

# Application of Protease and High-Intensity Ultrasound in Corn Starch Isolation from Degermed Corn Flour

Devon K. Cameron<sup>1</sup> and Ya-Jane Wang<sup>1,2</sup>

## ABSTRACT

Cereal Chem. 83(5):505–509

The conventional corn wet-milling process requires a long steeping time and has environmental and health concerns from the use of SO<sub>2</sub>. A recently proposed two-stage enzymatic milling procedure with the first stage of water soaking and coarse grinding of corn and the second stage of incubating with enzymes has been shown to reduce the soaking time and possibly eliminate the need for SO<sub>2</sub> addition. This current work explored the applications of protease and high-intensity ultrasound in the second stage of the two-stage enzymatic milling for corn starch isolation to further shorten the process time without use of SO<sub>2</sub>. The starch yield from sonication alone was 55.2–67.8% (starch db) as compared with

53.4% of the water-only control with stirring for 1 hr and 71.1% of the conventional control with SO<sub>2</sub> and lactic acid steeping for 48 hr. Protease digestion alone for 2 hr was not effective (45.8–63.9% yield) in isolating corn starch, but the starch recovery was increased to 61.2–76.1% when protease was combined with sonication. The preferred combination was neutral protease digestion for 2 hr followed by sonication at 75% amplitude for 30 min. The results demonstrated that combinations of high-intensity ultrasound and neutral protease could replace SO<sub>2</sub> and shorten the steeping time in the enzymatic wet-milling process for corn starch isolation.

The conventional corn wet-milling process involves the soaking of corn kernels in an aqueous solution containing 0.1–0.2% (w/v) SO<sub>2</sub> and 0.5–1.5% (v/v) lactic acid at 45–55°C for 24–40 hr (Shandera and Jackson 1996). Because the pericarp creates a barrier to the diffusion of water and SO<sub>2</sub> (Syarif et al 1987; Eckhoff and Okos 1989; Ruan et al 1992), the long steeping time is required to achieve sufficient release of starch granules from the protein matrix. The SO<sub>2</sub> provides a combination of antimicrobial, softening, and protein-dispersing characteristics that are essential for high starch yields; however, it also causes environmental and health concerns. Additionally, the long steeping time and use of chemicals have been shown to affect starch properties. For example, lactic acid can have an acid-thinning effect on starch granules, which can reduce pasting viscosities (Shandera and Jackson 1996; Pérez et al 2001). Reducing the steeping time could lower production and energy cost, increase productivity, and lessen the changes of starch properties.

Enzymes, such as proteases and cellulases, have been evaluated in the conventional corn wet-milling steeping process to reduce the steeping time and to further improve starch yield (Hassanean and Abdel-Wahed 1986; Caransa et al 1988; Steinke and Johnson 1991; Steinke et al 1991; Moheno-Perez et al 1999). These studies showed small but significant improvements over the conventional wet-milling with the addition of high doses of enzyme in addition to SO<sub>2</sub> during steeping. Recently, Johnston and Singh (2001, 2004), Singh and Johnston (2002), and Johnston et al (2003) demonstrated that enzymatic milling could reduce soaking time and possibly eliminate the need for SO<sub>2</sub> addition. They proposed a two-stage procedure with the first stage of water soaking and coarse grinding of corn and the second stage of incubating with enzymes. The grinding of hydrated corn kernels for size reduction removed the diffusion barriers because enzymes were not able to penetrate the kernels and break down the protein matrix surrounding starch granules (Singh and Johnston 2004). The enzyme diffusion rate increased in the hydrated ground corn, and the overall steep time was reduced to 4–6 hr. Proteases were more effective in starch recovery compared with other types of enzymes.

High-intensity ultrasound, which uses large power levels (10–1,000 W/cm<sup>2</sup> range), has been effective in isolating rice starch

from rice flour both alone and in combination with neutral protease in <3 hr (Wang and Wang 2004a,b). Rice flour treated with sonication at 75% amplitude (750W, 20 kHz) for 30 min and combinations of neutral protease and sonication produced significantly higher rice starch yields of 74.1–76.2% and 79.8–86.7% (starch db), respectively, compared with 71.6% from the conventional alkaline steeping method. The isolated rice starch from treatments combining neutral protease and sonication displayed similar gelatinization properties as measured by differential scanning calorimetry but slightly different pasting properties as measured by a Rapid Visco-Analyser when compared with the alkali-isolated treatments. Zhang et al (2005) tested the use of ultrasound to recover starch from degermed corn flour and hominy feed and reported significantly higher starch yields with sonication than with the control of water stirring only.

The objective of this study was to evaluate protease and ultrasound as alternatives in the second stage of the enzymatic milling process to further shorten the soaking time without the usage of SO<sub>2</sub>. Degermed corn flour was used as the model system to better compare the effectiveness of protease and sonication on corn starch isolation.

## MATERIALS AND METHODS

### Materials

Degermed yellow corn flour was purchased from ADM Milling (Milwaukee, WI). A high-intensity ultrasonic processor (750W, model, 20 kHz) with a three-quarter inch high gain probe was purchased from Sonics and Materials (Newtown, CT). Acid casein (purity 99.9%) was purchased from New Zealand Milk Products North America (Santa Rosa, CA). L-Tyrosine and trichloroacetic acid were purchased from EMD Chemicals (Gibbstown, NJ). Folin's reagent was purchased from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide, sodium bisulfite, and phosphoric acid were purchased from J.T. Baker (Phillipsburg, NJ).

Three types of proteases, provided by Amano Pharmaceutical (Nagoya, Japan) were studied. Neutral protease, N "Amano", in the dry powder form is from *Bacillus subtilis* with protease activity ≥150,000 units/g, optimum pH 7.0, and 55°C. Alkaline protease, P "Amano" 6, in the dry powder form is from *Apergillus melleus* with protease activity ≥60,000 units/g, optimum pH 8.0, and 45°C. Acid protease, "A", in dry powder form is from *A. niger* with protease activity ≥35,000 units/g, optimum pH 2.5, and 55°C. Three levels from each protease were evaluated at pH 7.0 and 50°C, and the selected amounts for neutral protease were 0.01, 0.03, and 0.05, which corresponded to activities of 1,500,

<sup>1</sup> Department of Food Science, University of Arkansas, Fayetteville, AR 72704.

<sup>2</sup> Corresponding author. Phone: 479-575-3871. Fax: 479-575-6936. E-mail: yjwang@uark.edu

4,500, and 7,500 units/g of flour (as-is basis), respectively. Selected amounts and activities chosen for alkaline and acid protease were slightly higher than those of neutral protease because the incubation pH was not the optimum pH for either enzyme.

### Starch Isolation

**Controls.** A conventional control was conducted by treating corn flour with SO<sub>2</sub> and lactic acid, which are used in the conventional corn wet-milling process. Degermed corn flour (100 g, as-is) was steeped in 200 g of deionized water with 0.5% (w/w) lactic acid, and 0.2% (w/w) SO<sub>2</sub> (as sodium bisulfite) at 50°C for 48 hr. A water-only control was also included for comparison by stirring the same amount of flour slurry at room temperature for 1 hr. After the steeping, the flour slurry was milled using a homemade centrifugal mill, passed through a 63- $\mu$ m screen, and centrifuged at 1,400  $\times$  g for 10 min. The centrifugal mill consisted of a cone-shaped rotor and stator to regulate the shear gap to produce intense friction on the flour for size reduction. The soft, top yellowish protein layer was carefully removed with a spatula, and the bottom starch layer was reslurried. This purification process of reslurrying, centrifugation, and protein removal was repeated two more times. The purified starch was dried in a forced-air oven at 40°C for 48 hr, ground using a mortar and pestle, passed through a 150- $\mu$ m sieve, and stored in a plastic jar at room temperature. The starch yield was calculated as the amount of starch recovered after drying by the amount of starch present in the degermed corn flour.

**High-intensity ultrasound.** Three factors were studied for starch isolation by high-intensity ultrasound: sonication amplitude, sonication duration, and temperature. Deionized water (200 g) was warmed to 30 or 42°C in a 500-mL reaction beaker by a circulator before the addition of the corn flour (100 g, as-is). The corn flour was stirred for 5 min before sonication began. The amplitude is related to the energy content, the greater the amplitude, the greater the distance the probe tip travels. The 100% amplitude setting for the three-quarter inch high gain probe used in this study was 61  $\mu$ m. The 6.5-cm probe tip was placed  $\approx$ 3 cm deep into the starch slurry. The sonication amplitude was set at 25, 50, or 75% with a pulsed time of 5-sec on and 5-sec off. The sonication duration was 15, 30, or 60 min, excluding the off time of pulsing sonication. The corresponding total time of sonication would be 30, 60, and 120 min, respectively. The maximum sample temperature was maintained at 40 or 50°C by the circulator and monitored by a temperature probe. After the treatment, the same procedure was followed for wet-milling, centrifugation, protein removal, drying, and storage as described above.

**Protease treatment.** Acidic, neutral, and alkaline proteases at concentrations of 0.01–0.25% (flour as-is) were applied to the corn flour (100 g, as-is) at 50°C for 2 hr with constant stirring to evaluate their effectiveness in isolating corn starch. Neutral protease at 0.03% was selected to be studied in combination with sonication because it produced the highest starch yield.

**Combinations of protease and sonication.** Three factors were studied for starch isolation from corn flour by combining sonication and protease: sonication duration, sonication amplitude, and sequence of application. The sonication amplitude was set at 25, 50, or 75% with 5 sec on and 5 sec off, and the maximum sample temperature was controlled at 50°C by the circulator. The sonication duration was set for 15, 30, or 60 min, which included only the on time. Sonication was applied before, during, or after protease digestion. Corn flour (100 g, as-is) was mixed with deionized water (200 g) in the reaction beaker. Neutral protease of 0.03% (w/w, flour as-is) was added to the corn flour at 50°C with constant stirring for 2 hr either before or after sonication at 50 or 75% for 15 or 30 min of on time. When sonication was applied during protease digestion, the total treatment duration, including both on and off time, was 60 or 120 min for 25% amplitude and 30 or 60 min for 50% amplitude. The 75% amplitude was not

employed when sonication was used during protease digestion to avoid potential inactivation of protease. Wang and Wang (2004b) reported that simultaneous sonication and protease digestion gave slightly lower starch yield possibly due to shorter reaction time or protease inactivation by sonication. After the treatment, the same procedure was followed for wet-milling, centrifugation, drying, and storage as before. Duplicates were performed for each control and experimental starch isolation sample.

### Chemical Composition and Physicochemical Properties of Isolated Starch

The moisture, protein, damaged starch, and total starch contents of the isolated starches were determined in duplicate following Approved Methods (AACC 2000). Samples (2 g) were placed in aluminum moisture dishes and dried at 130°C in a convection oven for 60 min according to Approved Method 44-15A. Crude protein was measured by micro-Kjeldahl according to Approved Method 46-13. Damaged starch content was determined using the spectrophotometric method of the Megazyme Starch Damage kit according to Approved Method 76-31. Total starch content was determined following the Megazyme Amyloglucosidase/ $\alpha$ -Amylase Method according to Approved Method 76-13.

The pasting characteristics were measured in duplicate at 7.5% (w/w) concentration using a Micro ViscoAmyloGraph (C.W. Brabender Instruments, South Hackensack, NJ) equipped with a 300-cmg cartridge and operated at a speed of 250 rpm. The starch slurry was heated from 50 to 95°C at a rate of 7°C/min, held at 95°C for 5 min, and cooled down to 50°C at a rate of 7°C/min. The breakdown viscosity is the peak minus trough viscosity, and the total setback is the final minus trough viscosity. The gelatinization properties were assessed in duplicate using differential scanning calorimetry (DSC) (model Pyris-1, Perkin-Elmer, Norwalk, CT). Starch ( $\approx$ 4 mg, db) was weighed accurately into an aluminum DSC pan and then moistened with 8  $\mu$ L of DI water using a microsyringe. The pan was hermetically sealed and allowed to stand for at least 1 hr before thermal analysis. Samples were heated from 25 to 130°C at a rate of 10°C/min. Enthalpy, onset, peak, and end temperatures were computed automatically. The scanning electron micrographs of isolated starches were taken with an XL30 ESEM (environmental scanning electron microscope) (FEI Corporation, Eindhoven, The Netherlands) at an accelerating voltage of 10 kV. Starch granules were sprinkled onto double-backed cellophane tape attached to a stub before coating with gold-palladium.

### Residual Protease Activity

The residual protease activity in the isolated starch was assayed following the procedure provided by Amano Pharmaceutical (Nagoya, Japan). Milk casein was used as the substrate for protease assay and prepared by heating 1.50 g of milk casein in 25 mL of 0.1M NaOH in a water bath at 90–95°C for 10 min. The solution was cooled to room temperature and adjusted to pH 7.0 with 0.033M phosphoric acid. Then 20 mL of 0.1M phosphate buffer (pH 7.0) was added and the solution was diluted to 100 mL. The neutral protease used in this study was diluted 60,000 times for activity measurement. Starch samples (1 g) were mixed with water to a final volume of 5 mL, and the protein content in the starch was used to determine the dilution factor to calculate the protease activity. A calibration curve was prepared with tyrosine at 0, 10, 20, 30, 40, and 50  $\mu$ g/mL in 0.1M HCl. Tyrosine standard solution (1 mL) was added to 5 mL of 0.4M Na<sub>2</sub>CO<sub>3</sub> and 1 mL of undiluted (fivefold) Folin's reagent. The solution was mixed and maintained at 37°C for 20 min; thereafter the absorbance was measured at 660 nm. The standard curve was constructed by plotting tyrosine concentration versus absorbance, and the slope was determined. For protease activity assay, 1 mL of casein substrate solution equilibrated at 37°C for 10 min was added with 1 mL of neutral protease solution or the supernatant from the starch

slurries and incubated at 37°C for 60 min. Two blanks were included with one containing 1 mL of DI water and 1 mL of 60,000-fold diluted neutral protease solution but no substrate, and the other containing 1 mL of DI water and 1 mL of the supernatant from the starch slurry without substrate. At the end of incubation, 2 mL of 0.4M trichloroacetic acid was added, the solution stood for 25 min, and then it was filtered through a filter paper (Whatman No. 42) to remove the precipitate. To 1 mL of each filtrate, 5 mL of 0.4M sodium carbonate and 1 mL of undiluted (fivefold) Folin's reagent were added. The solutions were mixed and stood at 37°C for 20 min before the absorbance was measured at 660 nm. One unit of protease activity is defined as the quantity of the enzyme to produce amino acid equivalent to 100 µg of tyrosine in 1 mL of filtrate under the conditions of the assay. Protease activity = (absorbance of sample – absorbance of both blanks) × (slope of the tyrosine standard curve) × (dilution factor of enzyme or protein in starch) × (1/100).

### Statistical Analysis

Experimental data were analyzed by using the GLM procedure (SAS Institute, Cary, NC), and Duncan's multiple range was used to compute the least significance differences at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Ultrasonic Treatments

The degermed corn flour used in the present study contained 5.9% protein, 67.9% total starch, and 2.35% damaged starch on a dry-weight basis. The starch yield, residual protein content, damaged starch content, and pasting properties of the isolated starches from the treatments of conventional control, water-only control, and high-intensity ultrasound alone are listed in Table I. The conventional control with 48 hr of steeping produced the highest starch yield but also the highest damaged starch content. The water-only control had the lowest starch yield and damaged starch content. The starch yield, residual protein, and damaged starch content of sonicated samples were 55.2–67.8%, 0.20–0.79% and 0.64–1.15% (db), respectively. When the sonication amplitude and duration increased, the starch yield was significantly improved, and the residual protein content was notably decreased under the same treatment temperature. The starch yield was not affected by the temperature (40 or 50°C), but the protein and damaged starch

contents varied with treatment temperature. In general, the residual protein content decreased with increasing sonication amplitude, duration, and temperature. However, the opposite trend was observed for the damaged starch content, presumably because a higher temperature coupled with a higher amplitude and a longer duration resulted in more damage to the starch granules. Similar trends were also observed for sonication-assisted isolation of rice starch reported earlier (Wang and Wang 2004a). The sonication treatment with 75% amplitude at 50°C for 30 min produced a comparable starch yield but a lower residual protein and damaged starch content as compared with the conventional control.

Zhang et al (2005) also observed significantly higher starch yields when corn flour was treated with ultrasound (100% amplitude, 20 kHz) than with water only for the same reaction time (30 min); however, they did not include a SO<sub>2</sub> control. They reported much higher starch yields than the present study, which was ascribed to the different methods used to separate protein from starch. Zhang et al (2005) used the tabling procedure described in Eckhoff et al (1996), whereas centrifugation was used in this study, by which more starch was lost to the protein fraction. Significantly higher starch yields relative to the conventional control was observed in rice starch recovery from rice flour (Wang and Wang 2004a) compared with the present study using the same sonication conditions, suggesting that differences exist between corn and rice flours in terms of interactions between starch and protein or susceptibility of protein to sonication.

**TABLE II**  
Starch Yield of Corn Starch Isolated by Different Proteases at Various Concentrations at 50°C for 2 hr<sup>a</sup>

| Treatment               | Activity (U) | Starch Yield (% starch, db) |
|-------------------------|--------------|-----------------------------|
| 0.05% Acid protease     | 1,750        | 47.4d                       |
| 0.15% Acid protease     | 5,250        | 46.9d                       |
| 0.25% Acid protease     | 8,750        | 57.9bc                      |
| 0.01% Neutral protease  | 1,500        | 54.3c                       |
| 0.03% Neutral protease  | 4,500        | 63.9a                       |
| 0.05% Neutral protease  | 7,500        | 58.4b                       |
| 0.03% Ikaline protease  | 1,800        | 47.3d                       |
| 0.09% Alkaline protease | 5,400        | 58.2bc                      |
| 0.15% Alkaline protease | 9,000        | 45.8d                       |

<sup>a</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

**TABLE I**  
Starch Yield, Protein Content, Damaged Starch Content, and Pasting Properties of Corn Starch Isolated by Different Methods<sup>a</sup>

| Treatment                         | Starch Yield (% starch db) | Protein (% flour db) | Damaged Starch (% flour db) | Viscosity (BU) |           |        |               |
|-----------------------------------|----------------------------|----------------------|-----------------------------|----------------|-----------|--------|---------------|
|                                   |                            |                      |                             | Peak           | Breakdown | Final  | Total Setback |
| Conventional control <sup>b</sup> | 71.1a                      | 0.33h                | 1.22a                       | 570ab          | 183a–c    | 737cd  | 348c          |
| Water-only control <sup>c</sup>   | 53.4f                      | 0.56d                | 0.59h                       | 599a           | 185ab     | 859ab  | 445a          |
| 40°C                              |                            |                      |                             |                |           |        |               |
| U30m25%amp <sup>d</sup>           | 55.6ef                     | 0.78a                | 0.99d                       | 481d           | 137f      | 739cd  | 395b          |
| U60m25%amp                        | 60.1de                     | 0.76a                | 0.97de                      | 596a           | 138f      | 864a   | 406b          |
| U15m50%amp                        | 61.6cd                     | 0.79a                | 0.93ef                      | 536bc          | 166de     | 701d–f | 331cd         |
| U30m50%amp                        | 62.3cd                     | 0.64c                | 0.67h                       | 589a           | 187ab     | 756c   | 354c          |
| U15m75%amp                        | 63.8b–d                    | 0.54de               | 0.77g                       | 559ab          | 142f      | 810b   | 393b          |
| U30m75%amp                        | 64.0b–d                    | 0.34h                | 0.81g                       | 575ab          | 184ab     | 744cd  | 353c          |
| 50°C                              |                            |                      |                             |                |           |        |               |
| U30m25%amp                        | 55.2ef                     | 0.78a                | 0.64h                       | 500cd          | 151ef     | 653ef  | 304de         |
| U60m25%amp                        | 63.0b–d                    | 0.52ef               | 0.97de                      | 541bc          | 163de     | 726cd  | 348c          |
| U15m50%amp                        | 59.9de                     | 0.71b                | 0.81g                       | 557ab          | 198a      | 651f   | 292e          |
| U30m50%amp                        | 63.1b–d                    | 0.50f                | 0.95de                      | 559ab          | 179b–d    | 719cd  | 339c          |
| U15m75%amp                        | 65.3bc                     | 0.40g                | 1.07c                       | 570ab          | 177b–d    | 729cd  | 336c          |
| U30m75%amp                        | 67.8ab                     | 0.20i                | 1.15b                       | 538bc          | 167c–e    | 706c–e | 335c          |

<sup>a</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

<sup>b</sup> Steeping with 0.2% (w/w) SO<sub>2</sub> solution and 0.5% (w/w) lactic acid at 50°C for 48 hr.

<sup>c</sup> Steeping in water with stirring at room temperature for 1 hr.

<sup>d</sup> U, ultrasound, m, minute, amp, amplitude.

The improved starch yield and increased damaged starch content for the samples treated with sonication relative to the water-only control imply that the acoustic cavitation of high-intensity ultrasound disrupted the protein matrix or the interactions between protein and starch, which helped starch recovery. At the same time, some starch granules were damaged from exposure to sonication. It was observed that separation of protein and starch fractions became easier after 30 or 60 min of sonication.

The isolated starch from the conventional control treatment showed a reduction in peak, final, and total setback viscosities relative to the water-only control, agreeing with Shandera and Jackson (1996) and Perez et al (2001). In their studies, the physicochemical

properties of the isolated starch were affected as a result of the annealing effect from the steeping conditions. The sonicated starch samples displayed similar pasting properties as the conventional control but lower final and total setback viscosities when compared with the water-only control. There was no clear trend among different treatments in terms of pasting properties.

For gelatinization properties, the onset temperature, peak temperature, and enthalpy of the isolated starch from the water-only treatment were 66.2°C, 72.2°C, and 10.9 J/g, respectively, whereas those of the isolated starch from the conventional control were 71.7°C, 74.8°C, and 10.6 J/g, respectively. The increased gelatinization temperatures were attributed to the annealing effect as

**TABLE III**  
Starch Yield, Protein Content, Damaged Starch Content, and Pasting Properties of Corn Starch Isolated by Different Combinations of Protease and Sonication<sup>a</sup>

| Treatment                         | Starch Yield<br>(% starch db) | Protein<br>(% flour db) | Damaged Starch<br>(% flour db) | Viscosity (BU) |           |       |               |
|-----------------------------------|-------------------------------|-------------------------|--------------------------------|----------------|-----------|-------|---------------|
|                                   |                               |                         |                                | Peak           | Breakdown | Final | Total Setback |
| Conventional control <sup>b</sup> | 71.1b–d                       | 0.33hi                  | 1.22b–e                        | 570b           | 183ab     | 737b  | 348b          |
| Water-only control <sup>c</sup>   | 53.4g                         | 0.56a                   | 0.59f                          | 599a           | 185ab     | 859b  | 445a          |
| U15min50%amp-P2h <sup>d</sup>     | 61.2f                         | 0.47b                   | 1.14e                          | 545b           | 170b–e    | 696bc | 321b          |
| U30min50%amp-P2h                  | 68.4c–e                       | 0.36fg                  | 1.32bc                         | 545b           | 169b–e    | 709bc | 333b          |
| U15min75%amp-P2h                  | 65.2ef                        | 0.37ef                  | 1.31b–d                        | 547b           | 166c–e    | 703bc | 322b          |
| U30min75%amp-P2h                  | 74.2ab                        | 0.30ij                  | 1.20c–e                        | 569b           | 179a–c    | 727bc | 337b          |
| PU30min25%amp <sup>e</sup>        | 72.0bc                        | 0.46bc                  | 1.15de                         | 544b           | 181a–c    | 680c  | 317b          |
| PU60min25%amp                     | 73.2ab                        | 0.40d                   | 1.24b–e                        | 568b           | 187a      | 716bc | 335b          |
| PU15min50%amp                     | 67.6de                        | 0.43c                   | 1.22b–e                        | 566b           | 175a–d    | 710bc | 319b          |
| PU30min50%amp                     | 66.7e                         | 0.39de                  | 1.22b–d                        | 561b           | 178a–d    | 712bc | 329b          |
| P2h-U15min50%amp <sup>f</sup>     | 67.5de                        | 0.34gh                  | 1.24b–e                        | 556b           | 174a–d    | 720bc | 338b          |
| P2h-U30min50%amp                  | 67.3e                         | 0.29j                   | 1.52a                          | 547b           | 162de     | 727bc | 342b          |
| P2h-U15min75%amp                  | 71.4bc                        | 0.25k                   | 1.37b–e                        | 544b           | 157e      | 724bc | 337b          |
| P2h-U30min75%amp                  | 76.1a                         | 0.24k                   | 1.52a                          | 563b           | 177a–d    | 736b  | 350b          |

<sup>a</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

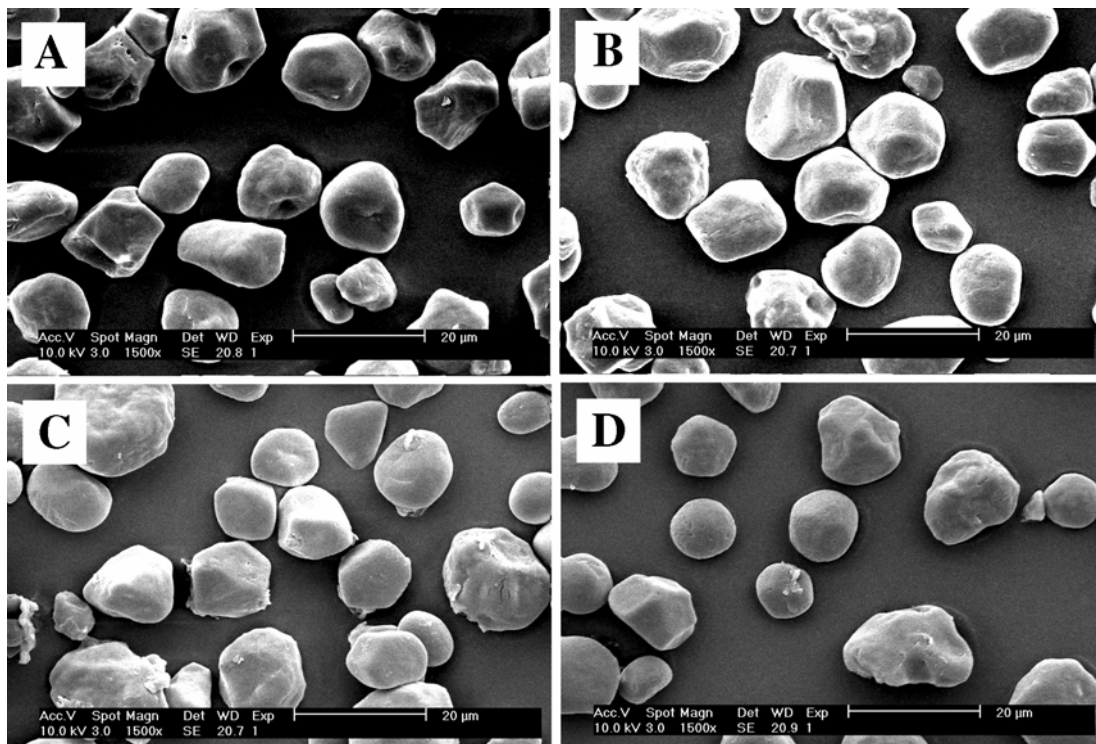
<sup>b</sup> Steeping with 0.2% (w/w) SO<sub>2</sub> solution and 0.5% (w/w) lactic acid at 50°C for 48 hr.

<sup>c</sup> Steeping in water with stirring at room temperature for 1 hr.

<sup>d</sup> Sonication 15 min at 50% amplitude and then protease digestion for 2 hr.

<sup>e</sup> Protease digestion and sonication 30 min at 25% amplitude concurrently.

<sup>f</sup> Protease digestion 2 hr and then sonication 15 min at 50% amplitude.



**Fig. 1.** Scanning electron micrographs of corn starch isolated by conventional control (A), water-only control (B), sonication at 75% amplitude and 40°C for 30 min (C), protease digestion for 2 hr followed by sonication at 75% amplitude for 30 min (D).

observed in the pasting properties. The gelatinization properties of the sonicated starch were similar to those from the water-only control, confirming the effect of annealing on starch properties.

### Treatments Combining Protease and Sonication

Proteases of different types (acidic, neutral, and alkaline) at different concentrations based on a similar range of activity were compared to select the best protease and concentration to be combined with sonication. The results showed that protease alone was not effective in improving corn starch isolation under the conditions with stirring at 50°C for 2 hr (Table II). Neutral protease at 0.03% was more effective in assisting starch isolation producing a starch yield of 63.6%, which was nevertheless much lower than the conventional control of 71.1%. Previously neutral protease at 0.03 or 0.05% was effective in isolating rice starch from rice flour, which produced a similar starch yield as the conventional alkaline steeping method (Wang and Wang 2004b).

Table III presents the starch yield, residual protein content, damaged starch content, and pasting properties of the corn starch isolated by combining 0.03% neutral protease with different sonication conditions. With increased sonication amplitude and duration, starch yield was significantly improved, residual protein content was greatly decreased, and damaged starch content was slightly increased, with the exception of the treatments where sonication and protease were employed together. The damaged starch content for all treatments was similar to or slightly higher than the conventional control of 1.22%. With simultaneous sonication and protease, treatments with 25% amplitude produced higher starch yields than treatments with 50% amplitude. These results indicate that the protease was possibly partially inactivated from exposure to higher intensity (50%) sonication, resulting in a lower starch yield. The protease digestion treatment followed by sonication at 75% amplitude for 30 min produced a higher starch yield (76.0%) than the conventional method (71.1%). Previously, combinations of protease and sonication produced starch yields 8–15% higher than the conventional method (Wang and Wang 2004b). The much improved starch yield could not be achieved for corn starch isolation with combinations of protease and sonication, probably because the majority of corn protein, zein, is hydrophobic, and protein-starch interaction is stronger in corn flour than in rice flour.

The starch isolated by combining protease and sonication generally displayed peak, breakdown, final, and total setback viscosities similar to the starch isolated from the conventional control. The gelatinization properties of the starch isolated from the combinations protease and sonication had ranges of 66.2–68.7°C for onset temperature, 71.7–72.8°C for peak temperature, and 11.6–12.4 J/g for enthalpy, which were similar to those of the starch from the water-only control except a slight higher enthalpy values.

The residual protease activity in the isolated starch samples was determined to ensure most protease was washed out during the process. The residual protease activity in isolated starches from all treatments had a range of 0.63–2.32 units/g of starch based on the equation described previously. This low residual protease activity probably would not cause adverse effects if the isolated starch is to be incorporated in most food systems.

### Starch Morphology from Different Treatments

The micrographs of starch from selective treatments as revealed by ESEM are shown in Fig. 1. No noticeable difference in starch appearance was observed among the different treatments, indicating the conditions used in the present study did not result in any change in starch surface structure.

## CONCLUSIONS

High-intensity ultrasound demonstrated the capability of isolating starch without causing undue starch damage within a short period of time. Combinations of neutral protease and sonication

were most effective in isolating starch from degermed corn flour with low residual protein, damaged starch, and protease activity. The preferred combination was neutral protease digestion for 2 hr followed by sonication at 75% amplitude for 30 min. The results of this study suggest that use of SO<sub>2</sub> can be eliminated, and the steeping process can be further shortened in the enzymatic milling process when combined with protease and sonication. The combination of protease and sonication can be incorporated in the second stage of enzymatic milling to increase the productivity and lessen changes of starch properties from the traditional wet-milling process.

## ACKNOWLEDGMENTS

Financial support received under USDA CSREES Award No. 2003-35503-12486 is gratefully acknowledged. We thank Linfeng Wang for assistance with scanning electron microscopy.

## LITERATURE CITED

- AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 44-15A, 46-13 76-13, and 76-31. The Association: St. Paul, MN.
- Caransa, A., Simell, M., Lehmussaari, A., Vaara, M., and Vaara, T. 1988. A novel enzyme application for corn wet milling. *Starch* 40:409-411.
- Eckhoff, S. R., and Okos, M. R. 1989. Diffusion of gaseous sulfur dioxide into corn grain. *Cereal Chem.* 66:30-33.
- Eckhoff, S. R., Singh, S. K., Zehr, B. E., Rausch, K. D., Fox, E. J., Mistry, A. K., Haken, A. E., Niu, Y. X., Zou, S. H., Buriak, P., Tumbelson, M. E., and Keeling, P. 1996. A 100-g laboratory corn wet-milling procedure. *Cereal Chem.* 73:54-57.
- Hassanean, A., and Abdel-Wahed, A. 1986. A new method to short the steeping period of corn grains. *Starch* 38:417-419.
- Johnston, D. B., and Singh, V. 2001. Use of proteases to reduce steep time and SO<sub>2</sub> requirements in a corn wet-milling process. *Cereal Chem.* 78:405-411.
- Johnston, D. B., and Singh, V. 2004. Enzymatic milling of corn: Optimization of soaking, grinding, and enzyme incubation steps. *Cereal Chem.* 81:626-632.
- Johnston, D. B., Singh, V., and Eckhoff, S. 2003. Use of enzymes to reduce steep time and SO<sub>2</sub> requirements in maize wet-milling process. U.S. patent 6,566,125.
- Moheno-Perez, J. A., Almeida-Domingues, H. D., and Serna-Saldivar, S. O. 1999. Effect of fiber degrading enzymes on wet milling and starch properties of different type of sorghums and maize. *Starch* 51:16-20.
- Pérez, O. E., Haros, M., and Suarez, C. 2001. Corn steeping: Influence of time and lactic acid on isolation and thermal properties of starch. *J. Food Eng.* 48:251-256.
- Ruan, R., Litchfield, J. B., and Eckhoff, S. R. 1992. Simultaneous and nondestructive measurement of transient moisture profiles and structural changes in corn kernels during steeping using microscopic nuclear magnetic resonance imaging. *Cereal Chem.* 69:600-606.
- Shandera, D. L., and Jackson, D. S. 1996. Effect of corn wet-milling conditions on starch functionality. *Cereal Chem.* 73:632-637.
- Singh, V., and Johnston, D. B. 2002. Pasting properties and surface characteristics of starch obtained from an enzymatic corn wet-milling process. *Cereal Chem.* 7:523-527.
- Singh, V., and Johnston, D. B. 2004. An enzymatic process for corn wet milling. *Adv. Food Nutr. Res.* 48:150-171.
- Steinke, J. D., and Johnson, L. A. 1991. Steeping maize in the presence of multiple enzymes. I. Static batchwise steeping. *Cereal Chem.* 68:7-12.
- Steinke, J. D., Johnson, L. A., and Wang, C. 1991. Steeping maize in the presence of multiple enzymes. II. Continuous countercurrent steeping. *Cereal Chem.* 68:12-17.
- Syarief, A. M., Gustafson, R. J., and Morey, R. V. 1987. Moisture diffusion coefficients for yellow dent corn components. *Trans. ASAE* 30:522-528.
- Wang, L., and Wang, Y.-J. 2004a. Application of high-intensity ultrasound and surfactants in rice starch isolation. *Cereal Chem.* 81:140-144.
- Wang, L., and Wang, Y.-J. 2004b. Rice starch isolation by neutral protease and high-intensity ultrasound. *J. Cereal Sci.* 39:291-296.
- Zhang, Z., Feng, H., Niu, Y., and Eckhoff, S. R. 2005. Starch recovery from degermed corn flour and hominy feed using power ultrasound. *Cereal Chem.* 82:447-449.

[Received October 10, 2005. Accepted May 5, 2006.]