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Carbohydrate Polymers 65 (2006) 371–380

Carbohydrate Polymers

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Influence of pH and xanthan gum addition on freeze-thaw stability of tapioca starch pastes

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Received 5 July 2005; received in revised form 15 January 2006; accepted 17 January 2006
Available online 20 March 2006

Abstract

Effects of pH (3, 7, and 9) and xanthan gum (XG) on freeze-thaw (FT) stability of tapioca starch (TS) pastes (6.0% or 24.0% w/w, total solids) were studied as a function of TS/XG mixing ratios (6.0/0.0, 5.7/0.3, and 5.4/0.6) and FT cycles (−20 °C, 22 h and 30 °C, 1 h up to five cycles). Syneresis results showed that xanthan was most effective in reducing the syneresis at pH 7 and this effect increased with increasing gum concentrations. At pH 3, however, xanthan was much less effective reflected by the highest syneresis produced within two FT cycles. DSC data demonstrated that neither transition temperatures nor enthalpies of the FT-TS/XG pastes were affected by pH and xanthan addition. Photographs of stained starch granules exhibited a phase separation between the added xanthan and starch polysaccharides at all pH values tested. SEM micrographs illustrated the largest ice crystals formation in the pH 3 pastes and in the 0.6% xanthan-added pastes of all pH values. Xanthan maintained textural properties of the tapioca starch pastes during FT treatments better at pH 7 and pH 9. Lower starch molecular weight (revealed by HPSEC) and a reduction in viscosity of xanthan solution caused by acid hydrolysis was probably the cause of the lower FT stability of starch pastes at pH 3.

Keywords: Tapioca starch; Xanthan gum; pH; Freeze-thaw stability; Syneresis; Texture

1. Introduction

Starch has been incorporated in many frozen foods (fruit fillings, soups, sauces, cream-based products, and frozen batter) either as main raw materials or as additives. As additives, starch contributes to the thickening and stabilizing effects and texture modification in food (Eliasson & Gudmundsson, 1996). However, when foods are frozen, the formation of ice crystals within the food matrix cause physical stress to food and upon thawing the melting of these ice crystals lead to moisture loss and the softening in food texture, which consequently affect the overall quality of frozen food (Rahman, 1999).

However, when starch-containing foods, i.e., starch pastes, are subjected to either storage or freezing and thawing conditions, their texture become more rigid resulting from association through hydrogen bonding of amylose and amylopectin components in starch (Funami et al., 2005). This process is termed retrogradation and is accelerated by freezing and thawing (Yuan & Thompson, 1998). The retrograded starch pastes eventually release water (syneresis).

Blending of hydrocolloids with starch aids processing and ameliorates changes in overall food quality during storage by synergistic interactions between starch and hydrocolloids. Hydrocolloids modify gelatinization and retrogradation behaviors of starch (Alloncle, Lefebvre, Llamas, & Doublier, 1989; Bahnassey & Breene, 1994; Christianson, Hodge, Osborne, & Detroy, 1981; Funami et al., 2005; Rojas, Rosell, & Benedito de Barber, 1999;

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doi:10.1016/j.carbpol.2006.01.029

Sudhakar, Singhal, & Kulkarni, 1996; Yoshimura, Takaya, & Nishinari, 1999), enhance freeze-thaw stability (Ferrero, Martino, & Zaritzky, 1993; Ferrero, Martino, & Zaritzky, 1994; Lee, Baek, Cha, Park, & Lim, 2002; Sudhakar et al., 1996) and modify textural properties (Liu, Eskin, & Cui, 2006; Mandala, Palogou, & Kostaropoulos, 2002; Yoshimura, Takaya, & Nishinari, 1998) of starch pastes.

Xanthan gum has been widely used in combination with starch in foods because it improves the physical properties of various starch (sweet potato, yam, corn starches, and wheat flour) pastes and gels such as decreasing retrogradation, syneresis (Ferrero et al., 1993, 1994; Lee et al., 2002; Mali et al., 2003) and increasing freeze-thaw stability of starch gels (Ferrero et al., 1993, 1994; Lee et al., 2002). Furthermore, xanthan gum not only minimizes syneresis of yam starch pastes kept under refrigerated conditions, but also prevents a marked increase in gel firmness of yam starch avoiding the undesirable effects of low temperatures on textural quality (Mali et al., 2003). The cryoprotecting effect of xanthan gum related to ice crystals' size formed in sweet potato starch gels induced by freeze-thaw (FT) treatments was investigated by Lee et al. (2002). Moreover, Ferrero et al. (1993, 1994) reported that xanthan gum did not affect ice crystal formation and amylopectin retrogradation, but it could prevent amylose retrogradation of corn starch and wheat flour pastes during frozen storage.

In food preparation, starch-based products can be submitted to different processing and storage conditions, which sometimes change the physical and/or chemical properties of these products, because they are strongly dependent on heat, pH, freeze-thaw, and shear conditions. The role of pH can be critical, but research on this topic is sparse. Mali et al. (2003) investigated the effect of pH (3, 5, and 6) on gelatinization of yam and tapioca starches. These authors reported that pasting properties of both starches were affected by pH. The effect of pH on stability of freeze-thawed food products should be of interest. However, up to the best of our knowledge the study on the influence of pH on freeze-thaw stability of tapioca starch–xanthan gum pastes has not yet been examined.

The aim of this study was to investigate the influence of pH and xanthan gum (XG) addition on FT stability of tapioca starch (TS) pastes as a function of TS/XG mixing ratios and number of FT cycles. Pastes and gels were characterized by syneresis measurement, differential scanning calorimetry (DSC), and texture profile analysis (TPA). In addition, light microscope (LM) photographs of the TS/XG pastes before and after FT treatments, scanning electron microscope (SEM) micrographs of the freeze-thawed TS/XG pastes, apparent viscosity of xanthan solutions and molecular size distribution of tapioca starch subjected to heat treatments at different pH values were also observed in line with the above investigations.

2. Materials and methods

2.1. Materials

A commercial TS was supplied by General Starch Co. Ltd, Bangkok, Thailand. Xanthan gum was obtained from Union Chemical 1986 Co. Ltd, Bangkok, Thailand. Hydrochloric acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Moisture contents of tapioca starch and xanthan gum were 11.9 and 10.3 % w/w, respectively.

2.2. Syneresis determination

Batches of 500 g TS/XG suspensions (6% w/w, dry basis) of three TS/XG mixing ratios; 6.0/0.0, 5.7/0.3, and 5.4/0.6 were prepared by dispersing the calculated amount of starch and gum in distilled water using a motorized stirrer for 1 h at room temperature (25 °C). The pH of suspensions was adjusted to 3, 7, and 9 using 1.0 N HCl or 1.0 N NaOH. 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. All the sample tubes were frozen in a −20 °C freezer for 22 h and then thawed in a water bath at 30 °C for 1 h. Three tubes were randomly selected for determination of syneresis, while the remaining tubes were then put back into the freezer for further freeze-thaw cycling up to five cycles. 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*g* for 15 min.

2.3. Thermal properties

Thermal characteristics of TS/XG systems were determined using differential scanning calorimetry (DSC 822^e, Mettler Toledo, Schwerzenbach, Switzerland). The total polysaccharides content of all suspensions was selected to be 24% w/w due to the sensitivity of the instrument. Suspensions of three TS/XG mixing ratios (6.0/0.0, 5.7/0.3, and 5.4/0.6) were adjusted to three pH values (pH 3, 7, and 9) using 0.1 N HCl or 0.1 N NaOH. Samples of 60–65 mg contained in 120 μl medium pressure crucibles were scanned from 25 to 120 °C with 10 °C/min heating rate. A sealed empty crucible was used as a reference. Sample rescanning was performed after five FT cycles under the same conditions as used in syneresis analysis and the scanning temperatures ranged from 10 to 120 °C with 5 °C/min heating rate. The DSC reported enthalpy and transition temperatures; onset (T_o), peak (T_p) and conclusion (T_c) temperatures of gelatinization and retrogradation. The enthalpies of starch gelatinization and melting were calculated in J/g of starch (dry basis). The samples were run in triplicate.

2.4. Microscopic examination

Gelatinized starch gels with and without xanthan gum obtained from the Brabender Viscoamylograph were microscopically examined before and after the freeze-thaw treatments (five cycles). The samples were put onto microscope slides and were stained with 0.02 N iodine solution (mixture of I:KI = 1:2), covered with cover glasses, and observed using a light microscope at 400× magnification. For the SEM observation, the freeze-thawed samples were freeze-dried and were mounted on aluminum stubs and then coated with gold-palladium. The morphology of the gel matrixes was examined under a scanning electron microscope (SEM S-2500, Hitachi Science Systems, Ibaraki, Japan) at 300× magnification with an accelerating voltage of 15 kV.

2.5. Molecular size distribution of the gelatinized tapioca starch

Fresh starch gels (6% w/w) of pH 3, 9, and unadjusted pH (control) obtained from the Brabender Viscoamylograph were diluted 15 times in order to obtain samples having 0.4% starch. The gels of pH 3 and pH 9 were neutralized using 0.1 N HCl or NaOH prior to dilution.

Molecular size distributions of amylose and amylopectin were characterized with high-performance size-exclusion chromatography (HPSEC) according to the method of Govindasamy, Oates, and Wang (1992). The system consisted of three SEC columns (Ultrahydrogel Linear, Ultrahydrogel 120, and Ultrahydrogel 120) connecting into series and maintained at 40 °C. A differential refractive index detector (Water 410 Differential Refractometer) was connected to the columns. The mobile phase was deionized water, which was filtered through a 0.45 μm cellulose nitrate filter and degassed by an ultrasonic bath (2210 Branson, USA). A flow rate of the mobile phase was 0.8 ml/min. The diluted starch gels (0.4% starch) were placed into an ultrasonic processor (Model VC 501, Sonic & Material Inc., CT) and ultrasonicated for 18 s to ensure a complete starch dissolution. The resulting gels were filtered through 8.0 μm cellulose nitrate filters prior to HPSEC injection of the 20 μl portion. HPSEC chromatograms reported retention times (RT) and peak areas related to the RT of standard dextran (Fluka Chemie AG, CH-9471 Buchs, Switzerland) with a range of molecular weight (Mn) and degree of polymerization (DPn) from 3260 to 500,500 μg/ml and 20 to 3090, respectively.

2.6. Viscosity measurement of xanthan gum dispersions

To determine effects of pH and heat treatment on viscosity of xanthan gum dispersion, the gum dispersion (0.2% w/w) was prepared using distilled water, adjusted to pH 3, 7, and 9, and then heated and cooled in the Brabender Viscoamylograph using the same conditions as in Section 2.2. Viscosity was measured at 25 °C with a Brookfield Programmable DV–II+ Viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) using a small sample adapter at a shear rate range of 0.4–106 s^{-1}. The viscosity of the pH 3 sample after neutralization was also measured.

2.7. Texture profile analysis (TPA)

The FT samples were prepared as described in Section 2.2. At the end of each FT cycle, the gel texture was determined using 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. These specimens were subjected to deformation levels of 50% of the original height for single compression cycle at room temperature (25 °C). Hemispherical probe (P/0.5HS) punched into the gel with a constant crosshead velocity of 1 mm/s. The textural parameters, i.e., hardness and rupture strength, were measured according to the definitions of Pons and Fiszman (1996). The hardness is defined as the peak force observed during the compression cycle, whereas the rupture strength is the first significant peak where the force falls off during the probe penetrates into the product.

2.8. Statistical analysis

SPSS (SPSS Inc., Chicago, IL) for Windows version 9.0 program was employed for analyzing the results obtained from three replications. Mean and standard deviations for each treatment were calculated. For the results of syneresis determination, gel texture and thermal analysis, analysis of variance (ANOVA) and Tukey's test were used to compare differences among the mean values at 0.05 level of confidence. A paired *t*-test was used for the analysis of apparent viscosity of xanthan gum dispersions at the 0.05 level of significance.

3. Results and discussion

3.1. Syneresis of freeze-thawed tapioca starch pastes with and without xanthan gum

Syneresis productions of freeze-thawed (FT) tapioca starch (TS)–xanthan gum (XG) pastes (6% w/w) of three TS/XG mixing ratios; 6.0/0.0, 5.7/0.3, and 5.4/0.6 at pH 3, 7, and 9 are shown in Fig. 1. A typical positive correlation between syneresis and number of freeze thaw cycle up to a certain level before leveling off was found in this study. This can be readily explained by the formation of additional starch retrogradation during the subsequent freezing and thawing process. Addition of xanthan gum resulted in a reduction of syneresis, however, this effect varied with pH values of the TS/XG pastes. At pH 7 (Fig. 1b) and pH 9 (Fig. 1c), the syneresis profiles of TS/XG pastes were

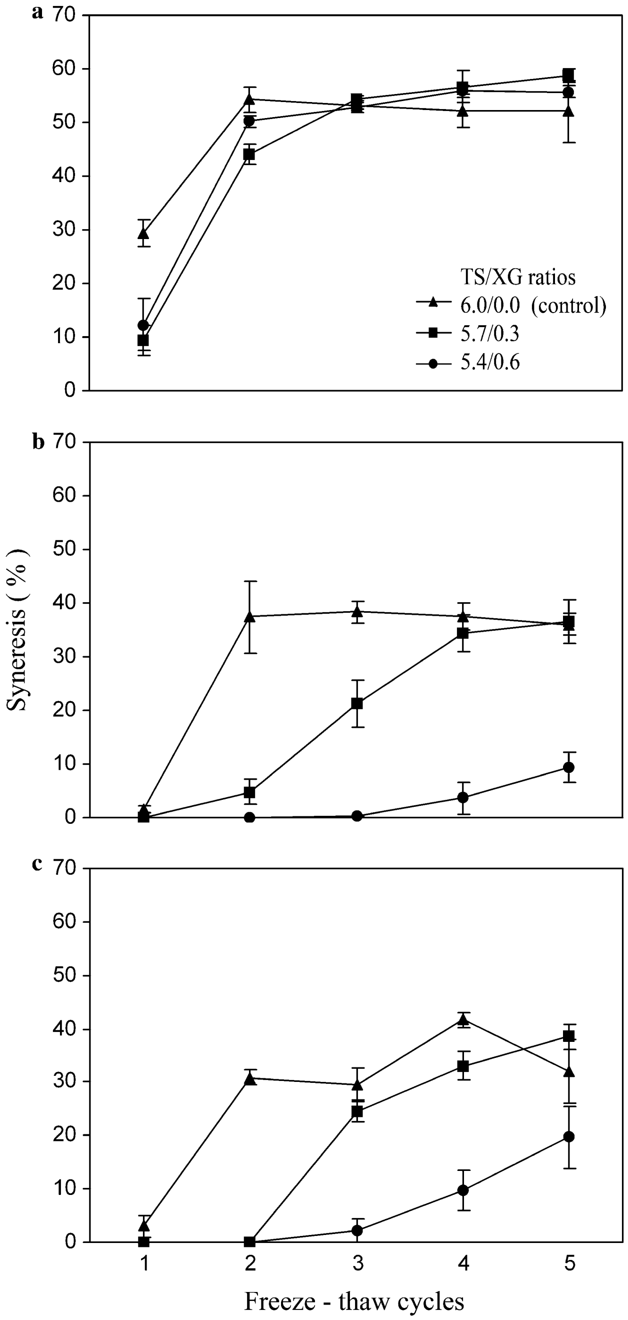

Fig. 1. Effect of number of freeze-thaw cycles on syneresis of tapioca starch (TS) pastes with and without xanthan gum (XG) (6% w/w, total solids content) gelatinized at (a) pH 3, (b) pH 7, and (c) pH 9. Error bars represent standard deviations.

similar. The syneresis was hardly detected after one FT cycle for all TS/XG pastes tested. The maximum syneresis values attained for the starch alone pastes (TS/XG = 6.0/0.0) were ~40% and these values decreased with increasing xanthan concentration up to 0.6% (TS/XG = 5.4/0.6). The 0.6% xanthan-added pastes seemed to be more stable at pH 7 than at pH 9, i.e., syneresis values after five FT cycles were ~10% and ~20% at pH 7 and pH 9, respectively.

At pH 3, pure tapioca starch paste gave up ~30% syneresis within one FT cycle, but the syneresis was substantially reduced to ~10% upon the addition of 0.3–0.6% xanthan gum (Fig. 1a). When the FT cycle was repeated twice, the syneresis of pure starch almost doubled, while xanthan gum-added pastes showed more than a four fold increase. These resulting syneresis values appeared to be the highest (55–58%) for all pastes tested and almost constant up to five FT cycles. The high syneresis values of the pH 3 samples, particularly the control, could be partly due to the soft texture of the gels (see Section 3.6) which provided less resistance to the deformation caused by centrifugation, and therefore a large amount of water can be separated. It is well recognized that the extent of syneresis measured by the centrifugation method is dependent upon the gel rigidity and elasticity as well as the extent of phase separation (Yuan & Thompson, 1998).

It is well known that during freezing, there is a progressive increase in local concentration of the starch as liquid water is converted into ice crystals, promoting intermolecular associations favored by hydrogen bonding which survive on thawing, to give the spongy texture and released water (syneresis). These effects contribute to the deterioration of the starch paste during freezing and thawing which can be reduced by adding small amounts of hydrocolloids (Ferrero et al., 1993, 1994; Lee et al., 2002).

Our finding that the syneresis decrease in xanthan-added starch gels was in good agreement with reported work on other starches, i.e., corn starch (Ferrero et al., 1994), corn starch and wheat flour (Ferrero et al., 1993), sweet potato starch (Lee et al., 2002), and yam starch (Mali et al., 2003). Moreover, in our view, the current work is of interest because it clearly reveals that the pH values of TS/XG suspensions during pasting significantly affected the freeze-thaw stabilizing capacity of xanthan gum, which has not been reported in the literature so far. Xanthan has been reported to hinder deteriorating effects from the repeated FT treatments by entanglement with starch molecules thus avoiding retrogradation (Ferrero et al., 1994). Moreover, the present study clearly revealed that phase separation between the added hydrocolloid and starch polysaccharides particularly amylose could be the cause of syneresis reduction (see Section 3.3). Xanthan was found to be most effective in improving the FT stabilization of the starch gel at pH 7, it was slightly less effective at pH 9 and far less effective at pH 3 which was probably due to molecular degradation of starch occurred during pasting under acidic and alkaline conditions (see Section 3.4).

3.2. Thermal properties of starch and freeze-thawed starch gels with and without xanthan gum

This experiment was carried out with an attempt to use differential scanning calorimetry (DSC) to assess the effects of pH and xanthan gum on pasting and freeze-thaw stability of tapioca starch pastes. For the DSC measurements, total polysaccharides concentration of 24% w/w was used and this concentration was much higher than those used in the other experiments (6% w/w) because the retrogradation process cannot be observed with such a low concentration with the DSC used. The transition temperatures and enthalpies of TS/XG suspensions and the corresponding freeze-thawed (five cycles) pastes at different pH values are summarized in Table 1. During pasting, the onset (T_o) and peak (T_p) gelatinization temperatures of starch with xanthan addition significantly ($P \leqslant 0.05$) shifted to higher temperatures whereas the gelatinization enthalpies were significantly lower than those of the starch alone at all pH values tested, which agrees with our previous study (Chaisawang & Suphantharika, 2006). However, when the comparison was made among the pure starch gelatinized at different pH values, the gelatinization enthalpy was significantly lower at pH 3 (13.0 J/g) and pH 9 (13.2 J/g) than at pH 7 (15.3 J/g). This indicated that hydrolysis of starch molecules occurred during acidic and alkaline pasting, which will be confirmed latter by the HPSEC results. Since the gelatinization enthalpy has been related to the melting of double helices (Cooke & Gidley, 1992), the reduction in gelatinization enthalpy could be interpreted as the occurrence of starch hydrolysis.

DSC results of the freeze-thawed pastes demonstrated much lower transition temperatures and enthalpies than those obtained during pasting. However, neither the transition temperatures nor enthalpies appeared to be affected by pH conditions and xanthan addition applied in this study. In general, it is well recognized that starch retrogradation can be considered as a two step process (Miles, Morris, Orford, & Ring, 1985), a 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. Transition enthalpies measured by DSC correspond to the heat involved in melting down the retrograded amylose and/or amylopectin. However, only amylopectin retrogradation could be quantified by DSC in the temperature range of 10–120 °C (Ferrero et al., 1994; Funami et al., 2005).

In our case, the transition enthalpies of the pastes subjected to five freeze-thaw treatment were very small and were in a range of 1.0–1.7 J/g. This results indicated that amylopectin retrogradation occurred to a very low extent. Thus, we can conclude that only amylose retrogradation was responsible for syneresis and textural changes during these FT treatments (up to five FT cycles) of starch pastes which in turn could be minimized by xanthan addition. Similar results have been reported in the cases of corn starch (Ferrero et al., 1994) and wheat flour (Ferrero et al., 1993). In addition, our results also clearly revealed that the centrifugation method (Fig. 1) was more sensitive than the DSC method (Table 1) for assessing the freeze-thaw stability of starch pastes, which agrees with those previously reported by Yuan and Thompson (1998).

3.3. Microscopic observations

This experiment was aimed to investigate the effects of pH and xanthan gum on microstructure of gelatinized starch pastes. In Fig. 2, the systems were examined microscopically staining for amylose after gelatinization and also after the freeze thaw cycles. As it can be seen for the gelatinized starch pastes without added xanthan, the stained starch granules and their fragments seem almost uncolored, i.e., pale blue (Figs. 2a–c). These granules had an irregular shape and were characterized by folds due to their efficient gelatinization in which amylose is considered to be totally leaked out from the granules during gelatinization. In the presence of xanthan gum, at all pH values tested, amylose leakage was hindered (Figs. 2d–f). Most swollen granules had preserved their shape. Some granules, primarily the smaller ones, had a blue core and a purple or red–brown shell, indicating that amylose had leaked from the outside layer with a definite depth and no indication of diffusion of amylose molecules from the inner area to the zone of

Table 1
Thermal properties of native and freeze-thawed (five cycles) tapioca starch (TS) with and without xanthan gum (XG) at 24% w/w total solids content (dry basis)

TS/XG mixing ratios	Native				Freeze-thawed			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
6.0/0.0, pH 3	65.2 ± 0.4^{d}	$75.4 \pm 0.0^{d,e}$	85.8 ± 0.2^{b}	13.0 ± 0.4^{b}	$45.4 \pm 0.5^{b,c}$	55.4 ± 0.2^{c}	$63.7 \pm 0.4^{b,c}$	$1.5 \pm 0.1^{a,b}$
5.7/0.3, pH 3	$68.0 \pm 0.2^{b,c}$	$76.5 \pm 0.3^{b,c,d}$	86.8 ± 0.4^{b}	12.8 ± 0.1^{b}	$46.3 \pm 0.2^{a,b}$	55.9 ± 0.1^{c}	$64.0 \pm 0.6^{b,c}$	$1.4 \pm 0.4^{a,b}$
5.4/0.6, pH 3	68.9 ± 0.2^{b}	$77.3 \pm 0.3^{b,c}$	87.3 ± 0.3^{b}	11.7 ± 0.3^{c}	$46.7 \pm 0.6^{a,b}$	$56.4 \pm 0.3^{b,c}$	$64.4 \pm 0.7^{a,b,c}$	$1.3 \pm 0.1^{a,b}$
6.0/0.0, pH 7	64.7 ± 0.1^{d}	74.7 ± 0.2^{e}	85.9 ± 0.3^{b}	15.3 ± 0.2^{a}	43.1 ± 0.8^{c}	56.3 ± 0.5^{c}	$64.9 \pm 0.8^{a,b,c}$	1.2 ± 0.2^{ab}
5.7/0.3, pH 7	67.4 ± 0.1^{c}	$76.2 \pm 0.4^{c,d,e}$	86.5 ± 0.2^{b}	13.0 ± 0.1^{b}	$45.3 \pm 0.7^{b,c}$	$56.4 \pm 0.5^{b,c}$	63.1 ± 1.3^{c}	1.2 ± 0.3^{ab}
5.4/0.6, pH 7	$68.7 \pm 1.2^{b,c}$	$77.6 \pm 1.6^{b,c}$	87.3 ± 3.8^{b}	12.8 ± 0.7^{b}	$46.3 \pm 1.1^{a,b}$	$58.6 \pm 1.3^{a,b}$	$65.6 \pm 03^{a,b}$	1.0 ± 0.2^{b}
6.0/0.0, pH 9	65.9 ± 0.3^{d}	$76.7 \pm 0.3^{b,c,d}$	88.6 ± 0.5^{b}	13.2 ± 0.5^{b}	40.6 ± 1.5^{d}	55.6 ± 0.3^{c}	$63.7 \pm 1.0^{b,c}$	$1.2 \pm 0.3^{a,b}$
5.7/0.3, pH 9	68.9 ± 0.3^{b}	78.1 ± 0.4^{b}	88.8 ± 0.5^{b}	13.2 ± 0.1^{b}	$45.4 \pm 0.5^{a,b,c}$	$57.1 \pm 1.8^{a,b,c}$	$63.9 \pm 0.5^{b,c}$	1.7 ± 0.2^{a}
5.4/0.6, pH 9	72.2 ± 0.2^{a}	83.3 ± 0.5^{a}	96.7 ± 0.6^{a}	10.9 ± 0.3^{c}	47.8 ± 0.9^{a}	58.9 ± 0.2^{a}	66.6 ± 1.0^{a}	$1.2 \pm 0.1^{a,b}$

T_o, T_p, T_c and ΔH are onset, peak, conclusion temperatures and enthalpy, respectively.
Assays were performed in triplicate.
Mean ± standard deviation values followed by different superscripts within the same column are significantly different ($P \leqslant 0.05$).

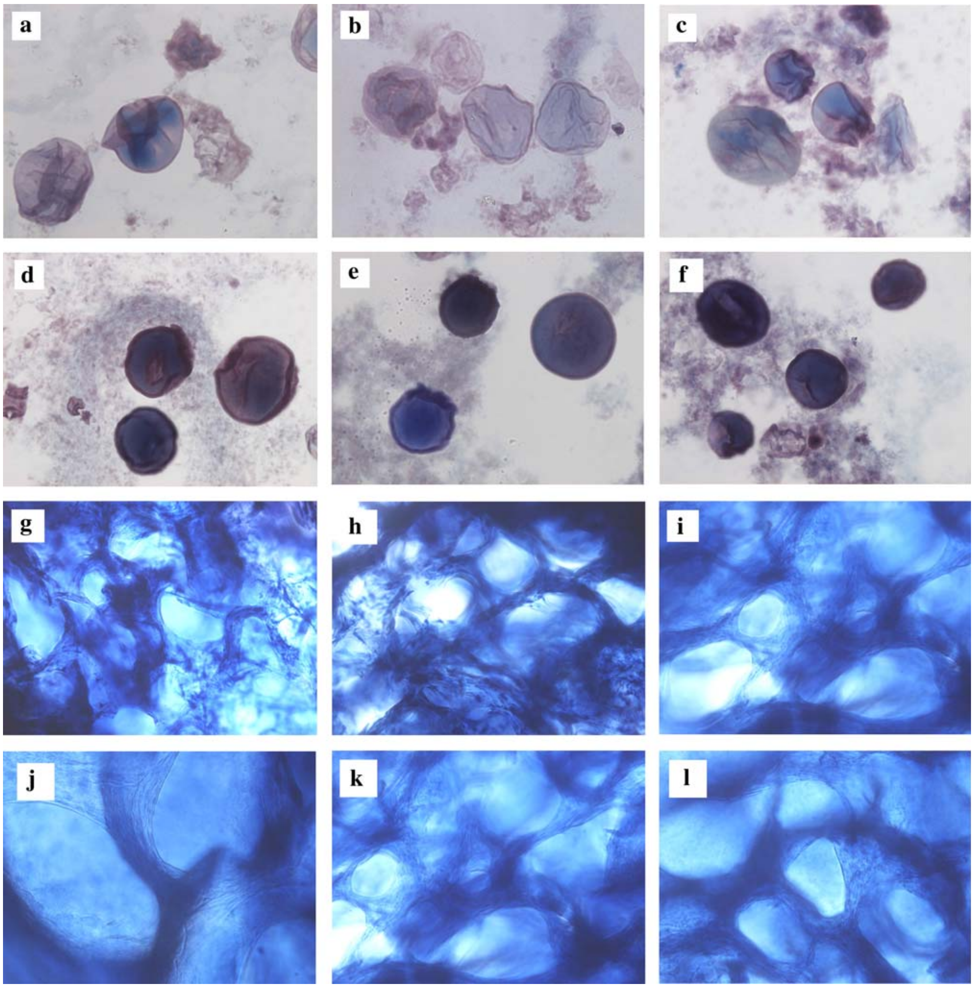

Fig. 2. Photographs of tapioca starch (TS) granules stained with iodine after gelatinization in the presence or absence (as control) of xanthan gum (XG) at different pH values and also after freeze-thawed (FT) for five cycles: (a–c) gelatinized TS/XG = 6.0/0.0 at pH 3, 7, and 9; (d–f) gelatinized TS/XG = 5.4/0.6 at pH 3, 7, and 9; (g–i) FT-gelatinized TS/XG = 6.0/0.0 at pH 3, 7, and 9; and (j–l) FT-gelatinized TS/XG = 5.4/0.6 at pH 3, 7, and 9, respectively (400× magnification).

leakage. These results clearly demonstrated evidence of phase separation between the added hydrocolloid and starch polysaccharides particularly amylose. A consequence of this is that the xanthan resulted in amylose being retained within the granules following gelatinization and consequently the association between amylose outside the granules was reduced. This phase separation phenomena also kept released amylose in its own domain preventing the formation of the continuous amylose network which dominated the gel strength and promoted syneresis. Similar results were previously shown that xanthan formed microphase-separated domains and was concentrated as a film wrapped around the granules which retained the shape of the gelatinized granules (Chaisawang & Suphantharika, 2006; Mandala & Bayas, 2004 ; Mandala et al., 2002).

After FT treatments, the gels had a sponge-like network with blue color indicating an amylose matrix and all starch granules disappeared (Figs. 2g–l). These findings indicate that the gelatinized starch granules were mostly disintegrated by the freezing and thawing processes. The macroscopic pores created by ice crystals in the frozen state can be seen after the ice had melted. Ice crystal sizes were visually estimated from the sizes of these pores.

Scanning electron microscope observation of the samples provides detailed information about the structures mentioned above (Fig. 3). The largest ice crystals formed were observed in the pH 3 pastes, whereas those of the pH 9 samples were only slightly larger than the ones found in the pH 7 samples. This could be mainly due to the extensive acidic degradation of starch (Fig. 4) and xanthan (Fig. 5) molecules during pasting which reduced the viscosity of the mixed pastes. Therefore, the highest molecular mobility of starch, xanthan and as well as water molecules would be expected which promoted starch retrogradation as evidenced by high syneresis (Fig. 1a) and ice recrystallization as evidenced by the large ice crystals formed (Figs. 3a, d, and g). The network membrane of the starch alone paste at pH 3 (Fig. 3a) was so thin and porous that it produced a very soft gel texture (Fig. 6a) in comparison with that of the pH 7 (Fig. 6b) and pH 9 (Fig. 6c) pastes. At pH 3, addition of xanthan reduced the porosity of the

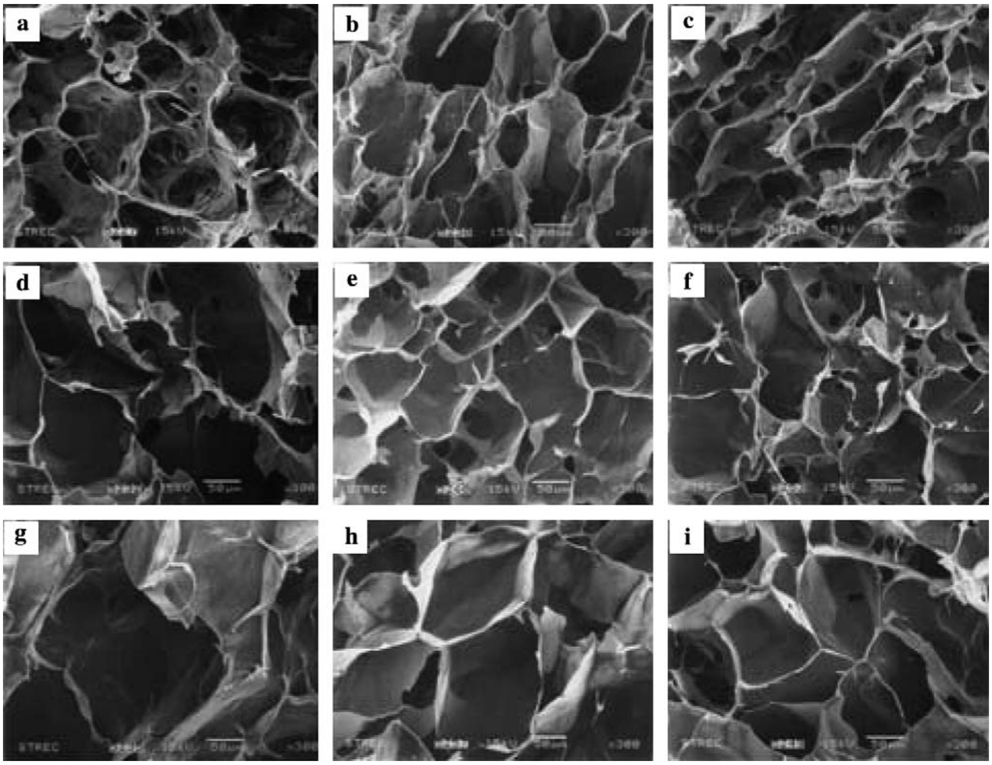

Fig. 3. SEM micrographs of freeze dried tapioca starch (TS) pastes with and without xanthan gum (XG) (6% w/w, total solids content) obtained after gelatinization at different pH values and freeze-thawed (FT) for five cycles: (a–c) TS/XG = 6.0/0.0 at pH 3, 7 and 9; (d–f) TS/XG = 5.7/0.3 at pH 3, 7 and 9; and (g–i) TS/XG = 5.4/0.6 at pH 3, 7, and 9, respectively (300×, Bar = 50 μm).

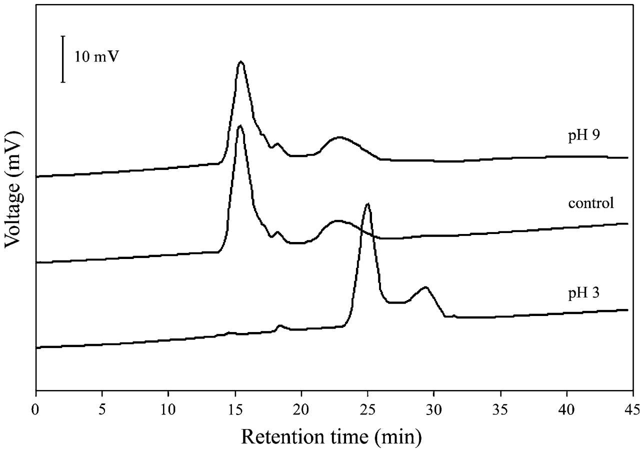

Fig. 4. High-performance size-exclusion chromatograms of tapioca starch pastes gelatinized at pH 3 and pH 9. The gelatinized starch obtained without pH adjustment prior to gelatinization was used as a control.

network membrane (Figs. 3d and g) and reinforced the gel strength (Fig. 6a).

Addition of xanthan gum, at all pH values tested, surprisingly resulted in formation of larger ice crystals, particularly at high xanthan concentration (TS/XG = 5.4/0.6), than those observed for starch pastes alone. This is due to the substitution of tapioca starch with xanthan at the total polysaccharides concentration of 6% w/w caused a reduction in the content of the gelling component, i.e., starch and a promotion of the phase separation between starch and xanthan molecules led to a reduction of gel formation of the starch and it was the starch gel which

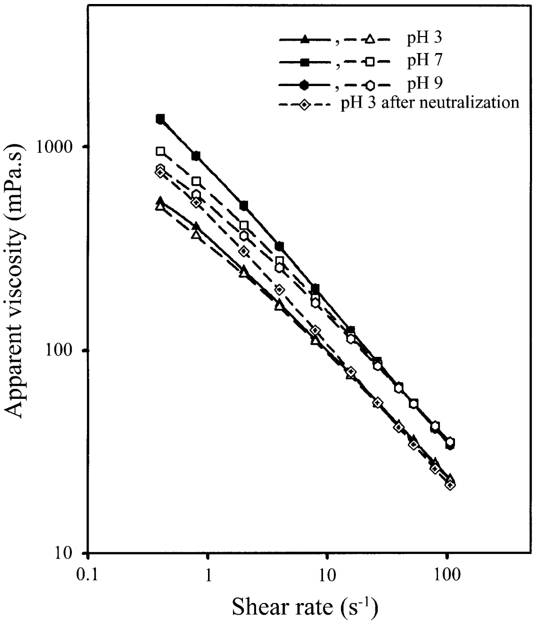

Fig. 5. Apparent viscosities of 0.2% w/w xanthan gum dispersions prepared at pH 3, 7, and 9 measured before (closed symbols) and after (open symbols) heat treatments and also after neutralization of the pH 3 sample. Measurements were made at 25 °C.

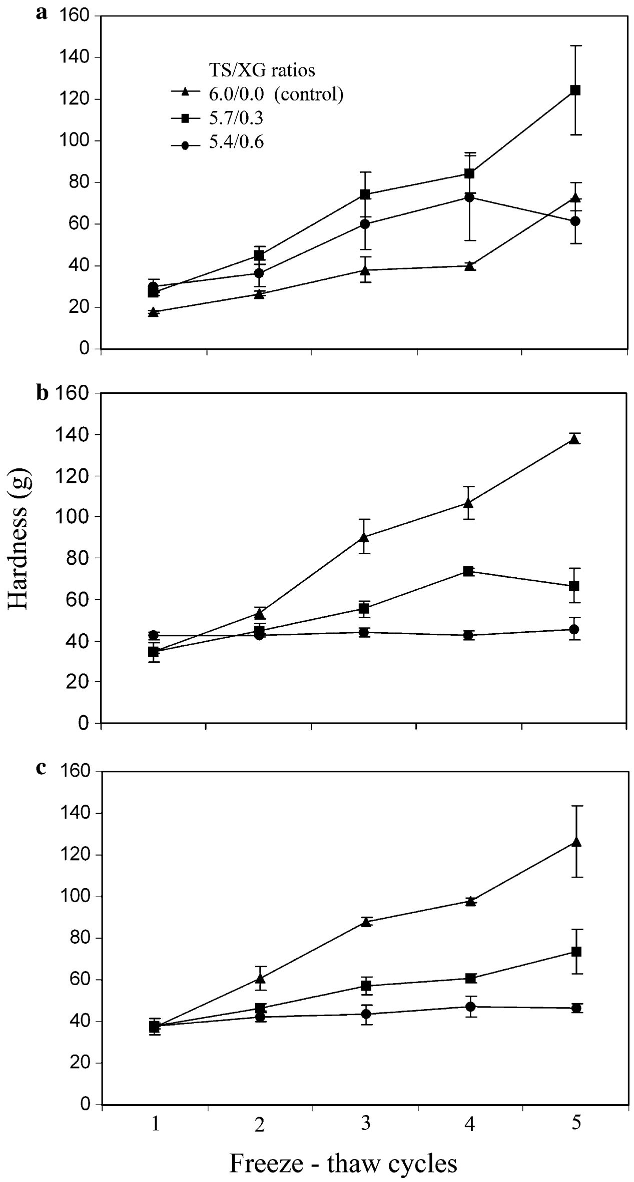

Fig. 6. Effect of number of freeze-thaw cycles on hardness of tapioca starch (TS) pastes with and without xanthan gum (XG) (6% w/w, total solids content) gelatinized at (a) pH 3, (b) pH 7, and (c) pH 9. Error bars represent standard deviations.

reduced ice crystal growth. These results are in good agreement with those previously reported by Ferrero et al. (1993, 1994) and Lee et al. (2002). Therefore, the effect of xanthan gum in reducing syneresis (Fig. 1) could not be associated with ice recrystallization (Fig. 3), but with the high water holding capacity of the gum which retains water during and after thawing.

3.4. Molecular size distribution of the gelatinized tapioca starch

The aim of this experiment was to examine the influence of pH during gelatinization on degradative changes in molecular size distributions of starch. Chromatograms of the gelatinized starch obtained without pH adjustment (control) analyzed by HPSEC showed a pattern with three distinctive peaks (Fig. 4): fraction I consisting of high molecular weight, mainly amylopectin molecules, fraction II consisting of intermediate size molecules, and fraction III composed of low molecular weight or linear amylose molecules. When the starch was gelatinized at pH 3, fractions I, II, and III markedly shifted to longer retention times or lower molecular weights, indicating that both amylose and amylopectin were simultaneously degraded by acid hydrolysis, which agrees with Wang and Wang (2001). These molecular changes would be responsible for the much higher syneresis (Fig. 1) and lower gel hardness (Fig. 6) which in turn lower freeze-thaw stability of the gels as compared with the control. On the contrary, starch gelatinized at pH 9 exhibited only a slightly larger peak shoulder of fraction I than the control indicating that only small fractions of amylopectin molecules were degraded at this pH value. These findings contribute to the results of the present study in which the freeze-thaw stability, expressed in terms of syneresis (Fig. 1), and textural properties (Figs. 6 and 7) of the pH 9 gels were comparable with those of the pH 7 samples.

3.5. Viscosity of the xanthan gum dispersions

The purpose of this experiment was to investigate the influence of pH and heat treatments on viscosity of the xanthan gum dispersions. As shown in Fig. 5, viscosities of xanthan solutions were hardly affected by heat treatments applied in this study at various pH values tested. The viscosities of pH 7 and pH 9 samples were identical at shear rate higher than 10 s^{-1}, whereas those of the pH 3 sample were significantly ($P \leqslant 0.05$) lower and did not change upon neutralization. These results indicated that xanthan molecules were degraded to some extents at pH 3 which, in turn, could adversely affect the freeze-thaw stability of the TS/XG gels. These findings are reflected by a slightly higher syneresis (after four FT cycles, Fig. 1a) and significantly higher hardness and rupture strength (after three FT cycles, Figs. 6a and 7a, respectively) of the TS/XG gels as compared with the control.

3.6. Textural properties

Textural changes occurred during freezing and thawing of starch pastes with and without xanthan gum are presented in Figs. 6 and 7 for gel hardness and rupture strength, respectively. In general, the results of hardness showed the same tendency as those of the rupture strength. Considering the pH 7 (Figs. 6b and 7b) and pH 9 (Figs. 6c and 7c) pastes, the starch alone pastes showed the most pronounced increase in both hardness and rupture strength with increasing the number of FT cycles, which led to gel hardening and consequently unacceptable texture. A higher xanthan gum concentration in the mixed pastes appeared to result in a lower change in textural properties during FT treatments. Incorporation of xanthan gum up to a level

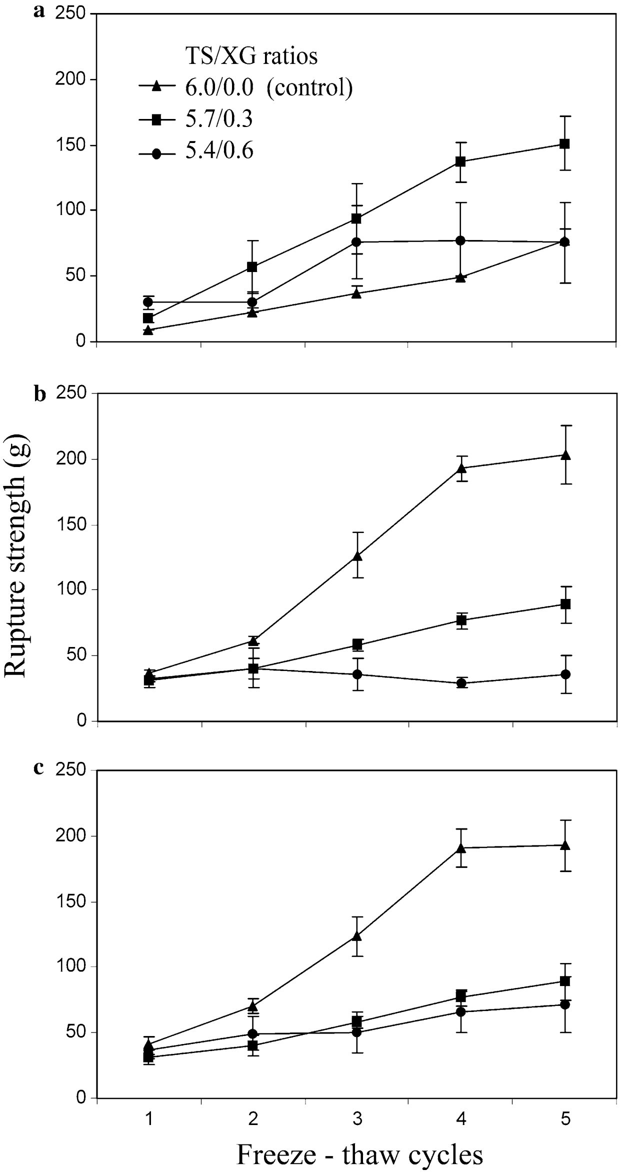

Fig. 7. Effect of number of freeze-thaw cycles on rupture strength of tapioca starch (TS) pastes with and without xanthan gum (XG) (6% w/w, total solids content) gelatinized at (a) pH 3, (b) pH 7, and (c) pH 9. Error bars represent standard deviations.

of 0.6% into tapioca starch is thus considered to be advantageous to maintain desirable textural properties and as well as to minimize syneresis (Figs. 1b and c) of the mixed pastes during FT treatments.

The opposite results, however, were observed for hardness (Fig. 6a) and rupture strength (Fig. 7a) of the pastes obtained at pH 3. A slight increase of these textural parameters with increasing the number of FT cycles was observed for starch pastes with and without xanthan gum. The starch alone paste exhibited the lowest values of these parameters, which could be due to an excessive acid hydrolysis of the starch molecules led to a weak network structure of the paste. The paste consisting of 0.3% xanthan gum (TS/XG = 5.7/0.3) gave the highest hardness and rupture strength values. As previously discussed in Sections 3.4 and 3.5, xanthan gum seemed to be more stable under acidic heat treatments than the starch itself. Therefore, the textural properties of the TS/XG pastes might be dominated by those of the pure xanthan. This hypothesis was confirmed by the work of Giannouli and Morris (2003), who found that much stronger gel networks of pure xanthan were formed, when xanthan solutions were frozen and thawed. Progressive increase in gel strength after successive FT cycles had been also reported. Addition of xanthan up to 0.6% (TS/XG = 5.4/0.6) slightly lowered the textural parameter values as compared with the 0.3% xanthan-added paste possibly due to the phase separation which predominantly occurred at a high gum to starch ratio.

4. Conclusions

This work clarified that FT stability of tapioca starch pastes was influenced by both pH and xanthan addition. Xanthan was most effective in enhancing FT stability of starch pastes at pH 7 and slightly less effective at pH 9. FT stability increased with increasing gum concentrations. At pH 3, however, xanthan was least effective led to the highest syneresis produced and textural changes during FT treatments. Low molecular weight of starch due to molecular degradation occurred during acidic and, to a much less extent, alkaline pasting coupled with low apparent viscosity of xanthan solutions at pH 3 were hypothesized to be the main causes.

Acknowledgements

This research work was partially supported by the Higher Education Development Project, Subproject: Graduate Study and Research in Agricultural Biotechnology, Ministry of Education. The authors thank Mettler-Toledo (Thailand) Ltd, Bangkok, Thailand for providing the differential scanning calorimeter (DSC) used in these experiments.

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