Preparation and Properties of Small-Particle Corn Starch[1]

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ABSTRACT

Small-granule starches are useful as fat substitutes and in the manufacture of degradable plastic films. But naturally occurring small-particle starches are expensive and difficult to isolate. We developed methods for breaking down granules of corn starch into small particles. We treated corn starch with acid under various conditions and then ball-milled it. The resultant starch particles had diameters similar to those of native small-granule starches such as amaranth (2 μm) and rice (5 μm). The particle sizes of the starches were determined with a Brinkmann particle size analyzer and an image analyzer. Particle size was correlated with average starch molecular size (degree of polymerization).

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Granule size is a characteristic property of starch. For example, corn starch has an average diameter of about 15 μm, wheat starch has a bimodal size distribution of 20–35 and 2–10 μm, potato starch has an average size of 40 μm, and rice starch has an average size of 5 μm. The particle sizes of starch granules have recently received much attention because of their important roles in determining both the taste and mouthfeel of fat substitutes (Daniel and Whistler 1990) and the tensile properties of degradable plastic films (Lim et al 1992). Daniel and Whistler (1990) reported that small-granule starch about 2 μm in diameter, or similar in size to the lipid micelle, had advantages as a fat substitute. Griffin (1989) suggested that small-granule wheat starch had potential as a filler for thinner degradable plastic film. Lim et al (1992) investigated the use of starches of different particle sizes in degradable plastic film. They reported a linear correlation between film thickness and particle size and an inverse linear correlation between tensile strength and particle size. Small-granule starches may also be used as face powder or dusting powder, as a stabilizer in baking powder, and as laundry-stiffening agents (the small granules may penetrate fabric and give a high gloss and stiffness after ironing).

Small-granule starches available in nature, including amaranth (diameter 1–2 μm), taro (2–3 μm), rice (5 μm), and small-granule wheat, rye, barley, and triticale (about 5 μm), are relatively difficult to isolate. Some other potential sources are unconventional: for example, starches from cow cockle, pigweed, canary grass, cattail roots, catchfly, and dropwort all have granule sizes ranging from 0.5 to 10 μm (Goering and Brelsford 1966, Goering 1967, Goering and Schuh 1967, Goering and Rigault 1968, Goering and Subba Rao 1969, Subba Rao and Goering 1970, Goering and DeHaas 1972, Goering 1978, Lempiainen and Henriksnas 1979). These starches are more costly than corn, native wheat, and potato starches.

X-ray diffraction patterns indicate that starch has a semicrystalline structure; the type and degree of crystallinity depend on variety (Kainuma and French 1971, 1972; Nikuni 1978; French 1984). Kainuma and French (1972) proposed that crystallites are present in starch granules along with amorphous or gel forms. If this proposed internal structure is correct, then native starch granules can be broken into pieces if the starch molecules are partially hydrolyzed in the amorphous regions and then mechanically ground. In this study, we developed methods for breaking down granules of naturally abundant and low-priced native corn starch into small particles by heterogeneous hydrolysis of starch granules in their amorphous regions. Glucose and maltooligosaccharides, by-products of the acid treatments, can be recovered and used for corn syrup or for fermentation substrate.

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MATERIALS AND METHODS

Normal corn starch was given to us by American Maize Products Co. (Hammond, IN). Other chemicals we used were reagent grade and were used without further purification.

Preparation of Small-Particle Starch

Absolute ethyl alcohol solution. Normal corn starch (909 g, dry starch basis [dsb]) was suspended in 100% ethyl alcohol (2 L) containing HCl (1.8%, w/w). A three-neck, round-bottom flask equipped with a Liebig condenser and a heavy-duty propeller mixer was used for treatments. The mixture was heated with a heating mantle to its boiling temperature (80°C), refluxed for 3 hr, and cooled to 25°C. The starch was then isolated by filtration, resuspended in distilled water (1 L), neutralized with 10% NaOH, drained, and washed twice with distilled water (1 L). The starch was then dehydrated with alcohol and dried in a forced-air oven (80°C) for 4 hr. The acid-treated starch was then milled (70 rpm for 8 hr) in a ball-mill (0.5-cm glass beads) in the presence of 100% ethyl alcohol (starch-alcohol ratio 1:1, w/w).

Aqueous alcohol solution. Normal corn starch (909 g, dsb) was suspended in an aqueous alcohol solution (70%, v/v; 2 L) containing HCl (2.5%, w/w). The same equipment described for the absolute ethyl alcohol treatment was also used for this treatment. The mixture was stirred (25°C) for 1.5 hr, heated to its boiling temperature (82°C), and refluxed for 2 hr. The previously described washing, drying, and milling process was then applied.

Aqueous solution. Normal corn starch (909 g, dsb) was suspended in distilled water (2 L) containing 4.3% (w/w) HCl. The previously described equipment was used. The mixture was heated, stirred (55 ± 2°C) for 4 hr, and washed, dried, and milled as previously described.

Gel-Permeation Column Chromatography

An Econo-column (1.5 [i.d.] × 80 cm, Bio-Rad Laboratories, Richmond, CA) packed with Bio-Gel P-6 gel was used to analyze the molecular size distribution of the small-particle starch. Starch was suspended in a 90% dimethyl sulfoxide aqueous solution, and the solution was stirred in a water bath (96°C) for 1 hr to dissolve the starch. The starch was then recovered by precipitation with excess alcohol and centrifugation (2,000 × *g*, 10 min) and was redissolved in boiling water for injection. The column was developed in the descending mode with degassed, deionized, and distilled water as the eluant. The flow rate was 21 ml/hr. Fractions of 2.3 ml each were collected and analyzed for total carbohydrate with an AutoAnalyzer (Bran & Lubbe, Elmsford, NY). Anthrone-sulfuric acid reagent was used for the total carbohydrate analysis (Wright and Gann 1966).

Degree of Polymerization

Degree of polymerization (DP) of the small-particle starch was calculated by dividing the total carbohydrate concentration (micrograms of glucose per milliliter) of a starch solution by its reducing value (micrograms of glucose per milliliter). Total carbohydrate was analyzed according to the phenol-sulfuric acid procedure described by Dubois et al (1956). Reducing value was analyzed according to the Somogyi-Nelson method (Nelson 1944, Somogyi 1945). Glucose standard solutions were used for both analyses.

Microscopy

Scanning electron micrographs were taken with a JEOL JSM-35 scanning electron microscope (Tokyo, Japan). Starch samples were sprinkled on adhesive tapes, attached to specimen studs, and coated with gold-palladium. Light micrographs were taken with a Nikon Labophot microscope (Tokyo, Japan) operating in polarization mode with crossed Nicol prisms.

Particle Size Analysis

Time-of-transition particle size analyzer. Starch (2.0 g) was added to distilled water (250 ml) in a Waring Blendor. The mixture was blended at low speed for 1 min, allowed to stand for 10 min without agitation, and blended again at low speed for 20 sec. Three drops of the clump-free homogenous suspension was immediately transferred to a glass cuvette containing isotonic buffered saline solution (NaCl, 8.6 g/L; KCl, 0.38 g/L; ethylenediamine tetraacetic acid, 0.4 g/L; 2-phenoxyethanol, 0.2 g/L; and deionized water). The sample was analyzed for particle size distribution (acquisition range 0.5–60 μm) with a Brinkmann 2010 particle size analyzer (PSA) (Brinkmann Instruments, Inc., Des Plaines, IL) digitally interfaced with an IBM personal computer (System 2, software version 4.1, type 0.7 SI + SH). The analyzer was calibrated with polystyrene DVB microspheres (19.5 $\pm$ 0.6 μm) (Duke Scientific Corp., Palo Alto, CA) as the standard.

The PSA employs time-of-transition analysis in a photo-defined zone. A laser beam rotating at a fixed frequency scans a particle-containing cell. Particle sizes are determined by computer analysis of the widths of interaction pulses, which are proportional to particle diameter. Because the laser beam diameter varies along the beam, deviations may occur if particles interact with the laser beam outside the focus spot, or photo-defined zone. Transition analysis is unaffected by parameters such as index of refraction, attenuation of continuous phase, and output power of the laser, which complicate other optical laser methods.

Image analyzer. The Iowa State University Image Analysis Service acquired images of the starch particles with a Zeiss SEM-IPS image analysis system (Zeiss-Kontron, Thornwood, NY; IBAS version 1.31). Samples of each starch were placed on a slide and viewed with a Zeiss axiophot microscope at $\times$125 magnification ($\times$100 by $\times$1.25 Optovar). Images were captured with a Sony 3 CCD color video camera. The internal scaling feature of the image analysis software was calibrated to measure in micrometers. The starch images were interactively discriminated and edited to separate any touching particles. These particles were then measured, and the area, maximum diameter, minimum diameter, and diameter of an equivalent circle were recorded.

TABLE I
Preparation of Small-Particle Starch[a]

Acid Treatment	Resistant Starch (Average DP[b])	Yield
EtOH (100%), HCl (1.8%), 80°C (3 hr)	48.9 $\pm$ 1.5	66 $\pm$ 6%
EtOH (70%), HCl (2.5%), 25°C (1.5 hr), 82°C (2 hr)	52.7 $\pm$ 2.1	74 $\pm$ 4%
H_2O, HCl (4.3%), 55°C (4 hr)	56.3 $\pm$ 1.2	80 $\pm$ 3%

[a] Data are averages of two replications.
[b] Degree of polymerization.

TABLE II
Particle Sizes Analyzed with the Particle Size Analyzer

Acid Treatment	Volume Density (μm)	Number Density (μm)
EtOH (100%), HCl (1.8%), 80°C (3 hr)	5.77 $\pm$ 2.82	1.18 $\pm$ 1.12
EtOH (70%), HCl (2.5%), 25°C (1.5 hr), 82°C (2 hr)	5.21 $\pm$ 2.40	1.39 $\pm$ 1.23
H_2O, HCl (4.3%), 55°C (4 hr)	8.60 $\pm$ 4.68	1.62 $\pm$ 1.75
Native corn starch	17.23 $\pm$ 7.94	5.63 $\pm$ 4.84

X-Ray Diffractometry

The X-ray diffraction pattern was obtained with copper, nickel foil-filtered, K_α radiation at GMT Labs (Minneapolis, MN). Operation was at 30 μA and 40 kV. Slits were 3°/0.15°, and scanning speed was 1°/min.

RESULTS AND DISCUSSION

Native starch granules displayed great resistance to mechanical force. Normal corn starch granules ball-milled for 12 hr retained integrity and showed no broken pieces when viewed under a microscope (*data not shown*). Sorghum starch granules milled with a McCrone micronizing mill were severely damaged but did not break into pieces (Craig and Stark 1984). This resistance can be attributed to intermolecular and intramolecular hydrogen bonding and to the entanglement of starch molecules, such as double helix formation between branch chains of amylopectin molecules and intertwining between amylose and amylopectin. Thus, granule integrity is better preserved in normal than in waxy starch (Lindqvist 1979, Jane et al 1986).

The particle size and the yield of small-particle starch (broken pieces) obtained by our hydrolysis and grinding procedures depended on the acid treatment conditions (Table I). Starch treated with 1.8% HCl (w/w) in absolute ethyl alcohol (80°C for 3 hr) yielded 66% small-particle starch, compared to 74 and 80% for the other two treatments. The yields reflected the loss of water-soluble glucose and maltooligosaccharides.

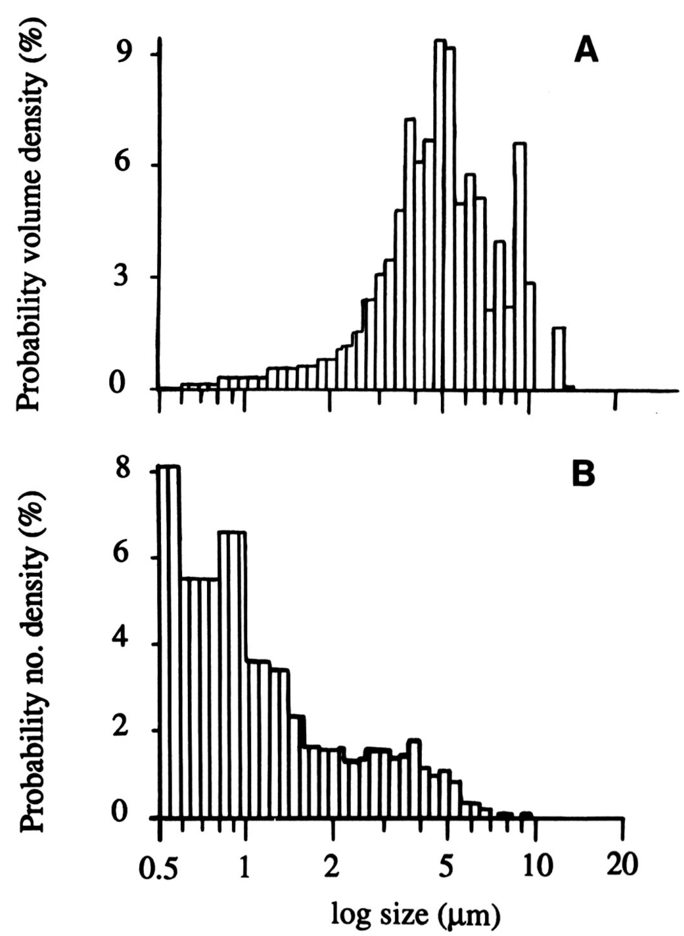

Fig. 1. Particle size distributions by probability volume density (A) and by probability number density (B) of small-particle corn starch treated with 70% EtOH and 2.5% HCl at 25°C for 1.5 hr and 82°C for 2 hr and measured with a Brinkmann particle size analyzer.

TABLE III
Particle Sizes Analyzed with the Image Analyzer[a]

Acid Treatment	Diameter (μm) Maximum	Minimum	Equivalent Circle
EtOH (100%), HCl (1.8%), 80°C (3 hr)	1.93 ± 1.11	1.30 ± 0.77	1.67 ± 0.97
EtOH (70%), HCl (2.5%), 25°C (1.5 hr), 82°C (2 hr)	2.20 ± 1.47	1.42 ± 0.94	1.80 ± 1.12
H_2O, HCl (4.3%), 55°C (4 hr)	1.43 ± 0.75	0.94 ± 0.46	1.21 ± 0.58
Native corn starch	11.60 ± 4.22	7.87 ± 3.12	9.57 ± 3.65

[a] Data are averages of 215 particles analyzed for each sample, with standard deviations.

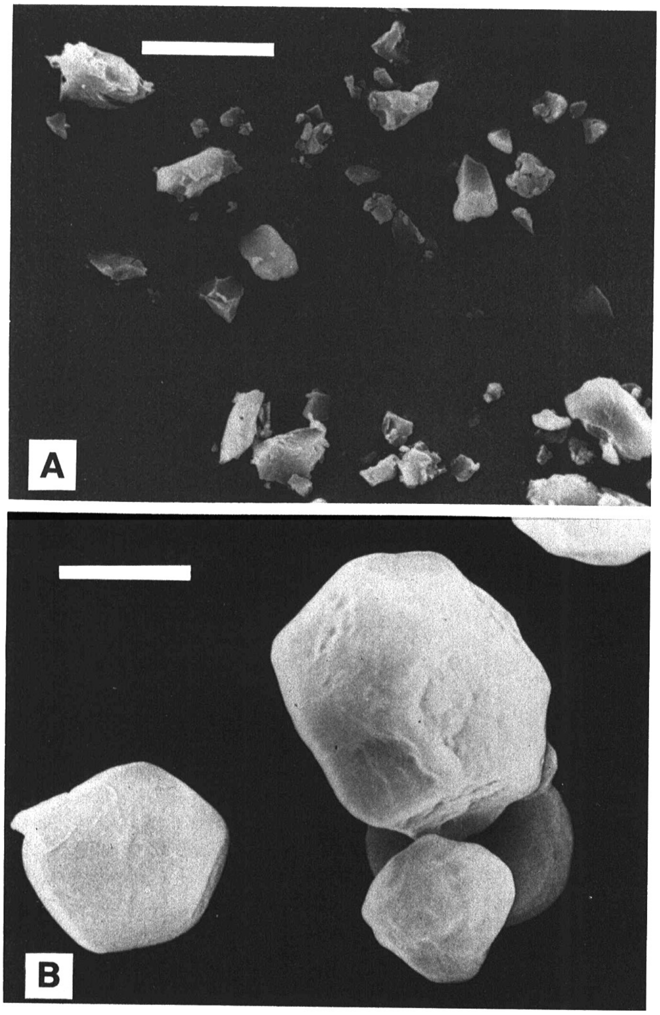

Fig. 2. Scanning electron micrographs of (**A**) the small-particle starch prepared as described in Figure 1 and (**B**) native corn starch. The bar stands for 10 μm.

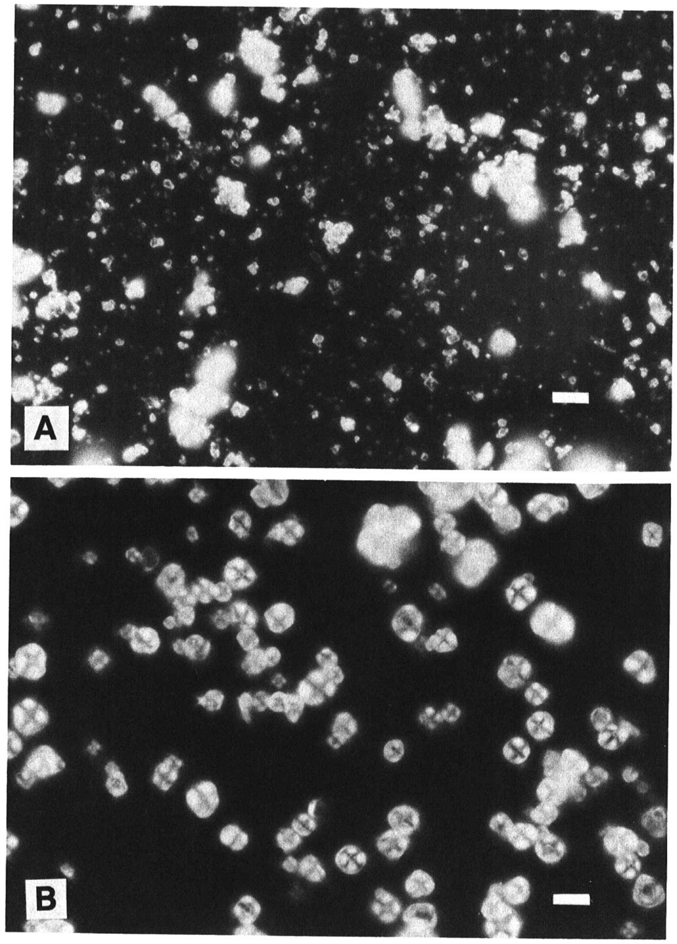

Fig. 3. Polarized light micrographs of (**A**) the small-particle starch prepared as described in Figure 1 and (**B**) native corn starch. The bar stands for 10 μm.

The particle sizes of the small-particle starches measured with the Brinkmann PSA (Table II and Fig. 1) and the image analyzer (Table III) correlated with starch DP (Table I). Particle sizes ranged from 1.2 to 1.6 μm (probability number density analysis) and from 5.2 to 8.6 μm (probability volume density analysis) as determined by the Brinkmann PSA and from 1.2 to 1.8 μm as determined by the image analyzer. The results from the PSA probability number density analysis (Fig. 1B) were in better agreement with those obtained from the image analyzer than the data from the PSA probability volume density analysis (Fig. 1A), which tended to be higher than the others. The difference may be attributed to the elimination of small particles with diameters less than 2 μm from the probability volume density analysis.

The average DP of the small-particle starches ranged from 49 to 56 (Table I). Gel-permeation column chromatograms (Bio-Gel P-6 column) showed a single peak in the molecular size distribution, suggesting an even and substantial hydrolysis; no large molecules remained (*data not shown*).

Scanning electron micrographs showed that the small-particle starch was smaller (about 2 μm in diameter) and more irregular (Fig. 2A) than the native corn starch (Fig. 2B). Ratios of the maximum to the minimum diameters of the small-particle starches varied between 1.48 and 1.55; these ratios are similar to that of native corn starch (1.47). Polarized light micrographs (Fig. 3) showed a strong birefringence of the small-particle starch compared with that of the native starch. The Maltese cross was lost in the small-particle starch as a result of lost symmetry and sphericity of the native starch granules. The strong birefringence suggested that crystalline structure was preserved in the starch, a finding confirmed by X-ray diffraction (Fig. 4). The small-particle starch produced a sharp A-type X-ray diffraction pattern. The intensity of the refraction was greater than that of native starch. This pattern was similar to that of Nageli dextrin, as reported by Kainuma and French (1972), and demonstrated that acid hydrolysis significantly reduced amorphous regions in native starch.

Both particle size and yield of the milled starch were affected by acid concentration, solvent media, hydrolysis temperature, and

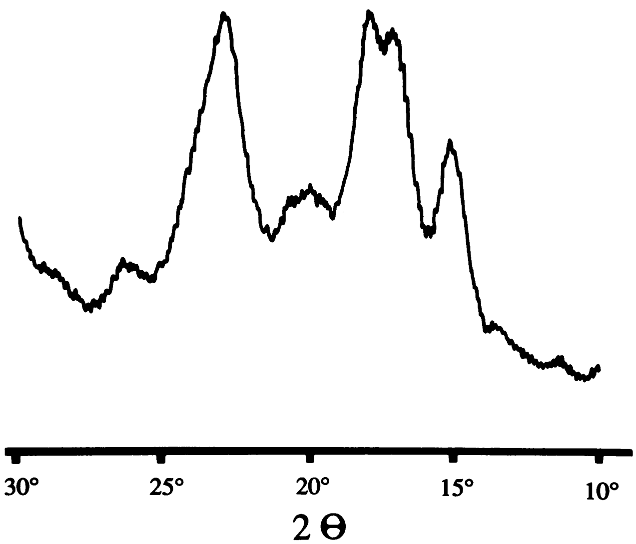

Fig. 4. X-ray diffraction pattern of the small-particle starch.

acid treatment period. Alcohols with longer hydrocarbon chains enhance acid hydrolysis (Ma and Robyt 1987) and thus decreased small-particle starch size and yield. Normal corn starch treated with propyl alcohol at 80°C and milled as described produced particle sizes smaller than 1 μm (*data not shown*). The yield of normal corn starch treated in an acid (4.3%) aqueous solution with 2 M Na_2SO_4 (70°C, 4 hr) decreased from 80% to 47%, and particle size decreased to less than 1 μm.

CONCLUSION

Acid hydrolysis and ball-milling methods were developed to prepare small-particle corn starch. Particle size, yield, and molecular size of the acid-resistant starch product depended on hydrolytic conditions. The small-particle starch was highly crystalline and retained X-ray diffraction pattern and birefringence. The particle sizes of the starches produced in this study are similar to those of naturally occurring small-granule starches from rice, wheat, taro, rye, barley, triticale, amaranth, cow cockle, pigweed, canary grass, cattail roots, catchfly, and dropwort.

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