A 100-g Laboratory Corn Wet-Milling Procedure

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ABSTRACT

A 100-g laboratory corn wet-milling procedure was developed in order to reduce sample size and labor time requirements for determining the milling characteristics of corn samples. The procedure gives starch yields statistically similar to those of a 1-kg laboratory procedure with a standard deviation in replicates of 0.36% when the replicates were performed during the same week, and 0.60% when replicates were performed weekly during the course of a year. In a properly equipped laboratory, the procedure can be performed at the rate of 40 samples per week using three trained personnel.

The feasibility of corn wet-milling facilities processing specific hybrids of yellow dent corn rather than mixed hybrid commodity corn has been enhanced by the use of biotechnology and genetic engineering in corn hybrid development. Identification of better wet-milling hybrids has been limited, in part, by the slow speed of widely used analytical milling techniques.

The milling quality of corn (millability) can be evaluated by using laboratory-scale milling procedures (Dimler et al 1944, Watson et al 1951, Watson et al 1955, Zipf et al 1959, Anderson 1963, White et al 1990, Steinke and Johnson 1991, Eckhoff et al 1993, Wehling et al 1993) that require samples of 300–1,500 g and are labor- and time-intensive. The amount of labor and time required often limits the number of experimental runs per analyst to four to six samples per week for procedures when mass balances are performed. To fully utilize the new hybrid development techniques, seed companies and wet millers need to evaluate a large number of samples in a short time to meet the decision-making requirements of current production procedures. There is a need for a laboratory-scale milling procedure that would be faster than the current laboratory procedures and that will utilize smaller amounts of corn (preferably 100 g or less).

The Pelshenke and Lindemann (1954) method mills 100–200 g of corn but is more complicated than the 300-g to 1-kg sample size procedures traditionally used. Germs are recovered from steeped corn by hand-dissection, which increases the time required to process the sample and also does not yield any information regarding the ease of separation of the germ or the ability of the germ to withstand mechanical forces. The procedure also requires the use of two centrifugation and washing steps before starch tabling, as well as two fiber washing steps. The procedure also lacks information on many details important to the use of the procedure, including the table slope, the amount of water used in each step, the rate of water addition on the starch table during final washing, and the specific gravity of the starch-protein solution before tabling. Although the Pelshenke and Lindemann procedure only uses a 100-g sample size, it is as time-consuming as alternative laboratory procedures. The procedure has a high degree of precision with a starch yield standard deviation of 0.2% at a starch yield of ≈69%.

The goal of this study was to develop and evaluate a 100-g sample size laboratory wet-milling procedure that incorporates aspects of the Pelshenke and Lindemann (1954) method but can be performed routinely with greater speed and similar precision.

METHODS AND MATERIALS

Description of the 100-g Wet-Milling Procedure

Steeping. Samples (wet weight) of corn (100 g) are placed in 500-ml Erlenmeyer flasks with 180 ml of steep solution and steeped in a 52°C water bath with no stirring or recirculation of the steep solution. Accurate measurement of the initial moisture content is critical for estimation of initial dry weight and is measured in triplicate using the 103°C, 72-hr forced-air oven procedure (AACC 1983). The length of steep time can be varied with 24 hr of steeping considered practical for maintaining a daily milling regime. The steep solution contains 2,000 ppm sulfur dioxide and 0.5% (w/w) lactic acid, although the level of sulfur dioxide and lactic acid can be adjusted.

At the completion of steeping, the steepwater is drained into a 250-ml graduated cylinder, and the unabsorbed steepwater volume is measured. The steepwater is dried for determination of solids using the two-stage drying procedure (AACC 1983). The two-stage procedure is preferred over a single-stage drying method because of the high percentage of water to be evaporated.

First grind. The steeped corn is milled in an equal volume of water using a Waring type blender (Dynamic Corp. of America, New Hartford City, CT) equipped with a 1-L glass container (jar) and stainless steel blades that have been ground to a radius edge. The blades are 6.3 cm long, 0.3 cm thick, and 1.0 cm wide with a pitch of ≈40°. The blender is equipped with a tachometer to monitor the rpm of the blades and is controlled by a variable transformer to maintain 7,500–7,600 rpm. The control of the rpm is important because the blenders vary as to the rpm at which they would operate under identical load. Control of the rpm also compensates for the natural loss of power as the blender ages.

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Germ plus coarse fiber washing. The slurry obtained after the first grind is transferred along with 500 ml of water to a tared standard testing sieve (U.S. No. 7, 2.80 mm) placed at the bottom of a 10-L bucket. The bucket was selected so that the sieve fitted snugly in the bottom of the bucket. The bucket and sieve arrangement is shaken (Ro-tap testing sieve shaker, W. S. Tyler Co., Cleveland, OH) or equivalent, with the tapping capabilities disengaged, for 5 min. During shaking, the slurry is periodically dispersed around the sieve using a spatula. The material retained on the sieve contains whole and large broken germ pieces and large pieces of the pericarp (coarse fiber). The shaking of the sieve acts to abrade endosperm material and the coarse fiber off of the germ as well as washing the germ and fiber. Small broken pieces of germ that passed through the sieve are recovered with the fine fiber. The tared sieve with the recovered germ and coarse fiber is dried using the two-stage drying method (AACC 1983) and the total weight of the germ and coarse fiber is measured by weighing the sieve and subtracting the initial empty weight of the sieve. The use of the tared sieve eliminates losses due to handling of the material.

Second grind. The degerminated slurry (that which passes through the No. 7 sieve) is finely ground in a plate mill (Quaker City model no. 4-E; The Straub Co., Warminster, PA). The necessary fineness of grind is achieved by increasing plate pressure in the mill until the motor begins to load noticeably. The slurry is then stirred and passed through the mill. After the degerminated slurry is passed through the mill, 250 ml of water is used to wash the mill, bucket, and miscellaneous equipment; this wash water is retained with the sample.

Preparation of the disk plates in the mill is very important for maintaining precision. New plates need to be ground down to where there is adequate contact surface between the plates to properly mill the sample. Low starch yields result when new plates are not adequately prepared. Experience has shown that the most expedient way to achieve the desired level of contact area is to grind the plates for 105 hr in the mill running only cooling water. Adequate pressure needs to be maintained on the plates to achieve the desired contact area and should be operated at the same level of motor loading as normally used when grinding corn. To assure that sufficient wear on the plates has been achieved, corn samples for which yield data has been estimated using the old plates, should be milled using the new plates. Sufficient replicates should be run using both the new and old plates to assure that the results are comparable. It has also been observed that different Quaker City mills grind the plates differently due to slight variations in the shaft location. It is recommended that the mill used to wear in a set of plates be used during the plate's service life.

The service life of the plates is ≈1,000 samples. Plates used past ≈1,000 samples have developed too much contact area and starch yields increase slightly (1–2%) with continued use. The major problems with using plates that have been too well ground down are that the time required to pass the sample through the mill increases dramatically and too much fine fiber ends up in the starch-protein fraction, which can negatively affect starch recovery (Eckhoff and Tso 1990).

Fine fiber separation. After the second grind, the slurry is allowed to settle undisturbed for 30–45 min, after which time ≈750 ml of water is decanted. The decanted slurry is transferred to a standard testing sieve (U.S. No. 200, 75 μm) fitted over a 7.5-L bucket (ACE Industries Inc. Grand Rapid, MI). This bucket-sieve arrangement is placed on the Ro-Tap testing sieve shaker (or equivalent), with the tapping capabilities disengaged, and shaken for 5 min while the slurry is washed using decant water.

During washing, a spatula is used to continuously disperse the slurry while the decant water is poured over the slurry. After washing with the decant water, the material on the sieve is washed by spraying with a wash bottle containing 500 ml of water.

The material retained on the sieve after washing is dewatered by pressing with the spatula, transferred into two tared weighing cups, and dried using the two-stage air oven procedure (AACC 1983). It would be possible to preweigh this sieve also and eliminate this transfer of solids, but the fine fiber has a tendency to dry on the 200-mesh sieve and block the screen. While it is recommended to periodically clean all sieves with an ultrasonic cleaner (USC 200-90; Haver & Boecker, Westfalen, Germany), drying the fine fiber on the 200-mesh sieve would necessitate daily ultrasonic cleaning.

Starch-protein separation. Starch-protein separation is achieved using tared 5.08-cm × 2.44-m aluminum channels as starch tables set at a slope of 0.0104 (cm/cm) as determined from a previous study (Singh 1995). The empty dry starch tables are weighed by suspending between two balances, one located on each end of the starch table. The sum of the two balances is the initial table weight. The table length was selected based on the need to move the tables around in the laboratory for weighing and ambient drying.

The specific gravity of the starch-protein mixture left after fine fiber removal is adjusted to 1.04–1.045 by allowing the mixture to decant for 1 hr. If the specific gravity is too high after decanting, adequate decant water can be added to achieve the desired specific gravity. The decanted liquid is used later in the washing of the recovered starch. The decanted slurry is pumped onto the starch table at the rate of 50–52 ml/min, and the overflow (containing primarily small or light granule starch and protein) is collected.

Immediately after the pumping of the starch-protein mixture is completed, the decanted water (specific gravity of ≈1.002) is pumped onto the table at the same pump rate. When washing with the decant water is finished, 125 ml of water is used to wash the sides of the bucket and is then pumped onto the table.

After tabling is completed, the starch is allowed to ambient air dry overnight (at least 6 hr) on the table. The air-dried starch table is then reweighed across the balances to give a wet starch weight. Starch is then scraped from the table into two tared 250-ml aluminum cups for moisture content determination using the 135°C, 2-hr forced-air oven moisture measurement procedure (AACC 1983). By knowing the moisture of the starch on the table and the total wet weight of the starch, total starch dry weight can be calculated. It is important to scrape as much of the starch off the table as is practical to ensure an accurate estimate of the moisture, but it is not necessary to recover all of the starch.

Because the two-stage drying procedure can damage the starch rheological characteristics, it is possible to only use a portion of the tabled starch to measure the moisture content (three 5-g replicates). This allows 40–50 g of starch to be available for other testing. However, care must be taken to ensure that the 15 g of starch used for moisture determination is representative of the whole starch sample.

Protein filtration. Overflow from the starch table is vacuum-filtered at 550–650 mm·Hg vacuum, using 25.3-cm dia. Buchner funnels and 24-cm dia. tared qualitative filter paper (Whatman International Ltd., Maidstone, England). The volume of liquid that passed through the vacuum filters (protein filtrate) is measured, and representative samples are retained for solids analysis. Solids on the filter paper are dried using the two-stage air-oven method (AACC 1983) to determine the total protein fraction dry weight.

The filtering of the protein fraction is tedious and laborious. For applications where the primary objective is a quantitative estimate of starch yield, the starch table overflow volume can be measured, and the solids content of the slurry measured, as is done with the steepwater. The slurry should be adequately stirred to ensure representative sampling and three 75-ml samples removed for solids analysis using the two-stage air-oven procedure (AACC 1983).

Germ recovery. As described earlier, the dried germ plus coarse fiber fraction was removed from the oven and weighed along with the sieve. Subtracting the weight of the sieve yields the total germ plus coarse fiber fraction dry weight. The dry matter on the sieve

is then gently hand-rubbed to disrupt any connected germ and fiber and an air jet is set at a pressure of 2,000 to 2,500 mm·Hg is used to aspirate the coarse fiber. The distance between the air jet and the bottom of the sieve should be maintained at ≈20–30 cm. The sieve is continuously shaken side to side while aspirating the coarse fiber. This procedure provided adequate airflow to remove the fiber but not the germ. Germ left on the sieve is reweighed to give an estimate of the total dry weight of recovered germ. Course fiber amount is determined by difference.

Sample Preparation

Yellow dent corn was sieved over a 4.76 mm (12/64 in.) round hole screen on a sieve shaker (Dean Gamet Mfg. Co., Minneapolis, MN) to remove foreign material and broken corn. Approximately 150 g was used for moisture content determination (AACC 1983) and the remaining corn was stored at 4°C until wet milled.

Comparison of the 100-g Procedure with the 1-kg Procedure.

A sample of corn representing a mixture of commercial hybrids was obtained locally and subdivided for milling. Five sublots were milled using the 1-kg procedure of Eckhoff et al (1993) and five sublots were milled using the 100-g procedure.

An unpaired, parametric, two tailed *t*-test (SigmaStat Statistical Analysis System, Version 1.00, Jandel Corp.) was used to compare the individual fraction means of the two groups.

Precision of Procedure

To estimate the procedural standard deviation of measurement, a sample of FR618 × FR600 hybrid corn was mechanically harvested during the 1993 season and ambient air-dried from ≈22% (wb) moisture to 14% (wb). Thirty, 100-g replicates were wet milled during a one-week period.

To estimate the stability of the procedure over time, samples of a single hybrid (FR1064 × LH59) were grown during the 1993 and 1994 crop years, mechanically harvested at ≈22% moisture content and ambient dried to ≈14% wb moisture. The corn was divided into 100-g sublots, sealed in plastic bags, and refrigerated until used. Weekly, during weeks when samples were being run, a sample of the hybrid was wet milled, and starch yield was used as an indication of the stability of the procedure over an extended time period.

RESULTS AND DISCUSSION

Yield Comparisons Between the 100-g and 1-kg Procedures

Mean fraction yields (Table I) from the 100-g and the 1-kg procedure showed no significant difference ($\alpha = 0.05$) for all fractions. Total recovery was over 98% for both procedures. The ability to account for 98% of the mass for the 100-g procedure was due to the extreme care taken to minimize the transferring of products between containers. The use of tared starch tables was probably most critical because in the 1-kg procedure, the recovery of the dry starch from the table is a tedious and time-consuming, and some loss is inherent. It is very difficult to get quantitative recovery of the starch from the table surface. A 98% recovery level in the 1-kg procedure means that 20 g can not be accounted for. In the 100-g procedure, 98% recovery means that 2 g can not be accounted for.

Steepwater solids levels were similar. There had been some concern that the static (no forced recirculation of the steepwater through the corn) steeping used by the 100-g procedure may not allow for adequate steeping. Recirculation of the steepwater in the 1-kg procedure is primarily to help maintain uniform temperatures with in the corn mass. Because of the small sample size used in the 100-g procedure and the intimate contact with the water bath, the temperature profiles will be more uniform than when a larger sample is used. Based upon the data presented here, as well as additional unpublished data from our laboratory, there seems to be no need to provide for recirculation of steepwater in the 100-g steeping procedure.

TABLE I
Product Yield Comparisons Between the 1-kg and 100-g Procedures[a]

Fractions	1-kg Procedure	100-g Procedure	Difference in Means
Steepwater	4.3 ± 0.2	4.3 ± 0.1	0
Germ	5.9 ± 0.2	5.2 ± 0.3	0.7
Fiber	9.8 ± 0.2	10.2 ± 0.5	–0.4
Germ + fiber	15.7 ± 0.3	15.4 ± 0.6	0.3
Starch	67.4 ± 0.9	67.3 ± 0.4	0.1
Protein	8.6 ± 0.4	8.8 ± 0.3	–0.2
Starch + protein	76.0 ± 1.1	76.1 ± 0.5	–0.1
Filtrate	2.3 ± 0.4	2.5 ± 0.1	–0.2
Total solids	98.3 ± 0.9	98.3 ± 0.4	0

[a] All yields are expressed in percentage dry basis ± one standard deviation.

There was no statistical difference between the two procedures as to the amount of recovered fiber. This was surprising, because the fine fiber fraction was recovered using a 200-mesh (75 μm) sieve in the 100-g procedure, while in the 1-kg procedure, a 325-mesh (50 μm) sieve is used, and in the 100-g procedure, the coarse fiber was recovered separately from the fine fiber. While both methods used reciprocating screen to work the fiber, the sieve shakers had different actions. Working of the fiber is very important in recovering starch from the kernel because there is always starch attached to the fiber that needs to be recovered by mechanical shearing.

Average germ yields differed by 0.68%, although they were not statistically different at a 5% level. Visual comparison indicated that the germ from the 100-g procedure was cleaner because the germ in the 100-g procedure was recovered by sieving rather than by floating and skimming. In samples where the germ is fragile and breaks apart easily during the first grind, the 1-kg procedure may recover the broken germ but the 100-g procedure will not. This problem has been observed in subsequent samples. However, for testing genetic differences, the 100-g procedure is more sensitive in delineating germ-recovery characteristics.

No significant differences were found between the starch yields recovered by the two procedures. The standard deviation for the 100-g procedure was less than that for the 1-kg procedure (0.40 vs. 0.89%). Standard deviation for starch yield using the 1-kg procedure was previously reported by Eckhoff et al (1993) as 0.31% and by Singh and Eckhoff (1995) as 0.14%, so this set of data had more variability than that previously observed with the 1-kg procedure. Other procedures have reported similar or higher standard deviations in the measurement of starch yield: 0.5% (Anderson 1963); 0.7% (Steinke and Johnson 1991); 0.4% (Steinke et al 1991); 2.24% (Wehling et al 1993); 2.90% (Fox et al 1992); and 0.96% (Shandera et al 1995). A standard deviation of ≈0.5% is required to delineate genetic differences between hybrid samples at a commercially significant level. Commercial hybrid samples will vary in starch yield by 10–18%. Eckhoff (1995) measured starch yield ranges of 54–72% for 131 commercial hybrid samples using the 100-g procedure, while Fox et al (1992) determined a range of 50.9–60.0% for 27 commercial hybrids. A standard deviation of 0.5% means that samples with starch yields that differ by 1.0% can be considered different with 95% confidence. A 1.0% increase in starch yield has an approximate increased value of $0.03 per bushel to a starch manufacturer, based upon differences in the value of starch and gluten feed.

No significant difference in protein yield was found between the two procedures. The 100-g procedure again had a lower standard deviation (0.28 vs. 0.43%). Eckhoff et al (1993) reported a standard deviation for protein yield of 0.31% and Singh and Eckhoff (1995) reported a value of 0.22% using the 1-kg procedure.

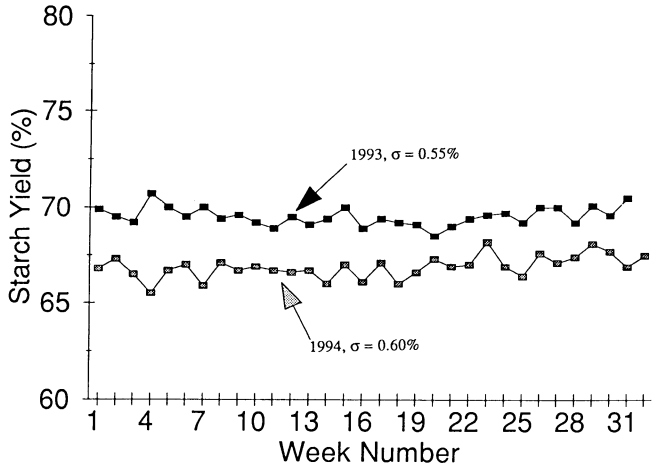

Fig. 1. Weekly starch yeilds for refrigerated samples of FR1064 X LH59 grown in two different years.

Anderson (1963) reported a standard deviation of 0.8%, while Steinke and Johnson (1991) and Fox et al (1992) reported values of 0.3 and 0.61%, respectively.

Precision of Procedure

Thirty replicates of the hybrid FR618 × FR600 averaged 67.5% starch yield with a standard deviation of 0.36%. This estimate of the precision of the procedure is comparable to other less replicated studies in our laboratory using the 100-g procedure, where standard deviations ranged from 0.25 to 0.60%. Singh (1995) wet-milled a sample of commercial corn (mixed hybrids) with the 1-kg procedure and found an average starch yield of 67.3% over five replicates with a standard deviation of 0.40%.

Figure 1 shows that the 100-g procedure maintained a standard deviation of 0.60% or less for each of the two years for which data was collected. There was a difference in the average starch yield for each of the two years: 1993 corn yielded an average of 69.5% and a standard deviation of 0.55%; 1994 corn yielded an average of 66.9% and a standard deviation of 0.60%. The precision of the procedure over time generally will be less than that of replicates made in a short time frame, as was observed in this study (0.36 vs. 0.60%). The results do show that there is very little drift in the procedure with time. Most importantly, the results show that the use of a refrigerated standard corn can provide the necessary quality control for the 100-g procedure.

Rate of Performing Procedure

Experience has shown that a properly equipped laboratory can process eight samples per day, five days per week, using three properly trained laboratory scientists. The necessary training of the millers is more extensive than required for the 1-kg procedure (Singh 1995). Additional training is needed because of the detailed care required to prevent loss of solids. Millers need to have strong laboratory skills to perform the procedure with adequate reproducibility.

While it is possible for just two millers to perform 10–12 milling runs per day using the procedure, the major bottleneck is the weighing of the various empty and full pans and sieves required for each run. Milling eight samples per day requires two people; a third individual is needed for weighing samples, entering the data on computer, and handling all purchase of disposables and correspondence. During the last two years, our laboratory has performed ≈1,500 milling runs per year using this procedure.

CONCLUSIONS

This procedure gives reproducible results for product yield with total solids recovery and yields statistically equivalent to those of the 1-kg procedure of Eckhoff et al (1993). The precision of this procedure was 0.36% for a hybrid run repeatedly during a one-week period and <0.60% when weekly replicates are run.

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