AN ENZYMATIC PROCESS FOR CORN WET MILLING

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I. INTRODUCTION: CORN WET MILLING INDUSTRY

In 2002, approximately 2.2 billion bushels of corn were processed in the United States for the production of food, fuel, and industrial products. Of that 2.2 billion bushels, 19.22 million tonnes (757 million bushels) were used for high fructose corn syrup (HFCS), glucose and dextrose, 6.34 million tonnes (250 million bushels) for pearl starch, 26.69 million tonnes (1051 million bushels) for fuel and beverage alcohol, and 4.75 million tonnes (187 million bushels) for cereals and other products (Corn Refiners Association, 2003). More than 50% of the corn processed in the United States is done so using the corn wet milling process.

Wet milling is a process in which wet (40–45% moisture content) corn kernels are fractionated in such a way that the individual components of corn kernels are separated, using an aqueous medium, in relatively pure

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form. From 25.4 kg (1 bushel) of corn, 14.3 kg (31.5 lbs) of starch can be produced, 6.1 kg (13.5 lbs) of corn gluten feed (animal feed), 1.2 kg (2.6 lbs) of corn gluten meal (animal feed), and 0.7 kg (1.5 lbs) of corn oil can be produced (National Corn Growers Association, 2002). Starch can be further converted into other high-valued products. From the 14.3 kg (31.5 lbs) of starch, another 15 kg (33 lbs) of sweetener or 9.5 liters (2.5 gal) of ethanol can be produced (National Corn Growers Association, 2002).

The corn wet milling process was developed in the early 19th century at which time alkali was used. The use of alkali was abandoned when the SO_2 process was invented in 1875. The alkali wet milling process was considered inferior to the SO_2 wet milling process because the steeping of whole corn kernels in the presence of alkali for 40–50 hr resulted in the pericarp being dissolved completely, in excessive starch solubilization, and in undesirable bacterial fermentation. Since then, many processing changes have been made and new technologies have been developed. New unit operations and equipment design have improved the conventional corn wet milling process greatly and made it more efficient. In the United States, there are currently 28 corn wet milling plants; more than half have been built in the last few decades (Johnson and May, 2003). During the last few years, growth in the corn wet milling industry has been 4 to 6% per year.

In the corn wet milling process, the use of SO_2 is very important. It breaks down the protein matrix that surrounds the starch particles and increases starch yield during milling. The use of SO_2, however, has some health and environmental problems associated with it. Previously, research has been done to develop alternative processing methods that do not require the use of SO_2 (Meuser and German, 1984; Meuser *et al*., 1985, 1989), but due to lower starch recovery or inferior separations, have not resulted in a commercialized wet milling process. Another alternative process developed for fractionating corn kernels that does not require the use of SO_2 is the sequential extraction process (SEP) (Chang *et al*., 1995; Hojilla-Evangelista *et al*., 1992). However, the SEP process is best suited for the production of ethanol and coproducts. An enzymatic corn wet milling process has been developed that shows the potential to reduce or completely eliminate the use of SO_2 and produce starch yields comparable to the conventional corn wet milling process. The objective of this article is to review the enzymatic corn wet milling process and to compare it to the conventional corn wet milling process.

The corn wet milling process involves taking apart a corn kernel into its individual components. To take apart a corn kernel for maximum and high-quality starch recovery, it is important to understand the structure and composition of the corn kernel.

II. STRUCTURE AND COMPOSITION OF THE CORN KERNEL

A corn kernel has four main parts: (1) tip cap, (2) pericarp, (3) germ, and (4) endosperm. Earl *et al.* (1946) gave the percentage component parts and the composition of these parts of dent corn kernels, as shown in Table I.

Endosperm constitutes the main part of the corn kernel and consists of 85 to 90% starch, 8 to 10% protein, and a small amount of oil and other compounds. Corn endosperm can be divided into two distinct parts: floury and horny endosperm. In floury endosperm, starch particles are round and are dispersed loosely in the protein matrix. In the horny endosperm, the protein matrix is stronger and starch particles are held more firmly. Starch granules are encased in the continuous protein matrix. The tighter setting in horny endosperm gives starch particles a polygonal shape. On average, the amount of horny endosperm in the corn kernel is twice that of the floury endosperm. However, this ratio is a function of the corn kernel protein content (Wolf *et al.*, 1952).

Germ (embryo) constitutes 11 to 12% of the corn kernel. It can be divided into three parts, of which one turns into leaves (plumule) and another turns into roots (radicle) when the kernel is planted. The third part (scutellum) provides high-energy oil to the plant for growth (Blanchard, 1992). The remaining parts of the corn kernel are the pericarp and tip cap. The pericarp is the dense outer layer of corn kernels consisting of layers of dead cells. One of these layers is a spongy tissue known as cross and tube cells, which facilitate the absorption of water into the kernel. Underneath the cross and tube cells is a layer of semipermeable cells known as the seed coat. The tip cap is the remaining fibrous material that connects the corn kernel to the cob. It is only through the tip cap and then through the cross and tube cells

TABLE I
PERCENTAGE COMPONENT PARTS AND COMPOSITION OF THESE PARTS OF DENT CORN KERNELS[a]

Corn kernel component	Dry weight of whole kernel (%)	Dry basis (%)				
		Starch	Protein	Oil	Ash	Sugar
Tip cap	0.8	5.3	9.7	3.8	1.7	1.5
Pericarp	5.3	7.3	3.5	0.98	0.67	0.34
Germ	11.5	8.3	18.5	34.4	10.3	11.0
Endosperm	82.3	86.6	8.6	0.86	0.31	0.61

[a]From Earl *et al.* (1946).

that water or other liquids can penetrate the kernels (Wolf *et al*., 1952). The cutinized outer layer of the pericarp prevents the absorption of water into the corn kernel.

III. CONVENTIONAL CORN WET MILLING PROCESS

Corn is delivered to the processing plant by rail car, truck, or barge. Currently, no rapid and precise methods are available to determine the wet millability (wet milling quality of the incoming corn). Corn for wet milling is usually purchased based on the United States Department of Agriculture (USDA) grain standards, which do not indicate directly the wet milling characteristics. Normally, #2 grade corn is purchased because of its price and availability and not because of quality. Different factors affecting the quality of grain for wet milling (Freeman, 1973) can be classified into three main categories: (1) hybrid or genetics (Anderson, 1962, 1965; Anderson and Griffin, 1962; Anderson and Pfeifer, 1959; Anderson *et al*., 1960; Watson and Yahl, 1967; Weller, 1987; Zehr and Eckhoff, 1995; Zehr *et al*., 1995, 1996), (2) environmental or growing conditions (Singh *et al*., 1996), and (3) postharvest handling (Brown *et al*., 1979; Lasseran, 1973; Le Bras, 1982; MacMasters *et al*., 1954, 1959; Singh *et al*., 1998b; Vojnovich *et al*., 1975; Watson and Hirata, 1962; Weller, 1987; Weller *et al*., 1989) and storage (Lasseran, 1973; Roushdi *et al*., 1979; Singh *et al*., 1998a).

The first and foremost operation in the corn wet milling process is cleaning to remove foreign material (sand, weeds, pieces of cob, and other cereal grains) and broken corn kernels, which restrict the flow of steepwater through corn kernels and result in understeeped corn and reduced separation performance (Blanchard, 1992; Johnson and May, 2003; Watson and Eckhoff, 2004). Broken kernels increase the amount of solids in steepwater (Wang, 1994) and can also release some starch into steepwater, which becomes gelatinized upon evaporation and makes steepwater viscous (Watson and Eckhoff, 2004), causing fouling of heat transfer surfaces (Madson and Manceaux, 1995). Reciprocating screens and aspiration are used to remove the broken corn and foreign material (BCFM). Cleaned corn is conveyed to steep tanks where it is steeped countercurrently (new corn with oldest steepwater) in 0.1 to 0.2% SO_2 at 48 to 52 °C for 24 to 36 hr. Steeping is accomplished in a battery of interconnected 6 to 18 stainless steel tanks, each of 254 to 635 tonnes/day (10–25,000 bu/day) capacity (depending on the plant size). Each steep tank is equipped with a pump to recirculate the steepwater or to move the steepwater to the next tank. Each tank, or a combination of 2 to 3 tanks, has a heat exchanger to maintain the temperature. Steeping is done to soften the corn kernels so that

subsequent milling and separation of corn components can be accomplished easily.

The conventional countercurrent steeping process can be divided into three distinct 8- to 12-hr duration stages (Watson and Eckhoff, 2004): (1) lactic acid-dominated stage, (2) sulfur dioxide diffusion stage, and (3) sulfur dioxide-dominated stage. In the first stage the soluble sugars leached from the corn kernel are fermented into lactic acid by *lactobacillus sp*. Several studies have been done to determine the role of lactic acid in the steeping process. Although the mechanism is not completely understood, a significant effect of lactic acid on increasing starch yields has been found (Eckhoff and Tso, 1991; Roushdi *et al*., 1981a,b; Singh *et al*., 1997, 1999a; Watson *et al*., 1951). Moreover, the effect of lactic acid on increasing the starch yield has been found to be hybrid dependent (Singh *et al*., 1997). It has also been observed that other weak or strong acids do not have the same effect of increasing starch yields as observed with lactic acid (Du *et al*., 1996; Kerr, 1950). There is something unique about lactic acid and there is probably some synergistic effect of lactic acid and SO_2 during steeping (unpublished data). Research has shown that lactic acid fermentation is not required to see its beneficial effects in increasing starch yields (Watson and Eckhoff, 2004) and the addition of externally produced lactic acid has the same effect as that produced by *in situ* fermentation.

The second stage of steeping is the sulfur dioxide diffusion stage in which sulfur dioxide diffuses with the water into the corn kernel through the base end of the tip cap and moves through the cross and tube cells of the pericarp to the kernel crown and then slowly into the horny endosperm. The second stage is diffusion limited because of the specific path required for water going into the kernel.

The third stage is the sulfur dioxide-dominated stage. During this stage the maximum amount of SO_2 is absorbed in the corn endosperm and cleaves the disulfide bonds in the protein matrix that encapsulates the starch granules and loosens up the protein matrix (Watson and Eckhoff, 2004). A balance between these stages has to be maintained in order to achieve optimum steeping. At the end of steeping, corn kernels have approximately 43–45% moisture content, leached out 6–6.5% of soluble solids (mainly from the germ), absorbed about 0.2–0.4 g of sulfur dioxide per kilogram, and become sufficiently soft so as to rupture when squeezed between the fingers (Watson and Eckhoff, 2004).

After steeping, corn is passed through attrition mills, which tear open the kernels and release the now rubbery germ. The objective is to release the maximum amount of germ with minimal germ damage and is usually done in two steps: a first grind and a second grind. The mills are equipped with one fixed and one rotating Devil's tooth plate, which mesh closely and are

designed specifically for corn (Blanchard, 1992; Johnson and May, 2003). Mill plates can be adjusted to different gap settings. The plate gap setting and revolutions per minute of the mill control the impact and shearing force on the kernels and, therefore, affects the quality of the germ recovered. Most of the germ and approximately 50% of the starch are released in the first grind. All of the ground slurry is collected in a tank (first grind tank) from where it is fed to the germ separation unit. Starch released at the first grind increases the density of the slurry in the first grind tank to a specific gravity of about 1.058–1.066 (8–9 Baumé). At this Baumé the germ, which contains about 45–55% oil, is lighter than the other corn components and, therefore, floats on top of the slurry and can be separated by density difference. Germ separation is done by passing the ground corn slurry under pressure through hydrocyclones, which are conical tubes ranging from 7.6 to 22.9 cm (3 to 9 in) in diameter and 0.91 to 1.37 m (3 to 4.5 ft) in length. The slurry is fed tangentially through the inlet port, causing a rapid swirling motion. The heavier particles (endosperm starch and fiber particles) are forced against the walls and come out through the underflow, whereas the lighter particles (germ) stay in the middle and are recovered through the vortex finder as the overflow (Blanchard, 1992).

Germ recovery is also done in two steps: primary germ separation and secondary germ separation. Each step has two sets of hydrocyclones, A and B, in which the volumetric ratio of overflow to supply (O/S) is different. A cyclones have an O/S ratio of 20%, and B cyclones have an O/S ratio of 30–50%. The underflow of A cyclones is fed to B cyclones. In primary germ separation, 80 to 85% of the germ is recovered and most of the remaining germ is recovered in the secondary germ separation. Any whole or broken germ not recovered in the secondary germ separation is lost in the slurry and is recovered later as a part of the fiber fraction. Oil released from broken germ ends up in the gluten (protein) fraction. The germ separation system is set up in recycle loops. Germ recovered with B cyclones is fed to A cyclones. Also, germ recovered from the secondary germ separation system is fed to the primary germ separation system. The only place from which germ is removed from a wet mill is overflow of the A cyclones of the primary separation system (Blanchard, 1992). The recovered germ is washed countercurrently over a set of screens to remove loose starch and protein and is dewatered in a germ press to 50 to 55% moisture content. Dewatered germ is subsequently dried to 2 to 4% moisture content and is processed further to recover corn oil, a valuable coproduct. If the corn oil is recovered at the plant site, germ meal is added later to the fiber fraction to produce corn gluten feed (CGF).

Corn slurry from underflow of the B cyclones of the secondary separation system is passed over screens (the third grind screens) to remove free starch

and protein from the endosperm and fiber fractions. This step reduces the load on the third degermination mill by removing approximately 50% of the solids. The third grind mill usually consists of two independently driven, grooved mill plates rotating in opposite directions. The plates together give a cutting action that reduces the amount of starch bound to fiber by 20 to 30% (Johnson and May, 2003). These mills are also known as refiner mills. Another type of third grind mill is the impact mill, or Entoleter mill. An impact mill has one rotating disc plate fitted with pins. The corn slurry fed to the mill is forced against the rotor and the stator pins to release starch and minimize disintegration of the fiber fraction. Refiner and impact mills give almost the same performance (Watson and Eckhoff, 2004).

The slurry, which contains starch, protein, and fiber, is passed through a series of five to six pressure-fed, or DSM, screens to separate fiber from starch and gluten. Usually a 120° concave wedge bar type of screen is used for fiber washing. The slurry is forced tangentially at fixed velocity across the screen surface. The concave surface and the velocity of the slurry across the surface provide the centrifugal force, which holds the slurry against the screen surface (Dorr-Oliver, 1990). The spacing between the wedge bars allows the starch and protein particles to pass through and the fiber particles to flow across the screen, with continuous dewatering and without clogging the screen. Usually the first fiber wash screen has a 50-μm spacing between the wedges. It is also known as the fiber block because it prevents fine fiber from passing through the screen and, therefore, prevents fine fiber from entering the centrifuges and the starch hydrocyclones. The subsequent four to five screens have a 75-μm spacing. With countercurrent washing, fiber coming off the last set of screens contains about 15 to 20% starch, of which about half is bound and half is free (Watson and Eckhoff, 2004). After washing, fiber is dewatered by centrifugal screens and screw presses to about 60% moisture content. Fiber is then dried partially, mixed with heavy steepwater, and dried further to about 10% moisture and mixed with germ meal (defatted germ) to make corn gluten feed. The final corn gluten feed contains 18 to 22% protein and 1.0% fat (Blanchard, 1992).

The starch and gluten (protein) slurry, known as mill starch or mill stream, is combined from the fiber wash and the underflow of the third grind screens. Normally, mill starch contains about 5–6% protein (db). Mill starch is passed through a set of degritting cyclones to remove any sand or other foreign material to prevent damage or blocking of centrifuge nozzles. After degritting, the mill starch, at a specific gravity of 1.043 to 1.058 (6 to 8° Bé), is concentrated to a specific gravity of 1.074 to 1.090 (10 to 12° Bé) by passing through centrifuges [mill stream thickeners (MST)] before the final starch separation (Blanchard, 1992). Concentrated mill starch is passed through another set of centrifuges [primary separators (PS)] to separate

the starch and the gluten fractions based on their density differences (1.5 g/cm^3 for starch particles vs 1.1 g/cm^3 for gluten particles). Primary centrifuges consist of a rotating bowl in which a stack of conical discs are separated by a distance of 0.4 to 1.0 mm, depending on the density of particles to be separated. On the periphery of the rotating bowl, there are 6 to 12 nozzles. The mill starch enters the rotating bowl from the top or the bottom. Due to centrifugal force, the heavier starch particles are forced toward the periphery of the rotating bowl and exit through the nozzles as underflow. The starch slurry coming out from the primary separator has a protein content of 2 to 4% and a specific gravity of 1.160 to 1.198 (35 to 42% dry solids). Lighter gluten particles move up between the discs and exit out as overflow. Gluten slurry from primary centrifuges comes out at a concentration of 15 to 30 g/liter (2 to 4 oz/gal) and contains about 68 to 75% protein (db). Routine maintenance is required to optimize performance.

The gluten slurry is concentrated from 15 to 30 g/liter (2 to 4 oz/gal) to 150 to 165 g/liter (20 to 22 oz/gal) by using another nozzle-bowl gluten thickener (GT) (Blanchard, 1992). Further dewatering of gluten is done with rotary vacuum filters, which consist of a rotary drum with a filter belt. The rotary drum dips partially into a trough of concentrated gluten; a vacuum is applied to build a cake on the belt surface. As the drum moves out of the trough, the vacuum sucks water out of the cake. The cake (approximately 60% moisture) is discharged from the belt onto a screw conveyer, and the belt is washed with high-pressure nozzles to remove the fine gluten particles and open the pores. Gluten dryers (flash or steam tube) further dry the cake to 10 to 12% moisture content to make corn gluten meal.

The starch slurry coming from the PS contains 2 to 4% protein. Further purification of starch is achieved by multistage countercurrent starch washing in 9 to 15 stages of small liquid cyclones to decrease the protein content to less than 0.35% db. These cyclones are similar to hydrocyclones used in germ separation, except the diameter and length of these cyclones are small. The diameter of these cyclones is about 10 mm and the length is about 152 mm. Because the capacity of individual cyclones is small, several hundred cyclones are mounted in parallel in a ''clamshell.'' The starch slurry is pressure fed at 690 to 896 kPa (100 to 130 psi) to the central compartment of the ''clamshell'' and then into the cyclones. Fresh water enters at the very last stage of starch washing and comes out as overflow and is added to the second to last stage of starch washing. Finally, water leaves the starch washing system as overflow from the first washing stage. The only place in a corn wet milling plant where fresh water is added is in the starch washing stage. Underflow of the last starch washing stage is the starch slurry at 60% moisture content, which is dried further to recover pearl starch or is processed to produce corn syrups.

Water leaving the starch washing stage picks up 25% of the total starch fraction, which is recovered by centrifuges, known as clarifiers. Before being fed to the centrifuges, this diluted starch slurry is cooled. Clarifier centrifuges concentrate the starch slurry to a specific gravity of 1.074 to 1.090 (10 to 12° Bé). This starch slurry is mixed back with the mill starch and fed to the PS. The clarifier centrifuge overflow is used primarily as wash water for the PS and the surplus is used for germ or fiber washing. Overflow of the MST is used mainly as steepwater after the addition of sulfur dioxide. GT overflow is used mainly for germ washing fiber washing, and rotary vacuum filter belt washing. Most of the U.S. wet milling plants use a four-centrifuge system (MST, PS, GT, and clarifier centrifuges); there are a few plants that operate with a three centrifuge system (no MST) Chiang and Lee (1995) presented a new design of centrifuge and a new process in which the efficient separation of starch and gluten can be achieved by only two-centrifuge systems. This process offers considerable savings in capital and operating costs.

IV. USE OF ENZYMES IN THE CONVENTIONAL CORN WET MILLING PROCESS

Enzymes have been used previously in the conventional corn wet milling steeping process to reduce the steeping time and the residual protein in the final starch fraction. Roushdi *et al.* (1981b) evaluated the use of different proteases (pepsin, papain, bromelain, and trypsin) in addition to SO_2 during the wet milling steeping process. No significant difference was observed in the residual protein in the final starch fraction from intact kernels. However, with broken kernels, a small but significant difference in the residual protein content of starch was observed with enzyme addition. Use of enzymes on grits (endosperm particles produced during dry milling) have also been investigated as either pretreatment for air classification (Spanheimer *et al.*, 1972) or to overcome the adverse effects of high-temperature drying on starch–gluten separation during subsequent milling (Eckhoff and Tso, 1991). Single enzymes or a combination of enzymes (cellulases, hemicellulase, xylanases, pectinases, and proteases) have also been tested during steeping to increase the starch yield and to reduce the steep time (Caransa *et al.*, 1988; Hassanean and Abdel-Wahed, 1986; Moheno-Perez *et al.*, 1999; Steinke and Johnson, 1991; Steinke *et al.*, 1991). These studies show small but significant improvements in the starch yield when high doses of enzyme were added during the steeping step. Most of these studies focused on using enzymes to provide increased starch yield in addition to the increase provided by sulfur dioxide. These studies were not aimed at removing or reducing the amount of SO_2 in the conventional corn wet milling process.

V. DEVELOPMENT OF THE ENZYMATIC CORN WET MILLING PROCESS

Most of the previous studies that showed improvements in starch yield with the addition of enzymes in the conventional corn wet milling process did not address adequately the specific enzymes responsible for those improvements. Initial work with the enzymatic corn wet milling process was done to reproduce the published results and to determine the specific enzymes responsible for improving starch yields. Several different enzymes and combinations of enzymes reported previously (Johnston and Singh, 2001) for their use in corn wet milling were tested using a precise 100-g laboratory conventional corn wet milling procedure (Eckhoff *et al.*, 1996). Results indicated no significant difference in starch yields when compared to the conventional procedure (0.2% SO_2 and 0.55% lactic acid) (Figure 1).

Due to the structure of the corn kernel (cutinized outer layer of the pericarp surrounding the corn kernel), the diffusion of water and chemicals inside the kernel is through a very specific pathway. Initial results with the use of enzymes during steeping (Figure 1) indicated that enzymes were not able to penetrate the kernels and break down the protein matrix surrounding starch particles. For enzymes to penetrate the corn kernel, it was necessary

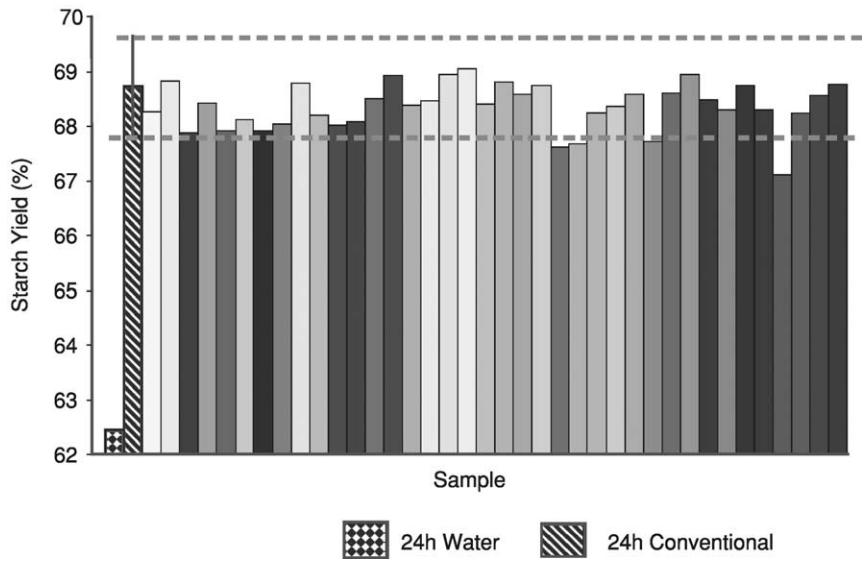

FIG. 1 Effect of different enzymes or combination of enzymes (cellulases, xylanases, cellobiases, β-glucanases, and proteases) on starch yield in the conventional corn wet milling process.

to do a size reduction to remove the diffusional barriers. However, any size reduction of kernels without adequate hydration could lead to germ damage. Germ is the most valuable component of the corn kernel. Germ damage in a corn wet milling plant has two disadvantages: (1) the value of germ is lost and (2) the oil released from the germ creates problems during the separation of other corn components.

Dailey (2000) studied the hydration of germ and endosperm in four different soaking solutions maintained at 52 °C (water; 0.55% SO_2; 0.5% lactic acid solution; and a mixture of 0.22% SO_2 and 0.5% lactic acid solution) and found that the type of soaking solution had no effect on the hydration rate. He reported that germ hydrates to more than 40% moisture content in approximately 3 hr irrespective of the soaking solution. When hydrated to approximately 40% moisture content, germ becomes rubbery and does not break when ground coarsely using degermination mills. Based on Dailey's work and initial results from the use of enzymes in the corn steeping process, a new enzymatic steeping procedure was developed in which kernels were initially soaked in water for hydration before size reduction (Johnston and Singh, 2001, 2003). After soaking, the kernels were cracked to disrupt the diffusional pathways and to allow the enzyme to penetrate the corn kernel. The ground corn slurry was incubated with the same enzymes and combinations of enzymes reported previously with intact kernels in the conventional corn wet milling steeping process. Also, with the new procedure, no SO_2 was added to the steep solution. The effects of enzyme addition were not apparent in the presence of SO_2 because SO_2 was giving beneficial effects. Results showed significant effects of enzyme additions on starch yields (Figure 2). With the enzymatic milling procedure, certain classes of enzymes gave starch yields comparable to those of the conventional wet-milled samples. A closer look at these enzymes showed that it was mainly the proteases giving the beneficial effects and, specifically, bromelain. Comparison of the enzymatic corn wet milling process with the conventional corn wet milling process showed that significantly higher amounts of starch (approximately 1.0%) and total gluten solids (approximately 3.5%) could be obtained with the enzymatic corn wet milling process (Table II). Further optimization of the bromelain concentration and incubation time showed that increases in enzyme concentration and incubation time improved starch yields significantly (Johnston and Singh, 2001).

Based on these results, several different commercial and experimental protease samples were obtained from enzyme companies and were tested for starch yield using the enzymatic corn wet milling process (Figure 3). Two commercial protease enzymes (enzymes A and C) gave starch yields comparable to the conventionally wet milled sample. Pasting properties, residual protein in starch, and surface characteristics of starch samples obtained from

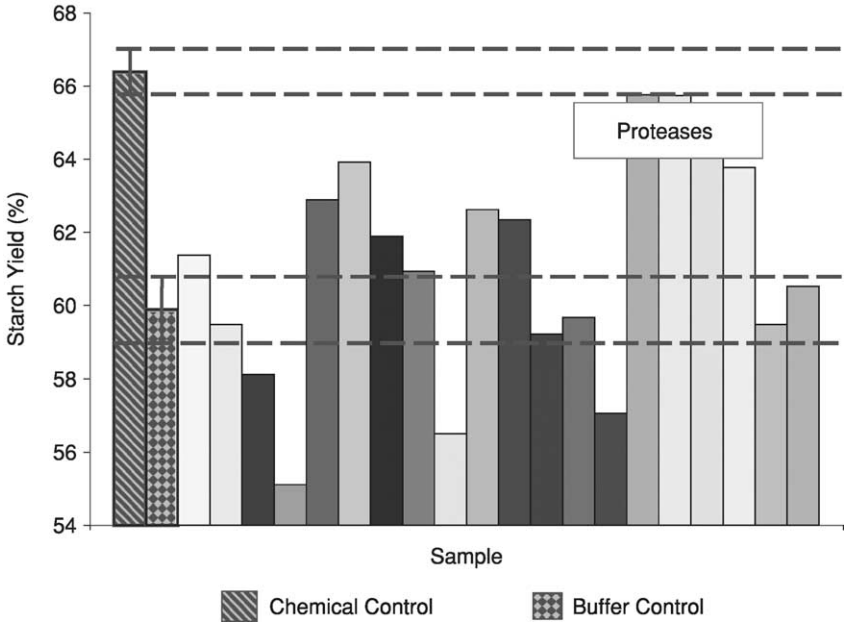

FIG. 2 Effect of different enzymes or combination of enzymes (cellulases, xylanases, cellobiases, β-glucanases, and proteases) on starch yield using the enzymatic corn wet milling procedure.

TABLE II
COMPARISON OF FRACTION YIELDS FROM THE ENZYMATIC AND CONVENTIONAL CORN WET MILLING PROCESS IN A 1-KG LABORATORY PROCEDURE

	Yields (%)		
Fractions	Enzymatic milling	Conventional milling	Difference in yields (%)
Soluble solids	0.12 B[a]	4.30 A	−4.18
Germ	6.15 B	6.73 A	−0.58
Fiber	9.83 B	10.20 A	−0.37
Starch	70.22 A	69.00 B	1.22
Total gluten	12.80 A	9.28 B	3.52
Total	99.13	99.51	

[a]Yields followed by the same letter within a row are not significantly different at a 95% confidence level.

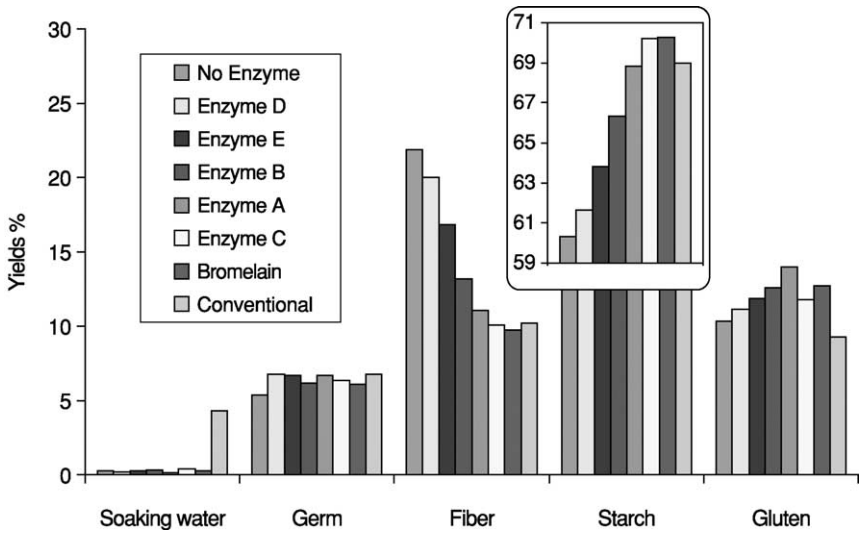

FIG. 3 Starch yields obtained with the use of different commercial protease enzymes and their comparison with the starch yield from a conventionally wet milled corn sample.

proteases were found to be comparable to the conventionally wet milled starch samples (Singh and Johnston, 2002).

VI. BENEFITS OF THE ENZYMATIC CORN WET MILLING PROCESS

The main benefit of the enzymatic corn wet milling process is that it is not a diffusion-limited process and does not require the use of SO_2. Therefore, no steeping is required and the total time required before conventional milling can be shortened to less than 6 hr. Conventional corn wet milling steeping is the most expensive, energy-intensive, and time-consuming unit operation in the corn wet milling process. The elimination of steeping and the reduction in time before milling could have a significant economic impact on the process economics of corn wet milling. Starch and protein yields from the enzymatic wet milling process are approximately 1.5 and 3.5% higher, respectively, when compared to starch and gluten yields from conventionally wet-milled samples. Increased starch yields with the enzymatic wet milling process come from reduction in the loss of starch in the fiber fraction and a cleaner separation of starch and protein fractions. Another potential benefit of the enzymatic wet milling process is the reduction in the amount of water

used in the corn wet milling process. These benefits could potentially reduce the capital cost of a new corn wet milling plant and reduce the operating cost of existing corn wet milling plants. Enzymatic corn wet milling technology potentially could allow existing plants to increase their milling capacity without increasing the steeping capacity of the plant.

VII. ISSUES WITH THE ENZYMATIC CORN WET MILLING PROCESS

The biggest challenge remaining for the enzymatic corn wet milling process is the cost of the enzymes. One of the commercial protease enzymes that works extremely well in the enzymatic corn wet milling process currently sells for approximately $15/lb. Preliminary economic analysis done with the enzymatic corn wet milling process shows that a 30-fold reduction in cost of the enzyme is required for the process to be economically comparable to the conventional corn wet milling process (Singh and Johnston, 2002). In this preliminary analysis, only the increase in prime product yield and removal of SO_2 was factored in. If all the potential benefits of enzymatic milling are factored in the analysis, such as elimination of steeping, steepwater evaporation, reduction in the amount of water used, and reduction in utilities, then only a 2- to 3-fold reduction is required in the cost of the enzyme. It is possible that several other commercial protease enzymes may be available that will provide similar results and are less expensive. Currently, the screening of commercial protease enzymes is ongoing.

Another issue that remains to be resolved with the enzymatic corn wet milling process is possible microbial problems in the soaking and milling unit operations. One of the roles of SO_2 in the conventional corn wet milling process is the control of microbial contamination. It is possible that if the SO_2 is eliminated entirely, as in the enzymatic milling process, some fungi or other microorganisms might grow and cause odor problems or the total microbial count in final starch might increase. It has been shown that a small amount of SO_2 (200–600 ppm) (enough to keep microbes under control), when used in conjunction with the protease enzymes, does not have any detrimental effect on the starch yield and starch quality (Johnston and Singh, 2001). Currently, research is underway at the Eastern Regional Research Center, Agricultural Research Service, United States Department of Agriculture and the University of Illinois to develop strategies for microbial control in the enzymatic corn wet milling process.

The protein obtained from the use of bromelain in the enzymatic corn wet milling process is different from that of the conventional corn wet milling process. Bromelain has a very wide spectrum of protease activities. Bromelain not only degrades the glutelin matrix that surrounds starch

particles, but also breaks down other classes of proteins in the corn kernels. Comparison of Sodium dodecyl sulfate–polyacrylaxide gel electrophoresis (SDS–PAGE) data for protein samples showed lower molecular weight peptides with bromelain when compared to the conventionally wet milled sample (Figure 4). Excessive breakdown of the protein fraction possibly could create recovery problems on the gluten belt filters (equipment used currently by the wet milling industry to recover protein). However, this problem is reduced greatly with the use of the commercial protease (mentioned previously) in the enzymatic corn wet milling process. Protein quality and profile, obtained with the commercial protease (using SDS–PAGE), is comparable to the protein quality of conventionally wet milled samples.

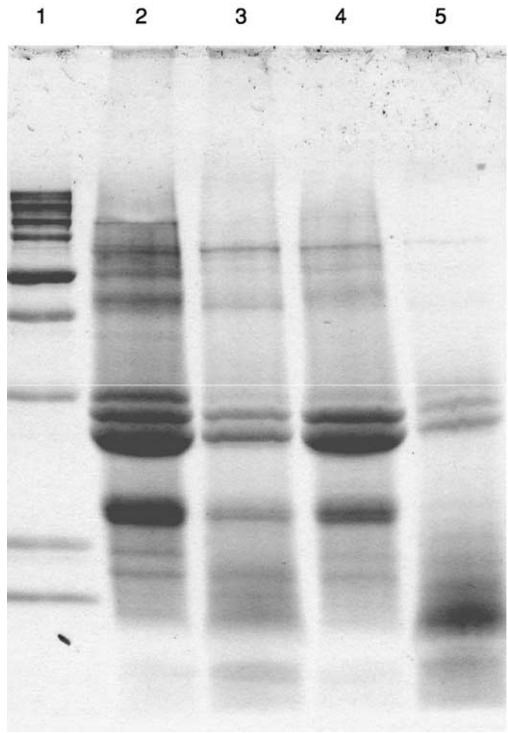

FIG. 4 SDS gel electrophoresis of insoluble gluten samples from laboratory corn wet milling. Lane 1: Molecular mass standards (250, 150, 100, 75, 50, 37, 25, 15, and 10 kDa). Lane 2: Enzymatic milling with commercial protease with added SO_2 and lactic acid. Lane 3: Enzymatic milling with commercial protease and no added SO_2. Lane 4: Conventional laboratory milling. Lane 5: Enzymatic milling using Bromelain and no added SO_2.

VIII. FUTURE OF THE ENZYMATIC CORN WET MILLING PROCESS

The enzymatic corn wet milling process has passed the proof of the concept stage. Scale-up studies (pilot plant and plant trials) are currently being planned with corn wet milling and enzyme companies. It is anticipated that this process will be adopted by corn refiners in the next 10 years. At the time of writing of this article, a commercial license for the enzymatic corn wet milling process is being negotiated.

Although the enzymatic corn wet milling process was developed for corn wet milling, it currently is being investigated for use in the dry grind ethanol process with additional modifications (Singh *et al*., 2003). In the dry grind process, the kernel is ground using a hammer mill. The dry granular material is mixed with water to form a slurry, which is cooked at approximately 160 °C using pressurized steam to break down the crystalline structure of starch granules. α-Amylase is added to break down starch polymers into short chain molecules, called dextrins, to form mash. The mash is held at an elevated temperature (~70 °C) for a short period of time, cooled to approximately 32 °C, and transferred into the fermentation vessel. Glucoamylase and yeast are added for simultaneous saccharification and fermentation. In the mash, glucoamylase breaks down the dextrins into sugars, such as glucose, while yeast ferments these sugars into ethanol. At the end of fermentation, the resulting beer is transferred to a holding tank called a beer well. From the beer well, the beer is transferred to a stripper/rectifier column to remove ethanol. Overflow from the stripper/rectifier column is an ethanol and water mixture, and underflow from the column is whole stillage (all nonfermentable components of corn, yeast, and water). The ethanol and water mixture is processed further through a distillation column and molecular sieves to remove remaining water from the ethanol. Whole stillage is centrifuged to produce thin stillage (water and soluble solids) and wet grains (suspended solids). Using an evaporator, thin stillage is concentrated into syrup and is mixed with the wet grains, which is dried to produce a coproduct with 12% moisture content. This coproduct is marketed as distiller dried grains with solubles (DDGS). The value of DDGS is lower relative to the coproduct value of germ, protein, and fiber from corn wet milling. Also, the traditional markets for DDGS utilization currently are near saturation in the United States. There is a need to reduce the volume of DDGS and diversify the markets for its utilization.

New process modifications have been developed for the conventional dry grind corn process such as quick germ (Singh and Eckhoff, 1996, 1997) and quick fiber processes (Singh *et al*., 1999b; Wahjudi *et al*., 2000). These

process modifications allow cost-effective removal of germ and pericarp fiber as coproducts at the beginning of the dry grind corn process. The quick germ process involves soaking kernels in water for a short period (12 hr) followed by a coarse grind and germ recovery by density separation using germ hydrocyclones. Maintaining the right density of the slurry is critical for effective germ separation. The quick fiber process requires increasing the density of the slurry further to allow pericarp fiber to float and using germ hydrocyclones to recover pericarp fiber (Singh and Eckhoff, 2001). Savings in capital costs can be realized by combining the quick germ and quick fiber processes. Benefits of the quick germ and quick fiber processes are (1) recovery of germ for corn germ oil and fiber for corn fiber oil; (2) removal of nonfermentable components (germ and fiber) from the fermenter, therefore, potentially increasing fermentation capacity of the process; and (3) removal of germ and fiber (Singh and Eckhoff, 1997; Singh *et al*., 1999; Taylor *et al*., 2001), which increases the protein content of the residual DDGS after fermentation.

Combining the quick germ and quick fiber processes for dry grind corn processing with the enzymatic corn wet milling process, followed by a process to recover cellular fiber, would allow dry grind ethanol producers to recover individual components of the corn kernel at a much lower capital cost when compared to a wet mill. Moreover, the unresolved issues observed with the enzymatic corn wet milling process are resolved when the enzymatic process is applied to dry grind. In the dry grind process, microbes are controlled by thermal sterilization and not SO_2 and, therefore, the microbial problem is not an issue. Dry grind ethanol plants are currently set up to recover soluble as well as insoluble proteins and, therefore, any breakdown of the corn proteins by the proteases is no longer a problem.

The commercial protease enzyme that worked well in the enzymatic corn wet milling process is currently used by the dry grind ethanol industry to increase the free amino nitrogen (food for yeast microorganisms) during the fermentation process. In the enzymatic dry grind ethanol process, the point of addition of this enzyme would change; otherwise, the dosage and its effect (on fermentation) should remain the same.

Enzymatic milling, when applied to dry grind, adds more value to the dry grind ethanol process, reduces the volume, and increases the protein content of DDGS. Singh *et al*. (2003) reported results from enzymatic milling applied to dry grind and showed that the protein content of DDGS increased to about 58% and that the volume of DDGS was reduced by more than 60%. It is very likely that enzymatic milling will be adopted by the dry grind ethanol industry prior to it being adopted by the wet milling industry.

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