Optimizing a Small-Scale Corn-Starch Extraction Method for Use in the Laboratory

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ABSTRACT

The objectives of this experiment were to determine the effects of altering starch extraction procedures designed for use in the laboratory on starch yield, protein content, and thermal properties. Public Corn Belt inbred line Mo17 was used in this study. The altered procedures that were examined included steeping time (24, 48, or 72 hr), numbers of corn kernels extracted (2, 5, or 10 kernels), and isolation method (sedimentation or centrifugation). Starch thermal properties were obtained by using differential scanning calorimetry (DSC). Starch yield and protein content were significantly different among the experimental treatments. In general, more kernels, and sedimentation rather than centrifugation, resulted in greater yields. Also, treatments involving more kernels or sedimentation rather than centrifugation, yielded starch with the lowest protein content. Starch extracted after steeping for 24 hr and purified by the sedimentation method had the lowest gelatinization onset temperature (by DSC) and the widest gelatinization temperature range among the treatments. The energy required to gelatinize starch did not differ among the treatments. The differences among treatments in onset temperature and temperature range were probably caused by annealing of starch that occurs over time, during steeping. Therefore, to obtain the purest starch quality, this study suggests that sedimentation is preferred over centrifugation, and 10 kernels is preferred over 2 or 5. Furthermore, soaking the seeds for less than 24 hr is preferred if minimal annealing is desired.

Common to all starch research is the initial step of extracting starch granules from the plant material. The extraction must be accomplished without significant modification of the starch granules and in sufficient quantities to permit various analyses. Wet-milling, the industrial process for extracting starch from corn grains, involves chemical, biochemical, and mechanical operations to separate corn into relatively pure fractions of starch, protein, germ, and fiber. The process involves softening the kernel in steepwater, followed by grinding. Fractions are separated by taking advantage of differences in the physical properties including density and particle size of the fractions (Singh et al 1997).

During extraction of corn starch, the grain is steeped in dilute sulphur dioxide (SO_2) or bisulfite solution (a form of aqueous SO_2) for more than 20 hr at 48–52°C. The SO_2 disrupts the protein matrix that surrounds starch granules by breaking inter- and intramolecular disulfide bonds, thus making the physical separation of starch and protein easier. The SO_2 also activates endogenous protease activity in the endosperm, which helps solubilize the protein matrix (Wahl 1969). The degree of protein peptidization in whole kernels increases over the 24-hr steeping period with increasing SO_2 concentrations (up to 0.4% tested) and higher steeping temperature (up to 55°C tested), resulting in increased starch granule release from the surrounding protein matrix. In commercial steeping, kernel degradation for starch release does not occur until kernels are exposed to SO_2 (Wagoner 1948). Bisulfite ions also can form sulfo-protein complexes (Boundy et al 1967). The naturally occurring *Lactobacillus* sp. can propagate at low (20–200 ppm) levels of SO_2 and will consume soluble materials that leach into the steepwater directly from the steeped corn or enter with the recycling of the water used in the processing (Watson 1984). Lactic acid, arising from fermentation of corn by the *Lactobacillus* sp. in commercial operations, further enhances separation of starch and proteins.

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Different extraction procedures affect the chemical composition and physical properties of starch. These changes in starch properties, and even in starch granule structure, resulting from the extraction procedure are a reflection of the nonrigid organization of starch granules (Singh et al 1997). For example, the reduction in yield of starch from samples pretreated with potassium metabisulphite, an accepted pretreatment in some extraction procedures, could be a result of mild oxidative degradation during pretreatment because sulphite cleaves the amylolytic linkages by way of its prooxidant activity (John et al 1999).

Laboratory wet-milling procedures can be used to evaluate wet-milling characteristics of new corn hybrids, the effect of harvest and drying methods on the milling efficiency of corn, and the use of different steeping and processing techniques on product yields. Laboratory procedures generally mill between 50 g and 2 kg of corn. These quantities, however, are still too large for the purpose of screening corn germplasm or developmental lines to identify unusual properties for selection of desirable lines for breeding purposes. In addition, traditional laboratory wet-milling procedures is time-consuming. Grinding the endosperm involves two steps: initial grinding with a mortar and pestle and then blending for at least 4 min in a commercial microblender (White et al 1990).

A single-kernel wet-milling starch-extraction process for use in the laboratory was designed by White et al (1990) and later modified by Krieger et al (1997). This method used 1–10 corn kernels (0.2–3.5 g). The corn kernels were steeped in sodium metabisulfite solution, the pericarp and germ removed by hand, and the endosperm blended with a a tissue homogenizer for 30 sec to further enhance the separation of starch and protein. The microscopic study showed that starch granules separated using the tissue homogenizer were intact and undamaged. The thermal properties measured with a differential scanning calorimetry (DSC) were similar to those of starch separated using the microblender (Krieger et al 1997). Additional evaluation of this procedure could further optimize conditions to obtain maximum starch yields with minimal residual protein in the shortest amount of time.

In the current study, the effects of starch extraction methods differing in steeping time (24, 48, or 72 hr), number of kernels (2, 5, or 10 kernels), or in isolation method (sedimentation or centrifugation) were examined. A goal of the project was to determine the procedures that would provide the maximum starch yield with minimum protein content in a short time, while still retaining the thermal properties characteristic of native starch, thus optimizing the process conditions designed for use in the laboratory.

MATERIALS AND METHODS

Materials

The public Corn Belt inbred Mo17 was grown and self-pollinated near Ames, IA, in 1998. Ears were harvested at full physiological maturity and dried at 37.5°C until the moisture content reached ≈12%. All seeds were stored at 4°C and 10% rh until analyzed.

Starch Extraction

Corn kernels were hand-picked and cleaned to remove foreign material, mold, and broken kernels before analysis. Kernels of whole corn (2, 5, or 10 kernels) were steeped in 5 mL of 1% sodium metabisulfite solution (≈0.67% SO_2) at 45°C for 24, 48, or 72 hr, followed by manual removal of the pericarp and germ with forceps. The separated endosperm was placed in a 50-mL centrifuge tube with 10 mL of distilled water and homogenized using a vortex type tissue homogenizer (Ultra-Turrax T25, 600W, Tekmar, Cincinnati, OH) at 20,500 rpm for 30 sec. The homogenized slurry was filtered by using a 30-µm nylon filter under vacuum with several washes, for a total wash water volume of 500 mL. Coarse and fine fibers and part of the protein were removed during filtration. The starch-protein mixture from the filtrate was further separated by either centrifugation or sedimentation. Each sample was separated three times, with 250 mL of distilled water used for each of the three separations. All treatments were performed in replicates of five, and the results averaged.

Centrifugation Procedure

The starch slurry was centrifuged (Sorvall RC 5B Plus Newtown, CT) at 5,219 × *g* force for 30 min. The supernatant was decanted, the protein layer was scraped off, and more water (250 mL) added to the partly cleaned starch, with centrifugation and decanting repeated three times. The resulting sediment was air-dried.

TABLE I
Effect of Extraction Method on Starch Yield and Protein Content

Isolation Method	Steeping Time (hr)	No. of Kernels	Starch Yield (%)	Protein Content (%)
Centrifugation				
	24	2	45.0a[a]	4.34a
	24	5	52.8a–d	3.32ab
	24	10	47.2a–c	3.30a–c
	48	2	45.3ab	3.86ab
	48	5	50.7a–d	3.31a–c
	48	10	53.5b–d	3.05a–d
	72	2	49.7a–d	3.15a–d
	72	5	56.9de	3.11a–d
	72	10	59.1e	2.21b–e
Sedimentation				
	24	2	52.2a–d	2.45b–e
	24	5	57.4de	1.90c–e
	24	10	56.8de	2.13c–e
	48	2	55.1cd	1.89c–e
	48	5	57.8de	1.74d–e
	48	10	56.7de	0.92e
	72	2	54.0cd	1.70de
	72	5	58.2de	1.21e
	72	10	63.8e	0.92e
	Averages among subcategories			
Centrifugation			51.1a	3.29a
Sedimentation			56.9b	1.65b
	24		51.9a	2.91a
	48		53.2a	2.46ab
	72		57.0b	2.05b
		2	50.2a	2.90a
		5	55.6b	2.43b
		10	56.2b	2.09b

[a] Values followed by the same letter within the same section of a column are not significantly different ($P < 0.05$).

Sedimentation Procedure

The starch slurry was allowed to settle at 4°C for 2 hr and the supernatant drained. The starch was rinsed with 250 mL of water, drained twice, and the resulting sediment air-dried.

Starch Yield

In this study, the dried material obtained from both procedures was referred to as starch, even though the material was not completely pure. The dry matter recovered after wet milling contained very minor amounts of protein, fiber, and other residues. Starch yield was determined as % yield = (dry weight of starch recovered from extraction × 100)/dry weight of whole corn kernels. The weights of dry starch and corn kernels were measured on the same balance (±0.01 g accuracy) to enhance accuracy (Mettler AE 104, Toledo, OH).

Moisture Content

The moisture content of the dried starch recovered from the extraction procedures was measured by using Approved Method 44-19 (AACC 2000).

Thermal Property Analyses

Transition temperatures and enthalpies associated with the gelatinization process of starch were determined by using differential scanning calorimetry (DSC, Perkin Elmer DSC 7, Norwalk, CT). A Perkin-Elmer DSC-7 analyzer (Norwalk, CT) equipped with thermal analysis software (Perkin-Elmer) was used. All experiments were run at a scanning rate of 10°C/min from 30 to 110°C. The samples were prepared with a water-to-starch ratio of 2:1. The actual dry weight of starch used ranged from 3.96 to 4.02 mg. All enthalpy calculations were based on the dry starch weight. Thermal transitions for gelatinization were characterized by T_o (onset temperature), T_p (peak temperature), T_c (conclusion temperature), and ΔH (enthalpy of gelatinization).

Protein Content

Protein content was determined by using a combustion nitrogen analyzer (Perkin Elmer Series II 2410). Combustion and reduction temperatures used in the experiment were 930°C and 640°C, respectively. CO_2 was used as purge gas. The compound ethylenediaminetetraacetic acid (EDTA) was used as a standard which was analyzed every three starch samples to calibrate the nitrogen analyzer. Starch (40–50 mg) was used for each measurement and each sample was analyzed in triplicate, with the results averaged. The protein conversion factor used was 6.25.

Statistical Analysis

A 3 × 3 × 2 complete factorial experimental design was used with three steeping times (24, 48, or 72 hr), three levels of kernel number (2, 5, or 10 kernels), and two isolation methods (sedimentation or centrifugation). Unless otherwise noted, samples were processed and analyzed in five replicates. Analysis of variance (ANOVA) was used to test the hypothesis that means were not different for the starch yield, protein content, and each of the DSC properties, and to test for main effects and interactions (including two-way and three-way interactions) between steeping time, numbers of kernels, and isolation method. Tukey's multiple range test was used to test for differences between groups ($\alpha = 0.05$). Calculations were performed with SAS v. 8.2 (SAS Institute, Cary, NC) for the Unix operating system.

RESULTS AND DISCUSSION

Overall Evaluation of Extraction Procedures on Starch Yield and Composition

The main effects of steeping time, number of kernels, isolation method, and two-way interactions (steeping time × number of kernels, steeping time × isolation method, and number × isolation method) were analyzed by using ANOVA for the General Linear Model (GLM). Three main factors (steeping time, number of kernels, and isolation method) affected starch yield and protein content significantly ($P < 0.01$). Only the interaction of steeping time and number of kernels was significant for starch yield ($P < 0.05$), indicating that the impact of number of kernels on the starch yield was affected by the steeping time.

The mean yield and protein content of starches extracted by using the various treatments are shown in Table I. Starch yield of 45.0–63.8% and protein content of the starch of 0.92–4.34% were significantly different among treatments ($P < 0.05$). Starch extracted from 10 kernels following soaking for 72 hr and separated using sedimentation resulted in the greatest starch yield with the lowest protein content in the starch, whereas starch extracted from two kernels following soaking for 24 hr and separated by using centrifugation resulted in the lowest starch yield and greatest protein content in the starch.

Interactions between effect of steeping time and effect of number of kernels on starch yield were elucidated by studying the effect of steeping time on the starch yield with different numbers of kernels (Fig 1A for sedimentation and 1B for centrifugation). In Fig 1A, curves represent the different number of kernels by sedimentation; in Fig 1B, curves represent the different number of kernels by centrifugation. The clear lack of parallelism of curves in both figures indicated interaction effects between number of kernels and steeping time on starch yield, which were significant. For the sedimentation method, yields from 2 and 5 kernels were minimally affected by increasing the steeping time from 24 to 72 hr. However, yields from 10 kernels were increased significantly by increasing steeping time from 24 to 72 hr. A similar trend also was observed for the centrifugation method. Overall, sedimentation resulted in greater starch yields and lower protein content than did centrifugation (Table I averages among subcategories).

Effect of Steeping Time on Starch Yield and Protein Content

Starch yield increased and protein content decreased as the steeping time increased for both centrifugation and sedimentation isolation methods when the methods were evaluated separately or together (Table I). The average starch yield at 48.3–55.2% with centrifugation and 55.5–58.7% with sedimentation, and the starch protein content of 3.65–2.82% with centrifugation and 2.16–1.28% with sedimentation for the three levels of kernels used in the extractions. The protein was probably more completely separated from starch when steeping time was >48 hr, resulting in higher starch yields and lower protein content (Wang and Johnson 1992).

Effect of No. of Kernels on Starch Yield and Protein Content

Increasing the number of kernels extracted at one time resulted in lower protein content in the starch and higher starch yields when using either isolation method (Table I). Increasing the number of kernels from 2 to 5 increased starch yield to 46.7–53.5% for centrifugation and 53.8–57.8% for sedimentation (data averaged for all steeping times shown in Table I). No significant increase for starch yield was observed with a further increase in the number of kernels from 5 to 10. As the number of kernels decreased from 10 to 2, the protein content increased from 2.85 to 3.78% for centrifugation and 1.32 to 2.01% for sedimentation (data averaged for all steeping times shown in Table I). Overall averages for all subcategories showed similar results.

Possible reasons were considered for the increased starch yield and decreased protein content as number of kernels increased. A smaller number of corn kernels could cause nonuniform blending within the 30-sec blending time, resulting in some unbroken large particles. Starch then would not be released from these large particles, which would affect starch yield and protein content. However, we did not observe any large particles from the residue left after filtration of starch by any of the methods, so we assume that the blending of 2, 5, or 10 kernels was complete.

Another possible reason for the increased starch yield and decreased protein content as number of kernels increased might have been incomplete separation of starch and protein with the 30-μm filter used in the methods. Starch particles are ≈10–30 μm in diameter, whereas, protein particles are typically 5–10 μm in diameter (Singh 1994). Although the size of the starch particles is greater than the size of the protein particles, the starch should pass through the filter, whereas the stickier proteins coat the filter cloth and bind with fiber that has not been adequately separated (Singh and Eckhoff 1996). In addition, corn protein agglomerates under certain conditions, which also would account for the protein being retained by the filter (Singh and Eckhoff 1996). The same amount of water was used to wash all the starch slurries. Thus, more protein may have been forced through the filter when fewer kernels were extracted, resulting in greater protein content in the recovered starch. In addition, starch might have been inadvertently removed with the wash water during both sedimentation and centrifugation. This loss would have a greater effect on the 2-kernel extraction than on the 10-kernel extraction, because of the small initial weight. A low starch yield in the 2-kernel extraction procedure would result.

Effect of Isolation Method on Starch Yield and Protein Content

Sedimentation resulted in starch with lesser protein content and greater starch yield than starch extracted by using centrifugation (Table I). For centrifugation, the average values of yield and protein content of starch over three steeping times and three levels of kernels were 51.1 and 3.29%, respectively; whereas for sedimenta-

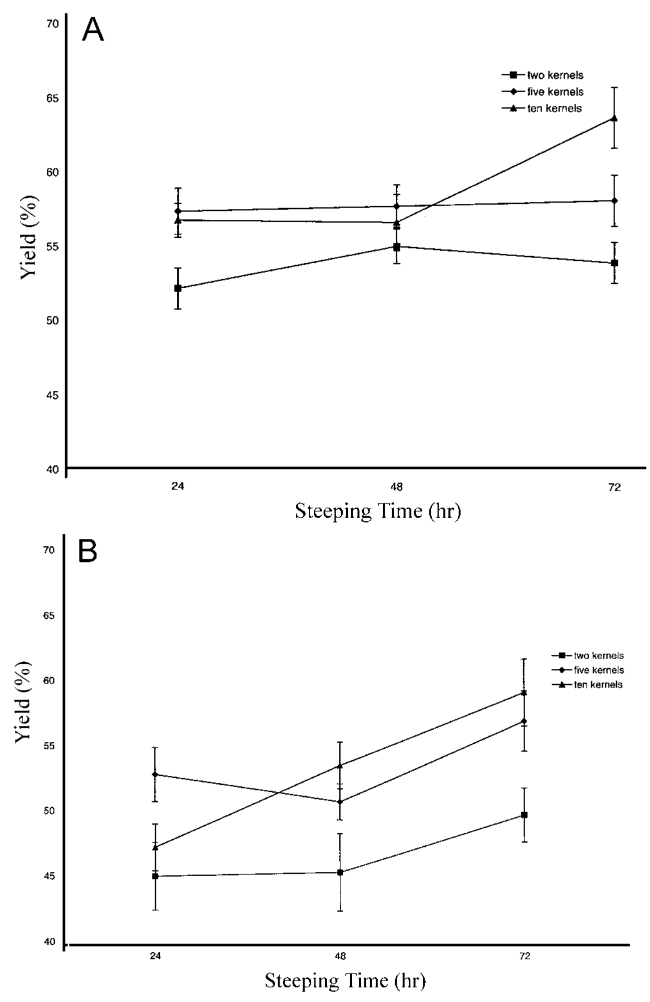

Fig. 1. Interaction between effect of number of kernels and steeping time on the starch yield. **A,** sedimentation method; **B,** centrifugation method.

TABLE II
Effect of Steeping Time on Gelatinization Characteristics of Starch as Determined by DSC[a,b]

	Steeping Time (hr)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	R (°C)
Centrifugation	24	67.9a[c]	72.0a	77.0b	11.88a	9.1c
	48	68.8b	72.5b	77.0b	11.74a	8.2a
	72	68.5b	72.4b	77.0b	11.69a	8.6ab
Sedimentation	24	67.8a	71.9a	76.5a	11.80a	8.7bc
	48	68.0a	71.9a	76.3a	11.79a	8.3a
	72	68.6b	72.5b	76.9b	11.61a	8.4ab

[a] T_o = Gelatinization onset temperature; T_p = gelatinization peak temperature; T_c = gelatinization conclusion temperature; ΔH = enthalpy of gelatinization; R = range of gelatinization temperature.
[b] Each value represents an average over the number of kernels (2, 5, and 10 kernels) because no significant effect of number of kernels on the DSC properties was observed.
[c] Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

tion, corresponding average values were 56.9 and 1.65%, respectively. Milled starch consists primarily of starch and corn protein particles that can be further separated by particle density differences in the isolation procedure. In commercial practices, centrifuges are used for primary starch-protein separations because of the greater average density of starch granules (1.5 g/cm^3) than of protein particles (1.1 g/cm^3) (Gausman et al 1952; Biss and Cogan 1988; Steinke and Johnson 1991). After centrifugation, the heavy starch fraction and the lighter protein fraction both form sediment, and the protein layer, which lies above the starch, is scraped off.

In the laboratory, centrifugation may not be an appropriate separation method when the amount of starch sample is small, especially for single- or small multikernel extractions. In such extractions, the protein fraction was partly dispersed within the starch fraction, with a blurred separation of the two fraction layers, making it very difficult to remove the protein layer. Furthermore, some starch might also be removed with the protein fraction. In contrast, during sedimentation, the heavy starch fraction settled to the bottom of the beaker and the lighter protein fraction remained suspended in the water, being removed during decanting.

Effect of Extraction Procedures on StarchThermal Properties

No significant main effects of number of kernels on the DSC properties were found, and therefore the DSC values were averaged for the purpose of discussing the effect of steeping time on the thermal properties of starch. Among all the thermal properties (Table II), T_o and R were most significantly affected by the steeping time. Some significant differences occurred for T_p and T_c. Starch from kernels steeped for 48 hr or more had greater T_o and a narrower R than did starch from kernels steeped for 24 hr. It is likely that the starch underwent annealing, which decreased swelling power and solubility and delayed the gelatinization (Krueger et al 1987; Fisher and Thompson 1997). Krueger et al (1997) studied the annealing of commercial corn and observed that annealing narrowed the gelatinization temperature range and increased peak temperature. Our results confirmed these results. These researchers concluded that annealing caused structural changes in the starch granules that affect their amorphous-crystalline relationships, forcing granules into a more crystalline orientation.

CONCLUSIONS

This study suggests that sedimentation is preferred over centrifugation for laboratory starch extractions, especially when the quantity of sample is small, because of the lowered starch-protein content and higher starch yields obtained with sedimentation. With a total wash water volume of 500 mL, 10 corn kernels in the extraction produced starch with higher yields and lower protein contents than did 2 or 5 corn kernels. A longer steeping time resulted in starch with lower protein concentration and higher yield. Soaking seeds for <24 hr is preferred to minimize annealing of starches and thus altering starch thermal properties.

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