Effect of Laboratory Batch Steeping pH on Starch Yield and Pasting Properties of Selected Corn Hybrids

L. Cabrales,[1] Y. X. Niu,[1] P. Buriak,[1] and S. R. Eckhoff[1,2]

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ABSTRACT

This study evaluated the effect of initial pH on percent of starch yield and pasting characteristics for a laboratory wet-milling procedure. Four commercial hybrids, selected because they have significantly different starch yield values, were laboratory wet-milled, and the pasting properties of the starch fractions were evaluated using a Rapid Visco Analyser (RVA). Percent starch yield (db) decreased when initial pH values were >4.0 but was unaffected by any lower initial pH values. The pasting properties of some of the selected hybrids were more sensitive to steepwater pH than others. There was an overall increase in peak, trough, and final viscosity as pH increased.

Steeping is the heart of the corn wet-milling process. Without proper steeping, the subsequent steps of the wet-milling process are more difficult, resulting in higher starch protein content and higher loss of starch into the coproducts. In steeping, the corn kernel is hydrated, causing differential swelling and making the germ and fiber separation more effective. Addition of sulfur dioxide (SO_2) cleaves endosperm disulfide bonds, increasing the release of starch from the protein matrix (Watson 1984). If the corn is oversteeped, the starch will have a lower viscosity, more endosperm protein will be solubilized and lost into gluten feed, and the germ will become slippery and difficult to dewater (Blanchard 1999).

In industrial countercurrent steeping, SO_2 is added to the oldest corn at levels of 0.1–0.2% and the process water pH drops to ≈2.5 but will rise to ≈3.5 as the SO_2 initially in the steepwater is absorbed and reacts with the corn (Blanchard 1999). At the newest corn end of the process, SO_2 decreases to a level that is ineffective in stopping endogenous *Lactobacillus* sp. from fermenting available sugars to produce 1–2% lactic acid. This results in a stable pH of 3.5–4.2 throughout the process (Blanchard 1999). In laboratory batch steeping (Eckhoff et al 1996), 0.2% SO_2 and 0.5% lactic acid are added at the start of steeping, dropping the pH to 2.3–2.9, causing an increase in pH throughout the steep period to pH ≈4.5. Addition of lactic acid in batch steeping increased the starch yield (Du et al 1996; Singh et al 1997; Sing et al 1999; Perez et al 2001; Manzoni et al 2002), making it a necessary part of the laboratory process. Haros et al (2004) found that steeping corn with lactic acid affected the pasting properties of the starch, yet was necessary for maximum starch yield for the single hybrid examined. Hydrolysis of the starch through acid thinning was deemed the cause of the decrease in pasting properties.

The 100-g wet-milling procedure used in our laboratory was established as a method to precisely estimate starch yield from different hybrids or processing conditions. This procedure is used as a standard for starch yield determination and is the basis for a recent starch yield calibration in use by industry. There is interest in evaluating the rheological properties of the resulting starch. To ensure that the batch steeping conditions result in starch that is of industrial standard, it was proposed to adjust the pH after the initial addition of SO_2 and lactic acid. The objective of this study was to evaluate the effect of batch steep pH on percent starch yield and the pasting characteristics of the resulting starch.

[1] Graduate student, research specialist, professor, and professor, respectively, Department of Agricultural Engineering, University of Illinois, Urbana, IL 61801.
[2] Corresponding author. Phone: 217-244-4022. Fax: 217-244-0323. E-mail: seckhoff@uiuc.edu

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MATERIALS AND METHODS

Four yellow dent corn hybrids selected for this study had starch yields of 62.4–69.0% starch yield (db) to represent industry variability in starting material. The corn hybrids were coded 6333, 66B33, 14V33, and 26D33. They were grown at the Agricultural Engineering Farm (Urbana, IL) and field-dried to ≈13% (wb) moisture content before harvesting. The corn samples were hand-cleaned to remove any broken kernels or foreign material, placed in plastic bags, and stored at 4°C until wet-milling. The whole kernel moisture content was obtained using the 103°C convection oven method (AACC International 2000).

The wet-milling procedure of Eckhoff et al (1996) was used. In addition to the 2,000 ppm of SO_2 and 0.5% (w/w) lactic acid added to the steep, a solution of 0.2*M* sodium bicarbonate (1*M* $NaHCO_3$, Certified, Fisher Scientific, Pittsburgh, PA) was used to adjust the pH. The control sample did not have any sodium bicarbonate solution added, and the pH level of the solutions from the different samples was 2.3–2.9. The amount of sodium bicarbonate added to the initial steep solution was adjusted to achieve pH 3.5, 3.75, 4.0, and 4.5. Each hybrid and pH combination was wet-milled in triplicate. Each starch replicate was lightly ground with a mortar and pestle to obtain a uniform powdered product.

The starch replicates from each hybrid and pH combination were mixed to make a single starch sample for each. Pasting properties of the starches were determined in triplicate with a Rapid Visco Analyser (model RVA-3D, Newport Scientific, Warriewood, Australia). The specific RVA protocol used evaluated a 5.5% (w/w) starch solution stirred at 160 rpm as it was heated from 55 to 95°C in 3 min and maintained at that temperature for 20 min. The samples were then cooled to 55°C in 3 min and held there for 9 min. The pasting profiles resulted in peak viscosity (PV), trough viscosity (TV), final viscosity (FV), setback viscosity (SV), breakdown viscosity (BV), pasting temperature (P_{temp}), and peak time (P_{time}). Setback is the difference between the final and trough viscosities and describes the degree of hardening of cooked starch during cooling. The breakdown is the difference between the peak viscosity and trough viscosities and is the measurement of the susceptibility of the cooked starch to breaking down with polymer solubilization and molecular alignment (Thomas and Atwell 1999).

Analysis of variance and the Duncan's multiple range test were used for data analysis (SAS Institute, Cary, NC). Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of pH and Hybrid on Starch Yield

The main effects of hybrid and pH and interaction of pH × hybrid were statistically significant ($P < 0.05$) (Table I). The significance of the interaction term suggests that the pH affected the starch yield of the selected hybrids differently. Closer inspection of the ANOVA results (Table I) shows that the sum of squares associated with the interaction term accounted for very little (1.8%) of the total variation. The effect of hybrid was more prominent than the pH treatment on percent starch yield. This is not surprising because our hybrids were selected to cover a range of starch yields. Similar results were reported by Shandera et al (1995) and Singh et al (1997), who found that hybrid effect on the wet-milling yields was more pronounced than that of the steep solution.

Three of the four hybrids showed no difference in starch yield in going from the control level to pH 4.0. The fourth (66B33) had a statistically significant drop in starch yield at pH 4.0. However the drop was only 0.7% yield. The low coefficient of variation of the procedure (CV < 1.0) constitutes a statistically insignificant difference. Increasing to pH 4.5 caused a decrease in starch yield of 1–3.7% (db) depending on the hybrid (Table II). The significant interaction between hybrid and pH can be attributed to this variation in the reduction in starch yield at pH 4.5. So while the interaction term is significant, it is unimportant.

The decrease in percent starch yield at pH 4.5 was lost to the fiber fraction of the sample. At pH 4.5, the fiber fraction increased by 1.4–4.6% depending on the hybrid used when compared with the control sample (Table II). Decrease in starch yield due to loss to the fiber fraction was also found in other studies (Roushidi et al 1981; Eckhoff and Tso 1991; Du et al 1996; Singh et al 1997).

In general, percent starch yield decreased at values above pH 4.0. The results provided evidence that adjusting the steepwater to a maximum of pH 4.0 would not significantly affect percent starch yield of most hybrids tested.

TABLE I
Analysis of Variance for Starch Yields of Four Hybrids at pH Concentrations of Control, 3.5, 3.75, 4.0, and 4.5

Source	df	Sum of Squares	Mean Squares	*F* Value
pH	4	61.619	15.405	62.07*[a]
Hybrid	3	447.217	149.072	600.66*
pH × Hybrid	12	9.713	0.809	3.26*
Error	40	9.927	0.248	

[a] *, Significant at $P < 0.05$.

Starch Pasting Properties

The addition of sodium bicarbonate solution to adjust the initial steepwater pH significantly affected the peak viscosity ($P < 0.05$) (Table III). The main effects of pH and hybrid were significant, but the interaction of pH × hybrid was not. There was no significant difference among the control, pH 3.5, and pH 3.75 in any hybrid tested, yet there was a significant increase in peak viscosity at values above pH 3.75 (Table IV). Three of the four hybrids had the highest peak viscosity occurring at pH 4.5 and one (6333) at pH 4.0. Adjusting the steepwater from the control to pH 4.5 increased the peak viscosity by 1.7–4.9% depending on the hybrid. Overall, the initial peak viscosity increased with increasing pH. Even though the control steep solution is a mild acid, the starch present underwent some acid thinning which accounted for the decrease in peak viscosity.

The main effects of pH and hybrid had a significant impact on the trough, breakdown, and final viscosity ($P < 0.05$) (Table III). The interaction of pH × hybrid was also significant, which suggests that the pH affected the trough, breakdown, and final viscosity of the selected hybrids differently. The trough viscosities at steepwater pH 4.0 and 4.5 were significantly higher than the control (Table IV). All of the selected hybrids except one (6333) had the highest viscosity at pH 4.5. The trough viscosity increased by 7.0–10.7% between the control and pH 4.5, depending on the hybrid, and there was an overall increase in trough viscosity as pH

TABLE II
Mean Wet-Milling Fraction Yields (% db) Obtained from Four Hybrid Corn Samples with Sodium Bicarbonate Solution Added to Steepwater (SW) to Adjust pH Levels

Hybrid	pH	SW Solids	Fiber	Germ	Starch	Gluten	Total
6333	Control	3.9	11.8	5.4	68.7a[a]	9.3	99.2
	3.50	3.9	11.4	6.1	69.0a	8.8	99.2
	3.75	3.8	11.8	5.7	69.3a	9.3	99.8
	4.00	3.7	12.4	5.8	68.9a	8.8	99.5
	4.50	3.6	13.2	5.8	67.7b	9.4	99.6
14V33	Control	3.8	13.4	5.8	67.2a	8.7	99.0
	3.50	3.7	13.8	6.0	67.5a	8.3	99.2
	3.75	3.7	14.2	5.9	67.6a	8.4	99.7
	4.00	3.6	14.8	5.8	67.1a	8.3	99.5
	4.50	3.4	16.6	6.1	65.1b	8.1	99.4
26D33	Control	4.1	15.9	5.5	62.7a	10.7	98.9
	3.50	4.0	16.1	5.6	62.6a	10.5	98.9
	3.75	3.9	17.2	5.6	62.4a	10.4	99.4
	4.00	3.9	17.9	5.7	61.5a	10.1	99.0
	4.50	3.7	20.5	5.7	59.0b	10.2	99.1
66B33	Control	3.9	15.2	4.6	64.8ab	10.8	99.3
	3.50	3.8	15.6	4.3	65.3a	10.6	99.6
	3.75	3.8	16.7	4.5	64.1bc	10.7	99.8
	4.00	3.7	17.8	4.5	63.4c	10.4	99.8
	4.50	3.5	19.7	4.8	61.5d	10.0	99.6

[a] Means followed by the same letter did not differ at $P = 0.05$.

TABLE III
Significance ($P < F$) of pH Levels and Hybrid Factors on Pasting Properties[a]

Factors	PV	TV	BV	FV	SV	P_{time}	P_{temp}
pH	*[b]	*	*	*	ns	ns	ns
Hybrid	*	*	*	*	*	*	*
pH × Hybrid	ns	*	*	*	*	ns	ns

[a] Peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), setback viscosity (SV), pasting temperature (P_{temp}), and peak time (P_{time}).
[b] *, Significant at $P < 0.05$; ns, not significant.

TABLE IV

Starch Pasting Properties of Corn Samples with Sodium Bicarbonate Solution Added to Steepwater to Adjust pH[a]

Hybrid	pH	PV (cP)	TV (cP)	BV (cP)	FV (cP)	SV (cP)	P_{time} (min)	P_{temp} (°C)
6333	Control	585a[b]	501a	84a	795a	294ab	4.4ab	94ab
	3.50	597ab	520b	77a	817b	297b	4.6a	94ab
	3.75	587a	513ab	75a	794a	282a	4.3bc	95c
	4.00	622c	538c	84a	879c	341c	4.2c	93a
	4.50	610bc	536c	74a	843d	307b	4.6a	95bc
14V33	Control	587ab	536ab	52a	793ab	258a	4.7a	94a
	3.50	577ab	524b	53a	779a	255a	4.5a	93a
	3.75	590ab	550ac	40a	791ab	242ab	4.8a	93a
	4.00	601bc	558c	44a	804ab	247ab	4.5a	94a
	4.50	616c	580d	36a	813b	233b	4.6a	94a
26D33	Control	612ab	457a	155a	786a	329a	3.7a	92a
	3.50	609ab	469a	140b	783a	314a	4.0bc	93a
	3.75	622ac	467a	155a	835b	368b	4.0a–c	92a
	4.00	639c	491b	147c	813c	322a	3.9ac	93a
	4.50	639c	506b	133b	828bc	322a	4.0bc	93a
66B33	Control	661ab	499a	162a	854ab	355ab	4.0a	92a
	3.50	641b	492a	149b	852b	360a	4.1a	94b
	3.75	646b	492a	154ab	835b	343bc	4.0a	93ab
	4.00	651b	515b	136c	846b	331c	4.0a	94ab
	4.50	672a	535c	137c	876a	341bc	4.0a	94b

[a] Peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), setback viscosity (SV), pasting temperature (P_{temp}), and peak time (P_{time}).
[b] Means followed by the same letter did not differ at $P = 0.05$.

increased. This means that the starch hot paste was thicker at the higher pH, suggesting that some acid thinning occurred at the lower pH levels.

Some of the selected hybrids are more susceptible to breakdown and have breakdown viscosities three times higher than other hybrids (Table IV). The steepwater pH did not have a significant effect on the hybrids with lower breakdown values (6333 and 14V33). The hybrids with larger values for breakdown viscosity (26D33 and 66B33) were significantly affected by the steepwater pH. The breakdown increased by 13.5–44.4% between the control and pH 4.5, depending on the hybrid, with an overall decrease in the breakdown viscosity as the pH increased.

The impact of steepwater pH on the final viscosity was different for the selected hybrids. For two hybrids (14V33 and 66B33), the steepwater pH did not significantly affect the final viscosity, yet others (6333 and 26D33) were significantly affected. As the starch paste cooled, the final viscosity increased, resulting in firmer gels at the higher pH levels. The final viscosity increased by 2.5–6.0% between the control and pH 4.5 values, depending on the hybrid, with an overall increase in final viscosity as pH increased.

The main effect of pH was not a significant factor for setback, peak time, or pasting temperature, suggesting that they were the pasting properties least affected by steepwater pH ($P < 0.05$) (Table III). The main effect of hybrid and the interaction term of hybrid × pH were significant factors for setback and pasting temperatures. The degree of hardening of the cooked starch was a larger function of the hybrid than the pH, yet only one hybrid (14V33) was not significantly affected by pH ($P < 0.05$). The pasting temperatures were not significantly affected by pH for most of the hybrids except 6333.

CONCLUSIONS

Starch yield was unaffected at initial pH 4.0 and below. There was an overall increase in peak, trough, and final viscosity as pH increased. Hydrolysis of the starch through acid thinning was deemed the cause of the decrease in pasting properties at the lower pH samples. The precision of the study may be high enough to establish statistical significance, but the effect on pasting properties may not be practically significant from an industrial standpoint. Yet, to minimize the effect of steeping pH on pasting properties while maintaining the highest possible percent starch, a steepwater at pH 3.75 is suggested.

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