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Influence of guar gum on granule morphologies and rheological properties of maize starch

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Abstract

The effect of guar gum on the microstructure and viscoelastic properties of maize starch was investigated using confocal laser scanning microscopy (CLSM) and dynamic viscoelastic measurements. CLSM showed that guar gum had little effect on the swelling behavior of starch granules during heating. It also indicated that guar gum tended to inhibit starch components from leaching out of starch granules during gelatinization, as the average molecular weight or concentration of added guar gum increased. Dynamic viscoelastic measurements showed that the mechanical loss tangent tan δ of the pastes increased with increasing concentration of added guar gum. In conclusion, guar gum inhibits starch components from leaching out of starch granules to the continuous phase of starch pastes during gelatinization, resulting in an increase in viscous characteristics in the system.

Keywords: Guar gum; Maize starch; Gelatinization; Confocal laser scanning microscopy; Dynamic viscoelastic measurements

1. Introduction

Starches and non-starch hydrocolloids are often used together in food systems to modify or control the texture and improve the stability of food products. The key to any starch use is primarily in the behavior of its pastes. Changes in the behavior of starch pastes can all be affected by the presence of non-starch hydrocolloids (Appelqvist & Debet, 1997). Therefore, it is important to understand the effects of non-starch hydrocolloids on starch properties such as gelatinization and retrogradation.

The changing of paste viscosity during heating of starch suspensions and the formation of gels during cooling are the fundamental functional characteristics of starch (Parker & Ring, 2001). In an excess of water, above a characteristic temperature known as the gelatinization temperature, starch granules lose their native crystalline order and swell irreversibly. Swelling is accompanied by the solubilization of amylose, while substantial solubilization of high molecular weight amylopectin is not observed (Miles, Morris, Orford, & Ring, 1985). Starch paste or gel structure is composite of a three-dimensional matrix of swollen gelatinized starch granules, embedded in a matrix of amylose (Morris, 1990). The gelatinized granules increase the stiffness of the amylose gel, and their size greatly influences the rheological properties of starch gels (Okechukwu & Rao, 1995; Tsai, Li, & Lii, 1997). For this reason, the structure of gelatinized starch granules has been studied by many analytical methods such as light microscopy (Atkin, Abeysekera, Cheng, & Robards, 1998; Conde-Petit, Nuessli, Handschin, & Escher, 1998; Han, Hamaker, & Lafayette, 2000; Obanni & BeMiller, 1995, 1996), electron microscopy (Fannon & BeMiller, 1992), differential scanning calorimetry (Cooke & Gidley, 1992; Crochet, Beauxis-Lagrave, Noel, Parker, & Ring, 2005; Liu & Lelievre, 1993; Tester & Sommerville, 2003) and X-ray dif-

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fraction (Prentice, Stark, & Gidley, 1992; Vermeylen et al., 2006).

It is widely recognized that gelatinizing starch dispersions in the presence of a non-starch hydrocolloid (e.g., guar gum, locust bean gum, xanthan gum, etc.) strongly influences the rheological properties of starch pastes and gels, such as viscosity and viscoelasticity (Alloncle & Doublier, 1991; Alloncle, Lefebvre, Llamas, & Doublier, 1989; Closs, Conde-Petit, Roberts, Tolstoguzov, & Escher, 1999; Rayment, Ross-Murphy, & Ellis, 1995; Sudhakar, Singhal, & Kulkarni, 1996). Funami et al. (2005a, 2005b, 2005c) investigated the effects of galactomannans on the gelatinization and retrogradation of wheat and maize starches. They reported that the rheological change in starch pastes with added guar gum was closely related to the amount of amylose leached out from starch granules to the continuous phase of the starch paste during gelatinization (Funami et al., 2005c). This result clearly pointed to the need for visualization of gelatinized starch granules for a better understanding of the effects of hydrocolloids on the behavior of starch pastes. However, rheological aspects of hydrocolloids, and the microstructural basis of their contribution, are still not clear.

Microscopic techniques are useful methods to study the structures of starch paste and gels (Atkin et al., 1998; Conde-Petit et al., 1998; Fannon & BeMiller, 1992; Han et al., 2000; Obanni & BeMiller, 1995, 1996). Among several microscopic methods, confocal laser scanning microscopy (CLSM) has an advantage for this purpose (Dürrenberger, Handschin, Conde-Petit, & Escher, 2001; Gonera & Cornillon, 2002; Rodríguez-Hernández, Durand, Garnier, Tecante, & Doublier, 2006; van de Velde, van Riel, & Tromp, 2002). The key feature of CLSM is the imaging of a single focal plane in the sample. This enables us to visualize a cross-section of starch granules without the need for complicated preparations such as fixing and dehydrating, allowing us to monitor starches in their original environment. van de Velde et al. (2002) showed that CLSM is not limited to native starch granules, but can also be used for the imaging of the gelatinization process and of starch pastes.

The objective of this research was to reveal the effects of a non-starch hydrocolloid (guar gum) on the structures of starch granules and pastes, and to correlate structures with the rheological properties of maize starch. CLSM was used to study the effects of guar gum on the starch gelatinization process and gelatinized granules in starch pastes. The dynamic viscoelastic properties of starch pastes were also investigated to examine the relationship between starch paste structure and its viscoelastic properties.

2. Experimental section

2.1. Materials

Normal maize starch (26% amylose) was obtained from Japan Maize Products, Co., Ltd., Tokyo, Japan. Four different molecular weights of guar gum were used. In accordance with average molecular weights, each sample was abbreviated as G3, G5, G7, G8, and their average molecular weights ($\times 10^5$ g/mol) were 20.1, 12.2, 4.7, and 0.02, respectively. Further details of these guar gum samples have been described in a previous study (Funami et al., 2005b). Fluorescein 5-isothiocyanate (FITC), rhodamine B (rhodamine) and safranin O (safranin) were purchased from Sigma (St. Louis, MO, USA).

2.2. Sample preparation

Guar gum was dissolved in distilled water using a disperser (T8 Ultra-Turrax, IAK Japan, Nara, Japan).

Samples for observation of starch granule swelling during heating were prepared as follows. Starch (5% w/w) was dispersed in a 0.5% (w/w) G3 guar gum solution or distilled water (as control). A constantly agitated starch suspension with or without added G3 guar gum was heated at a constant rate (1 °C /min) to target temperatures. The heated starch suspension (20 μl) was withdrawn and diluted with distilled water to a total volume of 1 ml, followed by the addition of rhodamine (15 μl; 2 $\mathrm{g l^{-1}}$). Starch granules were examined by confocal laser scanning microscopy (CLSM).

Starch pastes for CLSM observation and dynamic viscoelastic measurements were prepared as follows. Starch (5% w/w) was dispersed in guar gum solution or distilled water (as control). Starch suspensions with or without added guar gum were heated at a constant rate (1 °C /min) to 95 °C and constantly agitated to prevent sedimentation of the granules. When the viscosity of the suspension increased, agitation was stopped. The suspension was kept at 95 °C for 30 min.

For CLSM observation, pastes (500 μl) were stained by the addition of one of the following dyes: rhodamine (20 μl), FITC (20 μl), and safranin (30 μl). Stock solutions of fluorescent staining reagent were prepared by dissolving the appropriate amount in distilled water. Concentrations used were 2 $\mathrm{g l^{-1}}$ for rhodamine and FITC and 5 $\mathrm{g l^{-1}}$ for safranin. Paste samples were stored for at least 2 h at 10 °C before observation by CLSM. For comparison, starch pastes stored at 10 °C for 2 h and 24 h were examined by CLSM. No changes in CLSM images were encountered after storage for 24 h.

2.3. Confocal laser scanning microscopy

A Digital Eclipse C1 confocal laser scanning microscope equipped with an upright microscope (Eclipse E600) was used with Helium–Neon and Argon lasers (Nikon Co., Ltd., Tokyo Japan). Laser excitation of the fluorescent samples was at 543 nm for rhodamine and at 488 nm for FITC and safranin. The objective used for all experiments provided a 40× magnification. Digital images were acquired in 512 × 512 pixel resolution. To obtain the distribution and average diameter of granules, micrographs were analyzed using Image-Pro Plus 4.5 (Nippon Roper Co., Ltd., Tokyo, Japan).

2.4. Dynamic rheology

Dynamic viscoelastic measurements were conducted at 10 °C using an AR 550 rheometer (TA Instruments Japan, Tokyo, Japan). The lower plate had been previously maintained at 10 °C. The hot sample paste was placed between two plates in a cone-plate geometry (gap: 0.058 mm; cone angle: 2°; diameter: 60 mm) and left for 15 min to start measurements. The edge of the sample was covered with a thin layer of silicon oil to prevent water evaporation during measurements. Dynamic time sweep tests were performed at 0.5 Hz and 0.5% strain for 2 h. All measurements were conducted at least in triplicate.

3. Results and discussion

3.1. Effects of guar gum on starch granule swelling

The effects of temperature on the swelling of starch granules with or without added G3 guar gum were studied by CLSM. A 5% suspension of starch, with or without added 0.5% G3 guar gum, was heated at a constant rate (1 °C /min) to target temperatures. CLSM images of maize starch granules that were unheated, heated to 70 °C or 80 °C, with or without G3 guar gum, are shown in Fig. 1. Maize starch granules have a typically truncated shape and an internal cavity, which is in agreement with a previous report (van de Velde et al., 2002). The starch granule sizes increased with temperature (Fig. 1). The diameter of starch granules was measured by image analysis. Fig. 2 shows the diameter distribution of granules that were unheated, heated to 70 °C or 80 °C, with or without added G3 guar gum. The size of starch granules shifted to higher diameters and the size distribution tended to be over a broader range with higher temperatures. Fig. 3 shows the average diameter of starch granules with and without added G3 guar gum as a function of extraction temperature. The granules started to gelatinize at around 65 °C and swelled remarkably at temperatures between 68 °C and 75 °C. Kokini, Lai, and Chedid (1992) reported that corn starch gelatinizes and swells greatly at a temperature between 62 °C and 72 °C. The gelatinization parameters (onset, T_o; peak, T_p; and conclusion, T_c temperatures), as determined by differential scanning calorimetry (DSC), are characteristic of starches. Cooke and Gidley (1992) reported that the T_p of normal maize starch was 70.2 °C and the endotherms of DSC were independent of starch concentration up to 25% w/w in water. Khanna and Tester (2006) determined that the T_o, T_p, and T_c for 20–23% (w/w) normal maize starch suspensions were 64.1 °C, 70.2 °C, and 78.5 °C, respectively.

The present result (Fig. 3) obtained by CLSM shows little difference in the diameters of granules during swelling with or without added guar gum. Thus, it is likely that guar gum has no effect on the swelling of starch granules during heating. This result is consistent with previous reports. Shi and BeMiller (2002) investigated the effects of non-starch hydrocolloids on the viscosity and granule structure of normal maize starch suspensions during heating at a constant rate. A significant earlier onset of viscosity increase during heating was observed for a starch suspension in both carboxymethylcellulose (CMC) and guar gum solutions. Suspensions of normal maize starch, with or without added CMC, were heated on the hot stage of a microscope. No change in gelatinization temperature was observed. From their results, they concluded that the first-stage viscosity increase was not due to a lowering of the gelatinization temperature. Moreover, DSC is a useful method with which to investigate starch gelatinization temperatures. The results determined by DSC also support our present results that hydrocolloids had little or no effect on either

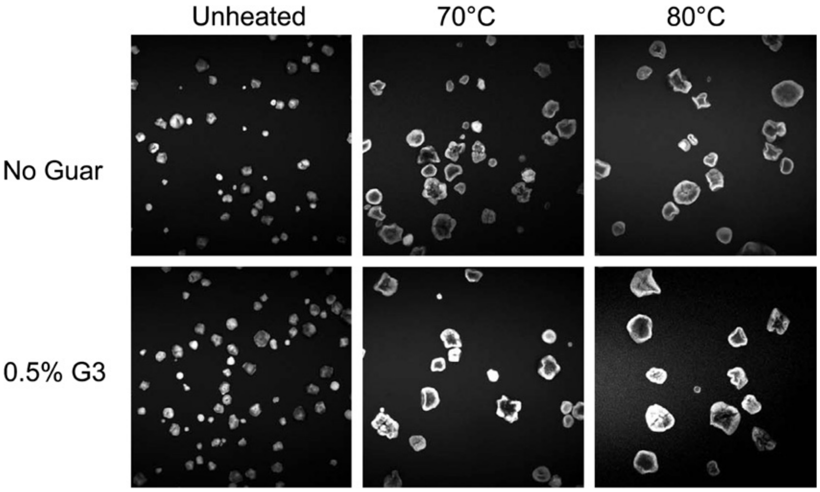

Fig. 1. CLSM images of maize starch granules. Starch granules that were unheated, heated to 70 °C or 80 °C, with or without added 0.5% G3 guar gum, are shown. Each image width is 318 μm.

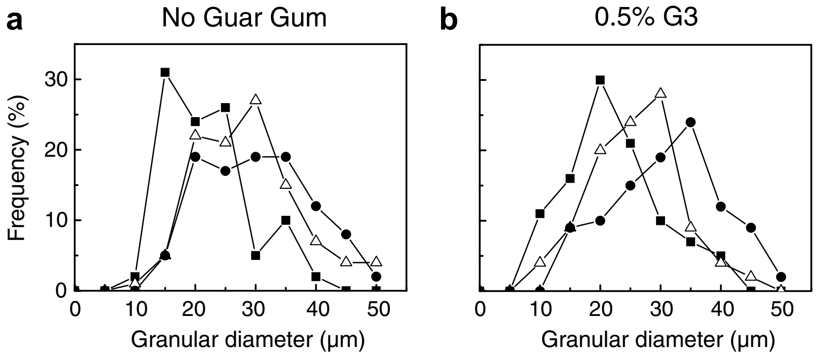

Fig. 2. The starch granule diameter distribution: (a) with added 0.5% G3 guar gum, (b) without guar gum, (■) unheated, (Δ) heated to 70 °C, and (●) heated to 80 °C.

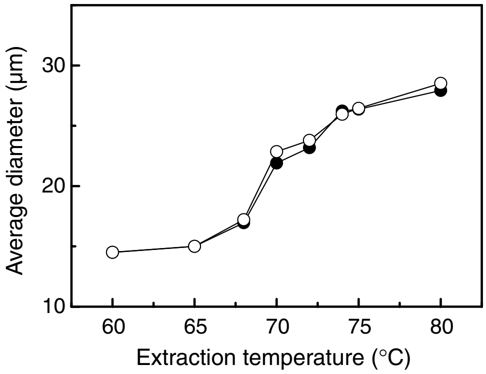

Fig. 3. Average starch granular diameter as a function of the extraction temperature: (○) with added 0.5% G3 guar gum, (●) without guar gum.

the onset or peak temperatures of starch gelatinization (Khanna & Tester, 2006; Tester & Sommerville, 2003; Yoshimura, Takaya, & Nishinari, 1996).

3.2. Effects of fluorescent dye on CLSM images

To find a suitable fluorescent dye for the observation of gelatinized starch granules by CLSM, 5% suspensions of starch with or without added 0.5% G3 guar gum, were heated at 95 °C for 30 min. These starch pastes were stained by three kinds of fluorescent dye; rhodamine, FITC, and safranin. Fig. 4 shows CLSM images of pastes, with or without added G3 guar gum, stained with these three fluorescent reagents.

Even though the images obtained with different fluorescent dyes were different, CLSM images with all dyes showed similar differences between pastes prepared with or without added guar gum. When maize starch granules were heated in water, many granule remnants, called starch ghosts, were observed. Starch ghosts are defined as gelatinized starch granule envelopes that form after the majority of internal starch polymers have been released (Atkin, Abeysekera, & Robards, 1998; Obanni & BeMiller, 1996). By contrast, when maize starch granules were heated in G3 guar gum solution, starch components tended to be inhibited from leaching out of the granules.

In the present study, we used three different fluorescent dyes to find a suitable fluorescent reagent. CLSM images showed that FITC was the most suitable fluorescent agent for the observation of gelatinized starch granules (Fig. 4). As a result, FITC has the advantage of being able to give better CLSM images for gelatinized starch granules. It was reported that rhodamine stained starches in a comparable manner to safranin (van de Velde et al., 2002). These two fluorescent reagents stain starch granules well, but they stain gelatinized starch granules less intensively (Figs. 1 and 4). The gelatinization of starch results in the loss of crystallinity, resulting in the loss of hydrophobic regions. It is likely that the contrast obtained by staining with rhodamine or safranin depends on the balance of hydrophobic affinities. By contrast, FITC gives good CLSM images for gelatinized granules, indicating that FITC has a strong affinity for swollen starch granules (van de Velde et al., 2002).

3.3. Effects of guar gum with different molecular weights on gelatinized starch granules

Suspensions of normal maize starch in solutions of four different molecular weights of guar gum, namely G3, G5, G7, and G8, were heated at 95 °C for 30 min, stained by the addition of FITC, and then observed by CLSM. Fig. 5 shows CLSM images of starch pastes with and without added guar gums with different molecular weights. The CLSM image of the paste with added G8 guar gum was similar to that without added guar gum, and starch ghosts were observed everywhere (Figs. 4 and 5). By contrast, when maize starch granules were heated in solutions of other guar gums with different molecular weights higher than G8, starch components tended to be inhibited from leaching out of the starch granules. This inhibition became more pronounced with increasing molecular weight of guar gum.

Funami et al. (2005c) investigated the effects of guar gums with different molecular weights on the rheological properties of normal maize starch by dynamic viscoelastic measurements. The mechanical loss tangent tan δ was determined for starch pastes, with and without added guar

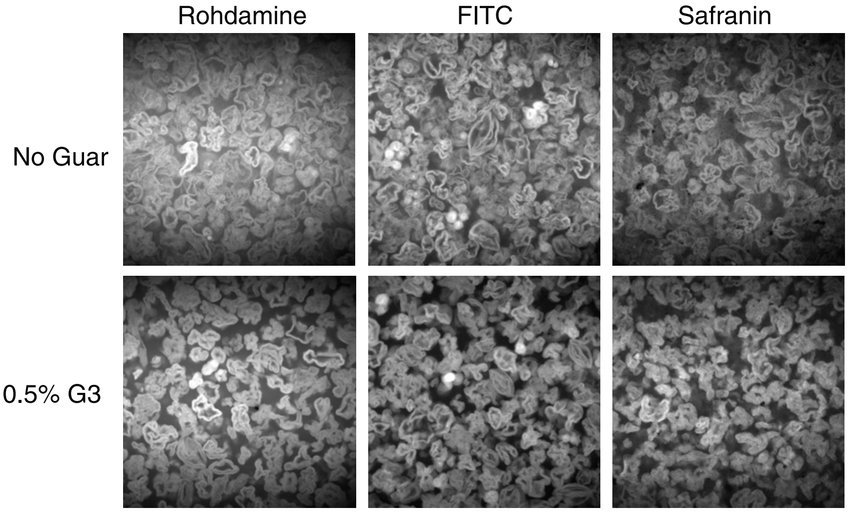

Fig. 4. CLSM images of maize starch pastes stained with rhodamine, FITC or safranin. Starch pastes were prepared with or without added 0.5% G3 guar gum. Each image width is 318 μm.

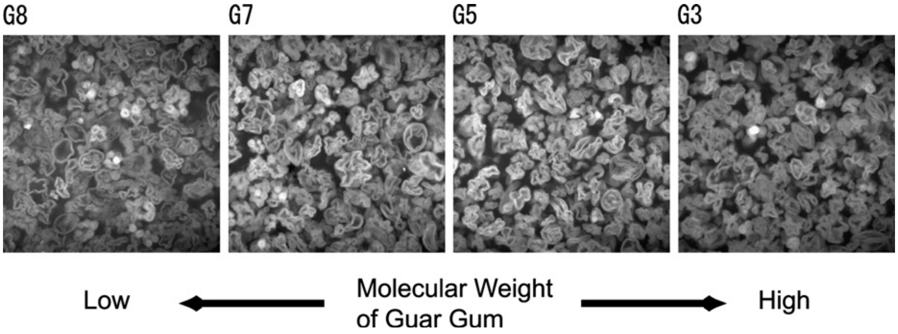

Fig. 5. CLSM images of maize starch pastes. Starch pastes were prepared with added 0.5% guar gum of different molecular weights. Each image width is 318 μm.

gum, after 24 h at 4 °C. The value of tan δ of the paste with added G8 guar gum was similar to that without added guar gum. The increase in tan δ of the pastes was observed as the molecular weights of added guar gum increased. This increase in tan δ indicates an increase in viscous characteristics in the system. In addition, they also reported that the increase in tan δ of the system related to a reduction in the amount of leachate (mainly amylase) from starch granules to the continuous phase of the starch pastes during gelatinization (Funami et al., 2005c).

Our study showed that guar gum with different molecular weights influenced gelatinized starch structure. The starch components tended to be inhibited from leaching out of the starch granules as the molecular weight of added guar gum increased. These results are in good agreement with the rheological properties reported by Funami et al. (2005c).

3.4. Effects of guar gum concentrations on gelatinized granules and rheological properties of starch pastes

Suspensions of normal maize starch in solutions of different G3 guar gum concentrations were heated at 95 °C for 30 min, stained by the addition of FITC and then observed by CLSM. Fig. 6 shows CLSM images of starch pastes with added G3 guar gum of different concentrations. The starch components tended to be inhibited from leaching out of the starch granules with increasing concentrations of added guar gum.

The effect of G3 guar gum of different concentrations on rheological properties was also investigated by dynamic viscoelastic measurements. Fig. 7 shows the time dependence of the storage modulus G' for starch pastes at different G3 guar gum concentrations. The initial value of G' became higher with increasing concentration of added guar gum. A similar synergistic effect has been reported previously. Some authors have observed synergistic interactions between a starch and a hydrocolloid (Alloncle et al., 1989; Shi & BeMiller, 2002). Others have hypothesized that this synergistic effect is due to phase separation (Closs et al., 1999; Mandala, Michon, & Launay, 2004). Promotion of intergranular association has also been proposed (Abdulmola, Hember, Richardson, & Morris, 1996).

By contrast, the increase in G' during measurements (2 h) was inhibited by increasing concentrations of added

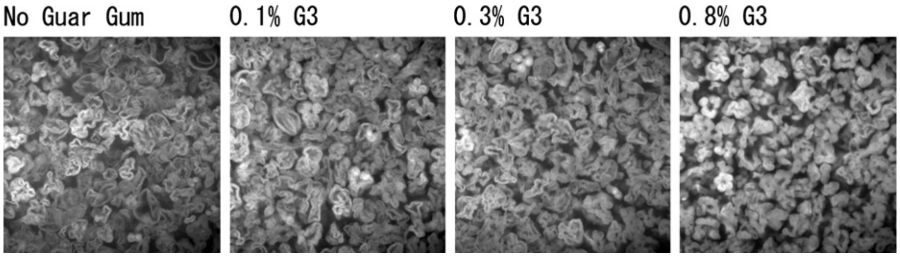

Fig. 6. CLSM images of maize starch pastes. Starch pastes were prepared with or without added G3 guar gum of different concentrations. Each image width is 318 μm.

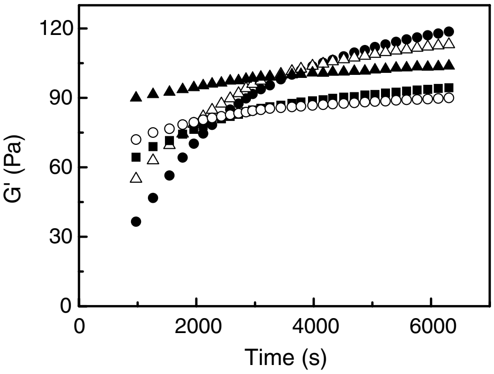

Fig. 7. The storage modulus G' as a function of time for maize starch pastes with or without added G3 guar gum of different concentrations. (●) No guar gum, (Δ) 0.1% G3, (■) 0.3% G3, (○) 0.5% G3, and (▲) 0.8% G3.

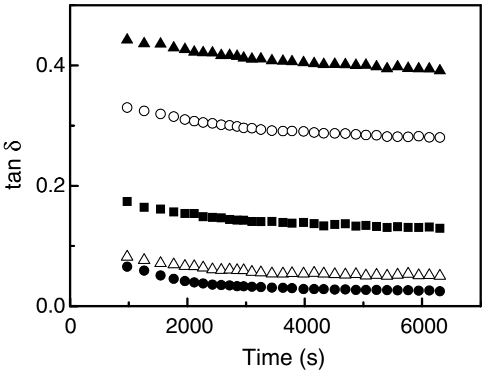

Fig. 8. Mechanical loss tangent tanδ as a function of time for maize starch pastes with or without added G3 guar gum at different concentrations. (●) No guar gum, (Δ) 0.1% G3, (■) 0.3% G3, (○) 0.5% G3, and (▲) 0.8% G3.

G3 guar gum (Fig. 7). Amylose was reported to be the main factor in the short-term (several hours) development of gel structure, while amylopectin was correlated with long-term (several days or weeks) development of starch gel structure (Miles et al., 1985; Morris, 1990). Dynamic viscoelastic measurements were performed for 2 h; therefore, the rheological result suggests that the inhibition of the increase in G' during measurements (Fig. 7) related to a reduction in the amount of amylose leaching from the starch granules to the continuous phase of the starch pastes during gelatinization.

Fig. 8 shows the time dependence of the mechanical loss tangent tan δ for starch pastes at different G3 guar gum concentrations. The increase in tan δ of the pastes was observed with increasing concentration of added guar gum. This increase in tan δ indicates an increase in viscous characteristics in the system. Similar results have been reported previously (Alloncle & Doublier, 1991; Kim, Lee, & Yoo, 2006; Kulicke, Eidam, Kath, Kix, & Hamburg, 1996). Kulicke et al. (1996) studied the viscoelastic properties of waxy rice starch in mixtures with galactomannans (guar gum and locust bean gum). An increase in the tan δ of the mixture of waxy rice starch and galactomannans was observed. Based on their rheological results, these authors suggested that the decrease in junction zones and the simultaneous increase in a physically effective loop with increasing galactomannan fraction resulted in an increase in the amount of viscous portions in the mixtures. Kim et al. (2006) investigated the effect of galactomannans of different concentrations on the rheological properties of rice starch. They also showed an increase in the tan δ of rice starch pastes caused by the addition of galactomannans.

CLSM images demonstrated that G3 guar gum tended to inhibit starch components from leaching out of starch granules. This inhibition became more pronounced with an increasing concentration of guar gum. In addition, dynamic viscoelastic measurements showed that an increase in the tan δ of the pastes is observed with an increasing concentration of added guar gum. This increase in tan δ indicates an increase in the viscous characteristics in the system. From these results, we conclude that guar gum inhibits starch components from leaching out of starch granules to the continuous phase of starch pastes during gelatinization, resulting in an increase in the viscous characteristics in the system.

In the present study, we showed the swelling process of starch granules during heating and the structure of gelatinized granules in maize starch pastes using CLSM. CLSM images provided useful information for a better understanding of the effects of non-starch hydrocolloids on the behavior of starch pastes. The structure of gelatinized granules plays an important role in determining the behavior of starch pastes. Therefore, this study should be widely applicable to understand starch-based food products.

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