

Pasting Properties and Surface Characteristics of Starch Obtained from an Enzymatic Corn Wet-Milling Process

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ABSTRACT

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Recently, we reported the development of an enzymatic corn wet-milling process that reduces or eliminates sulfur dioxide requirements during steeping, considerably reduces steep time, and produces starch yields comparable to that of conventional corn wet-milling. The best results so far, using the enzymatic corn wet-milling procedure, were achieved when a particular protease enzyme (bromelain) was used. In this study, pasting properties and surface characteristics of starch obtained from six different enzyme treatments (three glycosidases [β -glucanase, cellulase, and xylanase] and three proteases [pepsin, acid protease, and bromelain]) using the enzymatic corn wet-milling procedure were evaluated and compared with those from starch obtained using the conventional corn wet-milling procedure. Significant effects from enzymatic milling were observed on all the three starch pasting properties (peak, shear thinning, and setback).

The setback viscosities of starch from all enzyme treatments were significantly lower compared with those of the control sample, indicating that starch polymers from enzymatic corn wet-milling do not reassociate to the same extent as with the control. Comparison between bromelain treatment and the control sample showed that starch samples obtained from bromelain treatment are very similar to control starch in water-binding capacity, molecular breakdown, and time to swell when cooked in water. Significant effects from enzymatic milling were observed on the surface characteristics of starch granules. The glycosidase treatments, especially the β -glucanase samples, showed holes in the starch granules. No visual differences were observed in starch granules between bromelain and control samples.

Conventional corn wet-milling is a very capital- and energy-intensive process. A significant amount ($\approx 21\%$) of the capital and energy cost in a corn wet-milling plant is associated with the steeping process (unpublished data). Conventional steeping is also a very time-consuming process. It takes ≈ 24 – 36 hr to steep the corn kernels before they can be milled to yield starch and other coproducts. Conventional steeping requires adding a considerable amount of sulfur dioxide (SO_2) to disrupt the protein matrix surrounding the starch particles and to aid the separation of starch and protein during the subsequent milling. Because of its toxicity, the use of SO_2 is an environmental and health concern, and there is growing demand from environmental agencies to find alternatives to SO_2 wet-milling processes.

Recently, we reported development of an enzymatic corn wet-milling process, an alternative to the conventional process that does not require the use of SO_2 during steeping (Johnston and Singh 2001). The enzymatic corn wet-milling process consists of two steps: 1) size reduction of corn after brief water soaking of kernels, and 2) controlled incubation of the coarsely ground slurry produced (after size reduction) with enzymes. Size reduction of kernels removes the diffusional barriers that prevent the enzyme penetration into the intact corn kernels and the subsequent reaction with the endosperm protein matrix surrounding the starch particles. After the incubation step, the corn is processed by conventional wet-milling methods. Benefits of the enzymatic wet-milling process are that it drastically reduces or completely eliminates the use of SO_2 during steeping and reduces the steep time by 70% compared with the conventional corn wet-milling steeping process. Significant savings of capital and energy are also likely due to shorter steep times.

Johnston and Singh (2001) showed that starch yields obtained with the enzymatic process are comparable to the starch yields with the conventional wet-milling process. In this study, the starch obtained from the enzymatic wet-milling process was evaluated for its pasting properties and surface characteristics, which were compared with pasting properties and surface characteristics of starch from the conventional wet-milling process.

MATERIALS AND METHODS

A yellow dent corn hybrid (Pioneer 3394) grown during the 1999 crop season at the Agricultural Engineering Farm, University of Illinois at Urbana-Champaign, was used for the study. Corn samples were hand-cleaned to remove broken corn and foreign material, packaged in plastic bags, and stored at 4°C until wet milling. The whole kernel moisture content of the samples was measured using the 103°C convection oven method (Approved Method 44-15A, AACC 2000).

The two-step wet milling of corn samples using enzymes was done as described by Johnston and Singh (2001). The enzymes used were three glycosidases donated by a commercial enzyme company (β -glucanase, cellulase, or xylanase) and three proteases purchased from Sigma (pepsin, acid protease, or bromelain). Relevant activities of the enzymes are given in Table I. In addition to these enzyme treatments, one of the proteases (bromelain) was added with low amounts (200 or 600 ppm) of sulfur dioxide and its effect on starch yields was evaluated. Three different concentrations of bromelain (250, 500, and 1,000 mg) and four different incubation times (1, 2, 3, and 4 hr) were also evaluated for starch yields. To maintain pH

TABLE I

Enzyme Activity Profiles Measured at pH 4.5 in Sodium Acetate Buffer

Enzyme Preparation	Enzyme Activity (U/mg)	Amylase Activity (U/mg)	Native Starch Activity (U/mg)
Proteases			
Bromelain	500	trace	nd ^a
Pepsin	2,260 ^b	nd	nd
Acid protease	1.1 ^b	8,500	320
Glycosidases			
Cellulase	1,862	2,930	200
Xylanase	6,920	2,355	115
β -Glucanase	5,560	3,700	2,677

^a No activity detected

^b Activity units supplied by manufacturer.

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4.0 for enzyme treatments, 10 mL of 1M sodium acetate buffer was added. After enzymatic treatments, laboratory wet milling of samples was done using the 100-g corn wet-milling procedure as described by Eckhoff et al (1996). All enzyme treatments were compared with a control sample that was wet-milled using a conventional laboratory corn wet-milling procedure (Eckhoff et al 1996).

Amylase and native starch hydrolytic activities in the enzyme preparations were measured (Table I). Activity units were defined as the change in the reducing groups, equivalent to an increase in 1 µg of sugar/min, measured using the BCA assay method (Johnston et al 1998). The cellulase, xylanase, and β-glucanase assays used carboxymethyl cellulose, oat spelt xylan, and barley β-glucan, respectively. The amylase and native starch assays used gelatinized and ungelatinized corn starch as substrate and maltose as the standard sugar. Protease activity was determined as in Johnston and Singh (2001).

Pasting Properties

Starch pasting properties were determined using a Rapid Visco Analyser (RVA) (model RVA-4, Newport Scientific, Warriewood, NSW, Australia). A sample of starch was mixed with distilled water to achieve a 28-g sample with 4% dry solids content. A programmed heating and cooling cycle was used in which the RVA temperature was maintained at 50°C for 30 sec, increased at a constant rate over 2.5 min to 95°C, maintained at 95°C for 20 min, decreased at

a constant rate over 3 min to 50°C, and maintained at 50°C for 9 min. Peak, shear thinning, and setback viscosities as well as the peak time and peak temperature were recorded (Thomas and Atwell 1999). Each viscosity is measured in centipoises (cp) and two replicates per sample were analyzed.

Scanning Electron Microscopy

Scanning electron micrographs were taken to view the starch granules in three dimensions and to determine the shape and surface features of starch granules. Starch granules from all the enzyme treatments and the control sample were mounted on an aluminum stub using adhesive tape and then coated with a thin layer of gold by DC sputtering in an argon atmosphere for 3 min. Samples were examined and digitally imaged using a scanning electron microscope (model JSM 840A, JEOL USA, Peabody, MA) operated in the secondary electron imaging mode at an accelerating voltage of 10 kV and coupled to a digital image workstation (model Imix-SPARCS, Princeton Gamma-tech, Princeton, NJ). Five micrographs were taken for each starch sample at 1,000 and 5,000× magnification. All of the images for each sample showed representative results.

RESULTS AND DISCUSSION

We previously reported that bromelain, when added alone or with small amounts of sulfur dioxide (200–600 ppm) in a two-step enzymatic corn wet-milling procedure gave starch yields comparable to

TABLE II
Yield, Protein Content, and Pasting Properties of Starch from Enzymatic Laboratory Corn Wet-Milling Procedure^{a-c}

Enzyme Treatment	Starch Yield (%)	Protein in Starch (%)	Peak Viscosity (cP)	Shear-Thinning Viscosity (cP)	Setback Viscosity (cP)	Peak Time (sec)	Peak Temp. (°C)
β-Glucanase	53.5e	0.42a	515.5c	403.0c	168.5e	4.0a	89.3bc
Cellulase/cellobiase	58.5d	0.40ab	629.0b	474.0a-c	182.0de	4.0a	88.3bc
Xylanase	58.5d	0.41ab	731.0a	514.5a	214.0cd	3.8a	87.3c
Pepsin	62.9c	0.41ab	713.5a	496.0ab	240.5bc	3.8a	87.7c
Acid protease	62.6c	0.31a-c	556.5bc	404.0c	219.5b-d	3.9a	88.8bc
Bromelain	65.8ab	0.29a-c	628.0b	438.0bc	216.5b-d	3.9a	88.3bc
Bromelain + 200 ppm of SO ₂	65.3b	0.28a-c	580.0bc	447.0a-c	260.0b	4.0a	90.7ab
Bromelain + 600 ppm of SO ₂	66.4a	0.19c	589.0bc	448.5a-c	251.0bc	4.0a	89.3bc
Control	66.4a	0.22bc	573.0bc	431.5bc	308.5a	3.9a	92.5a

^a Starch yields and residual protein in starch for all enzyme treatments reported in Johnston and Singh (2001).

^b Starch yields, protein content in starch, and pasting viscosities are means of two observations.

^c Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

TABLE III
Pasting Properties of Starch Obtained from Enzymatic Corn Wet-Milling Procedure^{a,b}

Enzyme Treatment	Peak Viscosity (cP)	Shear-Thinning Viscosity (cP)	Setback Viscosity (cP)	Peak Time (sec)	Peak Temp. (°C)
Proteases	632.6a	446.0a	225.5b	3.85a	88.3b
Glycosidases	625.2a	463.8b	188.2b	3.92a	88.3b
Control	573.0a	431.5b	308.5a	3.9a	92.5a

^a Pasting viscosities are means of two observations.

^b Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

TABLE IV
Yield, Protein Content, and Pasting Properties of Starch from Change in Concentration and Incubation Time of Bromelain Enzyme^{a-c}

Enzyme Treatment	Starch Yield (%)	Protein in Starch (%)	Peak Viscosity (cP)	Shear-Thinning Viscosity (cP)	Setback Viscosity (cP)	Peak Time (sec)	Peak Temp. (°C)
Concentration (mg)							
250	64.0a	0.38a	684.0a	494.0a	232.0a	3.9a	88.3a
500	65.8a	0.29a	628.0a	438.0a	216.5a	3.9a	88.3a
1,000	65.4a	0.38a	645.5a	487.5a	258.0a	3.9a	88.9a
Incubation time (hr)							
1	62.9b	0.29a	691.0a	491.0a	252.0ab	3.8a	87.6a
2	63.8b	0.27a	629.0a	469.5a	259.0a	3.9a	89.4a
3	65.8a	0.29a	628.0a	438.0a	216.5b	3.9a	88.3a
4	66.2a	0.32a	605.0a	456.5a	240.0ab	3.8a	87.0a

^a Starch yields and residual protein in starch for all the enzyme treatments reported in Johnston and Singh (2001).

^b Starch yields, protein content in starch, and pasting viscosities are means of two observations.

^c Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

those of the conventional corn wet-milling procedure (Johnston and Singh 2001) (Table II). Similar increases in starch yields were not observed with other protease (pepsin or acid protease) or with glycosidases (β -glucanase, cellulase, or xylanase). Comparison of the pasting properties of starch obtained from the enzymatic corn wet-milling procedure (bromelain with no sulfur dioxide added) to pasting properties of starch from the conventional wet-milling procedure (control sample) showed no significant difference in the peak and shear-thinning viscosities, and time to peak. Significant differences were observed in the setback viscosity and peak temperature. Starch from bromelain enzyme treatment had lower peak temperature (88.3°C) and setback viscosity (216.5 cp) compared with the control sample (92.5°C and 308.5 cp).

When sulfur dioxide (200 or 600 ppm) was added in addition to bromelain enzyme during steeping, significant differences were observed between the setback viscosity of the control sample and setback viscosity of the samples with sulfur dioxide at 200 and 600 ppm. The setback viscosities of starch obtained with bromelain plus 200 or 600 ppm of sulfur dioxide were significantly lower (251–260 cp) compared with the setback viscosity of the control sample (308.5 cp). Significantly lower peak temperature (89.3°C) was also observed for the sample with 600 ppm sulfur dioxide compared with the peak temperature of the control sample (92.5°C).

These results suggest that starch samples obtained from the two-step bromelain enzyme treatment (with or without SO₂) are similar to the control starch in their water binding capacity, molecular breakdown, and time to swell when cooked in water. However, these starch polymers exhibit significant differences in their susceptibility to temperature (granules ruptured at slightly lower temperature) and in their ability to reassociate to form gels when compared with control samples. Amylase activity lowers the setback viscosity in sorghum starch (Beta et al 2000). However, no significant amylase or native starch hydrolytic activities were observed in the bromelain enzyme preparation (Table I). It is, therefore, difficult to ascertain the reasons for these differences in the peak temperature and setback

viscosities that were observed between the bromelain and the control samples. It is possible that sodium acetate buffer added to maintain pH 4.0 for all the enzyme treatments could have affected the setback viscosities of starch from all the enzyme-treated samples.

Significant differences were observed in all the three pasting viscosities (peak, shear-thinning, and setback) and peak temperature of the starch obtained with enzymes other than bromelain when compared with the control sample (Table II). The control sample also showed significant differences in the peak viscosity from that of the xylanase and pepsin samples and in shear-thinning viscosity from that of xylanase sample. The setback viscosities of all enzyme treatments were significantly lower compared with the control sample. This indicates that starch polymers in enzyme-treated samples do not reassociate to the same extent as the control sample.

Starch samples treated with all enzymes samples, except for bromelain with 200 ppm of SO₂, had a significantly lower peak temperature compared with the control sample, suggesting that starch granules of enzyme-treated samples are slightly more prone to pasting at lower temperature. Compared with control samples, no significant difference in any of the enzyme-treated samples was observed in time to peak, indicating that rate of granule swelling is the same in all samples. Lower peak temperature of starch samples obtained from a different enzymatic corn wet-milling process was also observed by Steinke et al (1991). They found that the peak and setback viscosities of enzyme samples were higher than for the conventional control samples. Steinke et al (1991) used multiple enzymes in their steeping system and, therefore, their results cannot directly be compared with this study. Radosavljevic et al (1998) compared alkaline treated with low alkaline and protease treatment for recovery of amaranth starch and showed that treatment with protease gave starch with low peak temperature and peak viscosity.

Grouping enzymes under proteases (pepsin, acid protease, and bromelain) and glycosidases (β -glucanase, cellulase, or xylanase) and comparing their pasting properties with those of the control sample showed that starch samples obtained with proteases had

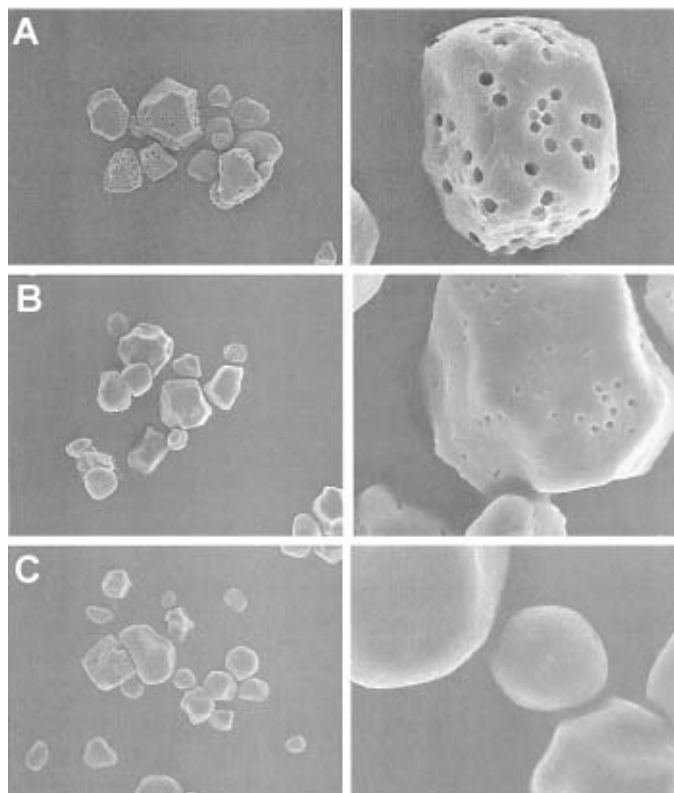


Fig. 1. Starch granules obtained from use of glycosidases in enzymatic corn wet-milling process. **A**, β -glucanase; **B**, cellulase; **C**, xylanase. Magnification: right 1,000 \times and left 5,000 \times .

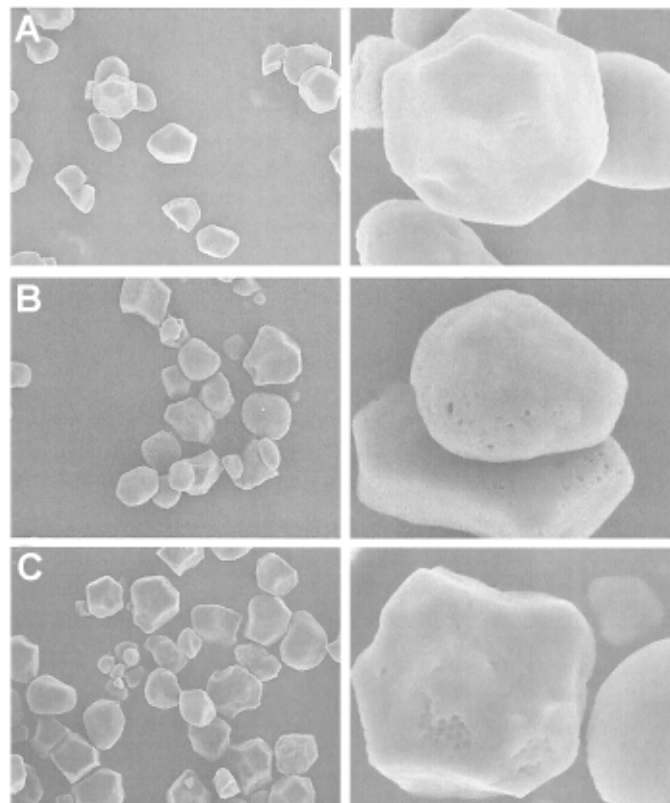


Fig. 2. Starch granules obtained from use of proteases in enzymatic corn wet-milling process. **A**, pepsin; **B**, acid protease; **C**, bromelain. Magnification: right 1,000 \times and left 5,000 \times .

significantly higher shear-thinning viscosities and significantly lower setback viscosity and peak temperature (Table III). Glycosidases had significantly lower setback viscosities and peak temperature compared with the control sample.

Effect of Change in Incubation Time and Concentration of Bromelain Enzyme

Johnston and Singh (2001) reported no significant change in starch yield or protein content in starch with change in concentration of the bromelain enzyme from 250 to 1,000 mg during incubation (Table IV). In this study, no significant difference was observed in any of the pasting properties or peak time and peak temperature of the starch samples with change in concentration of bromelain enzyme. A significant increase in starch yield with increase in the duration of incubation from 1 to 4 hr was observed (Johnston and Singh 2001). However, no significant difference in peak temperature, time to peak, or pasting properties was observed except for setback viscosity in which a small but significant difference was observed between 2 and 3 hr of incubation time. This is likely an anomaly because no significant difference was observed between 2 and 4 hr or 1 and 4 hr of incubation time.

Scanning Electron Microscopy

Starch granules obtained from the glycosidase enzyme preparations show a significant amount of holes on the surface of the granules (Fig. 1). These holes were significantly big, deep, and more predominant in the β -glucanase-treated samples compared with the other two glycosidase-treated preparations (cellulase and xylanase). Small holes were observed on the surface of the starch granules from the cellulase preparation. When comparing cellulase-treated with β -glucanase-treated samples, these holes were not as deep and were not found on every starch granule. Very small holes were observed on starch granules treated with xylanase enzyme. However, compared with β -glucanase and cellulase samples, holes

were observed on very few granules (Fig. 1). These holes on the surface of starch granules are probably due to the native starch and amylase activity of the enzyme preparations (Table I). Native starch activity of β -glucanase enzyme preparation is 1,200 and 2,000% more than the cellulase and xylanase preparations, respectively. Amylase activity of the β -glucanase enzyme preparation is also significantly higher compared with the other two enzyme preparations.

Micrographs of starch granules from different protease enzyme preparations showed significant holes only in the acid protease treatment, probably due to significant amylase and native starch degradative activity of the enzyme (Fig. 2). For pepsin and bromelain treatments, very few (one or two holes) were observed on a small number of starch granules. No significant amylase or native starch degradative activities were observed for pepsin and bromelain enzyme preparations (Table I). Besides holes, there are other surface features on starch granules such as indentations. These indentations on starch granules probably represent the places where protein bodies and other smaller starch granules were tightly packed with the granules inside the corn endosperm.

Comparison of samples treated only with bromelain enzyme (Fig. 2C) and bromelain enzyme with a small amount of SO_2 (Fig. 3A,B) with the control sample (Fig. 3C) showed no significant visual difference between the starch granules. Few holes (one or two) were also observed on a few starch granules from the control sample (conventional wet-milled). These holes are probably due to some endogenous amylase activity inside the corn kernel.

CONCLUSIONS

Significant effects of enzymatic wet milling was observed on all the three starch pasting properties (peak, shear thinning, and setback) of starch samples. Compared with a control sample (conventional wet-milled starch), significant differences were observed in the peak viscosity of xylanase and pepsin samples and shear-thinning viscosity of xylanase sample. The setback viscosities of all enzyme treatments were significantly lower compared with the control sample, indicating that starch polymers in samples from the enzymatic corn wet-milling do not reassociate to same extent as with the control sample. Comparison between the bromelain treatment and the control sample showed that starch samples obtained from the bromelain treatment are very similar to the control starch samples in their water-binding capacity, molecular breakdown, and time to swell when cooked in water. However, when the sample was cooled, starch polymers (from bromelain treatment) did not reassociate to the same extent as the control sample.

A significant effect of enzymes was observed on the surface characteristics of starch granules. The glycosidase enzymes, especially β -glucanase samples, showed holes in the starch granules. In protease enzyme samples, significant holes were observed only in acid protease treatment. These holes in starch granules are mainly due to the significant amylase and native starch degradative activities of the enzyme preparations. No visual differences were observed in starch granules between the bromelain and the control samples.

Overall, results of this study suggest that, with use of certain enzymes in an enzymatic corn wet-milling procedure, starch can be produced at the same yield and with quality equivalent closely comparable to the conventional wet-milling procedure. Slight differences in the setback viscosities of starch from enzymatic corn wet-milling procedure can be mitigated by chemical modification of starch to attain the desirable functionality.

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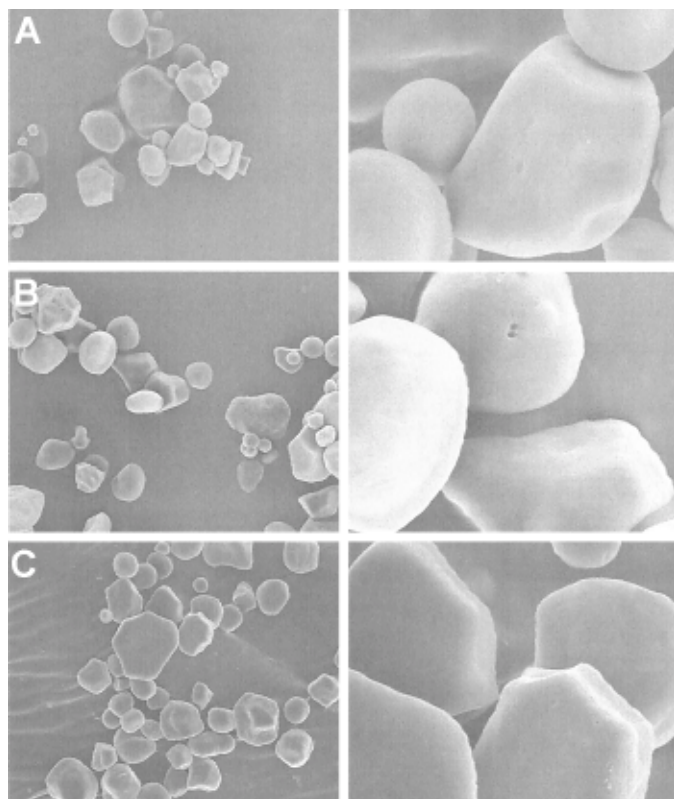


Fig. 3. Starch particle obtained from enzymatic and conventional corn wet-milling process. **A**, bromelain + 200 ppm of SO_2 ; **B**, bromelain + 600 ppm of SO_2 ; **C**, conventional corn wet-milling. Magnification: right 1,000 \times and left 5,000 \times .

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