

Reducing Steep Time by Adding Lactic Acid During Countercurrent Steeping of Corn with Different Initial Moisture Contents

P. Yang^{1,2} and S. R. Eckhoff¹

ABSTRACT

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Reducing corn steep time by adding lactic acid instead of relying on in situ fermentation was studied. Corn at two initial moisture levels (15 and 20%) was steeped for 18 hr in a countercurrent steep system. The initial SO₂ target concentration in steepwater was 2,000 or 3,000 ppm, while the initial lactic acid concentration in steepwater was 0, 0.28, or 0.55%. Adding lactic acid under all steeping conditions decreased steepwater pH, accelerated SO₂ absorption, and increased the amount of solids released from corn. Adding lactic acid during steeping also increased the first grind slurry density and made germ skimming easier than when no lactic acid was added. Starch yields for the hybrid used in this study under

all steep conditions were comparable to those from 24-hr steeping, except when steeping corn with an initial moisture content of 15% in ≈2,000 ppm of SO₂ alone. For the 20% moisture corn, adding lactic acid to fresh steepwater significantly improved the starch yield at ≈2,000 ppm of SO₂ for 18-hr steeping. At ≈3,000 ppm of SO₂, adding lactic acid did not increase the starch yield for the hybrid used. The protein content in starch was significantly lower when lactic acid was added. Pasting properties of starch were not affected by adding lactic acid. The hybrid used in this study had an initial moisture content of 20% and could be wet-milled without affecting starch yield, starch protein content, and pasting properties.

Industrial steeping, a capital- and energy-intensive step, is performed in a series of stainless steel tanks for 24–48 hr. Reducing steep time can have a favorable impact on the economics of the wet milling industry because capital costs and energy costs can be decreased, and existing facility capacity can be increased.

A proper balance between SO₂ level and steepwater pH enables rapid degradation of the glutelin matrix followed by the release of the starch granules within 20 hr of steeping (Biss and Cogan 1996). Corn kernels can be fully hydrated in ≈8–12 hr (Watson et al 1951; Anderson et al 1960, 1961; McKinney 1996). The longer steep times used by industry partially result from the length of time required for production of lactic acid from *Lactobacillus* fermentation. The positive benefits of lactic acid to wet milling have been discussed by many researchers (Cox et al 1944; Watson et al 1951, 1955; Roushdi et al 1981a; Watson 1984; Eckhoff and Tso 1991; Ruan et al 1992; Shandera et al 1995) and the effect of lactic acid is hybrid-dependent (Singh et al 1997). It is the fermentation product (lactic acid) and not the fermentation process itself that lowers the pH, increases the solubilization of protein, softens the cell walls, and facilitates the separation of components (Watson et al 1951; Roushdi et al 1979, 1981a,b).

The objectives of this study were to: 1) determine whether adding lactic acid into fresh steepwater can reduce steep time and improve or maintain product yields and quality, and 2) evaluate the effect of initial corn moisture content on steepwater profiles (SO₂ concentration, pH, total acidity, and solids content), wet milling yields, and starch pasting properties.

MATERIALS AND METHODS

Corn Sample

Pioneer hybrid 3394, harvested in 1998 at two different moisture levels, and cleaned using a 4.8-mm (12/64 in.) round hole screen was used in this study. Initial moisture contents of the corn (15.3 and 20.5%) were determined by the 103°C, 72-hr convection oven method (AACC 2000). Chemical composition (73.6% starch, 6.8% protein, 4.1% fiber, and 2.7% fat, dry basis) was determined using near-infrared transmittance (GrainSpec, Foss North America, Minneapolis, MN) by the Identity Preserved Grain Laboratory, Champaign, IL. Transmittance readings of 250 g were taken over a wavelength range of 800–1,100 nm.

¹ Postdoctoral research associate and professor, respectively. Department of Agricultural Engineering, University of Illinois, Urbana, IL 61801.

² Corresponding author. Fax: (217) 244-0323. E-mail: p-yang2@uiuc.edu

Steeping

Corn samples (1 kg ± 0.3 g, each tank) were steeped in a laboratory-scale continuous countercurrent system (Yang et al 1998) for 18 hr at 50 ± 2°C. The system was started with light steepwater obtained from a local wet-milling plant. Twelve tanks were used to steep corn at any given time, and the other four were used as a buffer so the system could be left for 6 hr without supervision (Yang et al 1998). The steepwater recycle rate was set at 5.0 mL/sec, and the light steepwater draw rate was 600 mL/kg. Initial SO₂ and lactic acid concentrations were independent variables, and total solids content in steepwater, total acidity, and pH of the steepwater, and the milling results were dependent variables. Initial SO₂ target concentrations in fresh steepwater were 2,000 or 3,000 ppm, while the initial lactic acid concentrations were 0, 0.28, or 0.55% (v/v).

The system was run for eight days to reach the state when steep variables including SO₂ concentration, solids content, total acidity, and pH of steepwater were stabilized (Yang and Eckhoff 1999). After the steep variables stabilized, two sets of steepwater samples were taken; the time between the two samplings was at least 24 hr. Steepwater profiles were generated and they included SO₂ concentration, solids content, total acidity, and pH of steepwater versus steep time. The SO₂ concentration was determined by titration with iodine solution (Eckhoff 1995b). Steepwater solids content was determined by a two-stage convection oven method (AACC 2000). Total acidity was determined using a titrimetric method (CRA 1991) by an autotitrator (model AT400, Lab Data Plus, Inc., Amherst, OH), and the pH of the steepwater was measured using the autotitrator. Corn samples with 15% initial moisture content were countercurrently steeped for 24 hr at 50 ± 2°C in 2,000 ppm of SO₂ and the average was used as a control.

Wet Milling of Steeped Corn

Three 1-kg (initial corn weight) samples of 18-hr steeped corn for each condition and the control samples steeped for 24 hr were milled using the procedure described by Eckhoff et al (1993). Average product yields were reported.

Starch protein content (N × 6.25) was determined by Silliker Laboratories Group, Inc., Cedar Rapids, IA, by Kjeldahl nitrogen content analysis (CRA 1991). Starch pasting properties were determined using a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood NSW 2102, Australia).

A starch slurry (5.5% dry solids content) was heated rapidly from room temperature to 50°C, heated at 18°C/min from 50 to 95°C, held at 95°C for 20 min, and cooled down to 50°C at 15°C/min. Pasting temperature, peak viscosity, breakdown, and final viscosity were recorded.

Statistical Analysis

Differences in the product yields were compared among corn samples steeped in different concentrations of SO₂, lactic acid, and different initial moisture contents in corn by analysis of variance (ANOVA) and Duncan's multiple range test (SAS Institute, Cary, NC).

TABLE I
SO₂ Concentration Decrease Rate and Solids Content Increase Rate When Steeping Corn with Different Initial Moisture Contents in Different Levels of SO₂ and Lactic Acid^{a,b}

Initial Moisture (%)	Steeping Conditions		SO ₂ Initial Decrease Rate (ppm/hr)	Slurry Density After First Grind (°Be)
	SO ₂ (ppm)	Lactic Acid (%)		
15	2,199	0	166 ± 3	7.2 ± 0.9
15	2,117	0.28	323 ± 28	8.5
15	2,076	0.55	402	8.5 ± 0.4
15	2,771	0	116 ± 11	7.3 ± 1.1
15	3,165	0.28	351 ± 16	8.5
15	3,010	0.55	379 ± 10	8.8 ± 0.4
20	2,155	0	145 ± 5	7.8 ± 0.4
20	2,005	0.28	210 ± 1	8.8 ± 0.4
20	2,005	0.55	419 ± 9	8.8 ± 0.4
20	3,015	0	345 ± 31	7.8 ± 0.4
20	3,015	0.28	389	8.4 ± 0.2
20	3,015	0.55	450 ± 4	9.0 ± 0.7

^a SO₂ initial concentration decrease rate was the decrease in SO₂ concentration every hour during the first 4.5 hr of steeping and was calculated as the slope of the regression line (SO₂ concentration vs. steep time).

^b Average of three replicates ± one standard deviation.

RESULTS AND DISCUSSION

Steepwater Profiles

SO₂ concentration decreased linearly during the first 4.5 hr of steeping under all of the steeping conditions (Figs. 1A–4A). The initial SO₂ concentration decrease rate was calculated as the slope of the linear regression line between SO₂ concentration and steep time during steeping. Increasing the amount of lactic acid increased the initial SO₂ decrease rate (Table I) and decreased the SO₂ concentration in each tank during steeping (Figs. 1A–4A). This result indicates that lactic acid accelerated absorption of SO₂ by corn and it is in agreement with Shandera et al (1995) and Biss and Cogan (1996).

When steeping corn in 2,000 ppm (target value) of SO₂ alone, the pH of the steepwater stabilized at 5.2–5.5 (Figs. 1B and 3B), while it was in the range of 4.5–5.1 when steeping corn in 3,000 ppm (target value) of SO₂ for both moisture levels (between steeping 3 to 16.5 hr) (Figs. 2B and 4B). It took 6 hr for the pH to stabilize when adding lactic acid into the fresh steepwater instead of 3 hr without adding lactic acid. As expected, the stabilized pH was lower when adding lactic acid than without the addition of lactic acid (Figs. 1B–4B).

Without adding lactic acid into fresh steepwater, total acidity of steepwater was in the range of 2.1–2.9 meq/100 g when steeping corn in 2,000 ppm (target value) of SO₂ alone (Figs. 1C and 3C). When increasing the SO₂ concentration to 3,000 ppm (target value), the total acidity increased to a higher range (4.8–6.4 meq/100 g) (Figs. 2C and 4C). The increase in total acidity without adding lactic acid was due to natural fermentation during steeping. When increasing the SO₂ concentration, more solubles were released (compare Figs. 2D to 1D and 4D to 3D) and, therefore, more substances were available for fermentation. With the addition of lactic acid, the

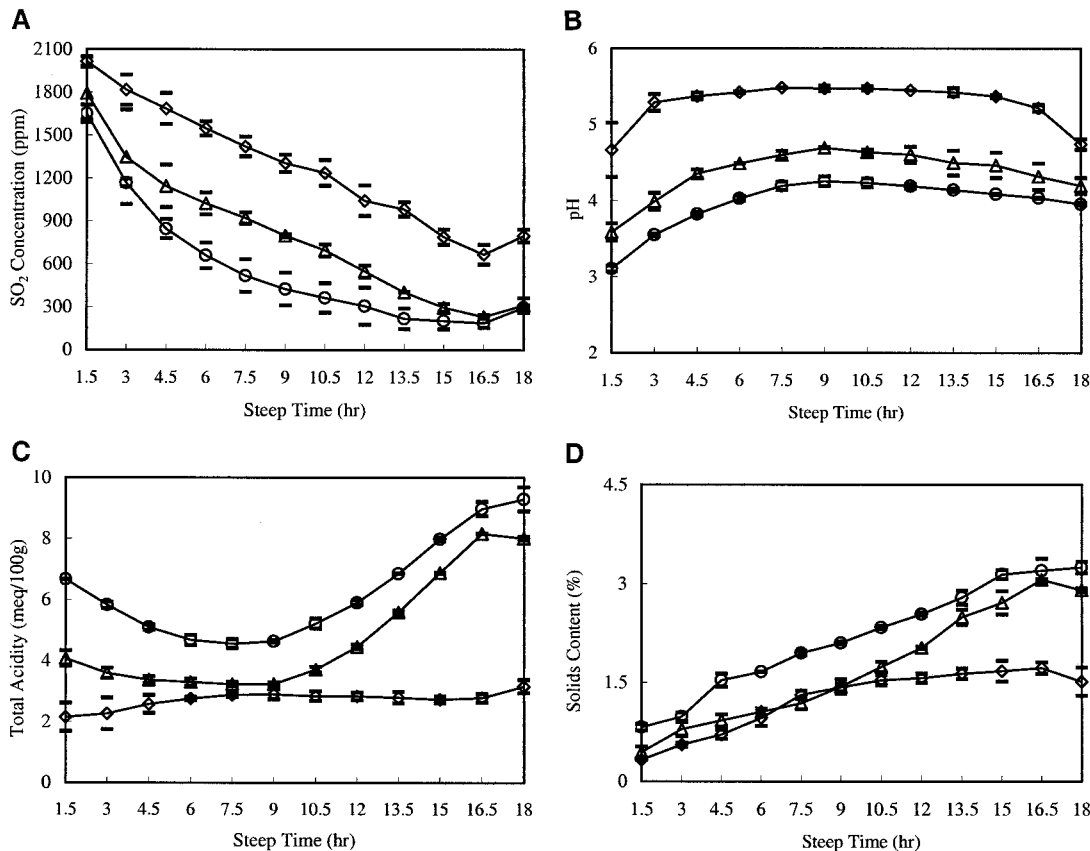


Fig. 1. Effect of lactic acid concentration on corn steepwater profiles when steeping corn with initial moisture content of 15% at 50°C for 18 hr in 2,000 ppm of SO₂. Steep time is the amount of time that new steepwater has come in contact with already steeped corn. Corn at 1.5 hr is oldest and corn at 18 hr is newest. A, SO₂ concentration. B, pH level. C, Total acidity. D, Solids content. ◇ = No lactic acid, Δ = 0.28% lactic acid, ○ = 0.55% lactic acid.

change in total acidity was caused by both added lactic acid and natural fermentation. When adding 0.28% lactic acid into steepwater, the total acidity tended to decrease slightly during the first 9 hr of steeping and then increase due to fermentation (Figs. 1C–4C).

When lactic acid was increased to 0.55%, the total acidity decreased during the first 9 hr of steeping and then increased. During the first 9 hr of steeping, lactic acid produced by in situ fermentation was not able to make up for that which was consumed

by the corn. Therefore, a decrease in total acidity for the first 9 hr was observed. The results indicate that it took ≈ 9 hr for fermentation to start during steeping. In summary, adding lactic acid decreased the pH of steepwater and increased the amount of SO_2 absorbed by corn, as indicated by an increased initial SO_2 decrease rate and an overall low SO_2 concentration in steepwater. Lactic acid probably increased the porosity of cellular membranes and softened the protein matrix in the vitreous regions of the corn kernel, which promoted absorption of SO_2 (Shandera et al 1995).

TABLE II
Wet-Milling Yields of Countercurrently Steeped Corn with Different Initial Moisture Contents Steeped for 18 hr in 2,000 or 3,000 ppm of SO_2 (target values) With and Without Lactic Acid

Initial Moisture (%)	SO_2 (ppm)	Lactic Acid (%)	Product Yields at Different Levels of Lactic Acid (%)					
			SWS ^a	Germ	Fiber	Starch	Gluten	Total
Control ^b	2,000	0	3.3ab ^c	7.3 ± 0.1a	10.2 ± 0.6c	69.3 ± 0.7a-c	9.9 ± 0.3ab	99.4 ± 0.5
15	2,199	0	2.4 ± 0.5b	6.9 ± 0.4ab	11.6 ± 0.6ab	67.9 ± 0.2d	9.5 ± 0.2bc	98.4 ± 0.9
15	2,117	0.28	3.3 ± 0.1ab	7.2 ± 0.2a	10.7 ± 0.1c	68.8 ± 0.5cd	9.3 ± 0.7bc	99.3 ± 0.1
15	2,076	0.55	3.0 ± 0.7ab	6.8 ± 0.2ab	10.4 ± 0.5c	68.9 ± 1.0a-d	9.9 ± 0.3ab	99.0 ± 0.7
15	2,771	0	2.8 ± 0.4ab	7.2 ± 0.2a	12.7 ± 0.3a	69.2 ± 0.6a-c	8.2 ± 0.7de	100.1 ± 0
15	3,165	0.28	3.2 ± 0.3ab	6.7 ± 0.6ab	10.9 ± 1.1bc	68.4 ± 0.6cd	8.5 ± 0.3c-e	99.5 ± 1.1
15	3,010	0.55	3.8 ± 0.5a	7.2 ± 0.2a	10.9 ± 0.5bc	69.4 ± 0.1a-c	8.7 ± 0c-e	100.0 ± 0.2
20	2,155	0	2.3 ± 0.3b	6.3 ± 0.2a-c	11.4 ± 2.0ab	68.2 ± 0.3cd	8.8 ± 0.1c-e	98.2 ± 0.7
20	2,005	0.28	2.3 ± 0.2b	6.2 ± 0.7a-c	10.3 ± 0.5c	69.8ab	9.5 ± 0.3bc	98.2
20	2,005	0.55	2.9 ± 0.8ab	5.8 ± 1.0c	10.1 ± 0.3c	70.1 ± 0.3a	10.9 ± 0.4a	99.2 ± 1.4
20	3,015	0	3.0 ± 0.4ab	6.2 ± 0.4a-c	12.5 ± 0.5a	69.2 ± 0.2a-c	8.0 ± 0.7e	98.7 ± 0.7
20	3,015	0.28	3.0 ± 0.7ab	6.5 ± 0.3a-c	10.6 ± 0.1c	69.7 ± 1.3ab	10.0 ± 1.0ab	100.7 ± 0.1
20	3,015	0.55	3.7 ± 0.5a	6.1 ± 0.7bc	10.9 ± 0.8bc	70.0 ± 0.1a	9.1 ± 0.2bcd	99.2 ± 0.5

^a Steepwater solids.

^b Control sample (15% moisture) was countercurrently steeped in 2,000 ppm of SO_2 for 24 hr at 50°C.

^c Values followed by the same letter in the same column are not significantly different ($P < 0.05$). Average of three replicates.

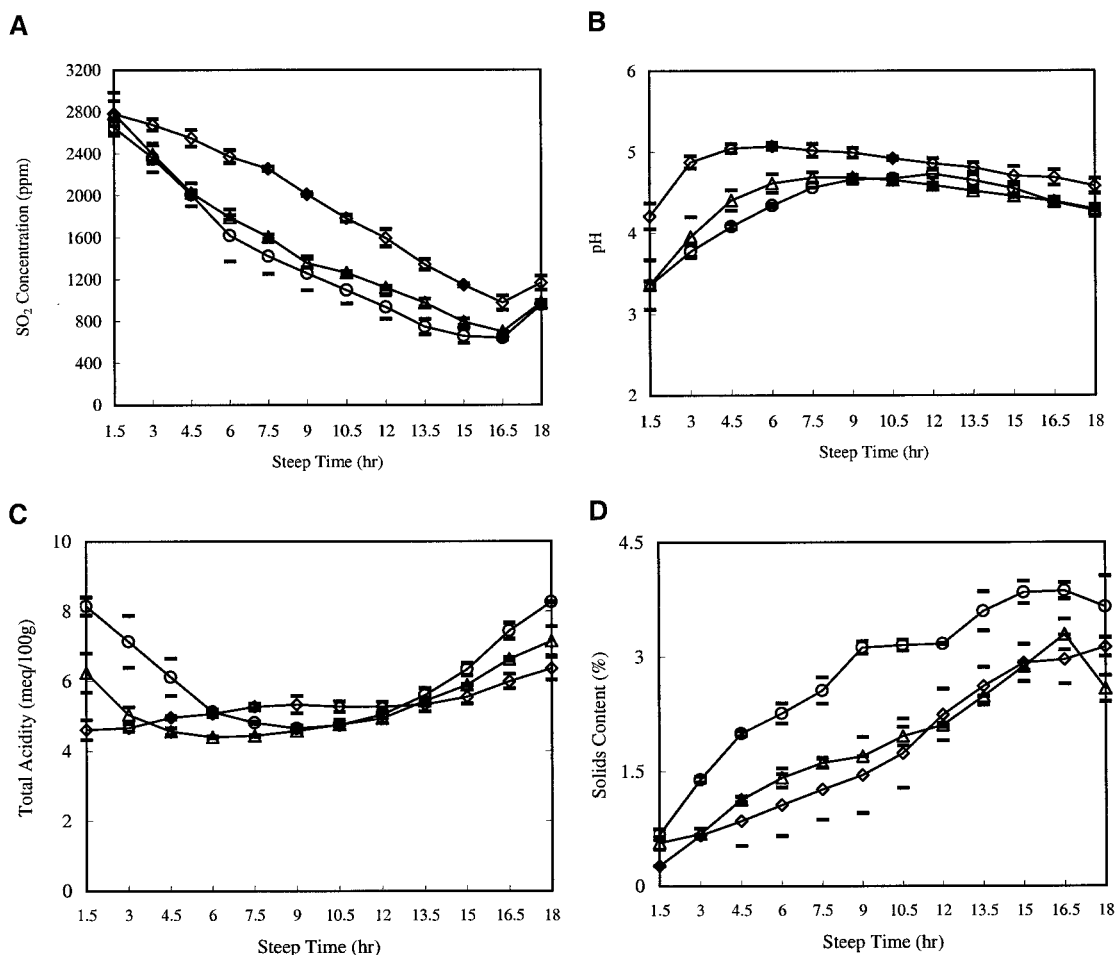


Fig. 2. Effect of lactic acid concentration on corn steepwater profiles when steeping corn with initial moisture of 15% at 50°C for 18 hr in 3,000 ppm of SO_2 . Steep time is the amount of time that new steepwater is in contact with already steeped corn. Corn at 1.5 hr is oldest and corn at 18 hr is newest. **A**, SO_2 concentration. **B**, pH level. **C**, Total acidity. **D**, Solids content. \diamond = No lactic acid, Δ = 0.28% lactic acid, \circ = 0.55% lactic acid.

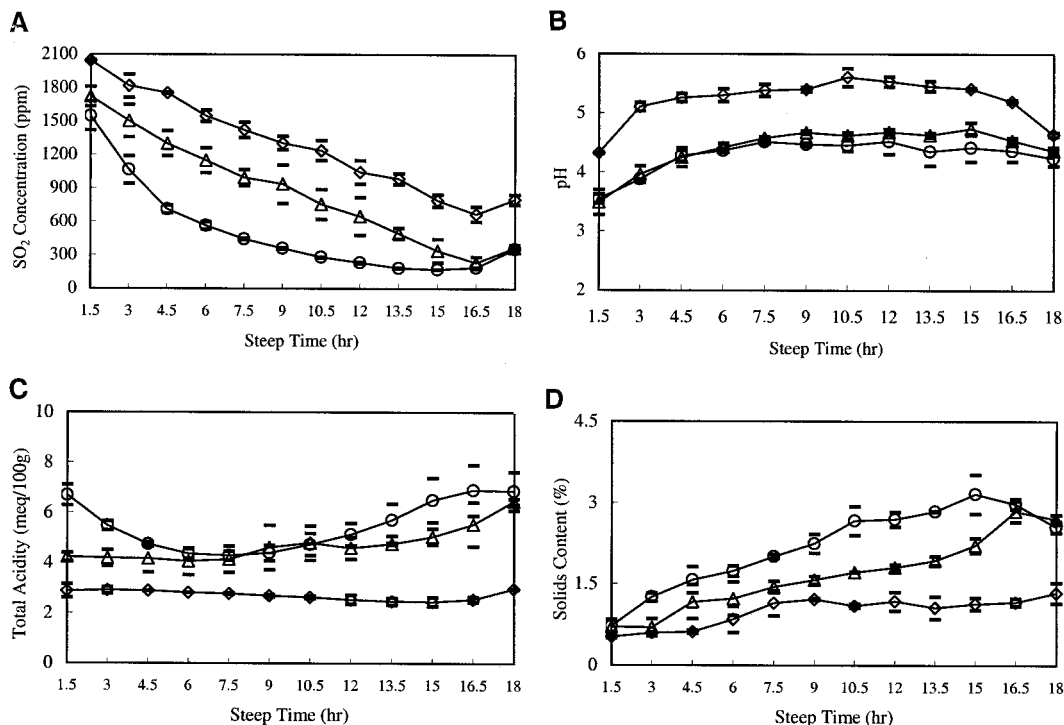


Fig. 3. Effect of lactic acid concentration on corn steepwater profiles when steeping corn with initial moisture content of 20% at 50°C for 18 hr in 2,000 ppm of SO₂. Steep time is the amount of time that new steepwater is in contact with already steeped corn. Corn at 1.5 hr is oldest and corn at 18 hr is newest. **A**, SO₂ concentration. **B**, pH level. **C**, Total acidity. **D**, Solids content. ◇ = No lactic acid, △ = 0.28% lactic acid, ○ = 0.55% lactic acid.

TABLE III
Protein Content in Starch Isolated from Corn Steeped in a Countercurrent System for 18 hr Using Different Levels of SO₂ and Lactic Acid^a

Initial Moisture (%)	Steeping Conditions ^b		Protein Content (%)
	SO ₂ (ppm)	Lactic Acid (%)	
15	2,199	0	0.4
15	2,117	0.28	0.3
15	2,076	0.55	0.3
15	2,771	0	0.4
15	3,165	0.28	0.4
15	3,010	0.55	0.3
20	2,155	0	0.4
20	2,005	0.28	0.3
20	2,005	0.55	0.3
20	3,015	0	0.4
20	3,015	0.28	0.3
20	3,015	0.55	0.3

^a Average of two replicates ± 0.02%.

^b Industrial data (Anderson and Watson 1982).

Under all of the steeping conditions, the SO₂ concentration at 18 hr of steeping was higher than that at 16.5 hr. Further study is needed to understand this phenomenon. Adding lactic acid also increased the amount of solids released from the corn into the steepwater because added lactic acid reduced the pH of the steepwater, which resulted in a larger amount of protein being solubilized (Biss and Cogan 1996).

Milling Yields of Steeped Corn

At 15% corn initial moisture content and 2,000 ppm of SO₂, starch yield from the 18-hr steep without the addition of lactic acid was significantly lower than that from the 24-hr steep (Table II). Adding lactic acid significantly improved the starch yield. Starch yield was significantly improved when increasing SO₂ concentration from ≈2,000 to 3,000 ppm when lactic acid was not added to fresh steepwater. This result is in agreement with other studies (Cox et al 1944, Watson et al 1951). When lactic acid was added to

fresh steepwater, starch yield was not significantly improved by increasing SO₂ concentration from ≈2,000 to 3,000 ppm.

At 20% initial moisture content, starch yield from the 18-hr steep, with or without lactic acid, was comparable to that from the 24-hr steep. With 18 hr of steeping, starch yield was improved significantly when adding lactic acid at ≈2,000 ppm of SO₂ but not at ≈3,000 ppm of SO₂ (Table II). This corn hybrid could be wet-milled when newly harvested with high initial moisture content to give the same or higher starch yield as corn with low initial moisture content.

When corn was steeped for only 18 hr, steepwater solids content level was lower from a practical perspective than that from a 24-hr steep except at ≈3,000 ppm of SO₂ with 0.55% lactic acid, although there was no statistical difference (Table II). Fewer solubles were leached out from the corn kernels and from the germ due to the short steep time. As a result, germ from the corn steeped for a short time was heavier than that from corn steeped for a long time (Lopes Filho 1995). Because of the short steep time, the density of the first grind slurry was lower when no lactic acid was added. The heavy germ and low density of the first grind slurry contributed to the difficulty in germ skimming. With the addition of lactic acid, the solids content in steepwater increased, the density of the slurry increased (Table I), and germ skimming was easier, as observed during the tests.

Adding lactic acid significantly decreased the fiber yield because lactic acid weakened endosperm cell walls during steeping (Roushdi et al 1981a) and resulted in a clean fiber fraction. For the corn with 15% initial moisture content, gluten yield was not changed by the addition of lactic acid but it decreased with an increase in the SO₂ level (Table II). For the corn with 20% initial moisture content, gluten yield increased when adding lactic acid into fresh steepwater; this would require further studies to explain.

Starch protein content ranged from 0.3 to 0.4% (Table III). The standard deviation for the protein measurement was 0.02% as reported by Silliker Laboratories Group, Inc., Cedar Rapids, IA. Adding lactic acid enhanced the separation between starch and gluten, as indicated by the low protein content in the starch isolated from corn steeped with lactic acid. Starch pasting temperature and the final

TABLE IV
Pasting Properties of Starch Isolated from Corn Steeped in a Countercurrent System for 18 hr
Using Different Levels of SO₂ and Lactic Acid

Initial Moisture (%)	Steeping Conditions			Pasting Properties		
	SO ₂ (ppm)	Lactic Acid (%)	Pasting Temperature (°C)	Peak Viscosity (cp)	Breakdown Viscosity (cp)	Final Viscosity (cp)
Control ^a	2,000	0	84 ± 0.5 ^{ab}	629 ± 9 ^{ab}	198 ± 3 ^{ab}	842 ± 8 ^{ab}
15	2,199	0	84 ± 0.2 ^a	602 ± 18 ^{a-c}	153 ± 6 ^{c-e}	906 ± 91 ^a
15	2,117	0.28	83 ± 5.9 ^a	516 ± 16 ^e	115 ± 12 ^e	836 ± 65 ^{ab}
15	2,076	0.55	85 ± 0.9 ^a	624 ± 16 ^{ab}	208 ± 6 ^a	855 ± 54 ^{ab}
15	2,771	0	84 ± 0.4 ^a	593 ± 30 ^{a-d}	165 ± 3 ^{b-d}	865 ± 66 ^{ab}
15	3,165	0.28	85 ± 0.9 ^a	627 ± 6 ^{ab}	177 ± 1 ^{a-c}	914 ± 29 ^a
15	3,010	0.55	85 ± 1.8 ^a	626 ± 18 ^{ab}	184 ± 1 ^{a-c}	867 ± 86 ^{ab}
20	2,155	0	88 ± 0.2 ^a	561 ± 28 ^d	137 ± 1 ^{d-e}	868 ± 1 ^{ab}
20	2,005	0.28	85 ± 2.6 ^a	625 ± 6 ^{ab}	193 ± 19 ^{ab}	883 ± 35 ^{ab}
20	2,005	0.55	83 ± 1.0 ^a	632 ± 1 ^a	200 ± 45 ^{ab}	850 ± 49 ^{ab}
20	3,015	0	84 ± 0.5 ^{ba}	625 ± 11 ^{ab}	215 ± 21 ^a	854 ± 5 ^{ab}
20	3,015	0.28	85 ± 1.3 ^a	591 ± 21 ^{b-d}	213 ± 2 ^a	814 ± 2 ^b
20	3,015	0.55	84 ± 0.2 ^a	574 ± 1 ^{cd}	183 ± 19 ^{a-c}	834 ± 16 ^{ab}

^a Starch from corn countercurrently steeped in 2,000 ppm of SO₂ for 24 hr at 50°C.

^b Values followed by the same letter in the same column are not significantly different (*P* < 0.05). Data were reported as averages of two replicates ± standard deviation.

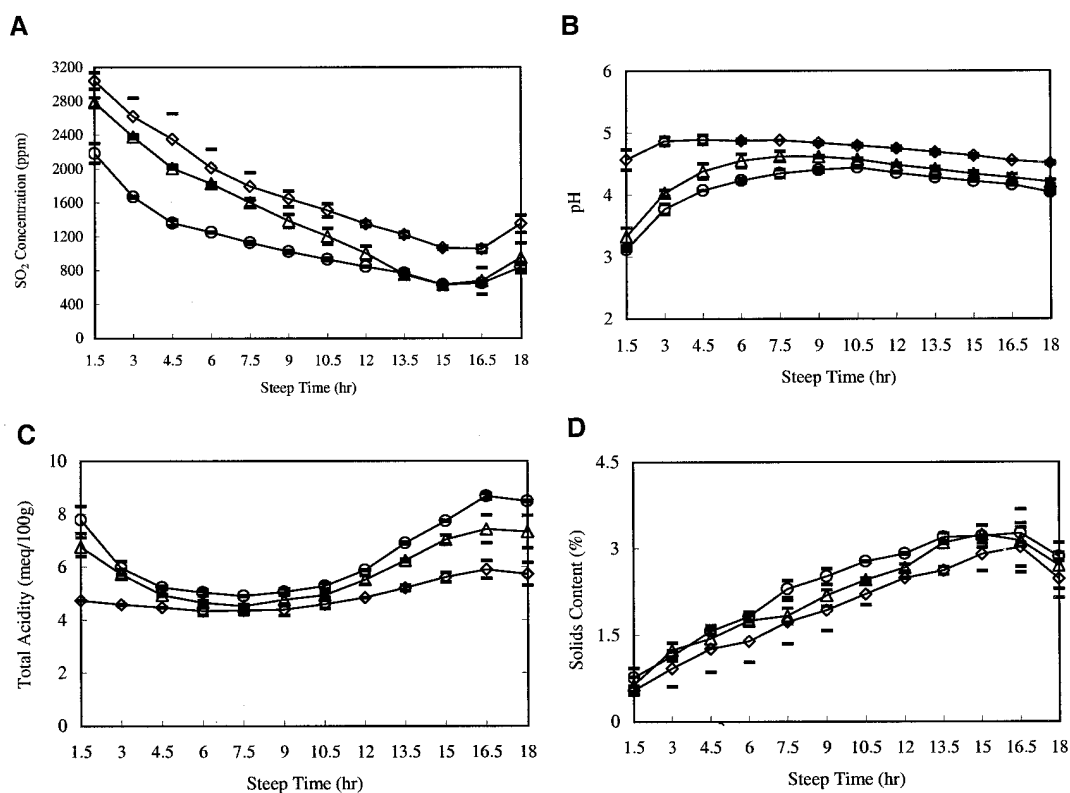


Fig. 4. Effect of lactic acid concentration on corn steepwater profiles when steeping corn with initial moisture of 20% at 50°C for 18 hr in 3,000 ppm of SO₂. Steep time is the amount of time that new steepwater is in contact with already steeped corn. Corn at 1.5 hr is oldest and corn at 18 hr is newest. **A**, SO₂ concentration. **B**, pH level. **C**, Total acidity. **D**, Solids content. ◇ = No lactic acid, Δ = 0.28% lactic acid, ○ = 0.55% lactic acid.

viscosity of starch were not affected by the initial corn moisture, the level of SO₂, or the level of lactic acid (Table IV). The peak viscosity from the testing conditions were similar to those from the control sample with three exceptions: 15% moisture corn steeped in ≈2,000 ppm of SO₂ with 0.28% lactic acid; 20% moisture corn steeped in ≈2,000 ppm SO₂ without lactic acid; and 20% moisture corn steeped in ≈3,000 ppm of SO₂ with 0.55% lactic acid. The breakdown viscosities from the testing conditions were similar to those from the control sample with the exception of 15% moisture corn steeped in ≈2,000 ppm of SO₂ with 0.28% lactic acid and 20% moisture corn steeped in ≈2,000 ppm of SO₂ without lactic acid. The samples that exhibited any differences were only marginally significant. Further study is needed to verify the results. The corn

hybrid used in this study can be processed after 18 hr of steeping at both 15 and 20% initial moisture levels with the addition of lactic acid. However, the reduction of steep time may be offset by the cost of the lactic acid. An economic analysis needs to be conducted.

CONCLUSIONS

Adding lactic acid decreased steepwater pH, accelerated SO₂ absorption, and increased the amount of solids released from corn. Starch yields for the hybrid used in this study from these steep conditions (≈2,000 or 3,000 ppm of SO₂ with 0, 0.28, or 0.55% lactic acid) were comparable to that from 24 hr of steeping except when

steeping corn with an initial moisture content of 15% in 2,000 ppm (target value) of SO₂ alone. Adding lactic acid to fresh steepwater significantly improved the starch yield when steeping corn with 20% initial moisture content in ≈2,000 ppm of SO₂. At ≈3,000 ppm of SO₂, adding lactic acid did not increase the starch yield for the hybrid used. Under all of the steeping conditions, adding lactic acid increased the first grind slurry density and made germ skimming easier than when no lactic acid was added. The protein content in starch was significantly lower when lactic acid was added. Pasting temperature and final viscosity of starch were not significantly affected by added lactic acid. In general, peak and breakdown viscosities were not significantly affected by added lactic acid either, with a few marginally significant exceptions. The hybrid used in this study could be wet-milled at a moisture level of 20% without affecting starch yield, starch protein content, and pasting properties.

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