

Steeping Maize in the Presence of Multiple Enzymes.

II. Continuous Countercurrent Steeping¹

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ABSTRACT

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A laboratory procedure for steeping maize, which closely simulates commercial practice in wet milling, was developed and used to determine the effects of multiple-enzyme treatment on wet-milling performance. Yellow dent maize was continuously and countercurrently steeped for 24 hr in a solution containing 1.25% of a multiple-enzyme preparation and 0.20% sulfur dioxide and then wet milled. Yields and protein contents of wet-milling fractions were compared with those of maize steeped for

48 and 24 hr in 0.20% sulfur dioxide alone. Reducing the steeping time without adding enzymes reduced starch yield and increased the protein content of the starch. Maize steeped with multiple enzymes and sulfur dioxide for 24 hr gave nearly the same yields and purities of fractions as maize steeped for 48 hr in sulfur dioxide alone. Thermal properties, pasting properties, and colors of the starch were unaffected by steeping in multiple enzymes and sulfur dioxide.

Watson (1984) and May (1987) have discussed modern industrial practices for wet milling of maize. Steeping is the first and most important step in the milling process. Improper or inadequate steeping fails to produce normal yields of starch (about 90% of the total starch) and low residual protein contents in the starch (0.40% protein or less). But steeping is, by far, the most time-consuming step in the process, typically requiring 36–52 hr. New approaches to steeping are being sought to make wet milling more cost-effective and to reduce the cost of maize starch and starch-derived products, such as sweeteners and ethanol.

One approach that has been considered is the addition of

enzymes, which was recently reviewed and studied by Steinke and Johnson (1991). They used a static batch-steeping procedure to evaluate the feasibility of adding multiple enzymes to steep water containing typical levels of sulfur dioxide to enhance starch separation and reduce steeping time. Steeping for 24 hr in a solution of multiple enzymes and sulfur dioxide produced milling results equivalent to those obtained by steeping for 48 hr in a 0.20% sulfur dioxide solution alone.

In industry, however, steeping is accomplished in a continuous, countercurrent manner by putting maize that has been steeped the longest in contact with downstream process water to which 0.10–0.20% sulfur dioxide has been added. Each subsequent steep tank receives steep water from the previous steeping tank, until the steep water (about 20 L/bu) finally exits the steeping tank containing maize that has been steeped for the least amount of time. Although Watson et al (1951) developed a laboratory system for continuous, static, batch-advanced steeping, most steeping studies (Cox et al 1944, Zipf et al 1950, Watson et al 1955a, Anderson 1963, Vojnovich et al 1975, Krochta et al 1981, Weller et al 1988) have used static batch procedures. These procedures do not simulate the industrial continuous, countercurrent flow

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of gradually declining sulfur dioxide concentration and increasing solubles concentration or the mixing brought about by countercurrent flow and recirculation of steep water within each steeping vessel. These differences may affect results and lead to erroneous conclusions. Thus, a laboratory steeping procedure that more closely simulates commercial practice would give more reliable predictions of what to expect in industry.

The objectives of this study were to develop a laboratory continuous, countercurrent steeping system, which closely simulates industrial practice; to determine whether the presence of multiple enzymes and sulfur dioxide enhances starch separation and facilitates reduced steeping time in continuous, countercurrent steeping; and to evaluate the effects of such steeping on the quality of starch.

MATERIALS AND METHODS

Steeping Treatments

Maize was given three steeping treatments. The first treatment, 48 hr of steeping with 0.20% sulfur dioxide, was used as the control. The second treatment involved the same control procedure except that steeping time was reduced to 24 hr to examine the effects of reduced steeping time. In the third treatment, maize was steeped with an experimental multiple-enzyme system in a 0.20% sulfur dioxide solution for 24 hr.

The 0.20% sulfur dioxide solution was prepared by diluting a 6% sulfurous acid solution. The multiple-enzyme system (Table I) consisted of cellulase, hemicellulase, pectinase, β -glucanase, and bromelin, as used in previous batchwise steeping trials of Steinke and Johnson (1991). All five enzymes were added in equal amounts (0.25%), regardless of their individual activities, to a 0.20% sulfur dioxide solution. The total enzyme concentration was 1.25%.

Steeping System

The continuous countercurrent steeping system developed in this study (Fig. 1) was based on commercial practice as described by Watson (1984) and May (1987). The steeping vessels (1-6)

were 1,200-ml jacketed glass vessels arranged in a battery of six vessels (three more are needed for loading and are waiting). Each vessel was stoppered and fitted with four Teflon tubes. Three tubes were inlets (one for recirculating within the vessel, another for incoming steep water from the previous vessel, the third for inflow of fresh steeping solution), and the fourth was an outlet for overflow into the next succeeding steeping vessel. The depth of the overflow tube into each steeping vessel was set to maintain 1,050 ml. The bottom of each vessel was funnel-shaped and fitted with a Teflon stopcock. A perforated acrylic disk was placed in the bottom of the vessel to hold the maize. The temperature was maintained at $50 \pm 2^\circ\text{C}$ by circulating water through the vessel jackets and by using a water bath coupled to a centrifugal pump.

Solution was advanced through the steeping vessels by positive pressure from the smaller peristaltic pump (B in Fig. 1) and by negative pressure from a second peristaltic pump (C). Solution was recirculated within the vessels by a 10-channel peristaltic drive (E). The system for delivering fresh steeping solution was automated. Inflow of steeping solution into each vessel was actuated by a normally closed two-way solenoid valve (I_1 - I_6) controlled

TABLE I
Sources and Properties of Enzymes Used in Steeping Maize

Enzyme	Source	Optimum pH Range	Optimum Temperature Range ($^\circ\text{C}$)	Activity (units/g)
Cellulase ^a	<i>Trichoderma viride</i>	4.0-6.0	50-60	150
Hemicellulase ^a	<i>Aspergillus niger</i>	3.0-6.0	30-80	25
β -Glucanase ^b (Ceremix 2 \times 1)	<i>Bacillus subtilis</i>	4.0-7.0	40-55	300
Pectinase ^b (Pectinex 3 \times 1)	<i>A. niger</i>	3.5-6.0	20-50	3,000
Bromelin ^a	Pineapple	4.0-5.5	45-55	3,000

^aPurchased from Sigma Chemical Co., St. Louis, MO.

^bPurchased from Novo Industri, Wilton, CT.

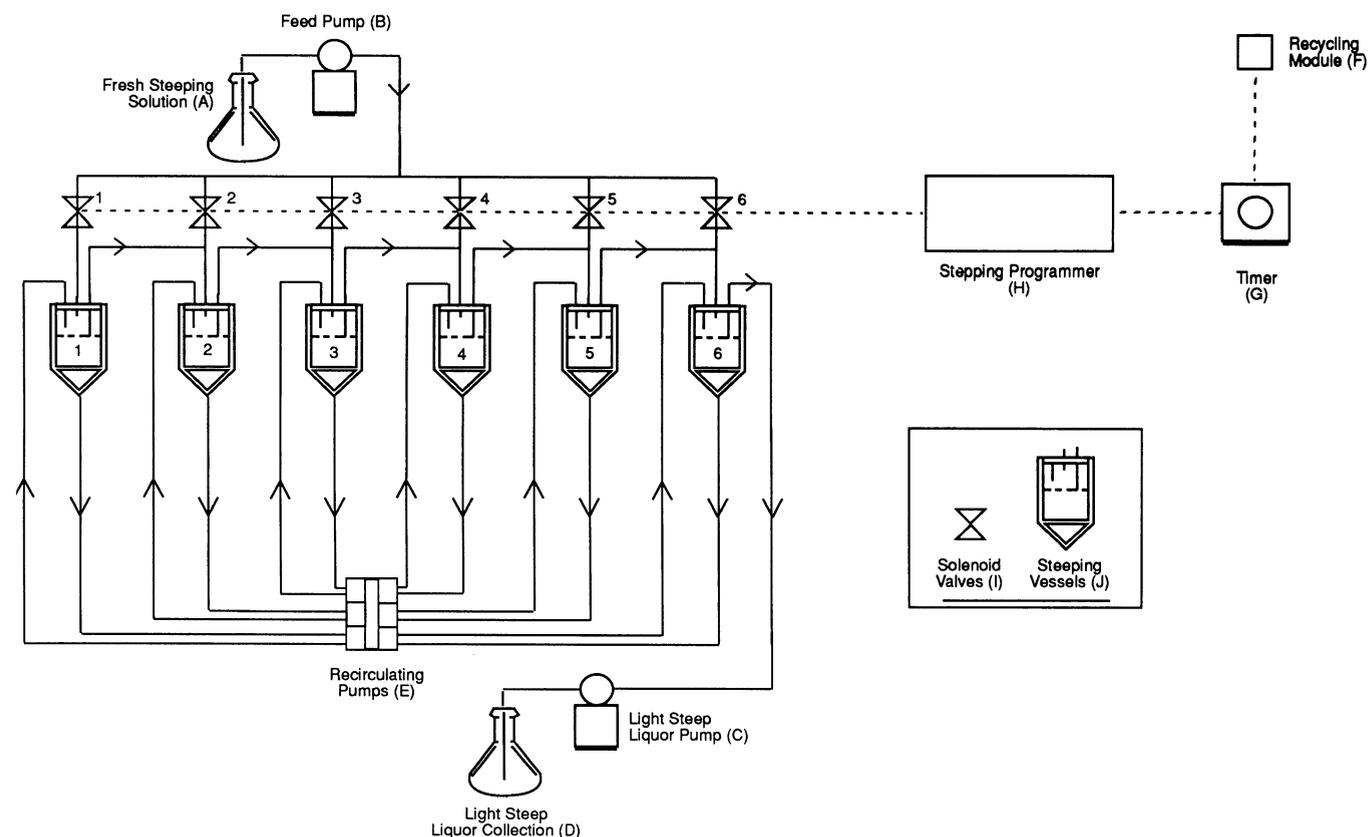


Fig. 1. Laboratory countercurrent steeping system.

by a timer and a recycle module. The solution was advanced to succeeding steeping vessels by an electromechanical multicircuit stepping programmer.

Steeping Procedure

Each vessel was filled with 300 g of Pioneer Hybrid 3475 (Pioneer Hi-Bred International, Inc., Johnston, IA) yellow dent maize harvested from an Iowa State University test plot during 1987. The maize was dried with forced air to approximately 15.5% moisture at room temperature and screened with a Carter dockage tester to remove foreign materials. Each steeping vessel was inoculated with 5 ml of steep liquor (Penford, Ltd., Cedar Rapids, IA) as it was brought on line to enable the establishment of lactic acid-producing microorganisms. However, we cannot be certain that the level of *Lactobacillus* fermentation was the same as is achieved in industrial practice.

The steeping solution entered the system from a 6-L Erlenmeyer flask (A), was delivered to the top of the first steeping vessel, traveled down through the grain until the steeping solution level rose to the bottom of the overflow tube, and overflowed into the top of the next steeping vessel. At the same time, steeping solution was recirculated within the steeping vessels at the rate of seven parts to one part of new solution or carryover from the previous steeping vessel. This gave a flux for recirculation that was the same as typically found in industry. Once the sixth vessel became filled with steeping solution, the step switch closed solenoid valve I₁ and opened solenoid valve I₂ to fresh steeping solution. This brought a new vessel on line, and the fully steeped maize in vessel I was removed from the system. Thus, six steeping vessels were on line at any one time. This sequence of events continued in a circular fashion around the battery of six vessels until equilibrium was reached. The system was considered in equilibrium when the pH values of the steeping vessels no longer changed. This required about 96 hr and discarding of the maize from the first 12 lots.

The 48-hr steeping period required 8 hr to fill each vessel. During the filling period, the steep water was also recirculated within the steeping vessel. After 8 hr, the steep water overflowed into the succeeding steeping vessel and so proceeded through the steeping vessels in 8-hr stages. After 48 hr (at which point the sixth steeping vessel in the series was full), and every 8 hr thereafter, a new steeping vessel came on line, and the oldest maize in the series was removed for wet milling. During start-up, the fresh solution flow rate was set at 1.7 ml/min until the sixth vessel was reached. At this time, the flow rate was increased to 2.2 ml/min and continued at this rate for the rest of the run to produce light steep liquor (246 ml per 300-g batch of maize). The light steep liquor was collected and stored at 4°C. The recirculation rate was maintained at 15.5 ml/min in each steeping vessel.

The 24-hr steeping treatment was run the same way except that the filling time was halved to 4 hr, and all flow rates were doubled to 3.4 ml/min for the first five vessels, 4.4 ml/min for subsequent vessels, and 31.1 ml/min for the recirculation rate. The same amount of light steep liquor (246 ml per 300-g batch of corn) was collected. The 24-hr method was used for both enzyme and nonenzyme treatments.

The temperature and pH of each steeping vessel were measured at the end of each 4- or 8-hr stage to monitor the system.

Milling Procedure

After steeping, the light steep liquor was drained, and the steeped maize was removed. Wet milling began immediately, following the method described by Steinke and Johnson (1991). Six samples from each treatment were evaluated for wet-milling properties.

Compositions and Yields of Wet-Milling Fractions

Light steep liquor was analyzed for pH and for sulfur dioxide and solids contents. Sulfur dioxide content was determined by titrating with a standard iodine-potassium iodide solution adjusted to be equivalent to 0.001 g of sulfur dioxide per milliliter (Watson et al 1955b). Soluble starch was used as an indicator. Solids con-

tents of steeping solutions and starch wash water samples were determined by drying 10-ml samples at 65°C for 24 hr and then drying to a constant weight in a vacuum oven at 65°C.

All milling fractions, except starch, were dried for 24 hr in an oven at 65°C followed by 8 hr in a vacuum oven at 65°C. Starch was dried in an oven at 40°C for 48 hr to minimize damage to the starch. The moisture contents of maize and dried fractions were determined by the vacuum oven method (AACC 1983). Fraction yields were determined on a dry-matter basis.

The nitrogen content of each fraction was determined by the macro-Kjeldahl method with a Kjeltec digestion and distilling system (Tecator, Inc., Hoganas, Sweden). The factor of 6.25 g of protein per gram of nitrogen was used to calculate the protein contents.

Starch Pasting Properties

The pasting properties of starch slurries from maize processed by the three steeping treatments were determined with a Brabender Viscoamylograph (C. W. Brabender Instruments, Inc., Hackensack, NJ) and modified methods of Mazurs et al (1957). Starch solutions (8%) composed of 40 g of dry starch and 460 g of distilled water were analyzed for peak pasting temperature (temperature at which there was an increase of 10 BU from the onset of gelatinization); peak viscosity, irrespective of the temperature at which the pasting peak was attained; viscosity when the paste reached 95°C; viscosity after the paste was cooked for 15 min at 95°C; and viscosity of the cooked paste during cooling (setback).

Thermal Properties of Starch

Differential scanning calorimetry (DSC) was used to study the thermal properties of starch processed by different steeping methods. A Perkin-Elmer DSC-7 calorimeter (Norwalk, CT) equipped with a thermal analysis data station was used. Samples (4 mg) of starch were weighed into aluminum sample pans with 8 μ l of water, and the pans were hermetically sealed. A pan containing only 8 μ l of water was used as a reference. Samples were heated at the rate of 10°C/min from 30 to 120°C. Heat of gelatinization, onset temperature, and peak temperature were computed. All samples were run in triplicate.

Starch Color

Starch color was measured with a Labscan 5100 Spectrocolorimeter (Hunter Associates Laboratory, Inc., Reston, VA). The Hunter *L*, *a*, *b* opponent color scale was used, and values were calculated relative to the Commission Internationale de l'Eclairage (CIE) 1964 10° Standard Observer.

RESULTS AND DISCUSSION

Comparison of Wet-Milling Results with Previous Studies

Table II shows the yields and protein contents of products from wet milling of maize by our continuous countercurrent steeping and wet-milling methods and the corresponding results from industry and previous laboratory methods. In previous batch systems, unlike in industry, the maize does not come in contact with gradually increasing concentrations of solubles and metabolites from *Lactobacillus* fermentation and decreasing concentrations of sulfur dioxide. Also unlike industrial systems, these batch systems are static, achieving little mixing. The countercurrent steeping method of Watson et al (1951) was also not truly continuous, but rather a static, batch-advanced, countercurrent system; nor was the steeping solution recycled within the steeping vessel, which limited mixing. These differences in method may affect chemical and biological changes during steeping and thereby also influence wet-milling characteristics.

Germ yields were comparable among the steeping methods, but batch steeping resulted in germs with higher protein contents (Table II), perhaps because of less mixing and, thus, less driving force for protein solubilization and leaching. Batch steeping results in higher fiber yields, whereas continuous countercurrent steeping, although closer to industry yields, may underestimate them. The

present continuous countercurrent steeping and milling procedures produced yields (64.9%) and purities (0.42% protein) of starch more typical of those found in industrial practice (67.5% and 0.30%, respectively). Most laboratory methods result in a small fraction, known as "squeegee" or "inseparables," that is difficult to separate into starch and gluten. This fraction is not separated in industry. If the squeegee fractions were included with starch, the yields and protein contents of the starch recovered with the present countercurrent steeping method would exceed industrial results (69.3% starch with 0.59% protein). All laboratory methods produced gluten with lower protein contents than industry, although results with the present method more closely approached those of industry.

The milling procedures used in the present study and in batch studies by Steinke and Johnson (1991) were identical, except for the steeping step. Much less variability about the mean yields and protein contents was observed in the present study, indicating that the results were more uniform and reliable with continuous countercurrent steeping. We believe that the steeping and milling procedures used in this study produced results that are more representative of those achieved by industry than previous simulations.

Steep Water Composition

Table III shows the pH profiles within the battery of steeping vessels for the three steeping treatments. Maize constituents have considerable buffering capacity. The control steeping solutions were more acidic than those in industry (pH 3.4–4.0) because starch wash water, with its attendant buffering capacity, is used to prepare sulfur dioxide solution for steeping (Watson 1984). When the steeping period was shortened from 48 to 24 hr, the steeping solutions became more acidic, especially in early steeping vessels, because of less time to leach buffering solubles. The added enzymes also have considerable buffering capacity, and the pH values rose considerably, especially in early steeping vessels.

Soluble solids profiles (less the weight of added enzymes) of the steeping solution are also shown in Table III. As expected, when steeping time was reduced from 48 to 24 hr, soluble solids leached from the grain into the steeping solution decreased substantially. However, when steeping for 24 hr was done in the presence of multiple enzymes and sulfur dioxide, the soluble solids

TABLE II
Yields and Protein Contents of Wet-Milling Fractions Produced by Industry and Various Laboratory Procedures

Fraction	Laboratory Steeping Procedure				
	Industrial Practice ^a	Batch		Countercurrent	
		Watson (1982) ^a	Steinke (1991) ^b	Watson (1951) ^c	Steinke (Present) ^d
Germ					
Fraction yield, %	7.5	6.2	6.6 ± 0.4	7.2	6.7 ± 0.1
Protein, %	12.0	23.7	17.6 ± 0.3		14.6 ± 0.3
Fiber					
Fraction yield, %	11.5	12.5	19.2 ± 1.9	8.4	10.7 ± 0.1
Protein, %	12.0	14.7	11.6 ± 0.8	10.8	9.9 ± 0.2
Starch					
Fraction yield, %	67.5	65.4	58.4 ± 0.7	62.8	64.9 ± 0.4
Protein, %	0.30	0.54	0.56 ± 0.05	0.36	0.42 ± 0.08
Gluten					
Fraction yield, %	5.8	8.1	5.2 ± 0.3	11.5	5.6 ± 0.1
Protein, %	65.8	42.9	56.0 ± 4.2	39.5	47.6 ± 1.2
Squeegee ^e					
Fraction yield, %		1.4	3.7 ± 0.4		4.4 ± 0.2
Protein, %		20.8	7.5 ± 0.7		3.2 ± 0.7
Solubles					
Fraction yield, %	7.5	7.1	7.2 ± 0.7	7.5	7.7 ± 0.7

^aAnderson and Watson (1982).

^bSteinke et al (1991); mean of six replicates plus or minus one standard deviation.

^cWatson et al (1951).

^dMean of six replicates plus or minus one standard deviation.

^eEquivalent to inseparables fraction. This fraction is not produced in industry and was not reported by Watson et al (1951).

content was similar to that of the 48-hr control. This suggests that the extent of cellular disruption was similar in these two treatments.

Higher levels of residual sulfur dioxide were retained in steeping solutions of earlier steeping vessels (Table III). This result indicated less extensive reduction of disulfide bonds in the protein matrix of maize steeped for 24 hr compared with maize steeped for 48 hr. Sulfur dioxide profiles were similar for the 48-hr control and the 24-hr treatment with multiple enzymes and sulfur dioxide, which suggested that the protein matrix was disrupted to a similar degree. However, some sulfur dioxide may react with the enzymes and be consumed.

Wet-Milling Characteristics

The maize wet-milling industry expects high starch yields (>65% of the dry weight), low protein content in starch (<0.4% db), high yields of gluten (5.7% db), and high protein content in gluten (60% on a 10% mb). The mean yields (dry-weight basis for six replicates) and protein contents for each of the wet-milling fractions from the three steeping treatments in this study are shown in Table IV. The yields and protein contents for the 48-hr control

TABLE III
Profiles of pH, Soluble Solids, and Sulfur Dioxide Levels in Steeping Solution in Laboratory Continuous Countercurrent Steeping

Steeping Treatment	Steeping Time (hr)	Steeping Vessel						
		F ^a	1	2	3	4	5	6 ^b
pH								
Control	48	2.20	3.50	4.02	4.10	4.34	4.80	4.74
Control	24	2.20	3.11	3.50	4.05	4.47	4.71	4.69
Enzyme	24	3.40	4.70	4.85	4.95	4.95	4.75	4.65
Soluble Solids (%)								
Control	48	0	0.63	1.21	1.81	2.38	3.01	3.30
Control	24	0	0.47	0.76	1.15	1.39	1.48	1.55
Enzyme	24	0	0.74	1.43	2.13	2.81	3.55	3.89
Sulfur Dioxide Levels (%)								
Control	48	0.20	0.13	0.12	0.11	0.10	0.08	0.06
Control	24	0.20	0.17	0.17	0.14	0.12	0.12	0.11
Enzyme	24	0.20	0.14	0.12	0.10	0.10	0.09	0.08

^aF denotes fresh steeping solution.

^bSteeping vessel 6 contains the newest corn.

TABLE IV
Yields and Protein Contents of Wet-Milling Fractions from Continuous Countercurrent Steeped Maize (db)

Fraction	48-Hr Steeping Control ^a	24-Hr Steeping ^a	
		Control	Enzyme
Germ			
Fraction yield, %	6.72 a	7.45 b	6.72 a
Protein, %	14.6 a	17.2 b	15.2 a
Total fiber			
Fraction yield, %	10.65 a	11.50 b	12.51 c
Protein, %	9.9 a	11.1 b	7.9 c
Starch			
Fraction yield, %	64.94 a	56.95 b	63.98 c
Protein, %	0.42 a	0.82 b	0.33 c
Gluten			
Fraction yield, %	5.57 a	6.34 b	5.80 a
Protein, %	47.6 a	44.4 b	50.1 c
Inseparables			
Fraction yield, %	4.44 a	10.78 b	2.35 c
Protein, %	3.2 a	1.8 b	4.5 c
Solubles ^b			
Fraction yield, %	7.68 a	6.98 b	8.64 c

^aMeans within a row followed by the same letter do not differ significantly at the 0.05 level according to Duncan's multiple range test.

^bComposite of starch wash solubles and steep liquor solubles (enzyme addition subtracted).

TABLE V
Pasting Properties of Laboratory-Isolated Starches^a

Steeping Treatment	Steeping Time (hr)	Peak Temperature (°C)	Peak Viscosity (BU)	Viscosity at 95°C (BU)	Viscosity at 95°C, 15 min (BU)	Setback	
						Viscosity (BU)	Time (min)
Control	48	72.0 a	798 ab	791 ab	664 ab	1,010 ab	74
Control	24	73.0 a	774 a	761 a	652 a	990 a	77
Enzyme	24	72.5 a	823 b	823 b	710 b	1,040 b	72

^a Means within a column followed by the same letter do not differ significantly at the 0.05 level according to Duncan's multiple range test.

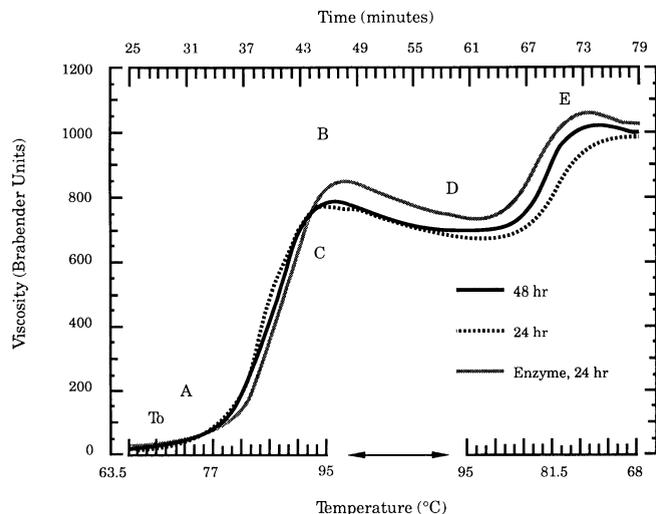


Fig. 2. Brabender viscoAmylograph curves of laboratory-isolated starches from continuous countercurrent steeping with and without multiple enzymes, showing peak pasting temperature (A), peak viscosity (B), peak viscosity at 95°C (C), extent of breakdown (D), amount of setback (E), and gelatinization onset temperature (To).

were nearly typical of industry practice, as has been previously discussed.

Wet-milling characteristics were significantly poorer in the 24-hr control than in the 48-hr control. Higher germ protein contents in the 24-hr control indicated less leaching of soluble protein into the steeping solution, which made germs denser and consequently more difficult to separate. Fiber yields were high in the 24-hr control, but not nearly as high (19.2%) as those observed in previous batch studies (Steinke and Johnson 1991). Starch separated from gluten very poorly in the 24-hr control, as indicated by the low starch yield, high level of protein contamination in the starch, high yield of inseparables, and low protein content of gluten. We observed many small intact pieces of horny endosperm after fine grinding of the 24-hr control that could not be expected to separate efficiently into starch and gluten.

Inclusion of multiple enzymes in the steeping solution greatly improved the wet-milling properties of maize when the steeping time was reduced to 24 hr. Both the yield and the protein content of the germ from the 24-hr treatment with multiple enzymes and sulfur dioxide were more typical of those from the 48-hr control treatment. Total fiber yield was the highest for the treatment with multiple enzymes and sulfur dioxide. Previous batch-steeping studies (Steinke and Johnson 1991) indicated that the increased yield of starch resulted from enhanced starch-fiber separation as well as enhanced starch-gluten separation. Enhanced starch-fiber separation was not observed in these continuous countercurrent steeping experiments. The benefit from adding enzymes to continuous countercurrent steeping solutions came from enhanced starch-gluten separation only.

Starch yields were much higher and yields of inseparables were lower (less than even the 48-hr control) in the 24-hr treatment with multiple enzymes and sulfur dioxide. Starch purity was significantly greater from maize steeped with multiple enzymes and sulfur dioxide for 24 hr than in maize steeped in sulfur dioxide solution alone for 48 hr. Gluten yields from the multiple enzyme

TABLE VI
Thermal Properties of Laboratory-Isolated Starches^a

Steeping Treatment	Steeping Time (hr)	Onset Temperature (°C)	Peak Temperature (°C)	Heat of
				Gelatinization (cal/g)
Control	48	67.2 a	73.2 a	3.00 a
Control	24	66.5 a	73.0 a	3.00 a
Enzyme	24	66.8 a	73.2 a	3.11 a

^a Means within a column followed by a common letter do not differ significantly at the 0.05 level according to Duncan's multiple range test.

TABLE VII
Chromatic Values of Laboratory-Isolated Starches^a

Steeping Treatment	Steeping Time (hr)	Chromatic Values	
		L	+b
Control	48	97.2 a	5.59 a
Control	24	96.8 b	6.56 b
Enzyme	24	97.2 a	5.89 a

^a Means within a column followed by the same letter do not differ significantly at the 0.05 level according to Duncan's multiple range test.

treatment were close to those of the 48-hr control, and the gluten had more protein. Soluble solids from the 24-hr treatment with multiple enzymes and sulfur dioxide were greater than those from either control treatment. We believe that the addition of multiple enzymes to steeping solution enhances starch-gluten separation when steeping is done under conditions that closely simulate industrial steeping and milling practices.

Starch Pasting Properties

The pasting characteristics of starch obtained from the three steeping procedures used to wet mill maize were determined (Table V and Fig. 2). All treatments gave starch with typical pasting properties of maize (Greenwood 1976). Samples did not differ significantly for any of the five critical points on the Brabender viscosity curves when the 48-hr control was compared with the 24-hr control. However, differences were significant between the 24-hr control and the 24-hr treatment with multiple enzymes and sulfur dioxide. Starch from the 24-hr control had a slightly higher peak temperature, lower peak viscosity, lower viscosity at setback, and delayed setback. The large differences in protein contents in starch of these two treatments were believed to be the cause of these differences in pasting characteristics. Differences observed in the pasting properties of starches obtained by 24-hr steeping with and without enzymes may be due to the adherence of hydrophobic protein to starch granules, which would affect the ability of the granules to swell.

Thermal Properties of Starch

The thermal properties of starch processed by the three treatments are shown in Table VI. The thermograms from all three treatments were similar to those previously obtained for laboratory-isolated maize starch (Krueger et al 1987). No significant differences between treatment values were found for gelatinization onset temperature, peak gelatinization temperature, or heat of gelatinization.

Starch Color

The chromatic dimensions for the degree of lightness (L) and the intensity of yellowness ($+b$) of starch processed by the three treatments are given in Table VII. The L values for the 48-hr control and the multiple-enzyme treatment were significantly higher (lighter colored) than those for the 24-hr control treatment. Correspondingly, the b values for the 24-hr control were also significantly higher, indicating more intense yellow color. These higher values are indications of higher levels of contaminating protein and associated pigments (carotenoids and xanthophyll). No significant differences in either L or b values were found when the starch from the 24-hr treatment with multiple enzymes and sulfur dioxide was compared with the starch from the 48-hr control treatment.

CONCLUSIONS

A laboratory maize-steeping system that closely simulates continuous countercurrent steeping in industry was developed and used to evaluate the feasibility of adding enzymes in steeping of maize to enhance separation of starch and reduce steeping time. Wet-milling results with maize steeped in this system more closely approach those of industry than those encountered in previous laboratory studies.

Reducing the steeping time from 48 to 24 hr without adding multiple enzymes to steeping solution (0.20% sulfur dioxide) adversely affected the wet-milling properties of maize. The addition of multiple enzymes to steeping solution in the continuous countercurrent steeping system enhanced starch-gluten separation. Nearly the same yields and protein contents of most wet-milled products were obtained from maize steeped for 24 hr with multiple enzymes and sulfur dioxide as were obtained from maize steeped for 48 hr in sulfur dioxide alone. The profiles for residual sulfur dioxide contents and soluble solids contents for the steep water of the 24-hr treatment with multiple enzymes and sulfur dioxide were more like those of the 48-hr control, suggesting that similar extents of kernel softening were achieved. The pasting, thermal, and color properties of the starch separated from maize steeped in the presence of multiple enzymes and sulfur dioxide for 24 hr were equivalent to those of starch from maize steeped in sulfur dioxide for 48 hr.

This study has not elucidated which of the five enzymes used play critical functional roles or how much of each enzyme is effective. The level of enzymes used probably exceeds commercially practical levels today, but as costs for enzymes come down, enzyme-enhanced separation processes may become economically attractive.

LITERATURE CITED

- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 44-40, approved April 1961; reviewed October 1976 and October 1982. The Association: St. Paul, MN.
- ANDERSON, R. A. 1963. Wet-milling properties of grains: Bench-scale study. *Cereal Sci. Today* 8:190.
- ANDERSON, R. A., and WATSON, S. A. 1982. The corn milling industry. Pages 31-61 in: *CRC Handbook of Processing and Utilization in Agriculture*, Vol. 2, Part 1. I. A. Wolff, ed. CRC Press: Boca Raton, FL.
- COX, M. J., MacMASTERS, M. M., and HILBERT, G. E. 1944. Effect of the sulfurous acid steep in corn wet milling. *Cereal Chem.* 21:447.
- GREENWOOD, C. T. 1976. Starch. *Adv. Cereal Sci. Technol.* 1:119.
- KROCHTA, J. M., LOOK, K. T., and WONG, L. G. 1981. Modification of corn wet milling steeping conditions to reduce energy consumption. *J. Food Process. Preserv.* 5:39.
- KRUEGER, B. R., KNUTSON, C. A., INGLETT, G. E., and WALKER, C. E. 1987. A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. *J. Food Sci.* 52:715.
- MAY, J. B. 1987. Wet milling: Process and products. Pages 377-397 in: *Corn: Chemistry and Technology*. S. A. Watson and P. E. Ramstad, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.
- MAZURS, E. G., SCHOCH, T. J., and KITE, F. E. 1957. Graphical analysis of the Brabender viscosity curves of various starches. *Cereal Chem.* 34:141.
- STEINKE, J. D., and JOHNSON, L. A. 1991. Steeping maize in the presence of multiple enzymes. I. Batchwise steeping. *Cereal Chem.* 68:7-12.
- VOJNOVICH, C., ANDERSON, R. A., and GRIFFIN, E. L., Jr. 1975. Wet-milling properties of corn after field shelling and artificial drying. *Cereal Foods World* 20:333.
- WATSON, S. A. 1984. Corn and sorghum starches: Production. Pages 417-468 in: *Starch: Chemistry and Technology*. R. L. Whistler and E. F. Paschall, eds. Academic Press: New York.
- WATSON, S. A., WILLIAMS, C. B., and WAKELY, R. D. 1951. Laboratory steeping procedures used in a wet milling research program. *Cereal Chem.* 28:105.
- WATSON, S. A., SANDERS, E. H., WAKELY, R. D., and WILLIAMS, C. B. 1955a. Peripheral cells of the endosperms of grain sorghum and corn and their influence on starch purification. *Cereal Chem.* 32:165.
- WATSON, S. A., HIRATA, Y., and WILLIAMS, C. B. 1955b. A study of the lactic acid fermentation in commercial corn steeping. *Cereal Chem.* 32:382.
- WELLER, C. L., PAULSEN, M. R., and STEINBERG, M. P. 1988. Correlation of starch recovery with assorted quality factors of four corn hybrids. *Cereal Chem.* 65:392.
- ZIPF, R. L., ANDERSON, R. A., and SLOTTER, R. L. 1950. Wet milling of grain sorghum. *Cereal Chem.* 27:463.

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