A Laboratory Wet-Milling Procedure to Increase Reproducibility and Accuracy of Product Yields

S. R. ECKHOFF,[1,2] K. D. RAUSCH,[3] E. J. FOX,[3] C. C. TSO,[3] X. WU,[4] Z. PAN,[3] and P. BURIAK[1]

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ABSTRACT

A study investigating the accuracy and reproducibility of a laboratory-scale wet-milling procedure was conducted using eight individuals with two levels of milling experience. Four individuals had extensive milling experience, and four had conducted a maximum of three replicate milling runs as training. Process variables were strictly controlled as part of the standard operating procedure. Results showed no significant differences between the two groups of technicians for yields of steepwater, germ, fiber, starch, gluten, and gluten filtrate ($\alpha = 0.05$). Recovery of initial dry solids was above 98% for both groups, with protein content of the starch consistently below 0.4% (db) of the starch fraction. Controlling variations in operator techniques, process water inputs, and length of individual process stages had a noticeable affect on reducing the variability of milling yields.

Laboratory wet-milling procedures can be used to evaluate wet-milling characteristics of new corn hybrids, effect of harvest and drying methods on corn millability, and use of different steeping and processing techniques on product yields. A laboratory-scale study can be conducted when a pilot-scale study would be too expensive or would require greater quantities of a particular hybrid than is available. Current laboratory corn wet-milling procedures presented in the research literature (Watson et al 1951, Anderson 1963, Steinke and Johnson 1991) have been shown to correlate well with industrial wet-milling yields. However, a large degree of "art" or technique is required to achieve reproducible and accurate results. The procedures have been shown to have difficulty in achieving consistent results between individual millers due to the amount of "art" in the procedure. For example, the amount of water used in the fiber-washing process has not been documented, varies with each miller, and influences starch yield.

Our experience with laboratory procedures indicated that training of personnel generally required one to two months of practice milling before reproducible results could be obtained. Furthermore, variability of results (lack of precision) between technicians has been attributed to differences in technique, which resulted in specific individuals being required to run all samples for a

[1]Associate professors, Department of Agricultural Engineering, University of Illinois, Urbana 61801. Mention of product or trade names does not imply endorsement by the University of Illinois.
[2]Author to whom correspondence should be addressed.
[3]Graduate research assistants, Department of Agricultural Engineering, University of Illinois, Urbana, 61801.
[4]Visiting scholar, Beijing Agricultural Machinery Academic Research Institute, Beijing China.

particular study.

As a result of these problems, the present work focused on developing a laboratory corn wet-milling procedure that would reduce variability of replicate milling runs conducted by an individual technician, remove the potential for differences in technique by controlling time intervals and process water inputs, and thereby diminish the differences in results obtained by different technicians. The standard procedure was revised in such a way as to control as many process variables as possible and document them in a standard operating procedure.

The objectives of this research were: 1) to evaluate the reproducibility of results among different technicians using the standard laboratory procedure, 2) to determine the mean variation between replicate milling runs conducted by the same technician, and 3) to identify major sources of variation in an improved laboratory wet-milling procedure.

MATERIALS AND METHODS

Laboratory Wet-Milling Method

In the following description, specific time periods for certain process steps are listed when considered important in controlling variation in milling results. However, the effect of varying times between each step was not fully investigated in this study; the times were fairly short, so as to allow a single technician to complete grinding and separation processes within 8–9 hr. As a result, this overall time constraint shortened the time lag between individual process steps to less than 30 min, unless specified otherwise.

Sample Preparation and Steeping

Corn samples of a single corn hybrid (FR618 × FR600) were batch dried at ambient temperature to 15% final moisture from an initial moisture content of 23% (moistures reported on percent wet basis, unless noted). Samples were sieved over a 4.76-mm round-hole sieve on a reciprocating Gamet shaker (Dean Gamet Mfg. Co., Minneapolis, MN) to remove foreign material and broken corn. One-thousand-gram samples of cleaned corn were batch steeped at 52°C for 36 hr in a steeping solution of distilled water, 0.2% sulfur dioxide, and 0.55% lactic acid (1,867 ml total solution). Sulfur dioxide was introduced into distilled water by bubbling gaseous SO_2 through water to form a concentrated solution, then diluting the solution to 0.2%. Lactic acid was then added to the steeping solution.

After steeping, the steepwater was drained out of the tank and immediately titrated to determine the residual SO_2 concentration of the steepwater. Titrations of initial and residual SO_2 followed standard titration methods (AOAC 1955, Eckhoff and Okos 1983). The volume of the steepwater at the end of the steep period was measured by using a 2,000-ml graduated cylinder.

First Grind

The objective of the first grind process was to detach the germ from the other corn kernel components without damaging the germ. Within 60 min after draining of the steepwater, the corn was ground in a Waring commercial-grade blender equipped with a 15-amp motor, a 5-L container, and blades that had been ground to form a radius edge (Dynamic Corp. of America, New Hartford City, CT). Blades measured 8.9 cm in length, 1.9 cm in width, and 2.4 mm in thickness.

The corn sample was divided into four equal-volume batches of approximately 500 ml. Each batch was then ground in the blender with an equal volume of distilled water for 3.0 min at 35% of full power, followed by 3.0 min at 40% of full power. A Powerstat transformer (model no. 116, Superior Electric company, Bristol, CT) was used to control motor speed. Use of various blenders and containers may require adjustment of power and time requirements to ensure adequate and consistent first grind without undue damage to the germ. For normal dent corn that was of good quality for milling, no whole kernels were left in the container at the end of first grind; this was used as a qualitative measure in establishing first-grind parameters for this procedure.

Germ Skimming

The germ skimming process allowed the germ component and other kernel components to be separated by density differences. After first grind, the coarsely ground slurry was transferred to a 10-L bucket for germ skimming. To control the specific gravity of the slurry, 0.5 L of distilled water was used to wash the remaining material from the blender into the bucket. For most regular dent corn samples, addition of dry starch is not necessary to increase the specific gravity of the slurry for germ floatation. Addition of starch is not desirable, since it alters the composition and characteristics of the starch obtained by the milling process. The coarsely ground slurry is intermittently stirred during skimming to aid germ floatation, then skimmed by hand using 14- and 18-mesh stainless steel screens. The amount of time required to skim the germ was recorded as a relative measure of ease of germ separation, but this measurement is subjective due to technician skill and experience. In addition, steep conditions and varying corn genotypes (i.e., regular dent, waxy, high-amylose, etc.) can have a large effect on the time required for skimming. When skimming was completed, the germ was rinsed with a wash bottle and 1.0 L of distilled water over a 1-mm round-hole screen to remove additional fiber and starch. The effluent from the germ washing was added to the degerminated slurry.

Second Grind

The degerminated slurry was finely ground by passing it through a Quaker City plate mill (model 4-E, The Straub Co., Hatboro, PA) having one stationary and one rotating plate. The corrugated plates (9.5-cm diameter) on this mill actually contact each other and must mate well to give consistent particle size. Since the corrugated plates are usually not perfectly flat when new, they must be broken in before use in the milling procedure by running them in the mill for approximately 10 hr or until properly mated. The plates are kept cool by wetting them periodically during the break-in period.

The fineness of the grind was adjusted by increasing plate-to-plate pressure in the mill until the motor began to load noticeably when the plates were wet. The plates do not heat appreciably during the grinding process due to the large amount of liquid passing between them. The slurry was stirred as it was being poured into the mill, and 0.5 L of distilled water was used to wash the mill, buckets, and miscellaneous equipment when the second grind was finished.

Fiber Separation

After the second grind was completed, the finely ground slurry was allowed to settle undisturbed for a minimum of 30 min before 2.0 L of water was decanted. The decanted slurry was placed on a Kason Vibrascreen shaker (61.0-cm diameter; model KM-1-SS, Kason Corp., Linden, NJ) equipped with a 325-mesh (43-m openings) stainless steel screen. The motion of the shaker was adjusted according to manufacturer's specifications to enhance dewatering of the slurry.

The fiber-starch-gluten mixture on the screen was slowly washed with the 2.0 L of decanted water and then with an additional 3.5 L of distilled water. The mechanical action of the vibrating screen and the rinse water removed starch and gluten from the fiber; hand mixing of the fiber while on the shaker allowed more complete removal of starch and gluten from the fiber fraction. Washing was deemed adequate when squeezing the fiber retained on the screen produced a clear liquid relatively free of starch and gluten. When the fiber washing was completed, the slurry that passed through the screen (the millstarch) was rescreened by hand using a 200-mesh (74-m openings) stainless steel screen to check for fiber leakage through the Kason shaker. The 325- and 200-mesh screens used in the procedure were items available at the laboratory; industry typically uses screens with 74-m openings for the fiber separation process. A constant 0.5 L of distilled water was used to clean fiber separation equipment and was added to the millstarch.

Starch Tabling

Millstarch consists primarily of starch and corn gluten particles that can be separated by differences in particle density using a starch table. The starch tabling procedure follows specifications from previous laboratory-scale research (Watson et al 1951, Anderson 1963, Watson 1984). Tables used were steel H-beams 10.6 cm wide that had been placed to form a 8.3-cm wide channel in which the millstarch flowed. In preparing the H-beams for use, excess rust was removed before they were painted with several coats of rust-inhibiting paint. Between each coat of paint, the beam channel was lightly sanded to improve the smoothness of the finish. For regular dent corn, the slope of the table was adjusted to have a drop of 5.7 cm over the 610-cm beam length.

The millstarch was allowed to settle for 45–60 min after the fiber washing process. Specific gravity was then adjusted to 1.04–1.045 (5.5–6.2 Bé) by decanting approximately 4 L of water. The millstarch was then pumped onto the upper end of the starch table at a rate of 300 ml/min using a peristaltic pump and spread across the width of the table to form a uniform, continuous sheet as it flowed down the table.

When all of the millstarch had been pumped onto the table, the 4 L of decant water was pumped onto the table at the same rate. When pumping was finished, the starch on the table was allowed to settle for 10 min, and then the entire table was gently rinsed with 1.0 L of distilled water using a wash bottle equipped with a small piece of tubing attached to the bottle spout. The tubing was held just above the table, and the surface of the starch cake was gently rinsed to avoid dislodging starch granules. The purpose of washing was to remove protein and other impurities on the surface of the tabled starch. All solids retained on the table were included in the starch fraction and were collected from the table by scraping up the starch after it had dried overnight (12–18 hr). Starch was collected using a plastic putty knife and a soft-bristled brush, so as to not damage the table surface while recovering as much starch as possible.

Gluten Filtration

Overflow from the starch table (the gluten fraction) was dewatered by being vacuum filtered through Buchner funnels (25-cm diameter) using qualitative filter paper (24.0-cm diameter, manufactured by Whatman International Ltd., Maidstone, England). Filtration was performed at a vacuum of approximately 560–660 mm of Hg. The volume of liquid that passed through the vacuum filters (gluten filtrate) was measured, and a representative sample was retained for solids determination.

Yield Determinations and Processing Time

Milling fractions (germ, fiber, starch, and gluten) were dried at 49°C for 24 hr in a Blue M convection-type drying oven (Blue M Electric, Blue Island, IL). A small sample from each fraction was placed in a Blue M convection-type drying oven at 103°C for 2 hr to determine the solids content of the fractions (AACC 1983). Technicians routinely recovered at least 98% of the initial dry solids of the corn sample.

Exclusive of steeping and drying of fractions, the processing time for a single individual conducting a normal milling run of regular dent corn was 8–9 hr; with experience, two runs could be conducted simultaneously within 12–14 hr.

Experimental Design of Reproducibility Test

A group of eight individuals participated in the laboratory-scale wet-milling study, with each individual conducting two replicate milling runs for a total of 16 observations. Four individuals were considered "experts" and had completed between 35 and 50 wet-milling runs before the study, whereas the other four "novices" had received initial verbal instructions, witnessed demonstrations, and conducted a maximum of three milling runs. The two levels of experience were used to determine the sensitivity of the milling process to differences in operator-developed techniques.

RESULTS AND DISCUSSION

Reproducibility of Procedure

The variety of corn milled in this study exhibited typical laboratory milling yields for this procedure, with germ, fiber, starch, and gluten yields of approximately 7, 10, 65, and 10% (dry solids basis), respectively (Table I). Sufficient steeping is indicated by the relatively low fiber and gluten yields and by low protein content in the starch. Furthermore, Table I shows little difference between milling yields obtained by expert and novice groups, each of which were means of eight replicates. In comparing the expert and novice yields using the least significant difference method, no significant differences were detected ($\alpha = 0.05$).

Using the standard deviation of each individual, four standard deviations for each group were averaged and divided by the mean group yield. This gave the mean individual coefficient of variation (CV) for the expert and novice groups. The mean individual CV measures the variability of individuals conducting milling runs, within the novice and expert groupings. The overall CV measures the variability among all individuals in the study. High recovery rates, low protein contents in starch, and relatively low CVs indicate that a relatively limited amount of training and experience were required to obtain reproducible results using this procedure.

There is some variation among all individuals, as indicated in Table I. The overall CV was calculated from all milling runs and is shown with the mean individual CV. Although variability among individuals is relatively high, individuals showed acceptable reproducibility when replicating their own work. This also indicates areas where procedural improvement or refinement may be necessary, such as in the fiber, gluten, and filtrate processes.

TABLE I
Variations in Laboratory Wet-Milling Results Among Expert and Novice Groups

Milling Fraction	Mean Group Yield[a]		Mean Individual Coefficient of Variation,[b] %		Overall Coefficient of Variation (%)
	Novice	Expert	Novice	Expert	
Steepwater	4.75 ± 0.26	4.63 ± 0.12	5.5	2.6	7.5
Germ	6.92 ± 0.14	7.06 ± 0.35	2.0	5.0	5.3
Fiber	10.10 ± 0.28	9.73 ± 0.25	2.8	2.6	13.2
Starch	65.00 ± 0.61	64.50 ± 0.65	0.9	1.0	1.5
Gluten	9.54 ± 0.95	10.30 ± 0.31	9.9	3.0	15.0
Filtrate	2.43 ± 0.35	2.21 ± 0.05	14.3	2.4	19.1
Solids recovery[c]	98.73 ± 0.61	98.43 ± 0.35	0.6	0.4	0.7
Protein in starch	0.33 ± 0.01	0.32 ± 0.01	2.9	3.9	6.2

[a]All yields are expressed on a percentage dry solids basis ± one standard deviation and are the mean of eight observations. The standard deviation shown is the mean of the individual standard deviations. There were no significant differences between yield means ($\alpha = 0.05$).
[b]Calculated using the mean of each of the individual standard deviations and dividing by the group mean.
[c]Percentage recovery of initial dry corn solids.

Mean variances of each mean yield for the expert and novice groups were compared using the least significant difference method ($\alpha = 0.05$). The mean variance was calculated using the variances of each individual within each group. No statistically significant differences were observed in the mean yields or mean variances between the two groups, indicating that the novice group could perform as well as the expert group with proper training.

The relatively high CV for gluten yields is attributed to differences in starch washing techniques. During the starch tabling process, the time from the end of the tabling procedure to the start of the starch washing was not controlled and varied among replicate milling runs and among technicians. It is thought that different amounts of time from the end-of-tabling to the start-of-washing will cause different amounts of clean starch to be rinsed from the starch table, causing variation in starch yields and especially in gluten yields.

Novice variability was relatively large for the gluten filtrate fraction. This is attributed to a relatively small mean yield; an increase in filtrate yield causes a much larger change in the CV than does the same change in other fractions, especially starch yield. The expert group had higher variation in germ yield than the novice group, perhaps caused by the novice group being more careful and patient than the experts. Since no time limit on germ skimming was set, novices may have skimmed longer, reducing variability between runs. Germ skimming was one of the processes that was difficult to control objectively.

Accuracy of Procedure

The yields observed in this study were comparable to the yield results from industry and from previous laboratory procedures. However, the laboratory procedure used a single set of operating conditions and a single corn hybrid, whereas in industry any number of conditions and hybrids are present at one time, making direct numerical comparison of yields inappropriate. The yields of steepwater and gluten filtrate are combined together as solubles for comparison with industry results (Table II). Differences in industrial yields reported in the literature are attributed to corn quality and variety and to numerous variations in equipment.

When compared with industry results, our laboratory procedure had lower germ, fiber, and starch yields and higher gluten yields. The lower germ yield is attributed to the hand skimming process, whereby mostly pure germ is recovered and fiber particles continue downstream. This results in a higher quality germ fraction but a lower yield. The lower starch and higher gluten yields of our laboratory procedure have a common link. In industry, starch and gluten are separated by a system of hydrocyclones that repeatedly refine the starch-gluten separation, using 2.1–2.5 L of water per kilogram of dry starch (May 1987). In contrast, the starch table used in this procedure is a single-pass separation that separates most of the starch from the gluten. Starch tabling usually gives high quality starch but lower protein concentrations in the gluten. This is a limitation of the starch tabling method; a high quality starch fraction is obtained in exchange for lower starch yield and protein concentration in the gluten. As mentioned earlier, the starch washing technique may also affect starch yield.

Since this method was based upon research reported by Watson et al (1951) and Anderson (1963), it was expected to have results similar to the results of these studies (Table III). The variation in starch yields between 67.3 and 62.8% is probably caused by differences in dent corn hybrids being milled and is not attributed to procedural differences. Based on experience with the present procedure, starch yields below 60–61% using this procedure could be attributed to a low starch content of the hybrid being milled, poor initial corn quality, or an error in performing the milling procedure. All milling fractions from the present procedure can be directly related to fractions obtained in industry, making comparison with industrial practice easier. There is no "inseparables" fraction as in the method reported by Steinke and Johnson (1991). The current procedure also has lower fiber and higher starch yields than the Steinke and Johnson method. In all of the procedures presented in Table III, the steep times are somewhat longer than today's industry norm of 24–36 hr.

Starch and gluten fractions obtained using the procedure in the present study will likely have different functionality than the same fractions from industry. The hydrocyclones used to wash the starch in industry tend to retain smaller starch granules than the starch tabling method; thus the starch obtained by these two methods will likely have different particle size distributions. The gluten from industry will likely have lower starch and higher protein contents than that from the laboratory procedure, giving the gluten different functional characteristics. Therefore, the

TABLE II
Comparison of Industry Yield Data Reported in the Literature with Data from the Current Laboratory Study

Milling Fraction	Anderson and Watson (1982)	Knight (1969)	Anderson (1963)	May (1987)	Current Laboratory Study
Solubles[a]	7.5	6.8	n/a[b]	7	7.0
Germ	7.5	7.3	n/a	7.9	7.0
Fiber	11.5	11.5	12.8[c]	13	9.9
Starch	67.5	68	64.3	66.0	64.8
Gluten	5.8	5.0	14.5	5.7	9.9
Recovery[d]	99.8	98.6	91.6	99.6	98.6

[a] Sum of steepwater and gluten filtrate fractions.
[b] Not available.
[c] Combination of fiber and germ fractions.
[d] Sum of the above rows.

TABLE III
Comparison of Laboratory-Scale Milling Studies

Milling Fraction	Present Study	Eckhoff and Tso (1991)	Steinke and Johnson (1991)	Watson (1984)	Anderson (1963)	Watson et al (1951)	Rubens (1990)[a]
Solubles	7.0[b]	6.2[b]	7.6[b]	7.6[b]	7.1[b]	7.5[b]	5.1[c]
Germ	7.0	6.0	6.6	7.3	...[d]	7.2	10.5
Fiber	9.9	8.8	19.2	9.5	18.7[e]	8.4	21.8
Starch	64.8	67.3	58.4	63.7	65.4	62.8	58.8
Gluten	9.9	9.8	8.9[f]	11.3[g]	8.1	11.5	7.6
Recovery[h]	98.6	98.1	100.3	99.4	99.3	97.4	103.8
Protein in starch, %	0.32	0.32	0.56	0.30	0.54	0.36	0.63
Steep times, hr	36	48	48	48	48	...	...

[a] Pilot-scale study.
[b] Sum of steepwater, gluten filtrate, and other process water fractions.
[c] Process water solubles were combined with the gluten fraction in this study.
[d] Not reported.
[e] Includes the germ fraction.
[f] Sum of gluten and "squeegee" starch (7.5% protein) fractions.
[g] Sum of gluten and "squeegee" starch (6.1% protein) fractions.
[h] Sum of all milling fractions.

characteristics of the starch and gluten obtained from the laboratory method should not be directly extended to those fractions obtained from an industrial process.

CONCLUSION

A study was conducted to determine the effect and extent of operator differences when using a standard laboratory wet-milling procedure. This study revealed many small variations in individual operator techniques that had not been detected before the study. This study stressed the importance of the amount of process water applied and the length of individual process stages in controlling variability of results.

Overall, this process produced milling fractions that had yields that could be compared with those of the wet-milling industry. The time requirements for training novice technicians was approximately one week plus one to three practice milling runs, requiring an additional week. Based on the overall mean individual CV, replicate milling runs conducted by the same operator had good reproducibility regardless of experience. There were no significant differences between milling yields obtained by expert and novice groups. The germ skimming process showed little variation between groups, despite its subjective nature.

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