Comparison of Wet-Milling Properties Among Maize Inbred Lines and Their Hybrids[1]

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ABSTRACT

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A 100-g laboratory wet-milling procedure was used to compare wet-milling properties among 15 maize inbred lines and 20 related hybrids. Analyses of variance indicated sufficient precision in measurement of wet-milling yields for detection of differences among genotypes (inbred lines, hybrids). Significant divergence of hybrid from mid-parent values indicated that larger kernels of hybrids were lower for germ and fiber yields, and higher for gluten yield and filtrate solids in comparison to their inbred parents. Gene action for starch yield and starch recovery appeared to be additive in nature. However, only the predictive model for starch yield (hybrid starch yield = 13.9 + 0.74[mid-parent starch yield]) was significant. Positive correlations between wet-milling starch yield and starch content of kernels estimated by near-infrared reflectance spectroscopy were consistent among inbred lines and hybrids (r = +0.90 and r = +0.67, respectively), suggesting near-infrared reflectance as a potential tool in breeding for hybrids with enhanced starch extraction properties. The correlations between grain hardness and starch yield were r = –0.77 and r = –0.66 for inbred and hybrid-based evaluations, respectively, indicating the need to overcome this negative relationship when developing hybrids with both high starch yield potential and postharvest grain quality.

Food and industrial uses of maize (*Zea mays* L.) grain are an important component of United States agriculture, representing approximately 20% of maize production (Anonymous 1994). Products from maize wet-milling, particularly those derived from starch, comprise the largest single nonfeed use. There have been growing concerns among members of the wet-milling industry about deficiencies in the current maize grain grading system, with a realization that variation in grain characteristics of hybrids used in production are resulting in reduced milling efficiency. Representatives from both the wet-milling and hybrid seed industries are currently establishing collaborations for evaluation and contract production of specific maize hybrids with enhanced milling properties. However, in order for the hybrid seed industry to fully address these needs, methods must be identified that would allow for rapid, large-scale evaluation of existing hybrids and development of new lines and hybrids with superior milling properties.

Several laboratory-scale wet-milling procedures have been developed (Dimler et al 1944, Zipf et al 1950, Watson et al 1951, Anderson 1963, Steinke and Johnson 1991, Eckhoff et al 1993). These procedures require 300–1,500-g samples and are extremely labor and time intensive, which preclude their use in large-scale hybrid maize breeding programs. A number of attempts have been made to identify rapid predictors of wet-milling properties. Quantification of millability has been proposed based on visual estimation of proportion of horny to floury endosperm (Watson and Hirata 1954), percentages of prime starch and cleanup residue (Watson and Hirata 1962), and separation of starch and gluten in graduated cylinders (Freeman and Watson 1969). Brown et al (1979) reported a strong correlation between steeping index, based on observations of steeped kernel sections, and starch recovery, and moderate correlations between steeping index and grain test weight, stress cracking, and viability. Weller et al (1988) related starch yield from wet-milling with starch content estimated by near-infrared reflectance (NIR) spectroscopy. Fox et al (1992) observed a negative relationship between starch yield and protein content by NIR, but no correspondence between starch yield and starch content. Brumm et al (1991) and Wehling et al (1993) have attempted to develop NIR calibrations for direct prediction of starch yield, with the latter group identifying reproducibility of the laboratory wet-milling procedure as a limiting factor.

More recently, a laboratory wet-milling procedure using 100-g samples has been developed with potential for rapid, large-scale evaluation of materials from maize hybrid breeding programs (Singh 1995). This procedure successfully duplicates yields of a 1,000-g laboratory protocol (Eckhoff et al 1993) on which it is based. The 100-g procedure offers advantages of low sample amount and high throughput potential, both of which are requirements for use in maize breeding scenarios. However, while laboratory wet-milling evaluations have used grain from hybrids, maize breeders are concerned with characteristics of inbred-parental lines as well as their F_1 hybrids. Because of the large grain sample requirements of previous laboratory wet-milling procedures, little information has been available regarding wet-milling properties of maize inbred lines relative to hybrids. To assist in hybrid development, more information is needed regarding possible effects of heterosis on wet-milling yields and their underlying quantitative genetic control.

This study used the 100-g laboratory wet-milling procedure to compare fraction yields from inbred lines with those from related hybrids, and to determine precision of yield measurements for detection of differences among genotypes (inbred lines, hybrids). Also studied were relationships among wet-milling yields, kernel composition and grain physical properties of maize inbred lines in comparison to their hybrids.

MATERIALS AND METHODS

Genotype Selection and Production of Grain Samples

Fifteen maize inbred lines were selected for grain analysis. Eleven of these lines were chosen based on their representation of germ plasm groups commonly used in United States maize breeding programs, and for their historical importance in hybrid pedigrees. The remaining four inbred lines were developed at Purdue University for enhanced grain hardness, and were included to increase the

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diversity of grain endosperm texture under study. Twenty F_1 hybrids derived from pair-wise crosses involving nine of these fifteen inbred lines also were included for comparison of milling properties among hybrid and inbred grain, and to study possible effects of heterosis. No compositional analysis or physical measurements of grain were employed to assist in selection of genotypes for use in this study.

All inbred lines and hybrids were grown in 1992 in a maize breeding nursery at the Purdue University Agronomy Research Center near West Lafayette, IN. Self-pollination was done by hand to maintain purity of each genotype. Inbred lines were grown in either two- or four-row plots to obtain quantities of grain necessary for replicate milling evaluations. Hybrids grown in single-row plots were adequate for the same purpose. Grain from all genotypes was harvested by hand, forced-air dried on the cob at low temperature (40°C) until reaching ≈10–12% moisture, and then hand-shelled. Shelled grain was stored in bulk at 4°C and used as stock for further wet-milling and quality analyses over a six month period.

Wet-Milling, Grain Physical Properties, and Kernel Composition

Laboratory wet-milling. Laboratory wet-milling evaluations used the 100-g procedure (Singh 1995), which focuses on relative yields of wet-milling fractions for germ, fiber, starch, gluten, steepwater solids, and filtrate solids. In preparation for wet-milling, grain samples of each maize genotype were divided into four subsamples: three weighing ≈100 g each and a fourth weighing 50 g. The smaller subsample was used for moisture content determination (AACC 1983), while the remaining 100-g partitions were stored at 4°C before milling evaluation. In most cases, only two of the three 100-g subsamples were laboratory wet-milled, providing an average of slightly over two replicates per genotype. Subsamples for milling were randomly selected, steeped at 52°C for 36 hr in 200 ml of steepwater containing 2,000 ppm SO_2/0.55% lactic acid, and then milled (Singh 1995). Wet-milling fractions were dried in a convection oven (Blue M Electric, Blue Island, IL) using a two-stage procedure (AACC 1983); initial drying at 49°C for ≈24 hr, followed by a higher temperature for a shorter length of time, depending on the fraction (Singh 1995). All yields of wet-milling fractions were reported on a dry weight basis (dwb).

Grain physical properties. Grain hardness, absolute density, and average kernel size were evaluated for each genotype using separate grain samples. Samples were equilibrated to ≈12.0% moisture by placing them in a conditioning chamber (model I-35L, Percival Inc., Boone, IA) at 27°C with 67% rh for 10–14 days. Relative grain hardness was measured using the Stenvert hardness testing method (Pomeranz et al 1985). Duplicate grain samples of 20 g (± 0.01) each were ground through a 2-mm screen on a Micro Hammer-Cutter Mill, type IV (Glen Mills Inc., Maywood, NJ), recording the time in seconds required to accumulate 17 ml of whole meal. Determinations of absolute kernel density were made using a gas displacement method (Chang 1988). Triplicate whole kernel samples of ≈100 g (± 0.01) each were weighed, and volume determinations made using a stereopycnometer (model MVP-1, Quantachrome Corp., Syosset, NY) with nitrogen as the displacing gas. Average kernel size was estimated by 1,000-kernel weight and 1,000-kernel volume. For measurement by weight, 100-kernel samples were weighed to within 0.01 g, and the result multiplied by 10 for conversion to 1,000-kernel values. The 1,000-kernel volumes were estimated indirectly by division of 1,000-kernel weight (g) with pycnometer absolute density ($g \cdot cm^{-3}$).

Kernel composition. Moisture, oil, protein, and starch content of ground grain samples were estimated using an NIR analyzer (model 6000, Dickey-john Corp., Auburn, IL) operated by ICI Seeds, Slater, IA. Triplicate grain samples were taken for each genotype. Calibrations for crude free fat, protein, and starch content were licensed from the Agricultural Engineering Department, Iowa State University, and were determined as described by Fox et al (1992). All measurements were reported at 15.5% moisture adjustment. Starch recovery from wet-milling was then estimated by adjusting NIR starch composition to % dwb and dividing % starch yield from milling by % dwb starch composition.

Statistical Analysis

Analyses of variance was performed using the SAS procedure GLM (SAS 1988). The data were treated as a completely randomized design, with analyses performed over the entire data set using a linear additive model partitioning variation among groups (inbred versus hybrid), variation among genotypes (inbred and hybrid) within groups, and variation among sample replicates within genotypes. Genotype and replication effects were treated as random, while group effects were considered fixed. F-tests for significance of variance were performed using mean square values for each source of variation. Mean squares for sample replicates were used as the denominator in F-test for significance of differences among genotypes. Mean squares for genotypes were used as the denominator in F-test for significance of differences among groups. Mean values of genotypes (average of sample replicates) were used to calculate ranges, standard errors, and significance of differences between inbred and hybrid group means using a two-tailed t-test.

For each trait, two methods were used to calculate divergence of hybrid values from those of their respective inbred parents: 1) the average difference between hybrid and mid-parent values, and 2) the average increase in hybrid value over the high-parent mean. Mid-parent values were calculated as an average of the two inbred line means representing parents of each hybrid. Divergence of hybrids from their respective mid-parent and high-parent values were used in the SAS procedure MEANS (SAS 1988) to test for significance of differences from zero by a two-tailed t-test. Significant average divergence between hybrid and mid-parent value was classified as mid-parent divergence, while significant average increase in hybrid value over its high-parent mean was classified as high-parent heterosis. Plant breeders typically define heterosis, or hybrid vigor, as an increase in hybrid value over some measure of the inbred parents (Fehr 1987). Because wet-milling yields may either increase or decrease when comparing hybrid grain to that of the inbred parents, significant mid-parent divergence was not strictly classified as mid-parent heterosis. Both mid-parent divergence and high-parent heterosis are indicative of nonadditive gene action when significantly different from zero (Falconer 1981).

Single-factor correlations were calculated among all traits using mean values of genotypes and the SAS procedure CORR (SAS 1988). Correlation coefficients were estimated using inbred line and hybrid data sets separately, and for all the genotypes combined. Only correlations derived from separate inbred and hybrid data sets are presented in tabular form.

RESULTS AND DISCUSSION

Analysis of Variance, Comparison of Inbred and Hybrid Means

Wet-milling yields. Analyses of variance revealed the majority of variation for yields of wet-milling fractions were due to differences among genotypes, inbred or hybrid (Table I). Highly significant F-values for testing of variation among genotypes indicated that variability among milling replicates within genotypes was relatively small by comparison. Therefore, the 100-g laboratory wet-milling procedure was precise in measuring fraction yields, even though only two milling replicates were performed for most genotypes. Only an estimate of total solid recovery, calculated as the sum of all milling yield fractions, failed to show significant variation among genotypes. For most wet-milling yields, >70% of the total variation could be accounted for by differences among genotypes, with R-square = 0.89 for starch yield. No significant variation was present among group designations (contrast of inbred vs. hybrid genotypes). R-square values associated with variation among groups were extremely small; the largest value was associated with gluten yield (*R*-square = 0.09).

Differences in average wet-milling yields among inbred line and hybrid groups were not significantly different (Table II). Mean yields of starch were somewhat low in comparison to industry standards, and corresponding gluten yields were high. Starch yields in this study ranged from 46.5 to 62.3% dwb over all genotypes. Gluten yields ranged from 11.7 to 23.8% dwb over all genotypes. Solids recovered from steepwater after 36 hr of steeping were <4.0% dwb on average, with little variability among genotype means. While it has been suggested that laboratory wet-milling procedures generally produce starch yields lower than those of commercial mills (Steinke and Johnson 1991, Fox et al 1992, Wehling et al 1993), these results may be due to the wide diversity of grain types chosen for wet-milling analysis rather than the analytical procedure itself. The 100-g laboratory wet-milling procedure used in this study also has produced starch yields averaging 66.5% in analyses of 131 commercial hybrids (Eckhoff 1995).

Kernel composition. No significant differences were detected among inbred line and hybrid groups for kernel components estimated by NIR (Table II). Average starch content was <68% dwb for both inbred line and hybrid categories, while average protein content was relatively high (≥11.0%). These results followed a trend we have noted in other experiments involving kernel component analysis. Hand-pollination in production of grain, like that used in this study, has resulted in significant increases in protein content for the same genotypes when compared with grain produced by open-pollination (unpublished data). Also, protein and starch contents have shown an inverse relationship. Therefore, increased protein content of grain used in this study may have contributed to lower than anticipated starch content and yield from wet milling.

Grain physical properties. Measurements of average kernel size, 1,000-kernel weight and 1,000-kernel volume, were significantly higher for hybrids compared to inbred lines (Table II). Measures of average grain density and hardness were not significantly

TABLE I
Results from Analyses of Variance for Yields of Wet-Milling Fractions

	Variation Among All Genotypes (Inbred and Hybrid)[a]			Variation Among Groups (Inbred versus Hybrid)[b]			Model
Fraction	**Mean Square**	***F*-Value**	***R*-Square[c]**	**Mean Square**	***F*-Value**	***R*-Square[c]**	***R*-Square[c]**
Steeping solids	0.3980	5.61***[d]	0.83	0.0001	0.00	0.00	0.83
Germ	2.2188	3.95***	0.77	1.2930	0.58	0.01	0.78
Fiber	8.1596	8.19***	0.87	6.6104	0.81	0.01	0.88
Starch	24.4146	9.73***	0.89	2.6991	0.11	0.01	0.90
Gluten	11.1109	4.54***	0.73	46.5786	4.19	0.09	0.82
Filtrate solids	0.6203	2.57**	0.69	0.9199	1.48	0.01	0.70
Total solids recovered[e]	1.7645	1.57	0.55	5.9224	3.36	0.05	0.60

[a] 33 Degrees of freedom (DF) associated with variation among genotypes, inbred lines and hybrids included. Variation among replicates within genotypes (37 DF) used as experimental error in *F*-test of significance.
[b] 1 DF associated with contrast among inbred and hybrid groups. Variation among genotypes used as experimental error in *F*-test of significance.
[c] Percentage of variation accounted for by differences among genotypes, differences among groups, and the total genetic model, respectively.
[d] *, **, and *** = Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
[e] Sum of yields from wet-milling fractions.

TABLE II
Average Yields and Recoveries of Wet-Milling Fractions, Kernel Composition and Grain Physical Properties of 15 Maize Inbred Lines and 20 Hybrids

	Inbred Lines			Hybrids			Among Inbred Lines and Hybrids	
Fraction/Property	**Mean**	**Range**	**SE[a]**	**Mean**	**Range**	**SE[a]**	**Mean Difference[b]**	**SE[a]**
Wet-milling yield/recovery, % dwb								
Steeping solids	3.9	1.4	0.1	3.9	1.4	0.1	+0.0	0.1
Germ	6.1	3.8	0.3	6.0	2.5	0.2	–0.1	0.3
Fiber	13.9	9.3	0.7	13.4	4.7	0.3	–0.5	0.7
Starch	56.2	13.0	1.0	55.6	14.7	0.8	–0.6	1.2
Gluten	15.7	7.5	0.6	17.3	10.5	0.6	+1.6	0.8
Filtrate solids	2.8	2.6	0.2	3.0	1.3	0.1	+0.2	0.2
Starch recovery[c]	84.8	14.5	1.0	82.8	18.4	1.0	–2.0	1.5
Total solids recovered[d]	98.7	3.6	1.0	99.2	3.0	0.2	+0.5	0.3
Composition[e]								
Crude free fat, %	3.8	1.2	0.1	3.8	1.0	0.1	+0.0	0.1
Protein, %	11.2	3.1	0.3	11.0	2.6	0.2	–0.2	0.3
Starch, %	56.7	3.4	0.3	57.0	2.7	0.2	+0.3	0.4
Starch, % dwb	66.8	4.0	0.4	67.1	3.1	0.2	+0.3	0.4
Physical property[f]								
1,000-grain weight, g	268	226	16	359	171	11	+91***[g]	19
1,000-grain volume, cm^3	203	181	12	268	134	9	+65***	15
Absolute density, $g{\cdot}cm^{-3}$	1.32	0.14	0.01	1.34	0.05	0.004	+0.02	0.01
Stenvert time, sec	22.9	17.3	1.2	24.0	12.2	0.7	+1.1	1.3

[a] Standard error of mean for inbred lines, hybrids, and differences between hybrid and inbred means, respectively.
[b] Average of differences between hybrid and inbred line means.
[c] Starch yield from wet-milling as % of starch composition, adjusted to dry basis.
[d] Sum of yields from wet-milling fractions.
[e] Adjusted to 15.5% moisture; starch also adjusted to dry basis.
[f] Grain equilibrated to 12.0% moisture.
[g] *** = Significantly different from zero at 0.001 probability level.

different between groups, although variability among genotypes appeared to be reduced in the hybrid group.

Hybrid: Inbred-Parent Divergence, Heterosis

Nine of 15 inbred lines were used as parents of the 20 hybrids tested in this study. Combinations of these nine inbred lines were used to calculate average mid-parent divergence (difference between hybrid value and mean of two inbred-parent values) and average high-parent heterosis (increase in hybrid value over that of the highest parent mean) for all traits measured (Table III). Significant mid-parent divergence was detected for wet-milling fraction yields of germ, fiber, gluten and filtrate solids, and for average kernel size measurements. Germ and fiber yields were significantly decreased in hybrids by 4.8 and 4.3% of mid-parent means, respectively. Yields of gluten and filtrate solids were significantly increased in hybrids by 13.1 and 15.4% of mid-parent means, respectively. The 1,000-kernel weight and 1,000-kernel volume expressed significant increase in hybrids by 36.0 and 34.7% over mid-parent means, respectively. Only measurements of kernel weight and kernel volume expressed significant high-parent heterosis (Table III). Average gluten yield from hybrid grain was equivalent to the average of the highest yielding parental inbred lines, but was not significantly greater. Yields of other hybrid wet-milling fractions that expressed significant mid-parent divergence were within the parental range.

These data suggest that larger kernels of hybrids are relatively lower in fiber and germ content as a percentage of the total. Also, the enlarged endosperm in hybrid grain may result in increased gluten yield over the average of the parental inbred lines. However, changes in milling yields from hybrid grain relative to their inbred-parents were not proportional to the highly significant increase in kernel size beyond that of either parent, as indicated by high-parent heterosis. Significant mid-parent divergence and high-parent heterosis were indicative of nonadditive gene action, making prediction of wet-milling yields from hybrid grain difficult based on milling evaluation of grain from parental inbred lines.

On average, mid-parent divergence for both starch yield and starch recovery were not significantly different from zero (Table III), suggesting additive gene action in expression of these traits. However, only the regression of hybrid starch yield on mid-parent value was significant (Table III footnote), with a predictive model of (hybrid starch yield = 13.9 + 0.74[mid-parent starch yield]).

Prediction of hybrid starch yield based on milling evaluation of the inbred-parents would be a useful property in development of maize hybrids for wet-milling application. Further research with additional genotypes would be warranted to confirm the additive nature of starch yield inheritance.

Comparison of Inbred and Hybrid-Based Correlations

Among wet-milling yields. Correlation coefficients (r) among starch and fiber yields, and among starch and gluten yields were significant for both inbred and hybrid data sets (Table IV). Inbred-based correlations were $r = -0.72$ and $r = -0.63$ for starch-fiber and starch-gluten relationships, respectively. Hybrid-based correlations were $r = -0.81$ and -0.91 for starch-fiber and starch-gluten relationships, respectively. The remaining significant correlation coefficients among yields of wet-milling fractions were not consistent between inbred line and hybrid analyses (Table IV). Inbred-based correlations tended to associate forms of dissolved solids recovery (steeping or filtrate) with one or more of the other fraction yields. Steeping solids were positively associated with fiber yield ($r = +0.62$) and negatively associated with starch yield ($r = -0.66$). Filtrate solids were negatively associated with germ yield ($r = -0.57$) and positively associated with starch yield ($r = +0.53$). The relationship between total solids recovery and filtrate solids was significant and positive ($r = +0.70$) for inbred lines. However, the same relationship in hybrids was essentially zero. Other inconsistencies involved significant, positive correlations among fiber and gluten yields, and among steeping solid and total

TABLE III

Measures of Hybrid: Inbred-Parent Divergence for Yields of Wet-Milling Fractions, Kernel Composition and Grain Physical Properties

Fraction/Property	Mid-Parent Divergence			High-Parent Heterosis		
	Mean[a]	SE[b]	Mid-Parent Mean[c] (%)	Mean[d]	SE[b]	High-Parent Mean[c] (%)
Wet-milling yield/recovery, % dwb						
Steeping solids	0.0	0.1	0.0	. . .	. . .	. . .
Germ	–0.3	0.1	–4.8**[e]	. . .	. . .	. . .
Fiber	–0.6	0.3	–4.3*	. . .	. . .	. . .
Starch	–0.7	0.7	–1.2[f]	. . .	. . .	. . .
Gluten	+2.0	0.7	+13.1**	+0.5	0.7	+3.0
Filtrate solids	+0.4	0.1	+15.4**	. . .	. . .	. . .
Starch recovery	–2.1	0.9	–2.5[g]	. . .	. . .	. . .
Composition, %[h]						
Crude free fat	0.0	0.1	0.0	. . .	. . .	. . .
Protein	–0.1	0.2	–0.9	. . .	. . .	. . .
Starch	+0.2	0.2	+0.4	. . .	. . .	. . .
Physical property[i]						
1,000-grain weight, g	+95	6	+36.0***	+54	8	+17.7***
1,000-grain volume, cm^3	+69	5	+34.7***	+37	6	+16.0***
Absolute density, $g \cdot cm^{-3}$	+0.01	0.004	+0.8	. . .	. . .	. . .
Stenvert time, sec	+0.7	0.7	+2.9	. . .	. . .	. . .

[a] Average difference between F_1 hybrid and mid-parent mean. Mid-parent values were from 9 inbred lines in 20 pairs representing parents of 20 F_1 hybrids. Each mid-parent value was calculated as an average of two inbred line means.

[b] Standard error of mean.

[c] Average mid-parent divergence expressed as percentage of mid-parent mean, and average high-parent heterosis expressed as percentage of high-parent mean, respectively.

[d] Average increase of F_1 hybrid mean over inbred parent with highest mean.

[e] *, ** and *** = Significantly different from zero at 0.05, 0.01, and 0.001 probability levels, respectively.

[f] Predictive model: (hybrid starch yield = 13.9 + 0.74[mid-parent starch yield]); probability of greater |T| = 0.03.

[g] Predictive model: (hybrid starch recovery = 46.6 + 0.44[mid-parent starch recovery]); probability of greater |T| = 0.28.

[h] Adjusted to 15.5% moisture.

[i] Grain equilibrated to 12.0% moisture.

solid recoveries in hybrid-based evaluations only.

These data support the commonly acknowledged negative relationships between wet-milling yield of starch and those of both fiber and gluten. Correlations involving other comparisons among wet-milling fraction yields were contradictory, and thus general conclusions could not be drawn. Correlation analysis combining all genotypes, inbred line and hybrid inclusive, produced coefficients intermediate to those shown in Table IV (data not shown), and provided no new conclusions.

Between wet-milling yields and kernel composition. In comparisons between wet-milling yields and kernel composition estimated by NIR, a general trend for relatively equal and opposite correlation coefficients was evident in comparisons involving starch and protein composition of both inbred lines and hybrids (Table V). For inbred-based analyses, significant correlations between starch content and wet-milling yields of fiber, starch, starch recovery and gluten were $r = -0.59$, +0.90, +0.80, and –0.54, respectively. Correlations involving inbred line protein content and yields of these same wet-milling fractions were $r = +0.51$, –0.86, –0.76, and +0.64, respectively. For hybrid-based analyses, significant correlations between starch content and wet-milling yields of fiber, starch, starch recovery and gluten were $r = -0.64$, +0.67, +0.50, and –0.57, respectively. Correlations involving hybrid protein content and yields of these same wet-milling fractions were $r = +0.67$, –0.67, –0.50, and +0.54, respectively. Significant and opposite correlation coefficients also were identified between steeping solids and starch or protein content of inbred lines, but not for hybrids. Statistical significance could not be assigned to any of the correlation coefficients involving crude fat free content and yields of wet-milling fractions; although most of these coefficients were ±0.50 for inbred line data.

The trend for opposite and similar relationships of NIR protein and starch contents with yields of wet-milling fractions differs from those observed by Fox et al (1992). The results of Fox and co-workers indicated significant correlations between protein content based on NIR measurement and wet-milling yields of starch, gluten and fiber, but no significant correlations for NIR starch. Differing results between this study and that of Fox et al (1992) may result from differences in wet-milling procedures, as NIR calibrations in both cases were from the same source. Wehling et al (1993) suggested that precision of the laboratory wet-milling reference method was a limiting factor in reliability of correlations between NIR calibrations and wet-milling starch yield. Analyses of variance (Table I) indicated good precision in measurements of wet-milling yields using the 100-g procedure (Singh 1995). We have also observed opposite and similar correlation coefficients involving NIR protein and starch contents with wet-milling yields in other maize genetic studies which have used the 100-g laboratory procedure (unpublished data). High correlation between starch content and starch yield from wet-milling of inbred lines ($r = +0.90$) suggests potential application of NIR-based measurements during inbred line development.

Between wet-milling yields and grain physical properties. Measures of average kernel size, 1,000-kernel weight and 1,000-kernel volume, were significantly correlated with wet-milling yields of fiber ($r = -0.47$ and –0.50, respectively) and starch ($r = +0.48$ and +0.51, respectively) in hybrids (Table VI). Correlation coefficients for these comparisons using inbred lines were similar in magnitude, but not significant at the same level of probability. Measures of absolute kernel density and Stenvert hardness were highly correlated with starch yield ($r = -0.72$ and –0.77, respectively) and starch recovery ($r = -0.84$ and –0.71, respectively) for inbred lines, while hybrid-based correlations for the same comparisons were significant for Stenvert hardness only. Failure to detect hybrid-based correlations involving absolute density may be due to reduced variability for this trait among hybrids as compared to inbred lines (Table II). A consistent relationship between increased Stenvert hardness and increased fiber yield was observed

TABLE IV
Correlation Coefficients Among Yields of Wet-Milling Fractions Based on 15 Maize Inbred Lines and 20 Hybrids

Hybrid-Based Correlations (Below Diagonal)	Inbred-Based Correlations (Above Diagonal)						
	Steeping Solids	Germ	Fiber	Starch	Gluten	Filtrate Solids	Total Solids Recovered[a]
Steeping solids	···	+0.08	+0.62*[b]	–0.66**	+0.21	–0.36	–0.23
Germ	–0.18	···	+0.14	–0.22	–0.27	–0.57*	–0.30
Fiber	+0.42	+0.22	···	–0.72**	+0.05	–0.40	+0.02
Starch	–0.35	–0.17	–0.81***	···	–0.63*	+0.53*	+0.42
Gluten	+0.36	+0.01	+0.58**	–0.91***	···	–0.10	–0.32
Filtrate solids	–0.16	–0.27	–0.09	+0.16	–0.25	···	+0.70**
Total solids recovered[a]	+0.51*	+0.30	+0.33	–0.14***	+0.23	–0.05	···

[a] Sum of yields from wet-milling fractions.
[b] *, ** and *** = Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

TABLE V
Correlation Coefficients Between Kernel Composition and Yields of Wet-Milling Fractions Based on 15 Maize Inbred Lines and 20 Hybrids

Wet-Milling Fraction Yield	Inbred-Based Correlations for Kernel Composition Factors			Hybrid-Based Correlations for Kernel Composition Factors		
	Starch	Protein	Crude Free Fat	Starch	Protein	Crude Free Fat
Steeping solids	–0.80***[a]	+0.76**	+0.46	–0.02	+0.01	+0.19
Germ	–0.02	–0.14	+0.52	–0.27	+0.32	–0.01
Fiber	–0.59*	+0.51	+0.48	–0.64**	+0.67**	+0.31
Starch	+0.90***	–0.86***	–0.48	+0.67**	–0.67**	–0.35
Starch recovery[b]	+0.80***	–0.76**	–0.50	+0.50*	–0.50*	–0.30
Gluten	–0.54*	+0.64*	–0.07	–0.57**	+0.54*	+0.33
Filtrate solids	+0.14	–0.08	–0.47	–0.10	+0.11	–0.10
Total solids recovered[c]	+0.27	–0.22	–0.24	–0.25	+0.24	+0.10

[a] *, ** and *** = Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
[b] Starch yield from wet-milling as % of starch composition, adjusted to dry basis.
[c] Sum of yields from wet-milling fractions.

TABLE VI
Correlation Coefficients Between Grain Physical Properties and Yields of Wet-Milling Fractions Based on 15 Maize Inbred Lines and 20 Hybrids

	Inbred-Based Correlations for Grain Physical Properties				Hybrid-Based Correlations for Grain Physical Properties			
Wet-Milling Fraction Yield	1,000-Kernel Weight	1,000-Kernel Volume	Absolute Density	Stenvert Time	1,000-Kernel Weight	1,000-Kernel Volume	Absolute Density	Stenvert Time
Steeping solids	–0.27	–0.34	+0.49	+0.67**[a]	–0.43	–0.43	–0.05	+0.24
Germ	+0.05	–0.03	+0.16	+0.14	–0.05	–0.08	+0.60**	+0.39
Fiber	–0.45[b]	–0.48[b]	+0.39	+0.59*	–0.47*	–0.50*	+0.30	+0.56*
Starch	+0.40[b]	+0.46[b]	–0.72**	–0.77***	+0.48*	+0.51*	–0.42	–0.66**
Starch recovery[c]	+0.15	+0.22	–0.84***	–0.71**	+0.50*	+0.52*	–0.39	–0.69***
Gluten	–0.13	–0.16	+0.48	+0.40	–0.51*	–0.53*	+0.41	+0.64**
Filtrate solids	+0.18	+0.25	–0.48	–0.76**	+0.36	+0.37	–0.31	–0.25
Total solids recovered[d]	+0.19	+0.26	–0.63*	–0.68**	–0.36	–0.38	+0.32	+0.41

[a] *, ** and *** = Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
[b] Significant at 0.10 probability level, for comparison with the same hybrid-based correlations.
[c] Starch yield from wet-milling as % of starch composition, adjusted to dry basis.
[d] Sum of yields from wet-milling fractions.

for both inbred lines ($r = +0.59$) and hybrids ($r = +0.56$).

Measures of average kernel size also were significantly correlated with starch recovery (positive) and gluten yield (negative) in hybrid comparisons, but not inbred lines (Table VI). Other inconsistencies in correlated factors between inbred lines and hybrids were significant relationships of density-germ yield and hardness-gluten yield for hybrids only, and significant relationships between grain hardness and forms of dissolved solids recovery for inbred lines only. For inbred-based comparisons, a significant positive correlation was detected between Stenvert hardness and steeping solids, while significant negative correlations were detected between Stenvert hardness and both filtrate solids and total solids recovery. Absolute density was negatively associated with total solids recovery for inbred lines only.

These data suggest that among hybrids, those with larger kernel size have decreased fiber and gluten yields, and increased starch yield and starch recovery. Only the negative relationship between kernel size and fiber yield was consistent with trends observed between inbred lines and their hybrids, as measured by mid-parent divergence (Table III). However, correlation coefficients for these comparisons were not large (approximately ±0.50), and did not increase when calculated across all genotypes, inbred and hybrid inclusive (data not shown).

The strongest and most consistent correlations among inbred line and hybrid evaluations were negative between grain hardness and starch yield from wet-milling. This supports the generalization that hybrids with increased grain hardness are less likely to produce high starch yields when wet-milled. Increased hardness was positively associated with NIR protein content, and negatively associated with NIR starch content (data not shown). However, a simple categorization of relatively hard versus soft grain endosperm is not the only issue of importance in wet-milling, as the ability to maintain postharvest grain quality must be considered. If possible, selection of grain endosperm texture in maize breeding programs should focus on achieving a degree of grain hardness necessary to maintain postharvest quality, yet contain a relatively low amount of protein to enhance prospects for high starch extraction during wet-milling.

CONCLUSIONS

Analyses of variance indicated that precision of measurements from the 100-g laboratory wet-milling procedure used in this study were sufficient for detection of genetic differences among maize inbred lines and hybrids. Analyses of mid-parent divergence revealed differences in wet-milling properties of grain from hybrids as compared to their inbred-parents, although not to the extent of heterosis producing increased kernel size. In general, the larger kernels of hybrids tended to produce less germ and fiber yields, and increased gluten and filtrate solids in comparison to grain from their inbred-parents. On average, starch yield and starch recovery expressed additive gene action between inbred lines and their F_1 hybrids. However, only the predictive model for hybrid starch yield was significant based on mid-parent values. Correlations between NIR starch composition and starch yield from the 100-g laboratory wet-milling procedure were positive, significant and consistent across inbred and hybrid-based evaluations. Significant negative correlations between grain hardness and starch yield or starch recovery also were consistent across inbred lines and hybrids. Other correlations among wet-milling yields, kernel composition and grain physical properties were inconsistent when comparing inbred lines and hybrids. These data suggest that caution be used when making general interpretations of wet-milling yields from grain of inbred lines with respect to potential hybrid combinations. However, the average value of inbred-parents may be useful in projecting hybrid starch yield from wet-milling. Furthermore, strong and consistent correlations between starch content estimated by NIR and starch yield from wet-milling suggest potential use of NIR measurements during inbreeding to produce new maize lines for testing in hybrid combinations. Also, the negative relationship between grain hardness and starch extraction during wet-milling must be overcome if hybrids with both high starch yield and adequate postharvest grain quality are to be developed.

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