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Output ที่ได้จากโครงการ

1. ผลงานวิจัยที่ตีพิมพ์ในวารสารวิชาการระดับนานาชาติ

1. **Charoenrein S**, Tatirat O and Muadklay J. Use of centrifugation – filtration for determination of syneresis in freeze-thaw starch gels**.** *Carbohydrate Polymers* **2008**; 73: 143-147. *(Impact Factor (2009) = 3.167)*

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5. **Charoenrein S**, Tatirat O, Rengsutthi K and Thongngam M. Effect of konjac glucomannan on syneresis, textural properties and the microstructure of frozen rice starch gels *Carbohydrate Polymers* (inpress). *(Impact Factor (2009) = 3.167)*

หมายเหตุ บทความที่ 1 เกิดจากการทดลองหาวิธีการตรวจหา % การแยกตัวของน้ำจากเจลสตาร์ชแช่เยือกแข็งและทำละลาย และพบว่าวิธีการใหม่นี้ลดการแปรปรวนของผลได้ดี จึงนำมาเขียนเป็นบทความ ส่วนบทความที่ 3 เกิดจากการสังเกตุในระหว่างการทดลองและเห็นว่าเป็นประเด็นที่น่าสนใจ จึงนำมาเขียนเป็นบทความ

2. การเผยแพร่ผลงานวิจัยในการประชุมวิชาการระดับนานาชาติ

1. **Charoenrein, S.** and Tatirat, O. 2007. Effect of konjac glucomannan on stability of frozen rice starch gel. 10th Asean Food conference. 21-23 August 2007. Kuala Lumpur, Malaysia.

2. **Charoenrein, S**., Tatirat, O. and Muadklay, J. 2008. New and simple method for determination of syneresis in freeze-thaw starch gels. 9th International Hydrocolloids Conference. 15-19 June 2008. Singapore.

3. **Charoenrein S**, Rengsutthi K and Tatirat O. 2010. Freeze–thaw stability and microstructure of starch gel: Influence of type of starches, sugars and freezing rate. XVIII International Starch Convention Moscow-Cracow 2010. 20-26 June 2010. Cracow, Poland.

3. การเผยแพร่ผลงานวิจัยในการประชุมวิชาการระดับชาติ

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4. การนำผลงานไปใช้ประโยชน์ด้านการเรียนการสอน

งานวิจัยนี้เป็นประโยชน์ต่อการเรียนการสอนด้านวิทยาศาสตร์และเทคโนโลยีการอาหารทั้งในระดับปริญญาตรีและบัณฑิตศึกษา

5. ผลิตนิสิตระดับปริญญาโท เอก จำนวน 3 คน

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วิทยานิพนธ์เรื่อง การปรับปรุงคุณภาพของเจลสตาร์ชข้าวแช่เยือกแข็งด้วยคอนยักกลูโคแมน-แนนและน้ำตาลชนิดต่างๆ

2. นางสาวณัฐสุดา ปรีชาธรรมวงศ์ ระดับปริญญาโท
วิทยานิพนธ์เรื่อง การปรับปรุงคุณภาพของเจลสตาร์ชข้าวแช่เยือกแข็งด้วยสตาร์ชมันสำปะหลังและแป้งข้าวเหนียว

3. นางสาวอรวรรร ตติรัตน์ ระดับปริญญาเอก (โครงการปริญญาเอกกาญจนาภิเษก)
วิทยานิพนธ์เรื่อง Influence of Extrusion Process on Physicochemical Properties of Konjac Glucomannan (เป็นงานวิจัยที่มีจุดเริ่มต้นจากผลงานวิจัยของโครงการนี้)

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Use of centrifugation–filtration for determination of syneresis in freeze–thaw starch gels

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Abstract

Several methods for measuring the freeze–thaw stability of starch gels can lead to inaccurate and imprecise estimates of syneresis due to partial reabsorption of separated water by spongy starch gels. This study evaluates a method that combines centrifugation with simultaneous separation of released water through a separator and filter paper. The evaluation procedure used low- and high-amylose rice flour gels treated to 5 freeze–thaw cycles. The traditional centrifugation method was unable to detect significant increases in syneresis ($p < .05$) of medium-amylose gel after 4 freeze–thaw cycles due to formation of a water reabsorbing spongy structure in 4–5 cycles. For high-amylose flour gel, which forms a spongy structure after the first freeze–thaw cycle, the traditional method did not detect significant change in syneresis values in any of the freeze–thaw cycles. In contrast, the centrifugation–filtration method, which actively separated released water and prevented its reabsorption, detected significant increases ($p < .05$) in syneresis with each cycle for medium-amylose flour gels. When using this method with high-amylose flour gel, we detected high syneresis values after the first cycle which stayed similar through 2–5 cycles indicating a progressive reduction in freeze–thaw stability of the samples which is consistent with the fact that high-amylose rice flour gels have less freeze–thaw stability than do gels made from medium-amylose flour. In conclusion, this study demonstrated that the centrifugation–filtration method measures syneresis with increased accuracy and precision. The authors recommend adoption of this method for determination of freeze–thaw stability in starch gels.

Keywords: Syneresis; Freeze–thaw; Stability; Starch; Gels

1. Introduction

As demand for ready-to-eat food products increases, a variety of frozen foods are continually launched into world markets. Upon freezing, however, water in the 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 deterioration of overall 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 occur 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 occurring during freezing and

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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). Repeated 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).

Several syneresis based measures for determination of the freeze–thaw stability of starches have been reported. However, the procedures for these methods have not been standardized. In general, methods for measuring syneresis fall into four groups. The most widely used group uses centrifugation (Lee, Kim, Park, & Lee, 2006; Schoch, 1968; Varavinit, Shobsngob, Varanyanond, Chinachoti, & Naivikul, 2002; Yeh & Yeh, 1993; Yuan & Thompson, 1998). In this method, the weight of water extruded by centrifugation at 1000–8000*g* is used to measure syneresis as the percent reduction of the original gel mass (Yeh & Yeh, 1993). However, the result of syneresis measurement taken after a fixed number of freeze–thaw cycles may lead to improper or misleading conclusions since some starch pastes subjected to several freeze–thaw cycles may partially reabsorb separated liquid upon standing for a short time at room temperature.

The second group uses vacuum filtration of the freeze–thaw gel samples through the filter paper with a constant weight placed upon the sample during filtration. The extent of syneresis is calculated as weight percent of water loss based on the initial water content in the gel (Lee, Baek, Cha, Park, & Lim, 2002).

The third group uses gravimetric drip of expelled water from the thawed gels. In this method the thawed starch gel was placed into a glass funnel, allowing the water to drip out for 2 h by gravity (Chen, Schols, & Voragen, 2003).

The fourth group uses the measurement of diameter of the released water front on the filter paper from contacting the freeze–thaw sample with controlled time. The extent of syneresis is calculated as difference in diameter of released water front of the freeze–thaw sample and unfrozen sample after a controlled time as compared with the diameter at the initial contact time (Ferrero, Martino, & Zaritzky, 1994).

The second, third, and fourth methods could partially prevent or eliminate the problem of re-absorption of extruded water; however, these methods either consist of complicated steps or require a long time to perform. Moreover, if the measurement conditions are not properly controlled, the results will lack precision.

In this paper, we present an alternative approach to separate the released water by combined method of low force centrifugation with simultaneous separation of the extruded water through a drilled hole separator and filter paper. Two types of rice flour with medium- and high-amylose content were used to verify our proposed techniques. Both rice flour pastes exhibited different frozen structure after being subjected to repeated freeze–thaw cycles. The traditional and widely used method with centrifugation was selected to compare with our proposed method. These two methods measure the released water after freeze–thaw starch gel was subjected to a centrifugal force.

2. Materials and methods

2.1. Materials

Two varieties of Thai rice Khao Dok Mali 105-KDML105 and Luang 11-L11 from the Kalasin province were selected for the study. The crude rice was stored for six months prior to milling in Karasin Rung Rueng Rice Mill Factory. Rice was wet milled and then dried at 45 °C for 5 h. Resulting flours were then ground in a hammer mill and passed through a 100 mesh sieve. The granule size of KDML 105 was 2.8–5.1 μm with mean diameter of $3.8 \pm .7$ μm and L11 was 2.3–5.6 μm with mean diameter of $3.9 \pm .9$ μm. KDML 105 and L11 rice flour contained 7.68 and 10.59% moisture and 17.58 and 32.48% amylose contents, respectively (AACC, 2000).

2.2. Flour gel preparation

Rice flour suspensions (9% total solid w/w wet basis) were prepared by mixing the starch in distilled water and stirring continuously at 250 rpm for 1 h followed by 200 rpm at 85 °C for 25 min. 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

Flour gel samples were frozen in chest freezer 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

Two methods for measuring syneresis were compared. For method 1, thawed flour gel samples were removed from the syringes and put in centrifuge tubes with closed screw caps. Samples were centrifuged at 8000*g* for 15 min. The supernatant was decanted and the residue was weighed. The percentage of syneresis was then calculated as follows:

$$\%\text{Syneresis} = \frac{\text{Weight of separated liquid from gel}}{\text{Total wt. of gel before centrifuging}} \times 100$$

For method 2, the syneresis was determined in the cylindrical plastic tube with filter paper (Whatman No. 41) on the drilled holes (.7 mm diameter, 13 holes) at the bottom. The cylindrical plastic tube was placed in centrifuge tubes as shown in Fig. 1. Centrifuge tube (28×104 mm) was weighed (wt_0). A single piece of Whatman No. 41 filter paper (24 mm diameter) was placed at the bottom of the cylindrical plastic tube with cover, after which the tube

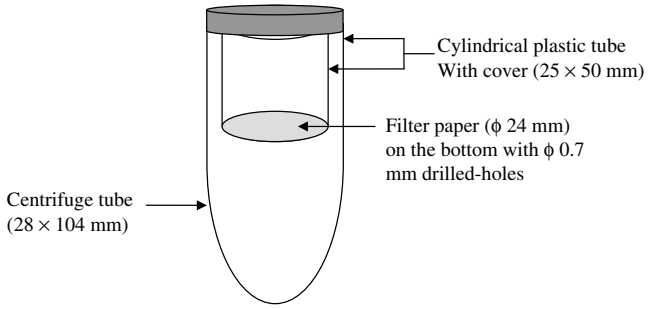

Fig. 1. Apparatus for centrifugation–filtration of gels (tube set).

set was weighed (wt_1). Thawed flour gels were added to each tube set (added into cylindrical plastic tube with cover) and the tube set with sample was weighed (wt_2) again. The tube was then centrifuged at 100*g* (centrifuge DAMON/IEC division) for 15 min. The cylindrical plastic tube with cover was pulled from centrifuge tube, before final weighing (wt_3). The liquid separated from starch gel was weighed and the syneresis percentage calculated as method 1. The data were reported as averages of five 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. 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. Structure of freeze–thaw gels

To elucidate the relationship between the syneresis and the structure of rice flour gels, the microstructure of freeze-thawed gels was examined using SEM. Specimen images are shown in Fig. 2. There were clear differences in microstructure in KDML105 and L11 flour gels after 1–3 freeze–thaw cycles. The freezing and thawing processes resulted in pores in the gels. However, for KDML 105, medium-amylose, flour gel treated with 1–3 freeze–thaw cycles appeared to have less well-defined pores embedded in a weak matrix and a texture that was similar in appearance to mashed wet tissue paper (Fig. 2a). In contrast, after 4–5 cycles, the texture of the flour gels changed to a sponge-like structure (Fig. 2b). The pores resulting from ice crystal formation and thawing were more clearly seen and the matrix surrounded pores were stronger due to increasing retrogradation of the starch matrix from repeated freeze–thaw cycles.

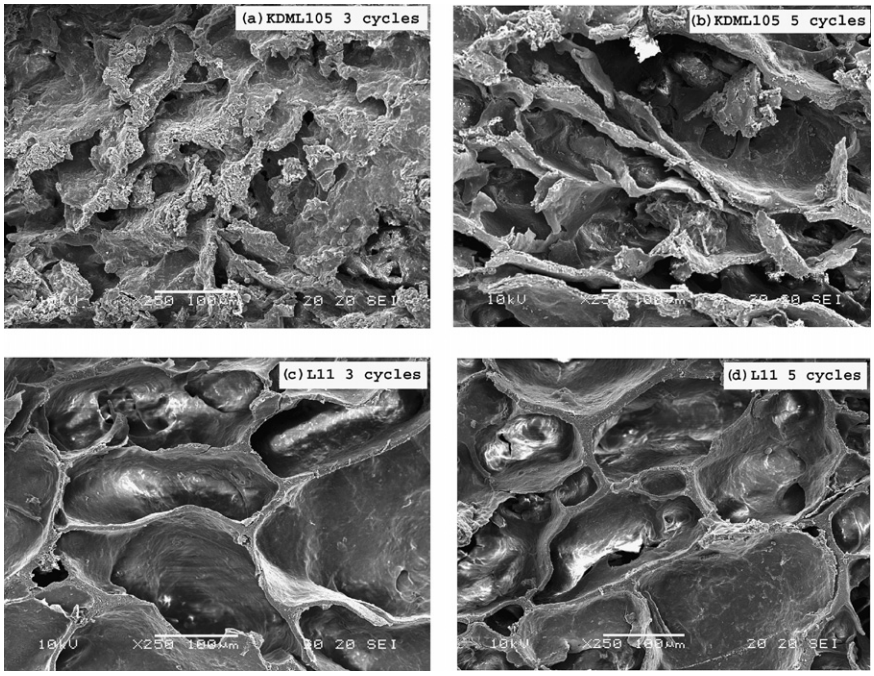

Fig. 2. SEM images of freeze–thaw rice flour gels. (a) KDML 105 after 3 cycles, (b) KDML 105 after 5 cycles, (c) L11 after 3 cycles, and (d) L11 after 5 cycles.

Table 1
Syneresis during freeze–thaw cycles for KDML 105 and L11 flour gels determined by method 1 (M1) and method 2 (M2)

Sample/method	Syneresis (%)[a]				
	1 cycle	2 cycle	3 cycle	4 cycle	5 cycle
KDML 105 M1	1.86 ± 2.53 a	14.67 ± 3.64 b	27.35 ± .56 c	7.65 ± 10.59 ab	6.98 ± 6.18 ab
KDML 105 M2	1.74 ± 1.49 a	3.75 ± .84 a	15.09 ± 6.07 b	32.79 ± 2.50 c	47.80 ± 2.48 d
L11 M1	3.79 ± 6.41 ns	5.62 ± 3.72	6.01 ± 3.86	6.03 ± 3.50	3.61 ± 3.05
L11 M2	59.46 ± 1.13 a	63.17 ± 1.36 b	64.86 ± 1.04 bc	66.16 ± .63 c	64.62 ± 3.20 bc

A significant letter in the same row of flour gel/method indicates the difference in each method ($p < .05$).
[ns]There is no significant difference ($p > .05$) among freeze–thaw cycle of each method.
[a] The values reported as means ± standard deviation.

Freeze-thawed L11, high-amylose, flour gels behaved differently. The well-defined spongy structure with termite gallery-like pores appeared after 1 freeze–thaw cycle, and there were no additional significant changes in frozen structure during 4 more cycles. The frozen structure of L11 flour gel cycles 3 and 5 is shown in Fig. 2c and d. The matrix surrounding the pores was thick and strong due to retrogradation of amylose.

3.2. Syneresis

Method 1 and method 2 show that both rice flour gels synerese after 1–5 freeze–thaw cycles. However, the results from each method differed (Table 1). Moreover, the standard deviations for results clearly show that method 1 results had much larger variation for both rice flour gels. This indicates that method 1 is less precise than method 2.

For KDML 105, medium-amylose rice flour, method 1 and method 2 resulted in similar syneresis after 1 freeze–thaw cycle. However, the results after 2–5 cycles using both methods were different. Method 1 resulted in significant increase of syneresis values ($p < .05$) from freeze–thaw cycles 1–3. However, after fourth and fifth freeze–thaw cycles the percentage of syneresis decreased. This decrease was likely from the changes in gel structure to sponge-like structure (Fig. 2b). This structure easily reabsorbed most of the extruded water if the water separation was too slow. Chen et al. (2003) state that the spongy structure made it difficult to measure the excluded water because, after centrifugation the sponge-like gel reabsorbed most of the separated liquid, which led to misleading results. Similarly, Yuan and Thompson (1998) also encountered the same problem in their research. Since the flour gel directly contacted the released water, separation of the water was difficult to control, leading to results with high standard deviations. In contrast, method 2 exhibited a continuous significant increase ($p < .05$) in the syneresis values with increasing freeze–thaw cycles. The flour gel with spongy structure from 4 to 5 freeze–thaw cycles could not reabsorb the extruded water back into the gel matrix since the extruded water was readily separated out from the gel and was collected at the bottom of the centrifuge tube. This result is reflected in the changes in gel structure from cycles 3 to 4 and 5 as shown in Fig. 2a and b.

For L11, high-amylose rice flour, method 1 resulted in nonsignificantly low syneresis values in all freeze–thaw cycles. This could be misleading. These rice flour gels showed a sponge-like structure since passing after the first freeze–thaw cycle and through the fifth freeze–thaw cycle (Fig. 2c and d). Again, method 1 could not separate extruded water fast enough before it was reabsorbed into the spongy gels. The large variation in results of each cycle was noticeable. On the other hand, method 2 resulted in high syneresis values after the first cycle and changed little through 2–5 freeze–thaw cycles. This indicates a reduction in freeze–thaw stability of the samples, which correlates to the fact that high-amylose rice flour gels are of less freeze–thaw stability than medium-amylose rice flour gels. Using method 1 could not clearly show this fact.

Another advantage of method 2 is that it used low centrifugal force of 100*g* which would not cause severe distortion to the freeze–thaw gel.

4. Conclusion

This study reported on the advantages of using a combination of centrifugation and filtration method for measuring syneresis in rice starch gels. This method has proved to be successful in determination of syneresis in freeze–thaw rice flour gels and offers important advantages over centrifugation method. The results obtained from medium- and high-amylose rice flour gels correlated well to the structure of freeze–thaw gels by SEM. Moreover, the results had less variation than those of traditional centrifugation method due to simultaneous separation of the released water.

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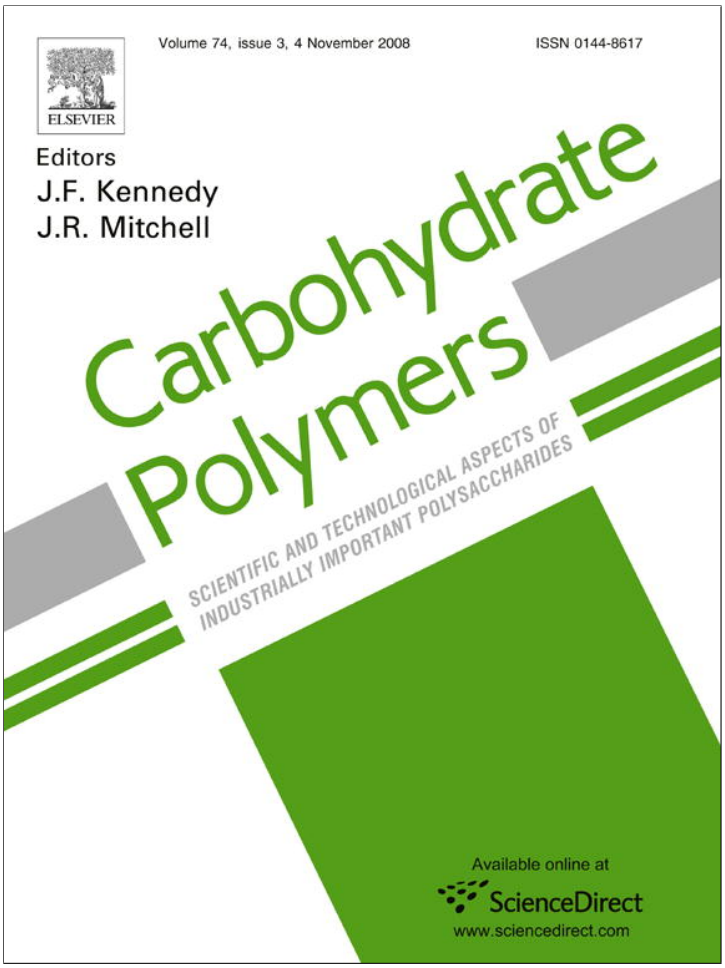

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Effect of sucrose on the freeze–thaw stability of rice starch gels: Correlation with microstructure and freezable water

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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.

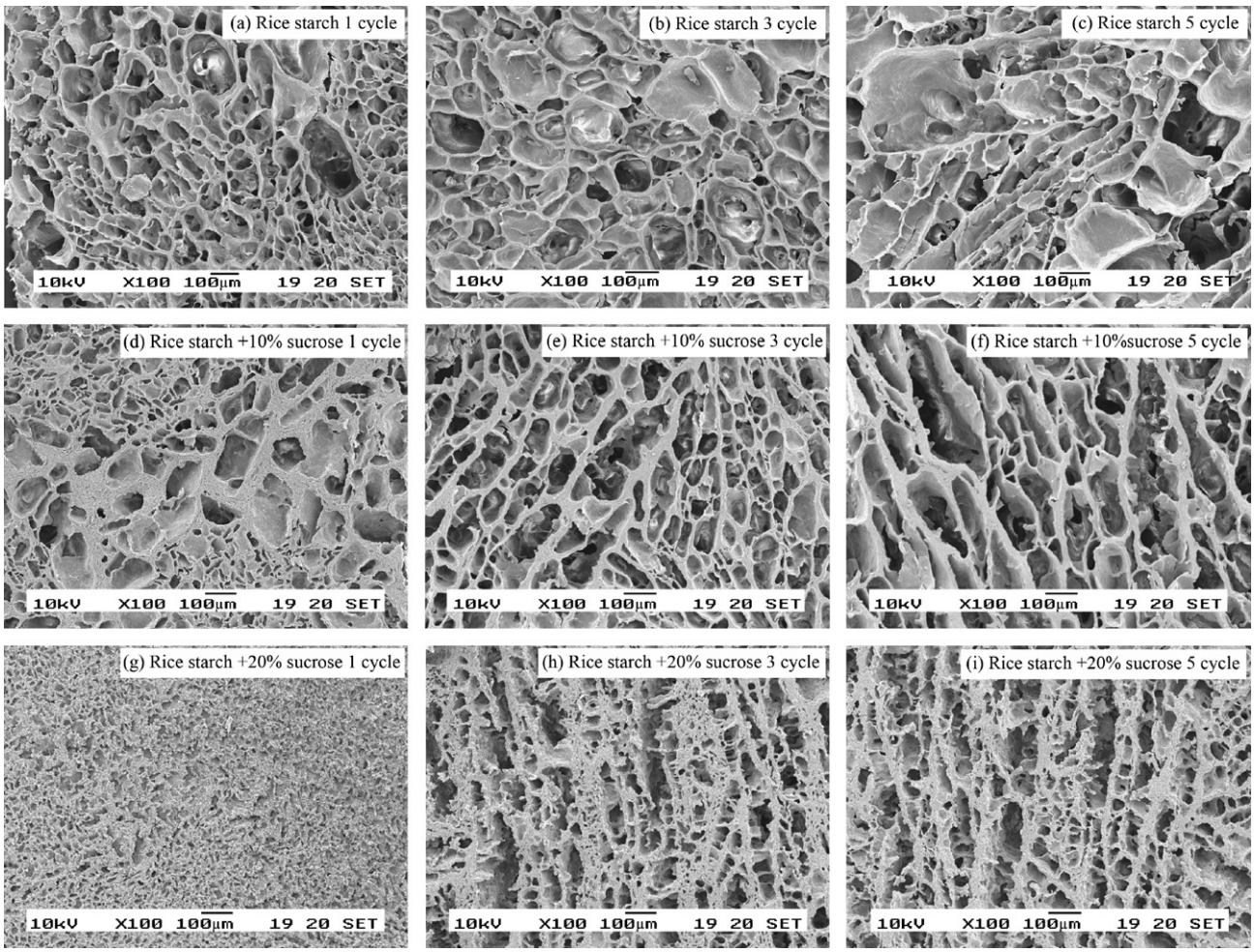

Fig. 1. SEM images of rice starch gels (8% w/w) containing sucrose (0%, 10% and 20%) after freeze–thaw for 1, 3 and 5 cycles (100×, Bar = 100 μm).

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).

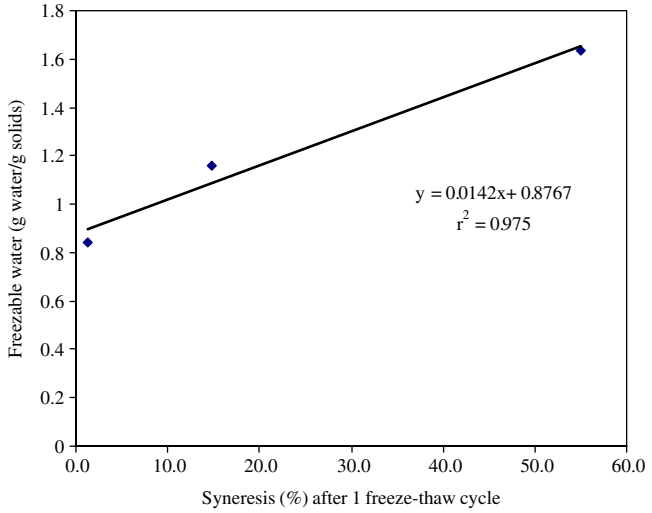

Fig. 2. Correlation between freezable water (g water/g solids) and syneresis (%) after 1 freeze–thaw cycle.

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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Undercooling associated with slow freezing and its influence on the microstructure and properties of rice starch gels

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ABSTRACT

It is well accepted that high undercooling or supercooling usually produces numerous small ice crystals. This paper shows that if heat transfer is not rapid enough, high undercooling causes non-homogenous sized ice crystals. Three freezing regimes (i.e. fast, slow and slow with undercooling) were used in this study. Fast freezing produced numerous homogeneously small ice crystals embedded in a thin rice starch gel matrix. This microstructure caused low % syneresis and hardness versus slow freezing's rather homogenous distribution of fewer large ice crystals embedded in a thicker gel matrix resulting in high % syneresis and hardness. However, slow freezing with undercooling produced non-homogenous clusters involving small and large ice crystals embedded in a very thick gel matrix. Starch retrogradation before ice formation played an important role in this frozen structure. The information gained from this study enhances understanding of the behavior of starch-based food during freezing and storage.

1. Introduction

Freezing has been recognized as an excellent and fairly widespread method of preserving food products, especially ready to eat meals which increasingly play an important role in today's lifestyle. The freezing process involves removing sensible and latent heat in order to lower product temperature generally to −18 °C or below (Delgado and Sun, 2001). During freezing, nucleation of ice occurs when the temperature of a food is lowered to the initial freezing point and this is followed by ice crystal growth.

Undercooling, sometimes called supercooling, is the phenomenon where the temperature of a solution or material is reduced below its freezing point without ice crystallization occurring (IIR, 2006). Undercooling is a non-equilibrium, metastable state, which is analogous to an activation energy necessary for nucleation process (Fernández et al., 2006). It is well accepted that the number of nuclei formed is directly proportional to the extent of undercooling reached in the sample before nucleation occurs (Burke et al., 1975; Gilpin, 1977). There are several investigations showing that a higher extent of undercooling causes smaller ice crystal formation in pressure shift freezing (Fernández et al., 2007; Fuchigami et al., 2002; Kalichevsky-Dong et al., 2000) and in conventional freezing (Miyawaki, 2001; Petzold and Aguilera, 2009). However, there has been no published data on undercooling induced by slow freezing and its impact on ice crystal size and the properties of starch gels.

The rate of freezing is also known to affect the retrogradation rate of starch gels. Slower freezing rates increase both starch molecular associations and precipitation (Jacobson and BeMiller, 1998) and result in a harder rice starch gel (Varavinit et al., 2002) and a harder texture in cooked pasta (Olivera and Salvadori, 2009) as well as high syneresis in tapioca starch gel (Muadklay and Charoenrein, 2008). The beneficial effects of faster freezing can be attributed to the prevention or retardation of the formation of retrogradation nuclei due to the rapid transition through the rubbery state during which both nucleation and the propagation of starch chain association occur (Ferrero et al., 1994).

In this study, we were able to consistently create an undercooling condition associated with slow freezing rice starch gels and then to compare the ice crystal size, microstructure, syneresis and texture of this frozen gel with fast and slow frozen gels. Syneresis and changes in texture are two of the most important properties of frozen starch-based foods which affect acceptability by consumers. The outcome of this research would be a benefit in gaining a greater understanding of the freezing process and its behavior in a model system as well as in frozen cooked rice or starch-based products.

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2. Materials and methods

2.1. Materials

Rice starch was supplied by Thai Flour Industry Co., Ltd. (Bangkok, Thailand). The amylose content of the rice starch was 37.50% as determined by the method of Hoover and Ratnayake (2001).

2.2. Starch gel preparation

The preparation of starch gel followed the method of Charoenrein et al. (2008). Rice starch suspension (8% total solid w/w wet basis) was heated to 80 °C for 25 min. The suspension was 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 using three systems. Systems (i) and (ii) used a cryogenic cabinet freezer (Minibatch 1000 L; Bangkok Industrial Gas Co., Bangkok, Thailand) which allowed the flow rate of liquid nitrogen to be adjusted creating a cold atmosphere of −80 °C and −20 °C respectively and (iii) used a chest freezer (Sanyo refrigerator, model SF-C1497) at −20 °C. The temperature inside this freezer after sample loading was around 10–12 °C before the freezer door was closed. During freezing, sample temperatures were recorded using thermocouples placed in the sample cylinders at surface and center positions. The temperature inside the freezer was also recorded. Temperature measurements for (i) and (ii) were recorded every 30 and 60 s respectively and every 10 min for (iii) using a data acquisition system. Each experiment was repeated twice. The frozen samples were first kept in a chest freezer at −20 °C for 24 h and were then thawed at room temperature (25 ± 2 °C) for 120 min. After thawing, they were removed from the syringes prior to performing the following tests.

2.4. Determination of frozen structure with scanning electron microscopy (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 a stub, coated with gold and observed using a JSM-5600LV microscope (JEOL, England). The accelerating voltage and the magnification are shown on the micrographs.

2.5. Syneresis measurement

Syneresis measurement followed the method of Charoenrein et al. (2008). The thawed starch gel samples were removed from their syringes and put in a cylindrical plastic tube with a perforated bottom which was covered with filter paper (Whatman No. 41). These tubes were then placed in centrifuge tubes and centrifuged at 100*g* (centrifuge CN-1050, MRC Ltd., Holon, Israel) for 15 min. The cylindrical plastic tube with cover was removed from the centrifuge tube, and the liquid which had separated from the starch gel was weighed. The percentage of syneresis was then calculated as the ratio of the weight of liquid separated from the gel to the total weight of the gel before centrifugation and multiplied by 100. The data were reported as the average of five measurements.

2.6. Texture measurement

The thawed rice starch gel was transferred from the syringe into a rectangular mold (about 150 × 40 mm and 30 mm deep which had a gap for sample cutting) and the middle of the gel was cut into a sample 20 mm in length. The texture was determined using the Texture Profile Analysis method (five replicates per treatment) with a Stable Micro System (TA-XT plus) Texture Analyzer. Samples were compressed with a 100-mm diameter probe at a test speed of 0.5 mm/s. The deformation level was 40% of the original sample height and the gels were compressed twice. Hardness was expressed as the maximum force exerted during the first compression cycle.

2.7. Statistical analysis

A completely randomized design was used. 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

Rice starch gel was used as a model system in this investigation because it is a common ingredient in foods. In addition to showing sensitivity to freezing rate (Jacobson and BeMiller, 1998; Ferrero et al., 1994; Muadklay and Charoenrein, 2008), it is semi-solid and hence eliminates the possibility of convective heat transfer. Thus, a relatively uniform distribution of ice crystals is produced. Moreover, the frozen structure of a starch gel can be easily illustrated (Charoenrein et al., 2008).

3.1. Freezing of rice starch gel

Fig. 1a–c shows the temperature–time freezing curves obtained experimentally for freezing rice starch gel under different freezing regimes. Fast freezing i.e. freezing at −80 °C in a cryogenic freezer lowered the sample temperature from 20 to −20 °C within 11 min (Fig. 1a). A small but noticeable freezing plateau (around 6 min) was observed after the sample reached the freezing point; however, no undercooling was observed. Fig. 1b shows the freezing curve for slow freezing at −20 °C in a cryogenic freezer with a freezing time of 59 min. It should be noted that the temperature inside the freezer was lowered to −20 °C in only 10 min due to the high efficiency of the freezer. A clear appreciable freezing plateau (around 30 min) was observed; however no noticeable undercooling was detected at this freezing rate. Fig. 1c shows a freezing curve for slow freezing at −20 °C in a chest freezer with a long freezing time of 307 min. It can be noted that the temperature inside the freezer after closing the freezer door was approximately 10 °C. This is due to the considerable length of time the chest freezer was open during sample loading and the lower efficiency of the chest freezer (as compared to the cryogenic freezer). The time required for the chest freezer temperature to reach −20 °C was about 100 min. In this freezing regime, clearly noticeable undercooling was observed before the freezing plateau was reached (at around 90 min). Our two separate experiments involving this freezing regime showed the same freezing curve pattern. The average gel surface temperature was lowered to about −7.5 °C before instantaneous ice formation took place. The surface temperature lowered slightly faster than the center temperature. After nucleation was initiated at the surface, a temperature increasing appeared due to the released latent heat into the still unfrozen gel from the frozen surface. Therefore a slightly higher undercooling temperature at the center was noticed. Freezing

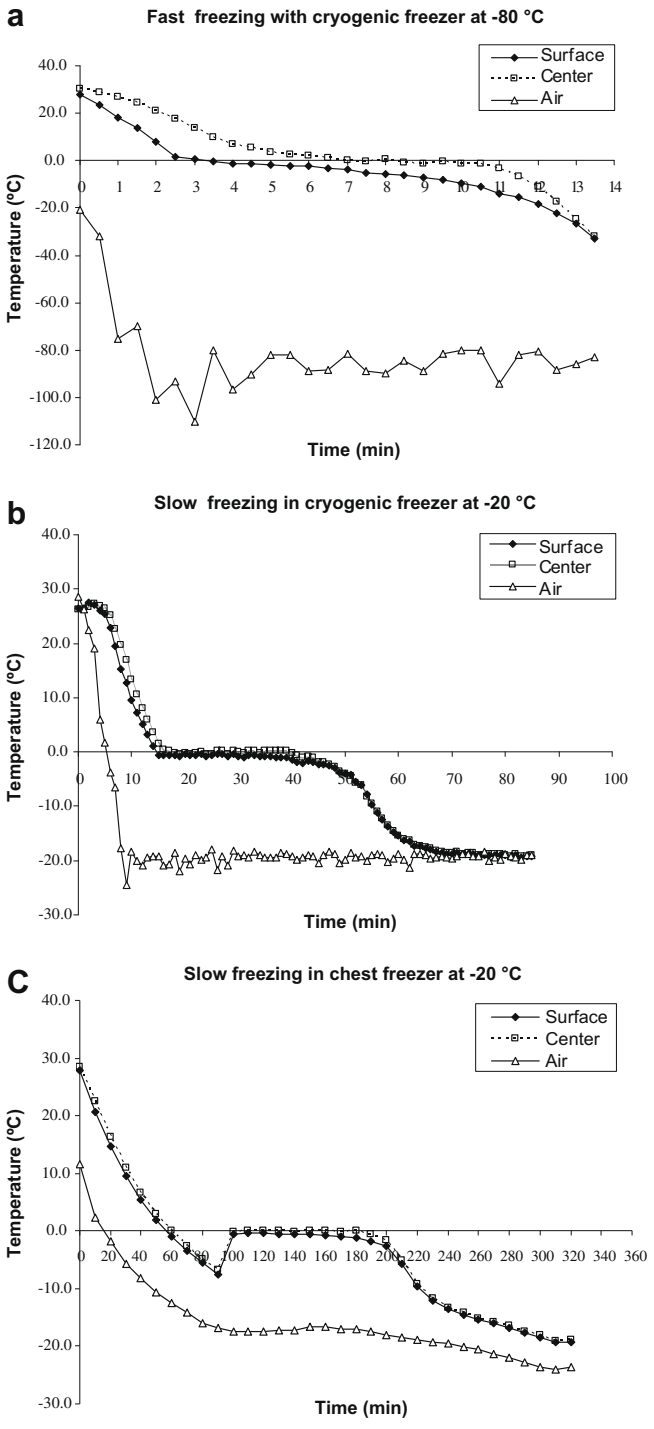

Fig. 1. Freezing curves of rice starch gels (8% w/w) at (a) −80 °C in cryogenic freezer, (b) −20 °C in cryogenic freezer, and (c) −20 °C in chest freezer.

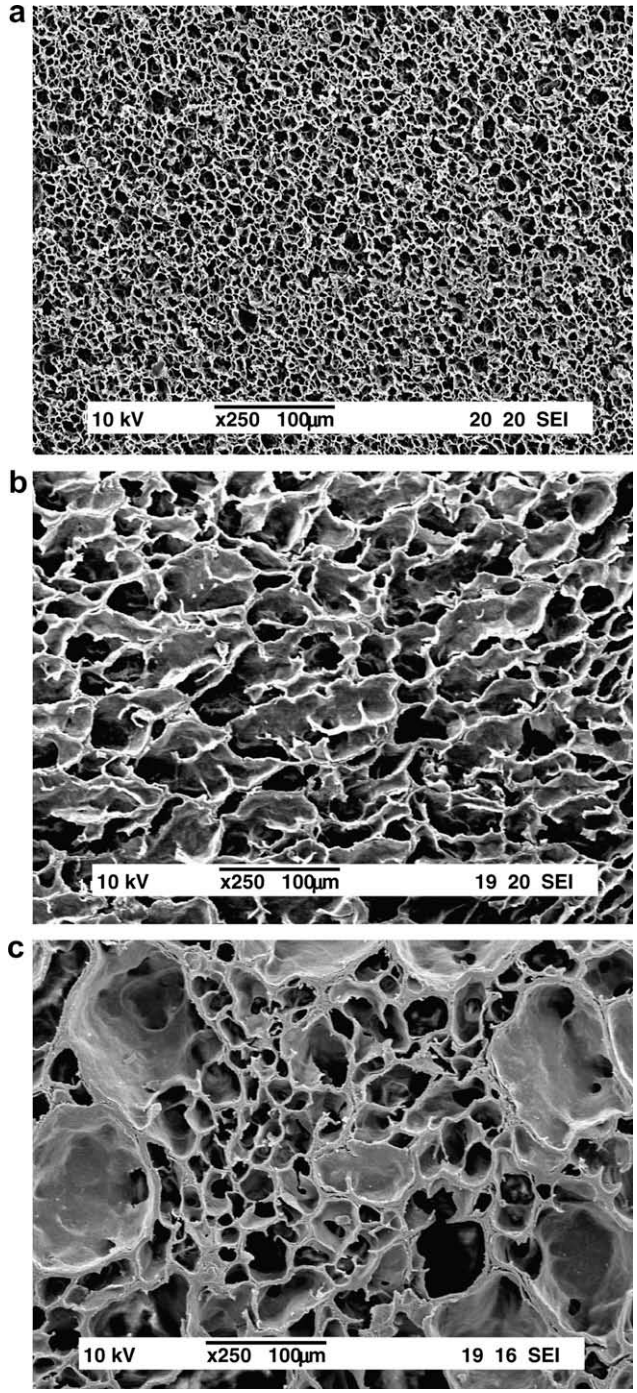

Fig. 2. SEM images of rice starch gels (8% w/w) after one freeze–thaw cycle (a) frozen at −80 °C in a cryogenic freezer, (b) frozen at −20 °C in a cryogenic freezer, and (c) frozen at −20 °C in a chest freezer (250×, bar = 100 µm).

profiles involving fast and slow freezing using the cryogenic freezer did not show undercooling and this might be because of the conditions under which these samples were frozen and the rate of heat transfer from the samples.

3.2. Frozen structure

The SEM images of frozen rice starch gels are shown in Fig. 2a–c. Ice crystals were visualized as holes left in the matrix. Fast freezing in the cryogenic freezer (−80 °C) produced, as expected, a large number of small ice crystals and each crystal was surrounded by a thin gel matrix (Fig. 2a). The shape of the ice crystals was spherical or oval and they were uniformly distributed throughout the sample. In contrast, slow frozen samples without undercooling contained a few large ice crystals embedded in a thicker gel matrix (Fig. 2b). A well-defined spongy structure with termite gallery – like pores was evident in this sample. It is surprising that the structure of samples that were slow frozen with undercooling in a chest freezer (Fig. 2c) was significantly different from that of fast and slow frozen gels. A less homogenous frozen structure was observed in this sample. It was expected, based on Fig. 1c, that high undercooling would

result in numerous small ice crystals in the frozen gel. However, the SEM micrograph showed otherwise. A cluster of small spherical ice crystals was surrounded by a few irregularly shaped large crystals within a very thick gel matrix. This frozen structure can be explained by its freezing profile (Fig. 1c). First, during cooling and undercooling which range in this sample from 10 to −7.5 °C, the gel temperature fell slowly (0.3 °C/min) which allowed amylose chains to retrograde to the highest extent as revealed by the presence of the thickest matrix around the ice crystals. In fact, several investigations have reported that low temperature accelerates retrogradation of starch gels or starch-based foods (Lin and Chein, 1995; Czuchajowska et al., 1998; Satmalee and Charoenrein, 2009). Second, although high undercooling is usually associated with a high nucleation rate (Reid, 1983) and consequently results in numerous small ice crystals (Miyawaki, 2001; Petzold and Aguilera, 2009), this phenomenon was not observed in this experiment. We understood that although a high nucleation rate was induced, with this slow freezing rate, the rate of heat removal was not sufficiently rapid to facilitate the growth of all nuclei into ice crystals. Instead, some ice nuclei might have melted due to the temperature increasing after releasing latent heat of ice crystallization while those remaining grew and became ice crystals. This explains the occurrence of non-uniform ice crystals surrounded by a very thick gel matrix.

3.3. % Syneresis

The determination of % syneresis from freeze–thaw starch gels is used to evaluate the ability of starch to withstand the undesirable physical changes which occur during freezing and thawing. Syneresis in a freeze–thawed gel is due to the increase in molecular associations between starch chains, in particular the retrogradation of amylose (Morris, 1990) which results in the expulsion of water from the gel structure (Saartratra et al., 2005). Thus the amount of water released due to syneresis is a useful indicator of the tendency of starch to retrograde (Karim et al., 2000).

Our previous study showed that medium-amylose rice flour (17.58%) gels had a significantly lower % syneresis after the first freeze–thaw cycle than did high-amylose rice flour (32.47%) gels (Charoenrein et al., 2008). This result implies that amylose plays an important role in the retrogradation associated with freezing and thawing.

Several studies on the effect of freezing rate on syneresis of freeze–thaw starch gels have been reported. Fast freezing was shown to result in lower syneresis (Muadklay and Charoenrein, 2008) or lower retrogradation (Jacobson and BeMiller, 1998; Ferrero et al., 1994) than slow freezing. The beneficial effects of fast freezing can be attributed to the prevention of starch retrogradation nuclei formation due to the rapid transition through the rubbery state during which both nucleation and propagation occur (Ferrero et al., 1994). In this study, we also found the same trend, that is, the fast frozen rice starch gel had a significantly lower % syneresis (18.02%) than the slow frozen starch gel (38.14%) as shown in Table 1. It was expected that the starch gel frozen at a slow freezing rate with undercooling freeze–thaw starch gel would have the highest % syneresis. However, this starch gel had the lowest % syneresis (1.92%) even though it retrograded to the greatest extent. This apparent contradiction could be due to the fact that the thawed water, especially that in the small pores surrounded with big pores, (Fig. 2c) was trapped in the thick matrix. In this study, we used a combination of centrifugation and filtration to simultaneously separate the released water from the freeze–thaw gels and to use this water as a measure of syneresis (Charoenrein et al., 2008). A low centrifugation force (100g) was used to minimize the impact of structural changes caused by high centrifugation force. However, the low centrifugation force applied in this study might not be sufficient to expel the water present in the thick matrix. When we removed these samples after centrifuging them and pressed them between our fingers, a large amount of water was released.

Table 1
Percent of water separated (syneresis) of rice starch gel at 8% (db) with different freezing rate. The samples were subjected to one freeze–thaw cycle.

Freezing rate	Syneresis (%)
Fast freezing (cryo −80 °C)	18.02 ± 1.49^{b}
Slow freezing (cryo −20 °C)	38.14 ± 0.69^{a}
Slow freezing (chest −20 °C)	1.92 ± 0.36^{c}

Mean values followed by the different letter are significantly different ($p < 0.05$).

Table 2
Hardness of rice starch gels with different freezing rate after subjecting to one freeze–thaw cycle.

Freezing rate	Hardness (N)
Fast freezing (cryo −80 °C)	3.92 ± 0.17^{c}
Slow freezing (cryo −20 °C)	5.95 ± 0.14^{b}
Slow freezing (chest −20 °C)	7.54 ± 1.98^{a}

Mean values followed by the different letter are significantly different ($p < 0.05$).

3.4. Texture

The textural properties of rice starch gels were studied after freezing and thawing. Table 2 shows that starch gels that were fast frozen and thawed were not as hard as were the slow frozen and thawed gels. Fast freezing took the starchy foods through the temperature zone for maximum staling faster than slow freezing (Kock et al., 1995; Muadklay and Charoenrein, 2008); therefore, much more starch retrogradation occurred during the slow freezing process which consequently resulted in the higher hardness values for the thawed gels. These results are consistent with the findings of Ferrero et al. (1994) which showed that increasing the freezing rate of 10% corn starch pastes decreased retrogradation. However, undercooling associated with slow freezing resulted in the highest hardness for the starch gels. The temperature range of this sample (10 to −7.5 °C) was lowered slowly (0.3 °C/min) which allowed amylose chains to retrograde to the greatest extent as revealed by the thickest matrix around the ice crystals in these samples. Several investigations have revealed that a temperature which is low but is above the glass transition temperature accelerates retrogradation or an increase in the hardness of starch gels and starch-based foods (Czuchajowska et al., 1998; Lin and Chein, 1995; Slade and Levine, 1986). This thick matrix caused a high hardness in the starch gel.

4. Conclusion

High undercooling can be achieved in rice starch gels using slow freezing in a chest freezer. However, in this study, with a low rate of heat transfer, a large number of the ice nuclei which formed subsequently melted and this resulted in a non-homogenous frozen structure. Clusters of relatively small ice crystals with some large ice crystals embedded in a very thick gel matrix resulted due to the large extent of retrogradation which occurred during the long cooling and undercooling period. This frozen structure caused a low % syneresis and high hardness which is different from starch gel properties seen in normal fast and slow freezing. However, further work on syneresis measurement with higher centrifugal force needs to be conducted to elucidate the relationship between

syneresis and microstructure of slow frozen rice gel with undercooling. This overall finding will assist in the understanding of changes in starch-based food during freezing and storage.

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