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Influence of maize starch granule-associated protein on the rheological properties of starch pastes. Part I. Large deformation measurements of paste properties ☆

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Abstract

Starch granule-associated proteins, mainly consisting of granule bound starch synthase (GBSS), were shown to influence the rheological properties of starch pastes measured under large deformation steady shear conditions. A normal, a waxy null mutant, and two GBSS-containing waxy mutant maize starches (waxy protein was synthesized with no GBSS activity) were isolated (isolation procedure I, IP I) and further purified (isolation procedure II, IP II) using the toluene method. Further purification removed additional residual proteins in starches including the majority of GBSS in the two GBSS-containing waxy starches, and increased the paste viscosity in all four starches, especially at high shear rates. IP I starch paste of the waxy null mutant exhibited higher viscosity at high shear rate than starch pastes of the GBSS-containing waxy mutants that was attributed to the absence of GBSS in the former. Shear sweep tests (up to a shear rate of 120 l/s) showed that the IP I GBSS-containing waxy mutant starches had less shear-induced breakdown than the waxy null mutant starch; removal of additional granule-associated protein in the former, including GBSS, caused an increase in shear-induced breakdown. This study showed that granule-associated protein reduced paste viscosity, and GBSS specifically reduced shear-induced breakdown of starch pastes.

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

1. Introduction

Maize starch is widely used in the food industry. Starches serve a variety of roles in foods and in food processing, mainly to produce viscous pastes and soft-textured gels (Whistler & BeMiller, 1997). Cooking behaviors of different starches are often compared using a Brabender Visco/Amylograph or rapid visco-analyzer (RVA). In these methods, a water suspension of starch is heated to 95 °C, held for a given time, and then cooled; the viscosity changes are recorded by the instrument. The viscosity of a starch suspension increases in the heating stage and reaches a peak. It then decreases during a 95 °C holding period, which is considered to be caused by shear-induced breakdown of the gelatinized starch granule structure. Paste breakdown is usually considered by the food industry to be an undesirable change. Waxy maize starch has a higher peak viscosity than normal maize starch, but it exhibits far more breakdown during holding than does normal maize starch (Whistler & BeMiller, 1997).

Viscosity of starch pastes is contributed by swollen gelatinized starch granules, and dispersed amylopectin and amylose (Evans & Haisman, 1979; Leelavathi & Indrani, 1987; Miller, Derby, & Trimbo, 1973; Svegmark & Hermansson, 1993). Under high shear, the gelatinized starch granule structure is broken down, which causes a drop in paste viscosity. The volume of swollen granules and their inherent deformability are important in determining the viscosity of starch pastes (Bagley & Christianson, 1982; Christianson & Bagley, 1983; Evans & Lips, 1992; Langton & Hermansson, 1989; Tsai, Li, & Lii, 1997).

Hamaker and Griffin (1993) and Hamaker, Griffin, and Moldenhauer (1991) proposed that rice starch granule-associated proteins provide the gelatinized starch granule a certain rigidity. Disruption of the polymeric structure of

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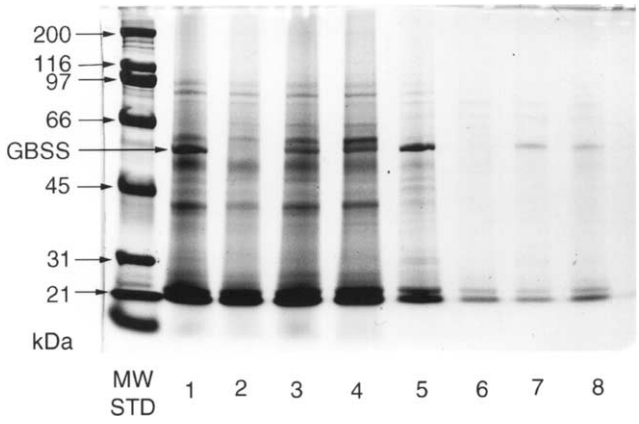

Fig. 1. SDS-PAGE of proteins in isolated (IP I, lane 1–4) and further purified (IP II, lane 5–8) starches. Starch GBSS is shown by an arrow. Genotypes: Lane 1 and 5—normal maize (EX 68), lane 2 and 6—waxy null maize mutant (*wx* 35), lane 3 and 7—GBSS-containing waxy maize mutant (*wx* 49), lane 4 and 8—GBSS-containing waxy maize mutant (*wx* 60).

proteins by dithiothreitol allowed the gelatinizing granules to swell to a greater extent than what was possible in its native state, but, when high shear stresses were applied, paste viscosity decreased more in the treated starch. Lim, Lee, Shin, and Lim (1999) showed that peak viscosity of rice starch pastes, measured by RVA, increased when isolated starch was purified to remove more proteins. In a study of mung-bean starch behavior using proteolytic enzymes, Oates (1990) suggested the possible existence of peptide cross-links within the amylopectin fraction that are responsible for maintaining the structure of gelatinized starch granules. Prentice and Stark (1992) found that gelatinized barley starch granule remnants or starch 'ghosts,' containing mostly amylopectin, were enriched with protein, suggesting the possible association of protein with amylopectin.

The granule-associated proteins in maize are thought to be composed of two distinct classes, the surface located zeins of 10–27 kDa and the granule-intrinsic proteins of 32 kDa or higher (Mu-Forster & Wasserman, 1998). The zeins that are removed easily by proteases, are considered to be deposited on the granule surface, while the proteins resistant to protease digestion are considered granule-intrinsic proteins (Mu-Forster & Wasserman, 1998). The 60 kDa waxy gene product, or granule bound starch synthase (GBSS), is considered to be strongly bound to starch granules (Mu-Forster et al., 1996; Nakamura, Yamamori, Hirano, & Hidaka, 1993). Although starch granule-associated proteins were implied to influence the paste behavior (Hamaker & Griffin, 1993; Hamaker et al., 1991; Lim et al., 1999), it was still not clear what is the contribution of the granule-associated proteins to the overall rheological properties of starch pastes. Additionally, functions of GBSS and the other granule-associated proteins have not been differentiated.

The overall objective of this research was to study the role of starch granule-associated proteins, particularly GBSS, on the overall rheological properties of starch pastes measured under large deformation steady shear conditions. The specific aims of this project were to investigate the role of starch granule-associate-proteins on viscosity and shear-induced breakdown of starch pastes, and to determine the functional contribution in rheological behavior from GBSS and the other granule-associated proteins.

2. Materials and methods

2.1. Materials

A normal maize starch genotype (EX 68) and three waxy maize isogenic mutant starches [waxy null mutant (mEX 68 *wx* 35), and two GBSS-containing waxy mutants (mEX 68 *wx* 49 and mEX 68 *wx* 60)] were provided by ExSeed Genetics LLC, USA. In *wx* 49 and 60, GBSS was synthesized, but had no synthase activity. The GBSS-containing waxy mutants were developed by ethyl methanesulfonate 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, 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, 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

Table 1
Protein contents of isolated starches and band intensity of GBSS

Protein contents	Isolation procedure (IP)	Normal maize (EX 68)	Typical waxy (*wx* 35)	GBSS-containing waxy (*wx* 49)	GBSS-containing waxy (*wx* 60)
Total protein content (%)	I	1.60 ± 0.00[a]	1.14 ± 0.06	1.53 ± 0.01	1.24 ± 0.02
	II	0.45 ± 0.03	0.06 ± 0.02	0.04 ± 0.01	0.08 ± 0.01
Band net intensity of GBSS	I	25753 ± 994	0 ± 0	10010 ± 14	11095 ± 700
	II	22463 ± 631	0 ± 0	3197 ± 251	1805 ± 26

[a] Values are means ± standard deviations.

AACC Method 44-19 (AACC, 1995), using a smaller sample size of 0.5 g.

2.3. Analysis of residual protein in isolated starch

Total residual protein contents in starches were analyzed using AACC Method 46-13 (AACC, 1995).

Protein extracts from isolated starches were analyzed by SDS-PAGE (minigel apparatus, Protean II, BioRad Corporation, Van Nuys, CA). Starches (15 mg) were dissolved in 0.6 ml sample buffer containing 2% 2-mercaptoethanol, 2% SDS, 10% glycerol (v/v), 66 mM Tris (pH 6.8), and 0.2 ml of 0.2% bromophenol blue. Suspensions were boiled for 20 min with intermittent stirring and 20 μl were loaded into each well of a polyacrylamide slab gel consisting of 4% stacking and 12% separating gels. The gel was run at a constant voltage of 150 V for about 30 min. Gels were stained using Gelcode Blue Stain reagent (Pierce, Rockford, Illinois) and photographed. Band intensities of the 60 kDa GBSS protein were analyzed using a Kodak Digital Science 1D program.

An experiment was conducted to test whether the inactive GBSS in *wx* 49 and 60 was embedded in the granule as in normal starch. Crude starches of GBSS-containing waxy starches were digested with thermolysin according to the method used by Mu-Forster et al. (1996). SDS-PAGE (not shown) of residual proteins before and after thermolysin digestion showed that GBSS in GBSS-containing waxy starch was not digested by thermolysin, which indicated that it is an integral protein in *wx* 49 and 60 starch granules.

2.4. Rheological measurements

Starch suspensions (7% w/v based on 7% moisture content) were prepared using purified water. Suspensions (1.2 ml) were transferred onto the center of the plate of a controlled stress 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 from 25 to 95 °C under a minimal shear rate of 5.6 l/s to minimize settling of the dispersion and to allow the starch to gelatinize and swell. Pastes were then cooled to 80 °C. Steady shear measurements were conducted at 80 °C using a range of shear rates of 5.6–542 l/s and the resulting flow curves were analyzed. For up–down shear sweep rheological measurements using the controlled rate mode, gelatinized starch pastes were sheared at 80 °C using a shear rate sweep from 5.6 to 120 or 542 l/s and then from 120 or 542 l/s back to 5.6 l/s (Tattiyakul & Rao, 2000). Flow curves were used to indicate relative differences in paste breakdown (Rao, Okechukwu, Da Silva, & Oliveira, 1997). Measurements were done at least in duplicate.

2.5. Light microscopy

Gelatinized starches were examined using a light microscope (Olympus Vanox-S Compound Microscope, Olympus America, Melville, NY) with a Spot RT digital camera (Diagnostic Instruments, 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. Proteins in isolated starch

SDS-PAGE analysis of protein extracts showed a more intense protein banding pattern in starches isolated using IP I than those isolated using IP II; major bands in IP I starches, except *wx* 35 were 60 kDa GBSS (see arrow) and residual zein proteins in the range of 20–24 kDa (Fig. 1). In IP II starches, most residual proteins were removed and there was partial loss of GBSS in the *wx* 49 and 60 mutants, even though GBSS is thought to be tightly bounded within starch granules in normal starch (Mu-Forster et al., 1996; Nakamura et al., 1993). Table 1 shows the loss of GBSS in the GBSS-containing waxy mutant starches with further purification, and similar concentrations of GBSS before and after further purification in normal starch. The difference in loss of GBSS between normal maize and the GBSS-containing waxy mutant starches after purification could possibly have been caused by differences in starch structure or distribution of GBSS in waxy and normal starches.

3.2. Steady shear measurements

Flow curves of waxy starch (*wx* 35, 49, and 60) pastes are

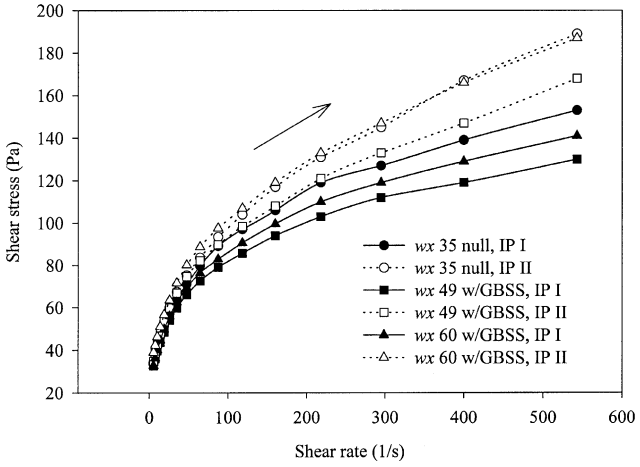

Fig. 2. Flow curves of gelatinized starch pastes. Waxy null (*wx* 35) and the GBSS-containing waxy (*wx* 49 and 60) starches were isolated (IP I) and further purified (IP II). Arrow indicates the direction of change of shear rates.

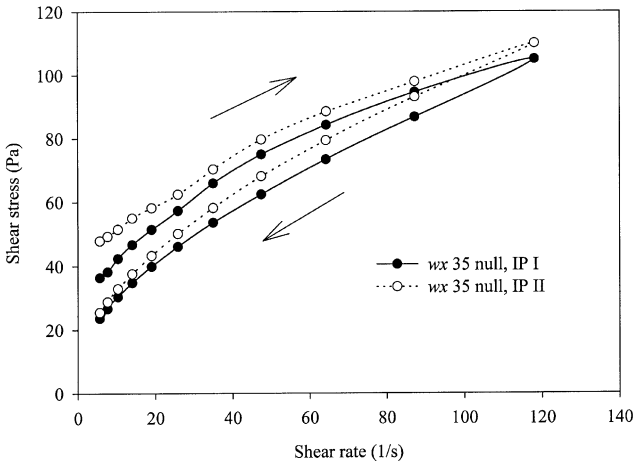

Fig. 4. Shear sweep measurements of gelatinized starch pastes of the waxy null mutant (*wx* 35) starch that were isolated (IP I) and further purified (IP II). Arrows indicate the directions of changes of shear rates.

illustrated in Fig. 2. Apparent viscosity is defined as the ratio between shear stress and shear rate, meaning a paste that has higher shear stress also has a higher apparent viscosity at the same shear rate. IP I waxy null mutant starch (*wx* 35) exhibited higher viscosity than did the GBSS-containing *wx*49 and 60 starches. Further starch purification (IP II) caused increases in viscosity in all three waxy starches, with the difference increasing at higher shear rates (Fig. 2). Gracza (1965), Lim et al. (1999) reported that purification of starch increases its paste viscosity as measured by the Amylograph and RVA at a fixed shear rate. Viscosities of gelatinized starches are influenced by the extent of swelling of gelatinized starch granules prior to their breakdown, as well as the expansion and dispersion of starch granule remnants after the granular structure is broken down. Higher swelling of gelatinized starch granules results in higher paste viscosity. Removal of residual proteins favored swelling of gelatinized starch granules and remnants, which in turn, increased paste viscosity.

3.3. Shear sweep tests

Shear-induced changes were observed in flow curves between the up cycle (increasing shear rates) and the down cycle (decreasing shear rates) of all starches tested (Figs. 3–8). These changes were caused by shear-induced structural breakdown and their magnitude was indicative of extent of breakdown. Thus, breakdown in this study is defined as area between the up and down shear sweep curves (see Table 2). The IP I waxy null mutant (*wx* 35) starch exhibited more shear-induced breakdown than the IP I starches of the two GBSS-containing waxy mutants (*wx* 49 and 60) (Fig. 3, Table 2). Because the major difference in proteins between the two starch types was the presence

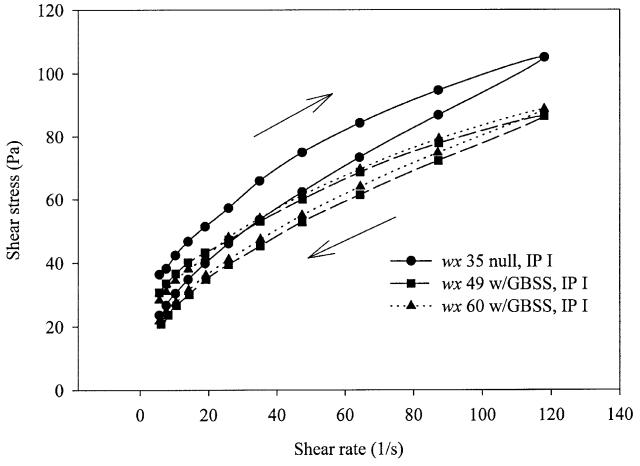

Fig. 3. Shear sweep measurements of gelatinized waxy starch pastes. Waxy null (*wx* 35) and the GBSS-containing waxy (*wx* 49 and 60) starches were isolated (IP I). Arrows indicate the directions of changes of shear rates.

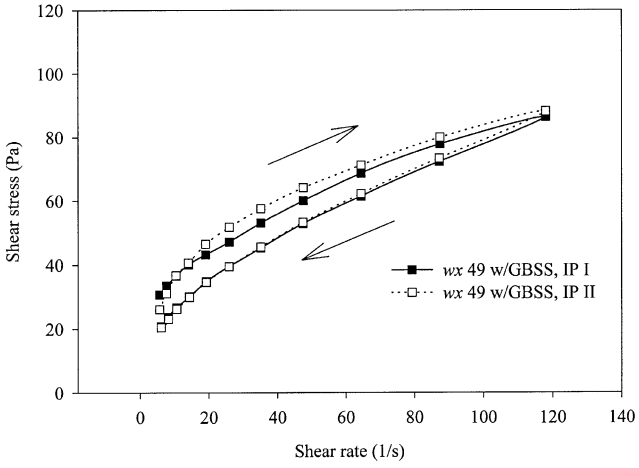

Fig. 5. Shear sweep measurements of gelatinized starch pastes of the GBSS-containing waxy mutant (*wx* 49) starch that were isolated (IP I) and further purified (IP II). Arrows indicate the directions of changes of shear rates.

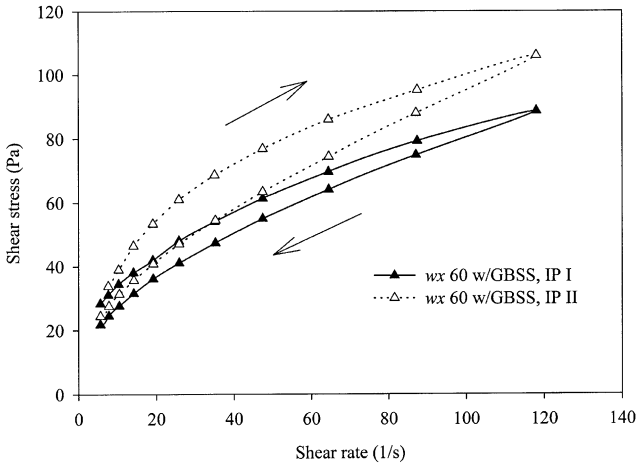

Fig. 6. Shear sweep measurements of gelatinized starch pastes of the GBSS-containing waxy mutant (*wx* 60) starch that were isolated (IP I), and further purified (IP II). Arrows indicate the directions of changes of shear rates.

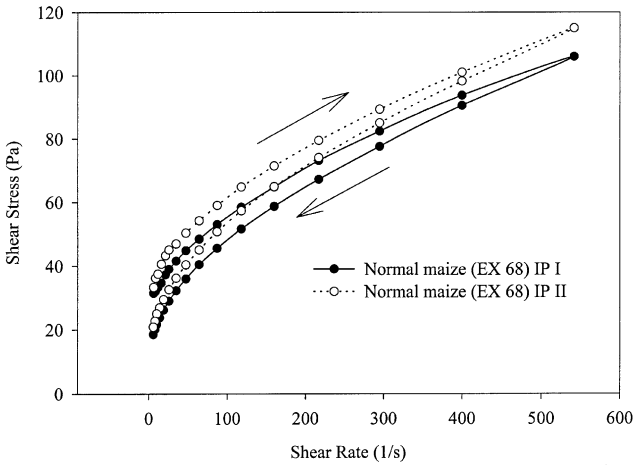

Fig. 8. Shear sweep measurements to 540 l/s of gelatinized starch pastes of normal maize (EX 68) that were isolated (IP I) and further purified (IP II). Arrows indicate the directions of changes of shear rates.

and absence of GBSS, this indicates that the presence of GBSS may reduce shear-induced structural breakdown.

Fig. 4 illustrates that the isolation procedures did not have a large effect on shear-induced breakdown of the waxy null mutant (*wx* 35) starch, which suggests that residual proteins other than GBSS did not much affect the rigidity of gelatinized starch granule and remnant structures. Removal of residual proteins from *wx* 49 and 60 after further purification (IP II), including most of the GBSS, caused greater shear-induced breakdown compared to flow curves of pastes of IP I starch (Figs. 5 and 6, Table 2). This suggests that GBSS strengthens the gelatinized starch granule and remnant structures, thereby reducing shear-induced breakdown of pastes. In normal maize starch (EX 68), there was no apparent shear-induced breakdown in IP I and IP II starches at shear rates up to 120 or 540 l/s (Figs. 7 and 8). The presence of a high level of GBSS in both IP I and II normal starches may have contributed to their low shear-induced breakdown.

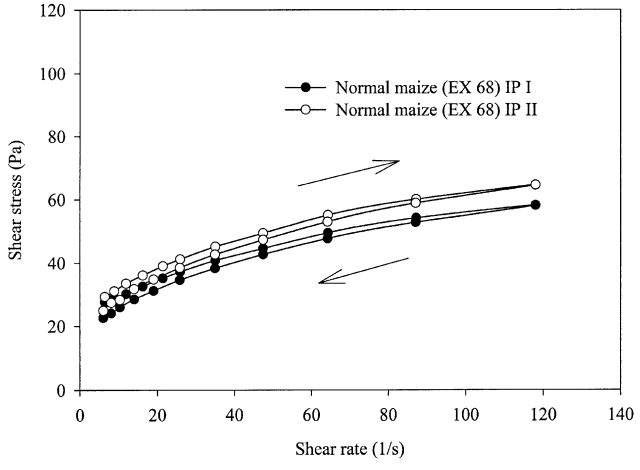

Fig. 7. Shear sweep measurements to 120 l/s of gelatinized starch pastes of normal maize (EX 68) that were isolated (IP I) and further purified (IP II). Arrows indicate the directions of changes of shear rates.

3.4. Microstructural basis of paste breakdown

The GBSS-containing waxy starches (*wx* 49 and 60) showed less shear-induced breakdown than the waxy null mutant (*wx* 35) starch (Fig. 3). However, waxy starches (*wx* 35, 49, and 60) subjected to extremely high shear rates (up to 540 l/s) exhibited similar shear-induced breakdowns (not shown), suggesting that GBSS in the waxy starches (*wx* 49 and 60) could not sustain that high shear rate. Presumably GBSS reduces paste breakdown through strengthening of gelatinized starch granule structure. If the shear rate is high enough to break down the gelatinized structure, the role of GBSS in the pastes would be limited considering its small amount. Light micrographs of sheared GBSS-containing waxy (*wx* 49) IP I and II starch pastes to 540 l/s showed nearly complete dispersion of gelatinized starch (Fig. 9(D) and (F)), while when sheared to only 120 l/s revealed gelatinized remnant structures (Fig. 9(C) and (E)). A comparison of micrographs of *wx* 49 IP I and II starch pastes showed that further purification resulted in greater starch breakdown with shear (Fig. 9(D) and (F)). Therefore, loss of GBSS appears to have caused gelatinized granules/remnants to become more fragile and prone to shear breakdown. Normal IP II starches, which retained GBSS, sheared at high rates showed gelatinized granule/remnant structure (Fig. 9(A) and (B)), suggesting that its comparably high GBSS content acts to stabilize structure.

4. Conclusions

In general, starch granule-associated proteins were shown to decrease the viscosity of pastes, and GBSS was specifically shown to reduce the shear-induced breakdown. Isolated starches (IP I) of a normal cornstarch, a waxy null mutant starch and two GBSS-containing waxy starches

Table 2
Paste breakdown represented by area between the up and down shear sweep curves (asterisks denote values in the same row that are significantly different at $\alpha = 0.05$)

Corn starch	Highest shear rate (l/s)	Areas	
		IP I[a]	IP II
Waxy null mutant (*wx* 35)	120	909[b,c] ± 21	1031 ± 42
Starch GBSS-containing waxy mutant (*wx* 49)	120	797* ± 19	1041 ± 73
GBSS-containing waxy mutant (*wx* 60)	120	575* ± 14	922 ± 172

[a] Starch was isolated using toluene method (IP I) and toluene method with further purification (IP II).
[b] Areas are relative values from Microsoft Origin Program.
[c] Values are means ± standard deviations.

were further purified (IP II). Further purification removed additional residual proteins in starches including the majority of GBSS in the two GBSS-containing waxy starches, resulting in pastes of higher viscosity, especially at high shear rates. When starch pastes were sheared using a shear sweep tests up to the shear rate of 120 l/s, the two GBSS-containing waxy starches showed less shear-induced breakdown than the starch paste of the waxy null mutant, indicating that GBSS reduces the shear-induced breakdown of starch pastes. Removal by further purification of residual proteins, including the majority of GBSS, in the two GBSS-containing waxy starches caused higher shear-induced

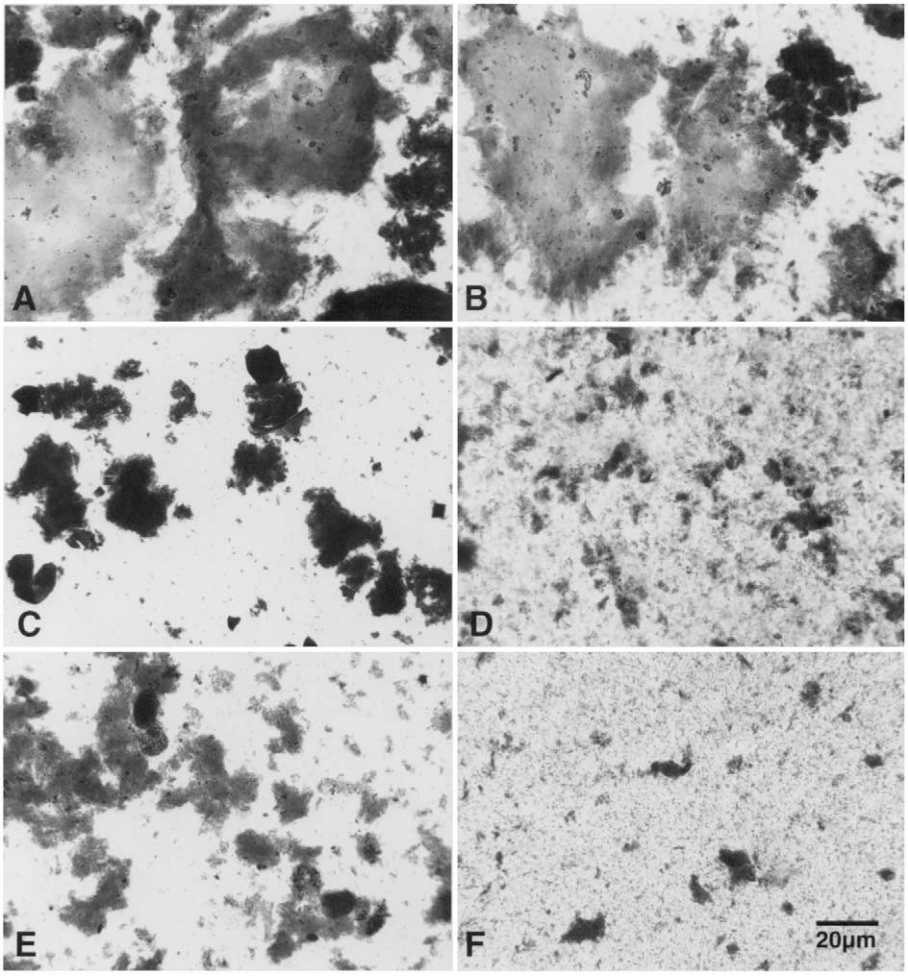

Fig. 9. Light microscopy of gelatinized pastes after shear sweeps of isolated (IP I) and further purified (IP II) starches. IP II normal maize starch pastes after shear sweep to 120 l/s (A) and to 540 l/s (B); pastes of IP I GBSS-containing waxy mutant starch (*wx* 49) after shear sweep to 120 l/s (C) and to 540 l/s (D); pastes of IP II GBSS-containing waxy starch (*wx* 49) after shear sweep to 120 l/s (E) and to 540 l/s (F). All micrographs have the same scale.

breakdown than in the waxy null mutant starch in which only non-GBSS residual proteins were removed. In normal maize starch in which GBSS was mostly retained after further purification, no discernible change in shear-induced breakdown was observed after further purification. The results indicate that GBSS reduces the shear-induced breakdown of gelatinized starches, which suggests that breakdown of waxy starch pastes could be reduced by increasing inactivated GBSS content.

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