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Improvement on the freeze–thaw stability of corn starch gel by the polysaccharide from leaves of *Corchorus olitorius* L.

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ABSTRACT

Effect of the polysaccharide from leaves of *Corchorus olitorius* L. (PLC) on the freeze–thaw (FT) stability of corn starch gel was studied. PLC was incorporated into the starch gel at 0.7% and total solid was adjusted to 6.0%. The syneresis was measured by the centrifugal-filtration method and, as a result, addition of PLC reduced effectively the syneresis of the starch gel even after 5 FT cycles, which was less than one third that of the normal starch gel. The rheological changes of the starch/PLC gel during the FT treatments were evaluated while the gel remained on the rheometer plate. The starch/PLC gel had less significant changes in the rheological parameters during the FT cycles than starch/guar gum or xanthan gum gel systems. SEM images showed that PLC stabilized the gel matrix surrounding pores, which would contribute to both a lower syneresis production and a higher stability in the rheological behavior at FT.

1. Introduction

Freezing is one of the most effective ways to preserve food quality. However, for food product developers, the difficult task is to design foods in such a way as to minimize the detrimental effects that freezing and thawing induce. Freezing of foods causes serious changes to structure, due to a combination of both the formation of ice crystals and the freeze-concentration of solutes and dispersed macromolecules in the unfrozen phase (Goff, 1992). In the case of starch-based foods, freezing and thawing changes their textural properties drastically due to amylose and amylopectin crystallization (retrogradation). There are at least two ways to prevent or retard the changes during freezing and thawing of starch-based foods. The first is shortening the time required for the freezing and thawing, which significantly affects the retrogradation rate (Muadklay & Charoenrein, 2008). There is less retrogradation at faster freezing rates. However, this way usually involves a high initial cost. In addition, the shape of the food must be as small and thin as possible in order to exchange heat efficiently. The second is to use food ingredients such as sugars and polymers. Addition of a large amount of sucrose, usually more than 20% (w/w), is known to retard starch retrogradation. But the sweetening effect of sucrose restricts applications to many foods. On the other hand, some kinds of food hydrocolloids improve the freeze–thaw (FT) stability by addition of small amounts of hydrocolloids, usually less than 1% (Norton & Foster, 2002). Therefore, there are numerous research reports about application of food hydrocolloids to stabilize the FT treatments. Charoenrein, Tatirat, Rengsutthi, and Thongngam (2011) reported the effect of konjac glucomannan on syneresis, textural properties and the microstructure of frozen rice starch gels. It was found that, when konjac glucomannan was incorporated into rice starch gels that were then subjected to repeated FT cycles, the hydrocolloid reduced the %syneresis and moderately increased gel hardness. Further, scanning electron microscopy of the gel showed that it contained smaller pores and less well-defined surrounding matrices than those without konjac glucomannan. In another study, xanthan gum improved the rheological properties of tapioca starch-based products (Pongsawatmanit & Srijunthongsiri, 2008). Application of novel hydrocolloids is still needed to satisfy the increasing demands for maintaining higher quality of food products by consumers as well as food industries, although effects of conventional hydrocolloids addition have been investigated.

Corchorus olitorius L. is native to Egypt and called Mulukhiyya. Stem of the plant is an important source of fiber known as jute in India and countries of the circumference. Leaves of the plant are so rich in nutrients that these are a very popular and important

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summer vegetable in Egypt, the Middle East, Southeast Asia, and Fareast Asia, including Japan. It is also well known that leaves of the plant contain water-soluble mucilage. Recently, we have reported the ammonium sulfate fractionation of the water-soluble polysaccharide extracted from the leaves of *C. olitorius* L. (PLC) that gave a high-viscosity preparation (Yamazaki, Kurita, & Matsumura, 2009). The aim of this study was to investigate the stabilizing effect of PLC on corn starch gel as a function of FT cycles.

2. Materials and methods

2.1. Materials and chemicals

Polysaccharide from the leaves of *C. olitorius* L. was prepared by the method previously reported (Yamazaki et al., 2009). Corn starch employed in this study was normal type, which means not waxy type, and purchased from Wako Pure Chemical Industry (Japan). High methoxyl pectin (HMP), whose degree of methylation was approximately 73.6% determined by the alcohol oxidase test (Yamazaki et al., 2009), was also purchased from Wako Pure Chemical Industry. Commercial xanthan gum (XG), guar gum (GG), locust bean gum (LBG) and sodium alginate were obtained from Taiyo Kagaku (Japan).

2.2. Corn starch/gum paste system preparation

Corn starch powder containing gum (0.7% gum, 6% total solids) was added to water with vigorous stirring. The mixture was stirred for an hour at room temperature and then it was pasted in a Rapid Visco-Analyzer (RVA, Model Super4, Newport Scientific Pty. Ltd. Instruments and Engineer, Australia). The sample was held at 50 °C for 6 min, heated to 95 °C at a constant rate of 9 °C/min and held at 95 °C for 7 min. Then the paste was used for the syneresis and the rheological measurements.

2.3. Syneresis measurement

Approximately 20 g of corn starch/gum paste system (95 °C) prepared with a RVA was poured into centrifuge tubes (117 mm × 35 mm, VectaSpin 20, Whatman, UK) and placed in an incubator at 25 °C for 2 h. The tubes were then frozen at −40 °C for 22 h in a freezer (NF-400SF3-A, NIHON FREEZER, Japan) and then thawed at 30 °C for 2 h in an incubator (MINI-SUBZERO, ESPEC, Japan). This FT cycle was repeated for up to 5 cycles. In the syneresis measurement, the water separation was measured via centrifugation and simultaneous separation (Charoenrein, Tatirat, & Muadklay, 2008) because starch gels tend to reabsorb a considerable amount of water. The thawed starch/gum gel system was removed from the centrifuge tube and put on an insert (20 mL capacity, 10 μm mesh bottom) of a VectaSpin 20. The insert was placed in the tube, which was then centrifuged at 100 × g for 15 min. The liquid separated from the corn starch/gum gel system was collected and the percentage of syneresis (%syneresis) was calculated according to Eq. (1). The data were reported as average of three measurements.

$$\%\text{Syneresis} = \frac{\text{Weight of separated liquid from gel}\quad(\text{g})}{\text{Total weight of gel before centrifuging}\quad(\text{g})} \times 100 \qquad (1)$$

2.4. Flow rheological measurements

Gums, other than locust bean gum (LBG), were dissolved in distilled water at room temperature by stirring overnight. Since LBG was only slightly soluble in cold water, it was first dispersed in distilled water at room temperature, and the mixture was heated at 100 °C for 10 min. Then, the solution was cooled to room temperature and stirred overnight. Flow behavior of the gum solutions was obtained with a rheometer (AR-G2, TA Instruments, DE, USA) with a cone (60 mm dia.; 2° angle) and a plate system. The gum solutions were sheared from 0.01 to 100 (1/s) at 25 °C.

2.5. Dynamic rheological measurements

Corn starch/gum paste systems (95 °C) prepared with a Rapid Visco-Analyzer were loaded between parallel plates (1 mm gap) with a Peltier temperature control system in a rheometer (AR-G2, TA Instruments, DE, USA). After trimming off the over-loaded portion of the samples around the plates, samples were held at 25 °C for 15 min to develop gel structure via amylose crystallization (Biliaderis & Zawistowski, 1990; Miles, Morris, Orford & Ring, 1985; Miles, Morris & Ring, 1985; Ring et al., 1987). Then small amplitude oscillatory rheological measurement was performed by frequency sweep at a constant deformation (2% strain). The 2% strain was in the linear viscoelastic region of the corn starch/gum gel systems tested. The samples were immediately cooled to −18 °C for 8 min and held for 1 min, and then heated to 25 °C at 20 °C/min and held for 1 min. Small amplitude oscillatory measurements (frequency sweep) were performed again. In the experimental conditions, it was confirmed that the dynamic measurement of the gel systems was not affected by the previous dynamic measurement (data not shown). More, we controlled the normal force of the rheometer plate to 0 (N) so as to freely change the gap during freezing and thawing. This enabled the rheometer plate not to separate from gels and not to slip during the measurements. The FT cycle was repeated for up to 5 cycles. The storage modulus (G'), loss modulus (G'') and loss tangent ($\tan\delta = G''/G'$) were obtained.

2.6. Scanning electron microscopy

Each freeze-thawed gel prepared as described in Section 2.3 was immediately lyophilized. Sample was cut with a microtome (model TH, Kenis, Japan), and the cut-surface was sputter-coated with a thin layer of gold-palladium alloy. Scanning electron microscopy (SEM) was performed using an S 4100 microscope (Hitachi, Japan). The accelerating voltage and the magnification were 5 kV and 100, respectively.

3. Results and discussion

3.1. Syneresis

The ability of starch to withstand the undesirable physical changes during freezing and thawing has been commonly termed “freeze–thaw” stability and can be used as an indicator of the tendency of starch to retrograde (Schoch, 1968). When a starch gel is frozen, the formation of ice crystals creates starch-rich regions in the gel matrix in which water remains unfrozen and in which chain associations to form thick filaments are facilitated. Upon thawing, bulk phase water is easily released from the polymeric network, a phenomenon known as syneresis (Karim, Norziah, & Seow, 2000). Therefore, the amount of syneresis has been assumed to be directly related to the tendency of a starch to retrograde. However, as measured in this research, it may also be related to the ability of the hydrocolloid to thicken the aqueous phase and/or to hold water. Thus the effects of HMP, sodium alginate, GG, LBG, XG, and PLC on the amounts of syneresis in the corn starch/gum gel system were compared and the results are shown in Fig. 1. Under the experimental conditions used, the control gel lost 58.7% of total weight of the gel after the first FT cycle and showed little change through subsequent FT cycles. Addition of HMP, sodium alginate and LBG

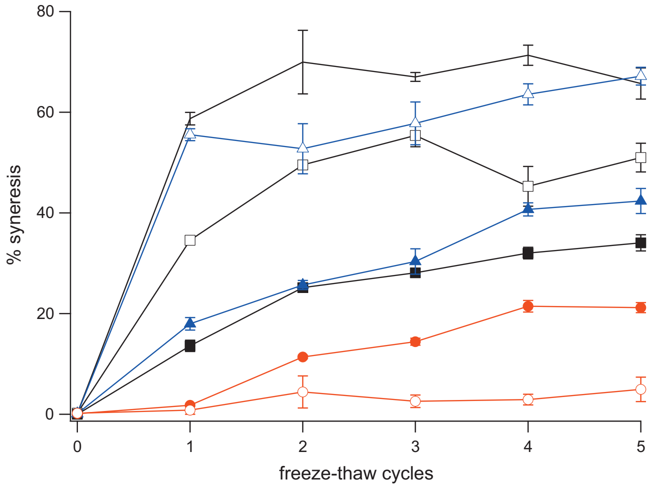

Fig. 1. Effect of number of freeze–thaw cycles on %syneresis of corn starch gels with and without gums: normal corn starch (solid line), guar gum (■), sodium alginate (▲), xanthan gum (○), locust bean gum (□), high methoxyl pectin (△), PLC (●). Reported values are the mean of three independent experiments and error bars represent standard deviations.

resulted in large amounts of %syneresis after 5 FT cycles, indicating that it was difficult to reduce syneresis of the corn starch gel at repeating FT cycles by addition of such gums. The %syneresis of the corn starch/gum gel systems containing GG increased steadily throughout 5 FT cycles to 34.1, although GG was somewhat effective in reducing syneresis (18.0%) at the first FT cycle. Addition of XG and PLC to the corn starch gel resulted in a significantly reduction of %syneresis at the first FT cycle (0.8% and 1.8%, respectively). PLC had the highest reducing effect among the gums except XG although %syneresis of the gel containing PLC slightly increased through cycles to 22.2% after 5 FT cycles.

Fig. 2 shows the flow behavior of the gum aqueous solutions tested (0.7% (w/w), 25 °C). There was obviously important relation between the syneresis result of corn starch/gum systems and the viscosity of the gum solutions: the gums had high viscosity (GG, PLC and XG) were advantageous to reduce %syneresis of the system. On the other hand, viscosity of PLC and XG were obviously greater than that of GG at lower-shear region. It is probable that strong psuedoplasticity of PLC and XG thicken the aqueous phase of the system more strongly and hold water more tightly. But there may not a cause-and-effect relationship: e.g., syneresis decrease in XG-added starch gel was in valid agreement with many literature findings (e.g., Lee, Baek, Cha, Park & Lim, 2002). They clamed that XG associates with amylose and reduces amylose-amylose interactions and, as a result, starch retrogradation would be retarded and syneresis also would be reduced. At the present state, it is not clear whether PLC would associate with amylose and/or amylopectin.

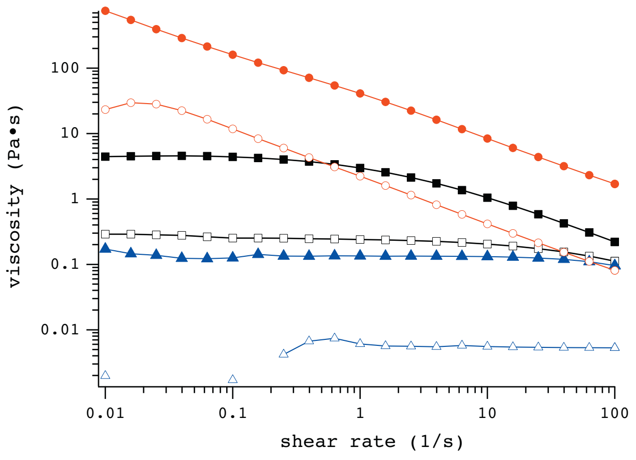

Fig. 2. Flow behavior of the gums (0.7%, w/w, 25 °C): guar gum (■), sodium alginate (▲), xanthan gum (○), locust bean gum (□), high methoxyl pectin (△), PLC (●).

3.2. *Rheological results*

Each rheological test was repeated more than three times. Given here are typical results. Based on the syneresis results as shown in Fig. 1, GG, XG and PLC were selected from the six gums tested to examine the effect of the FT treatment on the rheological behavior of the corn starch gel. In general, a material whose G' and G'' are frequency-independent over a large time scale, with $G' \gg G''$, is solid-like. In contrast, strong frequency-dependence suggests a material structure with molecular entanglements. Such material behaves more solid-like (lower tan δ) at higher frequencies and more liquid-like (higher tan δ) at lower frequencies (Kaur, Singh, Singh, & McCarthy, 2008). In this study, G' and G'' of the corn starch/gum gel systems were frequency independent over a large time scale, with $G' \gg G''$, suggesting that these systems behaved as a solid-like material (data not shown). Thus, changes in rheological behavior of the freeze-thawed gels were evaluated by determining the loss tangent (tan δ) obtained by frequency-sweep analysis.

For the freeze-thawed normal corn starch gel, the magnitude of tan δ decreased significantly after the first FT cycle, indicating that its overall structure became firmer and more solid-like (Fig. 3a). Following the first FT cycle, tan δ of the gel decreased gradually with increasing the FT cycles. The magnitude of tan δ without FT cycle would be mainly attributed to amylose crysatallization which involves rapid establishment of a cross-linked network of amylose chains since its process is so fast as to have almost finished during the initial aging (25 °C, 15 min) before the dynamic rheological measurement. On the other hand, amylopectin gelation is much slow process involving intra- and intermolecular chain association (Karim et al., 2000). Therefore, the significant decrease of tan δ after the first FT treatment would be mainly attributed to amylopectin crystallization.

There have been numerous research reports dealing with the rheological changes of starch gels induced by FT treatment. However, to the our knowledge, techniques of evaluating them seemed to be limited to only uniaxial compression methods such as texture analysis, e.g., Sae-kang and Suphantharika (2006). Dynamic rheological method has been mainly applied for evaluation of the magnitude of starch retrogradation during storage at low temperature. Uniaxial compression methods usually apply to relatively firm gels, and a large number of replicated samples of uniform dimensions are required to obtain acceptable reproducibility because heterogeneity of the rheological profile within and between samples could seriously affect the validity of the results obtained (Karim et al., 2000). The dynamic rheological method used in this study can be performed on very soft gels because they can be formed in the rheometer; furthermore, the freezing and thawing treatment can be controlled rapidly, continuously and precisely without removing gel from the plates of the rheometer. Thus the method presented in this study appears to be simpler and more attractive.

Tan δ of the corn starch/GG and XG gel systems without FT treatment were higher than that of the normal corn starch gel, indicating that addition of GG and XG to the corn starch gel resulted in its overall structure becoming weaker and less solid-like (Fig. 3b and c). There is considerable literature on the phenomenon that the presence of a hydrocolloid makes a starch gel less solid-like. These rheological changes will be disadvantageous to use in real food systems because the food texture of the composite gels may evoke a different one from the normal corn starch gel. Comparing GG and

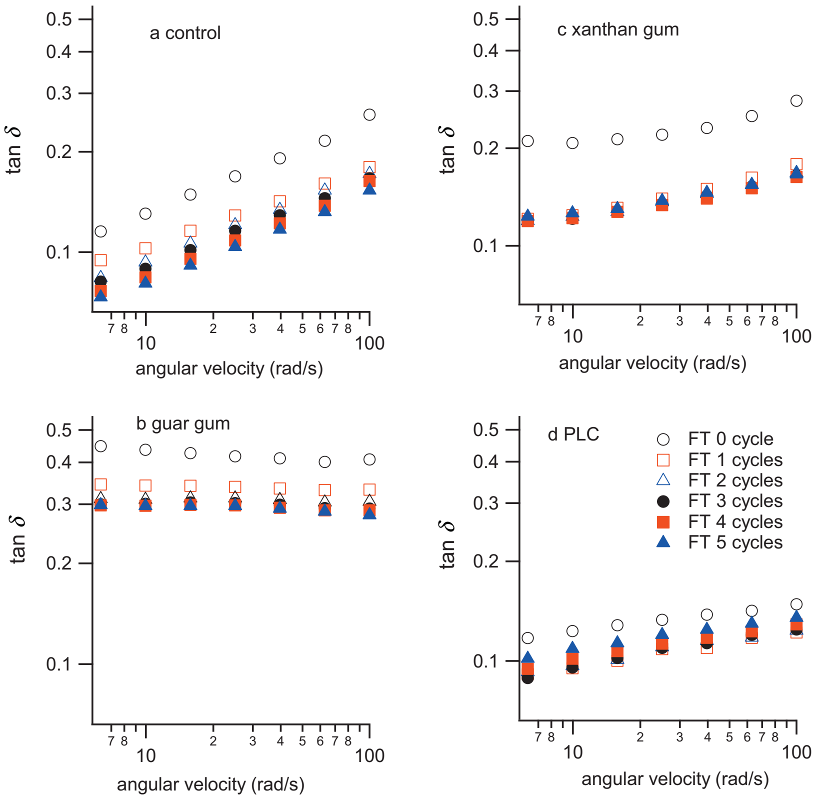

Fig. 3. Changes in the mechanical spectra of tan δ during the freeze–thaw cycles: (a), (b), (c) and (d) show the results of the gel system of normal corn starch, guar gum, xanthan gum, and PLC, respectively.

XG, the addition of GG obviously produced a greater effect than that of XG. Similar results were reported by other researchers, e.g., Christianson (1982) who found that XG had a marked effect on starch gel rigidity (increased), while GG had only a small effect. Because molecules of XG are rather rigid, while molecules of GG are flexible, BeMiller (2011) implied that the molecular structures of XG and GG play important roles of the viscoelastic properties of starch paste and/or gel. When the corn starch/GG and XG gel systems were freeze-thawed, tan δ of both of the systems decreased significantly at the first FT cycle, which is similar to the result of the normal corn starch gel. And then, for GG, the decrease of tan δ gradually decreased with FT cycles increasing (Fig. 3b) and, for the XG, the changes of tan δ were small (Fig. 3c).

Tan δ of the corn starch/PLC gel system during the FT cycles is shown in Fig. 3d. Without the FT treatment, the magnitude of tan δ of the corn starch/PLC gel system was similar to that of the normal starch gel at lower frequency range. Tan δ of the corn starch/PLC gel system was more frequency-independent than that of the normal corn starch gel, indicating that addition of PLC to the corn starch gel changed its rheological behavior to one that was more solid-like. These results also suggested that PLC associated with starch molecules and the PLC-starch molecule interactions were incorporated in the cross-linked network of corn starch gel. When the corn starch/PLC gel system was freeze-thawed, the decrease of tan δ was observed at the first FT cycle, as was the case with other gel systems. However, the decrease of tan δ appeared to be the least among the gel systems, including the normal corn starch gel. In addition, tan δ gradually recovered with increasing FT cycles. Therefore, the corn starch/PLC gel system not only exhibited a similar rheological behavior (tan δ) to the normal corn starch gel but the gel system was rather stable against the FT cycles.

On the other hand, the recovery of tan δ seen after second and subsequent FT cycles is attributed to the decrease of G' (data not shown). Unlike the corn starch/PLC gel system, G' of other corn starch/gum gel systems increased according to the number of the FT cycles (data not shown). These results strongly imply that repeating FT treatments promotes both starch molecule association and PLC-starch molecule dissociation. The first FT treatment would primarily promote the former, which would be more dominant than the latter, which would result in the significant decrease of tan δ. Following to the second cycles, the PLC-starch molecule dissociation would be more dominant than the starch molecule association, which would result in the increase of tan δ.

3.3. Scanning electron microscopy

When a starch gel is frozen, phase separation occurs with the formation of starch-rich regions. The extent of phase separation increases with an increase in the number of the FT cycles due to an increase in amylopectin retrogradation in the starch-rich phase (Yuan & Thompson, 1998). In addition, repeated FT cycles develop a thick fibrillar network in the spongy structure (Arunyanart & Charoenrein, 2008; Christianson, 1982; Lee et al., 2002; Sae-kang & Suphantharika, 2006). To elucidate the FT stabilizing effect of PLC on the corn starch gel, the microstructure of freeze-thawed gels was examined by SEM (Fig. 4). In the normal corn starch gel, the first FT treatment developed pores in the gel (Fig. 4a). After 3 FT cycles, the pore size were relatively larger and the matrix surrounding pores was thicker (Fig. 4b). Furthermore, after 5 FT cycles, the pore size was even larger and the matrix surrounding pores even thicker (Fig. 4c). On the other hand, the corn starch/PLC gel system produced rather larger pores than that of the normal corn starch gel after the first FT cycle (Fig. 4d), which implied larger ice crystal formations. Similar results were reported previously when XG was incorporated into tapioca starch (Sae-kang et al., 2006) and

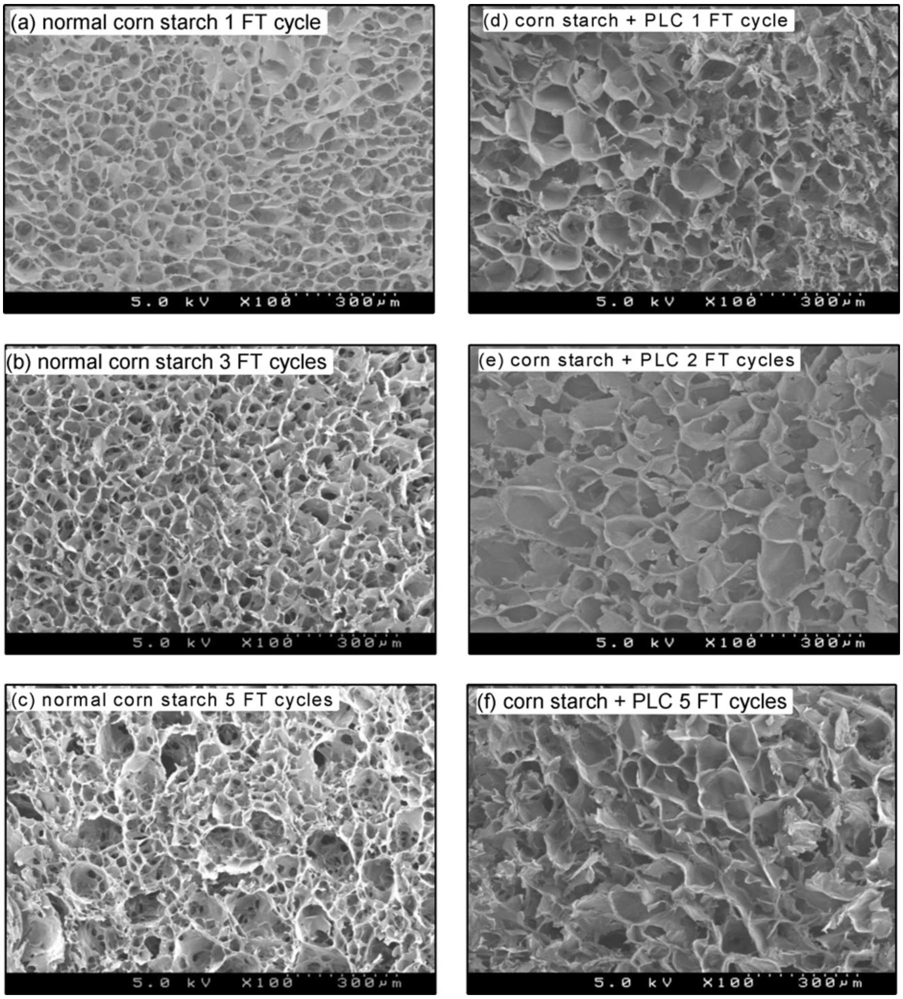

Fig. 4. SEM images of the normal corn starch and the corn starch/PLC gel system after freeze–thaw for 1, 3 and 5 cycles.

sweet potato starch (Lee et al., 2002) gels. It was assumed that addition of XG to starch resulted in a reduction of the gelling component by the substitution of starch with XG. With succeeding FT cycles, the pore size appeared to be larger slightly, while the matrix surrounding the pores did not exhibit any significant differences (Fig. 4e and f). Consequently, addition of PLC to the corn starch gel effectively stabilized the microstructure of the corn starch gel because PLC could maintain the matrix surrounding pores in the starch gels, which would contribute both a lower syneresis production and a higher stability in the mechanical behavior at FT cycles.

4. Conclusion

Addition of PLC reduced the %syneresis of a corn starch gel, though the effect was less effective than that of XG. However, the freeze–thaw stability should not be only evaluated by the high reduction of the %syneresis of the gel. In this paper, we introduced a new method to assess the rheological changes of the gel during the FT cycles. Conducting the FT cycles while the gel was on the plate of the rheometer allowed more precise and faster measurements for rheological changes than the conventional method. From the result, PLC gave a high rheological stability to the corn starch gel against FT treatment. These findings are expected to develop new starch-based foods which result in less syneresis and give less rheological changes against FT treatments.

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