

# Effect of a Cell-Wall-Degrading Enzyme Complex on Starch Recovery and Steeping Requirements of Sorghum and Maize

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## ABSTRACT

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The effect of a commercial cell-wall-degrading enzyme (CWDE) complex on the steeping time and starch yields of white regular sorghum (RSOR) compared with yellow maize (YMZ) was determined. An *in vitro* wet-milling method standardized to test dosages of 0–120 fungal  $\beta$ -glucanase units (FBG)/100 mL indicated that starch yields were significantly higher for YMZ than RSOR and increased proportionally as enzyme dosage increased. A factorial experiment with a level of confidence of  $P < 0.05$  was performed to study the effect of CWDE addition to coarsely ground grains for 4 hr after 20 or 44 hr of SO<sub>2</sub> steeping of whole grains. At both regular steep times, YMZ yielded significantly higher amounts of starch than RSOR. When steep times were compared, grains soaked for 48 hr produced 1.7% higher starch yields than counter-

parts treated for 24 hr. CWDE significantly increased starch yields and recoveries. Enzyme-treated grains yielded 2.5% more starch than counterparts steeped regularly. For both grains, the best wet-milling conditions to obtain the highest amount of starch were 48 hr of steeping and CWDE addition. Under these conditions, YMZ and RSOR yielded 66.9 and 66.6% starch, respectively. Starches obtained after the enzyme treatment at both steep times contained higher amounts of residual protein and ash compared with the untreated counterparts. Rapid viscoamylograph properties of YMZ and RSOR starches were not affected by the use of the CWDE nor the steep time. In comparison with RSOR starch, the YMZ starch initiated gelatinization at lower temperature, had less shear thinning and higher viscosity or setback at the end of cooling.

One of the main industrial uses of maize is for starch production. The wet-milling industry has grown because starch is bioenzymatically converted into high-fructose corn syrups (HFCS) and others used in soft drinks. Since the 40s, sorghum has been seen as a possible alternative to maize for starch (Watson et al 1951, 1955; Watson and Hirata 1955). Sorghum presents several disadvantages for wet milling such as the presence of peripheral endosperm which acts as a barrier against the penetration of the steep solution, a harder protein matrix and cross-linking which engulfs starch granules, thus lowering yields and starch quality. The leaching of phenolic pigments present in the pericarp, testa, and aleurone tissues produce off-colored starch (Rooney and Serna Saldívar 2000). Several researchers have found that sorghum generally yields  $\approx 10\%$  less prime starch than maize (Watson et al 1951, 1955; Norris and Rooney 1970; Watson 1984; Caransa and Bakker 1987). Once separated, sorghum starch can be used interchangeably with maize starch because both starches have almost identical viscoamylograph characteristics (Watson 1984; Moheno-Perez et al 1999).

In Mexico, sorghum is second to maize in terms of area planted and production, with an estimated production in 2001 of 5.5 million MT (FAO <http://apps.fao.org/>). Currently, the relative international cost of sorghum is  $\approx 90\%$  the price of maize. However, in Mexico, the differential has been higher because sorghum is used as animal feed, whereas maize is channeled directly for human foods. In 2001, the domestic cost of maize was  $\approx 20\%$  higher than sorghum (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación Centro de Estadística Agropecuaria. [www.sagar.gob.mx](http://www.sagar.gob.mx)). To fulfill demands for maize, during 2001 Mexico imported 5.54 million MT, mainly from the United States, with an estimated value of 0.5 billion dollars. Therefore, the utilization of sorghum starch and syrup production can lower maize importation and production costs. Pioneer research performed by Watson and Hirata (1955) and Watson et al (1955), in which sorghum was wet-milled in a

manner similar to that of maize, the authors stated that the major difference is the difficulty of separating sorghum starch and gluten. In addition, sorghum pericarp is more fragile than the maize pericarp, thus small pericarp particles impede the clear separation of the starch and protein and give the starch an off-color. Norris and Rooney (1970) studied the wet-milling properties of sorghum and found that the genotype influenced starch recoveries and quality. Peripheral endosperm content was significantly positively correlated with protein content of the starch and negatively related to starch yield and recovery. Steinke and Johnson (1991), Steinke et al (1991), and Johnston and Singh (2001) demonstrated that the use of proteolytic and cell-wall-degrading enzymes (CWDE) during wet-milling of maize significantly lowered SO<sub>2</sub> steeping requirements while maintaining similar starch yields. Recently, Johnston and Singh (2001) effectively used proteases to reduce steep time and SO<sub>2</sub> requirements in a two-stage wet-milling process. Wang et al (2000) tested a multiple enzyme cocktail with pectolytic, cellulolytic, hemicellulolytic, and proteolytic activities for wet-milling of sorghum and found that the enzymes slightly increased starch yield and produced a more refined starch when compared with grains steeped with SO<sub>2</sub>. However, the best wet-milling conditions were achieved with the use of SO<sub>2</sub> and lactic acid.

In a previous experiment, Moheno-Perez et al (1999) found that maize yielded amounts of starch similar to that of waxy sorghum, and both grains produced higher amounts of starch than regular or heterowaxy sorghums. The addition of a fiber-degrading-enzyme complex to whole grains did not improve the wet-milling process, with the exception of regular sorghum, where the CWDE reduced steep time while producing the same starch recovery.

The objectives of this experiment were to compare yields, composition, and viscoamylograph properties of starches extracted from regular sorghum (RSOR) with regular yellow maize (YMZ) and to determine whether the addition of commercial CWDE to coarsely ground grains increased starch yields or decreased steep time.

## MATERIALS AND METHODS

### Grain Utilized

A commercial YMZ with a relatively soft endosperm was used as the control treatment. The experimental white RSOR (ATX 631\*TX436), classed as Type 1, had an intermediate endosperm texture and regular endosperm. Grains were tested for test weight, 1,000 kernel weight (TKW) and density. Test weight (kg/hL) was measured with a Winchester bushel meter (Seedburo Equipment,

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Chicago, IL) according to Official U.S. Grain Standard Procedures. TKW was determined by weighing 100 randomly selected whole kernels; grain density was determined using an air comparison pycnometer (model 930, Beckman Instruments, Fullerton, CA) after weighing grain samples and determining volume by nitrogen displacement.

### CWDE Complex

A commercial CWDE complex (Viscozyme, Novo Nordisk, Princeton, NJ) with cellulolytic, hemicellulolytic, xylanase,  $\beta$ -glucanase, arabinase, and pectinase activities, and without proteolytic or amylolytic activities was utilized. The enzyme preparation was selected according to substrate specificity, pH level, and temperature of maximum activity and had an activity of 60 FBG/mL at 30°C as determined with the Somogyi Nelson test. The complex had a declared activity of 120 FBG/mL (1 FBG = 1  $\mu$ mol of glucose) or other reducing sugars liberated from 0.5% (w/v) barley  $\beta$ -glucan/min (Sigma, St. Louis, MO) at 30°C and pH 7.5.

### FBG Activity

The modified Somogyi Nelson method (Novo Nordisk) was used to determine FBG activity. The method uses barley  $\beta$ -glucans (0.5%, w/v) (Sigma) as substrate.  $\beta$ -Glucans are hydrolyzed by the enzymes yielding free glucose that is quantified by the Somogyi Nelson reagents. A glucose standard curve was built with solutions containing 0–0.2  $\mu$ g/ $\mu$ L.

### Steepwater Uptake

The steepwater uptake of 500-g samples of YMZ and RSOR throughout the 48-hr steeping at 50°C was determined by analyzing the moisture content (Approved Method 44-18, AACC 2000) of representative grain samples. Before moisture determination, the steeped grains were blotted between paper towels to remove surface steepwater. The steep solution was prepared by dissolving 1.48 g of sodium bisulfite (Productos Químicos Monterrey, Monterrey, Mexico), equivalent to 910 ppm of SO<sub>2</sub>, and 4.7 mL of 85% lactic acid (Mallinckrodt Baker, Paris, KY) in 1L of water.

### In Vitro Wet-Milling Studies

A bifactorial in vitro wet-milling study was designed to test the effect of grain type (YMZ or RSOR) and CWDE dose (0, 80, 90, 100, or 120 FBG) on starch yield. Grain (500 g) was steeped in 1L of 0.142% (w/v) (873 ppm of SO<sub>2</sub>) sodium bisulfite (Productos Químicos) and 0.4% (v/v) 85% lactic acid (Mallinckrodt Baker) solution at 50°C  $\pm$  1 for 44 hr. The steepwater was pH 4.9  $\pm$  0.1. After steeping, the solution was discarded and the grain was separated in lots of 200 mL. Each lot was coarsely ground for 2 min with 250 mL of distilled water in a Waring laboratory blender (model 31BL91, Cole Parmer Instruments, Vernon Hills, IL) using 50% of current intensity at a low velocity setting. The volume and pH level were determined in the ground sample. The slurry was homogenized and separated in volumes of 100 mL each. Dosages (0, 80, 90, 100, and 120 FBG) of CWDE were added to these volumes and incubated for 4 hr at 50°C  $\pm$  1. After incubation, enzyme activity was inactivated by freezing the aliquots at –20°C. All trials were made in triplicate. Each aliquot was then finely ground in the same laboratory blender at a high velocity setting (using 100% of total intensity of current) for 2 min. Coarse and fine fiber were separated using U.S. 40 and 100 sieves, respectively. The remaining starch slurry was left to sediment at 4°C for 24 hr. The water was then decanted and the remaining fraction transferred to 500 mL (69  $\times$  160 mm) polypropylene bottles (Beckman) for centrifugation (Beckman Avanti J-25I) at 4°C and 10,000 rpm (17,696  $\times$  g). The remaining water was decanted and the starch-gluten resuspended in distilled water and centrifuged again under the same conditions. After decanting the remaining water, the starch, gluten and inseparables were left to dry at room temperature and then the gluten and inseparables were removed

and discarded. The remaining starch was dried at 50°C for 12 hr in an air-forced oven and weighed. The starch dry solids content was determined by the gravimetric method (Approved Method 44-15A, AACC 2000).

### Laboratory Wet-Milling Studies

A trifactorial experiment with a complete randomized block design was used. The factors or levels studied were type of grain (YMZ or RSOR), steeping time (24 or 48 hr) and CWDE treatment (with and without). Thus, a total of eight different treatments (2 $\times$ 2 $\times$ 2) were compared. The experiment was replicated three times.

The wet-milling procedure was designed based on previous research (Watson et al 1951; Watson and Hirata 1955; Eckhoff and Tso 1991a,b; Eckhoff et al 1993) with a major modification of the procedure described by Moheno-Perez et al (1999). The CWDE complex was added for 4 hr to coarsely ground grains steeped for either 20 or 44 hr. Therefore, the total steep times were 24 and 48 hr. Batches of 500 g were steeped in 1L of steep solution at 50°C. The control steep solution was prepared by dissolving 1.48 g of sodium bisulfite (874 ppm of SO<sub>2</sub>) and 4.7 mL of 85% lactic acid in 1L of water. The initial steepwater was pH 4.9  $\pm$  0.1. Before enzyme addition, grains steeped for 20 or 44 hr were first coarsely ground at a low speed setting in a blender equipped with a speed regulator or autotransformer (Staco Energy Products, Dayton, OH) regulated to apply 50% of the current. Then, 300 FBG units were added to the coarsely ground grains and incubated at 50°C for 4 hr. The resulting material was finely ground in the same blender but at the highest speed setting for 2 min. The fiber-germ fraction was separated by first sieving and washing through a U.S. No. 40 mesh (coarse fiber) and then in a No. 100 mesh sieve (fine fiber). The starch was separated from the gluten by running the aqueous suspension over a stainless steel separation table (10 cm wide, 300 cm long, and 5 cm high) positioned at an angle of 0.7° (3.5 cm height and 300 cm long). Before tabling, the solids were kept in suspension by stirring at 100 rpm. The suspension was delivered to the gravity table using a peristaltic pump (Masterflex model 7518-10) delivering 125 mL/min. The solids retained on the first 270 cm of the table were considered prime starch, whereas those retained on the last 30 cm were starch associated with gluten (inseparables). The gluten fraction was collected, allowed to sediment for 5 hr, and then recovered by vacuum filtration through a Buchner filter equipped with Whatman No. 1 filter paper. Starch was dried at 50°C for 12 hr, while coarse and fine fiber, inseparables, and gluten at 60°C for 24 hr. The partially dried fractions were left on the counter for 6 hr to equilibrate and moisture content determined in an convection oven (Approved Method 44-15A, AACC 2000). Starch yield = starch wt (db)/grain wt (db)  $\times$  100 and starch recovery = starch wt (db)/grain starch wt (db)  $\times$  100.

### Grain and Starch Analyses

Grains were characterized for proximate composition according to Approved Methods 44-15A, 08-01, 46-13, 30-20, and 32-10 (AACC 2000) and starch assayed by an enzymatic procedure (Technicon Auto Analyzer II, Clinical Method No. SF4-0046) in which the polymer was hydrolyzed with amyloglucosidase (Sigma). The resulting glucose was colorimetrically quantified in an automated system. Refined starches were tested for protein and ash (Approved Methods 46-13 and 08-01, AACC 2000) and viscosity properties were determined with a Rapid Visco Analyser (RVA) (model 1170, Newport Scientific, Warriewood NSW, Australia) using a starch suspension with 10% solids. The heating profile was hold at 30°C for 2 min, heat to 90°C at 15°C/min (heating cycle), hold at 90°C for 4 min (high temperature hold), cool to 30°C at 15°C/min (cooling cycle) and hold at 30°C for 4 min (low temperature hold). Peak pasting viscosity, peak time, shear thinning (difference between peak viscosity and minimum viscosity during heating), viscosity at the beginning and end of cooling, and setback

(difference between final and minimum viscosities) during cooling were determined for each starch sample.

### Statistical Analysis

Data of in vitro and laboratory wet-milling studies was analyzed with analysis of variance (ANOVA) procedures using bifactorial and trifactorial experimental designs, respectively. For in vitro wet-milling, mean comparisons of individual (YMZ or RSOR, with or without enzyme addition) and mixed effects were run. Likewise, for laboratory wet-milling, mean comparisons of individual (YMZ or RSOR, 24 or 48 hr, with or without CWDE) and mixed effects were performed. Means of starch yield and recovery, starch protein and ash contents, by-product yields and RVA properties were compared using least significant difference ( $P < 0.05$ ) (SAS Institute, Cary, NC).

## RESULTS AND DISCUSSION

### Grain Composition

The RSOR kernels had higher test weight and density than YMZ (Table I). The maize utilized was the typical yellow dent

TABLE I

Physical Properties and Chemical Composition of Maize and Sorghum<sup>a</sup>

	YMZ	RSOR
Physical properties		
Test weight (kg/hL)	71.9	75.9
1,000 kernel wt (g)	312	32
Density (g/cm <sup>3</sup> )	1.26	1.36
Chemical composition <sup>b</sup>		
Moisture (%)	9.8	13.3
Protein (N × 6.25, %)	9.29	11.40
Ether extract (%)	4.31	3.13
Ash (%)	1.41	2.52
Crude fiber (%)	2.50	2.68
NFE (%)	82.49	80.27
Starch (%)	73.49	73.27

<sup>a</sup> YMZ = yellow maize, RSOR = regular sorghum. Each value is the average of at least two observations.

<sup>b</sup> Expressed on dry matter basis. NFE = nitrogen free extract calculated by difference (100 – protein – ether extract – ash – crude fiber).

TABLE II

Effect of Different Concentrations of Cell-Wall-Degrading Enzymes on In Vitro Starch Yields of Coarsely Ground Maize and Sorghum<sup>a,b</sup>

Grain	Treatment	
	Enzymatic Dose (FBG units) <sup>c</sup>	Starch Yield (%)
YMZ	0	44.10d
YMZ	80	44.13d
YMZ	90	44.52c
YMZ	100	44.65b
YMZ	120	44.70a
RSOR	0	29.21h
RSOR	80	29.29g
RSOR	90	29.33g
RSOR	100	29.75f
RSOR	120	31.02e
Grain type		
YMZ		44.42b
RSOR		29.52a
Enzyme Concentration (FBG units) <sup>c</sup>		
0		36.66a
80		36.71a
90		36.93b
100		37.20c
120		37.86d

<sup>a</sup> YMZ, yellow maize; RSOR, regular sorghum. Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

<sup>b</sup> Grain-enzyme concentration, grain type, and enzyme concentration values represent means of 2, 15, and 6 replicates, respectively.

<sup>c</sup> Fungal  $\beta$ -glucanase.

generally planted in the U.S. corn belt. Physical properties of YMZ were within that recommended for the wet-milling industry, although the test weight and density were slightly higher than the optimum (69.8 kg/hL and 1.26 g/cm<sup>3</sup>) (Watson 1984). The proximate composition of maize and sorghum was within values cited in the literature (Watson 1984; Serna Saldivar and Rooney 1995; Rooney and Serna Saldivar 2000). The regular sorghum grain had  $\approx 1\%$  more protein than maize but the starch content was similar because YMZ contained  $\approx 1.2\%$  more ether extract (oil) than RSOR.

### Wet-Milling

In in vitro wet-milling, the enzyme preparation had an activity of 60 FBG/mL at 30°C as determined with the Somogyi-Nelson test instead of the 120 FBG units declared by the supplier. YMZ yielded  $\approx 15\%$  more starch than RSOR (Table II). These results agree with previous investigations (Watson et al 1955; Watson 1984; Caransa and Bakker 1987; Moheno-Perez et al 1999). RSOR yielded less prime starch because it contains peripheral endosperm layers that delay steep liquor diffusion into the inner starchy endosperm and starch granules are more tightly covered by the protein matrix (Sullins and Rooney 1974). Zift et al (1950) found that the maximum sorghum starch recovery was obtained when the steep solution contained higher concentrations of SO<sub>2</sub> (0.25%) than the one utilized in this study (0.148%). Wang et al (2000) found no significant differences in sorghum starch yield when grains were steeped with 0.2 or 0.3% SO<sub>2</sub> and lactic acid. Dailey (2002) suggested that, at increasing lactic acid concentrations, more protein from the endosperm matrix is solubilized and higher amounts of starch are recovered. Addition of increasing amounts of CWDE significantly increased starch yields in both types of grains. However, in vitro starch recovery only increased 0.6 and 1.7% from maize and sorghum, respectively, treated with 120 FBG units of CWDE. This data shows that CWDE had a larger positive effect in RSOR than in YMZ.

In laboratory wet-milling, maize had a higher and faster SO<sub>2</sub> steepwater uptake than sorghum (Fig. 1). Most of the steep solution was absorbed during the first 10 hr. The maximum moisture content was achieved after 44 hr, and stayed practically the same until the end of steeping at 48 hr. At the end of steeping YMZ and RSOR contained 42.4 and 35.2% moisture, respectively. Sorghum absorbed less steep solution despite its lower TWK ( $\approx 1/10$  that of maize) or grain size. The lower uptake might be due to the waxy layer that covers the sorghum epicarp and the peripheral endosperm or subaleurone layer present in regular sorghums (Watson et al 1955; Sullins and Rooney 1974; Serna Saldivar and Rooney 1997; Rooney and Serna Saldivar 2000) that retarded SO<sub>2</sub> solution diffusion into the inner part of the endo-

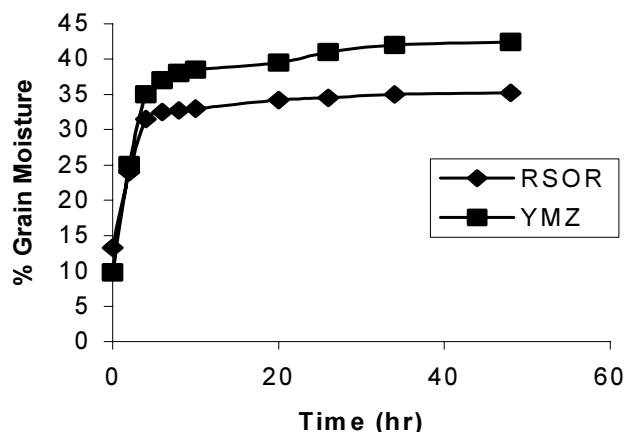


Fig. 1. SO<sub>2</sub> steep solution uptake on maize (YMZ) and sorghum (RSOR) kernels soaked for 48 hr at 50°C.

sperm. In addition, RSOR had a harder endosperm texture and higher test weight and density than YMZ (Table I).

Statistical analyses indicated that there were significant differences among the three variables studied (grain source, steep time, and steep treatment) (Table III). As expected, RSOR yielded  $\approx 2.5\%$  less starch than YMZ. These results agree with previous investigations (Watson 1984; Caransa and Bakker 1987; Moheno-Perez et al 1999). According to Mu-Forster and Wasserman (1998), starch granules from maize contain groups of polypeptides that are tightly associated with the starch matrix and zeins contain  $\approx 50\%$  of the granule associated proteins. These proteins are composed of two distinct classes, the surface localized zeins of 10–27 kDa and the granule intrinsic proteins of  $\geq 32$  kDa. Watson et al (1955) mentioned that sorghum yields less starch than maize because the starch granules are more tightly covered by the protein matrix, making the starch-gluten separation more difficult. Wall and Paulis (1978) determined that endosperm protein matrix and bodies, rich

in glutelins and prolamins, had higher amounts of disulfide bonds, more cross-linking, and thus are more difficult to extract than the corresponding proteins of maize. The higher cross-linking or tougher internal structure is the most logical explanation for the reduced starch yields observed for RSOR. An increase in  $\text{SO}_2$  or lactic acid concentration or the utilization of proteolytic enzymes could improve the sorghum starch refining process (Roushdi et al 1981; Steinke and Johnson 1991; Steinke et al 1991; Ling and Jackson 1991; Wang et al 2000). Starch yields obtained from sorghum in this study are similar or higher than those obtained earlier (Watson and Hirata 1955; Watson 1984; Kulkarni et al 1987; Wankhade et al 1989).

When steep times were compared, grains soaked for 48 hr showed higher starch yields and recoveries than counterparts treated for 24 hr. Grains steeped for 48 hr yielded 1.7% more starch than counterparts steeped for 24 hr. Results showed that the commercial CWDE complex significantly increased starch yields

**TABLE III**  
Effect of Grain Type, Steep Time, and Cell-Wall-Degrading Enzyme Treatment on Starch Yields and Composition<sup>a</sup>

Grain	Treatment		Starch Yield (%)	Starch Recovery (%)	Starch Composition		Co-Product Yield <sup>c</sup>			
	Time (hr) <sup>b</sup>	Enzyme			Protein	Ash	Germ Fiber	Gluten	Inseparables	Total
YMZ	24	W/O	63.6 <sup>cd</sup>	86.5 <sup>b</sup>	0.30 <sup>d</sup>	0.05 <sup>f</sup>	12.3 <sup>b</sup>	14.6 <sup>e</sup>	2.6 <sup>c</sup>	93.1
YMZ	48	W/O	64.5 <sup>c</sup>	87.7 <sup>b</sup>	0.28 <sup>e</sup>	0.05 <sup>g</sup>	11.4 <sup>c</sup>	13.7 <sup>f</sup>	2.2 <sup>cd</sup>	91.8
RSOR	24	W/O	61.4 <sup>d</sup>	83.8 <sup>c</sup>	0.31 <sup>d</sup>	0.15 <sup>b</sup>	13.4 <sup>a</sup>	16.8 <sup>a</sup>	5.0 <sup>a</sup>	96.6
RSOR	48	W/O	63.3 <sup>c</sup>	86.3 <sup>b</sup>	0.30 <sup>d</sup>	0.13 <sup>c</sup>	10.4 <sup>d</sup>	15.7 <sup>c</sup>	3.4 <sup>b</sup>	92.8
YMZ	24	CWDE	64.7 <sup>bc</sup>	88.1 <sup>b</sup>	0.35 <sup>b</sup>	0.08 <sup>d</sup>	10.5 <sup>d</sup>	14.7 <sup>e</sup>	2.0 <sup>de</sup>	91.9
YMZ	48	CWDE	66.9 <sup>a</sup>	91.0 <sup>a</sup>	0.34 <sup>c</sup>	0.08 <sup>e</sup>	10.1 <sup>e</sup>	13.4 <sup>g</sup>	1.7 <sup>ef</sup>	92.1
RSOR	24	CWDE	64.5 <sup>c</sup>	88.0 <sup>b</sup>	0.36 <sup>a</sup>	0.16 <sup>a</sup>	12.2 <sup>b</sup>	16.2 <sup>b</sup>	1.8 <sup>d-f</sup>	94.7
RSOR	48	CWDE	66.6 <sup>ab</sup>	90.9 <sup>a</sup>	0.36 <sup>a</sup>	0.16 <sup>a</sup>	9.4 <sup>f</sup>	15.0 <sup>a</sup>	1.3 <sup>f</sup>	92.3
Grain type										
			64.99 <sup>a</sup>	88.44 <sup>a</sup>	0.32 <sup>a</sup>	0.07 <sup>a</sup>	11.09 <sup>a</sup>	14.08 <sup>a</sup>	2.01 <sup>a</sup>	92.2
			63.84 <sup>b</sup>	87.14 <sup>b</sup>	0.34 <sup>b</sup>	0.15 <sup>b</sup>	11.33 <sup>a</sup>	15.96 <sup>b</sup>	2.97 <sup>b</sup>	94.1
Steep time										
			63.58 <sup>a</sup>	86.64 <sup>a</sup>	0.34 <sup>a</sup>	0.11 <sup>a</sup>	12.13 <sup>a</sup>	15.57 <sup>a</sup>	2.87 <sup>a</sup>	94.2
			65.26 <sup>b</sup>	88.94 <sup>b</sup>	0.32 <sup>b</sup>	0.10 <sup>b</sup>	10.29 <sup>b</sup>	14.46 <sup>b</sup>	2.11 <sup>b</sup>	92.1
Enzyme treatment										
			63.19 <sup>a</sup>	86.11 <sup>a</sup>	0.30 <sup>a</sup>	0.10 <sup>a</sup>	11.88 <sup>a</sup>	15.21 <sup>a</sup>	3.29 <sup>a</sup>	93.6
			65.65 <sup>b</sup>	89.47 <sup>b</sup>	0.36 <sup>b</sup>	0.12 <sup>b</sup>	10.54 <sup>b</sup>	14.82 <sup>b</sup>	1.69 <sup>b</sup>	92.7

<sup>a</sup> Mixed and individual effects values represent means of three and twelve replicates, respectively. YMZ, yellow maize; RSOR, regular sorghum; CWDE, cell-wall-degrading enzymes.

<sup>b</sup> Whole grains first steeped for 20 or 44 hr, followed by coarse grinding and 4-hr enzyme treatment.

<sup>c</sup> Solids lost in steep solution not quantified. Amount usually reported in literature 7–9% (Watson 1984).

<sup>d</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

**TABLE IV**  
Effect of Grain Type, Steep Time, and Cell-Wall-Degrading Enzyme Treatment on Viscoamylograph Properties of Refined Starches<sup>a</sup>

Grain	Treatment		Pasting Temp. (°C)	Time Peak Visc. (min)	Peak Visc. (RVU)	Shear Thinning <sup>c</sup> (RVU)	Viscosity (RVU)	
	Time (hr) <sup>b</sup>	Enzyme					Start Cooling	End Cooling
YMZ	24	W/O	75.9 <sup>bc<sup>d</sup></sup>	6.6a–c	285.5 <sup>ab</sup>	86.9 <sup>c</sup>	173.2 <sup>a</sup>	306.1 <sup>ab</sup>
YMZ	48	W/O	76.9 <sup>ab</sup>	6.8 <sup>ab</sup>	265.3 <sup>b</sup>	80.0 <sup>c</sup>	161.5 <sup>ab</sup>	297.9 <sup>bc</sup>
RSOR	24	W/O	75.9 <sup>bc</sup>	6.4 <sup>bc</sup>	321.1 <sup>a</sup>	163.2 <sup>a</sup>	122.7 <sup>cd</sup>	274.1 <sup>cd</sup>
RSOR	48	W/O	77.5 <sup>a</sup>	7.0 <sup>a</sup>	282.8 <sup>ab</sup>	135.5 <sup>a</sup>	139.6 <sup>bc</sup>	275.5 <sup>cd</sup>
YMZ	24	CWDE	75.3 <sup>c</sup>	5.9 <sup>d</sup>	304.1 <sup>ab</sup>	133.3 <sup>a</sup>	137.2 <sup>c</sup>	328.2 <sup>a</sup>
YMZ	48	CWDE	75.8 <sup>bc</sup>	6.3 <sup>cd</sup>	202.0 <sup>c</sup>	87.9 <sup>bc</sup>	97.1 <sup>e</sup>	275.4 <sup>cd</sup>
RSOR	24	CWDE	78.2 <sup>a</sup>	6.8 <sup>a</sup>	219.7 <sup>c</sup>	85.8 <sup>c</sup>	105.2 <sup>de</sup>	261.8 <sup>d</sup>
RSOR	48	CWDE	77.0 <sup>ab</sup>	6.6a–c	266.8 <sup>b</sup>	129.6 <sup>bc</sup>	109.8 <sup>de</sup>	269.1 <sup>d</sup>
Grain type								
			76.1 <sup>a</sup>	6.4 <sup>a</sup>	262.4 <sup>a</sup>	93.6 <sup>a</sup>	143.6 <sup>b</sup>	301.5 <sup>b</sup>
			77.1 <sup>b</sup>	6.7 <sup>a</sup>	274.4 <sup>a</sup>	131.9 <sup>b</sup>	118.0 <sup>a</sup>	270.6 <sup>a</sup>
Steep Time								
			76.2 <sup>a</sup>	6.4 <sup>a</sup>	282.7 <sup>a</sup>	118.1 <sup>a</sup>	133.5 <sup>a</sup>	291.6 <sup>a</sup>
			76.9 <sup>a</sup>	6.7 <sup>a</sup>	254.1 <sup>a</sup>	107.4 <sup>a</sup>	128.0 <sup>a</sup>	280.4 <sup>a</sup>
Enzyme Treatment								
			76.6 <sup>a</sup>	6.7 <sup>b</sup>	288.7 <sup>b</sup>	116.4 <sup>a</sup>	149.3 <sup>b</sup>	288.4 <sup>a</sup>
			76.6 <sup>a</sup>	6.4 <sup>a</sup>	248.1 <sup>a</sup>	109.2 <sup>a</sup>	112.3 <sup>a</sup>	283.6 <sup>a</sup>

<sup>a</sup> Mixed and individual effects values represent means of two and eight replicates, respectively. YMZ, yellow maize; RSOR, regular sorghum; CWDE, cell-wall-degrading enzymes; RVU, RVA units (1 = 10 cps).

<sup>b</sup> Whole grains first steeped for 20 or 44 hr followed by coarse grinding and 4-hr enzyme treatment.

<sup>c</sup> Drop of paste viscosity held at 90°C.

<sup>d</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

and recoveries. Enzyme-treated grains yielded  $\approx 2.5\%$  more starch than the control counterparts. The enzyme preparation hydrolyzed  $\beta$ -glucans, cellulose, and hemicellulose located in cell walls, thus providing an entry for the steep solution. Caransa et al (1988) added a commercial CWDE complex to whole corn and reported a 2.1% increase in starch recovery. Wolf et al (1952) found that carbohydrases have the greatest effect on the cross cells and aleurone layer. Wood and McRae (1978) reported that addition of CWDE to the steep solution provide access for penetration of  $\text{SO}_2$ /lactic acid and other enzymes. Roushdi et al (1981) concluded that lactic acid enhanced absorption and helped to solubilize proteins, thus positively affecting starch yields and starch quality. In a previous experiment conducted by Moheno-Perez et al (1999), the same enzyme complex was added to whole grains without any significant positive effect, whereas in this experiment, a positive effect was observed when enzymes were added to coarsely ground grains for 4 hr. In whole cereal grains, the water entry only occurs through the micropylar region or hilar layer, and it is likely that the CWDE complex could not enter readily through this tissue and thus was not effective during steeping of whole grains. As expected, milling of the steeped grains before starch refining achieved a better and more effective relationship between enzymes and substrate. Eckhoff and Tso (1991) and Watson and Sanders (1961) showed that starch release could be achieved within 6 hr of steeping if diffusion limitations are removed. Jayasena (1988) found that starch recovery in corn grits steeped with 0.1%  $\text{SO}_2$  for 6 hr was comparable to yields obtained from whole kernels steeped with 0.2%  $\text{SO}_2$  for 48 hr. Johnston and Singh (2001) also demonstrated that the application of enzymes to hydrated ground maize are effective in decreasing the steeping time and  $\text{SO}_2$  requirements.

For YMZ and RSOR the best wet-milling conditions to obtain the highest amount of starch were 48 hr of steeping and adding CWDE. The relative starch yield for the YMZ and RSOR steeped for 48 hr was 100 and 99.55%, respectively. However, grains soaked for 24 hr and with CWDE for 4 hr gave nearly the same yields and purity of fractions as the counterpart steeped for 48 hr without CWDE (Table III). In fact, although the difference was not significant ( $P > 0.05$ ), sorghum steeped for 24 hr and treated with CWDE yielded more starch than grains steeped for 48 hr without CWDE. Similar results and conclusions were observed in maize by Steinke et al (1991).

A comparison of protein and ash contents for the refined starches indicated that the process was more efficient for YMZ than RSOR (Table III). Starches from RSOR had higher protein and ash contents indicating that it was more difficult to separate the gluten that engulfed starch granules. Starch from kernels steeped for 48 hr had lower protein than starch from counterparts steeped for 24 hr. These observations agree with previous reports (Roushdi et al 1981; Watson 1984; Moheno-Perez et al 1999).

Enzyme-treated starches contained significantly higher amounts of residual protein and ash than the control starches. Some of the enzymes might have remained with the starch, thus increasing protein and ash contents. A thorough starch wash during refining might decrease amounts of these contaminants.

Table III shows the recovery of wet-milled products excluding soluble losses that were not quantified. In all grains and treatments, the recoveries were  $>91.8\%$ , indicating that the laboratory wet-milling procedure utilized was effective. Interestingly, YMZ steeped for 48 hr had lower wet-milled product recoveries than RSOR steeped for 24 hr, indicating higher losses of soluble compounds. More solids were recovered in grains steeped for 24 hr probably due to the lower amounts of soluble losses as indicated by Watson (1984) and Roushdi et al (1981). The same authors reported 7% of solids lost in the liquor of maize steeped for 48 hr. Soluble losses are mainly composed of soluble proteins such as albumins and globulins, and soluble sugars located in the germ. Maize usually has a higher germ to grain ratio than sorghum and

prolonged steeping probably allowed the diffusion of these compounds into the steep solution.

The amounts of gluten and inseparables were significantly higher for RSOR than for YMZ, indicating that higher amounts of starch remained with the gluten in sorghum (Table III). Grains steeped for 48 hr yielded less inseparables than counterparts steeped for 24 hr, indicating that a prolonged steeping favored reduction of disulfide bonds that liberated starch granules. The enzyme-treated grains also yielded fewer wet-milled co-products, especially inseparables, than untreated grains, indicating the effectiveness of CWDE in freeing starch. In these grains, fewer wet-milled products were recovered, probably because some of the hydrolyzed fiber compounds became soluble and therefore were lost in the steep solution.

The viscoamylograph properties of the refined starches are summarized in Table IV. The effect of steep time and treatment was not significant. The YMZ starch had a significant lower temperature to start gelatinization and viscosity after shear thinning than RSOR starch, indicating that YMZ starch granules were more prone to gelatinize (Zobel 1984) and were more stable during cooking. Also, YMZ starch had higher setback viscosity than RSOR starch, indicating a higher rate of retrogradation and cold paste consistency (Watson 1984).

## CONCLUSIONS

The enzyme complex added to coarsely ground grains for 4 hr improved starch yields. For maize and sorghum, the best wet-milling conditions to obtain the highest amount of starch were 48 hr steeping and utilization of the CWDE complex. The relative starch yield for the YM and RS steeped for 48 hr was 100 and 99.5%, respectively. However, grains soaked for 20 hr and treated with CWDE for an additional 4 hr gave yields and purity of fractions similar to those of the counterpart steeped for 48 hr without CWDE. For instance, sorghum steeped for 24 hr and treated with CWDE yielded amounts of starch similar to grains steeped for 48 hr without CWDE.

Compared with sorghum starch, the maize starch initiated gelatinization at lower temperature and had higher viscosity at both start and end of the RVA cooling cycle. In previous research (Moheno-Perez et al 1999; Wang et al 2000), addition of CWDE to whole sorghum or maize did not have any positive effect on starch yield and recovery. Therefore, the idea of coarsely grinding the presoaked grains was adequate because CWDE improved starch yields in both in vitro and laboratory wet-milling studies. Grinding allowed a better enzyme-substrate interaction and therefore improved starch yields and decreased steep time requirements. In commercial operations, grinding can be performed with the same plate or pin mills, and the ground material transferred to a holding tank with agitation before starting the starch refining process. The study shows that using CWDE can decrease steeping requirements without sacrificing prime starch yields and had a higher positive effect in sorghum than in maize. The enzyme process could decrease energy cost and capital investment and increase plant capacity.

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