Steeping Maize in the Presence of Multiple Enzymes. I. Static Batchwise Steeping[1]

J. D. STEINKE and L. A. JOHNSON[2]

Cereal Chem. 68(1):7-12

ABSTRACT

The feasibility of incorporating multiple enzymes in steeping solutions to reduce steeping time and enhance starch separation during wet milling of maize was investigated. Maize kernels were steeped batchwise in a 0.20% sulfur dioxide solution for 48 and 24 hr, in a sulfur dioxide solution containing an additional 1.25% of a commercial multiple-enzyme preparation (Novo SP249) for 24 hr, and in a sulfur dioxide solution containing an additional 1.25% of an experimental multiple-enzyme preparation (cellulase, hemicellulase, β-glucanase, pectinase, and bromelin) for 24 hr. Maize steeped for 48 hr in 0.20% sulfur dioxide exhibited normal wet-milling characteristics, but maize steeped in the same solution for only 24 hr showed poor starch-fiber and starch-gluten separations during wet milling. Maize steeped in sulfur dioxide plus either treatment of multiple enzymes produced fraction yields and purities comparable to those of maize steeped for 48 hr in 0.20% sulfur dioxide alone, suggesting that steeping maize in the presence of multiple enzymes and sulfur dioxide enhances separation of wet-milling fractions and may facilitate a reduction in steeping time.

Steeping maize in preparation for wet milling has not changed over the past 100 years. Although the process enables recovery of about 90% of the starch in the kernel, it is time-, capital-, and energy-intensive. Reducing steeping time from the normal 48 hr may reduce processing costs but creates serious problems in processing and product quality.

Some researchers have developed mechanical approaches to reduce steeping requirements (Gillenwater et al 1971, Roushdi et al 1979, Krochta et al 1981, Meuser et al 1985, Hassanean and Abdel-Wahed 1986); others have developed chemical and biochemical approaches (Grindel 1965, Krochta et al 1981, Neryng and Reilly 1984). Some of these modifications achieved adequate starch separation with less steeping time, but they required costly modifications of existing facilities, required pretreatment of maize kernels, increased starch leaching into the steep water, increased pollution, and/or increased energy use.

The role of *Lactobacillus* fermentation during steeping of maize has been controversial. Lactic acid produced by *Lactobacillus* may help soften the kernel (Cox et al 1944, Kerr 1950, Watson et al 1955) and degrade the protein matrix surrounding starch granules (Maslennikova 1970, Wahl 1970, Roushdi et al 1981b). However, these effects may be due to a variety of catabolic indigenous enzymes of the kernel and/or exogenous bacterial enzymes rather than the lactic acid. Wahl (1971) determined that indigenous proteolytic enzymes reach maximum activities during steeping. Wall and Paulis (1978) attributed poor starch-protein separation in maize that had been damaged by heat during drying to the inactivation of indigenous proteases.

The addition of proteolytic enzymes to enhance starch-protein separation has also been studied. The addition of papain, bromelin, and trypsin to degerminated corn improves the purity of glucose syrups (Van Twisk and Tegge 1968). Vojnovich et al (1960) subjected starch slurries to a solution of pepsin and papain for 2 hr and reduced protein content in starch from 1.27 to 0.42%. Wolf and Khoo (1975) were able to digest subcellular protein structures in thin sections of maize endosperm with pronase within 30-60 min. Spanheimer et al (1972) found that a variety of proteolytic enzymes increases protein solubility of maize grits. They also noted that a combination of bromelin and sulfur dioxide performs better than either treatment alone at the same pH.

Roushdi et al (1981a) studied the effects of alcalase and neutrase on steeping of intact, scratched, and broken maize kernels. Treating broken kernels with these enzymes increased water-solubility of maize protein, improved starch recovery, and decreased effective steeping time by 50%. No effects, however, were observed with scratched or intact kernels. The intact bran was believed to be impermeable to proteases.

Wolf et al (1952) found that carbohydrases have the greatest effect on the cross cells and aleurone. Wood and McRae (1978) reported that the granular areas in structures that contain cellulose are the most susceptible to degradation by carbohydrases. Therefore, the addition of enzymes that degrade cell walls and membranes, such as cellulase, hemicellulase, pectinase, and β-glucanase, to steeping solution may provide access for penetration of proteases and other enzymes.

The objectives of this study were to evaluate the feasibility of adding multiple enzymes to steeping solution to reduce steeping time and enhance starch separations, and to investigate the mechanisms of any improvement.

MATERIALS AND METHODS

Steeping Procedure

Pioneer Hybrid 3475 (Pioneer Hi-Bred International, Inc., Johnston, IA) yellow dent maize was dried with forced air at room temperature to about 15.5% moisture and screened with a Carter dockage tester (CEA Carter Day Co., Minneapolis, MN) to remove foreign materials. The laboratory steeping procedure of Watson et al (1955) as modified by Krochta et al (1981) was used. All steeping solutions were prepared from distilled water. A 300-g sample of maize and 600 ml of steeping solution were placed into a 1,000-ml beaker. The beaker was immersed in a water bath at 50°C. After steeping, the steeping solution was drained, measured, and analyzed for solids content.

Maize was steeped batchwise under four sets of conditions. In the first treatment, maize was steeped for 48 hr in 0.2% sulfur dioxide prepared by dissolving sodium bisulfite in deionized water. This treatment was used as a control. In the second treatment, the steeping time was reduced to 24 hr. The third treatment utilized a commercial multiple-enzyme preparation in a 0.20% sulfur dioxide solution for 24-hr steeping. The fourth treatment employed an experimental multiple-enzyme preparation in a 0.20% sulfur dioxide solution for 24-hr steeping.

The commercial multiple-enzyme preparation, SP249, was obtained from Novo Industri (Wilton, CT). This liquid enzyme product, produced from *Aspergillus niger*, degrades plant cell walls and has been recommended for use in maize wet milling.

[1]Journal paper J-13616 of the Iowa Agriculture and Home Economics Experiment Station, Ames 50011; Project 0178. Research was supported by the Center for Crops Utilization Research, the Corn Refiners' Association, the Iowa Corn Promotion Board, and the Iowa Agriculture and Home Economics Experiment Station.

[2]Graduate research assistant and professor, respectively, Department of Food Technology and Center for Crops Utilization Research, Iowa State University, Ames 50011.

SP249 contained pectolytic, cellulolytic, hemicellulolytic, and proteolytic activities and small amounts of saccharolytic activities (Table I). SP249 had optimum pH and temperature ranges of 3.5–5.5 and 40–50°C, respectively. The steeping solution for this treatment contained 1.25% of SP249 in a 0.20% sulfur dioxide solution.

The experimental multiple-enzyme system was composed of cellulase, hemicellulase, β-glucanase, pectinase, and bromelin. Their sources, optimum pH and temperature ranges, and activities are listed in Table I. Special care was taken to select carbohydrases with low levels of α-amylase activity. Equal weights of all five enzyme concentrates (0.25% of the steeping solution) were added to a 0.20% sulfur dioxide solution.

Milling Procedure

After steeping, the steeping solution was drained, and the maize was immediately wet milled as shown in Figure 1. The steeped maize was coarsely ground in a Waring Blendor (Dynamics Corp. of America, New Hartford, CT) to free the germs. Equal volumes of corn and water (about 100 cm^3) were measured into a 1,200-ml blender jar and blended for 2.0 min at one-third speed. Speed was controlled with a 115-V AC variable autotransformer set at 33%.

The coarsely ground slurry was transferred to a 2,000-ml beaker, and 100 ml of water was added to enable the germs to float. The slurry was stirred and allowed to settle for a few minutes. Then the germs were skimmed from the surface with a wire screen (20-mesh). The germs were removed from the screen with tweezers, placed in a 100-ml beaker, and washed three times with 100 ml of water each time. The wash water was returned to the degerminated maize slurry. The germs were dried, weighed for yield, and analyzed for moisture and protein contents.

The degerminated maize slurry was reblended, 400 ml at a time, in the Waring Blendor at full speed for 2.0 min. This fine grinding released the endosperm from the bran and helped release the starch from the protein matrix in the endosperm. Fiber was separated by pouring about 200 ml of the ground slurry onto a set of sieves (U.S. Standard No. 40 and No. 200 sieves and a pan). The sieves were then shaken for 5.0 min on a Ro-Tap Testing Sieve Shaker (W.S. Tyler, Inc., Mentor, OH) with tapping. The fraction retained on the No. 40 sieve was considered coarse fiber, the fraction remaining on the No. 200 sieve was considered fine fiber, and the material that passed through both sieves into the pan was considered mill starch.

The fractions were transferred to beakers with a rubber scraper. Fiber fractions were transferred to a piece of Spectra/Mesh nylon cloth (Fisher Scientific, Pittsburgh, PA) with 52-μm openings and washed by dunking the fiber-containing cloths into a series of three beakers each containing 400 ml of water for 1.0 min per beaker. As much water as possible was squeezed from the cloth into the last beaker after the washing cycle was completed. The fiber was removed from the cloth with a rubber scraper, dried, weighed for yield, and analyzed for moisture and protein contents. The wash water was combined with the mill starch in a 4,000-ml beaker, stirred, and allowed to settle for 24 hr at 4°C.

The last step in milling was starch-protein separation. Most of the wash water was decanted into a 4,000-ml beaker, leaving about 800 ml of mill starch and water. The mill starch slurry was transferred to a 1,000-ml beaker, stirred vigorously, and allowed to settle for 24 hr at 4°C. After 24 hr, as much water

TABLE I
Composition and Properties of Enzymes Used in Steeping Maize

Enzyme	Source	Optimum pH Range	Optimum Temperature Range (°C)	Activity (units/g)
Novo SP249[a]	*Aspergillus niger*	3.5–5.5	40–50	
Polygalacturonase				9,700
Pectinase				2,035
SPS-ase				24
Cellulase				917
β-Glucanase				152
Hemicellulase				288
Arabanase				98
Xylanase				86
Protease				1,120
Experimental				
Cellulase[b]	*Trichoderma viride*	4.0–6.0	50–60	150
Hemicellulase[b]	*A. niger*	3.0–6.0	30–80	25
β-Glucanase[a] (Ceremix 2 × 1)	*Bacillus subtilis*	4.0–7.0	40–55	300
Pectinase[a] (Pectonex 3 × 1)	*A. niger*	3.5–6.0	20–50	3,000
Bromelin[b]	Pineapple	4.0–5.5	45–55	3,000

[a]Purchased from Novo Industri, Wilton, CT.
[b]Purchased from Sigma Chemical Co., St. Louis, MO.

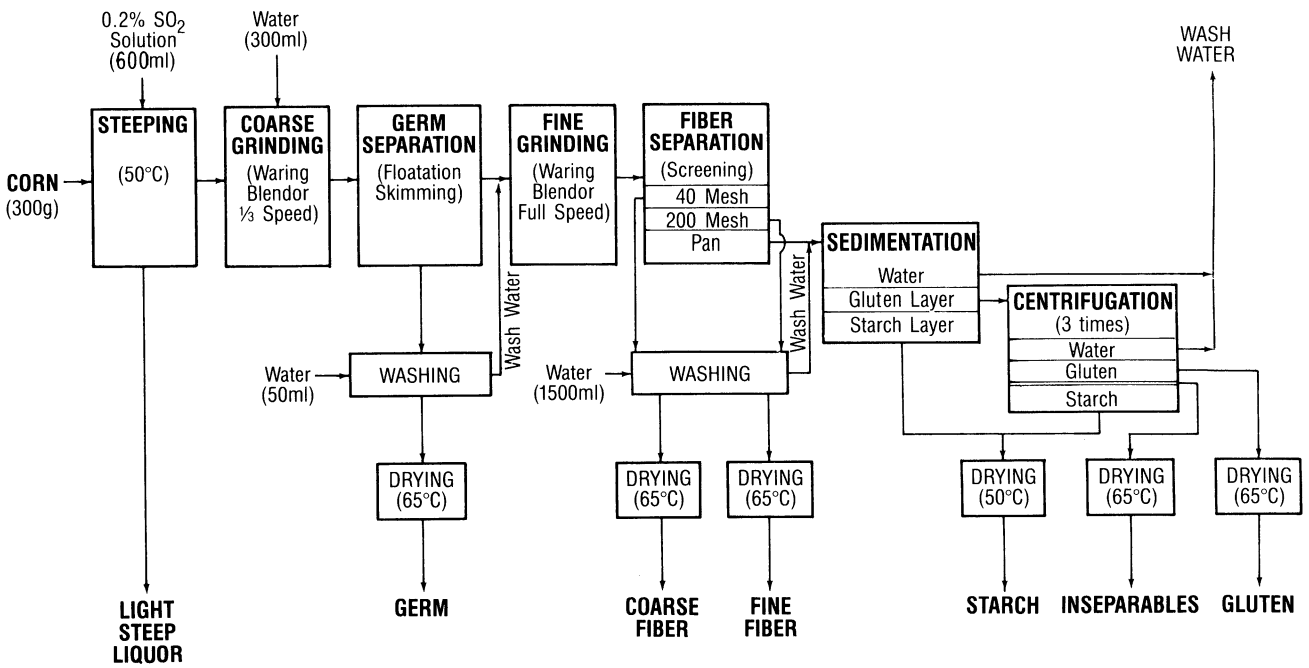

Fig. 1. Flow sheet for laboratory simulation of wet milling.

as possible was decanted into the 4,000-ml wash water beaker. The wash water was analyzed for solids and protein content.

The upper protein layer of the remaining slurry was carefully resuspended, poured off, and rinsed from the starch in the 100-ml beaker. The unpurified gluten fraction was then transferred to 250-ml centrifuge bottles and centrifuged for 30 min at 6,000 × *g* in a Sorvall Superspeed RC2-B refrigerated centrifuge (Newtown, CT). After centrifugation, the water was decanted and the protein layer was carefully scraped off the lower starch layer with a Scoopula. Between the two layers was an off-white layer composed of protein-bound starch. This layer was scraped off and collected as "inseparables." The bottom layer of starch was resuspended in water and combined with the starch in the 1,000-ml beaker separated by gravity in the cold room. The inseparable layer was resuspended, centrifuged, and separated from protein as already described. Two centrifugations were required to achieve clean starch-gluten separations for the control treatment.

Maize from all steeping treatments was milled in the same manner. Each treatment was replicated six times.

Protein Contents and Yields of Wet-Milling Fractions

All fractions except starch were dried for 24 hr in an oven at 65°C, then for 8 hr in a vacuum oven at 65°C. Starch was dried in an oven at 40°C for 48 hr to minimize heat damage to the starch. The moisture contents of maize kernels and dried wet-milled fractions were determined by the vacuum oven method (AACC 1983). The solids contents of spent steeping solution and starch wash water were determined by drying 10-g samples at 65°C for 24 hr and further drying to a constant weight in a vacuum oven at 65°C. Fraction yields were determined on a dry-weight basis.

The nitrogen content of each fraction was determined by the macro-Kjeldahl method with a Kjeltec digester and a distilling system (Tecator, Inc., Hoganas, Sweden). The amount of sample to be analyzed was adjusted to 10–50 ml of titrant (0.5–2.0 g of sample). The factor 6.25 g of protein per gram of nitrogen was used to calculate protein content.

Microscopic Examination of Steeped Kernels

Cross sections of steeped kernels were prepared for microscopic examination as described by Wagoner (1948). Photomicrographs were taken with a 35-mm camera attached to a trinocular microscope. The sections were magnified through 3.5× and 10× objectives, producing total magnifications of 8.75 and 25, respectively (the photographic eyepiece magnified the objective image 2.5 times).

TABLE II
Yields and Protein Contents of Maize Wet-Milling Fractions Produced from Industrial Practice and Laboratory Procedures

Fraction	Industrial Practice[a]	Laboratory Procedures: Watson's Batch Steeping[a]	Laboratory Procedures: Batch Steeping[b] (Present Study)
Germ			
Fraction yield, %	7.5	6.2	6.6 ± 0.4
Protein, %	12.0	23.7	17.6 ± 0.3
Fiber			
Fraction yield, %	11.5	12.5	19.2 ± 1.9
Protein, %	12.0	14.7	11.6 ± 0.8
Starch			
Fraction yield, %	67.5	65.4	58.4 ± 0.7
Protein, %	0.30	0.54	0.56 ± 0.05
Gluten			
Fraction yield, %	5.8	8.1	5.2 ± 0.3
Protein, %	65.8	42.9	56.0 ± 4.2
Squeegee[c]			
Fraction yield, %		1.4	3.7 ± 0.4
Protein, %		20.8	7.5 ± 0.7
Solubles			
Fraction yield, %	7.5	7.1	7.2 ± 1.0

[a] Anderson and Watson (1982).
[b] Mean of six replicates plus or minus one standard deviation.
[c] Equivalent to our "inseparables" fraction. This fraction is not produced in industry.

Separation of Fibrous Layers

Fifty steeped corn kernels were randomly selected from each treatment. The outer bran layer was removed by firmly grasping the kernel between two fingers at the crown, pinching the tip cap with two fingers of the other hand, and slowly pulling away a thin strip of bran. The sample was categorized as "attached" if the seed coat was removed along with the pericarp, or "detached" if the pericarp was removed alone.

Water Absorption and Diffusivity

We compared the rate of water absorption by maize steeped in experimental solutions of multiple enzymes and sulfur dioxide with that of maize steeped in 0.20% sulfur dioxide only. Diffusivity determinations were replicated three times.

Approximately 5 g of steeped corn was removed for moisture analysis every 6.0 min for the first hour, every 0.5 hr thereafter until the sixth hour, and after 7, 8, 9, 10, 12, 16, 20, and 24 hr. The kernels were blotted dry with a tissue to remove surface droplets, predried in an oven for 24 hr at 65°C, and tested for moisture content by the vacuum oven method (AACC 1983).

A Basic computer program model (Hsu 1984) was used to calculate diffusivity constants. Moisture at a given time, maximum absorption, and the average radius of a single kernel were used in the calculation. The average radius was determined from the average volume of three lots of 100 unbroken kernels. The volume of the kernels was determined with a Beckman model 930 air comparison pycnometer (Beckman Instruments, Irvine, CA).

RESULTS AND DISCUSSION

Comparison of Laboratory Wet-Milling Procedures

Wet-milling performance was evaluated on the basis of yields and purities of fractions. High starch yield, low protein content in starch, and high protein content in gluten were considered key indicators of good milling. Wet-milling results from industry, from Watson's laboratory batch steeping-milling procedure (Anderson and Watson 1982), and from our 48-hr control are compared in Table II.

The yield and protein content of the germ fraction from our laboratory simulation of wet milling compared favorably with results achieved in industry and with the laboratory results reported by Anderson and Watson (1982). However, we recovered a higher proportion (19.2%) of the grain solids as fiber than is typical in industrial practice (11.5%) or using Watson's laboratory method (12.5%). We cannot fully explain these differences at this time; the fiber recovered by our procedure appeared relatively free of endosperm. Possibly, the degerminated maize slurry should be ground more extensively before fiber removal.

Our starch yields were about 7–9% lower than those in industrial practice or laboratory simulation. The difference in starch yield between our method and the other two (industry practice and Watson's procedure) was nearly equivalent to the difference in yield of fiber. Our starch protein content (0.56%) was equivalent to that previously reported for laboratory simulation (0.54%). The gluten yield was more typical of industry, and the protein content was intermediate between those of industry and previous laboratory simulation. We also observed more squeegee (inseparables). The amounts of solubles leached into the steeping solution were nearly identical for all three procedures.

Some of the differences in yields and purities of the various wet-milling fractions from our laboratory simulation compared with those of industry and previous laboratory simulation may result from our use of a single variety of maize, whereas previous reports are for data on mill-run maize of unknown and mixed genetic and environmental backgrounds. The procedure used in this study reproduced relatively well, as evidenced by relatively low standard deviations (Table II), despite considerable subjectivity in some of the separations (e.g., protein-starch). We believe

our wet-milling procedure served well as the basis for comparing the effects of enzyme treatment with standard wet-milling practices.

Effect of Multiple Enzymes on Wet Milling

We encountered no difficulties in wet milling with maize steeped for 48 hr; however, evidence of incomplete steeping was discernible during the wet milling of maize steeped for 24 hr. Bran and pieces of endosperm remained attached to the germ, and many small pieces of horny endosperm remained in fiber fractions. Yields and protein contents of milling fractions recovered from maize steeped for 24 hr were significantly inferior to those for maize steeped for 48 hr (Table III). Most noticeable were higher fiber yield, lower starch yield, higher protein content in the starch, lower protein content in gluten, higher yield of inseparables, and higher protein content in the inseparables from maize steeped for 24 hr. We attribute these differences to incomplete reduction of the protein matrix by sulfur dioxide and consequently incomplete separation. These observations are consistent with industry claims that reducing the steeping period to less than 48 hr adversely affects wet-milling characteristics.

The yields and protein contents of most fractions recovered from maize steeped for 24 hr with either treatment of multiple enzymes and sulfur dioxide were similar to those of maize steeped with 0.20% sulfur dioxide alone for 48 hr and were significantly superior ($P > 95\%$) to those from maize steeped for only 24 hr with 0.20% sulfur dioxide alone (Table III). The solution containing the experimental multiple-enzyme preparation and sulfur dioxide gave better results than the solution containing the commercial multiple-enzyme preparation and sulfur dioxide solution in these laboratory trials. The yields and protein contents of the germs were not significantly different for the two multiple-enzyme treatments or the 48-hr control. The 24-hr treatment with the experimental solution of multiple enzymes and sulfur dioxide gave

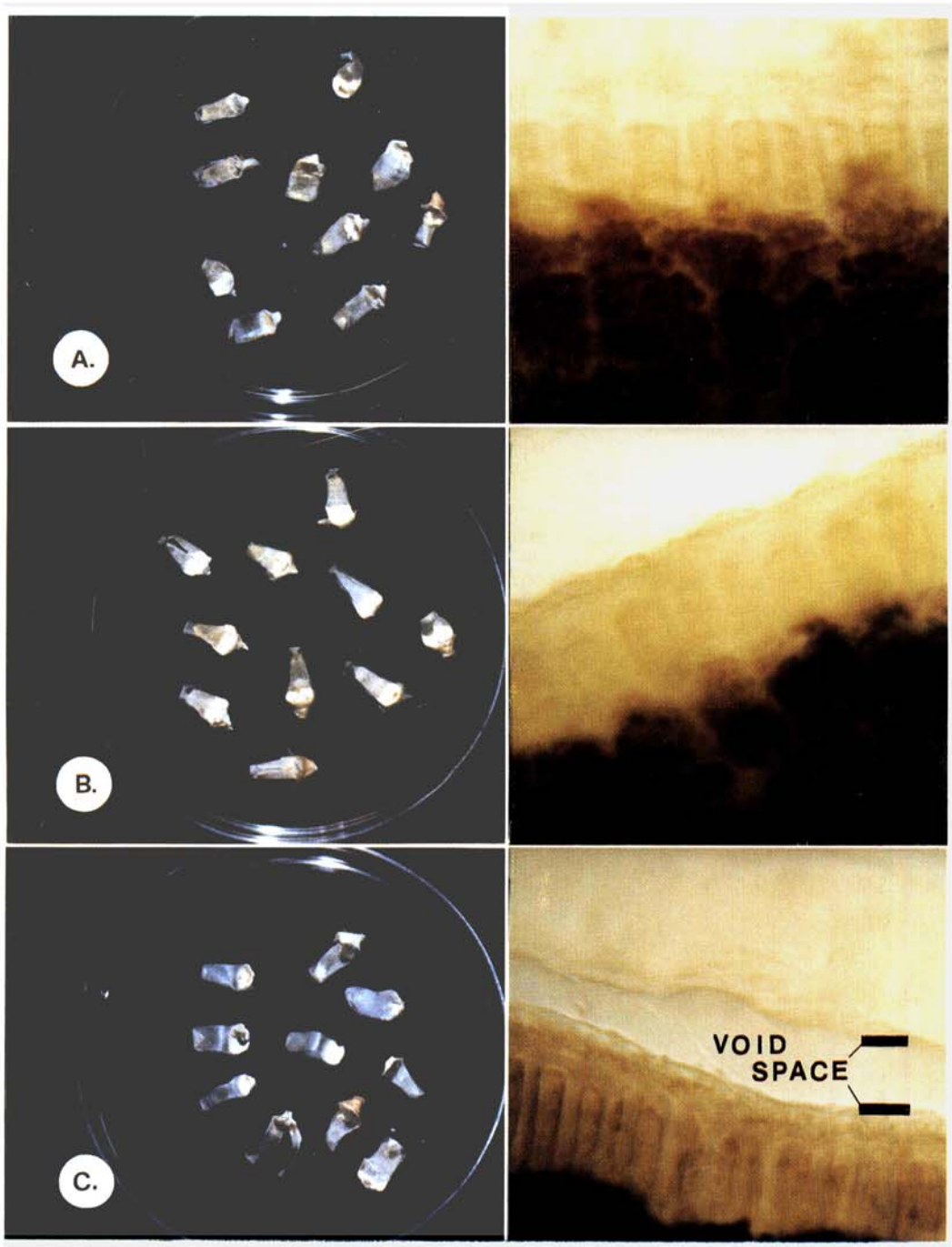

Fig. 2. Bran strips (left) and micrographs (right) of cross sections of kernels of control maize steeped for 48 hr **(A),** control maize steeped for 24 hr **(B),** and maize steeped in a solution of an experimental multiple-enzyme preparation and sulfur dioxide for 24 hr **(C).**

significantly lower fiber yield (17.6%) than either the 24-hr treatment with the solution of the commercial multiple-enzyme preparation and sulfur dioxide (20.1%) or the 48-hr control (19.2%). Starch yield and residual protein content of starch were also superior in the treatment with the solution of the experimental multiple-enzyme preparation and sulfur dioxide (57.7% and 0.52%, respectively) than those in the treatment with the solution of the commercial multiple-enzyme preparation and sulfur dioxide (54.9% and 0. 63%, respectively). Gluten yield was slightly higher in the experimental treatment (5.9%) than in either the 48-hr control (5.2%) or the commercial treatment (5.4%). Protein contents of gluten were not significantly different for any of the treatments except the 24-hr control, which was lower. We believe that the addition of multiple enzymes to the steeping solution improves the wet-milling performance of maize that has been steeped for less than the normal 48 hr by enhancing starch-fiber and starch-gluten separation.

None of the previously described indications of incomplete steeping were observed in either 24-hr treatment of multiple enzymes and sulfur dioxide solution. However, two obvious differences between the enzyme and nonenzyme treatments were noted. A petroleum-like odor, similar to that of butyl alcohol, emanated from the exit steeping solution (light steep liquor) from the treatments with solutions of multiple enzymes and sulfur dioxide but not from the control treatments. We did not attempt to identify the cause of this odor. Enzyme treatment also produced gray-black colors on the surfaces of the kernels, especially near the tip cap. These color changes were confined to the bran and did not carry through to the starch fractions.

Microscopic Examination of Steeped Kernels

The gray-black appearance of kernels steeped in the presence of enzymes and sulfur dioxide was attributed to enzymatic hydrolysis of one or more of the fibrous layers. Cross sections of several kernels from each treatment were examined for differences (Fig. 2). A void space was observed between the external layers of kernels steeped in solutions of multiple enzymes and sulfur dioxide (Fig. 2C) which interrupted the transmission of light to the yellow endosperm. The void space created in the pericarp of the kernel shown in Figure 2C was found near the cross-cell layer, which is one of the cellulosic layers most susceptible to carbohydrases. The void space was observed in most of the kernels steeped with enzymes, whereas the pericarps of the 48-hr and 24-hr control kernels were intact (Fig. 2).

TABLE III
Yields and Protein Contents of Wet-Milling Fractions from Batch-Steeped Maize

Fraction	48-Hr Steeping Control[a]	24-Hr Steeping[a]		
		Control	Commercial Multiple Enzymes	Experimental Multiple Enzymes
Germ				
Fraction yield, %	6.62 a	7.31 b	6.71 a	6.67 a
Protein, %	17.6 a	18.5 b	18.6 b	17.4 b
Total fiber				
Fraction yield, %	19.20 ab	25.74 c	20.12 a	17.63 b
Protein, %	11.6 a	13.1 b	12.8 b	11.8 a
Starch				
Fraction yield, %	58.44 a	48.73 b	54.87 c	57.72 a
Protein, %	0.56 ab	1.20 c	0.63 a	0.52 b
Gluten				
Fraction yield, %	5.16 a	5.92 b	5.41 a	5.85 b
Protein, %	56.0 a	42.8 b	56.8 a	57.4 a
Inseparables				
Fraction yield, %	3.73 a	7.07 b	5.33 c	4.51 a
Protein, %	7.5 a	10.3 b	8.6 a	6.1 c
Solubles[b]				
Fraction yield, %	7.22 a	5.88 b	7.62 a	7.55 a

[a]Values within a row followed by the same letter do not differ significantly at the 0.05 level according to Duncan's multiple range test.
[b]Composite of starch wash water and steeping solution.

Separation of Bran Layers

The bran was hand-dissected to confirm the action of the carbohydrases in the treatments with solutions of multiple enzymes and sulfur dioxide by observing how the bran peeled off the kernel. Of 50 kernels tested from each treatment, 60% of the kernels steeped for 24 hr in the solution of the experimental multiple-enzyme preparation and sulfur dioxide, 10% of the 48-hr control kernels, and none of the kernels from the 24-hr control had detached pericarps (i.e., the pericarp was removed alone and was transparent, as opposed to attached pericarps, which were opaque and where the seed coat, aleurone layer, and in some instances a portion of the endosperm were removed along with the pericarp). Ten representative samples from each treatment are shown in Figure 2.

Water Absorption and Diffusivity

No differences in absorption and diffusion of water into the kernel due to enzyme treatment were observed. The diffusivity constants were 4.913×10^{-3} cm^2/hr for maize steeped with the solution of the experimental multiple-enzyme preparation and sulfur dioxide versus 4.914×10^{-3} cm^2/hr for maize treated only with sulfur dioxide.

CONCLUSIONS

Incorporation of multiple enzymes into the steeping solution for batchwise steeping of maize enhanced separation of fractions in wet milling, especially when steeping time was less than the normal 48 hr. Reducing the steeping time by adding multiple enzymes to the sulfur dioxide solution seems to be technically feasible. However, the levels of enzyme activity used in these experiments probably exceed economically viable levels, and additional work is needed to ascertain the practical value of this approach to reducing steeping time.

The mechanism by which these enzymes enhanced starch recovery was not fully determined and also requires further investigation. Enzymes did not improve water diffusivity, but bran was separated more easily and completely after steeping in the presence of the enzymes used in this study. It is possible that the enzymes were not completely drained from steeped maize and were carried downstream into milling steps and were functional there. The feasibility of using enzymes in a laboratory countercurrent steeping system that more closely simulates industrial steeping practices, as well as the functional properties of the starch, are the subjects of a companion paper (Steinke et al 1991).

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., 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.

GILLENWATER, D. L., PFUNDSTEIN, G. B., and HARVEY, A. R. 1971. Edible starches and starch-derived syrups. U.S. patent 3,597,274.

GRINDEL, R. S. 1965. A comparison of a continuous steeping device to a conventional device. Starch/Stärke 17:298.

HASSANEAN, A., and ABDEL-WAHED, A. 1986. A new method to short the steeping period of corn grains. Starch/Stärke 38:417.

HSU, K. H. 1984. A theoretical approach to the tempering of grains. Cereal Chem. 61:466.

KERR, R. W. 1950. Chemistry and Industry of Starch. Academic Press: New York.

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.

MASLENNIKOVA, T. N. 1970. Zur Frage der Verwendung von Natriumdisulfit statt schwefliger Säure bei der Maisstärkeproduktion. Starch/Stärke 22:270.

MEUSER, F., GERMAN, H., and HUSTER, H. 1985. The use of high-pressure disintegration technique for the extraction of starch from corn. Pages 161-180 in: New Approaches to Research on Cereal Carbohydrates. R. D. Hill and L. Munck, eds. Elsevier: Amsterdam.

NERYNG, A., and REILLY, P. J. 1984. Laboratory wet milling of ensiled corn kernels. Cereal Chem. 61:8.

ROUSHDI, M., GHALI, Y., and HASSANEAN, A. 1979. Factors improving the steeping process of corn grain. Part I. Effect of steeping process, artificial drying, scratching, and storage. Starch/Stärke 31:78.

ROUSHDI, M., GHALI, Y., and HASSANEAN, A. 1981a. Factors improving the steeping process of corn grains. Part II. Effect of enzyme addition. Starch/Stärke 33:7.

ROUSHDI, M., FAHMY, A. A., and MOSTAFA, M. 1981b. Role of lactic acid in corn steeping and its relation with starch isolation. Starch/Stärke 33:426.

SPANHEIMER, J., FREEMAN, J. E., HEADY, R. E., and HEADLEY, V. E. 1972. Air classification of corn grits. I. Softening grits with enzymes and chemicals. Cereal Chem. 49:131.

STEINKE, J. D., JOHNSON, L. A., and WANG, C. 1991. Steeping maize in the presence of multiple enzymes. II. Continuous counter-current steeping. Cereal Chem. 68:12-17.

VAN TWISK, V. P., and TEGGE, G. 1968. Enzymatic degradation of starch in dry-degermed maize. Starch/Stärke 20:319.

VOJNOVICH, C., ANDERSON, R. A., and GRIFFIN, E. L., Jr. 1960. A note on the enzymatic reduction of protein in high-amylose corn starch. Cereal Chem. 37:576.

WAGONER, J. A. 1948. Microscopical observations and procedures used in a study of industrial corn steeping. Cereal Chem. 25:354.

WAHL, G. 1970. Biochemical-technological studies on wet processing of maize. Part III. Fermentation during the maize steeping process. Starch/Stärke 22:77.

WAHL, G. 1971. Biochemical-technological studies on wet processing of maize. Part 6. Examination of the proteolytic and lipolytic enzyme system of maize. Starch/Stärke 23:145.

WALL, J. S., and PAULIS, J. W. 1978. Corn and sorghum grain proteins. Adv. Cereal Sci. Technol. 2:135.

WATSON, S. A., HIRATA, Y., and WILLIAMS, C. B. 1955. A study of the lactic acid fermentation in commercial corn steeping. Cereal Chem. 32:382.

WOLF, M. J., and KHOO, U. 1975. Subcellular distribution and enzyme digestibility of endosperm proteins of amylomaize and normal corn. Cereal Chem. 52:771.

WOLF, M. J., BUZAN, C. L., MacMASTERS, M. M., and RIST, C. E. 1952. Structure of the mature corn kernel. Part II. Microscopic structure of pericarp, seed coat, and hilar layer of dent corn. Cereal Chem. 29:334.

WOOD, T. M., and McRAE, S. I. 1978. Synergism between enzymes involved in the solubilization of native cellulose. Pages 181-209 in: Hydrolysis of Cellulose. R. D. Brown, Jr. and L. Jurasek, eds. American Chemical Society: Washington, DC.

[Received July 31, 1989. Revision received July 5, 1990. Accepted July 8, 1990.]