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Effect of konjac glucomannan on syneresis, textural properties and the microstructure of frozen rice starch gels

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ABSTRACT

Repeatedly frozen and thawed rice starch gel loses quality. This study investigated how incorporating konjac glucomannan (KGM) in rice starch gel affects factors used to measure quality. When rice starch gels containing 0–0.5% KGM were subjected to 5 freeze–thaw cycles KGM reduced the % syneresis and moderate increases in gel hardness. SEM of freeze–thaw gels showed starch gel with KGM had smaller pores and less well-defined surrounding matrices than those without KGM. Moreover, CLSM of unfrozen gels without KGM showed densely aggregated swollen starch granules while those in gels with KGM were more evenly distributed. Furthermore, starch pastes with KGM showed higher viscosities than paste without KGM suggesting KGM inhibited granule association. These results suggest that KGM retards rice starch gel retrogradation induced by freeze–thaw treatment and that KGM is effective in preserving quality in freeze–thaw rice starch gels.

1. Introduction

As demand for ready-to-eat food products increases, a variety of new frozen foods are continually launched into world markets. Upon freezing, however, water in the food is transformed into ice and, as the ice separates out, the concentration of the unfrozen phase in contact with the ice increases. Both the ice formation and the increasing concentration of the unfrozen component result in physical stress on the food matrix (Reid, 1999; Reid, Kerr, & Hsu, 1994). When this frozen food is thawed for consumption, the moisture readily separates from the matrix and causes a change in the texture, drip loss, and often deterioration in overall quality (Rahman, 1999).

Starch-based frozen food products undergo textural changes related to amylose and amylopectin retrogradation and show syneresis after thawing. These changes attributed to starch retrogradation (Ferrero, Martino, & Zaritzky, 1994; Jacobson & BeMiller, 1998; Varavinit, Anuntavuttikul, & Shobsngob, 2000) may make such products unacceptable to consumers (Ferrero, Martino, & Zaritzky, 1993).

Hydrocolloids are commonly used to improve the texture and rheological properties of starch-based products (Shi & BeMiller, 2002) because, through the use of small quantities of hydrocolloids (Mali et al., 2003), products can be modified to have a higher viscosity and undergo less syneresis. Furthermore, hydrocolloids reduce starch retrogradation and improve gel stability in frozen starch gel systems (Ferrero et al., 1994; Lee, Baek, Cha, Park, & Lim, 2002). Ferrero et al. (1994) report that adding xanthan gum to corn starch pastes minimizes amylose retrogradation, syneresis and rheological changes after freezing. In addition, guar gum and locust bean gum were found to reduce syneresis in freeze–thaw corn starch and waxy *Amaranthus paniculatus* starch (Sudhakar, Singhal, & Kulkarni, 1996). In both of these studies, the authors base their conclusions on pasting properties and rheological data and they attribute this reduction to a slowing of retrogradation brought about by an interaction between the hydrocolloid and amylose. In another study, Ferrero and Zaritzky (2000), using oscillatory rheological measurements and visual observation, reported that the hydrocolloid might interact with amylose released outside the starch granule, inhibiting the development of a spongy matrix.

Konjac glucomannan (KGM) is a hydrocolloid obtained from the tubers of *Amorphophallus konjac* C. Koch. KGM is comprised of blocks of β-1,4-linked mannosyl and glucosyl residues, with approximately 5–10% acetylation. It has been recognized as GRAS (generally recognized as safe) by a consensus of scientific opinion since 1994 (Khanna & Tester, 2006; Takigami, 2000). The effects of KGM on gelatinization and retrogradation of maize starch (Yoshimura, Takaya, & Nishinari, 1996), and its effects on the rheological properties of maize starch gels (Bahnassey & Breene, 1994;

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Fanta & Christianson, 1996; Shelso, 1990; Yoshimura, Takaya, & Nishinari, 1998) have received the most recent attention.

No reports are as yet available on the use of hydrocolloids to reduce changes in frozen rice starch gel nor on the use of confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) techniques to study the gel's microstructure in order to understand the effect of the hydrocolloids on rice starch gels. Therefore, the objective of this study was to determine the effect of KGM in frozen rice gels by investigating their syneresis, textural changes and the correlation between these two properties. In addition, the microstructure of unfrozen and freeze–thaw gels were studied in order to gain a greater understanding of the interaction between KGM and starch. This research will make a contribution toward the improvement in the quality of frozen rice-based products.

2. Materials and methods

2.1. Materials

Rice starch was supplied by Choheng Rice Vermicelli Co., Ltd., Nakornpathom, Thailand. The amylose content and moisture content of rice starch was 31.58% and 11.41% respectively (AACC, 2000). Purified KGM (KGM ≥ 90%) was purchased from Diethelm Co., Ltd., Bangkok, Thailand.

2.2. Sample preparation

A rice starch suspension (8.0% (w/w) total solids) was prepared by stirring rice starch and water continuously at 250 rpm for 60 min. The suspension was gelatinized by placing it in a water bath at 80 °C for 25 min with continuous stirring at 200 rpm. Ten ml samples were then loaded into syringes (25 ml with a 20 mm diameter) and steamed for 9 min. Finally, the samples were placed in an incubator at 25 °C for 120 min.

Suspensions containing 0.3% and 0.5% KGM (8.0% (w/w) total solids) were prepared in two steps. KGM was sprinkled into 75% of the total suspension volume of water at room temperature and the mixture was stirred at 250 rpm for 120 min. The rice starch was suspended in the remaining 25% of the total suspension volume of water and was then added to the KGM solution. Both rice starch suspensions containing KGM (0.3 and 0.5%) were gelatinized using the same process as was used for the control rice starch suspension. Each experiment was repeated twice.

2.3. Freezing and thawing

Starch gel samples were frozen in a chest freezer at −18 °C for 22 h and then thawed at room temperature (25 ± 2 °C) for 120 min. This freeze–thaw cycle was repeated for up to 5 cycles. After thawing, gels were removed from the syringes prior to performing the following tests.

2.4. Syneresis measurement

Syneresis measurements followed the method of Charoenrein, Tatirat, and Muadklay (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.

2.5. Determination of the microstructure of frozen starch gel with SEM

The freeze–thaw rice starch gels with and without KGM 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.

2.6. Determination of the microstructure of unfrozen starch gel with CLSM

The unfrozen rice starch gels with and without KGM were cut into sections of 1–3 mm thickness using a razor blade. The sections were stained by immersion into FITC-dextran (fluorescein isothiocyanate dextran 0.01% (w/v) in distilled water) for 2 min followed by rinsing in distilled water for 3 times. The sample was mounted in a slide well and covered with a cover glass. Images were recorded using a confocal laser scanning microscope (Axio Imager MI, Carl Zeiss PTe Ltd., Germany). A HeNe laser with an excitation wavelength of 488 nm was used. CLSM digital images were acquired using the LSM 5 PASCAL program.

2.7. Pasting profile

The pasting properties of rice starch suspension (8%, w/w) with 0, 0.3 and 0.5% KGM were determined using a Rapid Visco-Analyzer (model RVA-4C, Newport Scientific Pty. Ltd., Warriewood, Australia). The slurry was held at 50 °C for 1 min, heated to 95 °C at a constant rate of 12 °C/min and then held at 95 °C for 2.5 min. It was subsequently cooled to 50 °C at the same rate and then held at 50 °C for 2 min. The data were reported as the average of triplicate measurements.

2.8. Texture measurement

The thawed rice starch gel was transferred from the syringe into a rectangular mold approximately 150 mm × 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 60% of the original sample height and the gels were compressed twice. Hardness was expressed as the maximum force exerted during the first compression cycle.

2.9. 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. Percent syneresis

The determination of % syneresis from freeze–thaw starch gels is used to evaluate the ability of starch to withstand the undesirable

Table 1
Syneresis values of rice starch gel (8.0% (w/w) total solid) containing KGM 0, 0.3 and 0.5% in each cycle.

Sample	Syneresis (%)				
	1 cycle	2 cycle	3 cycle	4 cycle	5 cycle
Rice starch	$62.5^{aA} \pm 0.8$	$66.2^{aB} \pm 0.6$	$66.0^{aB} \pm 0.3$	$66.6^{aB} \pm 1.1$	$69.9^{aB} \pm 0.4$
Rice starch + 0.3% KGM	$32.0^{bA} \pm 6.8$	$57.8^{aB} \pm 8.4$	$56.6^{bB} \pm 5.5$	$62.6^{aB} \pm 3.8$	$64.9^{aB} \pm 2.9$
Rice starch + 0.5% KGM	$21.5^{bA} \pm 6.4$	$44.6^{bB} \pm 2.9$	$43.5^{cB} \pm 3.3$	$49.0^{bB} \pm 0.0$	$49.7^{bB} \pm 2.2$

Mean values in each column with different superscripts (a–c) are significantly differ ($p \leq 0.05$). Mean values in each row with different superscripts (A and B) are significantly differ ($p \leq 0.05$).

physical changes which occur during freezing and thawing. Syneresis in a freeze–thawed gel is caused by an 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, Puttanlekb, Rungsardthong, & Uttapap, 2005). Thus the amount of water released due to syneresis is a useful indicator of the tendency of starch to retrograde (Karim, Norziah, & Seow, 2000). However, in starch gel containing ingredients which can bind to water molecules such as hydrocolloids or sugars, syneresis is reduced (Arunyanart & Charoenrein, 2008; Baker & Rayas-Duarte, 1998; Yoshimura et al., 1998).

The effect of KGM on the % syneresis in rice starch gels is presented in Table 1. From the first to fifth freeze–thaw cycles, both KGM at a 0.3 and 0.5% concentration significantly ($p \leq 0.05$) reduced the % syneresis. Freeze–thawed rice starch gels without KGM had a high syneresis value (62.5%) after the first cycle and showed little change through subsequent freeze–thaw cycles. High syneresis in this sample resulted from a high amylose content (31.6%) in the rice starch. Our previous study showed that medium-amylose rice flour (17.6%) gels had a significantly lower % syneresis after the first freeze–thaw cycle than did high-amylose rice flour (32.5%) gels (Charoenrein et al., 2008). This result implies that amylose plays an impor-

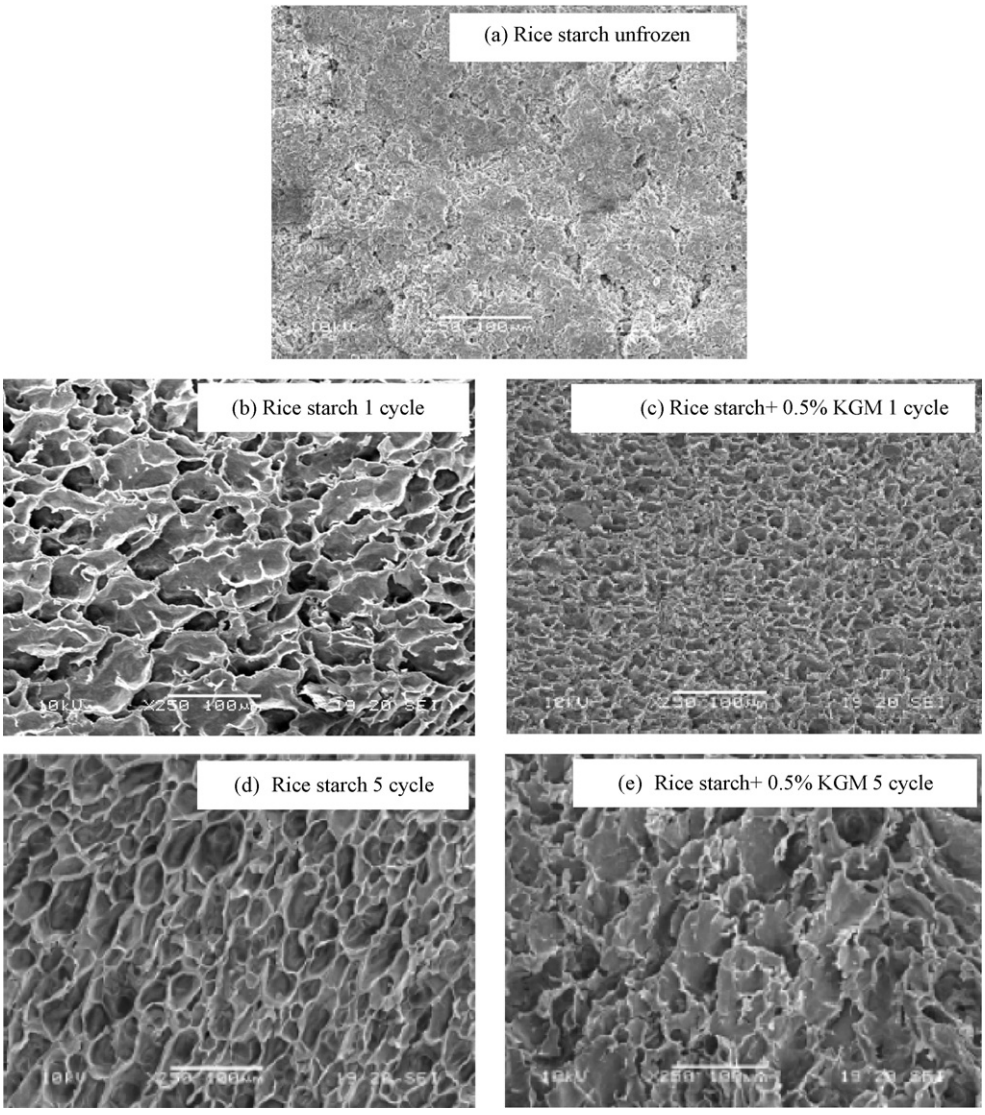

Fig. 1. SEM images of rice starch gels (8% (w/w) total solid) containing KGM 0 and 0.5% unfrozen and after one and five freeze–thaw cycles (250×, bar = 100 μm).

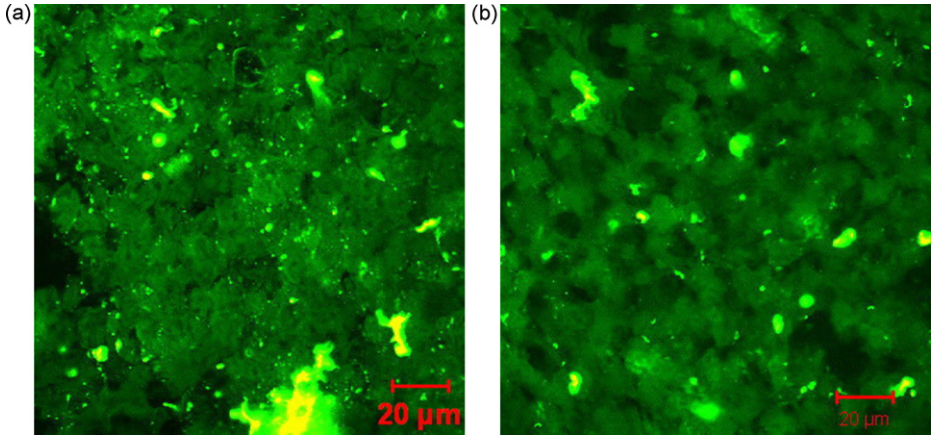

Fig. 2. CLSM images of unfrozen rice starch gels (8% (w/w) total solid) in the absence (a) and presence of 0.5% KGM (b) (bar = 20 μm).

tant role in the retrogradation associated with freezing and thawing.

However, freeze–thawed rice starch gel with KGM displayed a markedly lower % syneresis and behaved differently from gel that did not contain KGM. Starch gel containing 0.3% KGM which had shown 32.0% syneresis after the first freeze–thaw cycle, showed an obvious increase in its syneresis value to 57.8 after 2 freeze–thaw cycles. After that, the syneresis values changed only slightly through cycles 3–5. Starch gel containing 0.5% KGM which had 21.5% syneresis after the first cycle, showed an increase in syneresis value to 44.6%, after 2 freeze–thaw cycles. At this concentration also, after the second and through to the fifth freeze–thaw cycle, the percentage of syneresis increased only slightly.

The effects of other hydrocolloids on the reduction of syneresis in starch paste have been widely studied. It was shown that xanthan gum effectively reduces syneresis in freeze–thaw corn starch gel (Ferrero et al., 1994), and in high amylose and waxy corn starch gel (Weber, Queiroz, & Chang, 2008) while xanthan, alginate and guar gum reduces syneresis in freeze–thaw sweet potato starch gel (Lee et al., 2002). Muadklay and Charoenrein (2008) found that xanthan gum also reduces syneresis in freeze–thaw tapioca starch gel. It was hypothesized that the effects of hydrocolloids in the reduction of syneresis is due to the retardation of amylose retrogradation (Ferrero et al., 1993; Ferrero et al., 1994) and an increase in the viscosity of starch paste (Lee et al., 2002). Shi and BeMiller (2002) suggested more specifically that this effect was likely due to interactions between certain leached molecules, primarily between amylose and certain gums. In this experiment, where we studied the hydrocolloid konjac glucomannan, we used the microstructure of unfrozen and freeze–thaw rice starch gels examined by CLSM and SEM as described in Sections 3.2 and 3.3 and pasting properties as described in Section 3.4 to better understand the effect of this hydrocolloid in reducing syneresis.

3.2. Microstructure of freeze–thaw rice starch gels

To elucidate the relationship between syneresis and the addition of KGM to gels, the microstructure of freeze–thawed gels was examined using SEM. Images of treated specimens are shown in Fig. 1. The microstructure of rice starch gel before freezing is shown in Fig. 1a. Clear differences were observed in the microstructure of rice starch gels after 1 and 5 freeze–thaw cycles for both gels with and without KGM. All freeze–thaw starch gels developed a spongy structure which can be attributed to ice crystal formation and amylose retrogradation. 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 KGM added, the microstructure after the first freeze–thaw cycle was characterized by large pores in the gel (Fig. 1b). After the fifth freeze–thaw cycle, the starch gels still had a similar pore size but the matrix surrounding the pores were stronger and the pores were very clearly defined (Fig. 1c). These structural findings correlate well with the insignificant changes in syneresis values found after 1–5 freeze–thaw cycles of rice starch gel to which no KGM had been added. After 1 freeze–thaw cycle, the starch gels containing 0.5% KGM appeared to have smaller and less well-defined pores embedded in a weak matrix (Fig. 1d). After 5 freeze–thaw cycles, pore size in these gels had increased but the pores were still less well-defined than in starch gels without KGM and the matrix surrounding the pores were clearly weaker (Fig. 1c and e). In the rice starch gels containing added KGM and treated to multiple freeze–thaw cycles, the change in microstructure correlated closely with an increase in percent syneresis. The specimen images show that KGM effectively stabilized the microstructure of rice starch gels after the first freeze–thaw cycle but lost its stabilization power after successive freeze–thaw cycles. The microstructure of the unfrozen gel as described in Section 3.3 will demonstrate how KGM helps reduce syneresis and modify the microstructure of freeze–thaw starch gels.

3.3. Microstructure of unfrozen rice starch gel

During the preparation of rice starch gels, rice starch granules suspended in excess amounts of water (starch 8% (w/w) total solid) swelled upon heating. After agitation and subsequent steaming for 9 min, it is assumed that amylose molecules leach from the swollen rice starch granules creating granule ghosts. Granule ghosts are formed from starch granules when they are heated in water without or with little shear.

There have been several reported studies of starch granule ghosts during gelatinization (Atkin, Abeysekera, & Robards, 1998; Fannon & BeMiller, 1992; Obanni & BeMiller, 1995; Obanni & BeMiller, 1996). It is possible to visualize the influence of KGM on starch gel microstructure using CLSM and a fluorescent dye. Funami et al. (2008), found that FITC was the preferred fluorescent dye for observing gelatinized starch granules. However, in this study we found that FITC-dextran was the most suitable for this purpose. The starch was stained with FITC-dextran which was absorbed by the starch granules. Fig. 2a and b shows the optical sections of the rice starch gel and rice starch gel with 0.5% KGM. Fig. 2a shows swollen starch granules or starch ghosts of different sizes unevenly distributed throughout most of the volume fraction with the swollen starch granules tending to group together. Fig. 2b shows swollen starch granules in a different arrangement.

Table 2
Viscosity (RVA) profiles of rice starch (8% (w/w) total solid) with KGM addition 0, 0.3 and 0.5%.

Rice starch with KGM (%)	Viscosity (RVU)				
	Peak viscosity	Trough	Break down	Final viscosity	Setback
0.0	$127.92^{a} \pm 0.71$	$102.64^{a} \pm 0.28$	$25.46^{a} \pm 2.18$	$133.88^{a} \pm 0.18$	$31.42^{a} \pm 2.71$
0.3	$231.05^{b} \pm 5.48$	$164.29^{b} \pm 5.25$	$66.75^{b} \pm 0.24$	$285.25^{b} \pm 3.18$	$120.96^{b} \pm 2.06$
0.5	$330.50^{c} \pm 2.83$	$257.34^{c} \pm 6.24$	$73.17^{b} \pm 3.42$	$400.80^{c} \pm 2.30$	$143.46^{c} \pm 8.54$

Mean values in each column with different superscripts (a and b) are significantly different ($p \leq 0.05$).

The swollen starch granules are more evenly distributed and connected than that in Fig. 2a. We also observed that swollen starch granules in gel with 0.5% KGM had smaller size than those in gel that did not contain KGM. This finding corresponded to Ishihara et al. (2010) work which showed that swollen rice starch granules which were gelatinized in gum arabic and soybean soluble polysaccharide solutions had smaller size than those gelatinized in the absence of polysaccharides. We hypothesized that the viscous phase surrounding the swollen starch granules might be KGM interacting with leached amylose. The fact that starch granules in a starch gel with KGM are not densely packed might indicate a lower degree of retrogradation. This result correlates well with a lower % syneresis and a softer matrix around ice crystals than that observed in rice starch gel without KGM. Ferrero and Zaritzky (2000) also points out that when heating hydrocolloids and starch suspension, hydrocolloids interact with amylose released from the starch granules.

3.4. Pasting properties

In the RVA measurement, higher values for peak viscosity, breakdown, final viscosity and setback were observed in rice starch paste with KGM (Table 2). The higher the concentration of KGM, the higher the viscosity observed. Either a thickening effect from KGM or alternatively the interactions between KGM and swollen starch particles (Rojas, Rosell, & Benidito de Barber, 1999) might be responsible for this result. Funami et al. (2005) found that the addition of KGM to wheat starch was highly effective in increasing peak viscosity. Breakdown was also increased with the addition of KGM. The high final viscosity in starch paste containing KGM helps explain the influence of KGM on the microstructure of unfrozen starch paste as described in Section 3.3.

3.5. Texture

The textural properties of rice starch gels before and after freezing and thawing were studied and the results are shown in Table 3. It was found that before freezing and thawing, starch gel containing 0.3 and 0.5% KGM were not as hard as was starch gel without KGM.

Huang, Kennedy, Li, Xu, and Xie (2007) also reported that when present in unfrozen rice starch gels, KGM did not increase the hardness of the gels while guar gum and carrageenan significantly increased their hardness. The reason they proposed for this difference in hardness is that KGM is a kind of non-gelling polysaccharide that is not capable of forming an undivided network structure. Our textural results can be clearly elucidated by examining the microstructure of unfrozen gels using CLSM. It can be seen from Fig. 2a and b that KGM retarded the aggregation of swollen granules. After the first freeze–thaw cycle, hardness in all samples increased. Though retrogradation of starch molecules in starch-rich regions is enhanced during freezing and thawing (Yuan & Thompson, 1998) leading to an increase in the hardness of a gel, in rice starch gel without KGM, increase in hardness of the gel was much more obvious after freezing and thawing than in those gels containing 0.3 and 0.5% KGM (56, 18 and 13% respectively). As can be seen from SEM images of freeze–thaw gels (Fig. 1b–d), the spongy structure of rice starch gels with 0.5% KGM was weaker than that of those without KGM. This could explain the softer texture noted in freeze–thaw starch gels containing KGM. It should also be noted that, hardness of freeze–thawed starch gels containing 0.5% KGM was relatively similar to that of the unfrozen starch gels without KGM. KGM's effect of moderating the increase in gel hardness after freezing and thawing might be due to a reduction in the aggregation of swollen starch granules which resulted from their being located in a viscous KGM–amylose phase.

Table 3
Hardness of rice starch gel (8.0% (w/w) total solid) containing KGM 0, 0.3 and 0.5% before and after subjecting to one freeze–thaw (FT) cycle.

Sample	Hardness (N)	
	Before freezing	After 1 FT cycle
Rice starch	$3.323^{aB} \pm 0.091$	$5.193^{aA} \pm 0.761$
Rice starch + 0.3% KGM	$2.725^{bA} \pm 0.159$	$3.237^{bA} \pm 0.714$
Rice starch + 0.5% KGM	$2.644^{bA} \pm 0.116$	$3.292^{bA} \pm 1.389$

Mean values in each column with different superscripts (a and b) are significantly different ($p \leq 0.05$). Mean values in each row with different superscripts (A and B) are significantly different ($p \leq 0.05$).

4. Conclusions

The addition of KGM was shown to be an effective agent for the reduction of syneresis and for limiting (moderating, reducing) an increase in gel hardness in rice starch gels subjected to repeated freeze–thaw cycles. In this work, KGM was shown to be most effective in enhancing freeze thaw stability of starch gels at a 0.5% concentration because, during repeated freeze–thawing, at this concentration, KGM was able to retard the textural changes which result from a gel with a spongy structure. The microstructure of the unfrozen starch gel demonstrated that swollen starch granules form dense associations in rice starch gels containing no additives while in gels containing KGM, the swollen starch granules were less closely associated and consequently more evenly distributed. This research shows that KGM can be a useful additive for preserving quality in frozen rice starch-based food products.

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