

# Variability of Reaction Efficiencies and Pasting Properties of Acetylated Dent Corn Starch from Various Commercial Hybrids

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## ABSTRACT

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Genetic diversity in corn (maize) has contributed to variability in corn processing characteristics. Differences in acetylated dent corn starch pasting properties and reaction efficiencies due to hybrid were assessed. Ten dent corn hybrids grown during 1998 and nine dent corn hybrids grown during 1999 were wet-milled in the laboratory. Starch from each hybrid was modified using a laboratory-scale acetylation procedure. NaOH consumed and reaction time were recorded for each reaction. A Rapid Visco Analyser (RVA) was used to measure starch pasting properties. Acetyl content was measured by a

spectrophotometric method, from which reaction efficiency was calculated. Reaction efficiencies were observed at 35–56%. Overall reaction efficiencies for starch samples from 1998 hybrids were lower than samples from 1999 hybrids. Differences in peak viscosity, trough viscosity, final viscosity, setback, and pasting temperature were found among 1998 hybrids. Differences in trough viscosity, final viscosity, and breakdown were found among 1999 hybrids.

Genetic diversity of the world corn (maize) supply has increased over the past several years. Through use of hybridization and genetic engineering, scientists have developed hybrids yielding more than 200 bushels per acre with resistance to a variety of diseases and pests.

Currently, hybrids with benefits to corn processors are also being developed. For years, corn dry millers have used hybrids with large amounts of hard endosperm, often called food-grade hybrids, because of their ability to yield more flaking grits. Recently, hybrids with high amounts of extractable starch have been introduced to benefit the wet-milling industry. Corn hybrids containing starch that exhibits properties of modified starches, beneficial nutritional components, and high-value pharmaceuticals have been discussed as future uses of genetic modification in corn.

Hybrid effect on various corn-processing characteristics has been established in both corn wet milling and dry milling. Peplinski et al (1989) observed dry-milling fraction yields were hybrid-dependent. Zehr et al (1995) wet-milled several hybrids in the laboratory and found starch and coproduct yields were hybrid-dependent. Singh et al (1997) observed a hybrid effect on the starch yield increase of wet-milled hybrids due to lactic acid addition during steeping. Singh et al (1998) observed a hybrid effect on starch yield for hybrids subjected to a variety of postharvest handling procedures. Mathew et al (1999) found that both corn hybrid and growth environment affected corn curl properties and pet food extrudates processed from whole corn meal. Wilkins et al (2003) found a hybrid effect on several pasting properties of acetylated waxy corn starches as measured by rapid viscosity analysis.

No previous studies have been published on the hybrid effect on the acetylation and properties of dent corn starch acetates. Dent hybrids exhibit more genetic diversity than waxy hybrids, particularly in amylose content. Amylose content has an effect on native starch pasting temperature and other pasting properties (Jane et al 1999). Wilkins et al (2003) observed that differences in pasting properties among native waxy starches were observed after these starches were acetylated with a constant amount of acetic anhydride. An increased understanding of genetic effects on this aspect of corn processing should lead to more efficient processing of starch and related coproducts. In this study, we sought to determine how starch isolated from individual dent corn hybrids affected acetylation efficiency and pasting properties.

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## MATERIALS AND METHODS

### Wet Milling

Ten dent corn hybrids (samples 1–10) grown during the 1998 crop year and nine dent corn hybrids (samples 1–8 and 11) grown during the 1999 crop year were provided by a commercial seed company. Eight hybrids (1–8) were grown during both seasons. All hybrids were grown on the same research plots of a major agricultural products company. Each hybrid was available commercially during the two crop years.

Three 1,000-g samples of each hybrid (except 1999 samples of hybrid 3 and 1998 samples of hybrid 8, which had only two samples due to seed availability) were milled according to the procedure of Eckhoff et al (1993) to extract starch. Starch samples were organized into replicate blocks. Blocks a–c contained 1998 samples and blocks d–f contained 1999 samples. Each replicate block contained one replicate from each hybrid grown during that season (except block c, which did not contain hybrid 8, and block f, which did not contain hybrid 3). Hybrids were milled randomly within each block.

### Acetylation

Each hybrid starch sample was acetylated once using conditions described by Jarowenko (1986). Five samples of a commercial dent starch (C\*Gel 03420, Lot J1463-15, Cerestar USA, Hammond, IN) were laboratory-acetylated to assess reliability of the acetylation procedure. For each reaction, 180 g (db) of starch was combined with 514 g of water to obtain a solid-to-water ratio of 0.35. Starch slurry was mixed for 1 hr to fully suspend starch granules. At 30°C, slurry was adjusted to pH 8.2 with 1.5% (w/v) NaOH. Reaction temperature was controlled by placing the reaction vessel in a temperature-controlled water bath. By 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 rate of 0.24 mL/min while maintaining pH 8.0–8.4 with 1.5% NaOH. The amount of NaOH used and pH were measured for each reaction. The reaction was allowed to continue after acetic anhydride addition was completed and until slurry pH stabilized. A small amount (1.2–1.8 mL) of 6M HCl was added to the slurry with a pipette to lower to pH 6.0 to halt the reaction. The reaction time for each reaction was nearly constant. 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. The refiltered cake was dried at 49°C overnight.

### 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. In addition,

unmodified samples from each hybrid and block grown during 1999 were analyzed. Eight unmodified commercial dent samples (C\*Gel 04230, Lot J1463-15, Cerestar USA) were analyzed to determine repeatability of the RVA technique. The Staley-02 method was used (Anonymous 1997). For each 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. A solids content of 5% (w/w) was used for RVA analysis.

Acetyl content of each sample was determined using Corn Refiners Association Method C-2 (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

$$\% \text{ acetyl} = (\mu\text{g acetyl} \times 2500) / [\text{sample wt.} \times (1 - \% \text{ moisture in initial sample})] \quad (1)$$

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

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 replicate block were dependent variables; peak viscosity, trough viscosity, final viscosity, breakdown, setback, pasting temperature, and reaction efficiency were independent variables. An additional ANOVA was done on data from eight hybrids grown during both growing seasons (1–8) using the same independent variables and crop year as the dependent variable. In addition, linear correlations among pasting properties and reaction efficiency, pasting properties, and protein content, reaction efficiency, and protein content, and NaOH consumed and reaction efficiency were calculated using Excel 2000 software (Microsoft, Redmond, WA).

## RESULTS AND DISCUSSION

RVA analyses of unmodified commercial starch samples were repeatable. Standard deviations of viscosity measurements were <15 cP (COV ≤ 4.0%); pasting temperature standard deviation was 0.8°C (COV = 0.9%) (Table I). RVA analyses of laboratory-acetylated starch samples from a commercial source had greater variability than did RVA analyses of unmodified samples. Standard deviations of viscosity measurements were 8 cP for breakdown (COV = 3.2%) to 75 cP for final viscosity (COV = 7.5%). Pasting temperature and reaction efficiency had less variation, with standard deviations of 0.5°C and 1.8% (COV = 0.6 and 2.5%, respectively). The reason for large standard deviations (>40 cP) for final viscosity and setback is unknown; especially since reaction efficiencies of acetylated samples and RVA properties of unacetylated starch samples from commercial samples had little variability.

No differences in reaction efficiency (32.4–43.9%) were observed for starch samples obtained from 1998 hybrids (Table II). Differences among hybrids were observed in peak viscosity, trough viscosity, final viscosity, setback, and pasting temperature for acetylated samples produced from corn grown in 1998.

No differences in reaction efficiency (42.9–56.1%) were observed among starch samples obtained from hybrids harvested in 1999. Differences among hybrids were observed in trough viscosity, final viscosity, and breakdown for acetylated samples produced from corn grown in 1999 (Table III). No differences were observed among hybrids for other RVA properties. Differences among hybrids were observed in peak viscosity, trough viscosity, final viscosity, breakdown, and setback among unmodified samples from corn grown in 1999 (Table IV). No differences were observed in pasting temperature among unmodified samples from 1999 hybrids. Among 1999 hybrids, hybrid differences in pasting properties observed among acetylated samples also were observed among unmodified samples. Because differences in RVA properties among hybrids were present before acetylation, they were not due to factors associated with the acetylation reaction. Instead, these differences must be due to factors before acetylation such as wet milling, growing conditions, and genetics. These results are similar to those of a previous study on acetylated waxy corn starch (Wilkins et al 2003). In the previous study, differences in peak viscosity and setback were observed among un-

**TABLE I**  
Properties of Laboratory Acetylated Starch from a Commercial Source<sup>a</sup>

Properties	Unmodified	Acetylated
Viscosity (cP)		
Peak	535 ± 13	592 ± 40
Trough	360 ± 12	340 ± 34
Breakdown	175 ± 7	252 ± 8
Final	657 ± 15	1006 ± 75
Setback	297 ± 6	666 ± 56
Pasting temp. (°C)	92.3 ± 0.8	85.1 ± 0.5
Reaction efficiency (%)	na	71.7 ± 1.8

<sup>a</sup> C\*Gel 03420, Lot # J1463-15, Cerestar USA, Hammond, IN.

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

Properties	Hybrid									
	1	2	3	4	5	6	7	8	9	10
Viscosity (cP)										
Peak <sup>b</sup>	526 ± 26	510 ± 16	516 ± 4	573 ± 17	490 ± 22	481 ± 9	454 ± 21	449 ± 74	531 ± 16	550 ± 9
Trough <sup>b</sup>	368 ± 6	373 ± 13	308 ± 14	358 ± 20	301 ± 4	340 ± 15	327 ± 12	330 ± 45	312 ± 8	315 ± 10
Breakdown	158 ± 26	137 ± 5	208 ± 16	215 ± 18	189 ± 24	140 ± 11	127 ± 25	119 ± 29	220 ± 17	234 ± 16
Final	889 ± 54	905 ± 41	769 ± 23	874 ± 16	783 ± 12	858 ± 65	818 ± 9	814 ± 112	761 ± 9	817 ± 10
Setback <sup>b</sup>	521 ± 54	532 ± 37	461 ± 9	516 ± 7	482 ± 11	518 ± 60	492 ± 11	484 ± 67	450 ± 2	502 ± 10
Pasting temp. (°C) <sup>b</sup>	85.2 ± 0.0	86.5 ± 1.2	85.2 ± 0.0	79.3 ± 4.3	85.3 ± 1.3	86.8 ± 0.7	86.5 ± 1.2	87.6 ± 0.0	85.2 ± 1.3	87.6 ± 0.1
Reaction efficiency (%)	37.9 ± 1.9	35.2 ± 2.3	43.9 ± 5.2	35.6 ± 2.7	35.9 ± 2.9	36.6 ± 1.2	43.6 ± 1.5	38.5 ± 1.9	35.0 ± 0.3	32.4 ± 1.3

<sup>a</sup> Values reported as mean of three samples ± 1 standard deviation, except hybrid 8, which is mean of two samples.

<sup>b</sup> Significantly different among hybrids.

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

Properties	Hybrid								
	1	2	3	4	5	6	7	8	11
Viscosity (cP)									
Peak	519 ± 15	540 ± 25	527 ± 17	526 ± 3	526 ± 35	510 ± 20	539 ± 35	582 ± 39	508 ± 16
Trough <sup>b</sup>	398 ± 5	419 ± 11	352 ± 3	370 ± 9	344 ± 15	390 ± 19	397 ± 5	398 ± 9	361 ± 21
Breakdown <sup>b</sup>	121 ± 12	121 ± 22	175 ± 14	157 ± 8	182 ± 22	120 ± 9	141 ± 36	184 ± 31	147 ± 8
Final <sup>b</sup>	1101 ± 31	1123 ± 78	993 ± 15	961 ± 24	925 ± 54	1010 ± 54	1027 ± 39	1083 ± 104	1015 ± 63
Setback	704 ± 34	704 ± 72	641 ± 12	592 ± 19	581 ± 44	620 ± 39	629 ± 38	686 ± 96	654 ± 45
Pasting temp. (°C)	84.0 ± 2.1	85.7 ± 0.8	81.0 ± 2.6	83.6 ± 0.8	80.8 ± 4.5	84.4 ± 1.4	82.0 ± 1.9	82.9 ± 3.6	85.3 ± 0.1
Reaction efficiency (%)	55.6 ± 8.2	46.9 ± 10.6	53.9 ± 5.3	47.8 ± 5.4	46.2 ± 9.8	42.9 ± 4.4	51.7 ± 9.3	56.1 ± 12.2	47.4 ± 11.9

<sup>a</sup> Values reported as mean of three samples ± 1 standard deviation, except hybrid 3, which is mean of two samples.

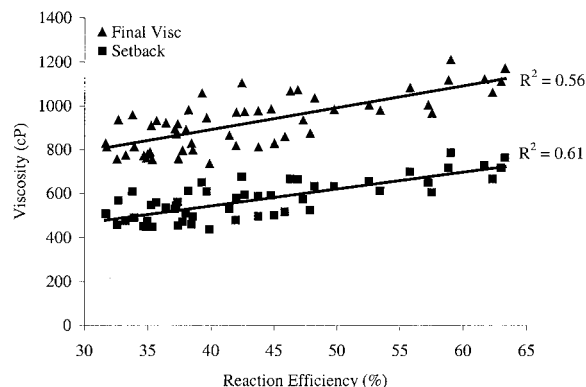
<sup>b</sup> Significantly different among hybrids.

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

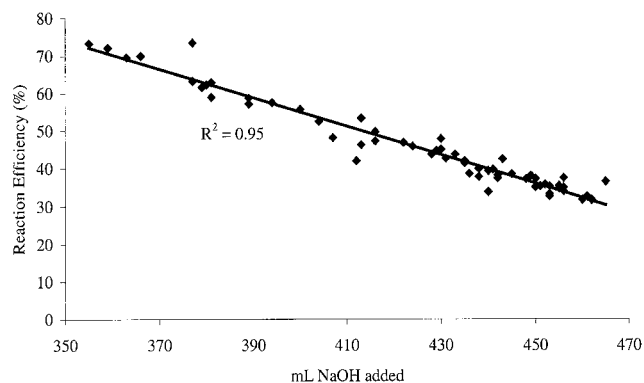
Properties	Hybrid								
	1	2	3	4	5	6	7	8	11
Viscosity (cP)									
Peak <sup>b</sup>	420 ± 33	452 ± 14	455 ± 12	431 ± 16	448 ± 28	463 ± 21	428 ± 31	498 ± 26	437 ± 17
Trough <sup>b</sup>	365 ± 23	640 ± 17	564 ± 27	618 ± 34	591 ± 31	674 ± 33	608 ± 34	653 ± 17	634 ± 41
Breakdown <sup>b</sup>	55 ± 9	67 ± 20	127 ± 9	99 ± 6	117 ± 15	92 ± 24	64 ± 10	116 ± 23	105 ± 4
Final <sup>b</sup>	616 ± 48	640 ± 17	564 ± 27	618 ± 34	591 ± 31	674 ± 33	608 ± 34	653 ± 17	634 ± 41
Setback <sup>b</sup>	251 ± 25	255 ± 7	235 ± 15	286 ± 23	261 ± 21	302 ± 20	245 ± 12	271 ± 13	301 ± 24
Pasting temp. (°C)	90.0 ± 1.3	91.3 ± 1.3	91.3 ± 1.2	90.1 ± 1.2	91.6 ± 1.3	90.0 ± 0.0	90.4 ± 0.6	90.8 ± 0.8	90.0 ± 0.0

<sup>a</sup> Values reported as mean of three samples ± 1 standard deviation.

<sup>b</sup> Significantly different among hybrids.



**Fig. 1.** Correlations of final viscosity and setback with reaction efficiency.



**Fig. 2.** Amount of NaOH consumed at different reaction efficiencies.

modified starch samples but not among acetylated samples. RVA data for unmodified samples from 1998 hybrids were unavailable.

Differences between crop years were found in peak viscosity, trough viscosity, final viscosity, setback, pasting temperature, and reaction efficiency (Table V). No differences between crop years were ob-

**TABLE V**  
Properties of Acetylated Starches Grown in Different Years<sup>a</sup>

Properties	Crop Year	
	1998	1999
Viscosity (cP)		
Peak <sup>b</sup>	502 ± 44	534 ± 31
Trough <sup>b</sup>	338 ± 30	385 ± 26
Breakdown	164 ± 39	149 ± 32
Final <sup>b</sup>	840 ± 62	1029 ± 83
Setback <sup>b</sup>	501 ± 39	645 ± 64
Pasting temp. (°C) <sup>b</sup>	85.2 ± 2.9	83.1 ± 2.6
Reaction efficiency (%) <sup>b</sup>	38.2 ± 4.1	49.9 ± 8.6

<sup>a</sup> Values reported as mean of 23 samples ± 1 standard deviation.

<sup>b</sup> Significantly different among crop years.

served in breakdown. Acetylated samples grown in 1999 had greater reaction efficiency, peak viscosity, trough viscosity, final viscosity, and setback than acetylated samples from 1998. Acetylated samples grown in 1999 also had lower pasting temperatures than 1998 samples.

Some variation in pasting properties within samples of the same hybrid was observed, particularly in final viscosity and 1998 hybrid 8 samples (only two 1998 hybrid 8 samples were analyzed) (Tables II and III). These variations were similar to those found in a previous study on waxy starch acetylation (Wilkins et al 2003). Variation in reaction efficiency within 1999 hybrids was greater than variations within 1998 hybrids (Tables II and III). All hybrids were acetylated according to the same procedure, and control samples acetylated at the same time showed a small variation in reaction efficiency (COV = 2.5%). Also, reaction efficiency showed low correlation with protein content (data not shown). Reasons for the increase in reaction efficiency variation with 1999 hybrids, as opposed to 1998 hybrids, are not known.

In a previous study involving waxy corn hybrids and starch acetylation, some differences in reaction efficiency or pasting properties among hybrids were observed (Wilkins et al 2003). Increased variability among dent hybrids compared with waxy hybrids probably is due to increased genetic diversity in dent corn. Starch from waxy hybrids is composed of nearly pure amylopectin, while starch from

dent hybrids can vary in amylose content. Jane et al (1999) determined that absolute amylose content and amylopectin branch chain length distribution had an effect on starch pasting properties. Variability in pasting properties for waxy hybrids primarily is based on amylopectin chain length distribution, whereas variability in dent hybrids can be attributed to both amylose content and amylopectin chain length distribution. Thus, hybrid effects on dent starch pasting properties should be more prevalent than hybrid effects on waxy starch pasting properties. Amylose content of each acetylated sample was not measured in this study.

Linear correlations were calculated between each pasting property and reaction efficiency using data from reactions performed for both crop years. All correlations used a 95% level of confidence. Setback showed the strongest correlation with reaction efficiency ( $R^2 = 0.61$ ), followed by final ( $R^2 = 0.56$ ) (Fig. 1), trough ( $R^2 = 0.33$ ) and peak viscosities ( $R^2 = 0.14$ ). Pasting temperature decreased with increasing reaction efficiency ( $R^2 = 0.35$ ). Breakdown showed almost no correlation with reaction efficiency ( $R^2 = 0.03$ ). Positive correlations between most of the pasting viscosities and reaction efficiency indicated that as reaction efficiency, which is related directly to acetyl content, increases, viscosity of acetylated starch also increases. In previous studies (Rutenberg and Solarek 1984; Liu et al 1997), peak viscosity of dent corn starch increased upon acetylation. For final viscosity and setback, results of this study were in contrast to Rutenberg and Solarek (1984), which showed acetylation decreased retrogradation and setback in dent corn starch. Acetylated starches in this study showed greater final viscosity and setback than their unmodified counterparts, similar to data on acetylated legume starches reported by Hoover and Sosulski (1985) and Comer and Fry (1978). The decrease in pasting temperature with increasing acetyl content follows other results previously reported (Schoch and Maywald 1956; Rutenberg and Solarek 1984; Liu et al 1997).

Linear correlations were made between each pasting property and unmodified starch protein content. Breakdown showed a small correlation with protein content ( $R^2 = 0.19$ ) (data not shown). All other pasting properties and reaction efficiency showed weak correlation with protein content with  $R^2 < 0.07$ .

A negative, linear correlation was observed between the amount of NaOH used in each acetylation reaction and reaction efficiency ( $R^2 = 0.95$ ) (Fig. 2). Starches that cannot react efficiently with acetyl groups consume increased amounts of NaOH. When starch reacts with acetic anhydride, two acetyl groups from each acetic anhydride molecule are released into aqueous solution. Ideally, one group reacts with a hydroxyl group on the starch molecule and the other combines with a sodium cation from NaOH to form sodium acetate (Jarowenko 1986). Starches that exhibit low reaction efficiency do not react with as many acetyl groups as do starches with higher reaction efficiencies, so more free acetyl groups remain in aqueous solution. Acetyl groups in aqueous solution form acetic acid, which decreases reaction pH; therefore, more NaOH is needed to react with free acetyl groups to maintain reaction pH in the optimal range. These results are similar to a previous acetylation study conducted with waxy corn starch (Wilkins et al 2003).

## CONCLUSIONS

Hybrid had no effect on the extent of acetylation, as measured by reaction efficiency. However, reaction efficiencies were observed to range from 35–56%. Reaction efficiencies for starch samples from 1998 hybrids were lower overall than samples from 1999 hybrids. Hybrid affected several pasting properties of both unmodified and acetylated samples. Differences among 1999 hybrids in pasting properties that were observed in acetylated samples also were ob-

served in unacetylated samples. Differences among 1999 hybrids in pasting properties appeared linked to factors prior to acetylation, similar to trends observed for waxy corn hybrids (Wilkins et al 2003). Crop year also had an effect on reaction efficiency and all pasting properties except for breakdown. Some variations among samples of the same hybrid were found in some pasting properties; these variations were similar to those found in a previous study on waxy starch acetylation (Wilkins et al 2003). Reaction efficiency variation within 1999 hybrids was greater than reaction efficiency variation within 1998 hybrids. Higher reaction efficiency resulted in greater pasting viscosities and lower pasting temperature. NaOH consumed in the acetylation reaction was correlated ( $R^2 = 0.95$ ) to reaction efficiency; increased reaction efficiency increased acetyl content of acetylated starches and decreased NaOH consumption.

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