

# Use of Proteases to Reduce Steep Time and SO<sub>2</sub> Requirements in a Corn Wet-Milling Process

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## ABSTRACT

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To eliminate the diffusion barriers associated with enzyme addition during conventional steeping, we have developed a two-stage milling procedure to evaluate the effects of enzyme addition on corn wet milling. The current study compares the effects of the addition of commercially available enzyme preparations during conventional steeping to their comparable addition in the two-stage procedure. Results are presented in terms of yields of fiber, starch, germ, and gluten. The results demonstrate that the application of enzymes to the normal steeping step of wet milling is not an effective means of decreasing the steeping time or sulfur dioxide

usage. Only when specific enzymes are added to the hydrated ground corn, using the modified two-stage procedure, are enzymes effective in decreasing the steeping time and sulfur dioxide requirements. The overall steeping time with the two-stage modified procedure ranges from 6 to 8 hr, representing a 67–83% reduction over the conventional process. The modified process greatly decreases, and possibly eliminates, the need for sulfur dioxide addition, while producing starch yields and quality equivalent to that from the conventional process.

Processing of corn by wet milling is a time-consuming, energy-intensive, and capital-expensive process. The step that prolongs the process is the steeping step. This stage requires 24–36 hr for completion, as well as considerable space and energy. The resulting time delay limits the processing capacity of the plant and adds substantially to new plant construction costs. It is estimated that 21% of the total energy and capital cost is used for the steeping operation (S. R. Eckhoff, *unpublished data*). In addition to the time required, the addition of sulfur dioxide is required in considerable amounts, and this chemical is an environmental concern. Researchers have attempted to develop alternative processes to decrease time, increase plant capacity, and eliminate sulfur dioxide requirements while maintaining product yields and quality. Much of the research has investigated the use of mechanical and chemical approaches. These approaches, however, require costly modification of existing facilities and pretreatment of kernels, which results in increased pollution or increased energy use (Grindel 1965; Gillenwater et al 1971; Krochta et al 1981; Roushdi et al 1981, Neryng and Reilly 1984; Meuser et al 1985; Hassanean and Abdel-Wahed 1986).

Early interest in the use of enzymes during steeping was focused primarily on decreasing the protein content of the starch for the production of medical grade glucose (Roushdi et al 1981). Preliminary studies added proteases (pepsin, papain, bromelain, and trypsin) to whole grains during the steeping process, but a decrease in the residual protein content in the produced starch was not observed (Roushdi et al 1981). There was only a statistical change in the protein content of the produced starch when broken grains were used (Roushdi et al 1981). That initial report proposed that the time required for steeping could be substantially decreased by using enzymes.

Other researchers investigated the use of proteases on corn grits (endosperm fraction obtained during dry milling) as either pretreatment for air classification (Spanheimer et al 1972) or to overcome the adverse effects of high-temperature drying on starch-gluten separa-

tions during subsequent wet milling (Eckhoff and Tso 1991). Previous attempts to increase starch yield or decrease the total steeping time have investigated the addition of multiple enzymes (cellulases, hemicellulase, xylanases, pectinases, and proteases) during steeping (Caransa et al 1988; Hassanean and Abdel-Wahed 1986; Steinke and Johnson 1991; Steinke et al 1991; Moheno-Perez et al 1999). These studies have yielded results suggesting that there may be some benefit to the addition of enzymes during steeping. The results, however, were generated utilizing laboratory-milling techniques with yields that vary significantly from commercial wet-milling standards. Additionally, the specific enzymes responsible for the improvements were not adequately addressed and the possibility of decreasing or eliminating sulfur dioxide addition was not investigated. Other research (Wolf et al 1952) has shown that the diffusion of chemicals and water inside the intact corn kernel is through a fixed pathway, making enzyme penetration inside the intact kernel unlikely.

The development of an alternative processing procedure that could reduce the steep time and decrease or eliminate the use of chemicals such as sulfur dioxide would have a significant impact on the corn wet-milling industry. The new process would appreciably decrease operational energy costs, increase plant capacity, and reduce the capital costs involved in the construction of new corn wet-milling facilities.

## MATERIALS AND METHODS

Protease enzymes (bromelain from pineapple stem; pepsin from porcine stomach mucosa; *Aspergillus* acid proteinase, Type XIII from *Aspergillus saitoi*) were purchased from Sigma. All other enzymes (xylanase, cellulase, cellobiase,  $\beta$ -glucanase) were supplied as gifts from manufacturers. A yellow dent corn hybrid (Pioneer 3394) grown during the 1999 crop season at the Agricultural Engineering Farm, University of Illinois at Urbana-Champaign, was used for the study. Corn samples were hand-cleaned to remove broken kernels and foreign materials. Samples were then packaged and stored at 4°C until used. The whole kernel moisture content of the samples was measured using a 103°C convection oven (Approved Method 44-15A, AACC 2000).

### Enzyme Activity Measurements

Protein content was determined according to Bradford (1976), with reagents purchased from Sigma, using bovine serum albumin as the protein standard. The carbohydrase activities were measured as an increase in reducing groups equivalents according to the procedure of Johnston et al (1998), in acetate buffer, pH 4.5, at 40°C. Activity units were defined as the change in reducing groups, equivalent to an increase of 1  $\mu$ g of sugar/min. The cellulase and  $\beta$ -glucanase assays used carboxymethyl cellulose and barley  $\beta$ -glucan

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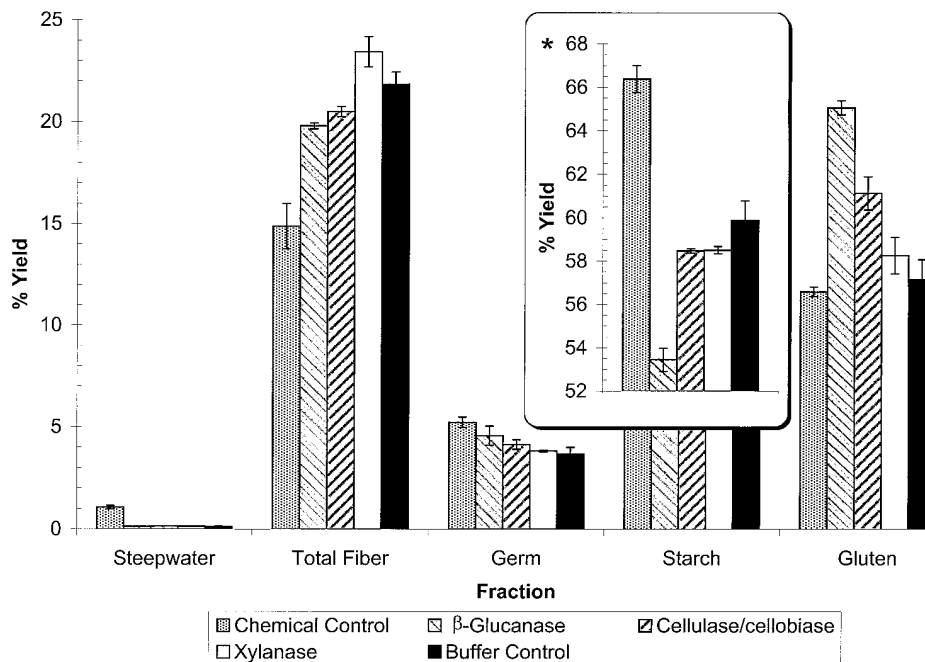
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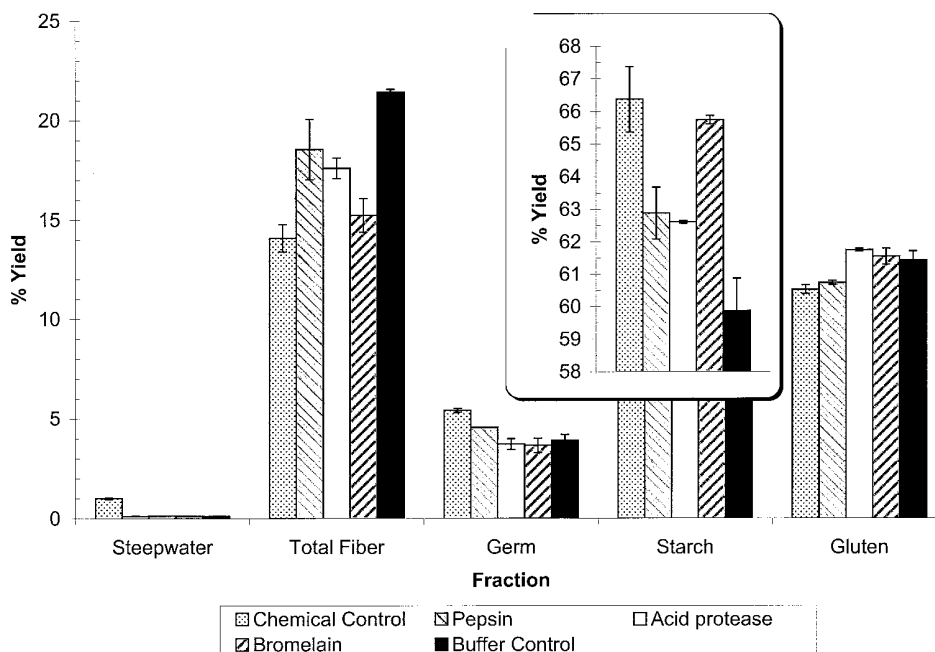
as substrates, respectively, and glucose as the standard sugar. Xylanase and hemicellulase assays used xylan and corn fiber gum, respectively, (Doner et al 1998) as substrates, and xylose as the standard sugar. The amylase and native starch assays used gelatinized and ungelatinized corn starch as substrate and maltose as the standard sugar. Protease activity was determined according to the modified method of Anson as described by Abe et al (1977). One protease unit is defined as the  $\Delta A_{280}$  of 0.001/min (1-cm light path) at pH 4.5 and 40°C, measured as trichloroacetic acid soluble products using hemoglobin as substrate in 10 mM cysteine.

### Wet-Milling Procedures

Conventional corn wet-milling was done using the 100-g laboratory corn wet-milling procedure of Eckhoff et al (1996). The two-stage modified steeping procedure was conducted as follows. Samples of corn (100 g) were placed in 500-mL Erlenmeyer flasks with 180 mL of water or steeping chemicals (0.2% SO<sub>2</sub> + 0.55% lactic acid). The corn was soaked for 3 hr at 48°C. The water was drained into a 250-mL graduated cylinder and this unabsorbed water volume was measured and then dried to determine total solids using the two-stage drying procedure (Approved Method 44-18,



**Fig. 1.** Comparison of fraction yields from corn samples steeped using individual hydrolase preparations, normal chemicals (chemical control; SO<sub>2</sub> at 2,000 ppm and 0.55% lactic acid) and buffer alone (buffer control; 0.05M acetate buffer, pH 4.0). Error bars represent  $\pm 1$  standard deviation from a duplicate average (quadruplicate for controls). Scale for graph is different from Figs. 2–5.



**Fig. 2.** Comparison of fraction yields from corn samples steeped using proteases, normal chemicals (chemical control; SO<sub>2</sub> at 2,000 ppm and 0.55% lactic acid) and buffer alone (buffer control; 0.05M acetate buffer, pH 4.0). Error bars represent  $\pm 1$  standard deviation from a duplicate average (quadruplicate for controls).

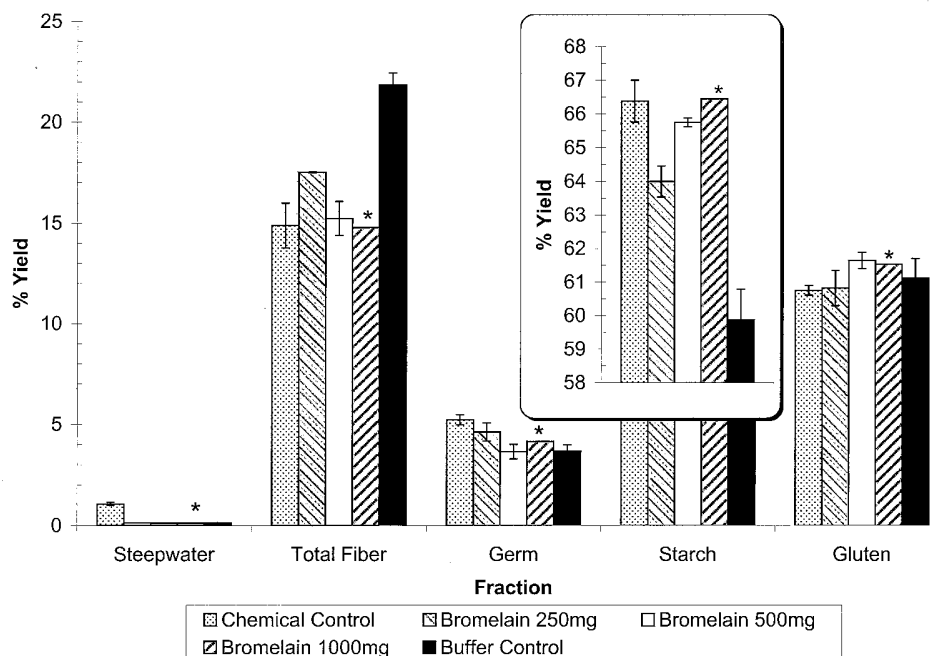
AACC 2000). The corn was then milled in an equal volume of water (v/v) using a Waring type blender. The slurry was then transferred to an Erlenmeyer flask and additional reagents added (enzyme, buffer, sodium metabisulfite, lactic acid). The flask was then incubated at 48°C (water bath) for 1–4 hr, with mixing at 30-min intervals. After incubation, the slurry was milled with the conventional wet-milling laboratory procedure as outlined by Eckhoff et al (1996).

### Incubation Conditions

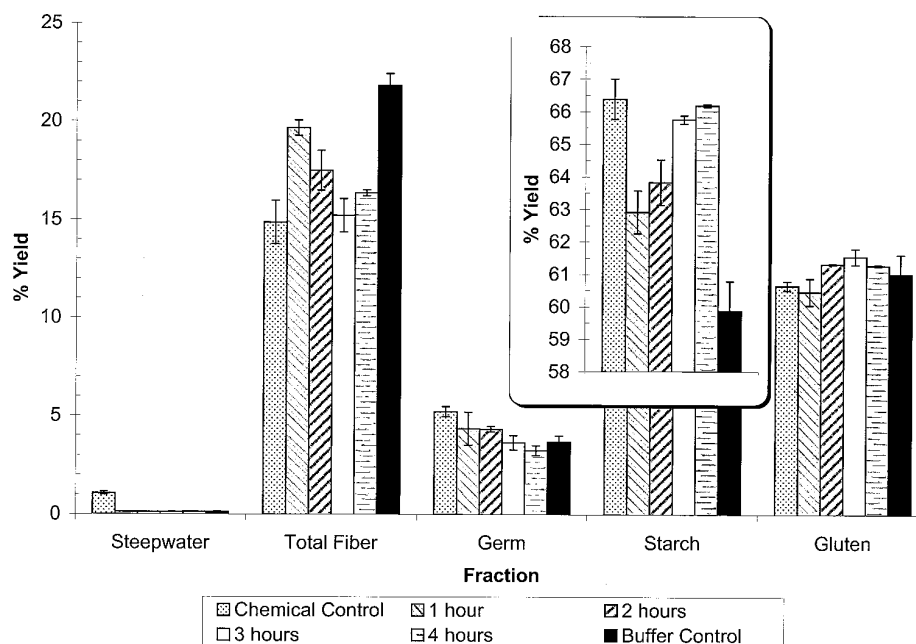
Normal steeping was done using the unmodified 100-g procedure (Eckhoff et al 1996) using 2,000 ppm of SO<sub>2</sub> and 0.55%

lactic acid and steeping for 24 hr at 52°C before milling. Enzymes and chemicals were added directly to the steeping solution. Enzyme treatments were done with the addition of SO<sub>2</sub> and lactic acid, with only lactic acid, and with no chemicals. Steeping times other than 24 hr were also tested.

Normal two-stage steeping was done using 2,000 ppm of SO<sub>2</sub> with 0.55% lactic acid added during the initial soaking step (3 hr). No additional chemicals were added during the second incubation procedure. Enzyme-treated samples using the two-stage procedure were soaked in water (no steeping chemicals, enzymes, or buffer) for the first step of the process. Following the first grind, 10 mL of



**Fig. 3.** Comparison of fraction yields from corn samples steeped using three concentrations of bromelain, normal chemicals (chemical control; SO<sub>2</sub> at 2,000 ppm and 0.55% lactic acid) and buffer alone (buffer control; 0.05M acetate buffer, pH 4.0). Error bars represent ±1 standard deviation from a duplicate average (quadruplicate for controls).



**Fig. 4.** Comparison of fraction yields from corn samples steeped using bromelain for 1–4 hr of incubation, normal chemicals for 3 hr (chemical control; SO<sub>2</sub> at 2,000 ppm and 0.55% lactic acid) and buffer alone for 3 hr (buffer control; 0.05M acetate buffer, pH 4.0). Error bars represent ±1 standard deviation from a duplicate average (quadruplicate for controls).

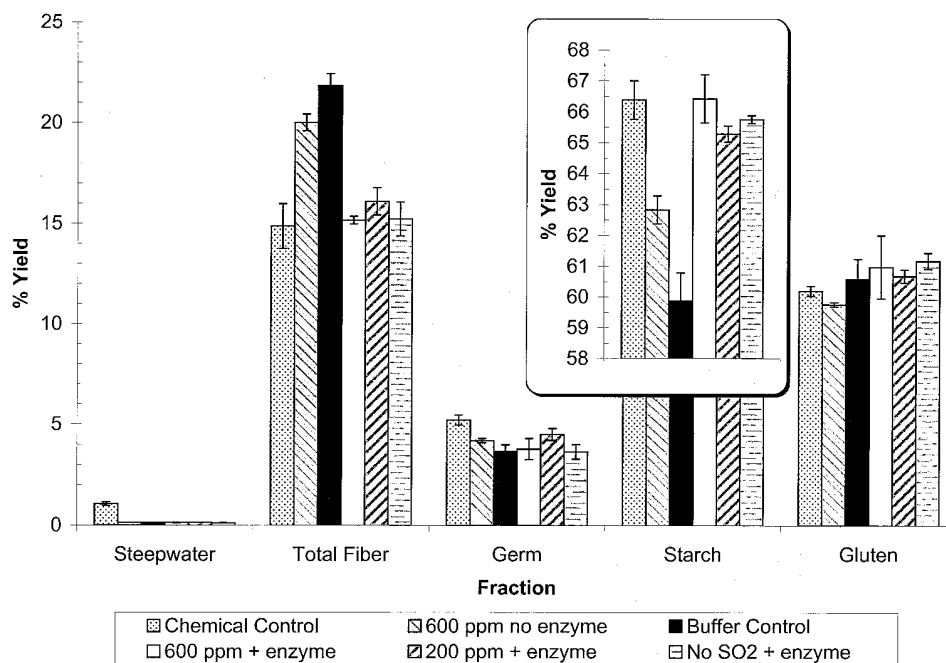
1M sodium acetate buffer, pH 4.0, was added to control pH level (final pH 4–4.5). Sodium metabisulfite was added to the samples to give a SO<sub>2</sub> equivalent concentration of 200, 600, or 2,000 ppm. Enzymes were added either as a dry powder (bromelain 250, 500, or 1,000 mg; pepsin 250 mg; *Aspergillus* acid proteinase Type XIII 250 mg) or as liquid (cellulases, xylanases, or β-glucanase, 5 mL). Control (buffer) and samples treated with SO<sub>2</sub> only were done identically but without any enzyme addition. Protein content of the starch was determined by a commercial analytical laboratory

(Silliker Laboratories Group, Chicago Heights, IL) using AOAC method 991.20 (AOAC 1990).

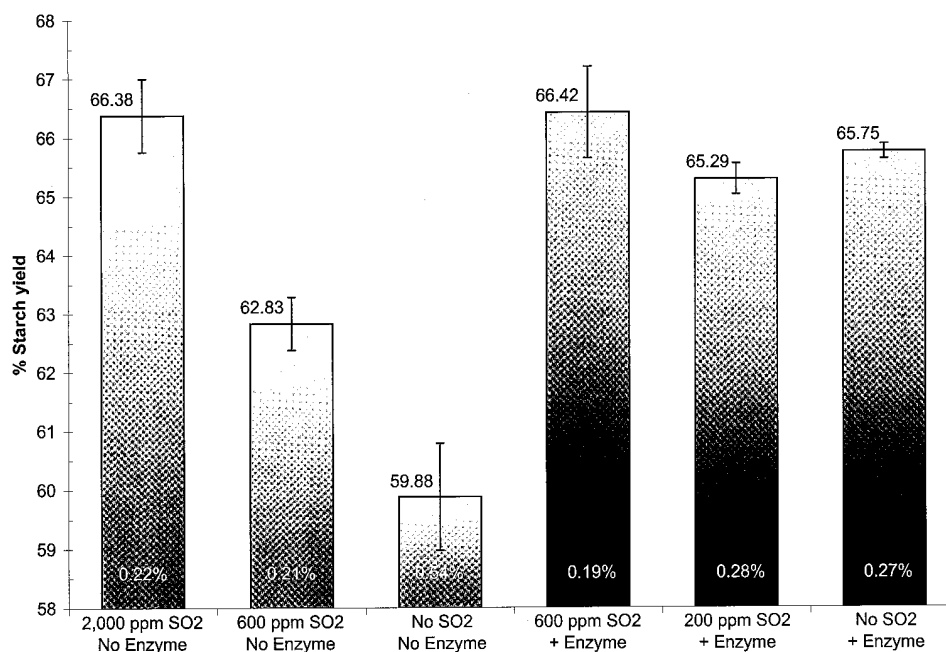
## RESULTS

### Conventional (One-Step) Procedure with Enzyme Treatments

The initial experiments were intended to replicate the published results for enzymes added during conventional steeping (Hassanean and Abdel-Wahed 1986; Caransa et al 1988; Steinke and Johnston



**Fig. 5.** Comparison of fraction yields from corn samples steeped using normal chemicals (chemical control; SO<sub>2</sub> at 2,000 ppm and 0.55% lactic acid), decreased SO<sub>2</sub> without enzyme (600 ppm, no lactic acid), decreased SO<sub>2</sub> with 500 mg of bromelain (200 and 600 ppm, no lactic acid), bromelain (500 mg) without SO<sub>2</sub> or lactic acid, and buffer alone (buffer control; 0.05M acetate buffer, pH 4.0). Error bars represent ±1 standard deviation from a duplicate average (quadruplicate for controls).



**Fig. 6.** Comparison of starch yields from corn samples steeped using normal chemicals (chemical control; SO<sub>2</sub> at 2,000 ppm and 0.55% lactic acid), decreased SO<sub>2</sub> without enzyme (600 ppm, no lactic acid), decreased SO<sub>2</sub> with 500 mg of bromelain (200 and 600 ppm, no lactic acid), bromelain (500 mg) without SO<sub>2</sub> or lactic acid, and buffer alone (buffer control; 0.05M acetate buffer, pH 4.0). Error bars represent ±1 standard deviation from a duplicate average. Percent values indicate the duplicate average protein content for corresponding sample.

1991; Moheno-Perez et al 1999) using the highly reproducible 100-g laboratory corn wet-milling procedure. Experimental treatments consisted of enzyme addition with a) addition of SO<sub>2</sub> and lactic acid; b) addition of lactic acid without SO<sub>2</sub>; and c) without addition of SO<sub>2</sub> or lactic acid. The 24-hr steeping experiments showed no improvement in starch yield with the addition of any of the enzyme combinations tested (results not shown). A small but statistically significant decrease in starch yields compared with buffered controls was observed with several samples.

### Two-Stage Procedure with Glycosidases

When the two-stage modified procedure was used (3-hr soak of intact kernels followed by coarse grinding and a 3-hr incubation of the ground slurry) with enzyme preparations similar to those used in the conventional (one-step) procedure, significant increases as well as decreases were observed for starch yields (results not shown). The mixtures of commercial preparations showing decreased starch yields were further tested to identify the specific component responsible. Figure 1 shows the fraction yields for three individual components ( $\beta$ -glucanase, cellulases, or xylanases). Although all three showed a significant decrease in starch yields when compared with the buffer control starch yield, the  $\beta$ -glucanase preparation was clearly identified as being the major component responsible for the extensive decrease in starch yields. The gluten yields were also elevated for the  $\beta$ -glucanase preparation, potentially indicating a loss of starch through enzymatic hydrolysis into the gluten fraction. None of the carbohydrases tested were helpful in increasing starch yields.

### Two-Stage Procedure with Proteases

Three different proteases (pepsin, acid protease, or bromelain) were tested individually and in combination with other hydrolases (cellulases, xylanases, and  $\beta$ -glucanase) using the two-stage procedure. Fraction yields for proteases without additional enzymes are shown in Fig. 2. Pepsin and the acid protease showed a significant improvement in starch yields over the buffer control; however, bromelain showed the largest improvement. There was also a significant decrease in the total fiber yield from the three proteases tested, as compared with the total fiber yield from the buffer control. There was no significant difference in the fiber yield between the chemical control and the bromelain-treated sample. Mixtures of proteases with the other hydrolases showed no additional improvement in starch yields over the use of protease alone; however, changes in the fiber and gluten fractions were observed (results not shown).

### Effect of Bromelain Concentration

Using the two-stage procedure, the effects of bromelain concentration on starch recovery were determined. Three levels of enzyme were evaluated (250, 500, and 1,000 mg/100 g of corn). Fraction yields are shown in Fig. 3. All levels tested showed improvements in starch yields over the buffer controls. Significant differences were observed between the 250- and 500-mg treatments. There was an

error found in the measurements made for the 1,000 mg samples and therefore the second replicate was not included in the analysis.

### Time Course of Bromelain Treatment

Using the two-stage procedure and the 500-mg application level of bromelain, a time course for the treatment was done. Samples were soaked for 3 hr in water (first step of modified procedure), followed by coarse grinding and enzymatic treatment for 1–4 hr before milling (second step of modified procedure). The fraction yields are shown in Fig. 4. The results clearly show a progressive increase in starch yields and a general lowering of total fiber with increased incubation times.

### Effect of SO<sub>2</sub> on Enzyme Treatments

To determine whether SO<sub>2</sub> levels (200–600 ppm) lower than what is used commercially (1,000–2,000 ppm) could be added to inhibit microbial growth without effecting enzyme activity (SO<sub>2</sub> is used in corn wet-milling plants to control microbial growth) during the modified milling process, samples were processed with and without SO<sub>2</sub> added during the incubation stage (Fig. 5). Samples treated with 600 ppm of SO<sub>2</sub> alone showed a small increase in starch yields compared with the buffer controls; however, the total fiber yield was significantly elevated and starch yields were significantly lower than for the bromelain-treated samples as well as the chemical controls. Samples that were treated with bromelain alone showed improved starch yields when compared with the starch yields of the buffer controls, as in previous experiments. Addition of both bromelain and SO<sub>2</sub> showed a further improvement in starch yields compared with the controls at the 600 ppm level; however, at the 200 ppm level, there was no additional improvement.

### Protein in Starch

The residual protein contents in the starch samples obtained from the milling studies was determined for bromelain-treated and SO<sub>2</sub>-treated samples. Figure 6 shows the results of the starch yields as well as the percentage of protein determined for each starch fraction. The protein content of the SO<sub>2</sub>-treated and enzyme-treated samples were all significantly lower than the buffer controls (no enzyme, no SO<sub>2</sub>). The differences between the individual samples, however, were not significant enough to make any additional conclusions about the effectiveness of protein removal using protease treatments. The combined effect of low level SO<sub>2</sub> plus the application of the protease enzyme does appear to be more effective at lowering the final protein content of the produced starch than either treatment alone.

## DISCUSSION

The first and most important operation in corn wet milling is the steeping process. The purpose of steeping is to soften the corn kernel and to break the disulfide bonds holding the protein matrix together. Steeping is a diffusion-limited process and is also one of

TABLE I  
Enzyme Activity Profiles Measured at pH 4.5 in Sodium Acetate Buffer

Preparation	Protein (mg/mL)	Enzyme Activity (U/mg)						
		Protease	Cellulase	$\beta$ -Glucanase	Xylanase	Hemicellulase	Amylase	Native Starch
Bromelain	0.337 <sup>a</sup>	500	trace	– <sup>b</sup>	–	40	trace	–
Pepsin	nd <sup>c</sup>	2,260 <sup>d</sup>	nd	nd	nd	nd	nd	nd
Acid protease	nd	1.1 <sup>d</sup>	nd	nd	nd	nd	nd	nd
Cellulase	42.30	28	1,862	7,700	626	494	2,930	200
Xylanase	13.58	14	trace	842	6,920	778	2,355	115
$\beta$ -Glucanase	23.90	270	594	5,560	926	774	3,700	2,677

<sup>a</sup> Protein content is in mg of protein/mg of powder.

<sup>b</sup> No activity detected.

<sup>c</sup> Not determined.

<sup>d</sup> Activity units supplied by the manufacturer.

the most capital- and energy-intensive parts of corn wet milling. The water and the steep chemicals ( $\text{SO}_2$  and lactic acid) diffuse into the corn kernel through the base end of the tip cap and move through the cross and tube cells of the pericarp to the kernel crown and into the endosperm (Wolf et al 1952). The  $\text{SO}_2$  in the endosperm interacts with the protein matrix that encapsulates the starch granules. The  $\text{SO}_2$  disrupts the protein matrix, which results in starch release during subsequent milling (Watson and Sander 1961). The slow penetration of  $\text{SO}_2$  in the endosperm and the long reaction time necessary to break down the protein matrix makes steeping a very time-consuming (24–36 hr) operation. Steeping times <24 hr, using the conventional process, result in poor starch yields and loss of starch to fiber and protein fractions.

Our results showed the application of enzymes (proteases or glycosidases) during the conventional steeping process produced no significant benefits. This is in agreement with Roushdi et al (1981), who showed benefits only when broken grains were used. Other researchers have shown that there can be some benefit to the addition of cell-wall-degrading enzymes during the initial steeping step (Roushdi et al 1981; Hassanean and Abdel-Wahed 1986; Caransa et al 1988; Steinke and Johnson 1991). In previously published research, enzymes were added to the conventional steeping step (with  $\text{SO}_2$  and lactic acid) for 12–24 hr and were likely removed in the light steep water before grinding. Considering the diffusion limitations for  $\text{SO}_2$  into the corn endosperm reported by Wolf et al (1952) and the comparative size of an enzyme relative to  $\text{SO}_2$ , it seems likely that using a procedure where the enzymes are added directly to the steeping solution will result in the removal of the enzymes before contact with the endosperm. It is our interpretation of the available information that the use of cell-wall-degrading enzymes may (given sufficient time) help to increase the penetration of  $\text{SO}_2$  into the endosperm but it is not involved with starch release. It is also possible that the enzyme preparations used contain significant protease contamination (if proteases were not added intentionally), which could have carried over in residual liquid into the first grind and may have had sufficient time to react with the endosperm during the additional processing to show some benefit.

To overcome the problem of enzyme penetration into intact endosperm, we modified the current steeping system into a two-stage process. The first stage is to hydrate the corn kernel in water (no steeping chemicals added) for 3 hr so that the germ is completely hydrated and becomes pliable enough that it does not break when the corn is coarsely ground. The second part of our steeping system involves treating the coarsely ground corn slurry with protease. After treatment, the corn is milled using normal corn wet-milling. This approach removes the diffusion barriers and allows the enzymes to penetrate the corn endosperm and react with the protein substrates.

Using this modified procedure, we tested most of the same enzyme preparations used in the conventional steeping experiments (enzymes added directly to the steeping solution). The changes in fraction yields were greater when compared with the yields from the conventional steeping method. The  $\beta$ -glucanase preparation gave a significant decrease in starch yield (7% lower) when compared with the buffer steeped control (Fig. 1). We investigated this and found it was due to amylase contamination of the  $\beta$ -glucanase preparation. The xylanase and cellulase preparations also showed some amylase activity and the relative levels appear to correspond to the decreases in starch yields and the increase in the gluten fraction. Hydrolyzing starch during processing would result in an increased mass determination in the gluten fraction.

The enzyme preparations that gave the most significant improvements in starch yields using the modified two-stage procedure were the proteases. The proteases for testing were chosen based on pH optima, temperature stability, and the enzyme's potential for retaining activity in the presence of  $\text{SO}_2$  (cysteine proteases). Retention of activity in  $\text{SO}_2$  was very important because low-level addition of  $\text{SO}_2$  would still be desired to prevent microbial contamination. Other proteases could be useful in the absence of  $\text{SO}_2$  or with

the use of another antimicrobial compound. The proteases alone were as effective or more effective than when mixed with other hydrolases (Fig. 2). This was somewhat surprising because we believed that hemicellulose-degrading enzymes would help release bound starch from the fiber. Starch yields from bromelain-treated samples were significantly higher than from samples treated with pepsin or acid protease. This was likely due at least in part to nonoptimal pH conditions used for pepsin and acid protease. It was necessary to use a compromised pH (where all are active but not necessarily optimally) to avoid having an excessive number of control samples.

Bromelain was selected for additional studies to determine whether yields could be improved further and to determine the minimum amount of enzyme necessary to maintain starch yield. Three different levels of bromelain (250, 500, and 1,000 mg using 3-hr soak and 3-hr incubation) were tested and four incubation times (1–4 hr using 500 mg of bromelain and a 3-hr soak) (Figs. 3 and 4). The 250-mg bromelain addition gave starch yields higher than the starch yields given by pepsin or acid protease tested previously (Fig. 2), but it was lower than the starch yields from the chemical control samples. The starch yield was higher than the 500-mg treatment incubated for 2 hr or less but not after longer incubations. Incubations longer than 3 hr were not tested using the 250-mg treatment; however, it is likely that yields would eventually reach chemical control yields, provided the enzyme is not inactivated.

A difference between the starch yields for 500- and 1,000-mg bromelain-treated samples was observed but statistical significance could not be assigned (only one data set for the 1,000-mg treatment). The total fiber yields were not significantly different between the 500- and the 1,000-mg bromelain-treated samples. Although not tested, it is unlikely that further gains could be made through the addition of additional bromelain.

The time course analysis (Fig. 4) showed greater starch yields with increasing time of incubation; however, the change per unit time decreases steadily, indicating a maximum value for starch yields. When we plotted the data on an XY graph and calculated the second-order best-fit equation (not shown), the maximum was 66.3% which is approximately equal to the chemical control yields.

We believe that the enzymatic reaction is nearing completion, and further gains could only be achieved by means such as addition of other enzymes with different specificity (Henrissat et al 1985; Sun and Henson 1990; Irwin et al 1993; Sisson and MacGregor 1994) or by removal of inhibition products (Mahoney et al 1984; Abe and Whitaker 1988; Chen et al 1999). It may be possible to improve starch yield by further increasing enzyme accessibility with additional grinding or by incubating the isolated fiber with a new enzyme.

The final series of experiments was done to determine the effects of low-level  $\text{SO}_2$  addition on the modified process during the incubation step. Microbial contamination could be a potential problem during the enzyme incubation stage of the processing.  $\text{SO}_2$  addition at levels of 200–600 ppm (depending on pH) could be effective at inhibiting microbial growth (Lewis 1989; Block 1995). As expected, we found that  $\text{SO}_2$  addition (without enzyme or lactic acid) gave some improvement in starch yields over controls (buffer only) (Figs. 5 and 6). The enzyme-treated samples all showed greater improvements in starch yields over samples treated with  $\text{SO}_2$  only. The combination of  $\text{SO}_2$  (600 ppm) with the enzyme showed the greatest improvement and was, on average, better than the chemical control samples. Protein determinations were made on the starch samples produced (Fig. 6) to determine whether the enzymatic treatments were adequately removing protein from the starch. The control starch sample (no  $\text{SO}_2$  and no enzyme) showed an average protein content of 0.54% and individual values as high as 0.7%, well above the 0.3% acceptance level. The enzyme-treated starch samples were all <0.28% and as low as 0.19% for the combined  $\text{SO}_2$  and bromelain treatment. It was clear from the data that the addition of  $\text{SO}_2$  to the enzyme incubation did not have a negative effect on the enzymatic activity but did give a slight improvement over using the enzyme alone.

Additional proteases that could be used in this process would need to possess activity and stability under the specific conditions used. These proteases would also need to hydrolyze the proteins surrounding the starch granules. Such enzymes would have specificity toward peptide linkages in glutelins, zein, and other minor corn endosperm proteins. Resulting peptides would then be separated during processing. The reaction conditions would need to consider enzyme concentration, pH, temperature, SO<sub>2</sub> tolerance (if used), and other enzyme-specific factors such as mineral or cofactor requirement. Further improvements in starch recovery may be made through selection of other enzymes and we will explore these possibilities.

This modified process presents a number of potential benefits that have not been specifically addressed but are likely outcomes of its application: 1) shorter steeping times could decrease energy cost and capital investment in steeping tanks; 2) shorter processing times could increase plant capacity; 3) the process could use broken as well as unbroken grains by grinding and adding them directly to the incubation tank or by soaking for a decreased time (relative to intact kernels) before adding to the milling stream, which would result in increased primary product output (starch) for the same corn input; 4) soak water from the modified process contains relatively low dissolved solids when compared with the conventional light steep water (~90% less) which could potentially be recycled by using membrane filtration, eliminating the need and expense for evaporators.

### CONCLUSIONS

This study confirmed that the application of enzymes to the normal steeping step of wet milling is not an effective means of decreasing the steeping time or SO<sub>2</sub> usage. It is likely that the enzymes do not adequately penetrate the intact kernel and therefore cannot degrade the starch-associated proteins. This study demonstrated that only when the enzymes are added to the hydrated ground corn are they effective in decreasing the steeping time. This approach removes the diffusion barriers and allows the enzymes to react with the endosperm-associated proteins that encapsulate the starch granules and loosen the starch-protein interactions. The overall steeping time with the two-stage, modified procedure is 6–8 hr, a 67–83% reduction over the conventional process. The corn kernel requires a hydration step of 2–4 hr at 48–52°C before grinding so that the germ is completely hydrated and becomes pliable enough that it does not break when the corn is coarsely ground. After grinding, the slurry must be incubated with an adequate protease enzyme for a sufficient period of time, with controlled pH and temperature, so that the enzyme can sufficiently degrade the proteins surrounding the starch granules. The modified process will substantially reduce or eliminate the addition of SO<sub>2</sub> while maintaining high product yields and quality. This change in procedure would have a significant effect on reducing environmental pollution.

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