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Influence of spent brewer's yeast β-glucan on gelatinization and retrogradation of rice starch

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Abstract

The effects of β-glucan (BG) prepared from spent brewer's yeast on gelatinization and retrogradation of rice starch (RS) were investigated as functions of mixing ratio and of storage time. Results of rapid visco-analysis (RVA) indicated that addition of BG increased the peak, breakdown, setback, and final viscosities, but decreased the pasting temperatures of the rice starch/β-glucan (RS/BG) mixtures. Differential scanning calorimetry (DSC) data demonstrated an increase in onset (T_o), peak (T_p), and conclusion (T_c) temperatures and a decrease in gelatinization enthalpy (ΔH_1) with increasing BG concentration. Storage of the mixed gels at 4 °C resulted in a decrease in T_o, T_p, T_c, and melting enthalpy (ΔH_2). The retrogradation ratio ($\Delta H_2/\Delta H_1$) and the phase transition temperature range ($T_c - T_o$) of the mixed gels increased with storage time, but this effect was reduced by the addition of BG. BG addition also slowed the syneresis of the mixed gels. Results of dynamic viscoelasticity measurement indicated that the addition of BG promoted RS retrogradation at the beginning and then retarded it during longer storage times. The added BG also retarded the development of gel hardness during refrigerated storage of the RS/BG mixed gels.

Keywords: Rice starch; β-Glucan; Gelatinization; Retrogradation; Brewer's yeast

1. Introduction

Starch has been incorporated in many ready-meals or precooked chilled foods either as main raw materials or as additives. As additives, starch contributes to the thickening and stabilizing effects and texture modification in foods (Eliasson & Gudmundsson, 1996). The gelatinization and retrogradation of starch are important properties in food processing, cooking, handling, distribution, and storage. Since they profoundly affect quality, acceptability, and shelf life of starch-containing foods (Biliaderis, 1991). On heating an aqueous suspension of starch up to and above a critical temperature, commonly referred to as the gelatinization temperature, granule swelling occurs, the crystalline structure arising from the association of amylopectin chains is lost, and amylose leaches into the solution. Upon cooling of the cooked starch pastes, retrogradation occurs when gelatinized starch begins to reassociate in an ordered structure under low energy input, as in freezing and chilling (Bao & Bergman, 2004). The retrogradation is the main cause of changes in the textural and digestibility characteristics of starch-containing foods during storage. Therefore, the technology of preparing starch-based products with extended shelf life requires effective retarding of starch retrogradation. Traditionally, starches are chemically modified to obtain the better properties demanded by the industry. However, the expensive and often questionable chemicals used will be banned in the health-conscious period.

Many investigations have been reported that hydrocolloids (gums) can alter the gelatinization and retrogradation characteristics of starch. Hydrocolloids such as guar gum, xanthan gum, carageenan, alginate, konjac glucomannan, locust bean gum, xyloglucan, β-glucan, etc., could be used

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in mixtures with starch to alter (increase or decrease greatly or slightly or no effect) apparent paste viscosity (Alloncle, Lefebvre, Llamas, & Doublier, 1989; Bahnassey & Breene, 1994; Chaisawang & Suphantharika, 2005, 2006; Christianson, Hodge, Osborne, & Detroy, 1981; Rojas, Rosell, & Benedito de Barber, 1999; Shi & BeMiller, 2002) and retard retrogradation rates of starch pastes during frozen (Ferrero, Martino, & Zaritzky, 1994; Lee, Baek, Cha, Park, & Lim, 2002; Sae-kang & Suphantharika, 2006) or chilled (Mali et al., 2003) storage.

From viewpoints of texture and stability modification, β-glucan (BG) is an interesting hydrocolloid (Thammakiti, Suphantharika, Phaesuwan, & Verduyn, 2004). The BG is attracting the increasing attention of the pharmaceutical and functional food industry because of its positive effects on human and animal health such as immune-stimulation, anti-inflammatory, antimicrobial, antitumoral, hepatoprotective, cholesterol-lowering as well as antifibrotic, antidiabetic and hypoglycemic activity (Zeković, Kwiatkowski, Vrvić, Jakovljević, & Moran, 2005), and because it is a natural polysaccharide found in microorganisms and plants. However, the BG from yeast (*Saccharomyces cerevisiae*) with low cost of production, simple extraction technology, and potential infinite supply will dominate the market for the foreseeable future (Zeković et al., 2005). Yeast BG is mainly composed of a backbone chain of β-(1–3) linked glucose units with a low degree of branching through β-(1–6) linkages. It is the alkali insoluble fraction of yeast cell walls (Jamas, Rha, & Sinskey, 1989).

Previously, we reported on a scheme for extracting BG of high purity from spent brewer's yeast (Thammakiti et al., 2004). Yeast BG is one ingredient that has demonstrated potential in improving the functional properties of food products, i.e., being used as a thickening, water-holding, or oil-binding agent, and emulsifying or foaming stabilizer (Romero & Gomez-Basauri, 2003; Thammakiti et al., 2004). We have also demonstrated the potential applications of spent brewer's yeast BG as an immunostimulant in shrimp feed (Suphantharika, Khunrae, Thanardkit, & Verduyn, 2003; Thanardkit, Khunrae, Suphantharika, & Verduyn, 2002) and a fat replacer in mayonnaise (Worrasinchai, Suphantharika, Pinjai, & Jamnong, 2006). Although there are a few reports on the effect of barley BG (Kim & Setser, 1992) and oat BG (Biliaderis, Arvanitoyannis, Izydorczyk, & Prokopowich, 1997) on gelatinization of wheat starch, however, to the best of our knowledge, there is no information available on the effect of spent brewer's yeast BG on gelatinization and retrogradation of rice starch (RS). Knowledge about how the spent brewer's yeast BG influences the gelatinization and retrogradation properties of RS is highly important for developing new product types and replacements for existing products.

This study aimed at investigating the effects of BG prepared from spent brewer's yeast on gelatinization and retrogradation characteristics of RS as functions of rice starch/β-glucan (RS/BG) mixing ratio and of refrigerated storage time by rapid visco-analysis (RVA), differential scanning calorimetry (DSC), syneresis measurement, dynamic shear rheometry, and texture profile analysis (TPA).

2. Materials and methods

2.1. Materials

Rice starch (30% amylose) was supplied by Cho Heng Rice Vermicelli Factory Co. Ltd., Nakhon Pathom, Thailand. Spent brewer's yeast slurry (a strain of *Saccharomyces uvarum*), a by-product from brewery was provided by Boonrawd Brewery Co. Ltd., Bangkok, Thailand.

2.2. Preparation of β-glucan

β-Glucan was prepared according to the procedure described previously (Thammakiti et al., 2004). Briefly, spent brewer's yeast slurry was adjusted to 15% solids content and pH 5 and then autolyzed at 50 °C for 24 h. Yeast cell walls were collected by centrifugation at 3565 × *g* for 10 min in a Beckman J-6M/E centrifuge (Beckman–Coulter, Inc., CA). The suspension of yeast cell walls (15% solids content) was homogenized by using a high pressure homogenizer (model Panda, Niro-Soavi, Parma, Italy) at 600–700 bars for six passes. Alkaline extraction was done by using five volumes of 1.0 N NaOH at 80 ± 5 °C for 2 h followed by an acid extraction with five volumes of 0.5 N acetic acid at 70 ± 5 °C for 1 h. The extracted cell walls from each step were centrifuged at 3565 × *g* for 10 min and then washed three times with distilled water. The obtained BG was a light-tan colored paste having a proximate composition in percentage (w/w) as follows: moisture 93.37, fat 0.07, ash 0.04, protein 0.38, and carbohydrate 6.13.

2.3. Determination of pasting properties

Pasting properties of RS/BG mixtures suspended in distilled water were determined by a Rapid Visco-Analyzer (model RVA-4C, Newport Scientific Pty. Ltd., Warriewood, Australia). Distilled water used in all experiments was added with 0.02%, w/w, sodium azide to prevent microbial spoilage of the stored samples. The RS/BG slurrys of 25 g were prepared by dispersing weighed amounts of RS and BG (dry basis) into distilled water at the total solids content of 6%, w/w, with four mixing ratios of RS/BG = 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0. Practically, BG was first dispersed in distilled water under magnetic stirring, then starch was slurried in the BG solutions. The dispersions were stirred with a spatula for sufficient duration to avoid the formation of lumps. The RS/BG suspensions were poured into aluminum containers and stirred manually using a plastic paddle for 20–30 s before insertion into the RVA machine. The heating and cooling cycles were programmed in the following manner. The slurry was held at 50 °C for 1 min, heated to 95 °C within 3 min 42 s and then held at 95 °C for 2 min 30 s. It was subsequently cooled to 50 °C within 3 min 48 s and held at 50 °C for 2 min, while maintaining a rotation speed of 160 rpm.

2.4. Differential scanning calorimetry measurement

Gelatinization temperatures and enthalpy of the RS with and without added BG were measured by a differential scanning calorimeter (DSC 822^e, Mettler Toledo, Schwerzenbach, Switzerland). The total solids content of samples was selected to be 12%, w/w (dry basis), due to the sensitivity of the instrument. The RS/BG mixtures with mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 were prepared following the method described above. After hydration for 1 h at room temperature, 10–15 mg of the well-stirred RS/BG dispersions were exactly weighed into 40 µl aluminum crucibles and hermetically sealed immediately to prevent moisture loss. Scans were performed from 25 to 100 °C at a controlled constant rate of 10 °C/min. A sealed empty pan was used as a reference and the DSC was calibrated using indium. The enthalpy and transition temperatures; the onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) were determined based on the first-run heating DSC curves. The gelatinization enthalpy was evaluated based on the area of the main endothermic peak and expressed in terms of J/g of dry starch using the equipment software. After the first-run heating, the gelatinized samples were cooled down and kept at 4 °C for 0, 1, 3, 7, 14, 21, 35, 49, and 63 days. The stored samples were heated again to study the effect of BG on retrogradation of the RS. The retrogradation ratio was calculated by dividing the re-gelatinization enthalpy (ΔH_2) in the second-run heating by the gelatinization enthalpy (ΔH_1) in the first-run heating (Kohyama & Nishinari, 1992).

2.5. Syneresis determination

Syneresis of the samples was estimated using a centrifugation method. Batches of 500 g RS/BG suspensions (6%, w/w, dry basis) of four RS/BG mixing ratios; 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 were prepared by dispersing the calculated amounts of RS and BG in distilled water (containing 0.02% sodium azide) using a motorized stirrer for 1 h at room temperature (25 °C). The suspensions were then gelatinized in a Brabender Viscoamylograph Type E (Duisburg, Germany). The heating temperature started from 30 to 90 °C, held at 90 °C for 15 min and then cooled down to 50 °C. The heating and cooling rates were 1.5 °C/min. The gelatinized mixtures (25 g) were transferred into 50 ml screw-cap plastic centrifuge tubes of 27 mm internal diameter and 115 mm height. The sample tubes were kept at 4 °C for 1, 3, 7, 14, 21, 35, 49, and 63 days. At each storage time, three tubes of the stored samples were randomly selected for each RS/BG ratio for determination of syneresis. The extent of syneresis (%) was determined as the weight percentage of the original weight of the gel released as liquid due to centrifugation at $1180 \times g$ for 15 min.

2.6. Dynamic viscoelastic measurement

The fresh RS/BG mixed pastes (3.5%, w/w) obtained from the RVA were cooled to room temperature (25 °C) and then kept at 4 °C for 0, 1, 3, 7, 14, 21, 35, 49, and 63 days. At each storage time the samples were determined for viscoelastic properties by using a rheometer (Physica MCR 300, Anton Paar GmbH, Stuttgart, Germany). The samples were placed into the rheometer measuring system (cone and plate geometry, 50 mm diameter, 1° cone angle, and 0.05 mm gap) which was equilibrated to 25 °C. Two steps of rheological measurements were performed: (1) deformation sweeps at a constant frequency (10 rad/s) to determine the maximum deformation attainable by a sample in the linear viscoelastic range and (2) frequency sweeps at a constant deformation (0.5% strain) within the linear viscoelastic range. The mechanical spectra were obtained recording the dynamic moduli G', G'', and $\tan\delta$ as a function of frequency.

2.7. Texture profile analysis

Compression tests of the RS/BG gels were carried out with a TA.XT2i Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) equipped with a Texture Expert for Windows Version 1 equipment software and a 5 kg load was used for force calibration. One cycle was applied, at a constant crosshead velocity of 1 mm/s, to a sample depth of 10 mm, then returned. Fresh RS/BG pastes (6%, w/w) weighed 25 g were poured into cylindrical containers (35 mm internal diameter and 65 mm height) and kept at room temperature (25 °C) for 2 h prior to measurement. To investigate the retrogradation behavior of the gelatinized pastes, fresh RS/BG pastes in cylindrical containers were kept at 4 °C for 1, 3, 7, 14, 21, 35, 49, and 63 days. All sample tubes were tightly covered with a moisture resistant laboratory film (Parafilm M®, Pechiney Plastic Packaging, Inc., Chicago, IL) to prevent water evaporation. The gel hardness was measured according to definition of Pons and Fiszman (1996) as the peak force observed during the compression cycle.

2.8. Statistical analysis

Data reported are means of triplicate determinations. The statistical analysis of the results was conducted by the analysis of variance (ANOVA) and Tukey's test using SPSS version 12.0 for Windows program (SPSS Inc., Chicago, IL). Significant differences were reported for $P \leqslant 0.05$.

3. Results and discussion

3.1. RVA pasting properties of the RS/BG mixtures

The pasting characteristics of the RS/BG mixtures at various mixing ratios determined by the RVA are shown in Fig. 1. Statistical analyses of all pasting parameters were also performed and are summarized in Table 1. The BG dispersion without starch was also run under the same RVA conditions to establish a control viscosity curve. The viscosity of the 1.0%, w/w, BG dispersion remained almost constant at 8 RVU. The high pasting temperature (94.9 °C) of the RS alone dispersion (RS/BG = 6.0/0.0) is in good agreement with those previously reported by Varavinit, Shobsngob, Varanyanond, Chinachoti, and Naivikul (2003). These authors found that normal rice starches containing 15–26% amylose exhibited the pasting temperatures in the range of 93–96 °C.

According to the RS alone, as a control, addition of BG resulted in significant ($P \leqslant 0.05$) increases in the peak, breakdown, setback, and final viscosities and peak times (time to reach the peak viscosity), but decreases in the pasting temperatures. This effect was more pronounced when BG concentration increased. The synergistic effect on the peak viscosity of the RS/BG systems was interpreted assuming that BG formed a network where swollen granules were immersed and that BG network absorbed water and reduced water availability for swelling of the granules, then higher amount of the swollen granules were produced when BG concentration was higher resulted in an increase in the peak viscosity. The increase in viscosity can make the shear forces exerted on the swollen granules in the shear field much larger than those encountered in starch/water suspensions (Christianson et al., 1981). This results in the loss of granule integrity and subsequent disruption leading to a reduction in the paste viscosity. The highest reduction of the paste viscosity of the RS/BG system with the highest BG concentration could be possibly due to its highest viscosity leading to the highest shear forces exerted on the swollen granules and the greatest extent of the granule disruption. The resultant drop in viscosity, which is defined as breakdown viscosity, is most evident in the starch/gum pastes indicating the possible breakdown of the granules and release more solubilized starch, primarily amylose. It appeared that strong associations of the solubilized starch with certain gums as pointed out by Christianson et al. (1981) could be responsible for the increases in setback and final viscosities and decreases in pasting temperatures of the RS/BG mixtures. Similar results were reported for other starch/gum systems (Bahnassey & Breene, 1994; Mali et al., 2003; Rojas et al., 1999).

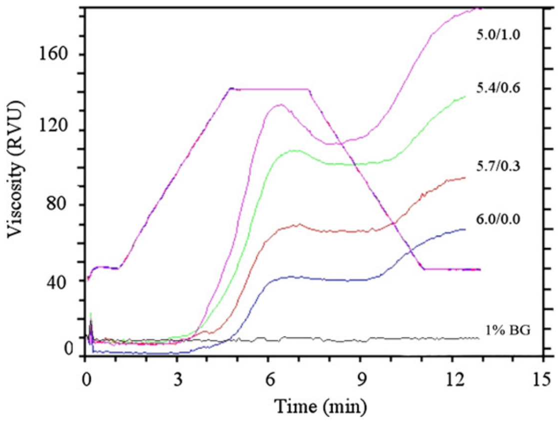

Fig. 1. Typical RVA pasting profiles of 1%, w/w, BG dispersion and 6%, w/w, RS/BG mixtures at mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0.

3.2. Thermal properties of the RS/BG mixtures

The thermal properties of the RS/BG mixtures and their corresponding retrograded gels determined by the DSC are summarized in Table 2. For the first run, the general trend was for the T_o, T_p, and T_c significantly ($P \leqslant 0.05$) shifted to higher temperatures, but ΔH_1 decreased as the concentration of BG in the system increased. The $T_c - T_o$ values, however, apparently were unaffected by the BG addition. A slight decrease in enthalpy and shift in the gelatinization temperature to a higher temperature in the presence of hydrocolloid and excess water can be interpreted as a result of the lower heating rates and the decreased mobility of water molecules as pointed out by Krüger, Ferrero, and Zaritzky (2003). These authors demonstrated negative correlations of the heat penetration rates and the mass transfer rates expressed in terms of water diffusivity versus the complex viscoelastic modulus ($G^* = \sqrt{G'^2 + G''^2}$) of the starch/gum mixtures during gelatinization. The presence of gums exhibiting higher values of the dynamic moduli (G^*, G', and G'') of the starch/gum mixtures resulted in a slight increase in the gelatinization temperatures (T_o, T_p, and T_c)

Table 1
Pasting properties of 6%, w/w, RS/BG mixtures at various ratios measured by the rapid visco-analyzer (RVA)

RS/BG mixing ratio	Peak viscosity (RVU)	Breakdown (RVU)	Setback (RVU)	Final viscosity (RVU)	Pasting temperature (°C)	Peak time (min)
6.0/0.0	42.6 ± 0.1^d	2.9 ± 0.5^d	29.2 ± 0.7^d	68.8 ± 0.8^d	94.9 ± 0.2^a	6.4 ± 0.1^c
5.7/0.3	70.4 ± 0.4^c	6.9 ± 0.3^c	32.9 ± 0.1^c	96.4 ± 0.6^c	94.5 ± 0.5^a	6.9 ± 0.0^b
5.4/0.6	109.4 ± 0.2^b	9.5 ± 0.1^b	40.3 ± 0.2^b	140.3 ± 0.7^b	89.0 ± 0.2^b	7.0 ± 0.0^a
5.0/1.0	133.4 ± 0.4^a	20.8 ± 0.1^a	72.3 ± 0.1^a	184.9 ± 0.4^a	82.3 ± 0.3^c	7.0 ± 0.0^a

Assays were performed in triplicate. Mean ± SD values in the same column with different superscripts are significantly different ($P \leqslant 0.05$).

Table 2
Gelatinization temperature, enthalpy, and retrogradation ratio for 12%, w/w, RS/BG mixtures at various ratios measured by the differential scanning calorimeter (DSC)

RS/BG mixing ratio	First run					Second run (21 days at 4 °C)					
	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH_1 (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH_2 (J/g)	$\Delta H_2/\Delta H_1$
6.0/0.0	70.0 ± 0.1^{d}	75.1 ± 0.1^{d}	78.3 ± 0.1^{d}	8.3 ± 0.1^{b}	10.1 ± 0.1^{a}	42.4 ± 0.5^{d}	54.8 ± 0.2^{c}	60.1 ± 0.0^{c}	17.7 ± 0.5^{a}	7.1 ± 0.3^{a}	0.70 ± 0.02^{a}
5.7/0.3	70.6 ± 0.0^{c}	75.4 ± 0.1^{c}	79.2 ± 0.0^{c}	8.6 ± 0.0^{a}	9.8 ± 0.1^{a}	44.9 ± 0.2^{c}	55.4 ± 0.1^{b}	60.4 ± 0.1^{b}	15.5 ± 0.2^{b}	5.6 ± 0.4^{b}	0.57 ± 0.04^{b}
5.4/0.6	71.3 ± 0.1^{b}	75.9 ± 0.1^{b}	79.6 ± 0.1^{b}	8.4 ± 0.1^{ab}	9.2 ± 0.2^{b}	46.2 ± 0.1^{b}	55.7 ± 0.0^{ab}	60.8 ± 0.1^{a}	14.6 ± 0.1^{c}	4.5 ± 0.4^{c}	0.49 ± 0.04^{bc}
5.0/1.0	71.9 ± 0.2^{a}	76.1 ± 0.1^{a}	80.3 ± 0.1^{a}	8.4 ± 0.1^{ab}	9.0 ± 0.2^{b}	47.8 ± 0.1^{a}	55.8 ± 0.1^{a}	61.0 ± 0.1^{a}	13.1 ± 0.1^{d}	3.9 ± 0.4^{c}	0.43 ± 0.05^{c}

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_1, gelatinization enthalpy; ΔH_2, re-gelatinization enthalpy; $\Delta H_2/\Delta H_1$, retrogradation ratio. Assays were performed in triplicate. Mean ± SD values in the same column with different superscripts are significantly different ($P \leqslant 0.05$).

measured by DSC which is in good agreement with our results presented in Fig. 7(a) and Table 2.

By comparing the DSC results of the gelatinization of RS/BG systems (Table 2) to their corresponding RVA results (Table 1), it was observed that the T_c values are lower than the RVA pasting temperatures for all samples tested, indicating that the melting of starch crystallites cooperatively in excess water precedes the first rise in viscosity where the rate of development of viscosity was the greatest. This result is expected and consistent with that reported by the other researchers (Yang, Datta, & Rao, 1997).

The reheating DSC data of the RS/BG retrograded gels stored for 21 days at 4 °C are also presented in Table 2. It is important to recognize that starch retrogradation can be considered as a two-step process, an initial short-term one related to amylose crystallization which is thermodynamically irreversible below 100 °C and a long-term one that involves amylopectin crystallization which is thermodynamically reversible (Miles, Morris, Orford, & Ring, 1985). Therefore, only amylopectin retrogradation could be quantified by DSC in the temperature range applied in this study (25–100 °C) (Ferrero et al., 1994). The tendency for the thermal properties to change with BG concentrations was similar to that observed during gelatinization (the first run) except for the $T_c - T_o$ values which significantly decreased with increasing BG concentration. However, the endothermic transition temperatures (T_o, T_p, and T_c) associated with melting of the retrograded starch occur at temperatures 18–28 °C lower than those for gelatinization of the starch granules. Starch retrogradation enthalpies (ΔH_2) were 30–57% smaller than the gelatinization enthalpies (ΔH_1). The retrogradation temperature ranges ($T_c - T_o$) were about twofold broader than the gelatinization range for a given sample. These results suggested retrogradation results in reassociation of the gelatinized starch molecules, but in less ordered and hence less perfect or stable forms than those present in the native starch granules (Karim, Norziah, & Seow, 2000). Furthermore, the retrogradation ratio, defined as the ratio of retrogradation enthalpy (ΔH_2) to that of the gelatinization enthalpy (ΔH_1), of the retrograded gels significantly decreased with increasing BG concentration, indicating that BG slowed the retrogradation rate of the RS during storage.

Time dependence of the transition temperatures (T_o, T_p, and T_c), retrogradation temperature ranges ($T_c - T_o$), and retrogradation ratios ($\Delta H_2/\Delta H_1$) of the retrograded RS/BG gels were plotted in Figs. 2–4, respectively. The T_o, T_p, and T_c markedly decreased during the first week of storage and slightly decreased during the rest of storage time. The decreases in these transition temperatures were in the following order: $T_o > T_p > T_c$ (Fig. 2), resulting in an increase in $T_c - T_o$ values with storage time (Fig. 3). The values of $T_c - T_o$ (Fig. 3) and $\Delta H_2/\Delta H_1$ (Fig. 4) for the RS/BG mixed gels increased during the first three weeks of storage as a result of amylopectin retrogradation and then remained practically constant for the rest of the

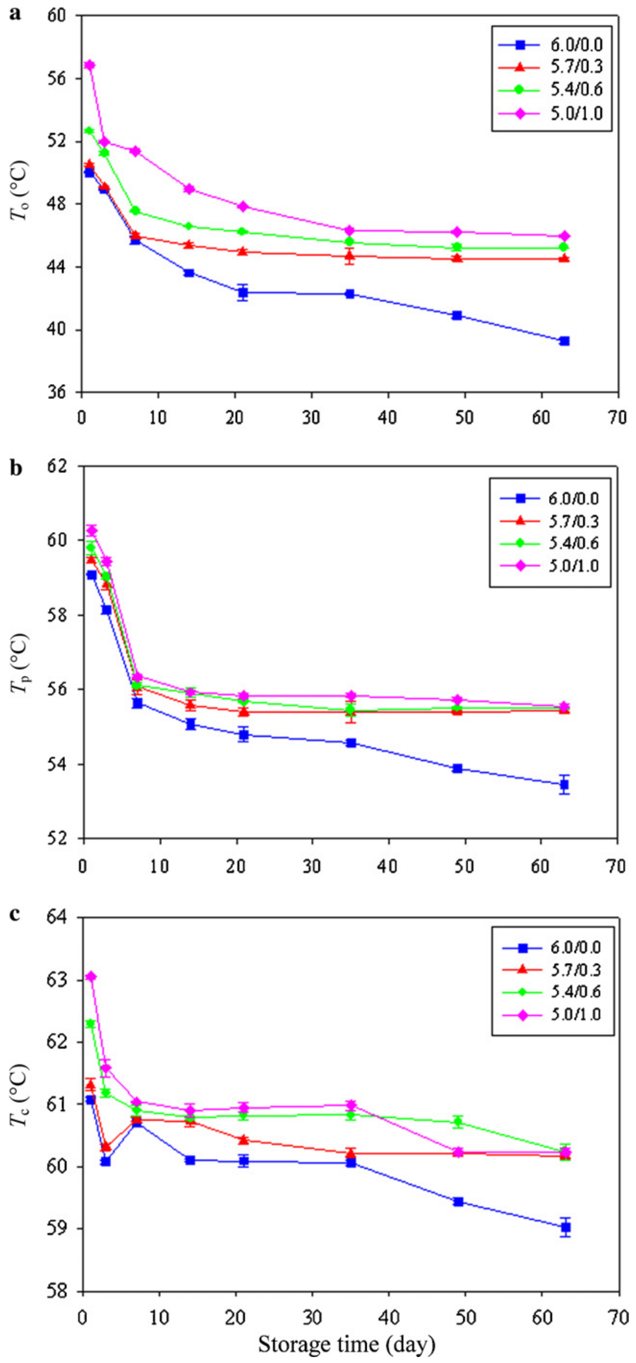

Fig. 2. Changes in (a) onset temperature, T_o, (b) peak temperature, T_p, and (c) conclusion temperature, T_c, of 12%, w/w, RS/BG mixed gels at mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 as a function of storage time at 4 °C. Error bars represent standard deviations.

storage period. The magnitude of both parameters decreased with increasing BG content indicating that BG retarded the retrogradation of the RS during storage.

Starch retrogradation is a non-equilibrium thermoreversible recrystallization process which is governed by a consecutive three-step mechanism of nucleation, propagation, and maturation (Slade & Levine, 1987). However, the storage temperature applied in this study (4 °C) favors the nucleation rather than the propagation of the crystallites which occurs at higher temperatures, i.e., 30–40 °C (Silverio, Fredriksson, Andersson, Eliasson, & Åman, 2000; Slade & Levine, 1991). In general, the rate-limiting step in the recrystallization process is nucleation (which is enhanced at lower temperatures) rather than propagation (which is enhanced at higher temperatures) (Slade & Levine, 1991). The T_o of the retrogradation endotherm concurs with the temperature where the least stable amylopectin crystallites formed during storage melt. Therefore, the marked decrease in T_o or even T_p means that there was a large amount of new and less stable amylopectin crystallites formed during storage, particularly during the first week (Fig. 2(a) and (b)). A slight decrease in T_c (Fig. 2(c)) which reflected the melting temperature of the most stable crystallites indicates that the propagation of amylopectin crystallites did not occur during storage.

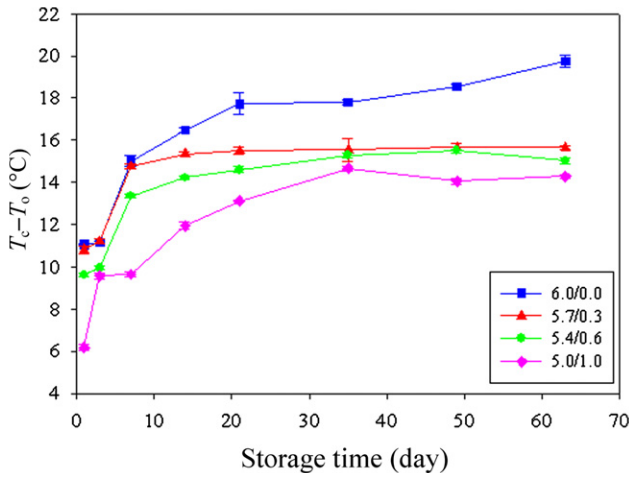

Fig. 3. Changes in transition temperature range ($T_c - T_o$) of 12%, w/w, RS/BG mixed gels at mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 as a function of storage time at 4 °C. Error bars represent standard deviations.

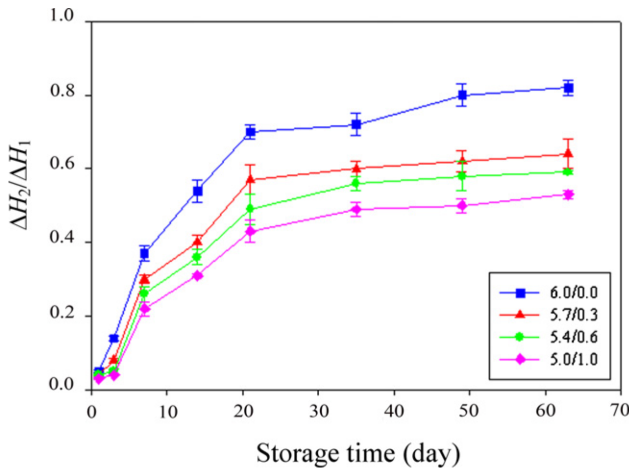

Fig. 4. Changes in retrogradation ratio ($\Delta H_2/\Delta H_1$) of 12%, w/w, RS/BG mixed gels at mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 as a function of storage time at 4 °C. Error bars represent standard deviations.

The increase in $T_c - T_o$ values during storage was observed (Fig. 3) and can be interpreted as a shift from a more homogeneous set of amylopectin crystallites with similar stability to a heterogeneous set with varying stability. As would be expected, the increase in $\Delta H_2/\Delta H_1$ values with storage time (Fig. 4) indicates that the more energy was required for melting a large amount of amylopectin crystallites formed during storage.

Because nucleation mechanism is a liquid state event which requires orientational mobility of the polymer chains in the amylopectin molecule (Slade & Levine, 1987), therefore addition of BG, which absorbed water, decreased the mobility of the starch chains and in turn retarded the retrogradation of the starch gels. The evidence was clearly demonstrated by the higher T_o (Fig. 2(a) and Table 2) and lower $T_c - T_o$ (Fig. 3 and Table 2) and $\Delta H_2/\Delta H_1$ (Fig. 4 and Table 2) of the RS/BG mixed gels as compared with the starch alone paste, indicating that a smaller amount of amylopectin crystallites formed in the pastes containing BG. This effect was more pronounced when the BG content increased. The $T_c - T_o$ and $\Delta H_2/\Delta H_1$ markedly increased during the first three weeks and then leveled off for the rest of the storage period which almost coincided with the syneresis profiles presented in Fig. 5.

3.3. Syneresis

The effect of BG on the syneresis of the RS/BG mixed gels with storage time at 4 °C is shown in Fig. 5. Addition of the BG markedly reduced syneresis of the RS/BG mixed gels in comparison with the control (RS/BG = 6.0/0.0). The degree of syneresis decreased with increasing BG concentration. The syneresis of the RS/BG gels markedly increased during the first three weeks of storage and the increment rates decreased afterwards.

Syneresis of starch gels during storage at low temperatures is due to increased molecular association of starch chains, amylose and amylopectin, which results in expulsion of water from the gel structure (Liu, Eskin, & Cui, 2006). The rate and extent of starch recrystallization are determined primarily by the mobility of the starch chains which depends on water availability of the system (Slade & Levine, 1991). Due to the high water-holding capacity of BG (Thammakiti et al., 2004), BG absorbed large amounts of water resulted in a decrease in the mobility of the starch chains and in turn the extent of recrystallization and degree of syneresis. This result is in good agreement with that demonstrated in the DSC study of the stored gels (Fig. 4 and Table 2).

The time dependence profiles of the syneresis of the RS/BG mixed gels (Fig. 5) apparently coincided with those of the $T_c - T_o$ (Fig. 3) and $\Delta H_2/\Delta H_1$ (Fig. 4) for each corresponding sample during the entire period of investigation (63 days). Therefore, an attempt was made to correlate these thermal properties to the syneresis for each sample during storage as shown in Fig. 6. Both $T_c - T_o$ (Fig. 6(a)) and $\Delta H_2/\Delta H_1$ (Fig. 6(b)) values increased almost linearly with the syneresis ($R^2 = 0.80$–0.96 for

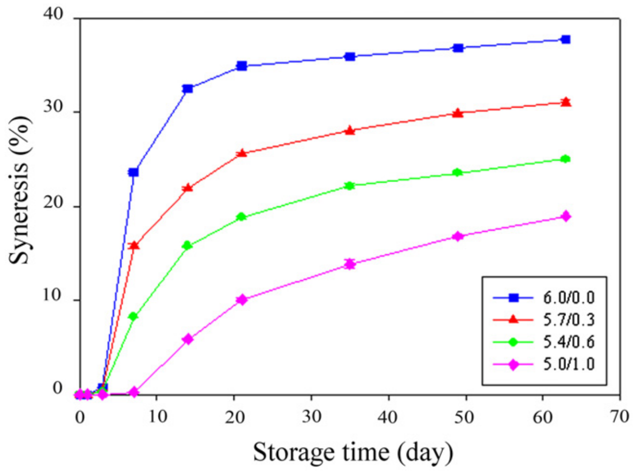

Fig. 5. Developments in syneresis of 6%, w/w, RS/BG mixed gels at mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 during storage at 4 °C. Error bars represent standard deviations.

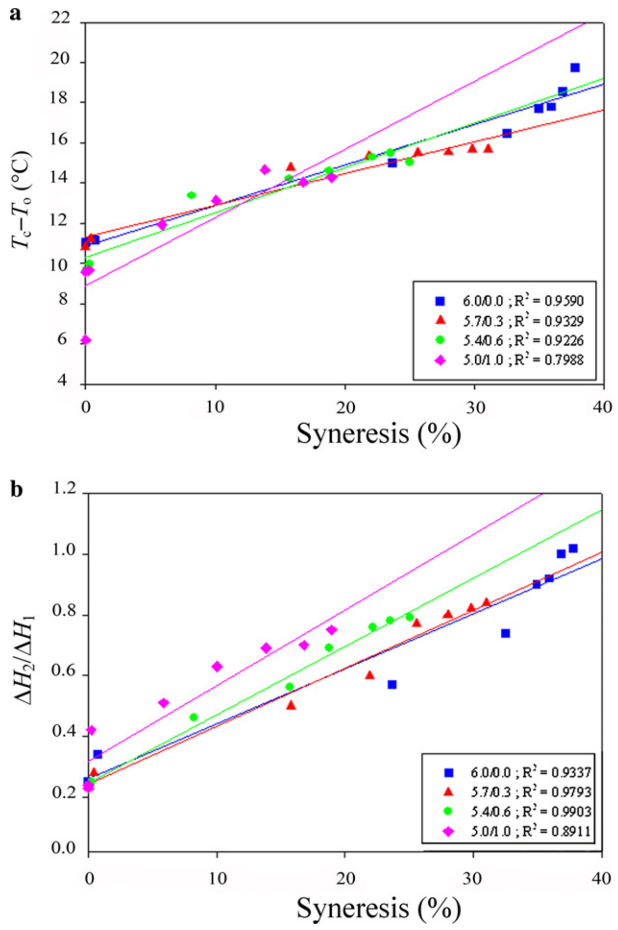

Fig. 6. Regressions of (a) $T_c - T_o$ versus syneresis and (b) $\Delta H_2/\Delta H_1$ versus syneresis for RS/BG mixed gels (12 and 6%, w/w, total solids for DSC and syneresis assays, respectively) at mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 during storage at 4 °C for 63 days. Full lines are linear regressions.

$T_c - T_o$; $R^2 = 0.89$–0.99 for $\Delta H_2/\Delta H_1$). Even though the data obtained from these two techniques could not be compared directly due to the difference in total solids content used, i.e., 12% for DSC assay versus 6% for syneresis assay, these correlations are useful for prediction of the extent of syneresis by using the DSC results and for understanding the retrogradation behavior of the RS/BG mixed gels.

3.4. Dynamic rheological properties

Dynamic mechanical spectra of the RS/BG mixed gels (3.5% total concentration) stored at 4 °C for 0 (i.e., immediately after gelatinization and cooled to room temperature (25 °C)), 7, 21, and 63 days are illustrated in Fig. 7. Oscillatory rheological assays could not be performed in the RS/BG systems with solids concentration higher than 3.5% because of the marked syneresis and the firmness of the samples, particularly the RS alone paste. Above this concentration the RS alone paste forms solid gels. These rheograms show that the storage modulus (G') was much larger than the loss modulus (G''), both moduli were almost frequency-independent except for G'' of the RS alone gel at 63 days storage which slightly increased with increasing frequency. A crossover between the two moduli was not observed throughout the frequencies accessed (ω = 0.1–100 rad/s). Moreover, the mechanical loss tangent ($\tan\delta = G''/G'$) values for the RS/BG gels are between 0.08 and 0.29 (Fig. 8(b)). This behavior may be classified rheologically as the intermediate between a weak gel and an elastic gel (Clark & Ross-Murphy, 1987).

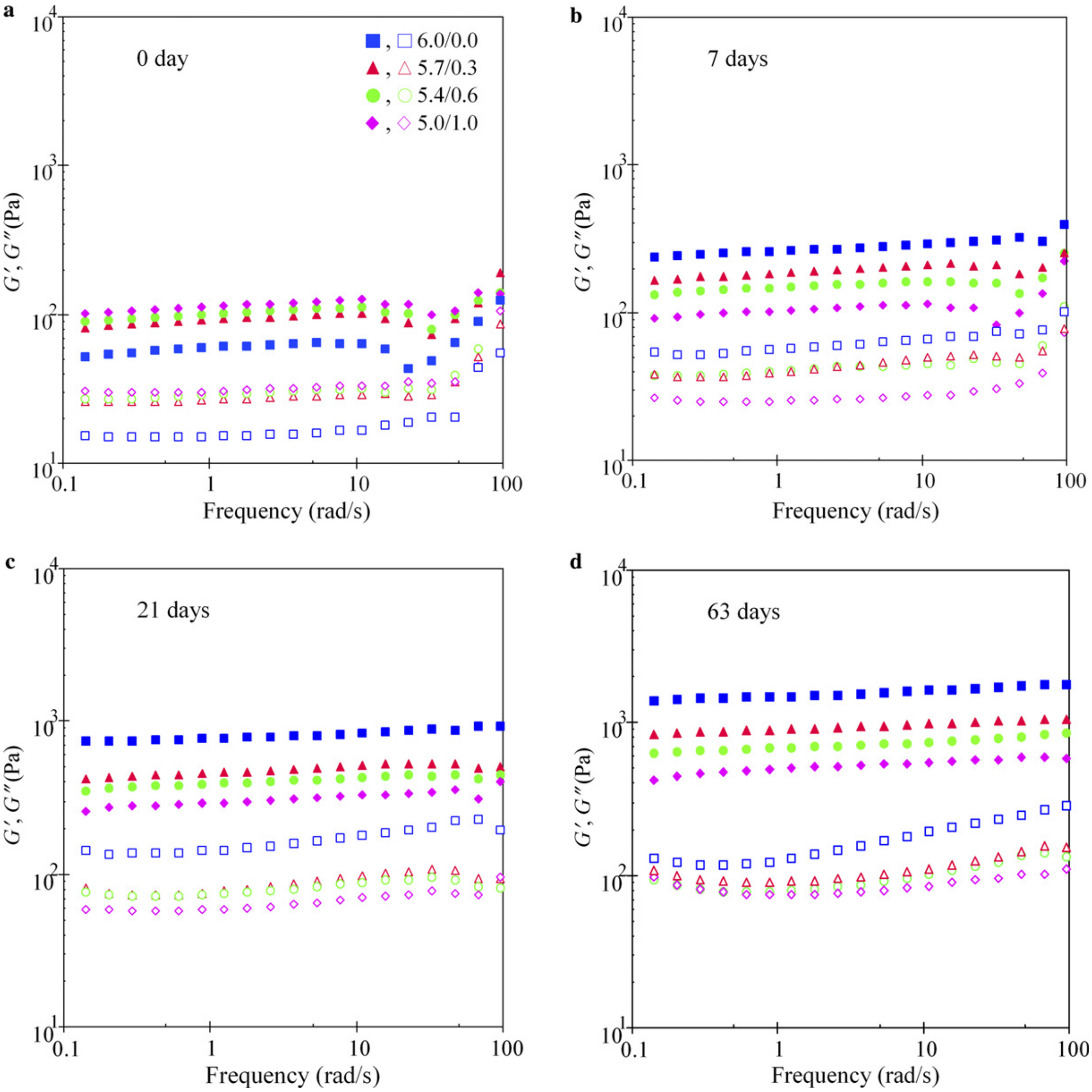

Fig. 7. Frequency dependence of storage modulus, G' (closed symbol) and loss modulus, G'' (open symbol) of 3.5%, w/w, RS/BG mixed gels at mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 (a) immediately after gelatinization and (b) 7, (c) 21, and (d) 63 days after storage at 4 °C. Measurements were made at 0.5% strain and 25 °C.

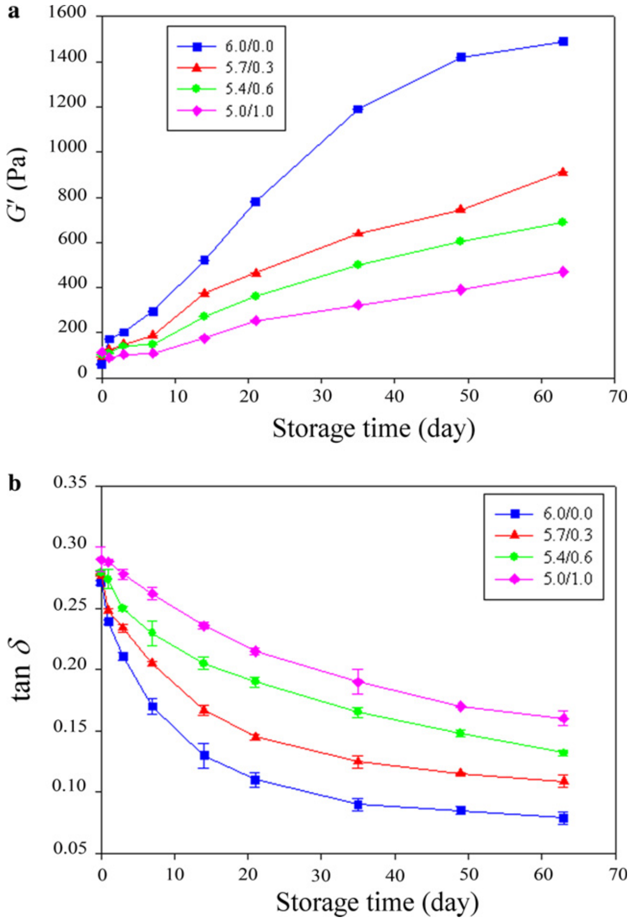

Fig. 8. Changes in (a) storage modulus,G' and (b) loss tangent, tan δ of 3.5%, w/w, RS/BG mixed gels at mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 as a function of storage time at 4 °C. Measurements were made at 1 rad/s, 0.5% strain and 25 °C. Error bars represent standard deviations.

For the freshly prepared RS/BG gels at 0 day storage (Fig. 7(a)), magnitudes of the G' and G'' increased with an increase in BG concentration. A similar tendency was reported for corn starch/konjac–glucomannan (Yoshimura, Takaya, & Nishinari, 1998) and rice starch/xanthan gum (Kim & Yoo, 2006) mixtures. This result is consistent with the previous RVA conclusion that BG promoted starch retrogradation initially as evidenced by the increase in setback viscosity with BG concentration (Table 1). Allonce et al. (1989) and Yoshimura et al. (1998) indicated that such acceleration of the gel formation process over short storage times can be attributed to the effective concentration of starch increased by the immobilization of water by gum resulting in the local interactions between amylose molecules more early and quickly.

The stored RS/BG gels exhibited the opposite trend in which the values of G' and G'' decreased with increasing BG concentration (Fig. 7(b)–(d)). This result indicates that the added BG retarded the formation of recrystallized amylopectin by reducing water availability and mobility of the starch chains as described earlier and in turn resulted in a weaker gel structure.

The conclusion can be made at this point that the BG promoted starch recrystallization (retrogradation) at the beginning and then retarded it during longer storage times. This tendency is in good agreement with those found in corn starch/konjac–glucomannan (Yoshimura, Takaya, & Nishinari, 1996, 1998) and rice starch/xanthan gum (Kim & Yoo, 2006) mixtures.

The G' and tan δ values for the RS/BG mixed gels as a function of refrigerated storage time are shown in Fig. 8. The G' values increased steadily with storage time (Fig. 8(a)) due to the development of a more firm structure (Fig. 9) attributed to starch retrogradation (Fig. 4). However, the magnitude of G' decreased with increasing BG content of the gels indicating that BG retarded gel network formation. Similar results were also reported for the mixed waxy maize starch/oat BG gels by Biliaderis et al. (1997).

The tan δ values, as expected, showed the opposite trend, i.e., decreased with storage time (Fig. 8(b)), indicating that the gels became more solid-like during storage. The values of tan δ increased with increasing content of BG. Mixtures with lower BG content are more solid-like than those with higher BG content. In studies on waxy rice starch/guar gum mixtures, it was also shown that guar gum changes the nature of the starch network from viscoelastic to more viscous-like, presumably by reducing the number of permanent cross-links between the amylopectin molecules (Kulicke, Eidam, Kath, Kix, & Kull, 1996).

3.5. Textural properties

Textural changes in terms of gel hardness occurred during refrigerated storage of the RS/BG mixed gels are presented in Fig. 9. The RS alone paste showed the most pronounced increase in gel hardness with increasing storage times, which led to gel hardening and consequently unacceptable texture. A higher BG concentration in the mixed gels appeared to result in a lower change in textural

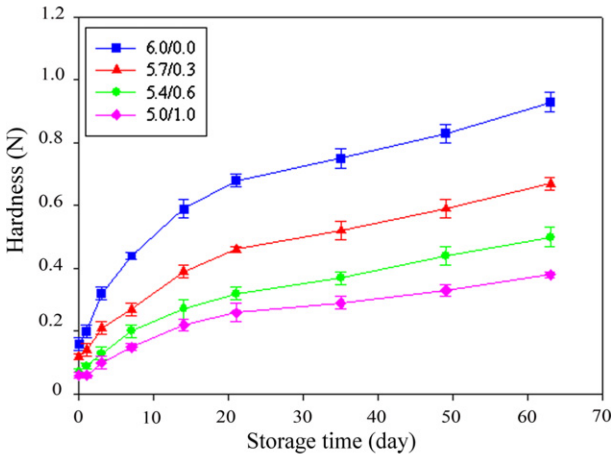

Fig. 9. Developments in hardness of 6%, w/w, RS/BG mixed gels at mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 during storage at 4 °C. Measurements were made at 25 °C. Error bars represent standard deviations.

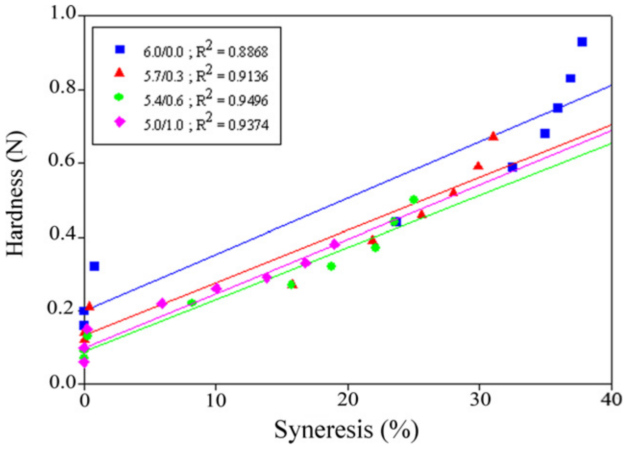

Fig. 10. Regression of gel hardness versus syneresis values for 6%, w/w, RS/BG mixed gels at mixing ratios of 6.0/0.0, 5.7/0.3, 5.4/0.6, and 5.0/1.0 during storage at 4 °C for 63 days. Full lines are linear regressions.

properties during storage. Incorporation of the BG up to a level of 1.0% into the RS is thus considered to be advantageous to maintain desirable textural properties and as well as to minimize syneresis (Fig. 5) of the gels during storage. This could be due to the hydrophilic character of the BG itself which minimized the RS retrogradation and water separation as previously described. This large deformation testing result is consistent with that of the small deformation oscillatory measurements (Fig. 8).

Due to the same total solids content used (6%, w/w), an attempt was made to correlate the retrogradation data obtained from syneresis assay (Fig. 5) and TPA analysis (Fig. 9) as shown in Fig. 10. As would be expected, the strong positive correlations ($R^2 = 0.89–0.94$) between the gel hardness and the syneresis values were observed for the RS/BG mixed gels during storage indicating that syneresis was responsible for gel hardening.

4. Conclusions

This work clarified that the gelatinization and retrogradation characteristics of the RS were largely modified by the spent brewer's yeast BG addition and the extent of this effect depended upon the BG content. The high thickening and water-holding capacities of the BG itself were hypothesized to be responsible for an increase in viscosity of the RS/BG mixed suspensions during pasting and a retardation of retrogradation, syneresis production, gel network formation and gel strength development of the RS/BG mixed gels during refrigerated storage. The effect was more pronounced at higher concentrations of BG. The spent brewer's yeast BG thus provides a potentially valuable addition to starch-based foods to restrict retrogradation of the starch.

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References

Alloncle, M., Lefebvre, J., Llamas, G., & Doublier, J. L. (1989). A rheological characterization of cereal starch–galactomannan mixtures. *Cereal Chemistry, 66*, 90–93.

Bahnassey, Y. A., & Breene, W. M. (1994). Rapid Visco-Analyzer (RVA) pasting profiles of wheat, corn, waxy corn, tapioca and amaranth starches (*A. hypochondriacus* and *A. cruentus*) in the presence of konjac flour, gellan, guar, xanthan and locust bean gums. *Starch/Stärke, 46*, 134–141.

Bao, J., & Bergman, C. J. (2004). The functionality of rice starch. In A.-C. Eliasson (Ed.), *Starch in food: Structure, function and applications* (pp. 271–282). Cambridge: Woodhead Publishing.

Biliaderis, C. G. (1991). The structure and interactions of starch with food constituents. *Canadian Journal of Physiology and Pharmacology, 69*, 60–78.

Biliaderis, C. G., Arvanitoyannis, I., Izydorczyk, M. S., & Prokopowich, D. J. (1997). Effect of hydrocolloids on gelatinization and structure formation in concentrated waxy maize and wheat starch gels. *Starch/Stärke, 49*, 278–283.

Chaisawang, M., & Suphantharika, M. (2005). Effects of guar gum and xanthan gum additions on physical and rheological properties of cationic tapioca starch. *Carbohydrate Polymers, 61*, 288–295.

Chaisawang, M., & Suphantharika, M. (2006). Pasting and rheological properties of native and anionic tapioca starches as modified by guar gum and xanthan gum. *Food Hydrocolloids, 20*, 641–649.

Christianson, D. D., Hodge, J. E., Osborne, D., & Detroy, R. W. (1981). Gelatinization of wheat starch as modified by xanthan gum, guar gum, and cellulose gum. *Cereal Chemistry, 58*, 513–517.

Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymer gels. *Advances in Polymer Science, 83*, 57–192.

Eliasson, A.-C., & Gudmundsson, M. (1996). Starch: Physicochemical and functional aspects. In A.-C. Eliasson (Ed.), *Carbohydrates in food* (pp. 431–504). New York: Marcel Dekker.

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*, 300–308.

Jamas, S., Rha, C.K., & Sinskey, A.J. (1989). Glucan composition and process for preparation thereof. U.S. Patent 4,810,646.

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

Kim, S. S., & Setser, C. S. (1992). Wheat starch gelatinization in the presence of polydextrose or hydrolyzed barley β-glucan. *Cereal Chemistry, 69*, 447–452.

Kim, C., & Yoo, B. (2006). Rheological properties of rice starch–xanthan gum mixtures. *Journal of Food Engineering, 75*, 120–128.

Kohyama, K., & Nishinari, K. (1992). Cellulose derivatives effects on gelatinization and retrogradation of sweet potato starch. *Journal of Food Science, 57*, 128–131 and 137.

Krüger, A., Ferrero, C., & Zaritzky, N. E. (2003). Modelling corn starch swelling in batch systems: Effect of sucrose and hydrocolloids. *Journal of Food Engineering, 58*, 125–133.

Kulicke, W.-M., Eidam, D., Kath, F., Kix, M., & Kull, A. H. (1996). Hydrocolloids and rheology: Regulation of visco-elastic characteristics of waxy rice starch in mixtures with galactomannans. *Starch/Stärke, 48*, 105–114.

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

Liu, H., Eskin, N. A. M., & Cui, S. W. (2006). Effects of yellow mustard mucilage on functional and rheological properties of buckwheat and pea starches. *Food Chemistry, 95*, 83–93.

Mali, S., Ferrero, C., Redigonda, V., Beleia, A. P., Grossmann, M. V. E., & Zaritzky, N. E. (2003). Influence of pH and hydrocolloids addition on yam (*Dioscorea alata*) starch pastes stability. *Lebensmittel-Wissenschaft und-Technologie, 36*, 475–481.

Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research, 135*, 271–281.

Pons, M., & Fiszman, S. M. (1996). Instrumental texture profile analysis with particular reference to gelled systems. *Journal of Texture Studies, 27*, 597–624.

Rojas, J. A., Rosell, C. M., & Benedito de Barber, C. (1999). Pasting properties of different wheat flour–hydrocolloid systems. *Food Hydrocolloids, 13*, 27–33.

Romero, R., & Gomez-Basauri, J. (2003). Yeast and yeast products, past, present and future: From flavors to nutrition and health. In T. P. Lyons & K. A. Jacques (Eds.), *Nutritional biotechnology in the food and feed industries. Proceedings of Alltech's 19th international symposium* (pp. 365–378). Loughborough, Leics, UK: Nottingham University Press.

Sae-kang, V., & Suphantharika, M. (2006). Influence of pH and xanthan gum addition on freeze–thaw stability of tapioca starch pastes. *Carbohydrate Polymers, 65*, 371–380.

Shi, X., & BeMiller, J. N. (2002). Effects of food gums on viscosities of starch suspensions during pasting. *Carbohydrate Polymers, 50*, 7–18.

Silverio, J., Fredriksson, H., Andersson, R., Eliasson, A.-C., & Åman, P. (2000). The effect of temperature cycling on the amylopectin retrogradation of starches with different amylopectin unit-chain length distribution. *Carbohydrate Polymers, 42*, 175–184.

Slade, L., & Levine, H. (1987). Recent advances in starch retrogradation. In S. S. Stivala, V. Crescenzi, & I. C. M. Dea (Eds.), *Industrial polysaccharides—The impact of biotechnology and advanced methodologies* (pp. 387–430). New York: Gordon and Breach Science.

Slade, L., & Levine, H. (1991). Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition, 30*, 115–360.

SPSS for Windows (2003). *User's manual*, version 12. Chicago, IL: SPSS Inc.

Suphantharika, M., Khunrae, P., Thanardkit, P., & Verduyn, C. (2003). Preparation of spent brewer's yeast β-glucans with a potential application as an immunostimulant for black tiger shrimp, *Penaeus monodon*. *Bioresource Technology, 88*, 55–60.

Thammakiti, S., Suphantharika, M., Phaesuwan, T., & Verduyn, C. (2004). Preparation of spent brewer's yeast β-glucans for potential applications in the food industry. *International Journal of Food Science and Technology, 39*, 21–29.

Thanardkit, P., Khunrae, P., Suphantharika, M., & Verduyn, C. (2002). Glucan from spent brewer's yeast: Preparation, analysis and use as a potential immunostimulant in shrimp feed. *World Journal of Microbiology & Biotechnology, 18*, 527–539.

Varavinit, S., Shobsngob, S., Varanyanond, W., Chinachoti, P., & Naivikul, O. (2003). Effect of amylose content on gelatinization, retrogradation and pasting properties of flours from different cultivars of Thai rice. *Starch/Stärke, 55*, 410–415.

Worrasinchai, S., Suphantharika, M., Pinjai, S., & Jamnong, P. (2006). β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. *Food Hydrocolloids, 20*, 68–78.

Yang, W. H., Datta, A. K., & Rao, M. A. (1997). Rheological and calorimetric behavior of starch gelatinization in simulation of heat transfer. In R. Jowitt (Ed.), *Engineering and food at ICEF 7/Part 2* (pp. K1–K5). London: Sheffield Academic Press.

Yoshimura, M., Takaya, T., & Nishinari, K. (1996). Effects of konjac–glucomannan on the gelatinization and retrogradation of corn starch as determined by rheology and differential scanning calorimetry. *Journal of Agricultural and Food Chemistry, 44*, 2970–2976.

Yoshimura, M., Takaya, T., & Nishinari, K. (1998). Rheological studies on mixtures of corn starch and konjac–glucomannan. *Carbohydrate Polymers, 35*, 71–79.

Zeković, D. B., Kwiatkowski, S., Vrvić, M. M., Jakovljević, D., & Moran, C. A. (2005). Natural and modified (1 → 3)-β-D-glucans in health promotion and disease alleviation. *Critical Reviews in Biotechnology, 25*, 205–230.