhibited retrogradation of rice starch gels. Lii, Lai, and Liu (1998) reported that sugars showed marked suppression effects on retrogradation of rice starch gel. Baker and Rayas-Duarte (1998) found that adding sugars to amaranth starch gels had varying results, but for the most part, sugars showed similar or increased stability when compared with a control freeze–thaw. Other researchers have found accelerated retrogradation

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with sugars. An increased rate of retrogradation was reported with the addition of sugars to corn and rice starch gels (Chang & Liu, 1991; Germani, Ciacco, & Rodrigues-Amaya, 1983). Maxwell and Zoble (1978) reported increased rate of crystallization in wheat starch gels with the addition of sugar. The majority of their research used differential scanning calorimetry technique to measure the extent of retrogradation due to amylopectin reorganization. This may not correlated well with the freeze–thaw stability of starch gels, which is mainly due to amylose re-association. Only two studies investigation the effect of sucrose on the freeze–thaw stability of starch gels have been reported in the literature (Ahmad & Williams, 1999; Baker & Rayas-Duarte, 1998).

In the present study, change in % syneresis (reflective freeze–thaw stability) of sucrose added rice starch gels were correlated with concomitant changes in microstructure observed by scanning electron microscopy. Freezable water of sucrose added to rice starch gels were determined and used to elucidate these results.

2. Materials and methods

2.1. Materials

Rice starch was supplied by Cho Heng Rice Vermicelli Factory Co., Ltd. (Nakorn Prathom, Thailand). The amylose content of the rice starch was 31.60% as determined by the method of Juliano (1971). Food-grade sucrose (Mitr Phol Sugar Co., Ltd., Supanburi, Thailand) was purchased from a local supermarket.

2.2. Starch gel preparation

The preparation of starch gel followed the method of Charoenrein, Tatirat, and Muadklay (2008). Rice starch suspensions (8% total solid w/w wet basis) containing 0%, 10% and 20% w/w sucrose were heated to 80, 82.5 and 87 °C for 25 min, respectively. The suspensions were then loaded into 10 ml syringes (20 mm in diameter) and steamed for 9 min. Finally, the samples were placed in an incubator at 25 °C for 2 h.

2.3. Freezing and thawing

Starch gel samples were frozen in a chest freezer (Sanyo refrigerator, model SF-C1497) at −18 °C for 22 h and then thawed at room temperature for 2 h. This freeze–thaw cycle was repeated for up to 5 cycles.

2.4. Syneresis measurement

Syneresis measurement was modified from methods of Charoenrein et al. (2008) and Baker and Rayas-Duarte (1998). The thawed starch gel samples were removed from syringes and put in the cylindrical plastic tube with filter paper (Whatman No. 41). The cylindrical plastic tube was placed in centrifuge tubes. The tube was then centrifuged at 100*g* (centrifuge CN-1050, MRC Ltd., Holon, Israel) for 15 min. The amount of liquid separated from the gel was measured in a burette. The percentage of syneresis was then calculated as the ratio of the amount of liquid separated (ml) to the total weight (g) of the gel before centrifugation and multiplied by 100. The data were reported as averages of three measurements.

2.5. Frozen structure by scanning electron microscope (SEM)

The freeze–thaw samples were cut and gradually dehydrated in 50%, 70%, 90% and absolute ethanol at room temperature for 24 h at each concentration and finally dehydrated using a critical point dryer. The cut surface samples were mounted on the stub, coated with gold and observed with a JSM-5600LV microscope (JEOL, England). The accelerating voltage and the magnification are shown on the micrographs.

2.6. Determination of freezable water

Freezable water is defined as water that can phase separate within the sample matrix and, when sufficiently cooled, from ice crystals detectable by DSC (Reid & Kerr, 1993). A differential scanning calorimetry (Pyris-1, Perkin Elmer, Norwalk, CT, USA) with Pyris™ operation software was used for determination of the amount of freezable water in starch systems. The instrument was calibrated with indium. The weight ratio between dry solids of starch and water remained constant (1:2.3), whereas the sucrose addition was either 1:2.7 or 2.25:2.7, based on the dry weight of starch. Rice starch (5.4 mg, dry basis) and sucrose (0, 2 or 4.5 mg) were placed in a stainless steel pan, and then a microsyringe was used to add distilled water (12.6 mg) until the starch–sugar mixture was fully wet. The water was allowed to evaporate on a balance, until the ratio between starch and water reached exactly 1:2.3, and then the pan was hermetically sealed. Using an empty pan as reference, the sample pan was cooled to −60 °C at 30 °C/min, and then held at that temperature for 20 min. Then heated to 160 °C at 10 °C/min. The sample was then cooled to −60 °C and held for 20 min once more, before heating to 160 °C at 10 °C/min again. Based on the known heat of fusion of ice of 334 J/g, freezable water (g water/g solids) was calculated from the area under the ice melting endotherm of the second scan. All measurements were done in duplicate.

2.7. Statistical analysis

We used a completely randomized design. The difference between means was determined using the Duncan's new multiple range test. All statistical analyses were performed using SPSS 12.0 for Windows.

3. Results and discussion

3.1. Syneresis

Freeze–thaw stabilities of starch gels were assessed by measuring liquid separated after freezing/thawing 1–5 cycles. The effect of sucrose on the amount of syneresis in rice starch gels is presented in Table 1.

In the first to fifth cycles, the analysis of variance shows that rice starch gels without sucrose and rice starch gels containing 10% and 20% sucrose significantly ($p \leqslant 0.05$) affects the percentage of syneresis of rice starch gels. Freeze–thawed rice starch gels no containing added sucrose had a high syneresis value (55%) after the first cycle and showed little change through subsequent freeze–thaw cycles. On the other hand, freeze–thaw rice starch gel with sucrose showed markedly lower in % syneresis and behaved differently from that of without sucrose addition. Starch gels containing 10% sucrose which had 14.84% syneresis in the first cycle, showed an obvious increase in syneresis value to 25.37 after 2 freeze–thaw cycle. After that the syneresis values changed slightly through 3–5 cycles. Starch gels containing 20% sucrose which had only 1.25% syneresis in the first cycle, showed a progressive increase in syneresis values, 4.64–25.20%, through 1–3 cycles. However, after fourth and fifth freeze–thaw cycles, the percentage of syneresis slightly increased. Our finding agreed with Baker and Rayas-Duarte (1998) who found that 10% and 20% sucrose addition into amaranth starch gels significantly improved the stability of

Table 1
Percentage of liquid separated (syneresis) of rice starch gels (8% w/w) containing sucrose 0%, 10% and 20% at each freeze–thaw cycle

Sample	Syneresis (%)*				
	1 cycle	2 cycle	3 cycle	4 cycle	5 cycle
Rice starch	55.02 ± 0.57^{aAB}	57.25 ± 0.39^{aC}	55.41 ± 0.41^{aB}	55.62 ± 0.39^{aB}	54.54 ± 0.27^{aA}
Rice starch + 10% sucrose	14.84 ± 2.16^{bA}	25.37 ± 1.53^{bB}	28.34 ± 2.70^{bBC}	31.05 ± 6.28^{bBC}	34.47 ± 5.25^{bC}
Rice starch + 20% sucrose	1.25 ± 0.36^{cA}	4.64 ± 1.20^{cB}	25.20 ± 2.54^{bC}	28.57 ± 1.41^{bD}	32.54 ± 1.80^{bE}

[a–c]Mean values in each column with different superscripts are significantly different ($p \leqslant 0.05$).
[A–E]Mean values in each row with different superscripts are significantly different ($p \leqslant 0.05$).
* The values reported as means ± standard deviation.

Table 2
The amount of freezable water in starch systems

Sample	Amount of freezable water (g water/g solids) in starch systems (gelled)*
Rice starch	1.638 ± 0.008^{a}
Rice starch + 10% sucrose	1.157 ± 0.005^{b}
Rice starch + 20% sucrose	0.842 ± 0.006^{c}

[a–c]Mean values in each column with different superscripts are significantly different ($p \leqslant 0.05$).
* The values reported as means ± standard deviation.

the starch gels after 2 cycles. However, after more repeated freeze–thaw cycles, they observe no significant differences in freeze–thaw stability between sugar added amaranth starch gels and control samples. The observed difference between Baker & Rayas-Duarte's results and ours is probably due to measurement method and starch types used in each experiment.

It is well known that when a starch gel is frozen, starch-rich regions are created in the matrix, where water remains partially unfrozen. High solid concentration in the regions facilitates the starch chains to associate forming thick filaments, whereas water molecules coagulate into ice crystals forming a separated phase. These effects contribute to spongy structure and released liquid or syneresis (Ferrero, Martino, & Zaritzky, 1993; Lee, Baek, Cha, Park, & Lim, 2002), which can be reduced by adding sucrose (Fig. 1). However, after increasing freeze–thaw cycles, starch gel containing sucrose increasing syneresis values. This indicates that acceleration of starch chain association and progressive larger ice crystals formation by repeated freezing and thawing reduce the influence of sucrose on freeze–thaw stability of starch gels.

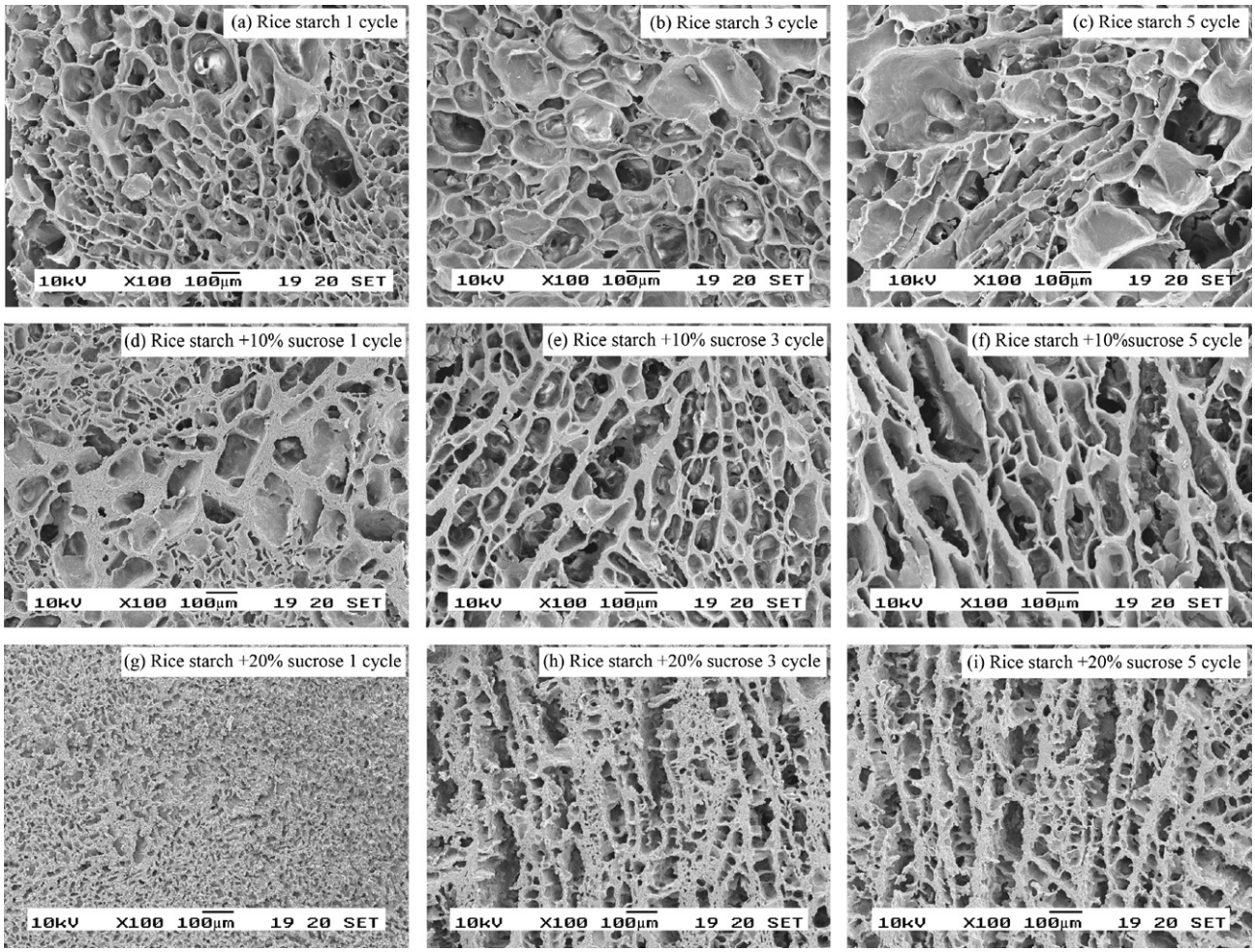

Fig. 1. SEM images of rice starch gels (8% w/w) containing sucrose (0%, 10% and 20%) after freeze–thaw for 1, 3 and 5 cycles (100×, Bar = 100 μm).

3.2. Structure of freeze–thaw starch gels

To elucidate the relationship between the syneresis and the microstructure of rice starch gels, the microstructure of freeze–thawed gels was examined using SEM. Images of treated specimens are shown in Fig. 1. Clear differences were observed in the microstructure of rice starch gels after 1–5 freeze–thaw cycles for both gels with and without added sucrose. All freeze–thaw treated starch gels developed a spongy structure which can be attributed to amylose retrogradation and ice crystal formation. Along with syneresis, a thick fibrillar network of starch gel was formed in the spongy structure during the repeated freeze–thaw cycles; similar findings were reported by Ferrero et al. (1993). In rice starch gel with no sugar added, the microstructure after the first freeze–thaw cycle produced pores in the gel (Fig. 1a). After the third and fifth freeze–thaw cycle, the starch gels had slightly larger pores but the matrix surrounding pores showed similar thickness (Fig. 1b and c). These structural findings correlate well with insignificant changes in syneresis values found after 1–5 freeze–thaw cycles of rice starch gel with no sucrose addition. After 1 freeze–thaw cycle, the starch gels containing 10% sucrose appeared to have smaller pores but have thicker matrix surrounded the pores (Fig. 1d). After 3 and 5 freeze–thaw cycles, pore size increased while matrix thickness decreased (Fig. 1e and f). In 20% sucrose systems after 1 freeze–thaw cycle, the matrix surrounded the pores was thickest and the pores were smallest (Fig. 1g–i). However, with succeeding freeze–thaw cycles, the matrix surrounding pores in the starch gels became thinner and more compact and the pores became larger (Fig. 1h and i). In the rice starch gels containing added sucrose and treated to multiple freeze–thaw cycles change in microstructure corresponded closely with increased percent syneresis.

The specimen images showed that sucrose effectively stabilized the microstructure of rice starch gels because sucrose could maintain the matrix surrounding pores in the starch gels. We speculate that increasing the concentration of sucrose in starch gels may retard amylose retrogradation by a mechanism that slows amylose–amylose re-association.

3.3. DSC studies of freezable water in starch systems

The amount of freezable water in starch systems significantly decreased ($p \leqslant 0.05$) with increasing sucrose concentration (Table 2).

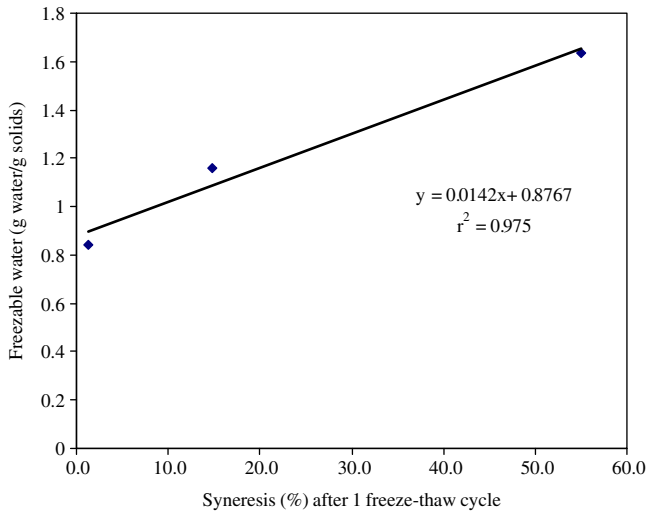

Fig. 2. Correlation between freezable water (g water/g solids) and syneresis (%) after 1 freeze–thaw cycle.

The results show that sucrose hydrated water in starch systems leads to lower amounts of freezable water. Thus, pores size resulted from ice crystals formation were smaller and the matrix surrounded pores were thicker.

Simple linear regression of freezable water on syneresis produced very high model fit ($r^2 = 0.975$) suggesting a linear relationship between the amount of freezable water and % syneresis after 1 freeze–thaw cycle (Fig. 2).

It can be noted that starch based frozen foods containing sucrose would show a low syneresis value after the first freeze–thaw cycle. However, if production, distribution, storage and consumer handling conducted without proper care and allowed the foods subjected to more than 2 freeze–thaw cycles, increase in syneresis as well as spongy structure can be noticeable.

4. Conclusions

The addition of sucrose was shown to be an effective agent for the reduction of syneresis in rice starch gels subjected to repeated freeze–thaw cycles. In this work, sucrose was most effective in enhancing freeze–thaw stability of starch gels at 20%. Therefore, sucrose could retard changes in the texture in rice starch gel to spongy structure during repeated freeze–thawing. Moreover, the amount of freezable water in starch systems was decreased with increasing sucrose concentrations, which correlated to gel microstructure and % syneresis. This research shows that sucrose can be a useful additive for preservation of the quality of frozen food products.

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