Wet-Milling and Dry-Milling Properties of Dent Corn with Addition of Amylase Corn

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ABSTRACT

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A transgenic corn (amylase corn) has been developed that produces an endogenous α-amylase that is activated in the presence of water and elevated temperature (>70°C). Wet- and dry-milling characteristics of amylase corn were evaluated using laboratory wet- and dry-milling procedures. Different amounts of amylase corn (0.1–10%) were added to dent corn (with the same genetic background as the amylase corn) as treatments. Samples were evaluated for wet- and dry-milling fraction yields using 1-kg laboratory procedures. Milling yields for all amylase corn treatments were compared with the control treatment (0% amylase corn or 100% dent corn). No significant differences were observed in wet- and dry-milling yields between the control and the 0.1, 1, and 10% amylase corn treatments. Most of the amylase activity (77%) in wet-milling fractions was detected in the protein fraction. In dry-milling, amylase activity (68.8%) was detected in endosperm fractions (fines, small grits, and large grits).

An amylase corn has been developed by transgenic technology that produces an endogenous α-amylase in endosperm that is activated in the presence of water and elevated temperature (>70°C) (Lanahan et al 2003). Amylase corn has been developed for the dry-grind process to aid the production of fuel ethanol and is being considered as a substitute for liquefaction enzymes currently used in the dry-grind corn process. In a conventional corn dry-grind process, exogenous α-amylases are added during liquefaction to break starch into dextrins. Use of these exogenous enzymes add to operating costs of a dry-grind ethanol plant. The expression levels of endogenous α-amylase in amylase corn are high; therefore, it is expected that only a small amount of amylase corn addition to regular dent corn is required to achieve adequate liquefaction. It is expected that use of amylase corn will potentially reduce operating costs in a dry-grind plant.

Production of amylase corn should be similar to production of any other genetically modified corn hybrid. Although current production and merchandising systems are very efficient in preserving the identity of corn hybrids when necessary, there is a small possibility for commingling of amylase corn with commodity corn typically processed by the corn wet-milling or dry-milling industries. In wet-milling processing, corn is fractionated into individual components of starch, protein, fiber, germ, and solubles in an aqueous medium (Johnson and May 2003). Starch is further processed to produce high-fructose corn syrup or ethanol. In wet-milling, protein, fiber, and solubles are mixed together or sold individually for animal food. Wet-milled germ is used for recovery of corn oil, which is mainly used as human food. In dry-milling, corn is dry-fractionated into grits (endosperm), germ, pericarp fiber, and flour (Duensing et al 2003). Grits are mainly used for producing breakfast cereals. Dry-milled germ, pericarp fiber, and flour are mixed together or sold individually for animal and human food.

Commingling of amylase corn with commodity corn could potentially affect the corn wet-milling and dry-milling processes. In the wet-milling process, corn is hydrated in dilute sulfurous acid for 24–36 hr at 52°C. Presence of water and temperature could activate α-amylase in amylase corn and affect wet-milling yields (solubles, germ, fiber, starch, and protein). In dry-milling, corn is tempered by adding warm water to increase moisture content to ≈22% and milled in a degerminator to fractionate germ, coarse fiber, and endosperm. During the dry-milling process, the corn temperature increases to ≈45°C, it is possible that α-amylase might become activated and affect dry-milling fraction yields.

The effect of amylase corn in conventional corn wet- or dry-milling processes is not known. The objective of this study was to evaluate conventional wet-milling and dry-milling processes when amylase corn is mixed with dent corn.

MATERIALS AND METHODS

Experimental Material and Design

Transgenic amylase corn and dent corn with the same genetic background were obtained from a commercial seed company (Syngenta Biotechnology, Inc., Research Triangle Park, NC). Corn samples were hand-cleaned to remove broken corn and foreign material, packaged in plastic bags, and stored at 4°C until processing. Whole kernel moisture content was measured using the 103°C convection oven method (Approved Method 44-15A, AACC International 2000). Two experiments evaluated performance of amylase corn in wet- and dry-milling processes. In the first experiment, four levels of amylase corn (0, 0.1, 1, and 10%) were added to dent corn as treatments, and samples were evaluated for yields in a 1-kg laboratory wet-milling procedure. In a second experiment, four amylase corn treatments (0, 0.1, 1, and 10%) were evaluated for yields in a 1-kg laboratory dry-milling procedure. Zero percent amylase corn treatment (100% dent corn) was the control treatment for both experiments.

Wet-Milling Procedure

Replicate corn samples (1 kg) were fractionated into starch, protein, germ, fiber, and steepwater solubles. Corn was laboratory wet-milled using a procedure developed by Eckhoff et al (1993). Starch, protein, germ, fiber, and soluble yields were reported as a percentage of the original sample dry weight on a dry basis. Moisture contents of fractions were determined using a two-stage convection oven method (Approved Method 44-18, AACC International 2000). To observe visual differences, milling fractions were placed in a convection oven at 60°C for 24 hr and discoloration (if any) was observed.

Dry-Milling Procedure

In the dry-milling procedure, replicate samples (1 kg) were tempered for 18 min after the addition of 8.5% (by weight) water to the corn. Tempered corn was passed through a horizontal drum

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TABLE I
Wet-Milling Yields Among Control (0% Amylase Corn) and 0.1, 1.0, and 10% Amylase Corn Treatments[a]

Fractions (%)	Control Treatment	Amylase Corn Treatments 0.1%	1.0%	10%
Steepwater	4.52 ± 0.21	4.40 ± 0.01	4.38 ± 0.18	4.82 ± 0.30
Germ	6.21 ± 0.22	6.35 ± 0.03	6.43 ± 0.37	6.74 ± 0.10
Fiber	12.36 ± 0.09	11.72 ± 0.11	11.98 ± 0.88	11.90 ± 0.12
Starch	67.24 ± 0.18	67.66 ± 0.29	67.33 ± 1.11	66.19 ± 0.75
Protein	10.25 ± 0.17	10.18 ± 0.28	10.16 ± 0.05	10.65 ± 0.25
Total	100.59 ± 0.18	100.31 ± 0.12	100.29 ± 0.82	100.30 ± 0.43

[a] Mean ± SD.

TABLE II
Dry-Milling Yields Among Control (0% Amylase Corn) and 0.1, 1.0, and 10% Amylase Corn Treatments[a]

Fractions (%)	Control Treatment	Amylase Corn Treatments 0.1%	1.0%	10%
Large grits	31.42 ± 1.89	33.23 ± 2.38	30.59 ± 1.81	28.73 ± 1.04
Small grits	29.88 ± 1.34	28.91 ± 1.69	31.79 ± 1.41	31.46 ± 0.09
Fines	18.01 ± 1.20	17.47 ± 0.53	16.65 ± 3.97	18.18 ± 0.07
Germ	13.02 ± 0.83	12.88 ± 0.09	13.32 ± 1.17	13.79 ± 0.37
Pericarp	7.45 ± 0.27	7.57 ± 0.11	7.64 ± 0.15	7.60 ± 0.22
Total	99.78 ± 0.46	100.06 ± 0.14	99.98 ± 0.27	99.76 ± 0.73

[a] Mean ± SD.

degerminator that impacts and abrades the corn, resulting in partial separation of germ and fiber from endosperm. The product was dried for 1 hr at 49°C to ≈15% moisture. A subsample (10–15 g) was used to determine moisture content using a two-stage convection oven method (Approved Method 44-18, AACC International 2000). Materials were sieved using a laboratory sifter (model P1202, Great Western Manufacturing, Leavenworth, KS). The fraction that passed over a standard 5-mesh sieve (+5 mesh, 4.0-mm openings) was roller milled to flatten the germ and aspirated (model 6DTA, Kice Metal Products, Wichita, KS) (0.4–0.5 in. of water vacuum) to remove the pericarp fraction. The material not removed by aspiration (heavy fraction) was sifted on a standard 10-mesh screen (1.68-mm openings) to remove the flattened germ particles. The remaining endosperm fraction was weighed and identified as large grit. The portion passing through the 5-mesh sieve also was roller milled, aspirated, and sifted on a 10-mesh sieve. The lighter material removed by the aspirator was added to the pericarp fraction. Heavier material from the aspirator was sifted on a standard 10-mesh sieve. Material passing over the 10-mesh sieve (primarily germ particles) was added to the germ fraction, and material passing through the 10-mesh sieve (primarily endosperm particles) was sifted on a standard 24-mesh sieve (0.707-mm openings). The material passing over the 24-mesh sieve were identified as small grit, and material passing through the 24-mesh sieve was called fines. Data were reported as a percentage of the original sample dry weight on a dry basis. To observe visual differences, milling fractions were placed in a convection oven at 60°C for 24 hr and discoloration (if any) was observed.

Residual Enzyme Activity in Wet- and Dry-Milling Fractions

The residual enzyme activity in the wet-milled fractions (starch, protein, germ, fiber, and solubles) and dry-milled fractions (large grits, germ, pericarp, small grits, and fines) was assayed using an amylase assay kit (Ceralpha HR, Megazyme International, Wicklow, Ireland). Dried samples were ground and suspended in 10× weight of 50 m*M* CAPS buffer, pH 10.0. The samples were adjusted to pH 10 as needed. Slurries were mixed at 55°C for at least 1 hr then centrifuged at 3,220 × *g* for 15 min. Aliquots from each supernatant were diluted serially in assay buffer (250 m*M* MOPS buffer, pH 7.0) to bring the enzymatic activity into the range of the Ceralpha HR assay. Enzyme assay (Ceralpha HR reagent, 50 μL) was mixed with diluted enzyme (50 μL) in 96-well PCR plates. An aliquot (25 μL) was removed and mixed with Tris base (1*M* × 175 μL). The PCR plates were then heated to 60°C for 20 min and cooled to 4°C. Heating and cooling were at the maximum rate of the thermocycler (PE 9700, PerkinElmer, Wellesley, MA). An additional 25-μL aliquot was removed from each assay and mixed with Tris base as above. The quenched samples were arrayed in a 96-well spectrophotometer plate and A_{400} wavelength was read using a spectrophotometer (Spectromax Plus, Molecular Devices, Sunnyvale, CA). The pathlength correction feature of the spectrophotometer was used to correct to the absorbance of a 1-cm sample. Samples that yield an absorbance of 0.5–1.5 are in the linear range of the assay. Units of enzyme (1 μmol of PNP produced/min) were determined using the known molar absorbancy of PNP (18,100 M^{-1} cm^{-1}) according to the instructions of the manufacturer. The dilution of the extract and the weight of extracted sample were used to provide a result in terms of units of amylase per gram dry weight (U/g).

Statistical Analysis

Wet- and dry-milling of all treatments was conducted with three replicates. Analysis of variance (ANOVA) and Duncan's multiple range test (SAS Institute, Cary, NC) were used to compare yield means among treatments. The level selected to show statistical significance was 5% ($P < 0.05$).

RESULTS AND DISCUSSION

Wet-Milling Fractions

No differences were observed in the steepwater solubles, germ, starch, protein, and fiber yields for 0.1, 1, and 10% amylase treatments compared with the control treatment (Table I). Total recovery of wet-milling fractions for each replicate was >99%, indicating negligible loss of material during milling. There were no qualitative differences in wet-milling characteristics such as germ floatation, fiber washing, fiber dewatering, or starch protein separation for any of the amylase corn treatments. No differences were observed in the appearance (discoloration or browning) of wet-milling fractions in any of the treatments after drying the samples in a convection oven. Fraction yields and wet millability of amylase corn treatments suggest that accidental mixing of amylase corn up to 10% with regular yellow dent corn will not have a significant effect on milling yields in a conventional wet-milling plant.

TABLE III
Amlyase Content (U/g) in Wet-Milling Fractions for Control (0% Amylase Corn) and 0.1, 1.0, and 10% Amylase Corn Treatments[a]

		Amylase Corn Treatments		
Fractions	**Control Treatment**	**0.1%**	**1.0%**	**10%**
Solubles	0.10 ± 0.14	0.14 ± 0.00	0.03 ± 0.04	0.06 ± 0.08
Germ	0.13 ± 0.10	0.10 ± 0.01	0.28 ± 0.33	1.12 ± 1.54
Fiber	0.06 ± 0.04	0.04 ± 0.01	0.31 ± 0.09	1.73 ± 0.45
Starch	0.00 ± 0.00	0.00 ± 0.01	0.07 ± 0.08	0.04 ± 0.01
Protein	0.10 ± 0.02	0.02 ± 0.40	3.68 ± 2.60	10.35 ± 3.76

[a] Mean ± SD.

TABLE IV
Amlyase Content (U/g) in Dry-Milling Fractions for Control (0% Amylase Corn) and 0.1, 1.0, and 10% Amylase Corn Treatments[a]

		Amylase Corn Treatments		
Fractions	**Control Treatment**	**0.1%**	**1.0%**	**10%**
Large grits	0.04 ± 0.01	0.04 ± 0.02	0.08 ± 0.05	2.13 ± 0.15
Small grits	0.01 ± 0.01	0.02 ± 0.01	0.09 ± 0.04	2.88 ± 0.72
Fines	0.03 ± 0.03	0.01 ± 0.01	0.07 ± 0.07	2.44 ± 0.49
Germ	0.09 ± 0.05	0.14 ± 0.09	0.63 ± 0.26	1.87 ± 0.83
Pericarp	0.00 ± 0.02	0.01 ± 0.01	0.20 ± 0.06	1.51 ± 0.46

[a] Mean ± SD.

Dry-Milling Fractions

No differences were observed in yields for large grits, germ, pericarp, small grits, and fines among the control and 0.1, 1, and 10% amylase corn treatments (Table II). Total yield of the dry-milling fractions for each replicate was >99%, indicating negligible loss of material during milling. No qualitative differences were observed in dry-milling characteristics such as degermination, sifting, or aspiration for any of the amylase corn treatments. No discoloration or browning was observed in dry-milling fractions in any of the treatments after drying the samples in a convection oven. Based on these results, accidental mixing of amylase corn with regular yellow dent corn will not have a significant effect on dry-milling yields in a conventional dry-milling plant.

Amylase Activity in Wet-Milling Fractions

Negligible amylase activities (<0.14 U/g) were detected in wet-milling fractions for control and 0.1% amylase corn treatments (Table III). Very low amylase activities (<3.68 U/g) were detected in the germ, fiber, and protein fractions for 1.0% amylase corn treatments. Negligible amylase activities (<0.07 U/g) were detected in the starch fractions for all wet-milling treatments. Amylase activity detected in protein, germ, and fiber fractions increased as the percent of amylase corn treatment increased from 1.0–10%. For 1 and 10% amylase corn treatments, most amylase activity (>77%) was detected in the protein fraction (10.35 U/g). These results suggest that most of the enzyme in the amylase corn is located in the corn endosperm and is recovered with the protein fraction during wet milling.

Amylase Activity in Dry-Milling Fractions

Negligible amylase activities (<0.14 U/g) were detected in dry-milling fractions for control and 0.1% amylase corn treatments (Table IV). Very low amylase activities (<0.63 U/g) were detected in dry-milling fractions from the 1.0% amylase corn treatment. Amylase activity in all fractions increased as the percent amylase corn treatment increased from 1.0 to 10%. For 1 and 10% amylase corn treatments, amylase activity was almost evenly distributed among the dry-milling fractions. These results from the dry-milling experiment indicate that most of the enzyme is located in the endosperm (also as observed in wet-milling fractionation) and is recovered with grits (small and large) and fines. For 10% amylase corn treatment, 68.8% of total enzyme activity was detected in the endosperm fraction (22.5, 26.6, and 19.6% for fines, small grits, and large grits, respectively).

Although no effect of amylase corn (up to 10%) was observed on wet- and dry-milling yields, it is possible that amylase corn could have an effect on final end products of wet- and dry-milling fractions. Quality measurements are warranted to determine these effects.

CONCLUSIONS

Presence of amylase corn (up to 10 %) in regular yellow dent corn resulted in no differences in yields for wet- or dry-milling processes. No differences were observed in wet- or dry-milling yields among the control and 0.1, 1.0, and 10% amylase corn treatments. Visually, no qualitative differences were observed in wet- or dry-milling characteristics for any of the amylase corn treatments. Most (>77%) amylase activity in wet-milling fractions was detected in the protein fraction and 68.8% of the amylase activity was detected in endosperm fractions during dry milling.

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