Influence of Lactic Acid on the Solubilization of Protein during Corn Steeping

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The role of lactic acid (LA) in corn wet-mill steeping is not well understood. Because LA is known to improve wet-milling starch yields and steepwater contains a large amount of proteinaceous material, one of the effects of LA in steeping may be to help break down the endosperm protein matrix. Protein solubilization was studied for four different steeping solutions containing LA, sulfur dioxide (SO_2), a combination of LA and SO_2, or no added chemicals at temperatures between 44 and 60 °C with steep times of up to 48 h. The accumulation of proteinaceous material in steepwater with time was sigmoidal regardless of the steeping chemicals or temperature. The initial slow rate of solubilization appeared to be due to incomplete kernel hydration. Significantly greater amounts of protein were released in the presence of LA than in its absence, with the greatest amounts found when steeping was performed with both LA and SO_2. The increase of proteinaceous material in steepwater containing LA was not due to low pH, because steeping solutions containing other organic and inorganic acids did not increase steepwater protein. The effect of LA concentration was also studied. In the absence of SO_2, higher concentrations of LA resulted in higher steepwater protein concentrations. The opposite trend was observed in the presence of SO_2. Similar steepwater protein concentrations were obtained with DL-lactic acid and L-lactic acid, indicating that the additional protein release was not sensitive to isomeric effects.

Keywords: *Corn; lactic acid; steeping; steepwater; wet milling*

INTRODUCTION

Steeping in the corn wet-milling process is critical to preparing the kernels for milling. During steeping, the kernels imbibe water and soften, and soluble carbohydrates and proteinaceous material diffuse into the steepwater. In commercial operations, steeping is conducted in a countercurrent manner, with the newest corn being exposed to the oldest steepwater and the oldest corn being contacted with the newest steepwater. Sulfur dioxide (SO_2) is added to the incoming steepwater, which helps to break the cross-linking disulfide bonds in the endosperm protein matrix and to limit bacterial growth. As the steepwater is cycled, the SO_2 concentration drops. As the SO_2 concentration drops, a natural *Lactobacillus* fermentation begins with the production of lactic acid (LA). The LA helps to maintain a low pH in the steepwater, which limits other microbial growth.

It is well established that SO_2 promotes the release of starch particles from the endosperm protein matrix (Cox et al., 1944). Although its effect on corn kernels is not well understood, LA produced during steeping is also reported to benefit the milling process. In addition to limiting bacterial growth, these benefits include increased kernel softening (Cox et al., 1944), increased rates of water absorption (Ruan et al., 1992), increased rates of SO_2 absorption (Shandera et al., 1995), improved gluten filtration rates (Watson, 1984), and reduced fouling of the steepwater evaporators (Watson, 1984). In addition and perhaps most importantly, several groups have reported improved starch recoveries when steepwater contains LA (Watson et al., 1951; Roushdi et al., 1981; Eckhoff and Tso, 1991; Du et al., 1996; Singh et al., 1997). In the most pointed investigation, Singh et al. (1997) reported increased starch yields of 3−12% for 18 different hard and soft dent varieties due to the addition of 0.55% LA to the steepwater.

Steepwater protein has been the focus of a few literature studies. Christianson et al. (1965) identified and quantified several nitrogenous compounds in industrial corn steep liquor. Protein (ethanol-insoluble material), peptides, and free amino acids constituted 84.8−86.3% of steepwater nitrogen; free ammonia, part of which is likely derived from amide degradation, constituted 5.3−6.9%; and quaternary nitrogen and heterocyclics contributed 3.1%. In recent studies, Hull et al. (1996a,b) reported on the protein, carbohydrate, and lipid composition of industrial and experimental steepwater. The authors concluded that proteinaceous material in industrial steepwater originates from the corn rather than from microbial fermentation because of the similarity of the amino acid distributions of steepwater and corn protein fractions. In addition, little protease activity was found, although they noted that the long duration, elevated temperature, and microbial activity of steeping would tend to support proteolytic activity. In industrial steeping, solubilization of initially insoluble kernel protein was found to occur during the initial 18−24 h (Biss and Cogan, 1988). The probable cause of this dissolution of structural protein was taken to be the introduction of SO_2. In addition, steepwater nitrogen has been correlated with starch yields in

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10.1021/jf990866s
Published on Web 03/28/2000

laboratory experiments that varied the exposure of the kernels to LA and SO_2 during a constant 24-h steeping cycle (Singh et al., 1999).

Initial experiments in our laboratory showed that there was a substantial increase in steepwater nitrogen when LA was added to batch steeps. In an effort to better the understanding of roles of LA in corn wet milling, detailed experiments were designed to study the appearance of protein (nitrogen) in steepwater. Both DL- and L-lactic acid (DL-LA and L-LA) were studied to test for isomeric differences, and other acids were investigated to test if the increase in steepwater nitrogen was primarily a pH effect.

EXPERIMENTAL PROCEDURES

Sample Preparation. The FR1064xLH59 yellow dent corn hybrid used in these studies was grown during the 1995 crop season at the University of Illinois Agricultural Engineering farm at Urbana−Champaign. The hybrid was chosen because of its availability and because Singh et al. (1997) showed that this particular hybrid yielded ~4% more starch when steeped in the presence of 0.55% LA. Upon arrival at the laboratory, the corn was stored in a cold room at 4 °C. Kernels were sifted through a screen with 7-mm-diameter holes and were hand-cleaned to remove foreign matter and broken kernels. Initial moisture content was determined by drying three 30-g corn samples in the oven at 105 °C for 72 h as specified in AACC (1995) Method 44-15A.

Chemicals. Citric acid (CA) (anhydrous) and DL-LA (85.6%) were obtained from Fisher Scientific Co. (Fair Lawn, NJ). Phosphoric acid (PA) (85.6%) and L-LA were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium metabisulfite (97.2%) was from J. T. Baker Co. (Phillipsburg, NJ). An effective 0.20% (w/v) solution of SO_2 was prepared by dissolving 3.053 g of sodium metabisulfite per liter of deionized water as described by Du et al. (1996).

Equipment. Steeping experiments were performed in two shaking water baths (model 3540, Lab-Line, Melrose Park, IL), each with a capacity for six 500-mL Erlenmeyer flasks. Samples were dried in a forced draft oven (model II Plus, Sanyo Gallenkamp, Leicester, U.K.). Steepwater nitrogen was determined by AACC (1995) Method 46-30 with a nitrogen analyzer (model FP-428, LECO Corp., St. Joseph, MI). A corn gluten standard was used for calibration.

Steeping Solutions. Protein solubilization was studied for solutions containing water, 0.50% LA (w/v), 0.20% SO_2 (w/v), and 0.50% LA plus 0.20% SO_2. Experiments were conducted at 52 (±1) °C with steeping times of 2, 4, 6, 8, 12, 16, 20, 24, 36, and 48 h and at 44 (±1) and 60 (±1) °C with steeping times of 6, 12, 24, 36, and 48 h. Additional experiments were conducted with steeping solutions containing concentrations of LA between 0.25 and 2.0% with and without 0.20% SO_2. These trials were conducted at 52 °C with steeping times of 24 and 48 h.

To determine if the increased solubilization of protein in 0.50% LA was primarily a pH effect, additional experiments were performed with CA and PA. These acids were studied at 52 °C and with steeping times of 24 and 48 h with and without the addition of 0.20% SO_2. Without SO_2, each of the solutions was prepared to have the same starting pH as the 0.50% LA solution (2.4 ± 0.1). The concentrations (w/v) of these solutions were 0.071% PA and 1.23% CA. Because the PA concentration was considerably lower than the 0.50% (w/v) LA solution, a 0.50% (w/v) PA solution was also studied (initial pH ~2.29). For solutions containing 0.20% SO_2, the same acid concentrations were used. The initial pH of these solutions varied between 2.0 for the 0.50% PA solution and 2.7 for the 0.071% PA solution.

Steeping Procedure. For each steep, the steeping solution (187 mL) was added to a 500-mL Erlenmeyer flask, which was sealed with Parafilm (American Can Co., Greenwich, CT) and placed in a water bath at the selected temperature. After the temperature had re-equilibrated (1−2 h), corn (100 g) was added and the flasks were resealed with Parafilm. The steeps were agitated at 160 rpm for the designated steeping time. Immediately after steeping, the steepwater was filtered through a 350-mL coarse sintered glass funnel. The filtrate volume and the mass of the hydrated corn kernels were measured. Depending upon the anticipated quantity of solids, three 10- or 25-mL aliquots of filtered steepwater were taken for nitrogen analysis. Each sample was concentrated in an aluminum pan by drying at 50 °C for overnight. The residue was quantitatively transferred with a small amount of water to a small tin foil, and the transferred sample was redried at 50 °C for at least 24 h before nitrogen determination. For each sample, the concentration of protein in the steepwater was calculated on the basis of percentage nitrogen, a nitrogen-to-protein conversion factor of 6.25, the mass of residue, and the volume of the aliquot. The mean of the individual determinations was taken as the result. The moisture content of the steeped kernels was determined by drying at 105 °C for 72 h.

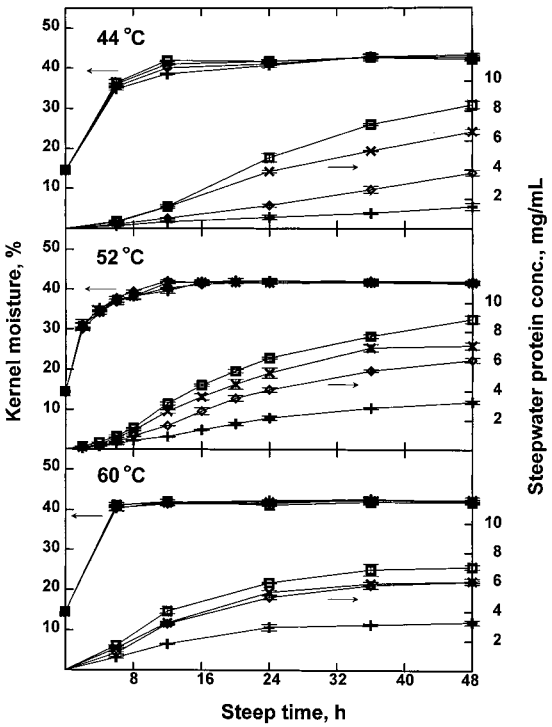

Figure 1. Corn kernel moisture and steepwater protein (nitrogen) during batch steeping at 44, 52, and 60 °C. Steeping solutions included water (+), 0.50% lactic acid (◇), 0.20% sulfur dioxide (×), and 0.50% lactic acid plus 0.20% sulfur dioxide (□).

Statistical Analyses. All experiments were conducted in triplicate. Standard deviations and coefficients of variance were calculated, and the data were subjected to SAS (Cary, NC) analysis of variance (ANOVA) procedures to test for significant differences among the different steep conditions. α was taken as 0.05, and a p value <0.05 was taken as indication of a significant difference.

RESULTS AND DISCUSSION

The initial moisture content of the kernels was 14.6 ± 0.1%, and the moisture content of the corn kernels increased during steeping (Figure 1). At 52 °C, kernel moisture equilibrated after ~16 h. At 44 °C, the hydration was complete after ~36 h, whereas at 60 °C, the hydration was close to completion by the first measured point (6 h). For all three temperatures the final moisture content was 42−43%. Minor differences between the chemical treatments at individual time points were present from the statistical analysis. These differences

were sporadic, and no general trends were indicated. Contrary to earlier reports (Fan et al., 1965; Ruan et al., 1992), the rate of hydration obtained from steeping dent corn with LA or SO_2 did not significantly exceed the rate from steeping in water.

The concentration of steepwater protein increased with steeping duration over the period of observation for all temperatures and steeping conditions studied (Figure 1). The protein concentration profiles were sigmoidal in shape. This dynamic response is typical of a process consisting of two steps in series, which suggested that the initial slow solubilization was due to incomplete kernel hydration. A high degree of kernel hydration is apparently necessary to provide a transport pathway for protein leaching. Unlike the hydration profiles, pronounced differences in the steepwater protein concentrations were apparent with the different steeping treatments. At 52 °C and for steeping times ≥6 h, the protein concentration was consistently in the order H_2O < 0.50% LA < 0.20% SO_2 < 0.50% LA/0.20% SO_2. At short steeping times (≤4 h), the protein concentrations in the H_2O, 0.50% LA, and 0.20% SO_2 steeps were not always significantly different. The combination of 0.50% LA and 0.20% SO_2, however, always gave significantly greater protein concentrations than the individual water, LA, or SO_2 treatments. Although Hull et al. (1996b) noted that steepwater protein generally increased with steep time, they did report a dramatic drop in the steepwater protein concentration in SO_2 steeps between 20 h (8.1 mg/mL) and 40 h (3.1 mg/mL). They also found the highest concentration of proteinaceous material in sterile water (11.3 mg/mL) compared with steeps treated with LA (5.5 mg/mL), SO_2 (3.1 mg/mL), and SO_2 with an added culture of *Lactobacillus* (10.2 mg/mL) at 40 h.

The results of the studies at 44 and 60 °C are also summarized in Figure 1. For longer steep times (24−48 h), the steepwater protein concentration was consistently in the same order at both temperatures as that found at 52 °C. For early time points a few differences were noted. At 6 h at 44 °C, the values obtained for the water and LA solutions were statistically similar, as were the values for the 0.20% SO_2 and 0.50% LA/0.20% SO_2 solutions at 6 and 12 h. Because kernel hydration rates increased with temperature, the sigmoidal shape of the protein solubilization curves was more pronounced at 44 °C and less pronounced at 60 °C.

Relative to the protein release induced by SO_2, the solubilization of kernel protein with LA was very sensitive to temperature. At 44 °C, the protein concentration for the SO_2 steepwater was approximately twice that of the LA steep throughout the observation period. At 52 °C the difference in concentration for these two solutions was much smaller, and at 60 °C little difference existed in the steepwater protein concentrations of these two steeping conditions.

At the later stages of the steeping process, the rates of protein release were greatly diminished at 60 °C. This is best observed by overlaying the temperature data (figure not shown). Steepwater protein was significantly greater at 52 °C than 44 °C for any of the steeping solutions. The same trend is found in comparing the 60 °C data with the 52 °C data at times <24 h. At 24 h, however, there was no significant difference in protein release between the 52 and 60 °C data for either the SO_2 or LA/SO_2 solution. At 36 h, there was no significant difference for these temperatures for the LA solution,

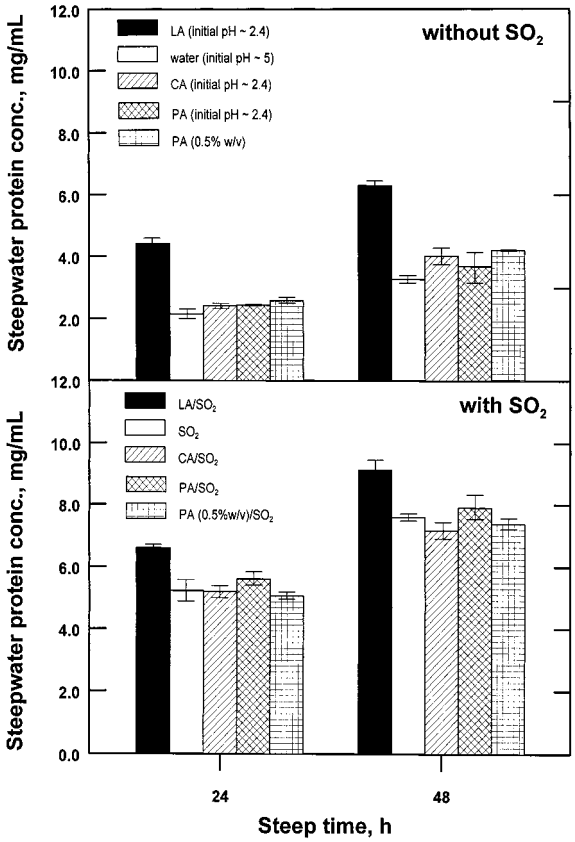

Figure 2. Effect of steepwater acids on the protein solubilization during corn steeping. Steeps were conducted at 52 °C for 24 and 48 h. Top and bottom panels show steeps conducted without and with 0.20% SO_2, respectively.

and solubilization in the SO_2 and LA/SO_2 solutions was significantly lower at the higher temperature. Regardless of the steeping solution, the solubilization of protein at 60 °C appeared to have reached a plateau after ∼36 h, whereas no plateau was observed at the lower temperatures. This effect is believed to result from increased starch granule swelling due to the elevated temperatures. At 25 °C, corn starch granules swell ∼9% in diameter with moisture uptake (Hellman et al., 1952). Initially, hydrated granules swell modestly with temperature, but as the temperature is raised a point is reached at which granule swelling increases dramatically, eventually leading to gelatinization (Collison, 1968). Although determination of the gelatinization temperature depends to a degree on the method used to detect the changes in the starch granule, by direct microscopic observation the initial temperature of gelatinization of cornstarch is reported to be 62 °C (Snyder, 1984). The granules, however, are likely to have swollen sufficiently at 60 °C in 24 h to occupy most of the interstitial space within the tightly packed peripheral region of the endosperm. Filling these voids would likely diminish the rate of transport of steeping chemicals and proteinaceous material into and out of the kernel endosperm.

Citric and phosphoric acids were also tested for the solubilization of protein at 52 °C (Figure 2). At both 24- and 48-h time intervals, the concentration of protein was consistently higher with the LA solution than with the other acid treatments. Hence, the enhanced solubilization of protein in 0.50% LA does not appear to be a pH effect. Although the differences were not as pronounced and small differences existed in the initial solution pH

values, a similar result was found when 0.20% SO_2 was included in the steepwater. The combination of SO_2 and LA always released more proteinaceous material from the kernel than the combination of SO_2 with any of the other acids.

In related research, Du et al. (1996) examined the effect of selected acids (phosphoric, acetic, citric, sulfuric, and hydrochloric) on corn wet-milling starch yields. Corn samples were steeped at 52 °C for 24 h in solutions containing 0.2% (w/v) SO_2 and 0.55% (v/v) of selected acid. The authors observed that the addition of any acid to steepwater containing 0.2% SO_2 increased starch yield by at least 3.6%, with the largest increases occurring with PA (5.8%) and LA (4.7%). Initial pH alone did not correlate with starch yield. However, the difference in final and initial steepwater pH did. No conclusion was drawn regarding this result, but it seems likely that the difference in final and initial pH was related to the concentration of released protein because solubilized protein would increase steepwater pH (see below). The difference in the results of this study and our observations that alternative acids alone do not enhance protein solubilization are difficult to explain, particularly since pH data from this earlier work suggest that more protein was released by the kernel. Hybrid effects may be partly responsible for this difference.

Steepwater pH generally increased with steep times for all three temperatures employed. Initial pH was lowest for the LA and LA/ SO_2 steeps and was highest for the water steep. Typically, the pH rise at 48 h was between 1.2 and 1.7 pH units regardless of the steeping additives. For the water and LA steeps, the pH rise correlated well with steepwater protein concentration. The coefficient of determination (R^2) was 0.93 for the water steep and 0.95 for the LA steep. For steeps containing SO_2, the pH initially increased rapidly and then increased slowly with increased protein concentration.

In laboratory steeping experiments with SO_2, high concentrations of steepwater LA have been reported to reduce starch yields for some hybrids (Singh et al., 1997). The effect of increasing the LA concentration on protein solubilization is shown in Figure 3. Without SO_2, increased LA levels in the steeps increased the concentration of leached protein, but at concentrations >1.25%, steepwater protein concentration was nearly constant. With SO_2, a small increase in protein concentration was found at low levels of LA (<0.75%) at 24 h. Otherwise, the general trend was to decrease protein concentration. Because of the dependence of the sulfurous acid−sulfite equilibrium on pH (King et al., 1981), this decrease likely occurs because of the additional protons contributed by the higher levels of LA. Because sulfurous acid is not effective in reducing disulfide bonds (Watson, 1984), the lower concentration of sulfite in these steeps inhibits the dissolution of endosperm protein. At LA concentrations ≥1.5%, lower protein concentrations were achieved in the steeps with SO_2 than with LA alone. The reason for this additional decrease in steepwater protein below the protein concentration for steeps containing only LA is unclear.

The increased rate of protein release in steeps containing LA must occur because of changes related to diffusional or chemical effects or from a combination of the two phenomena. Because the increased protein solubilization does not appear to be caused by a lowering

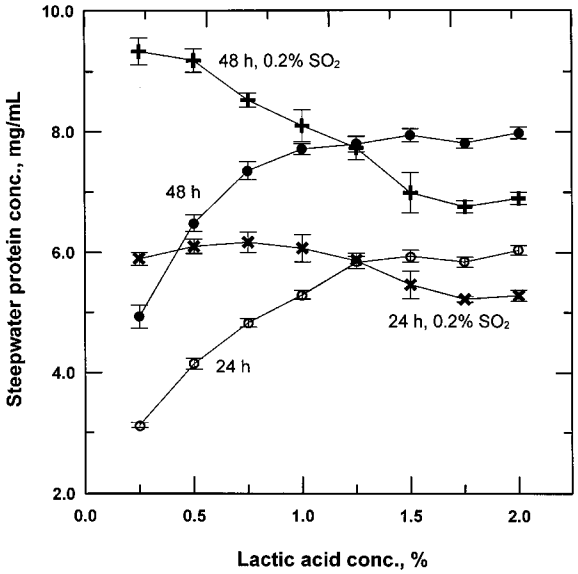

Figure 3. Effect of lactic acid concentration on protein solubilization at 52 °C. Steeps were conducted without SO_2 for 24 h (○) and 48 h (●) and with 0.20% SO_2 for 24 h (×) and 48 h (+).

Table 1. Steepwater Protein Concentration from Corn Steeps Containing L-LA and DL-LA at 52 °C in the Presence and Absence of SO_2

	steepwater protein concn[a] (mg/mL)	
treatment	24 h	48 h
0.50% L-LA	4.26 (0.07)	nd[b]
0.50% DL-LA	4.25 (0.02)	nd
0.50% L-LA + 0.20% SO_2	6.41 (0.01)	8.98 (0.32)
0.50% DL-LA + 0.20% SO_2	6.43 (0.23)	9.12 (0.28)

[a] Standard deviations in parentheses. [b] nd, not determined.

of the medium pH, the effect appears to be a direct effect related to LA. LA is known to be effective at solubilizing wheat proteins (Mangels and Martin, 1935; Shogren et al., 1969; Finney et al., 1982; Chakraborty and Khan, 1988), and low-pH lactate buffers are often used in electrophoresis studies of wheat protein fractions (Lookhart et al., 1982; Chakraborty and Khan, 1988).

Although a direct solubilization of the endosperm proteins is most likely the main cause of the increased protein release, we cannot completely eliminate the possibility that secondary mechanisms may also contribute to the increased solubilization. Endosperm cell walls are degraded during steeping (Earp et al., 1985), which may influence diffusional processes within the kernel and increase the rate of material released from the steeped corn kernel. It has not, however, been shown that LA contributes to this degradation. Acid degradation of the cellulosic and hemicellulosic components of the endosperm cell wall would be expected to be dependent on pH. Therefore, if this degradation contributed significantly to the rate of protein diffusional transport within the kernel, then other acids at similar pH values should have increased the rate of protein release. There have also been reports that endogenous proteases are active within corn endosperm. Proteases and protease inhibitors have been isolated from corn endosperm during germination (Abe et al., 1977, 1980), and Kerpisci (1984) has measured proteolytic activity in ground corn samples. In addition, protein solubilization from corn grits has been studied with enzyme and chemical treatments, and it was observed that some protein solubilization occurred even in the untreated

controls (Spanheimer et al., 1972). Endogenous proteases may be responsible for this effect. However, no studies indicate that LA enhances protease activity, and because similar levels of protein were found regardless of the chirality of the acid (Table 1), a direct activation of the proteases is unlikely.

CONCLUSIONS

Protein solubilization during steeping was affected by the presence of LA, SO_2, and the combination of the two in steepwater. The protein profiles were generally sigmoidal for all four steeping conditions, with the initial slow solubilization due to incomplete kernel hydration. The enhanced solubilization of protein in 0.50% LA is not primarily a pH effect because solutions of other acids at the same pH effected significantly lower protein release. The rate of solubilization of protein at 60 °C was greatly diminished after 24 h, which is believed to be due to starch granule swelling with temperature that further limits the transport of materials between the kernel and steepwater. Increasing the concentration of LA increased the amount of proteinaceous material leached from the corn kernel, but increasing the concentration of LA in the presence of 0.20% SO_2 reduced the concentration of leached protein. This phenomenon is believed to be due to a shift in the sulfurous acid−sulfite equilibrium toward the acid form when additional acid is added to the steeping medium. It is proposed that LA has a direct solubilizing effect on the corn protein matrix and that this effect is responsible for the increase in wet-milling starch yields found when corn is steeped with LA.

ABBREVIATIONS USED

CA, citric acid; LA, lactic acid; PA, phosphoric acid; SO_2, sulfur dioxide.

ACKNOWLEDGMENT

We thank S. R. Eckhoff (University of Illinois) for furnishing the corn used in this study and B. T. Vinyard (SRRC) for help with the statistical analyses.

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Received for review August 5, 1999. Revised manuscript received January 18, 2000. Accepted January 31, 2000. Mention of a trademark or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

JF990866S