Effect of Lactic Acid on Protein Solubilization and Starch Yield in Corn Wet-Mill Steeping: A Study of Hybrid Effects

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ABSTRACT

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To better understand the role of lactic acid (LA) in corn wet-milling, steeping studies were performed on different yellow dent corn hybrids using four different solutions containing LA, sulfur dioxide (SO_2), a combination of LA and SO_2, or no added chemicals. Although there was variation in protein solubilization among the hybrids, protein release was consistently higher when LA was included in the steepwater than when it was excluded (both with and without SO_2). Several groups have reported that starch recoveries are improved when steepwater contains LA. To explore the relationship between protein solubilization and starch yield as effected by LA, several yellow dent hybrids were steeped in 0.20% SO_2 and 0.50% LA-0.20% SO_2 solutions and milled to recover starch by a 100-g laboratory corn wet-milling procedure. In all instances, both starch yields and protein solubilization were enhanced in solutions containing LA. These results support the hypothesis that direct dissolution of the endosperm protein matrix by LA contributes to the improved starch recoveries.

Steeping in the corn wet-milling process is critical to preparing the kernels for milling. While it is well established that sulfur dioxide (SO_2) promotes the release of starch particles from the endosperm protein matrix (Cox et al 1944), the effect of lactic acid (LA) produced during steeping is not well understood. Some of the reported beneficial effects of LA include increased rates of water absorption (Ruan et al 1992), increased kernel softening (Cox et al 1944), increased rate of SO_2 absorption (Shandera et al 1995), and improved starch recoveries (Watson et al 1951; Roushdi et al 1981; Eckhoff and Tso 1991; Du et al 1996; Singh et al 1997; Haros and Suarez 1999).

Our initial experiments using a single yellow dent hybrid showed that there was a substantial increase in steepwater nitrogen (protein) when LA was added to batch steeps (Dailey et al 2000). Since the findings for one hybrid could have been atypical, steeping studies have been performed on an additional seven hybrids to evaluate the effect across a range of corn hybrids.

As stated previously, several groups have reported that starch recoveries improve when steepwater contains LA. 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. In addition, the data for one hybrid indicated a correlation between steepwater nitrogen and starch yield in laboratory experiments that varied the exposure of the kernels to LA and SO_2 during a constant 24-hr steeping cycle (Singh et al 1999). These results, coupled with our observations in protein solubilization (Dailey et al 2000), may suggest that improved starch recoveries occur because of dissolution of some of the endosperm protein components by LA. To further confirm or validate the correlation between enhanced starch yield and protein solubilization, eight yellow dent corn hybrids were steeped in solutions containing SO_2 and LA-SO_2 and milled to recover starch by a 100-g corn wet-milling procedure.

MATERIALS AND METHODS

All corn (yellow dent hybrids) used in these studies was stored at 4°C. Corn kernels were sifted through a screen with 7-mm diameter holes, hand-cleaned to remove foreign matter and broken kernels, and weighed out in 50-g or 100-g (wb) samples for subsequent studies. Initial moisture content was determined by drying three 15–30 g corn samples in the oven at 103°C for 72 hr (Approved Method 44-15A, AACC 2000). Hybrids I–VII were provided by Pioneer Hi-Bred International, Inc., Des Moines, IA and Hybrid VIII is FR1064xLH59 yellow dent corn hybrid (Dailey et al 2000). Hybrid IX (Purina Mills, Inc., St. Louis, MO) was purchased locally. Starch content of each hybrid was determined on a dry basis (db) by a polarimetric method (CRA 1980). Protein content of each hybrid was determined by Approved AACC Method 46-30.

Chemicals

Lactic acid (85.6%) was obtained from Fisher Scientific Co. (Fair Lawn, NJ). Sodium metabisulfite (97.2%) was from J.T. Baker Co. (Philipsburg, NJ). An effective 0.20% (w/v) solution of SO_2 was prepared by dissolving 3.053 g of sodium metabisulfite (97.2%) in 1,000 mL of deionized water (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 of kernels, steepwater residue, starch, and other components were dried in a forced air oven (VWR Scientific Products, model 1370 FM, Sheldon Manufacturing, Inc., Cornelius, OR). Steepwater nitrogen was determined by Approved AACC Method 46-30 with a nitrogen analyzer (model FP-428, LECO Corp., St. Joseph, MI). A corn gluten standard was used for calibration.

Steeping Studies

The following procedure applies for each of four soak solutions (deionized water; 0.50% LA [w/v]; 0.20% SO_2 [w/v]; 0.50% LA-0.20% SO_2 [w/v]). Each soak solution (100 mL) was added to an Erlenmeyer flask, the flask was sealed with Parafilm (American Can Co., Greenwich, CT) and placed in a shaker bath at 52 ± 1°C. After temperature equilibration was attained (0.5–1 hr), corn kernels (50 g) were added and the flask resealed with Parafilm. The flask was shaken at 160 rpm in the shaker bath for 24 hr, at the end of which, the flask was removed and the corn filtered through a 350-mL coarse sintered glass funnel. Three 10-mL aliquots of filtered steepwater were taken for nitrogen analysis. The aliquots were concentrated in aluminum weighing pans by heating in the oven at 52°C for 16–24 hr; the residues were transferred to tared tin foils and dried at 52°C for at least 24 hr. The dried residues were analyzed for nitrogen content. For each sample, the concentration of protein in the steepwater (mg/mL) was calculated based on a conversion factor of 6.25.

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100-g Wet-Milling Procedure

Corn samples were steeped as previously described with the following exceptions: only two soak solutions (0.20% SO_2 and 0.50% LA-0.20% SO_2) were used; volume was 180 mL; corn sample size was 100 g (wb). Following steeping, the corn was filtered through a funnel containing wire mesh. The volume of the filtrate was measured and three 10-mL aliquots of filtered steepwater were used for nitrogen analysis. Thereafter, a 100-g laboratory corn wet-milling procedure based on that of Eckhoff et al (1996) was followed. Components isolated were steepwater residue, germ + coarse fiber, fine fiber, gluten, and starch. All components were dried at 52°C for 24 hr and 135°C for 2 hr (AACC 2000) and recovery determined (dry basis).

Statistical Analysis

In the steeping studies, the experimental design for each hybrid was completely randomized with three replicates for four treatments. The treatments have a 2^2 factorial structure with two levels of LA (0 and 0.50%) and two levels of SO_2 (0 and 0.20%). A separate analysis of variance (ANOVA) was employed for each hybrid and treatment means for steepwater protein concentration were compared using the least significant different (LSD) test at $P \leq 0.05$ (Steel et al 1997). The 100-g milling studies measured starch recovery and solubilized protein for two steeping solution treatments (0.20% SO_2, and 0.50% LA-0.20% SO_2) were taken on eight hybrids. Measurements were averaged for each treatment-hybrid combination and thepaired *t*-test compared overall means. Using the eight hybrid means for each treatment, Pearson correlation coefficients were calculated to ascertain the relationship between starch recovery and solubilized protein.

RESULTS AND DISCUSSION

Steeping Studies

The results of the studies of solubilization of protein in steepwater for eight hybrids at 52°C are summarized in Fig. 1. Three determinations were made for each hybrid. The data for hybrid VIII are taken from a previous study in which 100 g of corn (wb) and 187 mL of steep solutions were used (Dailey et al 2000). The order of protein solubilization was deionized water < 0.50% LA ≤ 0.20% SO_2 < 0.50% LA-0.20% SO_2. Although there was variation in solubilization among hybrids, protein release was consistently higher when LA was included in the steepwater than when it was excluded (both with and without SO_2).

In the protein solubilization studies, ANOVA and mean comparisons were performed on each hybrid separately. The analysis revealed that 0.20% SO_2 had a greater effect on protein solubilization than 0.50% LA for all hybrids except Hybrid IV. Steepwater protein concentrations were significantly higher ($P \leq 0.05$) in 0.50% LA, 0.20% SO_2, and 0.50% LA-0.20% SO_2 steeping solutions than in water for all hybrids. In addition, steepwater protein concentrations were significantly higher in 0.50% LA-0.20% SO_2 than in either 0.50% LA or 0.20% SO_2 for all hybrids. Protein solubilization was significantly greater in 0.20% SO_2 than in 0.50% LA for six hybrids. For Hybrid III, steepwater protein concentration was higher in 0.20% SO_2 than in 0.50% LA ($P = 0.18$); for Hybrid IV, it was slightly higher in 0.50% LA ($P = 0.49$)

These results provide strong evidence that enhanced protein solubilization in steep solutions containing LA is a general effect. Our previous studies (Dailey et al 2000) showed that the increased solubilization was not primarily a pH effect since solutions of other acids at the same pH effected significantly lower protein release. A plausible explanation of why LA increases protein solubilization in steepwater can be based on the structure of LA as an α-hydroxy carboxylic acid. The enhanced protein solubilization may be attributable to intermolecular hydrogen bonding with one bond formed between the hydrogen of the α-hydroxy group and the oxygen of the peptide linkage and a second bond formed between the carbonyl group of lactic acid and the hydrogen of the peptide linkage.

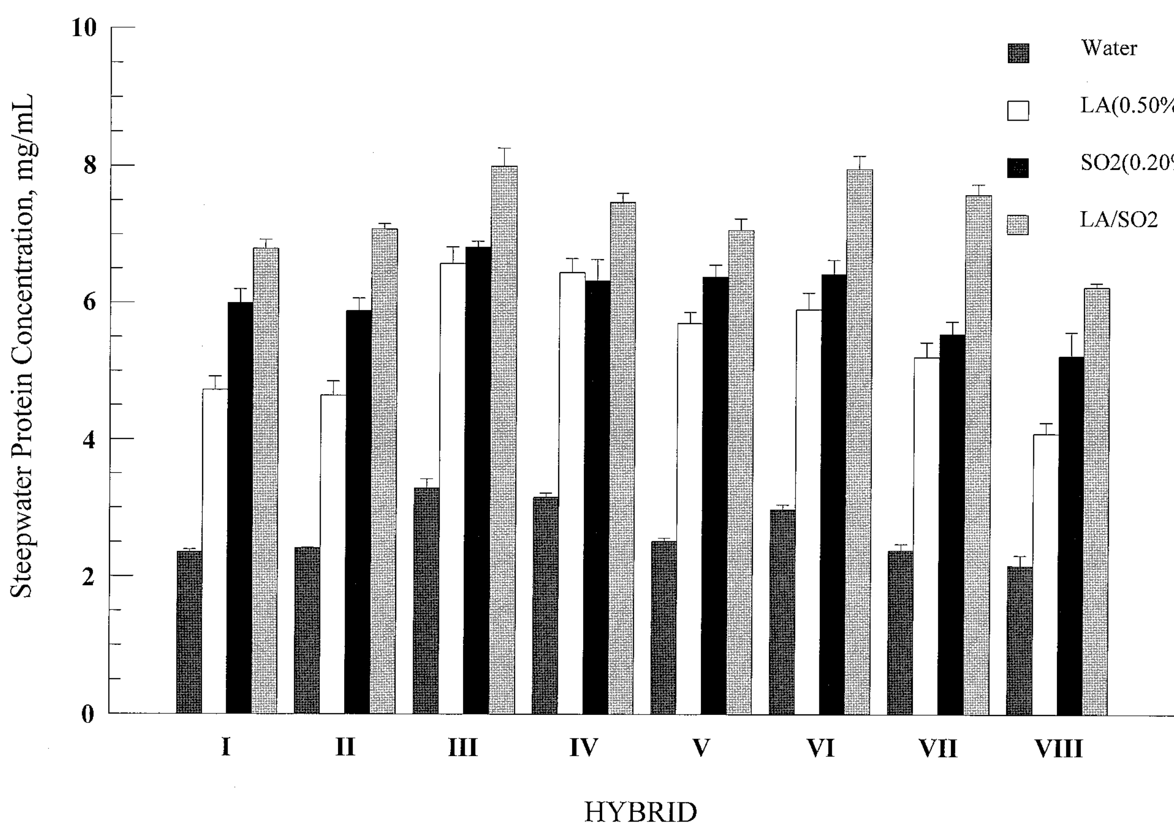

Fig. 1. Solubilization of protein from corn hybrids in four steep solutions. Standard deviations are shown as error bars. LA = lactic acid.

100-g Wet-Milling Studies

In the milling studies, yields of starch and the other components were calculated as a percentage of the dry weight of kernels used for each run. Total recovery of solids exceeded 97% for all experiments using SO_2 and 98% for all experiments using LA-SO_2 except for Hybrid II (96.8% and 97.9%). For all hybrids, the increase in starch yield due to the addition of LA to the steeping solutions resulted in a corresponding decrease in fiber. This observation is in agreement with other studies (Du et al 1996; Roushdi et al 1996; Singh et al 1997).

The analyzed starch content range of the hybrids was 68.6% (Hybrid II) to 73.0% (Hybrids I and IV). Consistent with these findings, Hybrid II gave the lowest starch yields and Hybrids I and IV gave the highest in the milling studies. Since the starch content of the eight hybrids (I–VII and IX) varied, the starch yields were converted to starch recoveries for comparison purposes. The starch recoveries obtained from the laboratory wet-milling procedure are illustrated in Fig. 2. Starch recoveries are a mean of three observations for Hybrid IX, four observations for Hybrid II (LA-SO_2 experiments), and two observations for all others. In all cases, the starch recovery obtained in LA-SO_2 steeping solutions exceeded that obtained in SO_2 steeping solutions. The range of increase in starch recovery was 1.7% (Hybrids IV and VII) to 3.8% (V).

Protein solubilized in the steepwater obtained in the wet milling of Hybrids I–VII and IX was determined in three different ways: steepwater protein concentration (mg/mL); mg of protein solubilized/g of corn (db); solubilized protein as a percentage of total protein content (db). The data obtained using the third determination are shown in Fig. 3. These solubilization data gave the best correlation with starch recovery.

Protein content (db) of the hybrids was 8.97% (Hybrid V) to 10.32% (Hybrid III). In all cases, the protein solubilized in LA-SO_2 steeping solutions exceeded that obtained in SO_2 steeping solutions. Percent increase in solubilized protein was 20.9% (II) to 51.3% (VII).

A paired *t*-test was used to compare the overall means for starch recovery and solubilized protein for the two treatments (0.20% SO_2, and 0.50% LA-0.20% SO_2). The eight hybrids were treated as replicates for the treatments. The means with standard error obtained for starch recoveries were 92.26 ± 0.18% for 0.20% SO_2, 94.73 ± 0.18% for 0.50% LA-0.20% SO_2. The corresponding data obtained for percentage protein solubilized were 9.43 ± 0.26% for 0.20% SO_2 and 12.60 ± 0.26% for 0.50% LA-0.20% SO_2. The differences were highly significant in both cases ($P \leq 0.0001$). The correlation coefficient was calculated to ascertain the relationship between starch recovery and solubilized protein: $r = 0.60$ ($P = 0.12$) for 0.20% SO_2; $r = 0.83$ ($P = 0.01$) for 0.50% LA-0.20% SO_2. With steeping solutions containing LA, the correlation was highly significant. These data suggest that increased protein solubilization is a major contributor to increased starch recovery, as facilitated by LA.

Although there is a strong correlation between starch recovery and protein solubilization when the eight hybrids are considered as a whole, the same conclusion cannot be made for an individual hybrid due to limited data points (two LA concentrations). The ratio of the percentage increase of protein solubilized to percentage increase of starch varied from hybrid to hybrid. The ratio ranged from 7.2 (Hybrid IX) to 30.5 (Hybrid VII). Excluding Hybrid VII, the ratio ranged from 7.2 to 15.8 (Hybrid IV).

CONCLUSIONS

Protein solubilization during corn wet-milling steeping was studied for four steeping solutions using eight yellow dent hybrids. Protein release was consistently higher when LA was included in the steepwater than when it was excluded, providing strong evidence that enhanced protein solubilization in steep solutions containing LA is

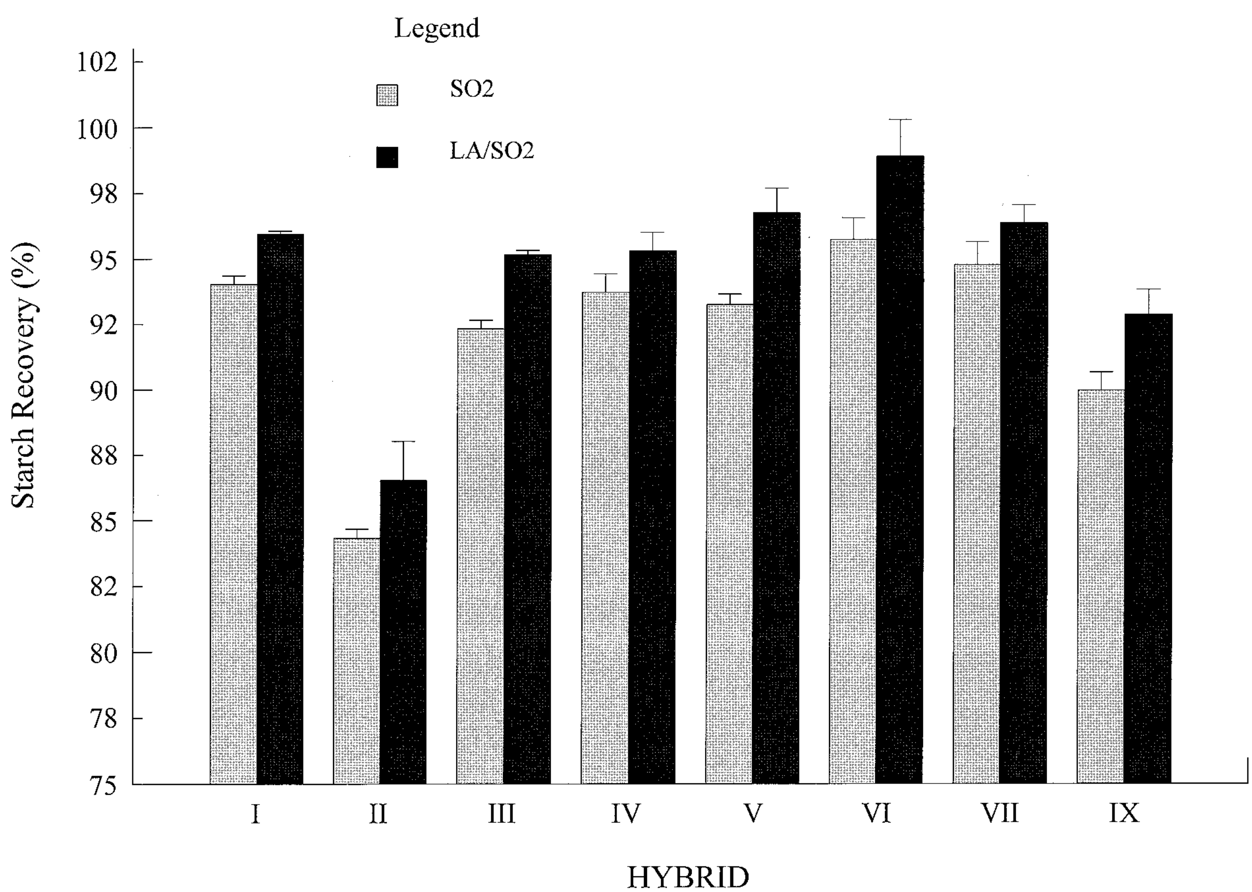

Fig. 2. Effect of addition of lactic acid (LA) to steep solution on starch yield. Standard deviations are shown as error bars.

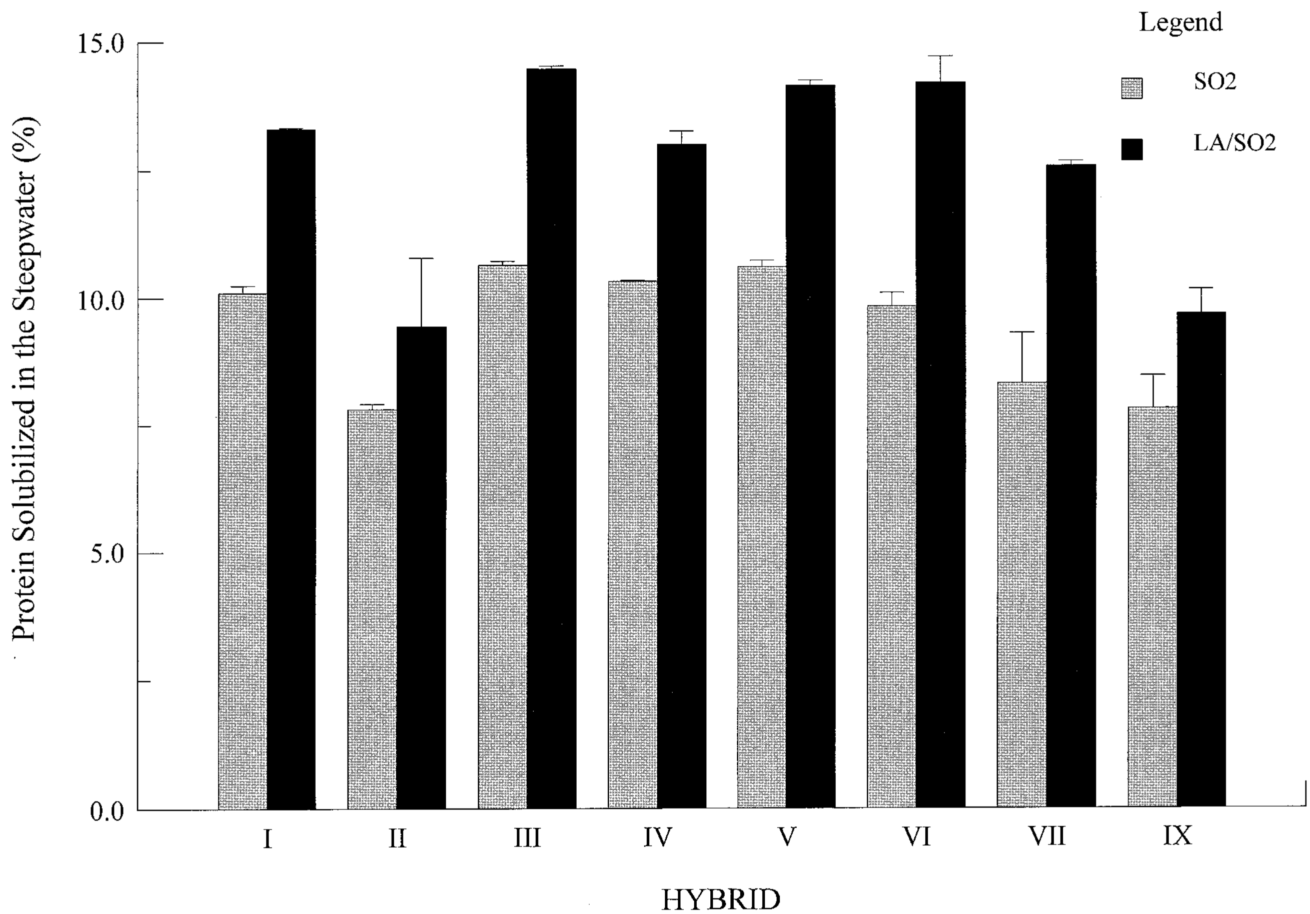

Fig. 3. Effect of addition of lactic acid (LA) to steep solution on protein solubilization. Standard deviations are shown as error bars.

a general effect. In laboratory wet-milling studies, eight hybrids were steeped in SO_2 and LA-SO_2 solutions. In all instances, both starch recoveries and protein solubilization were enhanced in solutions containing LA, reinforcing the premise 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 obtained when corn is steeping with LA. Future research will be directed toward fully delineating the correlation between enhanced starch recovery and protein solubilization using additional hybrids and lactic acid concentrations.

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