

# Variability in Starch Acetylation Efficiency from Commercial Waxy Corn Hybrids

M. R. Wilkins,<sup>1</sup> P. Wang,<sup>1</sup> L. Xu, Y. Niu,<sup>1</sup> M. E. Tumbleson,<sup>2</sup> and K. D. Rausch<sup>3,4</sup>

## ABSTRACT

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Raw material variability is common for starch processors and is responsible for increased processing costs. In this study, variability of starch acetylation due to hybrid influence was quantified. Six waxy corn (maize) hybrids from 1998 and five waxy corn hybrids from 1999 were wet-milled in the laboratory. Starch obtained from each hybrid was modified according to a laboratory-scale acetylation procedure. To evaluate reaction efficiency, reaction rate, acetyl content, pH, and amount of NaOH used were recorded for each reaction. After modification, a Rapid Visco Analyser (RVA) was used to characterize modified starches and determine differences in modified starches from different hybrids.

Using the same acetylation protocol, reaction efficiencies were observed at 47–73%. Reaction efficiencies were significantly lower for 1998 hybrids (50.0%) compared with the efficiency observed for the same hybrids grown in 1999 (62.7%). Acetylated starch from 1999 had increased peak, trough and final viscosities and increased reaction efficiency as compared with acetylated starch from 1998. Differences in setback were observed among 1998 hybrids for acetylated samples. Differences in trough and final viscosity were observed among 1999 hybrids for acetylated and native (unmodified) samples. Differences in breakdown among 1999 hybrids also were observed for native samples.

Modified starches are common ingredients in food and industrial products. Numerous physical and chemical modifications may be applied to starch to impart properties that are useful for particular applications. One common starch modification is acetylation, which is the esterification of starch polymers with acetyl groups to form starch acetates (Jarowenko 1986). Acetyl groups disrupt interactions among outer chains of amylopectin, the branched form of starch, and among amylose chains, the linear starch polymer (Jarowenko 1986). Decreased interactions prevent cloudiness and syneresis in aqueous starch dispersions stored at low temperatures (Rutenberg and Solarek 1984). Compared with native starches, acetylated starches also have lower gelatinization temperatures, higher peak viscosity, and reduced retrogradation upon cooling (Rutenberg and Solarek 1984). Corn (maize) starch obtained by wet milling is the predominant source of starch in the United States (Jarowenko 1986).

Final product inconsistency arises from three main factors: operator error, process equipment variability, and raw material variability. With increased sophistication in control systems and process equipment, operators are able to control reaction conditions more effectively. Therefore, variation in raw materials entering the process must be managed to control final product characteristics. Inconsistencies in modified starch properties force processors to increase reaction times and reagent amounts used in modification reactions. Reactions may need to be repeated to produce a product that meets customer specifications. Raw material variability increases capital costs and material costs for starch processors.

The source of corn hybrid influences numerous processes associated with corn milling. Peplinski et al (1989) dry-milled corn from several hybrids and observed that yields of milling fractions were hybrid-dependent. Using a laboratory wet-milling process, Zehr et al (1995) found starch and coproduct yields varied among different hybrids. During wet milling, Singh et al (1997) found that the increase of starch yield on addition of lactic acid to steep tanks was hybrid-dependent. Singh et al (1998) found that the effects of moisture content and drying temperature on starch yield during postharvest handling were hybrid-

dependent. De Boer (1991) investigated how starches from waxy and dull waxy corn reacted differently to several modifications including acetylation combined with cross-linking. No studies have been published that quantify how starches extracted from supposedly similar commercial waxy hybrids respond to acetylation alone. The objective of this study was to determine how starch from individual waxy corn hybrids affects acetylation efficiency and starch properties when acetylated by the same protocol.

## MATERIALS AND METHODS

### Wet Milling

Six waxy corn hybrids (1 through 6) grown during the 1998 crop year and five waxy corn hybrids (4 through 8) grown during the 1999 crop year were provided by a commercial seed company. Three hybrids (4, 5, and 6) were grown during both seasons. All hybrids were grown on the same plots. Each hybrid was available commercially during the two crop years.

Three 1,000-g replicate corn samples of each hybrid (except hybrid 2, which had only two replicates due to seed availability) were milled according to the procedure of Eckhoff et al (1993) to extract starch. Replicate starch samples were organized into milling blocks (a through f), each block containing one replicate from each hybrid grown during that season (except block c, which did not contain hybrid 5). Blocks a through c contained starch samples extracted from hybrids grown in 1998, blocks d through f contained starch samples extracted from hybrids grown in 1999. Hybrids were milled randomly within each block. Blocks a and b were milled six months before block c. Blocks d, e, and f were milled within a two month time frame.

TABLE I  
Properties of Waxy Starch from a Commercial Source<sup>a,b</sup>

Properties	Native (unacetylated)		Laboratory Acetylated	
	Mean	SD	Mean	SD
Reaction efficiency (%)	na	na	86	1
Viscosity (cp)				
Peak	627	16	695	22
Trough	240	18	343	18
Breakdown	387	16	352	30
Final viscosity	325	18	435	22
Setback	85	4	92	12
Pasting temp. (°C)	75.8	1	71.5	0.8

<sup>1</sup> Graduate fellow, academic professional, research specialist, and academic professional, respectively; Department of Agricultural Engineering, University of Illinois, Urbana, IL.

<sup>2</sup> Professor, Veterinary Biosciences, University of Illinois, Urbana, IL.

<sup>3</sup> Assistant professor, Agricultural Engineering Department, University of Illinois, Urbana, IL 61801.

<sup>4</sup> Corresponding author. E-mail: krausch@uiuc.edu. Phone: 217-265-0697. Fax: 217-244-0323.

<sup>a</sup> C\*Gel C04230, Lot 1463-15, Cerestar USA, Hammond, IN.

<sup>b</sup> SD, standard deviation; na, not applicable.

**TABLE II**  
Properties of Acetylated Samples from 1998 Hybrids<sup>a</sup>

Properties	Hybrid					
	1	2	3	4	5	6
Reaction efficiency (%)	46.6 ± 1.8	49.2 ± 0.1	58.2 ± 8.0	46.8 ± 5.0	55.8 ± 17.6	47.4 ± 1.5
Trough viscosity (cP)	355 ± 45	364 ± 30	379 ± 28	285 ± 21	351 ± 47	383 ± 63
Final viscosity (cP)	450 ± 27	443 ± 33	483 ± 35	364 ± 22	432 ± 51	487 ± 60
Setback (cP) <sup>b</sup>	95 ± 19	79 ± 3	104 ± 9	79 ± 10	81 ± 13	104 ± 3

<sup>a</sup> Values reported as mean of three replicates ± one standard deviation, except hybrid 2, which is the mean of two replicates.

<sup>b</sup> Significantly different among hybrids ( $P < 0.05$ ).

**TABLE III**  
Properties of Acetylated Starch Samples from 1999 Hybrids<sup>a</sup>

Properties	Hybrid				
	4	5	6	7	8
Reaction efficiency (%)	61.6 ± 4.5	68.1 ± 8.7	58.3 ± 5.0	61.4 ± 1.0	72.8 ± 5.9
Trough viscosity (cP) <sup>b</sup>	351 ± 16	414 ± 48	395 ± 45	350 ± 10	473 ± 13
Final viscosity (cP) <sup>b</sup>	440 ± 25	506 ± 60	494 ± 32	439 ± 25	573 ± 31

<sup>a</sup> Values reported as mean of three replicates ± one standard deviation.

<sup>b</sup> Significantly different among hybrids ( $P < 0.05$ ).

**TABLE IV**  
Properties of Native Starch Samples from 1999 Hybrids<sup>a</sup>

Properties	Hybrid				
	4	5	6	7	8
Trough viscosity (cP) <sup>b</sup>	255 ± 15	292 ± 30	276 ± 13	276 ± 13	327 ± 31
Breakdown (cP) <sup>b</sup>	503 ± 15	457 ± 15	489 ± 6	491 ± 21	443 ± 25
Final viscosity (cP) <sup>b</sup>	346 ± 20	391 ± 28	382 ± 19	372 ± 17	425 ± 33

<sup>a</sup> Values reported as mean of three replicates ± one standard deviation.

<sup>b</sup> Significantly different among hybrids ( $P < 0.05$ ).

## Acetylation

One starch replicate from each hybrid-block combination was acetylated using conditions described in Jarowenko (1986). To assess reliability of the acetylation procedure, 13 samples obtained from a single lot of commercial waxy starch (C\*Gel 04230, Lot J1463-15, Cerestar USA, Hammond, IN) also were acetylated. For each reaction, 180 g of starch were combined with 514 g of water to obtain a solid-to-water ratio of 0.35. The starch slurry was mixed for 1 hr to fully suspend starch granules. At 30°C, slurry pH was adjusted to 8.2 with 1.5% (w/v) NaOH. Using a peristaltic pump (model 77200-60, Cole-Parmer, Vernon Hills, IL) with 0.8-mm diameter tubing (Masterflex L/S 13, Cole-Parmer), 10.4 g (6% of solids) of acetic anhydride were added into the agitated slurry at a constant rate of 0.24 mL/min while maintaining a range of pH 8.0–8.4 with 1.5% NaOH. Reaction rate, amount of NaOH used, and pH were measured for each reaction. The reaction was allowed to continue after acetic anhydride addition was completed until slurry pH stabilized. A small amount (1.2–1.8 mL) of 6M HCl was added to the slurry with a pipette until it was lowered to pH 6.0 to halt the reaction. The slurry was vacuum-filtered through Whatman #3 filter paper. The cake was mixed with 500 mL of distilled water and refiltered to remove salts. Refiltered cake was dried at 49°C overnight. Blocks a and b were acetylated within a two-week period but block c was acetylated three months later. Blocks d, e and f were acetylated within a six-week period.

## Starch Analyses

Each acetylated starch sample was analyzed using a Rapid Visco Analyser (RVA) (model RVA-4, Newport Scientific Pty. Ltd., Warriewood, Australia) to determine sample pasting properties. Corresponding native samples from each hybrid and milling block also were analyzed. To determine repeatability of the RVA technique, 10 native waxy samples from a commercial source (C\*Gel 04230) were analyzed. The Staley-01 method (Anonymous 1997) was used to analyze the samples with one modification. A solids content of 4% (w/w) instead of 4.5% was used due to irregularities during measurement of

breakdown when 4.5% was used. For each RVA analysis, the sample was held at 50°C for 30 sec, heated at a constant rate (0.30°C/sec) to 95°C over 2.5 min, held at 95°C for 20 min, cooled at a constant rate (0.25°C/sec) to 50°C over 3 min and held at 50°C for 9 min.

Acetyl content of each sample was determined using Method C-2 of the Corn Refiners Association (CRA 1993). Each sample was measured in duplicate using 0.3 g of sample for each measurement. The amount of acetyl in each sample was calculated using a calibration curve developed using a spectrophotometer (Spectronic 20 Genesys, Spectronic Unicomb, Rochester, NY) and several solutions of known acetyl content. Sample absorbance was measured and the amount of acetyl in the sample was calculated using the calibration curve. Acetyl percent (CRA 1993) and degree of substitution (DS) (Wurzburg 1964) were calculated as

$$\% \text{ acetyl} = (\mu\text{g of acetyl} \times 2,500) / [\text{sample wt} \times (1 - \% \text{ of moisture in initial sample})] \quad (1)$$

$$\text{DS} = (162 \times \% \text{ acetyl}) / [4,300 - (42 \times \% \text{ acetyl})] \quad (2)$$

Reaction efficiency (RE) (Ed DeBoer, *personal communication*) was calculated as

$$\text{RE} = \% \text{ acetyl} / 6 \times (43/102) \quad (3)$$

This equation shows that only one acetyl group from each molecule of acetic anhydride is available for reaction (Rutenberg and Solarek 1984). Reaction efficiency measured how effectively starch samples reacted with acetyl groups. Each starch sample was reacted with the same amount of acetic anhydride. Protein content of each unacetylated sample was determined using Approved Method 46-13 (AACC 2000).

## Statistical Analyses

Analysis of variance (ANOVA) at a 95% confidence level was performed using the mixed procedure in SAS release 8.12 (SAS Institute, Cary, NC). Hybrids were separated by crop year and analyzed. Hybrid and milling block were dependent variables; peak

viscosity, trough viscosity, final viscosity, breakdown, setback, pasting temperature, and reaction efficiency were independent variables. An additional analysis of variance was conducted on data from the three hybrids grown during both growing seasons (4, 5 and 6) using the same independent variables and crop year as the dependent variable.

## RESULTS AND DISCUSSION

Properties of laboratory-acetylated starch from commercial sources used to determine precision of the acetylation procedure are shown in Table I. Mean reaction efficiency for commercial samples was 86% with a coefficient of variation (COV) of 1%. The reaction efficiency for starch from commercial sources was higher than that of starch from experimental sources; however, starch from other production lots may have shown different efficiencies. The commercial-scale drying process used may have affected reaction efficiency of commercial starch. Standard deviation of viscosities were 12–30 cP for laboratory-acetylated starch from commercial sources, while pasting temperature standard deviation was 0.80°C. Standard deviations of viscosities were 4–18 cP for unmodified starch produced commercially, while pasting temperature standard deviation was 1.0°C. Both the acetylation procedure and RVA analysis were deemed reliable and consistent.

Acetylated samples exhibited greater peak, trough, and final viscosities than native samples, and had lower breakdown and pasting temperatures than native samples (Table I). There were no differences in setback among acetylated and native samples.

Reaction efficiency variation was not significant for starch samples obtained from 1998 hybrids (Table II). Differences were observed in setback among acetylated starches produced from corn hybrids grown in 1998. No differences were observed for any other properties among 1998 acetylated starches, and no differences in any properties were observed among native starches produced from 1998 hybrids (data not shown).

Reaction efficiency varied from 58.3 to 72.8% for starch samples obtained from hybrids grown in 1999 (Tables III and IV). Differences among hybrids in trough and final viscosities were observed for acetylated starches from corn grown in 1999. Differences in trough viscosity, breakdown, and final viscosity were found among hybrids

for 1999 native starches. Reaction times were measured for each reaction and did not have an effect on any properties or reaction efficiency. Properties that exhibited a hybrid effect in acetylated samples generally exhibited an effect in corresponding native samples. The only exception to this was setback in acetylated starches from 1998 hybrids. No differences were seen between native and acetylated starches in setback. Variability in RVA properties was the result of factors occurring before the modification procedure.

Among 1998 hybrids, samples from block c were milled later than samples from other blocks. Block c samples exhibited lower gluten yields (Fig. 1) and lower reaction efficiencies than for other 1998 samples (Fig. 2). There were no differences in protein content of starch among blocks (data not shown). If data from block c are removed from the 1998 hybrid data set, differences among hybrids are found for peak viscosity, trough viscosity, breakdown, and reaction efficiency. No blocking effects were observed among 1999 hybrids (blocks d through f). A large amount of variation was observed among samples of the same hybrid for some properties, particularly reaction efficiency. This variation indicated native starch samples from the same hybrid had different properties even though they came from kernels that were genetically similar. This variation was likely due to differences in laboratory wet-milling and should be investigated with additional research.

Three hybrids were grown during the 1998 and 1999 crop years (Hybrids 4, 5 and 6). Data from these hybrids were compared by crop year to observe effects on starch acetylation due to growing conditions. All hybrids were grown at the same location. Differences between crop years were observed in reaction efficiency, peak viscosity, trough viscosity, and final viscosity for both acetylated and native starches (Table V). For each of these variables, 1999 samples had greater values than did 1998 samples. Because similar variation was seen in properties both before and after acetylation, variation among acetylated starches was due to differences in starch samples before acetylation, and not factors related to acetylation itself.

Factors such as environment and the milling process appeared to have an effect on starch properties, as well as hybrid. This is especially true concerning replicates from the same hybrid. Some hybrids had large standard deviations for some properties, even before acetylation. This suggests that even though the laboratory wet-milling procedure used has been shown to have consistent starch and coproduct yields (Eckhoff et al 1993), the procedure

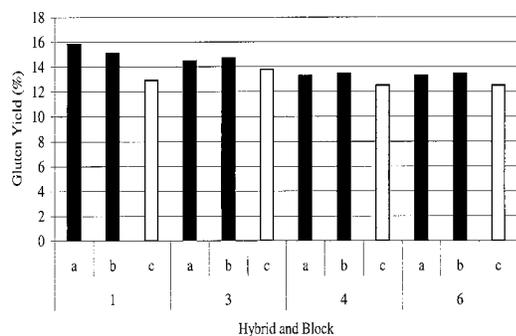


Fig. 1. Gluten yields of 1998 hybrids.

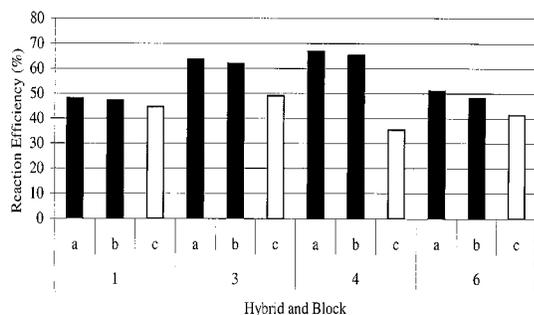


Fig. 2. Reaction efficiencies of 1998 hybrids.

TABLE V  
Properties of Acetylated Starches from Hybrids  
Grown in Both Crop Years<sup>a</sup>

Properties	1998	1999
Peak viscosity (cP) <sup>b</sup>	759 ± 23	826 ± 23
Trough viscosity (cP) <sup>b</sup>	340 ± 59	386 ± 44
Final viscosity (cP) <sup>b</sup>	428 ± 67	480 ± 47
Reaction efficiency (%) <sup>b</sup>	50.0 ± 10.1	62.7 ± 7.0

<sup>a</sup> Values reported as mean of three replicates ± one standard deviation.

<sup>b</sup> Significantly different for crop year ( $P < 0.05$ ).

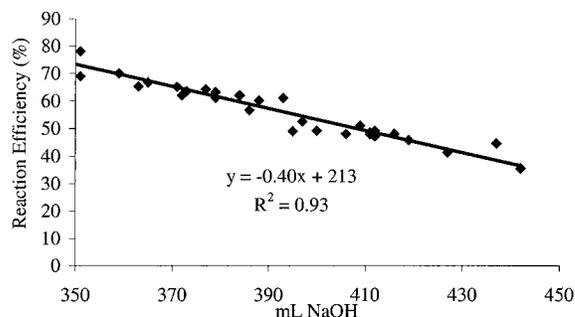


Fig. 3. Correlation of reaction efficiency and amount of NaOH added to the reaction.

may produce starch with variable pasting and reaction properties. Environment was not a factor for starch properties of hybrid replicates from a single crop year because all hybrids were grown at the same location.

Reaction efficiency was correlated with RVA properties to establish whether a relationship between acetyl level and RVA properties existed. This was determined on RVA values of acetylated samples. For RVA values, little correlation ( $R^2 < 0.25$ ) was found between reaction efficiency and any RVA property. RVA properties did not depend on acetyl level, but rather factors such as hybrid, environment, and milling. Reaction efficiency was correlated ( $R^2 = 0.93$ ) with NaOH usage during acetylation (Fig. 3). Acetic anhydride reacts with a starch alkoxy group to form the acetate ester and sodium acetate. Acetic anhydride also reacts with a hydroxide ion to form two sodium acetate molecules. Hydroxide ions are used up, which lowers pH (Jarowenko 1986). There was no hybrid effect on NaOH usage.

## CONCLUSIONS

Hybrid had an effect on the extent of acetylation, as measured by reaction efficiency, and certain properties of acetylated waxy starches as measured by RVA. Environmental conditions (crop year) and milling also appeared to have an influence on reaction efficiency and starch characteristics. Using the same acetylation protocol, reaction efficiencies were observed at 47–73%; reaction efficiencies were significantly lower for 1998 hybrids (50.0%) compared to efficiency observed for the same hybrids grown in 1999 (62.7%). Variation in reaction efficiency led to varying degrees of acetylation and thus to variability in RVA properties of modified starch samples. Acetylated starch from 1999 had increased peak, trough and final viscosities and increased reaction efficiency as compared with acetylated starch from 1998. Differences in setback were observed among 1998 hybrids for acetylated samples. Differences in trough and final viscosity were observed among 1999 hybrids for acetylated and native (unmodified) samples. Differences in breakdown among 1999 hybrids were observed for native samples. Additional data using more growing seasons is needed to firmly establish the effect of environment. Variation in starch reaction efficiency can cause variation in a modification process by requiring increased reaction times and increased usage of reactants, such as NaOH, to achieve a constant acetyl level in the final product. Variation in wet milling may affect starch pasting properties and reaction characteristics of starch entering the acetylation reactor. Variability in acetylated starches was the result of factors occurring

before acetylation, not the acetylation reaction itself. Additional research is needed to determine the relative effects of hybrid, growing conditions and milling.

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## LITERATURE CITED

- American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Method 46-13. The Association: St. Paul, MN.
- Anonymous. 1997. Newport Scientific Method Staley-01. Page 3 in: A Global Revolution in Food Starch Quality Analysis. Foss North America: Eden Prairie, MN.
- CRA. 1993. Standard Analytical Methods of the Member Companies of the Corn Refiners Association, 7th ed. Method C-2. The Association: Washington, DC.
- De Boer, E. D. 1991. Chemically modified derivatives of starch from a new genetic variety of corn. *Cereal Foods World* 36:631-639.
- Eckhoff, S. R., Rausch, K. D., Fox, E. J., Tso, C. C., Wu, X., Pan, Z., and Buriak, P. 1993. A laboratory wet-milling procedure to increase reproducibility and accuracy of product yields. *Cereal Chem.* 70:723-727.
- Jarowenko, W. 1986. Acetylated starch and miscellaneous organic esters. Pages 55-77 in: *Modified Starches: Properties and Uses*. O. B. Wurzburg, ed. CRC Press: Boca Raton, FL.
- Peplinski, A. J., Paulsen, M. R., Anderson, R. A., and Kwolek, W. F. 1989. Physical, chemical, and dry-milling characteristics of corn hybrids from various genotypes. *Cereal Chem.* 66:117-120.
- Rutenberg, M. W., and Solarek, D. 1984. Starch derivatives: Production and uses. Pages 311-388 in: *Starch Chemistry and Technology*, 2nd Ed. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: New York.
- Singh, V., Haken, A. E., Niu, Y. X., Zou, S. H., and Eckhoff, S. R. 1997. Hybrid-dependent effect of lactic acid on corn starch yields. *Cereal Chem.* 74:249-253.
- Singh, V., Haken, A. E., Paulsen, M. R., and Eckhoff, S. R. 1998. Starch yield sensitivity of maize hybrids to drying temperature and harvest moisture content. *Starch* 50:181-183.
- Wurzburg, O. B. 1964. Acetylation. Pages 286-288 in: *Methods in Carbohydrate Chemistry*, Vol. IV. R. L. Whistler, ed. Academic Press: New York.
- Zehr, B. E., Eckhoff, S. R., Singh, S. K., and Keeling, P. L. 1995. Comparison of wet-milling properties among maize hybrid inbred lines and their hybrids. *Cereal Chem.* 72:491-497.

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