

Physico-Chemical Characteristics of Media-Milled Corn Starch

CHUNG-JEN CHEN,^{†,‡} YU-CHIN SHEN,[†] AND AN-I YEH^{*,†}

[†]Graduate Institute of Food Science and Technology, National Taiwan University,
 1 Roosevelt Road, Section 4, Taipei, Taiwan, and [‡]Food Industry Research & Development Institute,
 331 Shih-Pin Road, Hsinchu, Taiwan

Nano/submicrometer scale corn starch particles have been prepared by using media milling. Molecular weight and pasting properties of milled starch were determined by HPSEC-MALLS-RI and an oscillatory rheometer, respectively. After 30-min of milling, the number-average diameter of the starch particle was reduced from 9.61 to 0.26 μm . Particle size was further reduced by extended milling. Microscopic observation confirmed the presence of particles smaller than 100 nm. As starch granules were being disintegrated, the mechanical energy imparted by media milling resulted in 43.7% of starch damage and 55% degree of gelatinization during the initial 30 min. In the meanwhile, the weight average molecular weight was reduced from 9.98 to 7.63×10^6 g/mol. The pasting properties were significantly altered by milling, which resulted in lower onset and peak temperature, and reduction in breakdown and set back. The importance of molecular weight on the physicochemical properties of milled starch was discussed.

KEYWORDS: Starch; media milling; nano/submicrometer; pasting properties

INTRODUCTION

Starch is a renewable, biodegradable, edible polymer as a major storage polysaccharide in plants with natural abundance next to cellulose and chitin. Starch plays an important role in food products, either as a major component or as a food additive. The functional properties of starch, such as pasting characteristics and swelling, are greatly affected by the structure which determines its application in food processing. Native starch has been modified to meet industrial needs using chemical or physical methods, such as substitution, cross-linking, polymerization, oxidation, hydrolysis, dextrinization, and pregelatinization. However, starch is quite inert to reactions due to its high molecular weight and granular crystallinity. Decreasing particle size would be beneficial for increasing the reactivity of starch (1), particularly reducing the size to the nano/submicrometer scale. Before achieving that goal, it is necessary to explore the feasibility for preparing nano/submicrometer starch particles and to understand related physicochemical properties.

Small-size starch particles have been prepared using acidic or enzymatic hydrolysis by removing the amorphous region, then inducing the fragmentation of starch granules (2, 3). The main drawbacks of hydrolysis are long process time and low yield. Small-size starch particles can be synthesized in water-in-oil microemulsions (4). However, polymerization in a microemulsion is complicated and requires a lot of chemicals. Ball mills have been used for reducing starch particle size, but only in micrometer scale (5). The media mill is one of the various types derived from a stirred ball mill and was introduced commercially in 1948 by Du Pont as a sand mill, primarily applied as a pigment grinder in the

paint and lacquer industry (6). Using more densely packed and rapidly rotated media improves the efficiency of comminuting (7). Development in the grinding chamber and the high efficiency temperature control system (8) minimize the thermal impact on materials. Date and Patravale (9) have pointed out that media milling has been one of the popular methods to prepare nanoparticles in the drug industry. Very little literature is concerned with the application of media milling in food. Chocolate appears to be the first material being manufactured by related technologies. Alamprese et al. (10) have reported the optimum operating conditions of a ball mill refiner for chocolate. The feature of media milling is to prepare hydrophobic materials at nano/submicrometer scales, suspended in water with reasonable shelf life. As in a suspension, nanoparticles will not float into the air and thus do not exhibit a hazard to the environment and human respiration system.

Both the crystallinity and percentage of damage are found to affect the functional properties of starch such as reduced size. Several researchers have reported the effects of milling on the functional properties of starch in the micrometer scale by decreasing crystallinity. The micronization results in an increase in cold water solubility (11) and a decrease in gelatinization temperature, heat for gelatinization (12, 13), and viscosity (14). Starch granules are mechanically damaged and partially fragmented by shearing and impact forces exerted by grinding media. Removal of surface material and the loss of starch order/integrity have resulted in an increase in water absorption/solubility (14) and reactivity (15).

Literature is available on the change of functional properties of starch granules treated by size reduction in a micrometer scale. Nonetheless, there is little information on the preparation and functional properties of starch particles in the nano/submicrometer scale. To evaluate the suitability of nano/submicrometer

*To whom correspondence should be addressed. Tel: +886-2-3366-4121. Fax: +886-2-2362-0849. E-mail: yehs@ntu.edu.tw.

starch particles for specific application, it is essential to understand the related physicochemical properties of milled products. The objectives of this article were to explore the feasibility of preparing nano/submicrometer corn starch particles by media milling and to understand the related structural and pasting properties after size reduction.

MATERIALS AND METHODS

Materials. Corn starch (S-4126) was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA) and was stored in a desiccator (25 °C) until use. The amylose content was specified as 27%. Two enzymes, pullulanase (P-5400) and β -amylase (A-7005), for determining the degree of gelatinization were also purchased from Sigma-Aldrich, Inc.

Media Milling. The nano/submicrometer corn starch particles were prepared according to the method of Yeh et al. (16). A media mill (MiniPur, Netzsch-Feinmahltechnik GmbH, Selb, Germany) with a driving motor of 0.94 kW was utilized to prepare samples. Media (yttria-stabilized tetragonal zirconia) of 0.2 mm were placed at a 70% v/v filling ratio in the milling chamber (200 mL). A starch suspension (5% w/v) was loaded into a jacket-cooling tank and then fed at a flow rate of 800 ± 5 mL/min into the milling chamber by a circulation pump. The temperature of the suspension was maintained below 30 °C by a circulation system. Milling was conducted continuously for 180 min, and samples were taken at 0, 30, 60, 90, 120, 150, and 180 min for analyses.

Particle Size. The particle size distributions of milled samples were determined by using a dynamic light scattering particle size analyzer (Nanotrak 150, Microtrac Inc., Largo, FL, USA) with the detecting range from 0.8 nm to 6.5 μ m. The instrument was calibrated with deionized water. Before measurement, all of the samples were diluted 10-fold, subjected to mild stirring, and then degassed by sonication for 5 min at 100 W and 42 kHz (Branson 3510R-DTH, Branson Ultrasonic Corp., Danbury, CT, USA). Volume-average (V_m) and number-average (N_m) diameters of particles were obtained using the software provided by Microtrac Inc. The equations for calculating V_m and N_m are as follows:

$$V_m = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (1)$$

$$N_m = \frac{\sum n_i d_i}{\sum n_i} \quad (2)$$

where n_i is the number of particles with the diameter d_i . All of the measurements were done in triplicate, and the average data with standard deviation were reported. The size reduction rate was used to evaluate the efficiency of milling and was calculated as follows:

$$\text{size reduction rate} = (N_{m, M_t} - N_{m, M_t+30})/t \quad (3)$$

where N_{m, M_t} was the number-average diameter of the sample being milled for t minutes. The size of the native starch granule was determined using an enhanced laser light diffraction particle size analyzer (LS 230, Beckman Coulter, Inc., Fullerton, CA, USA) with a small volume module at 25 °C since most of starch granules were greater than 6.5 μ m. The procedures were the same as those described above. V_m and N_m were also calculated using eqs 1 and 2 but based on the static laser scattering (SLS) measurement principle.

Morphology. *Scanning Electron Microscopy (SEM).* A piece of glass was attached to a specimen stub using double-sided metallic tape, then drops of milled samples were spread on it and dried by natural air circulation. For native starch, granules were sprayed on a stub previously covered with double-sided adhesive. The stub and sample were coated with gold-palladium using an ion sputtering device (E-101, Hitachi Co. Ltd., Tokyo, Japan) under vacuum. The gold-coated stub and samples were examined using a scanning electron microscope (Hitachi S-800, Hitachi Co. Ltd., Tokyo, Japan) operating at 20 kV and 1000/30000 magnification.

Transmission Electron Microscopy (TEM). Milled starch was centrifuged at 10000g for 20 min. Drops of the supernatant were spread onto a carbon-coated microscope grid (01800-F, Ted Pella, Inc., Redding, CA, USA) and dried by natural air circulation at 25 °C. Transmission electron microscopy was performed using a Hitachi H-7100 TEM (Hitachi Co.

Ltd., Tokyo, Japan) at an acceleration voltage of 75 kV and 50000 magnifications. Only the image for 180-min milled starch was reported.

Starch Damage. Degree of starch damage (DM) was determined by a spectrophotometric method (AACC International approved method 76-31) (17) using a starch damage assay kit (K-SDAM) purchased from Megazyme (Wicklow, Ireland). As a high level of starch damage was expected, the procedure was slightly modified using less starch (25 mg) and 5 dilution folds. All of the measurements were done in triplicate. The degree of starch damage (DM) was calculated according to the following equation:

$$DM (\%) = \Delta E \times F/W \times 40.5 \quad (4)$$

where ΔE was the absorbance read against the reagent blank, F was the conversion from absorbance to micrograms, W was the weight in milligrams of the sample analyzed, and 40.5 was the conversion factor with 5-fold dilution.

Degree of Starch Gelatinization. Degree of starch gelatinization (DG) was determined by using β -amylase-pullulanase (BAP method) as described in the literature (18, 19). All of the measurements were done in triplicate.

Molecular Weight. The molecular weight of starch was determined according to the method of Lin et al. (20) by using high-performance size-exclusion chromatography (TSK GMPW column, Tosoh, Tokyo, Japan) equipped with multi angle laser light scattering (model Dawn EOS, Wyatt Technology Inc., Santa Barbara, CA, USA) followed by a refractive index detector (RI, OPTILAB DSP, Wyatt Technology Inc., Santa Barbara, CA, USA) (HPSEC-MALLS-RI) with a dn/dc value of 0.147 mL/g. The chromatography was performed at 70 °C with an eluent made of deionized water containing 0.2 N NaNO₃ and 0.02% NaN₃ at a flow rate of 30 mL/h. The instrument constants for RI and MALLS were determined by pullulan molecular weight standards (peak molecular weight of 2.28 and 4.73×10^4 g/mol). The weight (M_w) and number-average molecular weight (M_n) were calculated by using ASTRA 4.73 software (Wyatt Technology Inc., Santa Barbara, CA, USA) based on MALLS and RI signals. All samples were run in triplicate. The number-average degree of polymerization (DP_n) was calculated by dividing M_n by the anhydrous glucose (molecular weight of 162 g/mol). The polydispersity index ($PDI = M_w/M_n$) was also calculated.

Pasting Properties. The pasting properties of native and milled starch were measured using an oscillatory rheometer with a starch pasting cell (AR2000ex, TA Instruments, New Castle, NJ, USA) according to the method of Park et al. (21). The operation procedure was the same as that for a rapid visco analyzer (RVA). The sample (about 30 mL at 4.8% w/w solid content) was placed in the aluminum canister and then was stirred at 16.76 rad/s (160 rpm) with an impeller fixture. The pasting process was started by holding the suspension at 50 °C for 5 min (stage 1), followed by raising the temperature to 95 at 10 °C/min (stage 2), holding at 95 °C for 6 min (stage 3), then cooling to 50 at 10 °C/min (stage 4), and finally holding at 50 °C for 5 min (stage 5). The rheometer was operated using TA Advantage Software, version 4.0, and the RVA curve was recorded. Parameters including on-set temperature, peak viscosity, peak temperature, trough viscosity, final viscosity, breakdown, and setback were determined. The ratio of breakdown to peak viscosity was defined as the relative breakdown. The ratio of setback to trough viscosity was defined as relative setback. All measurements were done in triplicate, and the average data were reported.

Flow Behavior. The flow behavior of two samples (heated and unheated) were explored by using the method of Yoo and Yoo (22). The procedure for determining pasting properties was considered as a heating process for preparing heated samples, which (28 mL) were transferred to a concentric cylinder system with a geometry of DIN. The rheometer (AR2000ex, TA Instruments, New Castle, NJ, USA) was operated at 25 °C using steady mode with the shear rate being increased from 0 to 300 s⁻¹. For unheated samples, the native corn starch or milled starch suspension was transferred to the concentric cylinder system for the measurement as described previously. Starch concentrations in all samples was 4.8% w/w. All of the data were fitted to various models, including Newtonian, Power law, Casson, Bingham, and Herschel-Bulkley, by employing the software (Rheology Advantage, TA v4.7.4) built in the instrument. The values of standard error (SE) from fittings were used as the criteria for selecting an appropriate model (23). The Herschel-Bulkley

model was selected to describe the flow behavior of the sample, and the apparent viscosity ($\eta_{a,100}$) at a shear rate of 100 s^{-1} was also reported.

$$\text{Herschel-Bulkley model } \sigma = \sigma_0 + k\dot{\gamma}^n \quad (5)$$

where σ is the shear stress (Pa), σ_0 is the yield stress (Pa), k is the consistency index ($\text{Pa}\cdot\text{s}^n$), $\dot{\gamma}$ is the shear rate (s^{-1}), and n is the flow-behavior index (dimensionless).

Swelling Power (SP) and Water-Soluble Index (WSI). The swelling power (SP) and water-soluble index (WSI) were determined in triplicate by modifying the method of Tsai et al. (24). Eight milliliter starch suspensions were placed into a centrifuge tube with a coated screw cap. The weight (W_d) of dry starch in the sample was measured after being dried to constant weight in an air oven at 105°C . The tubes were heated at 30, 50, 70, and 90°C in a water bath for 30 min. The tube was immediately cooled to 25°C in an iced water bath and then centrifuged ($3000g$) for 15 min. The supernatant was decanted and dried to constant weight (W_i) using the same method as that for W_d . The remainder in the tube was the sediment and was weighed (W_s). WSI and SP were calculated as follows:

$$\text{WSI} = (W_i/W_d)$$

$$\times 100\% \text{ (soluble solid (g)/dry mass of the whole starch sample (g))} \quad (6)$$

$$\text{SP} = W_s/(W_d - W_i) \text{ (hydrated solid (g)/dry solid in precipitate paste (g))} \quad (7)$$

Statistical Analysis. Statistical analysis was conducted using SAS version 9.1 for Windows (SAS Institute Inc., Cary, NC, USA). Data were expressed as the mean \pm standard deviation and analyzed using analysis of variance (ANOVA), followed by the Duncan's multiple range test to determine the significance of the differences among the data obtained.

RESULTS AND DISCUSSION

Size and Morphology. The volume-average diameter (V_m) of a native starch granule was $17.32 \pm 4.21 \mu\text{m}$ with a number-average diameter (N_m) of $9.61 \pm 2.17 \mu\text{m}$. Obviously, all native starch granules were in the micrometer scale. During the initial 30-min milling, most of the particles were greater than $6.5 \mu\text{m}$, which was beyond the measurement range of the dynamic light scattering particle size analyzer (Nanotracer 150). The measurement by using an enhanced laser light diffraction particle size analyzer (LS 230, Beckman Coulter, Inc., Fullerton, CA, USA) based on static laser scattering showed that the average particle size was decreased linearly by milling from 0 to 30 min. However, it was very difficult to compare the data from two instruments based on different principles. Thus, only the data from Nanotracer 150 were reported for the milling time longer than 30 min. When the milling was conducted from 30 to 180 min, V_m was decreased from $1.43 \pm 0.16 \mu\text{m}$ to a minimum of $0.70 \pm 0.05 \mu\text{m}$ at 120 min, and then was increased to $1.16 \pm 0.17 \mu\text{m}$ (Figure 1). The increase in V_m was likely due to the aggregation induced by van der Waals and electrostatic forces (25, 26). Nevertheless, N_m was decreased continuously from 0.26 ± 0.03 to $0.11 \pm 0.04 \mu\text{m}$ during the same milling period. It appeared that a small portion of particle numbers was involved with aggregation. The aggregation could be minimized by adding proper emulsifiers, which is not discussed in this article. V_m was generally greater than N_m as in the results from eqs 1 and 2. In terms of the effectiveness of milling, the size reduction rate at 30 min was the maximum ($0.312 \mu\text{m}/\text{min}$) and then dropped to $0.001\text{--}0.002 \mu\text{m}/\text{min}$ as the milling was continued. It implied that more energy was required to conduct size reduction for fine particles (25).

Native corn starch granules (Figure 2a) did not exhibit any difference from those in the literature (5, 13). After 120-min of milling, there exist a lot of fine particles as illustrated in Figure 2b.

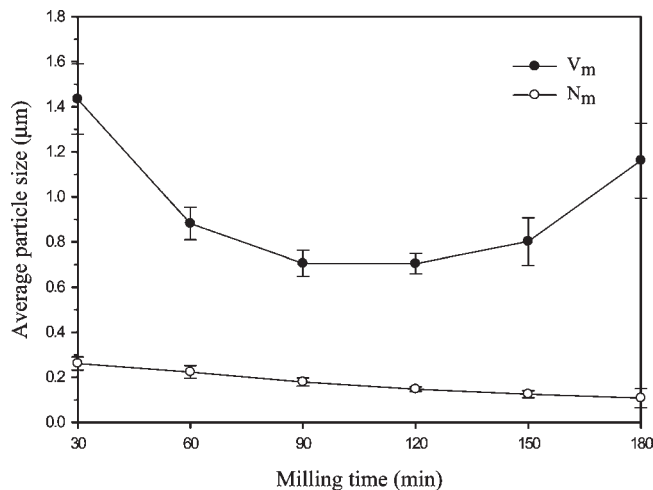


Figure 1. Particle size of the corn starch particle at selected milling time. V_m , volume-average diameter; N_m , number-average diameter.

No starch granules were observed. Starch agglomerated due to the sample preparation for SEM. Enlargement of the circled portion in Figure 2b revealed that most of particles were smaller than 100 nm (Figure 2c). The TEM observation confirmed that particles were smaller than 100 nm (Figure 2d). Nevertheless, particles agglomerated to form a string-like structure. It appeared that there were no starch chains connected between particles since milling resulted in the breakdown of starch molecules. Thus, the agglomeration was mainly due to physical forces. Gallant et al. (27) have postulated the presence of a blocklet with a diameter of $20\text{--}500 \text{ nm}$ in the starch granule. Dang and Copeland (28) illustrated the arrangement of the blocklet (about 100 nm) in rice starch granules on the basis of the observation from atomic force microscopy. However, it needed more study to confirm that the nanoparticle in Figure 2 was the blocklet since the shape was not oval as reported by the investigators above. The SEM and TEM observations and particle size analysis confirmed that it was feasible to prepare nano/submicrometer corn starch particles using media milling. Stabilization of the milled product is an important issue for commercially producing nano/submicrometer starch particles and is worth further study.

Physico-Chemical Properties. Both the degree of starch damage (DM) and degree of gelatinization (DG) were increased significantly during milling due to high shearing force (25, 26). As discussed previously, milling resulted in size reduction. The change in DM and DG of milled starch was associated with the size reduction (Figure 3). Both DM and DG increased linearly ($r^2 \geq 0.89$) as the number-average diameter of the starch particle being decreased (inset of Figure 3). DM was increased to 43.7% as the number-average diameter was reduced to $0.26 \mu\text{m}$ (corresponding to 30-min milling), and then was raised to 69.2% (180-min milling), which was slightly greater than that (66.0%) resulting from 8-h dry ball milling for maize starch (12). Mechanical damage caused by milling, possibly with local heat, resulted in the progressive loss of starch order and thus facilitated gelatinization. It was reasonable to observe that the increase in DG was associated with DM. DG was remarkably increased to about 55% as the number-average diameter was reduced to $0.26 \mu\text{m}$, then was increased to 95% as the number-average diameter was reduced to $0.11 \mu\text{m}$ (corresponding 180-min milling). The high DG indicated that media milling could be a method of preparing pregelatinized starch with ultrafine particles.

As the particle size was reduced by milling, the molecular weight was also reduced. When the number-average diameter was

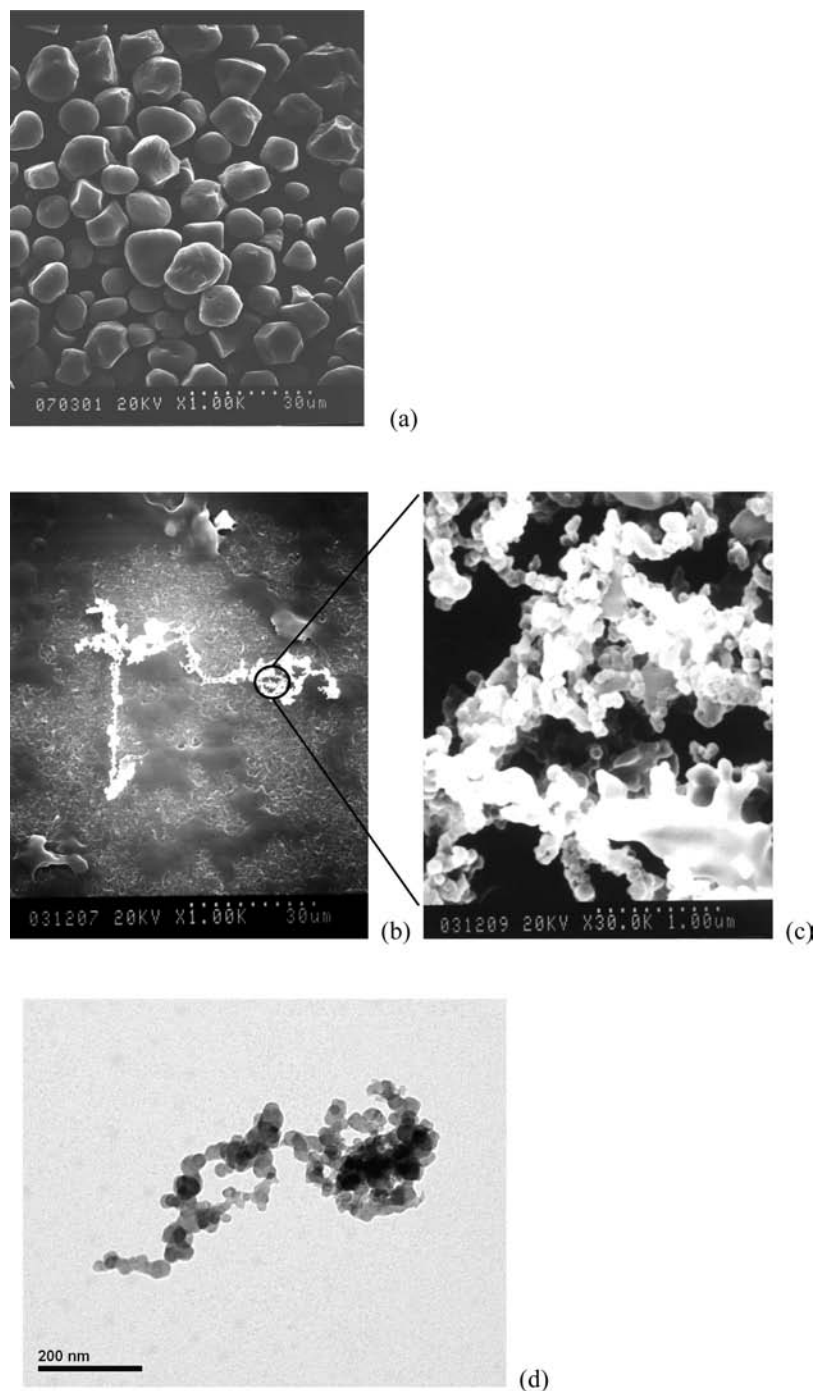


Figure 2. Scanning electron micrographs of (a) native starch and (b) 120-min milled starch; (c) enlargement of the circled area in b; and transmission electron micrographs of (d) 180-min milled starch supernatant.

reduced to $0.11 \mu\text{m}$ (corresponding to 180-min milling), M_w was decreased from 9.98 to 2.41×10^6 g/mol, and M_n was reduced from 4.30 to 0.28×10^6 g/mol. DP_n was also decreased from 26.54 to 1.74×10^3 (Figure 4). As illustrated by the inset in Figure 4, both M_w and DP_n were decreased linearly ($r^2 \geq 0.96$) with the size reduction. The data showed that it was feasible to prepare low molecular weight starch particles in the nano/submicrometer scale by physical means. The degradation of starch molecules could be due to the local heat associated with the high shearing force (29). At cryo-conditions, the mechanical force has been considered as a predominant effect on starch during milling (30). In this study, water was used for circulation and exhibited a cooling effect during milling. However, the presence of local heat

cannot be completely excluded in this study. Both shearing force and local heat could attribute to the degradation of starch molecules. The correlation between DP_n and particle size was similar to that for starch granules in the micrometer scale (5). The polydispersity index (PDI) was increased from 2.32 (native starch granule) to 4.22 (90-min milling) and then reached 8.56 after being milled for 180 min. PDI was increased as starch molecules were degraded during milling. The disruption at the glycosidic linkage of starch molecules lead to changes in the structure and properties (such as loss of crystallinity, decrease of enthalpy, and change in GPC profile) of starch granules (13).

Pasting Properties. Compared with native starch, all of the measurements, including onset and peak temperature, peak,

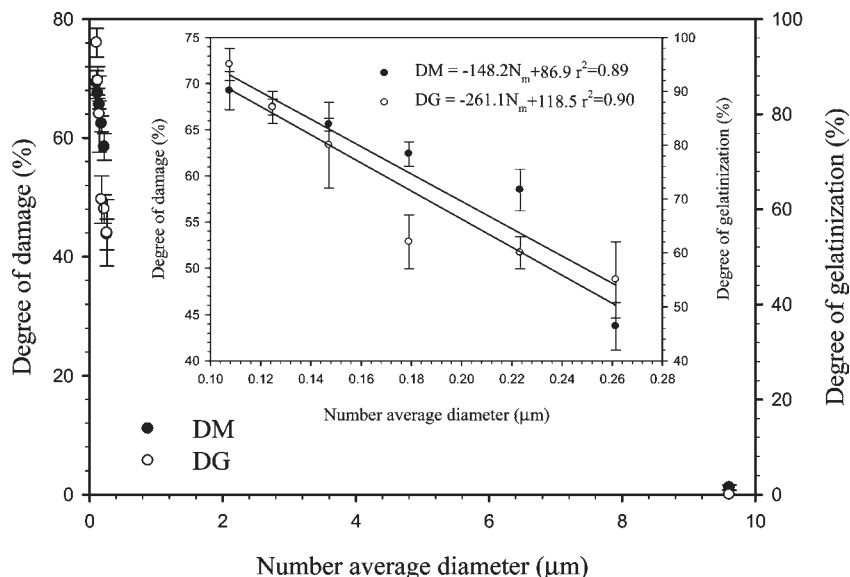


Figure 3. Degree of damage (DM) and gelatinization (DG) of starch as functions of number-average diameter (N_m).

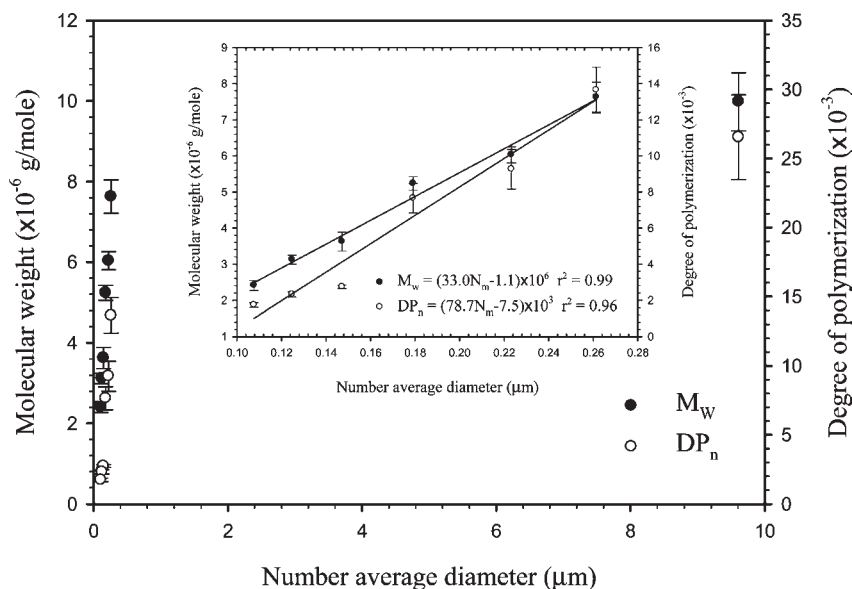


Figure 4. Molecular weight (M_w) and degree of polymerization (DP_n) of starch as functions of the number-average diameter (N_m).

trough and final viscosity, relative breakdown, and relative setback, were lowered by size reduction (Table 1). For the native corn starch (zero milling time), the pasting properties were similar to those in the literature (31). The onset temperature was lowered gradually from 79.8 to 74.9 °C as milling was conducted from 0 to 90 min. Nevertheless, the onset temperature became nondetectable when milling time was longer than 90 min. As discussed previously (Figure 3), milling longer than 120 min resulted in DG greater than 80%, which resulted in greater initial viscosity for milled starch than native starch. The increase in viscosity over 20 s was less than 2.5 cps when the temperature was raised from 50 to 90 °C during stage 2. Thus, it was difficult to determine the onset temperature even using the first derivative method proposed by Juhász and Salgó (31). Thirty-minute milling resulted in a remarkable drop in peak viscosity from 170.6 to 52.8 cps due to the degradation of the starch molecule, as only 0.1% of bonds in starch molecules need to be ruptured to depress the slurry viscosity by half (32). After 120-min of milling, the peak viscosity reached a plateau of 28 cps. The drop in peak viscosity was similar

to that resulting from the micronization of maize starch by cryomilling (30). Size reduction to the micrometer or nano/submicrometer scale resulted in low peak viscosity. Low onset temperature and low peak temperature indicated the ease of swelling of the starch particle at low temperature due to the disintegration of the starch granule. The breakdown (= peak viscosity – trough viscosity) was dramatically reduced from 49.3 to 7.3 cps by 30-min of milling. However, continued milling did not significantly alter the breakdown. Native corn starch yielded the greatest relative breakdown (28.9%) among the samples. It appeared that the milled starch was more shearing and thermal stable than native starch. There also existed a dramatic drop in setback (= final viscosity – trough viscosity) from 86.1 to 24.0 cps by 30-min of milling. The setback was decreased gradually as the milling was continued. Similar to the relative breakdown, the native corn starch yielded the greatest relative setback (70.9%) among all the samples. The relative setback was decreased to 26.2% after 180-min of milling. Native starch appeared to yield more gelling capability or retrogradation than the milled starch.

Table 1. Pasting Properties^{a, b} of Milled Starch Suspensions

milling time (min)	onset temperature (°C)	peak temperature (°C)	peak viscosity (cp)	trough viscosity (cp)	final viscosity (cp)	relative ^e breakdown (%)	relative ^f setback (%)
0	79.8 ± 0.7 a	93.8 ± 0.9 a	170.60 ± 25.74 a	121.35 ± 12.80 a	207.40 ± 33.52 a	28.9	70.9
30	77.3 ± 1.3 ab	93.4 ± 0.7 a	52.83 ± 1.87 b	45.54 ± 2.31 b	69.57 ± 1.37 b	13.8	52.8
60	75.7 ± 0.9 b	92.2 ± 0.1 ab	35.65 ± 0.01 bc	30.15 ± 0.52 c	43.89 ± 1.13 bc	15.4	45.6
90	74.9 ± 0.6 b	91.1 ± 1.4 b	30.14 ± 0.17 bc	24.91 ± 0.45 c	35.59 ± 0.10 c	17.4	42.9
120	ND ^c	87.6 ± 0.2 c	28.10 ± 0.55 bc	23.19 ± 0.04 c	31.37 ± 0.23 c	17.5	35.3
150	ND	50.7 ± 0.8 ^d	27.15 ± 0.19 c	21.75 ± 0.05 c	27.77 ± 0.45 c	19.9	27.7
180	ND	51.1 ± 1.3 ^d	28.27 ± 0.47 bc	20.61 ± 0.23 c	26.00 ± 0.27 c	27.1	26.2

^a Mean ± standard deviation. ^b Different lower case letters (a–d) in the same column indicate that the data are significantly different at $p < 0.05$, analyzed by Duncan's multiple range test. ^c ND: not detectable (the increase in viscosity over 20 s was less than 2.5 cP during heating, the stage 2). ^d The greatest viscosity was recognized as peak viscosity to identify the corresponding peak temperature for stage 2. ^e Relative breakdown (%) = breakdown/peak viscosity, where breakdown = peak viscosity – trough viscosity. ^f Relative setback (%) = setback/trough viscosity, where setback = final viscosity – trough viscosity.

Table 2. Rheological Properties^{a, b} at 25 °C of Starch before and after Heating^c

milling time (min)	before heating			after heating		
	yield stress ^d (Pa)	consistency index, k (Pa · S ⁿ)	flow behavior index, n	yield stress (Pa)	consistency index, k (Pa · S ⁿ)	flow behavior index, n
0	0.0525 ± 0.0009 a	0.0010 ± 0.0001 a	1.15 ± 0.01 a	5.2950 ± 2.8256 a	1.9267 ± 0.5094 a	0.52 ± 0.15 a
30	0.0613 ± 0.0032 a	0.0016 ± 0.0002 a	1.15 ± 0.05 a	1.3460 ± 0.2346 b	0.3953 ± 0.2063 b	0.65 ± 0.08 ab
60	0.0501 ± 0.0006 a	0.0036 ± 0.0006 ab	1.04 ± 0.01 b	0.2393 ± 0.1398 b	0.0975 ± 0.0187 b	0.75 ± 0.02 bc
90	0.0403 ± 0.0016 a	0.0069 ± 0.0015 c	0.94 ± 0.03 c	0.1144 ± 0.0144 b	0.0404 ± 0.0030 b	0.81 ± 0.02 bc
120	0.0168 ± 0.0174 a	0.0066 ± 0.0017 c	0.96 ± 0.04 c	0.0676 ± 0.0072 b	0.0280 ± 0.0085 b	0.81 ± 0.05 bc
150	0.0257 ± 0.0301 a	0.0052 ± 0.0012 bc	0.98 ± 0.04 bc	0.0523 ± 0.0056 b	0.0141 ± 0.0003 b	0.88 ± 0.09 cd
180	0.0330 ± 0.0381 a	0.0044 ± 0.0009 bc	1.01 ± 0.03 bc	0.0423 ± 0.0078 b	0.0057 ± 0.0038 b	0.99 ± 0.07 d

^a Mean ± standard deviation. ^b Different lower case letters (a–d) in the same column indicate that data are significantly different at $p < 0.05$, analyzed by Duncan's multiple range test. ^c Procedures for RVA were used as the heating of samples. ^d The ascending shear data were analyzed for the flow behavior.

Molecular weight appeared to be an important factor affecting the pasting properties. Peak viscosity and setback were found as exponential functions of the molecular weight of starch with r^2 of 0.82 and 0.96, respectively.

$$PV = 12.27e^{0.223M_w \times 10^{-6}} \quad (8)$$

$$SB = 2.01e^{0.350M_w \times 10^{-6}} \quad (9)$$

where PV was the peak viscosity and SB was the setback.

On the basis of eq 8, the influence of molecular weight reduction on peak viscosity was estimated. For example, at high molecular weight (such as 9.98×10^6 g/mol), 10% reduction in molecular weight resulted in 47% decrease in peak viscosity. Nevertheless, at low molecular weight (such as 3.63×10^6 g/mol), 10% reduction in molecular weight only resulted in about 9% reduction in peak viscosity. Similar to peak viscosity, there existed a dramatic drop in setback with the reduction in molecular weight during initial milling. The milled starch exhibited a setback much lower than that of native starch due to the degradation of starch molecule. Thus, milled starch yielded much lower gelling capability than native starch. The reduction in pasting properties was similar to that of the size reduction for potato starch in the micrometer scale (33). The results indicated that size reduction could be used to modify the functional properties of starch particles.

Without heating, the apparent viscosity of native starch was the lowest one (2.6 cps) among all the samples due to a lack of swelling. When M_w was reduced to 5.24×10^6 g/mol (corresponding to 90-min milling), the apparent viscosity was increased to 5.8 cps, which was the greatest one among samples. Both the number of particles and DG were increased as the reduction in M_w along with the milling. Thus, the apparent viscosity was increased as M_w was reduced. The result was in agreement with the size reduction of a starch colloid in the micrometer scale by using

high-pressure homogenization (34). Upon further reduction in M_w , the apparent viscosity was decreased slightly. After heating, the native corn starch suspension exhibited the greatest viscosity (371.9 cps) among samples. There existed a remarkable drop in viscosity (86.3 cps) at a M_w of 7.63×10^6 g/mol (corresponding to 30-min of milling). The apparent viscosity (AV) decreased exponentially (r^2 of 0.98) with the reduction in M_w due to milling.

$$AV = 1.548e^{0.530M_w \times 10^{-6}} \quad (10)$$

where AV was the apparent viscosity of the sample after heating.

Some other factors (including particle size distribution, particle–particle interaction, and packing fraction of particles and the viscosity of the continuous phase) might affect the rheological properties of the starch suspension. Nevertheless, the molecular weight was found as an important factor in this study. Generally, heating or gelatinization results in an increase in viscosity of the starch suspension. When M_w was reduced to 2.41×10^6 g/mol, heating did not alter the apparent viscosity of milled starch. This unique property would be beneficial for applications which require high starch content with low viscosity.

From fitting the data of heated samples to various rheological models, SE for the Herschel–Bulkley model ranged from 1.3 to 9.0. The fitting of the Newtonian, Power law, Casson, and Bingham model for heated samples yielded SE values ranging from 14.1 to 233.2, 3.7 to 11.8, 4.0 to 14.5, and 3.7 to 29.8, respectively. The fitting for the Herschel–Bulkley model yielded the lowest SE value among all of the models tested. Thus, rheological data fitted Herschel–Bulkley model well, which was also used for the nonheated sample. Without heating, all of the samples exhibited a low yield stress and consistency index (Table 2) due to low viscosity. There was no clear trend of the change in yield stress and consistency index with milling time. The native starch suspension exhibited a flow behavior index of 1.15. The flow behavior indices of milled samples (particularly milling

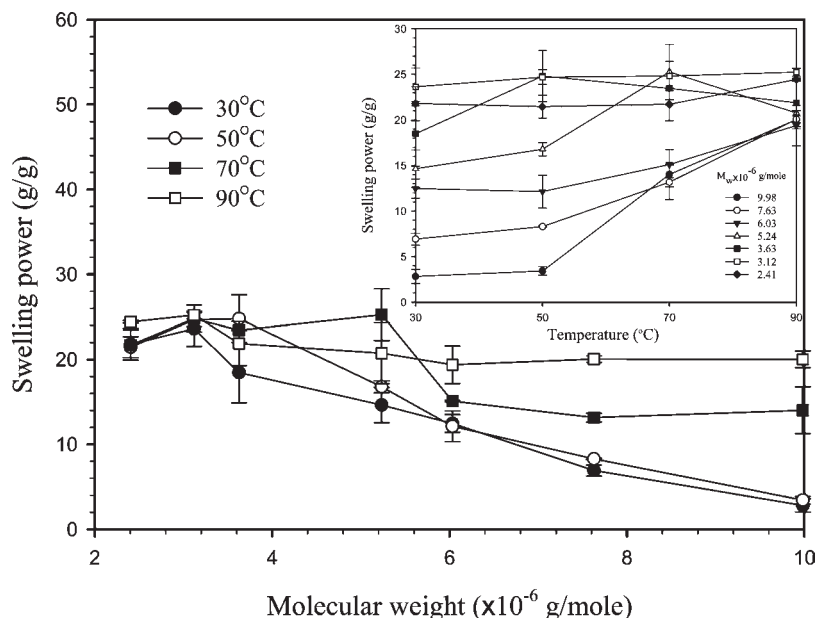


Figure 5. Swelling power (SP) of starch with different molecular weights (M_w) at different temperatures.

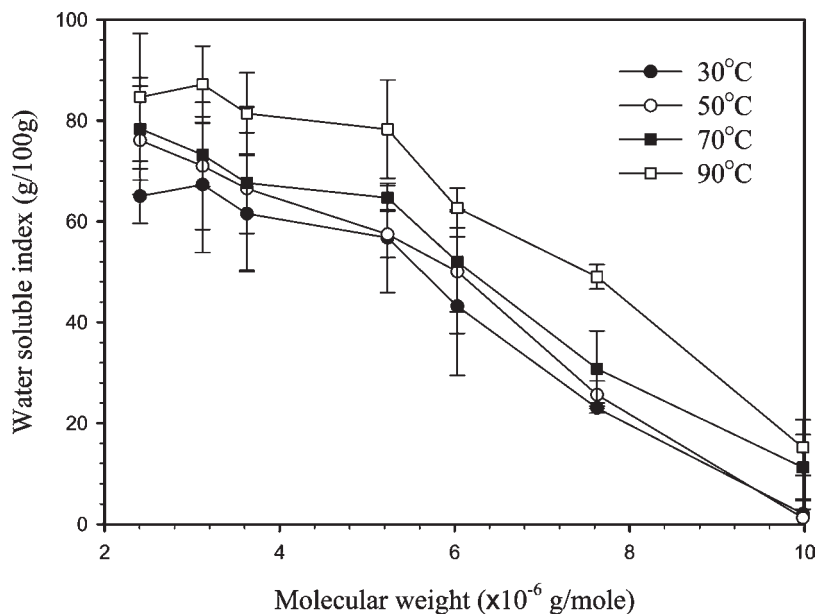


Figure 6. Water-soluble index (WSI) of starch with different molecular weights (M_w) at different temperatures.

time no less than 60 min) were close to unity, which indicated that the milled starch could exhibit Newtonian flow behavior. After being heated, the native starch paste exhibited shear thinning behavior with a flow behavior index of 0.52. Nevertheless, continuous milling resulted in an increase in the flow behavior index. A flow behavior index of 0.99 was reached at 180 min of milling. The heated native-starch paste exhibited the greatest yield stress and consistency index among all the samples. Both yield stress and the consistency index were decreased due to the degradation of the starch molecule during milling. The consistency index decreased exponentially (r^2 of 0.98) with the reduction of M_w , which was the combined effect of molecular degradation and the loss of granule integrity caused by mechanical damage.

$$CI = 0.001e^{0.735M_w \times 10^{-6}} \quad (11)$$

where CI was the consistency index.

Milling resulted in the disruption of an entangled network of starch molecules and imparted a thinning effect on the viscosity of heated samples (22). This was similar to the effect of high-pressure homogenization on the flow behavior of starch pastes (35).

Swelling Power and Water-Soluble Index. The swelling power (SP) was increased as M_w being reduced (Figure 5). Milled starch exhibited greater SP than native starch, particularly at temperatures lower than 90 °C. The milled starch with M_w of 3.12×10^6 g/mol exhibited the greatest SP among all the samples at four different temperatures (inset of Figure 5). Further reduction in M_w to 2.41×10^6 g/mol resulted in a slight decrease in SP due to the fact that M_w was so low as to lose the capability of trapping water. Raising the temperature from 30 to 90 °C resulted in a significant increase in SP for high M_w starch (such as native starch) but did not significantly alter the SP for the milled starch with M_w lower than 3.12×10^6 g/mol. For the samples with M_w no less than 5.24×10^6 g/mol, there existed a significant increase in SP at

temperatures higher than 70 °C. At 90 °C, the milled starch with low M_w (3.12 and 2.41×10^6 g/mol) exhibited slightly greater SP than other milled starch and native starch. At 30 °C, native starch (M_w of 9.98×10^6 g/mol) exhibited a SP of 2.8 g/g. Nevertheless, the milled starch with M_w of 3.12×10^6 g/mol yielded a SP of 23.6 g/g. Milling resulted in the ease of swelling of starch at low temperature. The decrease in starch crystallinity facilitated hydration and swelling, although progressive fracture of amylopectin gradually diminished the swelling potential (36). The increase in surface area due to size reduction might be another reason for high SP of milled starch.

Media milling resulted in the increase in DM as well as the reduction in M_w . Thus, the water-soluble index (WSI) was increased as M_w was reduced (Figure 6). For example, at 90 °C, WSI (49.0 g/100 g) of milled starch (M_w of 7.63×10^6 g/mol) was three times as that (15.2 g/100 g) for native starch (M_w of 9.98×10^6 g/mol) due to the increase in starch damage. The greatest WSI (87.2 g/100 g at 90 °C) was obtained when M_w was further reduced to 3.12×10^6 g/mol. Raising the temperature from 30 to 90 °C resulted in the increase in WSI. In addition to M_w , high WSI was associated with a high degree of starch damage by milling (such as cryo-milling) (30). The soluble material was mostly low molecular weight amylopectin (LMWAP) and small amounts of amylose (37, 38). The generation of LMWAP would result in the increase of PDI discussed previously. The molecular weight of starch has been found as an important factor affecting the physicochemical properties of milled samples.

ABBREVIATIONS USED

DM, degree of starch damage; DG, degree of gelatinization; PDI, polydispersity index; SE, standard error; SP, swelling power; WSI, water-soluble index; PV, peak viscosity; SB, setback; AV, apparent viscosity; CI, consistency index; LMWAP, low molecular weight amylopectin.

NOMENCLATURE

V_m = volume-average diameter (μm)
 N_m = number-average diameter (μm)
 M_w = weight average molecular weight (g/mol)
 M_n = number average molecular weight (g/mol)
 DP_n = number average degree of polymerization
 $\eta_{a,100}$ = apparent viscosity at shear rate 100 s^{-1} (cp)
 σ = shear stress (Pa)
 σ_0 = yield stress (Pa)
 k = consistency index ($\text{Pa} \cdot \text{s}^n$)
 $\dot{\gamma}$ = shear rate (s^{-1})
 n = flow-behavior index
 W_d = weight of dry starch (g)
 W_1 = weight of dry supernatant (g)
 W_s = weight of sediment (g)

LITERATURE CITED

- Bertolini, A. C.; Souza, E.; Nelson, J. E.; Huber, K. C. Composition and reactivity of A- and B-type starch granules of normal, partial waxy, and waxy wheat. *Cereal Chem.* **2003**, *80*, 544–549.
- Putaux, J. L.; Molina-Boisseau, S.; Momaur, T.; Dufresne, A. Platelet nanocrystals resulting from the disruption of waxy maize starch granules by acid hydrolysis. *Biomacromolecules* **2003**, *4*, 1198–1202.
- Kim, J. Y.; Park, D. J.; Lim, S. T. Fragmentation of waxy rice starch granules by enzymatic hydrolysis. *Cereal Chem.* **2008**, *85*, 182–187.
- Xiao, S.; Liu, X.; Tong, C.; Liu, J.; Tang, D.; Zhao, L. Studies of poly-L-lysine-starch nanoparticle preparation and its application as gene carrier. *Sci. China, Ser. B: Chem.* **2005**, *48*, 162–166.
- Jane, J.; Shen, L.; Wang, L.; Maningat, C. C. Preparation and properties of small-particle corn starch. *Cereal Chem.* **1992**, *69*, 280–283.
- Stehr, N. Recent developments in stirred ball milling. *Int. J. Mineral Process.* **1988**, *22*, 431–444.
- Gao, M.; Forssberg, E. Prediction of product size distributions for a stirred ball mill. *Powder Technol.* **1995**, *84*, 101–106.
- Merisko-Liversidge, E.; Liversidge, G. G.; Cooper, E. R. Nanosizing: a formulation approach for poorly-water-soluble compounds. *Eur. J. Pharm. Sci.* **2003**, *18*, 113–120.
- Date, A. A.; Patravale, V. B. Current strategies for engineering drug nanoparticles. *Curr. Opin. Colloid Interface Sci.* **2004**, *9*, 222–235.
- Alamprese, C.; Datei, L.; Semeraro, Q. Optimization of processing parameters of a ball mill refiner for chocolate. *J. Food Eng.* **2007**, *83*, 629–636.
- Huang, Z. Q.; Xie, X. L.; Chen, Y.; Lu, J. P.; Tong, Z. F. Ball-milling treatment effect on physicochemical properties and features for cassava and maize starch. *C. R. Chim.* **2008**, *11*, 73–79.
- Morrison, W. R.; Tester, R. F.; Gidley, M. J. Properties of damaged starch granules. II. Crystallinity, molecular order and gelatinization of ball-milled starches. *J. Cereal Sci.* **1994**, *19*, 209–217.
- Tamaki, S.; Hisamatsu, M.; Teranishi, K.; Adachi, T.; Yamada, T. Structure change of maize starch granules by ball-mill treatment. *Starch/Stärke* **1998**, *50*, 342–348.
- Martínez-Bustos, F.; López-Soto, M.; San Martín-Martínez, E.; Zazueta-Morales, J. J.; Velez-Medina, J. J. Effects of energy milling on some functional properties of jicama starch (*Pachyrhizus erosus* L. Urban) and cassava starch (*Manihot esculenta* Crantz). *J. Food Eng.* **2007**, *78*, 1212–1220.
- Huang, Z. Q.; Lu, J. P.; Li, X. H.; Tong, Z. F. Effect of mechanical activation on physicochemical properties and structure of cassava starch. *Carbohydr. Polym.* **2007**, *68*, 128–135.
- Yeh, A. I.; Huang, Y. C.; Chen, S. H. Effect of particle size on the rate of enzymatic hydrolysis of cellulose. *Carbohydr. Polym.* **2010**, *79*, 192–199.
- American Association of Cereal Chemists. *Approved Methods of the AACC*, 9th ed., Method 76-31; AACC: St. Paul, MN, 1995.
- Kainuma, K.; Matasunaga, A.; Itagawa, M.; Kobayashi, S. New enzyme system - β -amylase - pullulanase - to determine the degree of gelatinization and retrogradation of starch or starch products. *J. Jpn. Soc. Starch Sci.* **1981**, *28*, 235–240 (in Japanese).
- Matsunaga, A.; Kainuma, K. Studies on the retrogradation of starch in starchy foods. Part 3. Effect of the addition of sucrose fatty acid ester on the retrogradation of corn starch. *Starch/Stärke* **1986**, *38*, 1–6.
- Lin, J. H.; Lee, S. Y.; Chang, Y. H. Effect of acid-alcohol treatment on the molecular structure and physicochemical properties of maize and potato starches. *Carbohydr. Polym.* **2003**, *53*, 475–482.
- Park, I. M.; Ibáñez, A. M.; Zhong, F.; Shoemaker, C. F. Gelatinization and pasting properties of waxy and non-waxy rice starches. *Starch/Stärke* **2007**, *59*, 388–396.
- Yoo, D.; Yoo, B. Rheology of rice starch-sucrose composites. *Starch/Stärke* **2005**, *57*, 254–261.
- Ahmed, J.; Ramaswamy, H. S. Effect of high-hydrostatic pressure and temperature on rheological characteristics of glycomacropetide. *J. Dairy Sci.* **2003**, *86*, 1535–1540.
- Tsai, M. L.; Li, C. F.; Lii, C. Y. Effects of granular structure on the pasting behaviors of starches. *Cereal Chem.* **1997**, *74*, 750–757.
- Stenger, F.; Mende, S.; Schwedes, J.; Peukert, W. Nanomilling in stirred media mills. *Chem. Eng. Sci.* **2005**, *60*, 4557–4565.
- He, M.; Wang, Y.; Forssberg, E. Parameter effects on wet ultrafine grinding of limestone through slurry rheology in a stirred media mill. *Powder Technol.* **2006**, *161*, 10–21.
- Gallant, D. J.; Bouchet, B.; Baldwin, P. M. Microscopy of starch: evidence of new level of granule organization. *Carbohydr. Polym.* **1997**, *32*, 177–191.
- Dang, J. M. C.; Copeland, L. Imaging rice grains using atomic force microscopy. *J. Cereal Sci.* **2003**, *37*, 165–170.
- Barron, C.; Bouchet, B.; Della Valle, G.; Gallant, D. J.; Planchot, V. Microscopical study of the destructuring of waxy maize and smooth

- pea starches by shear and heat at low hydration. *J. Cereal Sci.* **2001**, *33*, 289–300.
- (30) Dhital, S.; Shrestha, A. K.; Gidley, M. J. Effect of cryo-milling on starches: functionality and digestibility. *Food Hydrocolloids* **2010**, *24*, 152–163.
- (31) Juhász, R.; Salgó, A. Pasting behavior of amylose, amylopectin and their mixtures as determined by RVA curves and first derivatives. *Starch/Stärke* **2008**, *60*, 70–78.
- (32) Stevenson, D. G.; Jane, J. L.; Inglett, G. E. Structure and physico-chemical properties of starches from sieve fractions of oat flour compared with whole and pin-milled flour. *Cereal Chem.* **2007**, *84*, 533–539.
- (33) Ren, G.; Mao, Z.; Li, D.; Zhou, Y. Functional Properties and Microstructure of Micronized Potato Starch. *ASABE Meeting Presentation Paper, No. 066114*, **2006**.
- (34) Liu, D.; Wu, Q.; Chen, H.; Chang, P. R. Transitional properties of starch colloid with particle size reduction from micro- to nanometer. *J. Colloid Interface Sci.* **2009**, *339*, 117–124.
- (35) Che, L. M.; Wang, L. J.; Li, D.; Bhandari, B.; Özkan, N.; Chen, X. D.; Mao, Z. H. Starch pastes thinning during high-pressure homogenization. *Carbohydr. Polym.* **2009**, *75*, 32–38.
- (36) Tester, R. F. Properties of damaged starch granules: composition and swelling properties of maize, rice, pea and potato starch fractions in water at various temperatures. *Food Hydrocolloids* **1997**, *11*, 293–301.
- (37) Stark, J. R.; Yin, X. S. The effect of physical damage on large and small barley starch granules. *Starch/Stärke* **1986**, *11*, 369–374.
- (38) Glennie, C. W.; McDonald, A. M. L.; Stark, J. R. Some observation on damage in commercial starch preparations. *Carbohydr. Res.* **1987**, *170*, 263–268.

Received for review June 1, 2010. Revised manuscript received July 20, 2010. Accepted July 21, 2010. This study is part of a research project (NSC 95-2221-E-002-037) sponsored by the National Science Council of the Republic of China. The financial support is greatly appreciated.