

Corn Wet Milling with a Commercial Enzyme Preparation¹

DU LING and D. S. JACKSON²

Corn wet milling is an industrial process that separates the corn kernel into its starch, protein, germ, and fiber fractions. Commercially, corn kernels are steeped for 48 hr in a solution (50°C) of SO₂ and lactic acid (produced by bacteria). Steeping takes place in a countercurrent batch system (May 1987). High starch recovery is the primary goal, but 100% of the analytically determined starch cannot be separated from the corn kernel using current wet-milling techniques. Caransa et al (1988) pointed out that some starch is bound to the bran and some is lost in the gluten fraction.

Commercially available enzyme systems are widely used to manufacture sweeteners from starch; different enzyme systems have potential applications in wet milling. U.S. patent 3,928,631 was issued to Freeman et al (1975) for a process that uses glucoamylase (EC 3.2.1.3) to degrade starch in the bran fraction. This step allows for more efficient bran dewatering. Caransa et al (1988) added a commercially available mixture of cellulose, hemicellulose, pectin, and phytin degrading enzymes to whole corn and steep water; they reported a 2.1 percentage unit increase in starch recovery. They also tried to reduce steeping times by steeping for 6 hr, coarse-grinding the corn, adding enzyme, and steeping for an additional 4 hr. The twice-steeped corn was then finely ground and the components separated. Starch yields were similar to those obtained using conventional 48-hr steeping without enzymes.

Although laboratory workers have shown some applications for enzymes in wet milling, impure commercial enzyme systems may degrade starch. Because laboratory wet milling is labor-intensive and time-consuming, a limited study was conducted to determine whether significant yield advantages resulted from adding a commercially available cellulosic enzyme system during steeping or just before component separation (after fine grinding). The effect of the commercial enzyme and treatment conditions on starch molecular weight profiles were also determined.

MATERIALS AND METHODS

Corn Samples and Enzyme Complex

Yellow dent corn (Pioneer 3780) grown in Nebraska during 1988 was stored at -4°C until used. Corn moisture was determined by drying for 72 hr at 103°C according to the AACC Method 44-15A (AACC 1983). To determine starch content, ground corn was autoclaved for 90 min and subsequently treated with glucoamylase for 48 hr. Total glucose was measured with a YSI model 27 glucose analyzer (Yellow Springs Instrument Co. Inc., Yellow Springs, OH), and multiplied by 0.9 to determine starch content.

A commercial enzyme complex (Cytolase 123, lot 802) with cellulase, β -glucanase, and arabinoxylanase (pentosanase) activity (100 GCU/ml) was obtained from Genencor, Inc. (San Francisco, CA). The complex is a fermentation product of *Trichodema longibrachiatum*. Corporate literature indicates that Cytolase 123 is essentially free of amylase, protease, and lipase enzymes.

Laboratory Wet Milling

Clean grain (300 g) was steeped for 24 or 48 hr in a solution of lactic acid (4.71 ml of 85% lactic acid per liter of steep solution) and sodium bisulfite (1.48 g per liter of steep water) (Watson et al 1955). Both chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). After steeping, grain drained from the steep water was placed in a Waring Blendor (blades reversed) with 250 ml of water. The steeped corn was ground for 1.5 min at the low speed setting. The ground grain, with and without 1 ml of enzyme complex, was placed in an oven set at 50°C for 1, 2, or 3 hr.

Bran and germ were collected together as overs on a U.S. No. 30 sieve (600 μ m), and the starch-protein slurry was the throughs of a U.S. No. 230 sieve (63 μ m). Starch was collected on an aluminum beam (3.05 \times 0.15 m, 10 \times 0.5 ft) and washed with water until clean. Fractionated bran (with germ) and starch were dried in a forced-air oven at 55°C until their weights remained constant; a subsample was removed and dried at 103°C for 18 hr to obtain a dry weight. Percent starch (or bran) fraction yields were calculated by dividing the dry weight of starch (or bran) by the original dry weight of corn and multiplying by 100. Protein content ($N \times 6.25$) in starch samples was determined by a semiautomated Kjeldahl system (Kjeltec 1026, Tecator Co., Herndon, VA). Molecular weight profiles of starch were determined by high-performance size-exclusion chromatography as outlined by Jackson et al (1989).

A randomized complete block design was used. Each treatment combination (24 or 48 hr; 1, 2, or 3 hr in a 50°C oven; 0- or 1-ml enzyme complex) was repeated three times. Statistical analysis was done using analysis of variance and Duncan's multiple range test with the SAS system for personal computers (SAS 1987).

RESULTS AND DISCUSSION

The corn had a moisture content of 13.1 (0.63% coefficient of variation [CV]). Total starch content was 72% on a dry weight basis (1.6% CV).

Although several batch samples can be steeped simultaneously, most laboratories cannot grind and table (separate) more than 1-2 samples at a time. When samples (with and without enzyme) were refrigerated at 4°C after steeping but before grinding, no significant differences were noted in starch or bran yields between samples refrigerated for 0, 24, and 168 hr ($\alpha = 0.05$). Thus, steeped corn can be refrigerated without significantly influencing starch yields. When the mixture of cellulase, -glucanase, and arabinoxylanase (Cytolase 123) was added to the steep water, mean yields of combined bran and germ fractions were not significantly different ($\alpha = 0.05$). However, starch yields were significantly different, but the mean starch yield increased by only 1.2 percentage units when enzyme was used. Since contact between the starchy endosperm and the cellulosic pericarp layers occurs within interior kernel layers, substrate-enzyme contact was minimized when whole corn was steeped with enzymes. The enzyme solution would initially follow the course of water migration into the kernel, namely, from the tip cap to the germ and endosperm, probably via the cross-cell layer (May 1987, Watson 1987). Bran and starch separation would likely be facilitated by more direct (less hindered) enzyme contact between the bran-starch interface within the corn kernel.

When enzyme was added after grinding the steeped kernels

¹Journal Series 9176, Agricultural Research Division, University of Nebraska-Lincoln.

²Research assistant and assistant professor, Cereal/Oilseed Science and Technology Laboratory, Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE 68583-0919.

TABLE I
Treatment Conditions and Starch Yields of Enzyme-Treated Wet-Milled Corn

Treatment Conditions			Starch Yield ^b (%)
Steep Time (hr)	Enzyme ^a	Incubation Time (hr)	
48	yes	3	66.8 a
		2	66.1 b
24	yes	1	65.6 b
		3	65.2 b
48	no	3	63.6 c
		2	63.6 cd
24	no	3	63.1 cde
		1	62.7 def
48	no	2	62.5 efg
		1	62.3 fg
48	no	1	61.8 g

^aYes = 1 ml, no = 0 ml.

^bMeans with the same letter are not significantly different ($\alpha = 0.05$).

TABLE II
Treatment Conditions and Bran Yields^a of Enzyme-Treated Wet-Milled Corn

Treatment Conditions			Bran Yield ^c (%)
Steep Time (hr)	Enzyme ^b	Incubation Time (hr)	
48	no	2	12.8 a
		1	12.8 a
24	no	1	12.7 a
48	no	3	12.7 ab
24	no	2	12.6 ab
		3	12.5 abc
24	yes	3	12.1 bcd
48	yes	1	12.0 cd
24	yes	2	11.9 d
48	yes	2	11.9 d
		3	11.8 d
24	yes	1	11.8 d

^aIncludes gum residue.

^bYes = 1 ml, no = 0 ml.

^cMeans with the same letter are not significantly different ($\alpha = 0.05$).

and the ground mixture was allowed to incubate at 50°C for 1–3 hr, starch yields increased significantly ($\alpha = 0.05$, Table I). The bran fraction yield was slightly reduced (Table II). In no cases were starch yields for steeping 48 hr with no enzyme significantly higher than yields for steeping 24 hr with enzyme. Steeping for 24 hr plus 3 hr of enzyme incubation resulted in significantly greater starch yields than the 24-hr steep plus 3 hr without enzymes (69.8% versus 67.5%). As expected, incubation without enzyme (which is equivalent to increased steeping time) appeared to increase starch yields slightly. In general, all enzyme-treated corn had lower bran yields, although no discernable pattern relative to steep and incubation times emerged. Since the primary activity of the enzymes is on components that predominate in bran, it is to be expected that bran yields would be reduced.

According to Watson and Hirata (1955), starch purity is usually assessed by measuring its residual protein; levels below 0.5% are acceptable. Mean starch protein contents did not exceed 0.36%, and the overall mean was 0.27% (16% CV). No significant differences ($\alpha = 0.05$) in residual protein were found between any treatment pair (enzyme versus no enzyme).

High-performance size-exclusion chromatography was used to characterize the molecular weight profile of the wet-milled starch. Figure 1 shows chromatograms of wet-milled starches steeped 48 hr (chromatograms of corn steeped for 24 hr were identical) with and without enzyme treatment. No depolymerization was

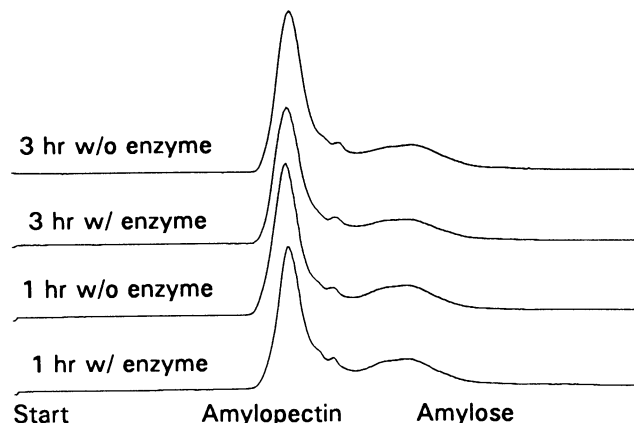


Fig. 1. Chromatographic profiles of wet-milled starch steeped for 48 hr with and without 1 or 3 hr of cellulosic enzyme treatment.

evident, and the chromatograms are similar to those obtained for commercially prepared regular corn starch (Jackson et al 1989). In addition, the peak areas per unit weight of starch were not significantly different ($\alpha = 0.05$). This confirms that the commercial enzyme preparation was essentially free of starch-degrading enzymes, the steeping and incubation procedure did not support excess activity of endogenous amylases found naturally in the environment, and the purity of the starches was not reduced by enzyme treatment.

The overs on the U.S. No. 230 sieve consisted of small quantities of finely ground bran, germ pieces, and endosperm chunks that passed through the U.S. No. 30 sieve. The composition and weight of this fraction would have reflected the changes in other fraction yields. Although not done for this limited study, a more detailed analysis of this fraction would provide supporting data on the changes that occur during enzyme steeping.

Although results obtained from batch wet milling are not necessarily indicative of a countercurrent commercial system, the improvements in fraction yields are significant enough to warrant further study to characterize more closely the effect of enzyme addition and process variables. Since laboratory wet milling is a time-consuming, labor-intensive process, studies with limited scope can encourage more rapid progress and indicate under what circumstances more detailed studies are warranted.

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