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ScienceDirect
Carbohydrate Polymers 73 (2008) 143–147
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

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

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Received 4 August 2007; received in revised form 31 October 2007; accepted 8 November 2007
Available online 17 November 2007

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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0144-8617/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2007.11.012

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

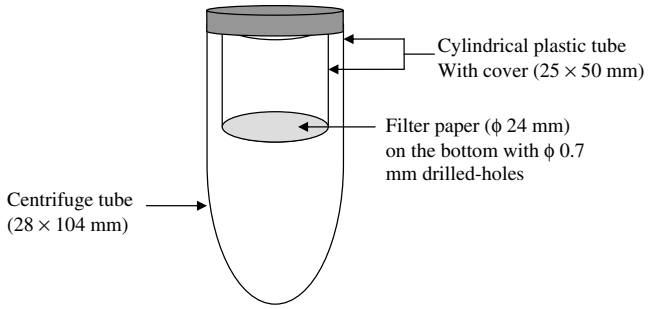

Fig. 1. Apparatus for centrifugation–filtration of gels (tube set).

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.

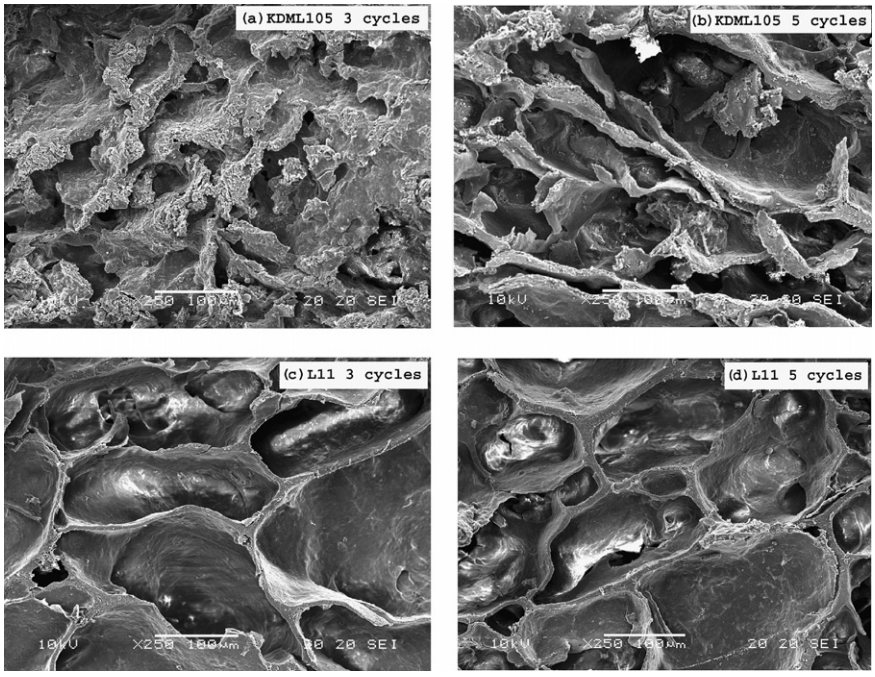

Fig. 2. SEM images of freeze–thaw rice flour gels. (a) KDML 105 after 3 cycles, (b) KDML 105 after 5 cycles, (c) L11 after 3 cycles, and (d) L11 after 5 cycles.

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.

Acknowledgements

Financial support from the Thailand Research Fund under project RSA 5080014 is acknowledged. The authors thank Andrew J. Mullard for reviewing the manuscript and Thunyaboon Arunyanart, Yupadee Poapun, and Sajjawat Charoenrien for SEM and graphic advice.

References

AACC. (2000). *Approved Methods of the American Association of Cereal Chemists*, 10th ed., American Association of Cereal Chemists, St. Paul, MN.

Chen, Z., Schols, H. A., & Voragen, A. G. J. (2003). Physicochemical properties of starches obtained from three varieties of Chinese sweet potatoes. *Journal of Food Science, 68*, 431–437.

Eliasson, A.-C., & Kim, H. R. (1992). Changes in rheological properties of hydroxypropyl potato starch pastes during freeze–thaw treatments. 1. A rheological approach for evaluation of freeze–thaw stability. *Journal of Texture Studies, 23*, 279–295.

Ferrero, C., Martino, M. N., & Zaritzky, N. E. (1994). Corn starch–xanthan gum interaction and its effect on the stability during storage of frozen gelatinized suspensions. *Starch/Stärke, 46*(8), 300–308.

Karim, A. A., Norziah, M. H., & Seow, C. C. (2000). Methods for the study of starch retrogradation. *Food Chemistry, 71*, 9–36.

Lee, M. H., Baek, M. H., Cha, D. S., Park, H. J., & Lim, S. T. (2002). Freeze–thaw stability of sweet potato gel by polysaccharide gums. *Food Hydrocolloids, 16*, 345–352.

Lee, K. Y., Kim, Y.-R., Park, K. H., & Lee, H. G. (2006). Effects of a-glucanotransferase treatment on the thermo-reversibility and freeze–thaw stability of a rice starch gel. *Carbohydrate Polymers, 63*, 347–354.

Morris, V. J. (1990). Starch gelation and retrogradation. *Trends in Food Science and Technology, 1*, 2–6.

Radley, J. A. (1976). *Industrial Uses of Starch and its Derivatives*. London: Applied Sciences Publishers.

Rahman, M. S. (1999). Food preservation by freezing. In M. S. Rahman (Ed.), *Handbook of Food Preservation* (pp. 259–284). New York: Marcel Dekker.

Saartratra, S., Puttanlekb, C., Rungsardthong, V., & Uttapap, D. (2005). Paste and gel properties of low-substituted acetylated canna starches. *Carbohydrate Polymers, 61*, 211–221.

Schoch, T. J. (1968). Effects of freezing and cold storage on pasted starches. In D. K. Tressler, W. B. van Arsdel, & M. J. Copley (Eds.). *The Freezing Preservation of Foods* (Vol. 4, pp. 44–56). Westport, CT: AVI.

Varavinit, S., Shobsngob, S., Varanyanond, W., Chinachoti, P., & Naivikul, O. (2002). Freezing and thawing conditions affect the gel stability of different varieties of rice flour. *Starch/Stärke, 54*, 31–36.

Wu, S. H., & Seib, P. A. (1990). Acetylated and hydroxypropylated distarch phosphates from waxy barley: paste properties and freeze–thaw stability. *Cereal Chemistry, 67*, 202–208.

Yuan, R. C., & Thompson, D. B. (1998). Freeze–thaw stability of three waxy maize starch pastes measured by centrifugation and calorimetry. *Cereal Chemistry, 75*, 571–573.

Yeh, A.-I., & Yeh, S.-L. (1993). Some characteristics of hydroxypropylated and cross-linked rice starch. *Cereal Chemistry, 70*, 596–601.