

# Effect of Steeping Conditions (Sulfur Dioxide, Lactic Acid, and Temperature) on Starch Yield, Starch Quality, and Germ Quality from the Intermittent Milling and Dynamic Steeping Process (IMDS) for a Brazilian Corn Hybrid

M. S. J. Manzoni,<sup>1</sup> S. N. Kronka,<sup>2</sup> and J. F. Lopes-Filho<sup>1,3</sup>

## ABSTRACT

Cereal Chem. 79(1):120–124

Effect of lactic acid, SO<sub>2</sub>, temperature, and their interactions were assessed on the dynamic steeping of a Brazilian dent corn (hybrid XL 606) to determine the ideal relationship among these variables to improve the wet-milling process for starch and corn by-products production. A 2×2×3 factorial experimental design was used with SO<sub>2</sub> levels of 0.05 and 0.1% (w/v), lactic acid levels of 0 and 0.5% (v/v), and temperatures of 52, 60, and 68°C. Starch yield was used as deciding factor to choose the best treatment. Lactic acid added in the steep solution improved the starch yield by an average of 5.6 percentage points. SO<sub>2</sub> was more avail-

able to break down the structural protein network at 0.1% than at the 0.05% level. Starch-gluten separation was difficult at 68°C. The lactic acid and SO<sub>2</sub> concentrations and steeping temperatures for better starch recovery were 0.5, 0.1, and 52°C, respectively. The Intermittent Milling and Dynamic Steeping (IMDS) process produced, on average, 1.4% more starch than the conventional 36-hr steeping process. Protein in starch, oil content in germ, and germ damage were used as quality factors. Total steep time can be reduced from 36 hr for conventional wet-milling to 8 hr for the IMDS process.

Brazil is the third largest worldwide corn producer, after United States and China. The wet-milling industry is the second largest consumer of corn in Brazil only exceeded by animal feed (Abimilho, available from world wide web at <http://www.abimilho.com.br>).

Wet-milling is an industrial process that involves chemical, biochemical, and mechanical operations to separate corn into relatively pure fractions of starch, gluten, germ, and fiber (Singh et al 1997b). The first and foremost operation in corn wet-milling is steeping, which softens the corn kernel and breaks the disulfite bonds within the protein matrix (Mehra et al 2000). During steeping, corn kernels are soaked in dilute sulfurous acid solution at 48–53°C for 24–48 hr (Kerr 1950). Sulfur dioxide (SO<sub>2</sub>) and elevated steeping temperatures are used to control the growth of putrefactive microorganisms and aid the degradation of kernel structure to enhance milling. The SO<sub>2</sub> cleaves disulfite bonds in the corn endosperm protein matrix that encapsulate starch granules (Watson 1984), dispersing the endosperm protein which enhances starch release (Watson and Sanders 1961). The use of warm temperatures helps disperse protein and accelerate corn hydration rate, which reduces steeping time (Cox et al 1944; Fan et al 1965).

Lactic acid is usually formed by bacterial fermentation in commercial steeps and is often added to steepwater used in laboratory batch steeping (Shandera et al 1995). The role of lactic acid in steeping is not completely understood. Cox et al (1944) reported that lactic acid softened the kernel and increased the effectiveness of SO<sub>2</sub>. Previous workers (Eckhoff and Tso 1991; Du et al 1996; Lopes Filho et al 1997; Singh et al 1997a; Haros and Suarez 1999) reported decreased starch yields in batch steeping when lactic acid is not used. Earp et al (1985), under microscopic examination, found holes and pits in the endosperm cell wall structure when corn was steeped with lactic acid and SO<sub>2</sub>. However Shandera et al (1995) studied interactions between lactic acid, SO<sub>2</sub>, and temperature in steep solutions and reported that excessive use of lactic acid (lowering the steep solution to below pH ≈ 2.5) decreases the overall millability of the steeped corn kernel.

Among all of the corn wet-milling unit operations, steeping is the most time-consuming and is very energy- and capital-extensive.

Reducing steep time will decrease energy cost, increase plant capacity, and reduce the capital cost involved in the construction of new wet-milling plants (Mehra et al 2000). A number of studies have attempted to find a steeping method that would decrease steeping time by increasing the rate of water and SO<sub>2</sub> penetration into the corn kernel (Powell and McGeorge 1975; Chwalek 1980; Huster et al 1983; Meuser et al 1985; Hassanean and Abdel-Wahed 1986; Eckhoff and Tso 1991; Muthukumarappan and Gunasekaram 1992).

One alternative steeping procedure is the intermittent milling and dynamic steeping (IMDS) process proposed by Lopes Filho et al (1997). In the IMDS process, after an initial short soak period, the kernels are cracked to reduce the diffusional barriers that inhibit the penetration of steep chemicals. Unlike conventional steeping, the kernel soak is performed in short time (2 hr) with hydration of the germ being the main objective. Breaking open the kernel after this initial hydration phase minimizes germ damage and retains the ease and purity of germ recovery associated with wet milling. Cracking kernels to open them up and dynamic steeping with intermittent milling to enhance germ separation and reduce particle size are the two other major steps of the process. The steep time can be reduced from 24–48 hr in the conventional process to 5 hr for the IMDS process, maintaining quality and quantity of starch and by-products similar to that of the conventional process. Mehra et al (2000) reported that starch and gluten yield increased by 1.6 and 4.26%, respectively, and germ recovery was 0.54% lower and slightly broken (2–4%) when comparing IMDS and the conventional steeping process. However, the use of IMDS for new hybrids such as Brazilian corn produced under environment and harvesting conditions different from the U.S. dent corn, demands adjustment of the variables of the process to achieved better milling properties. Although the production of this kind of corn in Brazil is mainly for animal feed (the industry also uses it for wet-milling purposes). The objective of this work was to determine the best steeping conditions for a Brazilian dent corn using the IMDS process. The variables considered were SO<sub>2</sub> and lactic acid concentrations and steeping temperature. The best conditions were chosen according to starch yield response. Starch and germ quality were also considered in the study.

## MATERIALS AND METHODS

### Sample Preparation

A Brazilian dent corn (hybrid XL 606) grown during 1996 and 1997 crop seasons at the Agricultural CFM, São Paulo, was mechanically harvested at ≈18% (wb) moisture content. The corn was

<sup>1</sup> Former graduated fellow and professor, respectively. Dept. Eng. Alimentos–UNESP–Rua Cristovão Colombo, 2265-15054-000 S.J. Rio Preto, SP–Brasil.

<sup>2</sup> Professor, Dept. Ciências Exatas–UNESP–Rodovia Carlos Tonanni, Km 5– 14870-000 Jaboticabal, SP–Brasil.

<sup>3</sup> Corresponding author. Phone: 017-221-2254. E-mail: [lopes@eta.ibilce.unesp.br](mailto:lopes@eta.ibilce.unesp.br)

handled in the laboratory of the Food Engineering Department at São Paulo State University. It was sieved over a 4.8-mm round-hole sieve, and foreign material and broken kernels were removed manually. One half of the corn was dried at ambient air temperature and the other half dried at 45°C to 13% wb moisture content. The two parts were mixed together equally before storage. For each test run, ≈1,200 g of corn was packaged in plastic bags and stored at 8–10°C. Moisture content of the corn were determined by using Approved Method 44-15A (AACC 2000). The mean moisture content of the corn at the time of experimental runs was 12.4% wb.

### Effect of Lactic Acid, SO<sub>2</sub> Level, and Temperature in Dynamic Steeping

A 2×2×3 factor experimental design was used with IMDS. Two SO<sub>2</sub> levels of 0.05 and 0.1% (w/v), two lactic acid levels of 0 and 0.5% (v/v), and three temperature levels of 52, 60, and 68°C made up the experimental design. The steepwater consisted of 0.05 and 0.1% SO<sub>2</sub> produced by dissolving 1.4 and 2.8 g of sodium metabisulfite, respectively, in 1,600 mL of water, with or without the addition of 0.5% (v/v) lactic acid. Three replicates were done for each test.

The procedures, apparatus, and materials necessary for soaking, cracking, and dynamic steeping of the grains were acquired and assembled according to Lopes Filho et al (1997).

### Soak and Steeping

Cleaned corn (1,000 g) was soaked in 1,867 mL of water for 2 hr at 60°C. At completion of the soak period, soak water was drained and solids were determined using a two-stage air oven Approved Method 44-18 (AACC 2000). Soaked corn was then cracked by passing it through a 10-cm diameter × 30-cm wide smooth roll roller mill. A triple-pass method was used for the roller mill with a gap setting of 3.99 mm (0.16") for the first and second passes, and 3.06 mm (0.12") for the third pass. The triple-pass system was selected based on preliminary tests, which identified it as the best for minimizing germ damage. The kernels were dropped into the nip of the cylinders rotating in opposite directions. Cracked kernels were collected at the bottom of the rolls, and 80 mL of water was used to rinse the cylinders after cracking to increase total mass recovery.

Dynamic steeping, with a total time of 6 hr, was accomplished inside a plastic bottle equipped with a speed agitator (100–150 rpm) using each of the 12 treatment conditions. During dynamic steeping, intermittent milling was performed by placing all material into a commercial blender with blades dulled to a radius edge to prevent slicing of the germ (Eckhoff et al 1993). The time (20–60 sec) of milling inside the blender and the speed (1,360–3,465 rpm) of the blender were tested in preliminary tests. The combination time and speed for the most complete kernel cracking with minimum germ damage was 20 sec at full motor power (3,450 rpm) after 2 hr of the beginning of dynamic steeping and for 60 sec at the same speed after 4 hr from the beginning of dynamic steeping. At the end of the 6 hr of dynamic steeping, the germ was recovered by skimming with 18- and 14-mesh pieces of steel screen (Eckhoff et al 1993). During germ skimming, the time required for germ recovery was kept at 50 min to provide a fair comparison of germ yield. Recovered germ was rinsed with 1L of water over a 0.4-mm round-ole sieve. After washing, the germ was dried at 49°C for 24 hr. All other process steps followed the laboratory wet-milling batch procedure. The steepwater from dynamic steeping was used as process water in subsequent milling steps. Yields of starch, germ, fiber, gluten, and solids from the soak water and filtrate were measured and reported on a dry solids basis (Eckhoff et al 1993).

### Comparison of IMDS and Conventional Laboratory Wet-Milling Procedures

Based on the first experiment, the best conditions of lactic acid and SO<sub>2</sub> levels and steeping temperature were identified. A second experiment was done with single measurement in which fraction

yields from the IMDS process and the conventional corn wet-milling process were compared for the same hybrid. For comparison, three 1,000-g corn samples were batch steeped for 36 hr at 52°C in 0.1% (w/v) SO<sub>2</sub> and 0.5% (v/v) lactic acid using the conventional procedure (Eckhoff et al 1993). For the IMDS process, 1,000-g samples of cleaned corn were used as described previously.

### Starch and Germ Quality

Protein in starch, oil content of the germ, and damage to germ were used as quality factors. Protein in starch was determined by Approved Method 46-11A (AACC 2000). The factor N × 6.25 was used to convert nitrogen to protein. Oil content of the germ was measured using the Bligh-Dyer (1959) method. Percentage of germ damaged was determined by sieving germs for 4 min through a stack of four standard sieves of 7, 9, 10, and 16 mesh on a shaker (Produteste 2708), and weighing the fractions on each sieve. For all germ samples tested, no whole germ passed through the 9-mesh sieve. Particles on the 10 and 16-mesh sieves and pan were considered to be pieces of broken germs. The percentage of damage was determined by adding the weight of germ pieces in the 10 and 16-mesh sieve and in the pan, and dividing by total dried germ weight.

### Statistical Analysis

Yields of starch, germ, fiber, gluten, and soluble solids (soak water plus filtrate solids) were submitted to analysis of variance (ANOVA), with unfolding of degrees of freedom of the 12 treatments for the IMDS process. Effect of lactic acid, SO<sub>2</sub>, temper-

**TABLE I**  
Corn Fraction Yields (%) from Intermittent Milling and Dynamic Steeping (IMDS) of Wet-Milled Corn Steeped With and Without Lactic Acid<sup>a</sup>

Corn Fractions	Lactic Acid	
	0.0%	0.5%
Germ	5.14b	6.53a
Fiber	14.39a	10.70b
Starch	55.71b	61.27a
Gluten	18.42a	15.32b
Soluble solids	4.68b	5.39a

<sup>a</sup> Mean values followed by the same letter in the same row are not significantly different ( $P < 0.05$ ).

**TABLE II**  
Corn Fraction Yields (%) from Intermittent Milling and Dynamic Steeping (IMDS) of Wet-Milled Corn Steeped in Different SO<sub>2</sub> Concentrations<sup>a</sup>

Corn Fractions	SO <sub>2</sub>	
	0.05%	0.1%
Germ	5.79a	5.87a
Fiber	13.38a	11.71b
Starch	58.05b	58.92a
Gluten	16.43b	17.32a
Soluble solids	4.93b	5.14a

<sup>a</sup> Mean values followed by the same letter in the same row are not significantly different ( $P < 0.05$ ).

**TABLE III**  
Corn Fraction Yields (%) from Intermittent Milling and Dynamic Steeping (IMDS) of Wet-Milled Corn Steeped in Different Temperatures<sup>a</sup>

Corn Fractions	Temperature		
	52°C	60°C	68°C
Germ	5.55b	5.65b	6.30a
Fiber	11.85b	12.58ab	13.20a
Starch	60.65a	60.24a	54.57b
Gluten	15.35b	15.16b	20.10a
Soluble solids	5.11a	5.12a	4.86b

<sup>a</sup> Mean values followed by the same letter in the same row are not significantly different ( $P < 0.05$ ).

**TABLE IV**  
**Analysis of Variance for Corn Fraction Yields from Intermittent Milling and Dynamic Steeping (IMDS) of Wet-Milled Corn Steeped**

Source <sup>a</sup>	<i>F</i> value				
	Germ	Fiber	Starch	Gluten	Soluble Solids
SO <sub>2</sub> (S)	0.22	40.25* <sup>b</sup>	4.59*	5.90*	7.38*
Lactic acid (A)	67.25*	197.02*	188.31*	71.77*	85.94*
Temperature (T)	7.58*	8.85*	93.62*	77.67*	5.13*
S×A	0.01	1.42	0.23	1.35	0.15
S×T	2.18	0.47	1.55	1.62	0.62
A×T	1.21	1.25	2.67	0.68	1.94
S×A×T	1.81	0.60	3.84*	6.62*	0.07

<sup>a</sup> Under two levels of SO<sub>2</sub> (S), two levels of lactic acid (A) and three levels of temperature (T) (2×2×3 factors).

<sup>b</sup> \* = Significant at *P* < 0.05.

**TABLE V**  
**Comparison of Corn Fraction Yields from Intermittent Milling and Dynamic Steeping (IMDS) Process and Conventional Corn Wet-Milling Process<sup>a</sup>**

Corn Fractions	Method	
	IMDS	Conventional
Germ	6.12b	6.96a
Fiber	9.18a	8.77a
Starch	64.96a	63.70b
Gluten	13.75a	12.39b
Soluble solids	5.47b	7.41a

<sup>a</sup> Mean values (3 replicates) followed by the same letter in the same column are not significantly different (*P* < 0.05).

ature, and their interactions were considered. The mean values of yield fractions were compared by Tukey's test at a 5% level. Starch yield was the main factor to determine lactic acid use, SO<sub>2</sub> level, and the best temperature during steeping in IMDS.

After defining the best treatment or treatments, the yields from these treatments were compared with those yields means obtained by conventional method. Starch and germ quality tests were performed with the results of the better tests. The means were also compared by Tukey's test at a 5% level.

## RESULTS AND DISCUSSION

### Effect of Lactic Acid During Dynamic Steeping

Significant increase in starch yield was observed when lactic acid was used (Table I). The average starch yield difference between steeping with and without lactic acid was 5.6%. In conventional steeping, Earp et al (1985), Shandera et al (1995), and Watson (1967) observed that lactic acid promoted softening of grains pericarp and endosperm, possibly due to small holes caused by the acid on the cell walls. This increases cellular membrane porosity as well as softening protein matrix in vitreous regions of the kernel. Thus, more SO<sub>2</sub> is absorbed which increased cellular concentration of SO<sub>2</sub> inside the kernel, increasing endosperm starch release. For the IMDS process, the lactic acid action is probably faster because of barriers reduction and constant agitation of the kernels. Du et al (1996) found that starch yield increased from 59.1 to 63.8% (4.7% difference) when 0.55% lactic acid was added to steeping solution. Similar results were obtained by Eckhoff and Tso (1991), who reported that starch yield increased from 64.9 to 69.1% (4.2% difference) with the addition of 0.55% lactic acid to the steeping solution.

The lowest fiber content was observed with lactic acid (Table I), indicating lower residual starch in the fiber. Singh et al (1997a) reported that increases in starch yields by ≈3–12% (depending on hybrids) were due to the addition of lactic acid because of the effect of the acid on leaching starch from the fiber during fiber wash step. This increase in starch yield with decreased fiber fraction is in agreement with other studies (Roushdi et al 1981; Lopes Filho et al 1997).

Statistical analyses showed that germ yield was significantly affected by the addition of lactic acid (Table I). At the end of

steeping, the slurry had higher density (≈10 Bé) when lactic acid was used than without it. The higher slurry density improves germ recovery by skimming due to better germ flotation. These results are in agreement with a previous study by Lopes-Filho et al (1997), who reported that lactic acid helps increase starch released from the endosperm after the first grind.

High gluten yield was recorded when steeping without acid (Table I), which indicates loss of starch into the gluten fraction. This can be observed in the increased protein yield and decreased of starch yield.

Total soluble solids (soak water solids plus filtrate solids) released were higher when lactic acid was used (Table I). This affirmation is in agreement with that obtained by Du et al (1996), who evaluated the changes in wet-milling characteristics caused by the use of different acids and concluded that the major difference between samples steeped with SO<sub>2</sub> alone and those steeped with lactic acid is in the total solubles recovered from steepwater and filtrate. For SO<sub>2</sub> alone, the amount of solubles was low (5.04%) compared with those of added acids (6.10–7.39%). Other researchers (Wang 1994; Lopes Filho et al 1997) reported that as the concentration of lactic acid increased, larger amounts of solids were released.

### Effect of SO<sub>2</sub> in Dynamic Steeping

The starch and gluten yields increased and fiber decreased when 0.1% SO<sub>2</sub> was used compared with 0.05% SO<sub>2</sub> (Table II). The increased starch yield was 0.9 percentage points, on average. The SO<sub>2</sub> was more available to break down the structural protein network at 0.1% (higher level) compared with 0.05%. Eckhoff and Tso (1991) reported that the increased cellular concentration of SO<sub>2</sub> ions increases endosperm starch release. Lopes Filho et al (1997) also reported increased starch and gluten yields and decreased fiber yield when 0.1 and 0.15% SO<sub>2</sub> was used, compared with 0.05% for 3 hr of steeping.

Larger amounts of solids were released when 0.1% SO<sub>2</sub> was used (Table II). As for starch, higher concentration of SO<sub>2</sub> optimized the reactions in the endosperm of grain. Watson (1984) confirmed that the extraction of the soluble solids substances also occurred by reaction of the endosperm proteins with diffusive SO<sub>2</sub>. There was no statistical difference in the germ yield between samples steeped with 0.05 or 0.1% SO<sub>2</sub> (Table II).

### Effect of Temperature in Dynamic Steeping

The recovery of starch decreased and gluten and fiber increased when steeping at 68°C (Table III). The low starch recovery, as well as the higher gluten and fiber yields, can be associated with the gelatinization of some starch granules that could occur at 68°C, which is close to the temperature of starch (≈72°C). Leach et al (1959) affirmed that steeping temperatures may alter starch within the kernel, and steeping at very high temperatures can gelatinize starch and result in difficult milling properties. Shandera and Jackson (1996) concluded that elevated steeping temperatures partially annealed native starch granules within the steeping kernels. These authors also reported that viewed by

**TABLE VI**  
**Starch and Germ Quality of Corn Steeped for Different Temperatures with 0.1% SO<sub>2</sub> and 0.5% Lactic Acid**  
**Using Intermittent Milling and Dynamic Steeping (IMDS) and Conventional Process<sup>a</sup>**

SO <sub>2</sub> (%)	Lactic Acid (%)	Temperature (°C)	Protein Content of Starch (%)	Germ Damage (%)	Germ Oil (%)
0.1	0.5	52	0.40ab	10.12b	42.74b
0.1	0.5	60	0.38ab	9.62b	43.12b
0.1	0.5	68	0.42a	9.73b	40.83b
Conventional	0.36b	4.91c	48.91a		

<sup>a</sup> Mean values (3 replicates) followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

scanning electron microscopy, more surface irregularities occurred on starch granules of corn steeped at a 57°C and 1.5% lactic acid than on corn steeped at 43°C or 0.2% lactic acid. The separation of starch-protein also could have been difficult due possible denaturation of the protein at high temperature (68°C), prejudicing the total release of starch from protein network and fiber fraction. There was no statistical difference in starch and gluten yield between samples steeped at 52 and 60°C. The lower fiber yield was obtained at 52°C, where lower amounts of starch aggregated at the fiber.

Statistical differences were observed in germ yield between steeping at 68°C, and at 52 and 60°C. The yields were similar for 52 and 60°C. The difference of germ yields was 0.7 percentage points. Fan et al (1965) reported that warm temperatures accelerates the water absorption rate by helping open and expand the channels inside the grain, with the rate of water diffusion increasing as temperature increases (<60°C). Soluble substances, located predominantly in the germ, are leached, lowering the density of the germ and thereby facilitating germ separation by flotation (Watson et al 1951; Freeman 1973).

Low amount of soluble solids were released to steep solution at 68°C as compared with 52 and 60°C. Watson (1984) reported that one-half of the steepwater solubles come from the germ and the other half from the endosperm. Our results confirm Watson's affirmation; as more soluble solids leave the germ, its recuperation is easier by flotation due to density reduction. When germ yield at 68°C was higher than for 52 and 60°C, we assume that more soluble solids left from the germ due to diffusion increases at higher temperatures. On the other hand, we observed a poor separation of starch-gluten at 68°C. In this case, part of the soluble solids was retained in the starch-gluten complex.

#### **Interactions of SO<sub>2</sub>, Lactic Acid, and Temperature in Dynamic Steeping.**

Table IV shows a significant interaction between SO<sub>2</sub>, lactic acid, and temperature on starch and gluten yields. Unfolding this interaction was necessary for better understanding of their effects.

For starch yield, SO<sub>2</sub> had significant effect only for treatments with lactic acid at 68 and 52°C, in which 0.1% SO<sub>2</sub> increased the yield. Lactic acid increased starch yield for all treatment combinations. There was no significant difference in starch yield between 52 and 60°C, but for these temperatures the yield was higher than those at 68°C for any lactic acid and SO<sub>2</sub> combination.

For gluten yield, SO<sub>2</sub> had significant effect only for treatments without lactic acid at 60°C and with lactic acid at 68°C, in which 0.1% SO<sub>2</sub> increased gluten yield. For SO<sub>2</sub> levels and temperatures combinations (except 0.1% SO<sub>2</sub> at 68°C) dynamic steeping without lactic acid increased gluten yield. There was no significant difference in gluten yield between 52 and 60°C, but for these temperatures the gluten yield was lower than those obtained at 68°C for any lactic acid and SO<sub>2</sub> combination.

#### **Factor Combination**

The analysis of variance (ANOVA) (Table IV), using the 2×2×3 factor experimental design to study the SO<sub>2</sub>, lactic acid, and temperature effects and their interactions, showed that lactic acid is vital for IMDS process. Its presence increased the starch yield, the most important fraction, as well as affected all other fraction

yields (Table I). Steeping without lactic acid resulted in an incomplete break down of structural protein network, confirmed by low starch yields and high gluten and fiber yields. In such case, the addition of 0.5% lactic acid in the IMDS process for Brazilian dent corn (hybrid XL 606) and others similar hybrids is proposed.

The 68°C temperature was inappropriate once starch yield was reduced by inefficient starch separation from protein fraction. There was no significant difference in the yields of other fractions between 52 and 60°C, therefore steeping at 52°C is suggested as energy consumed is reduced with a consequent decrease in operation costs.

The unfolding of the interaction of the three factors for the starch yield showed significant effect on the starch yield of the combination with lactic acid at 52°C, in which 0.1% SO<sub>2</sub> produced more starch than the 0.05% SO<sub>2</sub>. This combination is recommended as the best treatment when using IMDS process for the semi-hard hybrid XL 606. The efficacy of this treatment is confirmed by the highest starch yield obtained. These steeping conditions are similar to those used in the conventional process.

#### **Comparing IMDS and Conventional Laboratory Wet-Milling**

There was statistical difference in wet-milling fractions (except for fiber yield) between samples steeped by IMDS and conventional wet-milling procedure (Table V). The fraction yields for starch and gluten from the IMDS process were 1.3 and 1.4% higher, respectively, compared with the starch and gluten yields from the conventional wet-milling procedure. These results were in agreement with previous studies done by Lopes Filho et al (1997) and Mehra et al (2000). The higher yields of the IMDS can have two main reasons. The first is that steepwater is used as process water in subsequent milling steps. More soluble solids remain in the steepwater and are recovered with the fractions. The second and more important reasons is that the action of component solution is more efficient because, during dynamic steeping, the material is agitated which promotes faster interaction between steeping mash and components solution. SO<sub>2</sub> and lactic acid acting on reduced particles of the grains improve release of starch granules from protein network and enhance fiber fraction purification (less starch is lost with this fraction). The germ yield obtained from the IMDS process was 0.8% lower than that from the conventional corn wet-milling process. The lower germ yield is directly related to high germ damages observed in the IMDS process (germ damage for IMDS process was 4.9 percentage points higher than for conventional process). Mehra et al (2000) reported that the germ yield obtained from the IMDS process when compared with that obtained from conventional was 0.5% lower.

#### **Starch and Germ Quality**

Protein content of starch ranged from 0.36 for the control to 0.42% for steeping at 68°C (Table VI). There was no significant difference for the 0.5% lactic acid and 0.1% SO<sub>2</sub> concentrations among the temperatures tested. Also, there was no statistical difference in protein content of starch among samples steeped at 52 or 60°C by IMDS process and that obtained from conventional process. For all laboratory wet-milling, protein content in starch was within the industry standard levels (0.35–0.50%). Singh et al (1997b) affirmed that although the wet-milling industry targets are ≈0.30% protein in starch, 0.50% protein is generally accepted for normal dent corn. Some researches using recirculated batch steeping for wet-milling

(Anderson 1963; Watson 1984; Eckhoff and Tso 1991; Eckhoff et al 1993) and starch tables for starch-protein separation reported protein in starch ranges of 0.30–0.64%. The protein content of 0.30% is achieved because industry uses countercurrent washing of the starch to remove additional protein.

The average germ damage obtained from the IMDS process was 4.9% greater than that from the conventional corn wet-milling process. This result is in agreement with the study done by Mehra et al (2000), who reported that germ recovered for this method was also slightly more broken (2–4%) than that from the conventional corn wet-milling.

There was no statistical difference in germ oil content for all IMDS treatments. The germ oil content was 40.83–43.12% (Table VI). There was higher oil content in the germ obtained from conventional wet-milling ( $\approx 6.7\%$ ) than for the IMDS treatments. Lopes Filho et al (1997) reported that increased retention of solubles in the germ due to the difference between steeping times (36 hr for conventional process and 8 hr for IMDS process) is probably the reason for lower oil content. The values reported by these researchers were 42 and 47% for the IMDS and conventional process, respectively. However Watson et al (1984) affirmed that broken germs lose oil, which is absorbed by gluten and cannot be recuperated.

## CONCLUSIONS

Lactic acid during dynamic steeping increased starch yield and decreased fiber fraction by 5.6 and 3.7 percentage points, respectively. Lactic acid also increased germ yield by 1.4 percentage points. Increasing SO<sub>2</sub> levels from 0.05 to 0.1% in the steep solution starch yield increased by an average of 0.9 percentage points. There was no significant difference in starch yield between 52 and 60°C, but starch yield for these temperatures was higher ( $\approx 6\%$ ) than those obtained at 68°C for all lactic acid and SO<sub>2</sub> combinations. Treatment with addition of 0.5% lactic acid and 0.1% SO<sub>2</sub> at 52°C is proposed as the best for IMDS process for the Brazilian dent corn (hybrid XL 606) considered in this study. Comparison of the IMDS with conventional wet-milling process showed that starch and gluten yield increased by 1.3 and 1.4%, respectively, for the IMDS. There was no statistical difference of protein in starch obtained by the IMDS process (0.40%) or by the conventional process (0.36%). Germ yield from IMDS process was 0.8% lower than from the conventional process. Germ damage for IMDS was higher (4.9%) than for conventional process and germ oil content was  $\approx 6.7$  percentage points lower. Soluble solids yields decreased  $\approx 2$  percentage points for IMDS process. The great advantage of the IMDS process over conventional steeping confirmed in this study is that IMDS requires total combined soak and steeping times of 8 hr compared to 36 hr for conventional steeping, with a better starch recovered using IMDS.

## ACKNOWLEDGMENTS

We acknowledge the financial support from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP); Project No. 98/13760-1, and Mr. J.J. De Mílio for their valuable contribution.

## LITERATURE CITED

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Methods 44-15A, 44-18, 46-11A. The Association: St. Paul, MN.

Anderson, R. A. 1963. Wet milling properties of grains: Bench-scale study. *Cereal Science Today* 8:190-195.

Bligh, E. G., and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911-917.

Chualek, V. P. 1980. Combined dry wet-milling process for refining corn. U.S. patent 4,181,748.

Cox, J. M., MacMasters, M. M., and Hilbert, G. E. 1944. Effect of the sulfuric acid steep in corn wet milling. *Cereal Chem.* 21:447-465.

Du, L., Li, B., Lopes-Filho, J. F., Daniels, C. R., and Eckhoff, S. R. 1996. Effect of selected organic and inorganic acids on corn wet-milling yields. *Cereal Chem.* 73:96-98.

Earp, C. F., McDonough, C. M., and Rooney, L. W. 1985. Changes in the microstructure of the corn kernel during the wet milling steeping process. Progress Report. Corn Refiners Assoc.: Washington, DC.

Eckhoff, S. R., and Tso, C. C. 1991. Wet-milling of corn using gaseous SO<sub>2</sub> addition before steeping and the effect of lactic acid on steeping. *Cereal Chem.* 68:248-251.

Eckhoff, S. R., Rausch, K. D., Fox, E. J., Tso, C. C., Wu, X., Pan, Z., and Buriak, P. 1993. A laboratory wet milling procedure to increase reproducibility and accuracy of product yields. *Cereal Chem.* 70:723-727.

Fan, L. T., Chen, H. C., Shellenberger, J. A., and Chung, D. S. 1965. Comparison of the rates of absorption of water by corn kernels with and without dissolved sulfur dioxide. *Cereal Chem.* 42:385-396.

Freeman, J. E. 1973. Quality factors affecting value of corn wet milling. *Trans. ASAE.* 16:671-678, 682.

Haros, M., and Suárez, C. 1999. Effect of chemical pretreatments and lactic acid on rate of water absorption and starch yield in corn wet-milling. *Cereal Chem.* 76:783-787.

Hassanean, A., and Abdel-Wahed, A. A. 1986. A new method to short the steeping period of corn grains. *Starch* 38:417-419.

Huster, H., Friederich, M., Hoepke, C. H., and Salzuflen, B. 1983. Method of producing starch from grain products by the wet process. U.S. patent 4,416,701

Kerr, W. R. 1950. *Chemistry and Industry of Starch*, 2nd Ed. Academic Press: New York.

Leach, H. W., McCowen, L. D., and Schoch, T. J. 1959. Structure of the starch granule. I. Swelling and solubility patterns of various starches. *Cereal Chem.* 36:534-544.

Lopes-Filho, J. F., Buriak, P., Tumbleson, M. E., and Eckhoff, S. R. 1997. Intermittent milling and dynamic steeping process for corn starch recovery. *Cereal Chem.* 74:633-638.

Mehra, S., Singh, V., Tumbleson, M. E., and Eckhoff, S. R. 2000. Effect of mill plate setting and number of dynamic steeping stages for an intermittent milling and dynamic steeping (IMDS) process for corn. *Cereal Chem.* 77:209-212.

Meuser, F., German, H., and Huster, H. 1985. The use of high-pressure disintegration technique for extraction of starch from corn. Page 161 in: *New Approaches to Research on Cereal Carbohydrates*. R. D. Hill and L. Munck, eds. Elsevier Science Publishers: Amsterdam.

Muthukumarappan, K., and Gunasekaram, S. 1992. Above atmospheric hydration of corn. *Trans ASAE* 35:1885-1889.

Powell, E. L., and McGeorge, G. G. 1975. Process for recovery of starch and corn oil from corn. U.S. patent 3,909,288.

Roushdi, M., Fahmy, A. A., and Mostafa, M. 1981. Role of lactic acid in corn steeping and its relation with starch isolation. *Starch* 33:426-428.

Shandera, D. L., Parkhurst, A. M., and Jackson, D. S. 1995. Interactions of sulfur dioxide, lactic acid, and temperature during simulated corn wet milling. *Cereal Chem.* 72:371-378.

Shandera, D. L., and Jackson, D. S. 1996. Effect of corn wet-milling conditions (sulfur dioxide, lactic acid, and steeping temperature) on starch functionality. *Cereal Chem.* 73:632-637.

Singh, V., Haken, A. E., Niu, Y. X., Zou, S. H., and Eckhoff, S. R. 1997a. Hybrid-dependent effect of lactic acid on corn starch yields. *Cereal Chem.* 74:249-253.

Singh, S. K., Johnson, L. A., Pollak, L. M. Fox, S. R., and Bailey, T. B. 1997b. Comparison of laboratory and pilot-plant corn wet-milling procedures. *Cereal Chem.* 74:40-48.

Wang, D. 1994. Effect of broken and pericarp damaged corn on water absorption and steepwater characteristics. MS thesis. University of Illinois: Urbana, IL.

Watson, S. A., Willians, C. B. and Wakely, R. D. 1951. Laboratory steeping procedures used in a wet milling research program. *Cereal Chem.* 28:105-118.

Watson, S. A., and Sanders, E. H. 1961. Steeping studies with corn endosperm sections. *Cereal Chem.* 38:22-33.

Watson, S. A. 1967. Manufacture of corn and mill starches. In: *Starch Chemistry and Tecnology*, Vol. II. R. L. Whistler and E. F. Paschall, eds. Academic Press: New York.

Watson, S. A. 1984. Corn and Sorghum Starch. In: *Starch Chemistry and Technology*. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: Orlando, FL.

[Received February 20, 2001. Accepted August 20, 2001.]