Carbohydrate Polymers 49 (2002) 323–330

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Influence of maize starch granule-associated protein on the rheological properties of starch pastes. Part II. Dynamic measurements of viscoelastic properties of starch pastes

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Received 2 June 2001; revised 27 September 2001; accepted 29 September 2001

Abstract

The influence of granule-bound starch synthase (GBSS) on viscoelastic properties of gelatinized starch pastes was studied using a normal, a waxy null, and two GBSS-containing waxy mutant maize starches (waxy protein was synthesized with no GBSS activity). The four starches were isolated using the toluene method (isolation procedure I) (IP I) and were then further purified using an extended period of washing (isolation procedure II) (IP II). Dynamic rheological measurements of IP I gelatinized starches showed that storage moduli (G') were higher in the two GBSS-containing waxy starches than in the waxy null mutant starch not containing GBSS. The total non-GBSS granule-associated proteins of the three waxy starches were similar. Further removal of granule-associated proteins by purification decreased the G', with decreases higher in the two GBSS-containing waxy mutants than in the waxy null mutant starch. This was interpreted to be due to the removal of GBSS during purification of the former. High shear broke gelatinized IP I waxy and normal maize starches starch granule or remnant structure, which caused a decrease in G'. Cox–Merz plots showed that the elastic properties of the starch pastes were reduced by removal of starch granule-associated proteins.

Keywords: Starch granule-associated proteins; Granule-bound starch synthase; Rheology

1. Introduction

Starch granule-associated proteins have been shown to influence endosperm texture, and gelatinization and pasting properties of starch (Greenwell & Schofield, 1986; Hamaker & Griffin, 1993; Morrison, Greenwell, Law, & Sulaiman, 1992). Wheat proteins found on the surface of wheat starch granules influence the elastic properties of gelatinized starch causing an increase in the storage modulus (G') (Eliasson & Tjerneld, 1990). Starch granule-associated proteins in rice have been proposed to increase the rigidity of gelatinized starch granules. Hamaker and Griffin (1993) showed that disruption of the structure of starch granule-associated protein by dithiothreitol allowed the gelatinizing granule to swell to a greater extent than was possible in its native state, but the swollen granules broke apart more easily when high shear stress was applied.

A number of factors have been shown to influence the G' of starch pastes and gels. Tsai, Li and Lii (1997) showed that G' of waxy maize starch increased after cross-linking even though the swelling power decreased. This implied that G' of starch pastes is affected by the rigidity of gelatinized starch granules. Heating a rice starch and water suspension slowly decreased G' until 50 °C, and a rapid decrease was observed at higher temperatures (Tako & Hizukuri, 1999). G' was shown to be strongly dependent on starch concentration; when cross-linked waxy starch concentration increased from 3 to 4%, G' increased from 15 to 75 Pa and when increased from 4 to 5%, G' increased from 75 to 105 Pa (Rao, Okechukwu, Da Silva, & Oliveira, 1997). G' was also positively correlated with amylose content and gel strength of maize starch (Case et al., 1998).

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0144-8617/02/$ - see front matter
PII: S0144-8617(01)00348-4

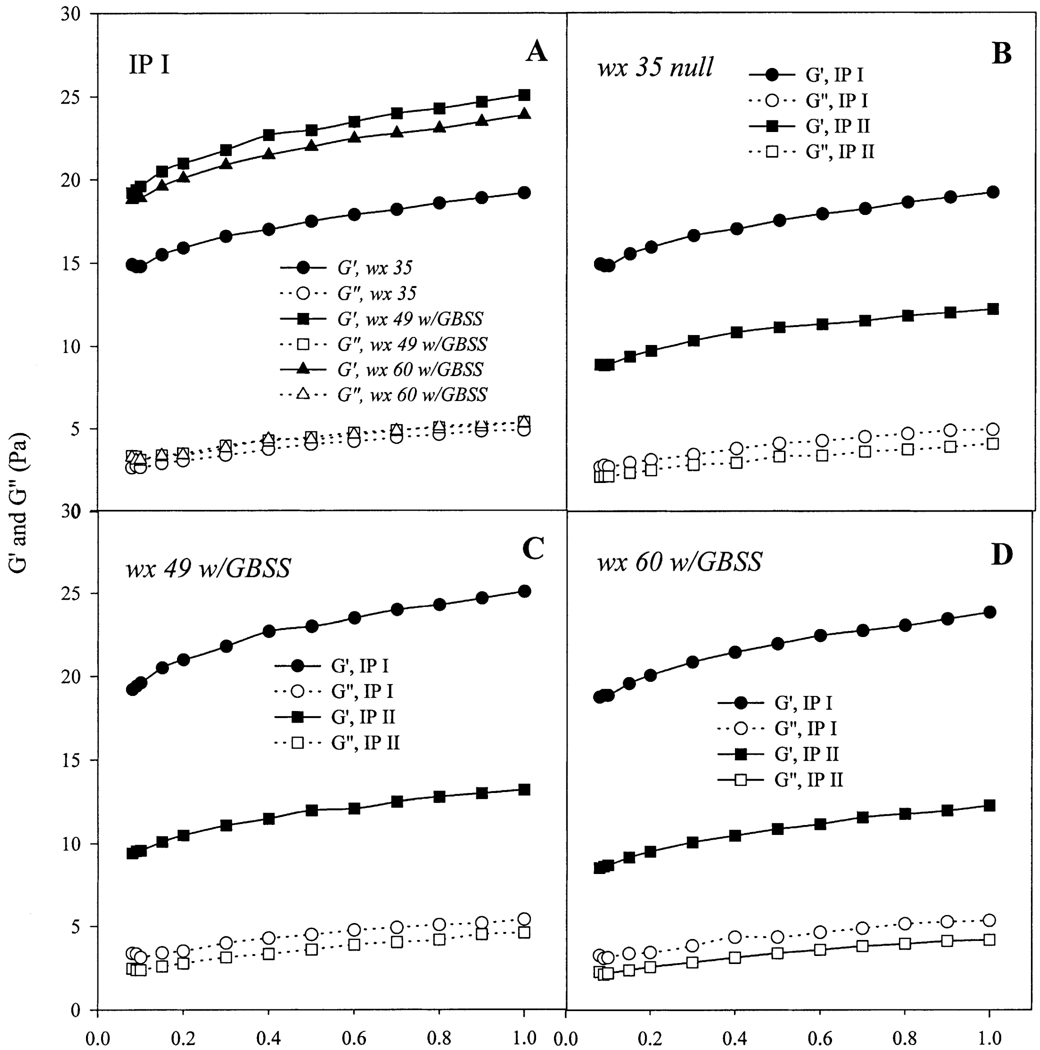

Fig. 1. Dynamic measurements of gelatinized waxy starch pastes. The waxy null mutant (*wx* 35) and the GBSS-containing waxy (*wx* 49 and *wx* 60) starches were isolated (IP I) and further purified (IP II).

The granule-associated proteins in maize starch were suggested to be composed of two groups, the surface located zeins of 10–27 kDa and the granule-intrinsic proteins of 32 kDa or higher (Mu-Forster & Wasserman, 1998). The 60 kDa starch granule-bound starch synthase (GBSS) was by far the major granule-associated protein in normal maize starch, though it is not observed in waxy maize starch (Goldner & Boyer, 1989). Amylose content is positively correlated to GBSS content (Hamaker, Griffin, & Moldenhauer 1991; Kuroda, Oda, Miyagawa, & Seko, 1989; Yamamori, Nakamura, & Kuroda, 1992) and is regulated by the waxy gene (Miura, Tanii, Nakamura, & Watanabe, 1994; Sivak, Wagner, & Preiss, 1993; Wang et al., 1995). GBSS is embedded in starch granules (Mu-Forster et al., 1996; Nakamura, Yamamori, Hirano, & Hidaka, 1993), which suggests that it may affect the behavior of the gelatinizing starch granules.

The overall objective of this study was to investigate the influence of starch granule-associated proteins on viscoelastic properties of pastes. The specific aims of the study were to (1) obtain information regarding the effect of GBSS and the other starch granule-associated proteins on storage and loss moduli of gelatinized starch (2) study the microstructural basis of functions of granule-associated proteins on viscoelastic properties of pastes.

2. Materials and methods

2.1. Materials

A normal maize genotype (EX68) and three waxy maize isogenic mutants, a waxy null mutant (*wx* 35), and the two GBSS-containing waxy mutants (*wx* 49 and *wx* 60) were provided by ExSeed Genetics LLC, USA. In the two GBSS-containing waxy mutants, GBSS was synthesized but had no synthase activity. The GBSS-containing waxy mutants were developed by ethyl methanesulfonate

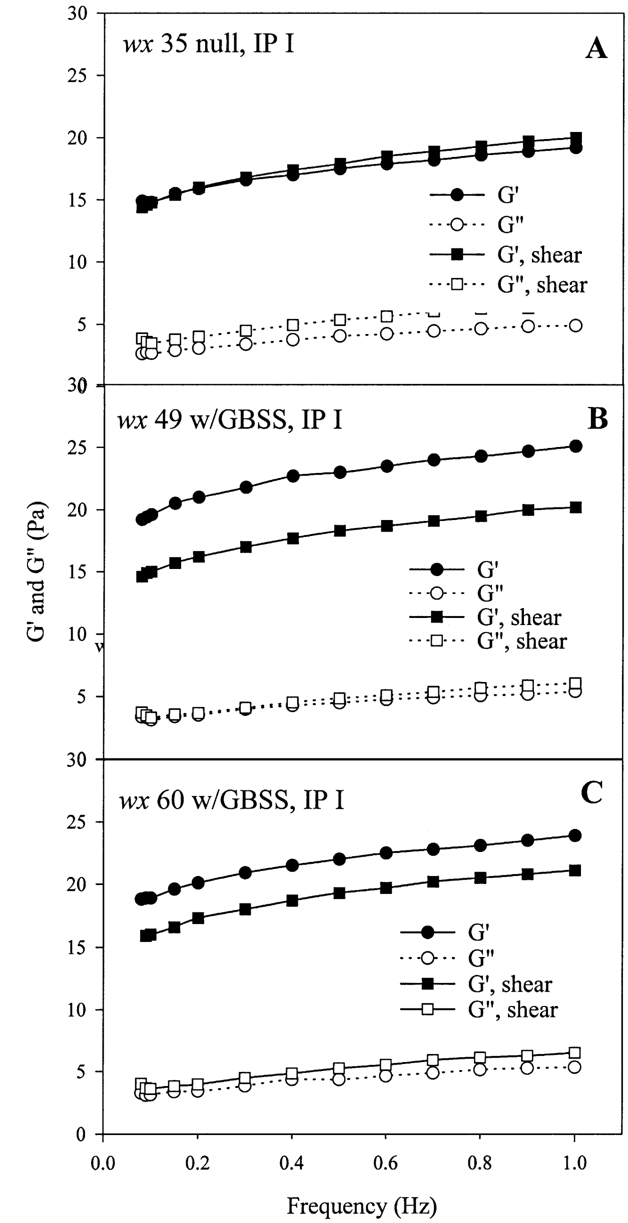

Fig. 2. The influence of shear on the dynamic measurements of isolated (IP I) waxy starches. Dynamic measurements were conducted before and after shear at 118 l/s for 5 min in waxy null mutant (*wx* 35) and the GBSS-containing waxy (*wx* 49 and *wx* 60) starches.

mutation, and its 60 kDa protein was recognized by GBSS antibodies.

2.2. Isolation of starches

Maize starches were isolated according to the toluene method described by Banks and Greenwood (1975). Maize kernels were softened by soaking for 48 h in 0.02 M acetate buffer (pH 6.5) containing 0.01 M mercuric chloride at 40 °C. Samples were rinsed with water and homogenized in a blender for 2.5 min. Homogenized suspensions were passed through a sieve with 63 μm openings. The material left on the screen was again homogenized for 1.5 min and passed through the sieve. The procedure was repeated three times to release the starch granules completely. The suspension was centrifuged and the solids collected. The solids were then mixed with toluene and water (1:10 ratio) and shaken for 10 h on a wrist shaker. The suspension was centrifuged at 1800*g* in a bucket centrifuge (AccuSpin FR, Beckman Coulter, Inc., Fullerton, CA) and the liquid, with the denatured protein at the water/toluene interface was discarded. Solids were again washed with the mixture of toluene and water, shaken for 15 min, and centrifuged. This was repeated five times. The isolated starch was washed with purified water (NANOpure II, Barnstead Co., Boston, MA) and dried at 40 °C. This procedure was called isolation procedure I (IP I).

In isolation procedure II (IP II), the isolated starch from procedure I (before drying) was further shaken with a mixture of toluene and water for 8.5 h. This was followed by another three washes with the toluene/water mixture for 15 min, and then centrifuged to collect the starch. The purified starch was washed with water and dried at 40 °C. Isolated starches were gently ground and passed through a 125 μm sieve. Moisture content was determined using AACC Method 44-19 (AACC, 1995), using a smaller sample size of 0.5 g.

2.3. Analysis of granule-associated proteins in isolated starch

The method and results of electrophoretic analysis of granule-associated proteins in isolated starch are described in Part I (Han, Campanella, Guan, Keeling, & Hamaker). The granule-associated protein contents of EX68, *wx* 35, *wx* 49 and *wx* 60 were 1.6, 1.14, 1.53 and 1.24% for IP I starches, and 0.45, 0.06, 0.04 and 0.08% for IP II starches, respectively. The band net intensity of GBSS of EX68, *wx* 35, *wx* 49 and *wx* 60 were 25753, 0, 10010 and 11095 for IP I starches, and 22463, 0, 3197, and 1805 for IP II starches, respectively. IP II normal maize starch (EX68) retained its GBSS after purification, while a large proportion of GBSS in the two GBSS-containing waxy mutants was removed during further purification.

2.4. Rheological measurements

Starch suspensions (7% w/v on a 7% moisture base) were prepared using purified water, and 1.2 ml were transferred onto the center of the plate of a controlled stress/strain rheometer (ReoLogica Instruments AB, Sweden). Measurements were conducted using a cone and plate system with a cone of 4 cm diameter and 4° angle. Water evaporation was prevented using a solvent trap. Starch suspensions were heated at a rate of 10 °C/min under a shear rate of 5.6 l/s from 25 to 95 °C to allow the starch to gelatinize and swell, and then cooled to 80 °C. Dynamic measurements were performed using a frequency sweep from 0.08 to 10 Hz in

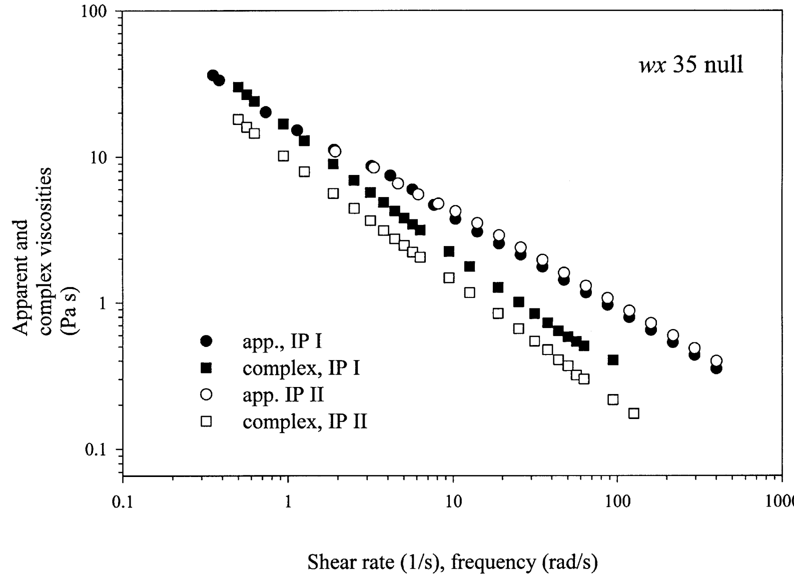

Fig. 3. Cox–Merz plot of the isolated (IP I) and further purified (IP II) waxy null mutant maize starch (*wx* 35).

the linear viscoelastic range as determined in a strain sweep test. Apparent viscosities were measured at shear rates of 0.08–400 l/s. Measurements were conducted at least in duplicate.

2.5. Light microscopy

Gelatinized starches were examined using a light microscope (Olympus Vanox-S Compound microscope, Olympus America, Inc., Melville, NY) with a Spot RT color digital camera (Diagnostic Instruments, Inc., Sterling Heights, Michigan) after staining with a 0.2% iodine solution (2 g iodine and 20 g potassium iodine to 1 l of water).

3. Results and discussion

3.1. Dynamic measurements of gelatinized starch

Starch suspensions were gelatinized by heating from 25 to 95 °C to allow the starch to gelatinize and swell and then cooled to 80 °C. Dynamic measurements were performed to determine the viscoelastic properties of starch pastes. Storage moduli (G') of gelatinized starches of the two GBSS-containing waxy mutants (*wx* 49 and 60) were higher than the G' of the waxy null mutant starch (*wx* 35) for IP I starches, while G'' of the three waxy starches were almost the same (Fig. 1A). Because the total non-GBSS granule-associated protein contents of the three waxy starches were

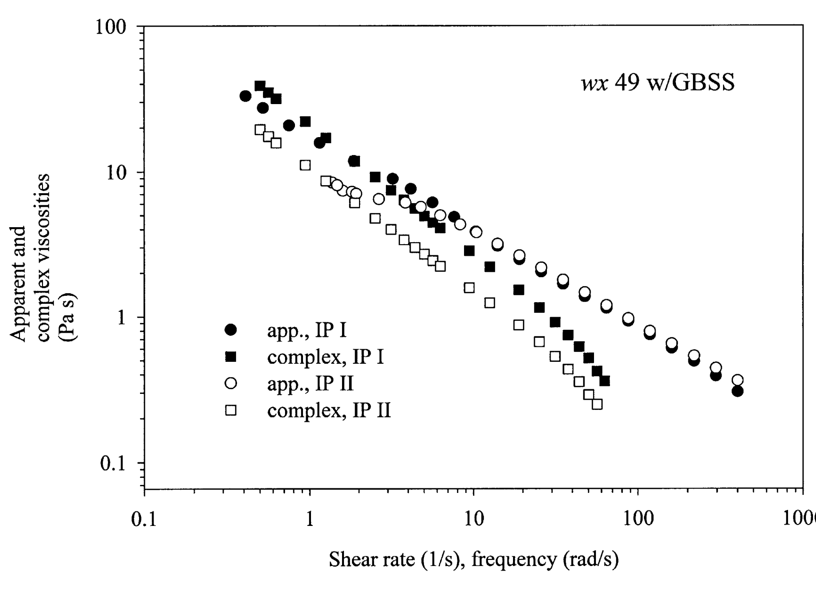

Fig. 4. Cox–Merz plot of the isolated (IP I) and further purified (IP II) GBSS-containing waxy maize starch (*wx* 49).

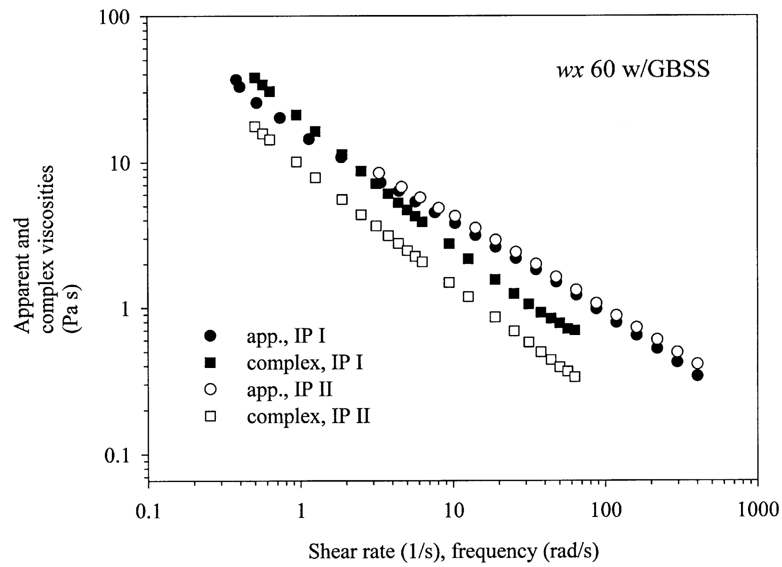

Fig. 5. Cox–Merz plot of the isolated (IP I) and further purified (IP II) GBSS-containing waxy maize starch (*wx* 60).

similar as shown in Part I (Han et al.), the GBSS protein itself was responsible for the increased G' in waxy mutants 49 and 60. Further purification of waxy starches caused a decrease in G' that was larger in the GBSS-containing waxy starch (Fig. 1B–D), indicating that removal of GBSS, in particular, in the latter caused the greater decrease of G' (Fig. 1C and D) than in the waxy null mutant starch (Fig. 1B). After significant removal of GBSS in the *wx* 49 and 60, the values of G' were similar in all three waxy starches (Fig. 1B–D).

The G' of gelatinized starches of normal maize were much higher than those of the waxy maize starches (not shown). This was likely the result of amylose and the rigid granular structure of gelatinized normal starch. Reddy, Subramanian and Bhattacharya (1994) reported that G' and G'' values were highly correlated with amylose content of starch in rice.

3.2. Influence of shear on viscoelastic properties of gelatinized starches

Starch suspensions were gelatinized by heating from 25 to 95 °C to allow the starch to gelatinize and swell, and then cooled to 80°C. Gelatinized starches were then sheared at a shear rate of 118 l/s for 5 min to disrupt the gelatinized starch granule structure. Dynamic measurements were performed to determine the viscoelastic properties of gelatinized starches before and after shear (Fig. 2A–C). Shear had no effect on the G' of gelatinized waxy null mutant starch (Fig. 2A), but significantly reduced the

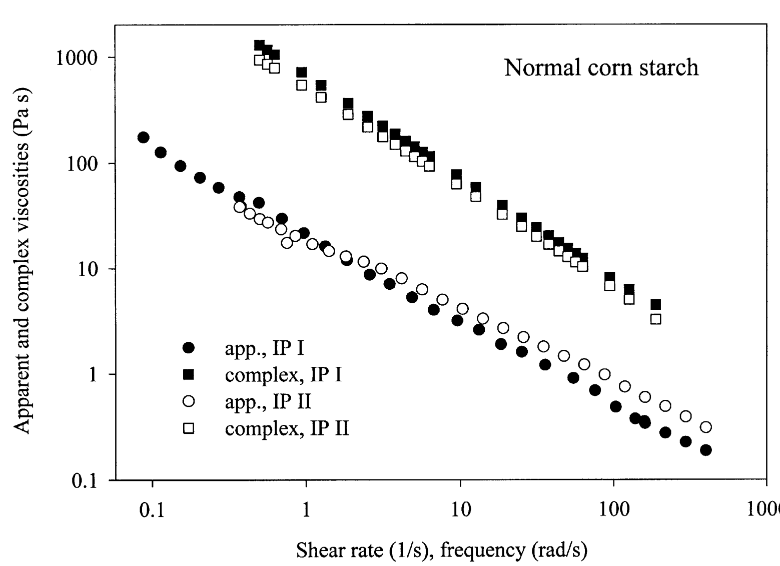

Fig. 6. Cox–Merz plot of the isolated (IP I) and further purified (IP II) normal maize starch (EX 68).

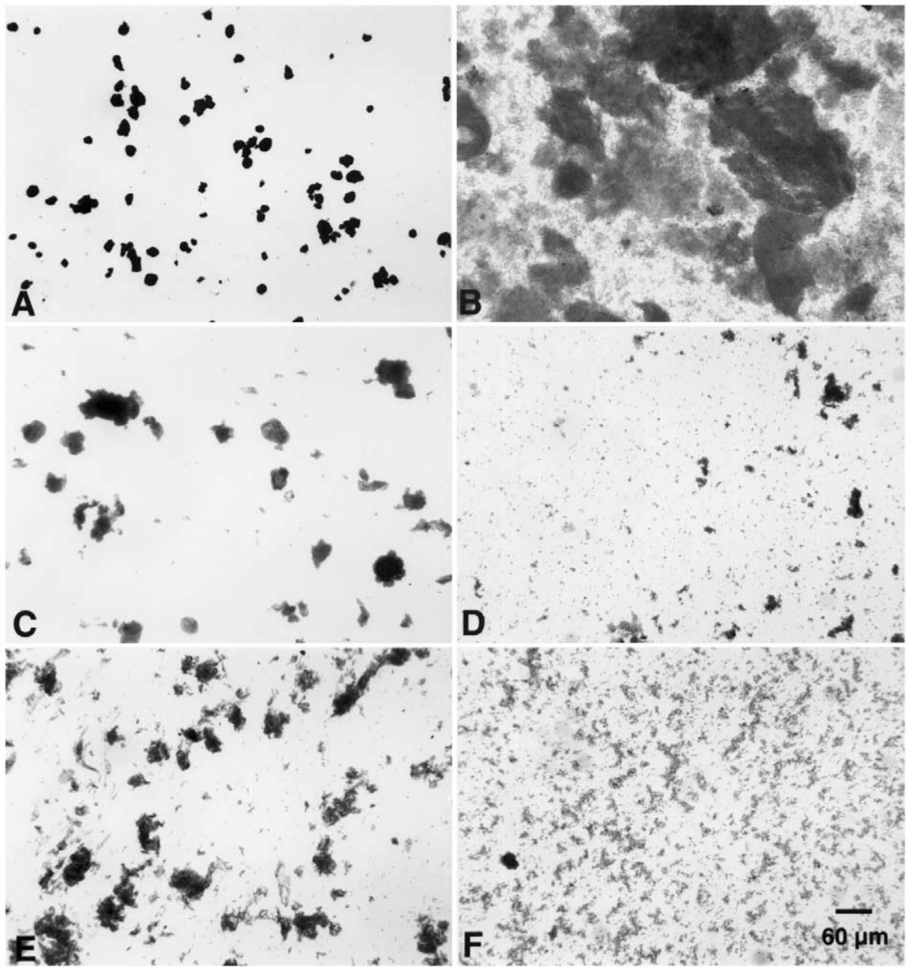

Fig. 7. Light microscopy of gelatinized starches. Gelatinized purified starch (IP II) of normal maize before (A) and after (B) shear; gelatinized isolated starch (IP I) of the GBSS-containing waxy maize (*wx* 49) before (B) and after (C) shear; gelatinized starch of the GBSS-containing waxy maize (*wx* 49) with further purification (IP II) before (B) and after (C) shear.

G' values of gelatinized starches of the two GBSS-containing waxy mutants (Fig. 2B and C). The values of G' were similar in three waxy starches after shear. Changes in G'' were not significant in any of the three waxy starches. The proposed explanation for these observations is that GBSS influences the viscoelastic properties of starch pastes through increasing the rigidity of gelatinized starch granules. When the shear was high enough to break down the gelatinized starch granules, the GBSS itself had limited effect considering its small amount in the waxy mutants. In the waxy null mutant starch that does not contain GBSS, G' was lower with little structure even before applied shear. Shear did not significantly change the G' of gelatinized waxy starches with further purification (IP II) (not shown).

In normal maize starch, shear decreased G' in starches without and with further purification (not shown). The proposed explanation is that gelatinized starch granular structure, partially maintained by GBSS, exists in both unpurified and purified normal starch, and shear disrupts the granular structure, which leads to decrease of G'.

3.3. Cox–Merz plot of gelatinized starch granules

The Cox–Merz relationship states that $\eta^*(\omega) = \eta(\dot{\gamma})|_{\omega} = \dot{\gamma}$; where η^* is the complex viscosity, η is the apparent shear viscosity, ω is frequency of oscillation (rad/s), and $\dot{\gamma}$ is shear rate (l/s). Deviation from the Cox–Merz rule indicates a gel-like structure of gelatinized starch (Rao, Okechukwu, Da Silva, & Oliveira, 1997; Ross-Murphy, 1984). If complex viscosity plotted vs. frequency is consistently higher than the apparent viscosity plotted versus shear rate, elastic gel-like structure exists (Silva, Oliveira, & Rao, 1997). Complex viscosities of the three waxy maize IP I starches were lower than their apparent viscosities (see Figs. 3–5), while complex viscosities of normal maize IP I and II starches were consistently higher than apparent viscosities (Fig. 6). This indicates that normal maize gelatinized starch exhibits higher elastic behavior or gel-like than those of waxy starches.

The plots also showed that the elastic nature of the gelatinized starch, measured by ratios of complex viscosity versus apparent viscosity, decreased after removal of granule-associated proteins. Further purification of starches (IP II) increased the apparent viscosities at high shear rates, but differences were not obvious at low shear rates. Decreases in complex viscosity were more pronounced than the increases in apparent viscosity in waxy starches after further purification (Figs. 3–5). Decreases in complex viscosity were greater in the gelatinized starches of the two GBSS-containing waxy mutants than in the waxy null mutant starch (Figs. 3–5). These decreases were likely caused by removal of the majority of GBSS in GBSS-containing waxy mutants during further purification.

3.4. Light microscopy of gelatinized starch granules

When normal maize IP II starch was heated in a rheometer at a minimal shear rate (5.6 l/s), granule structure was retained (Fig. 7A). These gelatinized starch granules sheared at a high rate of 118 l/s for 5 min showed lost in granule structure; swollen remnants were observed (Fig. 7B). The GBSS-containing waxy maize mutant (*wx* 49) IP I starch swelled to a greater extent than the normal maize starch when gelatinized at minimal shear rate (Fig. 7C), and was reduced to small pieces when sheared at high shear rate (Fig. 7D); this was accompanied by a decrease in G' (Fig. 2B). Further purification (IP II) of the same starch, with concomitant removal of GBSS, increased the breakdown of gelatinized starch granules even with gelatinization at low shear (Fig. 7E), and broken gelatinized starch granules were dispersed at high shear (Fig. 7F). Overall, the micrographs support the hypothesis that rigid gelatinized structures increase G', and that GBSS provides some structural rigidity.

4. Conclusions

Granule-associated proteins, and GBSS in particular, influenced the gel-like structure or viscoelastic behavior of pastes measured by G' and G'' and Cox–Merz plots. G' decreased when starches were further purified, and this decrease was greater in the starches of GBSS-containing waxy mutants, in which the majority of GBSS was removed, than in the waxy null mutant starch that did not contain GBSS. The contribution of GBSS in the IP I starches was influenced by shear. Shear broke gelatinized starch granule or remnant structures that caused a decrease in G' in IP I GBSS-containing waxy starches and normal maize starch. Cox–Merz plots showed that complex viscosity was much higher than apparent viscosity in gelatinized starch of normal maize, while, contrarily, apparent viscosities were somewhat higher than complex viscosities in waxy starches. The plots also showed that the elastic nature of gelatinized starch measured as the ratio of complex viscosity vs. apparent viscosity decreased by removal of granule-associated proteins. These experiments clearly show that GBSS provides more elasticity in gelatinized pastes, and support our hypothesis that the protein contributes to the rigidity of swollen, gelatinized starch.

Acknowledgements

We thank D.M. Sherman of the Microscopy Center in the School of Agriculture at Purdue University for assistance and use of the facility and equipment.

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