

# Batch Steeping of Corn: Effects of Adding Lactic Acid and Sulfur Dioxide at Different Times on Starch Yields, Protein Contents, and Starch Pasting Properties<sup>1</sup>

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## ABSTRACT

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The effect of adding lactic acid and sulfur dioxide at different times from the start of batch steeping on corn starch yields was studied. Five commercial hybrids were steeped with 0.5% lactic acid or 0.2% sulfur dioxide added over the first 15 hr of steeping and wet-milled following a 100-g corn wet-milling procedure. No significant differences were observed in starch yields when lactic acid was added to the steep solution (SO<sub>2</sub> and water) from 0 hr (start of steeping) to 15 hr. Addition of SO<sub>2</sub> to the steep solution (lactic acid and water) resulted in significantly higher average starch yields when SO<sub>2</sub> was added between 5 and 15 hr compared with addition at 0 hr (SO<sub>2</sub> and lactic acid for full 24 hr of steeping). Based on the results of the first experiment, a second experiment was done in

which one of five original hybrids was steeped for 24 hr, during which lactic acid or SO<sub>2</sub> was added until 23.9 hr (i.e., 5 min before milling) after the start of steeping. Similar results were found in the second experiment. Residual protein in starch samples did not exceed 0.85%. Steep-water protein content decreased with delays (16–20 hr) in adding either chemical to the steep solution. A significant effect on starch pasting properties of chemicals and duration of chemicals in steepwater was observed. Testing these findings using a larger scale (1,000 g) corn wet-milling procedure produced results similar to those obtained with the 100-g corn wet-milling procedure.

Different forms of sulfur dioxide (bisulfite and sulfite) cleave disulfide bonds in the corn endosperm protein matrix that encapsulates starch granules (Watson 1984). The result is dispersion of the endosperm protein matrix and enhancement of starch release (Watson and Sanders 1961). However, the pH of steepwater affects the form of SO<sub>2</sub> (sulfurous acid: bisulfite or sulfite) present in steepwater. Under acidic conditions, more sulfurous acid, which has little effect on disulfide bonds, and less bisulfite and sulfite are present (King et al 1981), long steep periods are required for protein rupture (Boundy et al 1967), and acid-soluble protein in the corn kernel disintegrates and is solubilized in steepwater (Watson 1984).

During the early stages of conventional steeping, corn is exposed to high levels of lactic acid and low levels of SO<sub>2</sub>. The concentration of lactic acid slowly decreases with corresponding increases in SO<sub>2</sub> during the course of steeping, and during the later stages of steeping, corn kernels are exposed to high levels of SO<sub>2</sub>. The high concentration of lactic acid present at the beginning of steeping reduces the pH of steepwater and therefore may reduce the action of existing low levels of SO<sub>2</sub> in disintegrating the protein matrix. Even though the pH inside the kernel controls the distribution of sulfite and bisulfite, the lower pH in steepwater ultimately reduces internal pH (Kerr 1950).

If we were to reverse the order of added steeping chemicals (i.e., expose corn kernels to high levels of SO<sub>2</sub> at the beginning of steeping and introduce lactic acid near the end), the steeping medium would be less acidic and therefore could enhance SO<sub>2</sub> action on disulfide bonds, resulting in higher starch yields.

Research has shown that lactic acid fermentation is not required in the steeping process for lactic acid to have the beneficial effect of increasing starch yields (Watson 1984). Addition of externally produced lactic acid has the same effect. Short steep times and new technologies that reduce steep time, such as gaseous SO<sub>2</sub> (Eckhoff and Okos 1990, Eckhoff and Tso 1991, McKinney 1996) or intermittent-milling and dynamic-steeping processes (Lopes Filho 1995), may require addition of externally produced lactic acid to the steep

system. Therefore, it is important to determine the time at which lactic acid should be added to the steep system to maximize increases in starch yields.

The effect that relative time differences in addition of lactic acid and SO<sub>2</sub> have on starch yields and quality is unknown. Shandera and Jackson (1996) showed that the levels of lactic acid and SO<sub>2</sub> in steepwater have an effect on starch quality. They also suggested that relative time differences in exposure of corn kernels to steep chemicals (lactic acid and SO<sub>2</sub>) during batch and countercurrent steeping may produce differences in starch quality.

Relative time differences in exposure of corn kernels to steep chemicals and the effects on wet-milled corn starch yields should be tested on a countercurrent steep system to be more realistic. However, due to the difficulty in testing on a countercurrent system, and to maintain more precise control over the time chemicals were added, a batch steep system was preferred. The objective of our study was to determine the effect of adding lactic acid and sulfur dioxide at different times during batch steeping on corn starch yields, protein contents, and starch pasting properties.

## MATERIALS AND METHODS

Four yellow dent corn hybrids (S61, S84, S231, and S348) were obtained from a commercial seed company. One yellow dent corn hybrid, FR1064×LH59, grown during the 1995 and 1996 seasons at the University of Illinois at Urbana-Champaign also was used in the study. All hybrids were hand-cleaned to remove broken corn and foreign material, packaged in plastic bags, and stored at 4°C until wet-milling. Whole-kernel moisture content was measured by the 103°C convection oven method (AACC 1995).

Analysis of hybrid oil, fiber, starch, and protein content (Table I) was performed using near-infrared (NIR) spectroscopy at the Identity Preserved Grain Laboratory, Illinois Crop Improvement Association, Champaign, IL. The NIR instrument (GrainSpec, Foss Electric, Brampton, ON, Canada) had a fixed filter whole-grain analyzer that operates on a transmittance mode and wavelength range of 800–1,100 nm. Five readings per 250-g sample of each hybrid were taken for analysis. Calibration of the NIR instrument was based on the wet-chemistry standard methods of the Corn Refiners Association (CRA 1980). Oil, crude fiber, protein and starch calibrations of the NIR instrument were based on methods A-6, A-8, A-18, and A-20, respectively.

Three factorial experiments were performed in duplicate with each treatment to study the effect on starch yields of adding lactic acid and sulfur dioxide at different times during steeping. Wet-

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milling of samples in the first two experiments was performed using the 100-g laboratory wet-milling procedure of Eckhoff et al (1996), and the third experiment was performed using the 1,000-g procedure of Eckhoff et al (1993). All experiments were done with a 24-hr steep at 52°C. The complete experimental design is given in Table II.

To confirm that the results of the first two experiments were not artifacts of the wet-milling procedure scale, a third experiment was done in which the same hybrid (FR1064×LH59 from the 1996 crop) was tested under five steep conditions (Table II).

All of the steepwater samples from the second experiment were analyzed for total protein content. Steepwater (50 mL) from each experiment was pipetted into weighed bottles, frozen to -80°C, freeze-dried for 48 hr, and reweighed. Weighed portions of dried residue (≈200 mg) were enclosed in tinfoil and analyzed for nitrogen content with a nitrogen analyzer (model FP-428, Leco Corp., St. Joseph, MI) (AACC 1995). Steepwater protein content was estimated by a conversion factor of 6.25. Residual protein content in starch from all samples in the second experiment also was analyzed with the Leco nitrogen analyzer.

Pasting properties of starch from the second experiment were determined with a Rapid ViscoAnalyser (model RVA-3D, Newport Scientific, Warriewood, Australia). Pasting profiles were recorded on 28-g samples of 8% (db) starch with a slight modification of the RVA standard 2 protocol. In this protocol, samples are stirred at 960 rpm for 60 sec to disperse starch, followed by constant stirring at 160 rpm. After equilibrating at 50°C, the sample is heated at 6°C/min to 95°C, maintained at 95°C for 5 min, and cooled at 6°C/min to 50°C. Peak, trough, final, setback, and breakdown viscosities were recorded, as well as pasting temperature and time required to reach peak viscosity.

Analysis of variance and Duncan's multiple range test were used (SAS Institute, Cary, NC) for data analysis. The level selected to show statistical significance was 5% ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### Experiment 1

No significant differences were observed in starch yields of three of five hybrids when lactic acid was added from 0 to 15 hr after steeping began (Table III). Two of five hybrids (S61 and S84) showed significantly lower starch yields when lactic acid was added to the steep solution at 15 hr than at 0 or 5 hr. Although statistically significant, the differences were not practically important. The mean starch yields across all five hybrids were not significantly different when lactic acid was added to the steep solution from 0 to 15 hr (Table III).

The effect on starch yields of adding SO<sub>2</sub> from 0 to 15 hr after steeping began was not significant in four of five hybrids. Only one of five hybrids showed a significantly lower starch yield when SO<sub>2</sub> was added at the very beginning of steeping (0 hr) (Table IV). Mean starch yields across all five hybrids were significantly lower (0.7–0.9%) when SO<sub>2</sub> was added at 0 hr than at 5, 10, or 15 hr (Table IV).

The results of the first experiment suggest that if SO<sub>2</sub> is present, there is no effect of time of lactic acid addition on starch yield

(from 0 to 15 hr). On the other hand, if lactic acid is present, late addition of SO<sub>2</sub> (from 5 to 15 hr) increases starch yields.

### Average pH Profiles of Corn Hybrids with Addition of Lactic Acid and SO<sub>2</sub> to Steep Solutions at Different Times

Figures 1 and 2 show the average pH values for five hybrids during batch steeping. With addition of 0.55% lactic acid to the steep solution, solution pH dropped by ≈2.5 (Fig. 1). As steeping progressed, pH increased and reached 4.0–4.5. Addition of 0.2% SO<sub>2</sub> to the steep solution marginally (0.1) increased steepwater pH at 0 and 5 hr (Fig. 2). Changes in steepwater pH were negligible when SO<sub>2</sub> was added at 10 and 15 hr. For all conditions (different lactic acid and SO<sub>2</sub> addition times), the steep solution pH at the end of steeping reached 4.0–4.5.

### Experiment 2

No significant differences were observed in starch yields with addition of only lactic acid to steepwater at different times (0–23.9 hr). When compared with the starch yield of the control sample (no chemicals in the steep solution), no significant differences were observed in starch yields for any lactic acid addition time, except when lactic acid was added at 0 hr or 23.5 hr (30 min before milling) (Table V). Lactic acid addition at 0 and 23.5 hr produced 3.9 and 2.4% higher starch yields, respectively, compared with that of the control sample (Table V). However, when comparing starch yields among different lactic acid addition times (Table V), no statistically significant differences in starch yields were observed.

With addition of only SO<sub>2</sub> to steepwater, no significant differences were observed from 0 to 16 hr. Starch yields significantly decreased by ≈2.4% when SO<sub>2</sub> was added after 16 hr. Compared with the starch yield of the control sample, starch yields for all SO<sub>2</sub> addition times were significantly higher (1.7–5.2%). At 0 hr, addition of only SO<sub>2</sub> to the steep solution had more effect on increasing starch yield than addition of only lactic acid when compared with the control sample (Table IV).

The more significant effect of SO<sub>2</sub> on increasing starch yields compared with lactic acid agrees with other research studies (Cox et al 1944, Watson and Sanders 1961, Watson 1984) done on the corn wet-milling steeping process. However, two interesting facts were observed: 1) lactic acid alone helped increase starch yield by

TABLE II  
Experimental Design for Studying Effects on Corn Starch Yields of Lactic Acid (LA) and SO<sub>2</sub> Added to Steepwater at Different Times<sup>a</sup>

Hybrid <sup>b</sup>	Chemical Added	Time Added (hr)
Experiment 1 FR1064×LH59 (1995); S61; S84; S231; S348	0.2% SO <sub>2</sub> added to steep containing 0.55% LA and water 0.55% LA added to steep containing 0.2% SO <sub>2</sub> and water	0, 5, 10, 15
Experiment 2 FR1064×LH59 (1996)	0.55% LA added to water 0.2% SO <sub>2</sub> added to water 0.55% LA added to steep containing 0.2% SO <sub>2</sub> and water 0.2% SO <sub>2</sub> added to steep containing 0.55% LA and water No chemicals (water only)	0, 8, 16, 20, 23, 23.5, 23.9
Experiment 3 FR1064×LH59 (1996)	0.55% LA added to water 0.2% SO <sub>2</sub> added to water 0.55% LA added to steep containing 0.2% SO <sub>2</sub> and water 0.2% SO <sub>2</sub> added to steep containing 0.55% LA and water No chemicals (water only)	0, 8, 22

<sup>a</sup> All experiments were done with 24-hr steep at 52°C.

<sup>b</sup> Experiments 1 and 2: 100-g wet-milling procedure; experiment 3: 1,000-g wet-milling procedure.

TABLE I  
Analysis of Corn Hybrids Using Near-Infrared Transmittance<sup>a</sup>

Hybrid	Oil	Fiber	Starch	Protein
S61	4.30	2.92	71.49	6.68
S84	3.53	2.04	73.10	6.50
S231	3.45	2.29	72.86	5.79
S348	4.16	2.72	69.65	7.27
FR1064×LH59 (1995)	4.32	1.94	71.83	9.70
FR1064×LH59 (1996)	4.84	2.36	69.41	9.52

<sup>a</sup> All yields are expressed as % dry solids and are means of five observations.

≈1.6–4.0%, depending on addition time, compared with the control sample (statistical significance was observed only for 0 and 23.5 hr); and 2) SO<sub>2</sub> and water added to the steep solution for as little as 8 hr produced significantly similar starch yields when compared with samples that contained SO<sub>2</sub> for 24 hr.

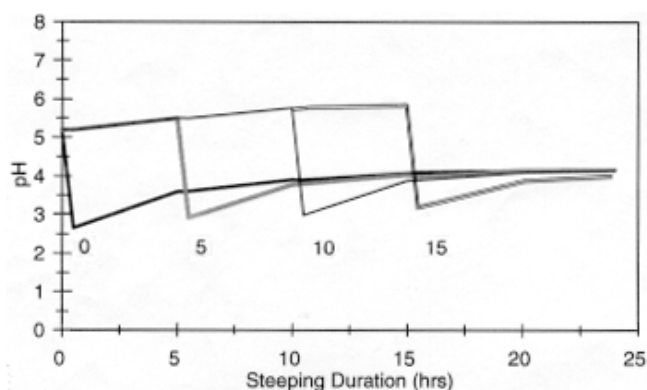
For lactic acid added to SO<sub>2</sub> and water at different times (Table VI), the average starch yields obtained at all times, except 23.9 hr, were statistically comparable (i.e., the difference was <2 standard deviations) to the starch yield obtained from the control sample (normal steeping, both SO<sub>2</sub> and lactic acid were present at 0 hr). However, when comparing starch yields between different addition times of lactic acid to SO<sub>2</sub> and water, some statistically significant effects were observed in starch yields, but they did not follow a pattern.

When SO<sub>2</sub> was added to lactic acid and water at different times during steeping, results similar to those obtained in the first experiment were observed. The starch yield obtained when SO<sub>2</sub> was added to the steep solution at 8 hr was significantly higher (1.3%) compared with the control sample (normal steeping, addition of SO<sub>2</sub> to lactic acid and water at 0 hr). At all other SO<sub>2</sub> addition times (16–

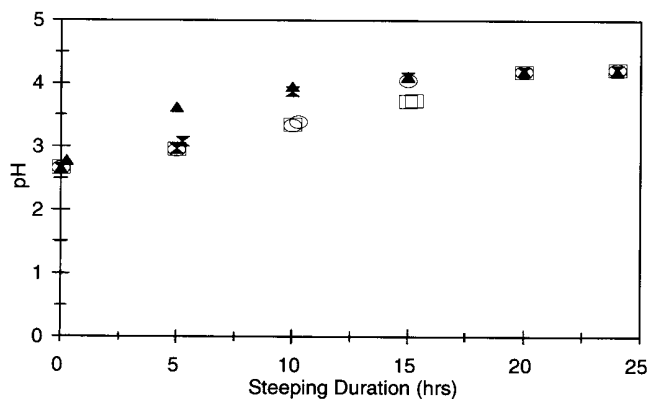
23.9 hr), no significant differences in starch yields were observed with respect to the control sample (Table VI).

It is difficult to ascertain why the average starch yields obtained with SO<sub>2</sub> added at very late stages (30–5 min before milling) were statistically comparable to the starch yield obtained with normal steeping. Watson and Sanders (1961) showed that, with all diffusion barriers removed for SO<sub>2</sub> to penetrate into the corn kernel and react with the disulfide bonds, 2–4 hr of reaction time is required to disrupt the protein matrix for starch release. However, it is not possible to compare the two studies because Watson and Sanders (1961) did not add any lactic acid to their steep medium.

The results again show that when lactic acid is already present in the steep solution, adding SO<sub>2</sub> a little later (≈8 hr) produces higher starch yields compared with SO<sub>2</sub> added at the very beginning or end. Also, when lactic acid is present, SO<sub>2</sub> can be added to the steep solution at 23.9 hr (5 min before milling), and starch yields that are significantly similar to the control sample (normal steeping, addition of SO<sub>2</sub> to lactic acid and water at 0 hr) can be obtained. When SO<sub>2</sub> is already present in the steep solution, lactic acid can be added at any time during steeping for all practical purposes.



**Fig. 1.** Effects of addition of lactic acid to steepwater containing SO<sub>2</sub> and water at different times (0, 5, 10, and 15 hr) during steeping on average pH profiles of five corn hybrids.



**Fig. 2.** Effects of addition of SO<sub>2</sub> to steepwater containing lactic acid and water at different times (▲, ✕, ○, □ = 0, 5, 10, 15 hr) during steeping on average pH profiles of five corn hybrids.

**TABLE III**  
Wet-Milling Fraction Yields<sup>a</sup> (%) Obtained from Five Corn Hybrids After Addition of 0.55% Lactic Acid (LA) to Steepwater (SW) Containing Corn, Water, and 0.2% SO<sub>2</sub>

Hybrid	Time LA Added (hr)	SW Solids	Fiber	Germ	Starch <sup>b</sup>	Gluten	Total
FR1064×LH59	0	3.7	12.9	6.8	65.6a	10.3	99.3
	5	3.6	12.9	6.9	65.5a	10.1	98.9
	10	3.4	12.9	6.5	66.0a	10.5	99.3
	15	3.3	13.3	6.5	65.5a	10.4	99.1
S61	0	3.5	14.3	4.0	69.9a	8.6	100.2
	5	3.4	14.1	3.9	69.2ab	8.9	99.5
	10	3.3	14.2	3.6	69.2ab	9.6	99.9
S84	15	3.0	13.7	4.1	68.9b	9.2	98.9
	0	3.7	14.3	4.3	66.4ab	10.9	99.5
	5	3.7	13.8	3.5	66.9a	11.0	98.9
S231	10	3.5	14.7	3.8	66.0b	10.7	98.6
	15	3.3	15.0	3.6	65.6b	11.3	98.8
	0	3.7	13.8	4.2	68.8a	9.1	99.7
S348	5	3.7	13.8	4.0	68.4a	8.9	98.7
	10	3.4	14.2	3.9	68.6a	8.8	98.9
	15	3.2	14.6	4.0	68.3a	9.0	99.1
Hybrid average	0	3.6	13.4	5.0	67.9a	9.7	99.6
	5	3.5	13.7	5.4	67.5a	9.0	99.2
	10	3.3	13.7	5.4	67.8a	9.5	99.7
	15	3.1	13.7	4.7	67.5a	10.1	99.1
Hybrid average	0	3.6	13.7	4.9	67.7a	9.7	99.7
	5	3.6	13.7	4.7	67.5a	9.6	99.0
	10	3.4	13.9	4.6	67.5a	9.8	99.3
	15	3.2	14.1	4.6	67.2a	10.0	99.0

<sup>a</sup> All yields are expressed as % dry solids and are means of two observations.

<sup>b</sup> Average starch yields followed by the same letter within a hybrid are not significantly different at 95% confidence level.

### Protein Contents of Starch and Steepwater

Residual protein contents in all starch samples (Tables V and VI) were within the acceptable limits for the 100-g wet-milling laboratory procedure. The procedure results in starch fractions that have higher protein levels because the starch is not rinsed by hand as is done in the 1,000-g wet-milling procedure. Most starch samples taken at later stages (within 30 min of milling) of chemical (SO<sub>2</sub> or lactic acid) addition had lower residual protein contents when compared with the control samples (Table VI).

Protein content in steepwater decreased as duration of chemical presence in steepwater decreased (Tables V and VI). The decrease in protein content in steepwater corresponded to a decrease in starch yields (coefficient of determination = 0.72). If the duration of either chemical in water was <4–8 hr (addition of chemicals at 16–20 hr), there was no significant change in the protein content of the steepwater (Table V). Also, after 20 hr, when lactic acid was added to SO<sub>2</sub> and water, or SO<sub>2</sub> was added to lactic acid and

water, no significant drop was observed in the protein content of steepwater (Table VI).

### Starch Pasting Properties

Addition of lactic acid to water, SO<sub>2</sub> to water, and both lactic acid and SO<sub>2</sub> to water significantly affected peak pasting viscosities (Table VII). Reduced peak viscosities of starch samples were observed when lactic acid or SO<sub>2</sub> was added to water as compared with starch samples obtained by steeping corn in plain water. Peak viscosities were reduced further when both lactic acid and SO<sub>2</sub> were added. Longer exposure of corn to each chemical (earlier chemical addition) during steeping, especially lactic acid and to a lesser degree SO<sub>2</sub>, lowered peak viscosities (Table VII). Peak viscosity was significantly reduced when lactic acid was present in steepwater because lactic acid causes acid thinning (due to depolymerization of amylopectin) of starch granules (Shandera and Jackson 1996). This also is true for starch samples recovered by laboratory batch

**TABLE IV**  
Wet-Milling Fraction Yields<sup>a</sup> (%) Obtained from Five Corn Hybrids After Addition of 0.2% SO<sub>2</sub> to Steepwater (SW) Containing Corn, Water, and 0.55% Lactic Acid

Hybrid	Time SO <sub>2</sub> Added (hr)	SW Solids	Fiber	Germ	Starch <sup>b</sup>	Gluten	Total
FR1064×LH59	0	3.7	12.8	6.8	65.0a	10.8	99.1
	5	3.6	12.5	7.0	65.7a	10.6	99.4
	10	3.5	12.4	6.5	66.0a	10.5	99.0
	15	3.4	12.5	6.4	65.7a	10.7	98.8
S61	0	3.5	14.6	4.1	68.6a	8.6	99.5
	5	3.3	13.6	4.1	69.5a	8.6	99.1
	10	3.3	14.3	3.7	69.1a	9.1	99.5
	15	3.1	14.2	3.7	68.7a	9.2	99.0
S84	0	3.7	14.1	4.2	66.0c	10.8	98.8
	5	3.7	14.2	3.2	67.3ab	10.6	99.0
	10	3.7	13.4	3.6	67.7a	10.3	98.6
	15	3.5	13.9	3.6	66.8b	10.9	98.7
S231	0	3.7	14.5	4.1	67.9a	9.0	99.3
	5	3.5	13.4	4.0	69.2a	8.6	98.8
	10	3.4	14.0	3.9	69.1a	8.5	98.9
	15	3.3	13.7	4.2	69.1a	8.7	98.9
S348	0	3.6	13.5	5.2	67.9a	9.1	99.3
	5	3.5	12.8	5.0	68.2a	9.6	99.1
	10	3.5	12.8	5.3	68.3a	9.5	99.4
	15	3.5	12.8	4.6	68.6a	10.0	99.4
Hybrid average	0	3.7	13.9	4.9	67.1a	9.7	99.2
	5	3.7	13.3	4.7	68.0a	9.6	99.1
	10	3.5	13.4	4.6	68.0a	9.6	99.1
	15	3.4	13.4	4.5	67.8a	9.9	98.9

<sup>a</sup> All yields are expressed as % dry solids and are means of two observations.

<sup>b</sup> Average starch yields followed by the same letter within a hybrid are not significantly different at 95% confidence level.

**TABLE V**  
Wet-Milling Fraction Yields<sup>a</sup> (%) and Protein Contents<sup>b</sup> in Steepwater (SW) and Starch Obtained from Corn Samples with No Chemicals Added (Control) and with Lactic Acid (LA) or SO<sub>2</sub> Added to Water

Steeping Treatment	Time Added (hr)	SW Solids	SW Protein <sup>c</sup>	Fiber	Germ	Starch <sup>d</sup>	Starch Protein	Gluten	Total
Control		1.6	2.8e	22.3	5.7	59.9b	0.52	7.3	96.8
LA added to water	0	2.7	5.0a	18.3	5.4	63.8a	0.55	8.1	98.4
	8	2.3	3.5c	19.9	6.4	61.7ab	0.47	7.4	97.7
	16	2.0	3.0de	20.6	5.6	61.9ab	0.45	7.5	97.6
	20	2.0	2.9de	21.0	5.4	62.1ab	0.55	7.7	98.1
	23	2.1	...	21.9	6.1	61.8ab	0.54	7.2	99.2
	23.5	2.1	...	21.8	5.0	62.3a	0.59	7.4	98.7
	23.9	2.1	...	22.3	5.7	61.5ab	0.36	7.3	98.8
SO <sub>2</sub> added to water	0	1.6	2.8e	22.3	5.7	59.9d	0.52	7.3	96.8
	8	2.5	4.2b	15.2	6.1	65.1a	0.85	8.5	97.5
	16	2.2	3.4c	16.8	6.7	64.0ab	0.58	8.2	97.8
	20	2.1	3.1d	17.8	5.8	64.2a	0.65	8.2	98.0
	23	2.1	2.9de	19.1	5.8	62.6c	0.73	8.1	97.6
	23	2.2	...	21.3	6.5	61.6c	0.57	6.9	98.6
	23.5	2.2	...	20.1	5.5	62.7bc	0.49	7.9	98.4
	23.9	2.2	...	21.1	6.1	61.7c	0.52	7.4	98.6

<sup>a</sup> All yields are expressed as % dry solids and are means of two observations.

<sup>b</sup> All protein contents are expressed as % dry solids and are means of four observations.

<sup>c</sup> Average steepwater protein contents followed by the same letter are not significantly different at 95% confidence level.

<sup>d</sup> Average starch yields followed by the same letter within a treatment are not significantly different at 95% confidence level.

**TABLE VI**  
**Wet-Milling Fraction Yields<sup>a</sup> (%) and Protein Contents<sup>b</sup> in Steepwater (SW) and Starch Obtained from Corn Samples**  
**Containing Lactic Acid and SO<sub>2</sub> (Control) and Samples with Lactic Acid or SO<sub>2</sub> Added to SW Containing Water and the Other Chemical**

Steeping Treatment	Time Added (hr)	SW Solids	SW Protein <sup>c</sup>	Fiber	Germ	Starch <sup>d</sup>	Starch Protein	Gluten	Total
Control		3.2	6.4a	14.0	6.0	67.2ab	0.64	8.6	99.0
LA added to water and SO <sub>2</sub>	8	3.2	5.8c	14.0	6.4	66.9a-c	0.53	8.3	98.6
	16	2.9	5.0e	14.2	6.5	66.5b-d	0.52	8.6	98.7
	20	2.9	4.5f	14.0	5.9	67.5a	0.78	8.7	99.0
	22	3.0	4.5f	13.9	5.6	67.1ab	0.69	8.9	98.6
	23	3.0	...	15.6	6.7	66.0cd	0.63	8.4	99.7
	23.5	3.0	...	14.5	5.6	67.5a	0.61	9.1	99.7
	23.9	3.0	...	16.4	5.7	65.7d	0.60	8.8	99.6
Control		3.2	6.4a	14.0	6.0	67.2bc	0.64	8.6	99.0
SO <sub>2</sub> added to water and LA	8	3.2	6.4a	13.3	5.6	68.5a	0.49	8.8	99.4
	16	3.2	6.1b	12.7	5.8	68.0ab	0.54	9.0	98.7
	20	3.1	5.5d	14.0	6.0	67.1bc	0.70	8.7	98.8
	22	3.1	5.5d	13.3	5.7	67.8ab	0.64	8.8	98.6
	23	3.2	...	14.3	5.5	67.7ab	0.45	9.3	99.9
	23.5	3.1	...	14.9	6.1	66.6c	0.48	8.9	99.6
	23.9	3.1	...	16.2	5.3	66.3c	0.51	8.8	99.6

<sup>a</sup> All yields are expressed as % dry solids and are means of two observations.

<sup>b</sup> All protein contents are expressed as % dry solids and are means of four observations.

<sup>c</sup> Average steepwater protein contents followed by the same letter are not significantly different at 95% confidence level.

<sup>d</sup> Average starch yields followed by the same letter within a treatment are not significantly different at 95% confidence level.

**TABLE VII**  
**Starch Pasting Properties<sup>a</sup> of Corn Samples with Lactic Acid (LA) and SO<sub>2</sub> Added to Steepwater**  
**Processed Under Different Steeping Treatments and Times<sup>b,c</sup>**

Steeping Treatment	Time Added (hr)	Peak Viscosity (RVU)		Breakdown Viscosity (RVU)		Setback Viscosity (RVU)		Peak Time (min)	
Water	0	185.6	A	91.4A	A	104.9	A	7.5	D
LA added to water	0	171.3c	D	72.2c	C	92.0b	C	8.3a	B
	8	174.5b		77.8b		95.4ab		8.2b	
	16	175.7ab		80.4ab		96.4a		8.1b	
	20	177.7a		83.5a		98.4a		8.1b	
SO <sub>2</sub> added to water	0	173.7b	B	82.0a	B	99.6a	B	7.9a	C
	8	177.0a		82.7a		98.3a		7.8b	
	16	175.5ab		82.5a		98.6a		7.8b	
	20	176.2ab		82.8a		99.4a		7.8b	
LA added to water and SO <sub>2</sub>	0	168.2ab	C	70.0a	D	87.9ab	D	8.3a	A
	8	168.3ab		68.4a		89.5a		8.3a	
	16	170.4a		70.2a		84.6b		8.3a	
	20	166.0b		66.77a		84.5b		8.3a	
SO <sub>2</sub> added to water and LA	0	168.2a	C	70.0a	D	87.9a	D	8.3a	A
	8	166.2ab		69.0a		86.4ab		8.3a	
	16	164.5b		71.7a		81.5b		8.3a	
	20	164.3b		67.7a		83.4ab		8.3a	

<sup>a</sup> All pasting properties are means of five observations.

<sup>b</sup> Average pasting properties followed by the same lowercase letter within a treatment are not significantly different at 95% confidence level.

<sup>c</sup> Treatments followed by the same uppercase letter within a pasting property are not significantly different at 95% confidence level.

steeping. During laboratory batch steeping, when lactic acid is added, pH drops to ≈2.5. However, during conventional continuous counter-current steeping, pH rarely drops to <4.0 (Watson 1984). Because SO<sub>2</sub> also lowers the pH of steepwater, it may have the same effect as lactic acid in lowering peak viscosity.

Breakdown viscosities of starch samples also were significantly affected by addition of chemicals. When both lactic acid and SO<sub>2</sub> were added, breakdown viscosities were significantly reduced compared with lactic acid or SO<sub>2</sub> alone in water or water alone. Lower breakdown viscosities with addition of lactic acid and SO<sub>2</sub> again were probably due to the slight acid-thinning effect of these chemicals on starch. The reduction in polymer chain length (linear character of chain) allowed hydrogen bonding and imparted stability (Blanchard 1992), resulting in lower breakdown viscosities. No effect of time was observed on breakdown viscosities when only SO<sub>2</sub> was added or when both chemicals were present. However, when only lactic acid was added, longer exposure significantly reduced breakdown viscosities (Table VII). A trend similar to that

observed with starch breakdown viscosities was observed with setback viscosities (Table VII).

Peak time significantly increased when chemicals were added to steepwater. When both lactic acid and SO<sub>2</sub> were added, no effect of exposure time on peak time was observed. The increase in peak time was lowest when SO<sub>2</sub> alone was added to the steep solution. An effect of exposure time was observed on peak time when lactic acid or SO<sub>2</sub> alone were added to the steep solution. Peak time increased slightly when SO<sub>2</sub> or lactic acid were present in the steep solution for 24 hr.

### Experiment 3

The results of the 100- and 1,000-g procedures were similar (Table VIII). No effect of time of lactic acid addition on starch yields was observed. When SO<sub>2</sub> was present, starch yield significantly increased as compared with the control sample. However, no effect of time of SO<sub>2</sub> addition was observed for up to 22 hr. When SO<sub>2</sub> was added to lactic acid and water, again no significant differences

**TABLE VIII**  
**Wet-Milling Fraction Yields<sup>a</sup> (%) Using 1,000-g Procedure from Controls,<sup>b</sup> Samples with Lactic Acid (LA) or SO<sub>2</sub> Added to Water, and Samples with LA or SO<sub>2</sub> Added to Steepwater (SW) Containing Water and the Other Chemical**

Steeping Treatment	Time Added (hr)	SW Solids	Fiber	Germ	Starch <sup>c</sup>	Gluten	Total
Control (no chemicals)		2.2	20.2	6.6	60.9a	6.8	96.6
LA added to water	0	4.0	18.0	6.5	60.0a	9.9	98.5
	8	3.4	19.0	6.3	60.6a	8.6	97.9
	22	2.7	21.6	6.3	59.7a	7.3	97.6
Control (no chemicals)		2.2	20.2	6.6	60.9b	6.8	96.6
SO <sub>2</sub> added to water	0	3.3	16.9	6.4	63.4a	8.2	98.2
	8	2.9	17.5	6.4	63.4a	7.7	97.9
	22	2.7	15.6	6.3	63.1a	9.9	97.6
Control (both chemicals)		4.6	10.4	6.6	68.3a	8.9	98.7
LA added to water and SO <sub>2</sub>	8	4.3	11.3	6.7	67.5a	8.8	98.6
	22	3.9	14.1	6.8	65.0b	8.9	98.7
Control (both chemicals)		4.6	10.4	6.6	68.3a	8.9	98.7
SO <sub>2</sub> added to water and LA	8	4.4	9.5	6.6	69.3a	8.5	98.2
	22	4.1	10.4	6.7	67.3a	10.0	98.5

<sup>a</sup> All yields are expressed as % dry solids and are means of two observations.

<sup>b</sup> Contained no LA or SO<sub>2</sub> or contained both chemicals.

<sup>c</sup> Average starch yields followed by the same letter within a treatment are not significantly different at 95% confidence level.

were observed at addition times of 8 or 22 hr compared with the control sample. When lactic acid was added to SO<sub>2</sub> and water, no significant differences in starch yields were observed at addition time of 8 hr compared with the control sample. However, a significant drop of 3.3% (compared with the control sample) in starch yield was observed when lactic acid was added at 22 hr.

### CONCLUSIONS

Relative time differences in addition of lactic acid and SO<sub>2</sub> to the steep solution had significant effects on starch yields. Addition of SO<sub>2</sub> to the steep solution (lactic acid and water) showed significantly higher starch yields for five corn hybrids when SO<sub>2</sub> was added between 5 and 15 hr compared with addition at 0 hr. No effect of time of lactic acid addition (0–15 hr) was observed in starch yields for five corn hybrids. Extended testing of addition of lactic acid and SO<sub>2</sub> to the steep solution up to 23.9 hr for one of the original five corn hybrids showed similar results. We found that when lactic acid was already present in the steep solution, adding SO<sub>2</sub> a little later (≈8 hr) produced higher starch yields compared with addition at 0 hr. Also, when lactic acid was present, SO<sub>2</sub> could be added to the steep solution at 23.9 hr (5 min before milling), and starch yields significantly similar to those of the control sample (normal steeping, addition of SO<sub>2</sub> to lactic acid and water at 0 hr) were obtained.

No unusually high residual protein contents were observed in starch samples obtained when SO<sub>2</sub> or lactic acid was added at a very late stage in the steeping process. Residual protein in the starch samples did not exceed 0.85%. Most starch samples obtained when SO<sub>2</sub> or lactic acid was added at later stages (within 30 min of milling), in fact, contained less residual protein compared with control samples.

Starch pasting properties were affected by addition of chemicals. Longer exposure of corn to lactic acid lowered peak and breakdown viscosities. A minimal effect of time (duration of chemicals in steepwater) was observed on peak and breakdown viscosities when SO<sub>2</sub> alone was added or when both chemicals were present. An effect of chemicals similar to that observed with breakdown viscosities was observed with setback viscosities. An effect of exposure time was observed on peak time when lactic acid or SO<sub>2</sub> alone was added to the steep solution.

Our results suggest that different times of lactic acid and SO<sub>2</sub> addition to the steep solution may help increase corn starch yields without affecting the residual protein contents in starch and cause only slight modifications in starch pasting properties. These findings can be useful for determining the best time to add lactic acid or SO<sub>2</sub> for short steeping or alternative corn wet-milling technol-

ogies that may require addition of externally produced lactic acid to the steep system. Relative time differences in addition of lactic acid and SO<sub>2</sub> to the steep solution should be tested on a counter-current steep system to confirm the results obtained with batch steeping and determine other factors such as microbial growth.

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