Wet-milling characteristics of propionate-treated high-moisture corn

by

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INTRODUCTION

The wet-milling industry plays a significant role in modern American society; starch produced by corn wet milling now dominates food and industrial starch markets. Corn sweeteners, derivatives of corn starch, have become the dominant sweetener in the beverage industry. Wet corn mills need a steady year-round supply of high-quality corn and corn must be dried to 14% moisture or less for safe long-term storage. When energy prices get high, as occurred in the early 1970s, some farmers turn to chemical applications to inhibit deterioration by molds. Propionic acid has become the preferred treatment and provides safe storage for over a year when properly applied to high-moisture.

Because over 10% of the corn produced in this country is consumed by the corn wet-milling industry, it is important that propionic acid treatments not adversely impact wet-milling characteristics. There are no published reports on the wet-milling characteristics of propionic acid-treated high-moisture corn. Acid-preserved corn may allow much shorter steeping time because of the higher moisture content already present. In order to utilize propionic acid-preserved corn, it is necessary to understand the effects of propionic acid on wet-milling operations. The objectives of this study were:

(1) to determine the effects of propionic acid on the wet-milling process and quality of its products;

(2) to explore solutions, if any adverse effects on the wet-milling process were observed.

LITERATURE REVIEW

Corn Kernel And Its Components

Kernel Structure

Corn kernels are composed of four parts: bran, endosperm, germ (or embryo), and tip cap. Bran, also known as pericarp, is the smooth, dense, outer covering of the kernel. Botanically, the pericarp is the combination of the fruit coat and seed coat. The endosperm has three major parts: the aleurone layer, the floury endosperm, and the horny endosperm. The embryo includes the scutellar and the embryonic axis. The tip cap connects the kernel to the cob.

Like other cereal crops, corn is rich in starch (61-73% mfb) and also contains protein (6-12% mfb), fat (3.1-5.7% mfb), fiber (8.3-11.9% mfb), and other minor components such as minerals and sugars (Watson, 1987). The amount of each component can be affected by genetic and environmental factors. These components are not equally distributed among the different anatomical parts of the corn kernel. The bran and the tip cap are mainly composed of fiber. The endosperm is the major source of starch and insoluble protein. According to Earle et al. (1946), 98% of the starch and 74% of

the protein is in the endosperm. The germ consists of 50-60% oil (accounting for about 83% percent of total kernel lipid), 30-40% protein (the majority is water- or salt-soluble), and 10% sugar.

When corn is subjected to wet milling, the germ is first separated from the other parts and crushed for oil. The bran and the aleurone layer of the endosperm and the tip cap are then separated as a fiber fraction and used for livestock feed. The remaining endosperm is separated into starch and gluten (Watson, 1984a).

Corn Starch

Starch exists in corn kernels as granules which range in size from 5 to 25 μm. Starch granules from floury endosperm are larger than starch granules from horny endosperm. The shape of the granule as observed with a light microscope resembles that of the corn kernel itself. The granular structure can be destroyed by such factors as high temperature and high pH. Gelatinization is a characteristic phenomenon of starch, which involves swelling, hydration, and solubilization of starch molecules in the presence of water and high temperature. This results in the loss of crystallity of the native granule.

Starch can be "damaged" by mechanical action during milling (Evers and Stevens, 1987). Starch damage can be

detected by staining (Jones, 1940), plane-polarized light microscopy (Sandstedt and Schroeder, 1960), differential scanning calorimetry (Stevens and Elton, 1971), and X-ray diffraction techniques.

In wet milling, steeping conditions can change starch properties (Takeda et al., 1988). At low pH's in steeping, starch can be acid-modified. Acid-modified starch has the same granular appearance, similar birefringence but lower hot-past viscosity than native starch (Rohwer and Klem, 1984).

Like starch from other cereals, corn starch is composed of two anhydroglucose polymers, amylose and amylopectin. The ratio of the two polymers varies depending on the corn variety and environment factors. Corn with the highest ratio of amylose to amylopectin is known as high amylo-maize, which has 70% amylose and 30% amylopectin. Corn with the lowest ratio is termed waxy corn, which has nearly 100% amylopectin. The ratio of the two polymers affects the physical and chemical properties of starch. Starch with high amylose content has lower hot viscosity and retrogrades easily; and starch with higher amylopectin has higher hot viscosity and retrogrades less.

Corn Protein

Corn proteins are classified into storage proteins and cytoplasmic proteins. The corn proteins can also be

classified into albumins, globulins, prolamins (zein), and glutelins on the basis of solubility. In corn endosperm, the amounts of each protein are: 8.0% albumin, 9.0% globulin, 39.0% prolamin, and 40.0% glutelin. The amounts of these proteins in the germ are quite different: 30.0% albumin, 30% globulin, 5% prolamin, and 23% glutelin (Lazstity, 1979). Another accepted fractioning method is the Landry-Moureaux method, by which glutelins can be further divided into three subgroups by using three different extraction solutions.

The storage proteins in the endosperm exist as protein bodies and protein matrix. The latter embeds starch granules (Watson, 1987). Zein is essential to the formation of protein bodies.

Corn Preservation

In modern agricultural practice, corn is generally harvested early to avoid field losses of grain due to weather. Harvesting equipment operates optimally when the moisture ranges from 18 to 22%. Excessively dry corn is more easily broken by combines. However, corn at the normal moisture level for optimum harvesting is prone to mold deterioration. High-moisture corn needs to be handled and stored properly to prevent fungal growth and nutritional losses. Corn can be stored in two ways, dry storage and wet storage. Freshly

harvested corn with high moisture content needs to be dried to safe moisture content, about 14%, for long-term storage. In developing countries, drying is done using solar energy. In developed countries, the drying is usually done with artificially heated air from burning fossil energy. With increasing costs for fuel and periodic fuel shortages, a lot of attention has been focused on finding alternative storage methods to reduce drying costs. Controlled atmosphere and chemical preservation are two major means of wet storage. Controlled atmosphere preservation includes fumigation, oxygen depletion, and pheromones. The chemicals popularly used to control growth of molds in grain are volatile organic acids and their salts, such as propionic, acetic, and isobutyric (Sauer et al., 1975). Propionic acid and mixtures of propionic and acetic acids are marketed to prevent mold growth and spoilage in corn containing up to 30% moisture (Sauer and Burroughs, 1973).

Corn Drying and Its Effects on Wet Milling

The frequency of processing problems in wet milling of artificially-dried corn has been increasing. These problems include: difficult and incomplete grinding with consequential loss of starch in by-product feed streams; poor germ separation leading to lower oil recovery; poor color and high fatty acid content of the oil; and poor starch and gluten

separation resulting in lower starch recovery and purity (MacMaster et al., 1959).

During artificial drying, especially at high temperatures (>80°C), a number of undesirable changes occur. Brown et al. (1979) showed that corn dried at high temperature had lower germination rates, higher amounts of kernel stress cracking, and lower test weight. These changes may lead to poor wet-milling performance. These researchers studied alternative drying methods to avoid the adverse effects of drying on wet milling. Lower temperatures (<60°C) for longer periods were considered, but mold growth during longer periods was a prevalent problem. Another significant change observed in artificially-dried corn was decreased protein solubility, which could also contribute to the adverse effects of drying on corn wet-milling quality (McGuire and Earle, 1958). Recently, Weller (1987) studied the effects of varieties, harvest moisture and drying temperature on starch recovery. He showed that starch recovery decreased as both harvest moisture and drying air temperature increased. Some have attributed this to heat denaturation of native enzymes. These enzymes are believed to play important roles during steeping (Steinke, 1988).

A method developed by Baird et al. (1950) made it possible for wet millers to test at the time of purchase whether the corn had been exposed to high temperature. The

method used 2,3,5-triphenyltetrazolium to test corn viability. Lower viability indicated more severe heat exposure. Glutamic acid decarboxylase activity has also been used as an index of heat damage during artificial drying (Gloria and Linko, 1962).

Artificial drying plays an important role in food and grain preservation, but the wet-milling industry has shown a great deal of concern about the high frequency of severely heat-damaged grain. Furthermore, cost of artificial drying is high and dependent on the world petroleum market. Therefore, alternative grain storage methods may be beneficial to both the wet-milling industry and to corn growers.

Acid Preservatives

In 1945, Cameron reported that the treatment of grain with butyric acid prevented growth of molds. Since then, volatile organic acids, such as formic, acetic, and propionic, have also been found to be effective in prohibiting mold growth. It had been believed that it was not commercially feasible to use these chemicals for long-term corn storage until the late 1960s when British Petroleum Ltd. demonstrated the usefulness of propionic acid for long-term storage of high-moisture grain. Sauer et al. (1975) conducted a very comprehensive comparison of chemical preservatives. Propionic acid was found to be the most consistently effective mold inhibitor for corn and sorghum with moisture contents of

18-24%. Isobutyric, acetic, and formic acids followed in order of effectiveness. Salt forms of these acids were less effective.

In a subsequent report by the same group of researchers (Sauer et al., 1975), it was stated that the efficacy of methylene bis propionate was equal to or slightly better than propionic acid when applied to corn harvested at 23% and 29%. Treatments of 23% moisture corn with 0.7% methylene bis propionate and of 29% moisture corn with 0.9% methylene bis propionate kept corn mold-free for more than eight months. Methylene bis propionate did not become as popular as propionic acid because the former is water insoluble, which made the application less practical.

Another potential high-moisture grain preservative is ammonia. Bothast et al. (1974) demonstrated the ability of ammonia to inhibit grain storage molds such as *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and *Rhizopus*. A solution composed of ammonia, urea, biuret, and urease can release ammonia constantly to the grain environment during storage (Cauwenberge et al., 1981).

A number of researchers have evaluated the effects of these acids on animal growth. It has been shown that propionic acid has no adverse effects on animal health and performance (Jones et al., 1970). Organic acid-treated, high-

moisture grain provides a readily acceptable feedstuff which is capable of supporting high levels of milk production by dairy cows or rapid gains in body weight by growing-finish animals (Jones et al., 1974). The Food and Drug Administration and the Environment Protection Agency have approved propionic acid, acetic acid, isobutyric acid and ammonium isobutyrate for use as preservatives in grain used for livestock feeds. But, grain treated with preservatives is still not approved for processing into human foods (Wilcox, 1985).

Wet-Milling Quality of Acid-Preserved Corn

There are no published reports on the effects of these preservatives on wet-milling characteristics of chemically-preserved corn which is of great concern to wet millers. A number of potential disadvantages for propionic acid-treated high-moisture corn have been discussed (Freeman, 1973): transportation costs could be higher as a result of transporting at higher moisture contents; acids may be corrosive to handling, storage, and milling equipment; the level of carotenoid pigments may be reduced; the germ oil may be oxidized during storage; pollution problems could be increased. However, it has also been suggested that less steeping time may be required to achieve good wet-milling properties with propionic acid-treated high-moisture corn

(Freeman, 1973).

However, Freeman (1973) concluded that acid preservation was likely to be confined to storage of grain destined for feed use. If this method of storage becomes widely practiced, it will be necessary to break this utilization limitation. Therefore, the effects of acid treatment on wet-milling characteristics need to be studied.

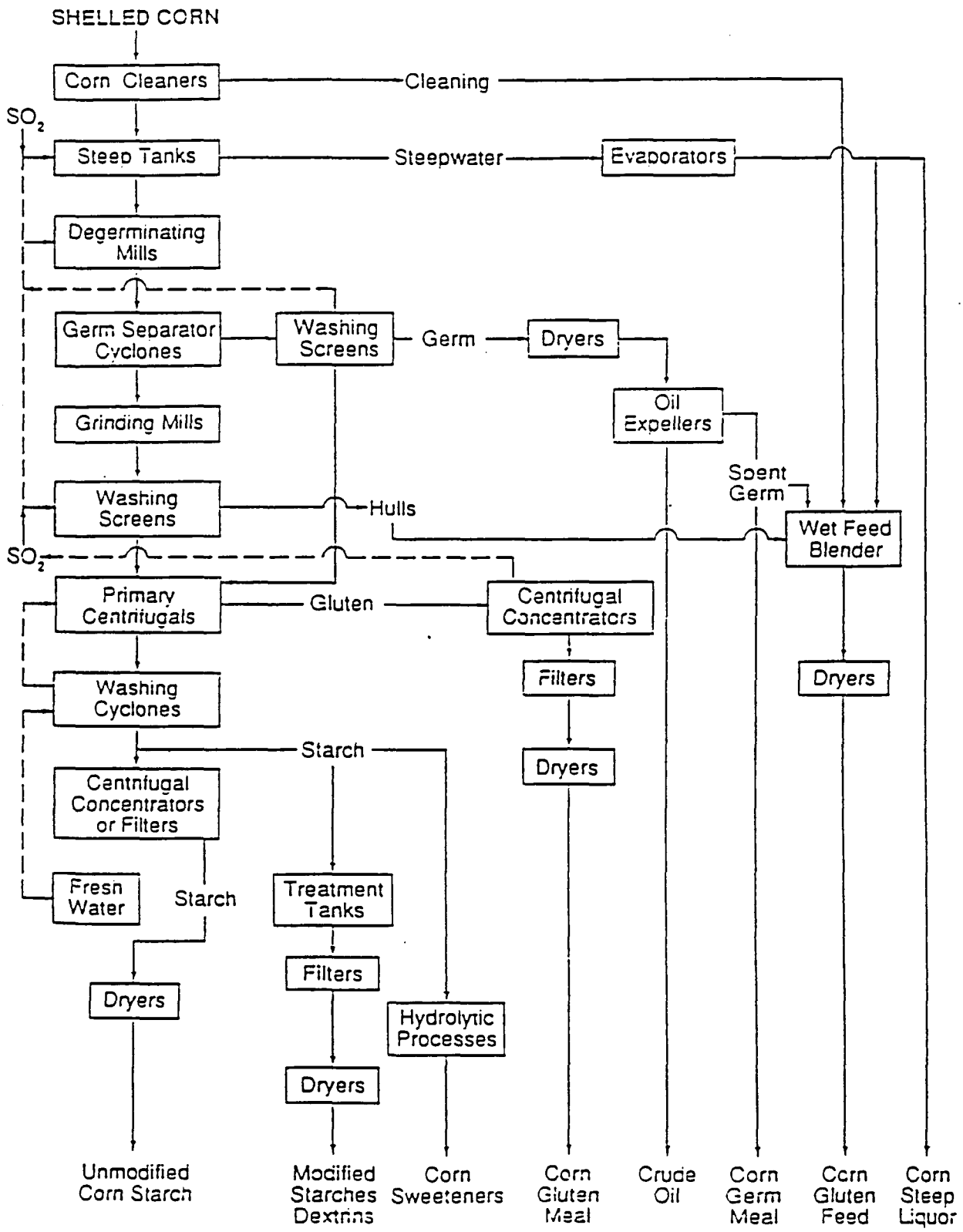
Corn Wet-Milling Process

The corn wet-milling process consists of several steps to convert corn to end products of starch, oil, and feeds. The process includes steeping, degermination, fiber removal, starch and gluten separation, and starch purification as shown in Figure 1.

Steeping

Clean shelled corn needs to be soaked in dilute (0.1-0.25% sulfur dioxide) sulfurous acid solution at about 50°C for 48 hr before going to grinding and separation operations. Steeping is more than a simple soaking process. A number of changes occur during corn steeping. Kernels become soft, moisture increases from storage level (10-20%) to saturation (about 45%), 3-6% soluble solids are lost to the steeping solution, and the parts of the corn kernel become

Figure 1. Flow diagram of the wet milling process (Johnson, 1988)


SHELLED CORN
Corn Cleaners
Cleaning
SO_2
Steep Tanks
Steepwater
Evaporators
Degerminating Mills
Germ Separator Cyclones
Washing Screens
Germ
Dryers
Oil Expellers
Grinding Mills
Spent Germ
Washing Screens
Hulls
Wet Feed Blender
SO_2
Primary Centrifugals
Gluten
Centrifugal Concentrators
Filters
Dryers
Washing Cyclones
Starch
Dryers
Centrifugal Concentrators or Filters
Fresh Water
Starch
Treatment Tanks
Filters
Dryers
Hydrolytic Processes
Dryers
Unmodified Corn Starch
Modified Starches Dextrins
Corn Sweeteners
Corn Gluten Meal
Crude Oil
Corn Germ Meal
Corn Gluten Feed
Corn Steep Liquor

easier to separate.

Sulfur dioxide (SO_2) was initially used to prevent the growth of bacteria. Later it was learned that SO_2 is more than a preservative. With SO_2 in the steeping solution, corn kernels become softer and easier to mill. The most important effects of SO_2 were first illustrated microscopically by Cox et al. (1944). They found that the matrix protein in which starch is embedded disintegrated when SO_2 was in the steeping solution. This enhanced starch and gluten separation. This finding was confirmed by a later study of Watson et al. (1951). They postulated that the mechanism was that the SO_2 functioned as a reducing agent breaking down the disulfide bonds in the matrix protein. Recently, Krochta et al. (1981) showed that as the SO_2 concentration increased, mill-starch yield increased.

Lactic acid is also believed to play an essential role in corn steeping, especially in softening the kernel. However, there is little scientific evidence concerning the function of this organic acid. It may be that exoenzymes produced by *Lactobacillus* are active during steeping. It is not known if propionic acid inhibits the growth of *Lactobacillus* and whether propionic acid has similar effects as lactic acid during steeping.

The steeping system used by the current wet-milling industry is a countercurrent system instead of single batch steeps. The system normally consists of 6-12 tanks, each tank having the capacity of 2,000-13,000 bu (May, 1987). The fresh steeping water, which has the highest SO_2 concentration (0.1%-0.2%) and lowest solids content, contacts the corn that is about to leave the system. The solution is circulated within a tank and passes through to the next one. Finally the solution contacts the newest corn before leaving the system with reduced SO_2 (0.01%) and increased solids (7%) contents (Watson, 1984).

When propionic acid-treated high-moisture corn is steeped in a countercurrent system, the acid in the corn would be extracted in the early steeps and would be removed with the steep liqour. There should not be much residual acid in latter steeps or in the starch.

There have been several attempts to shorten the steeping time and reduce volume of steeping solution (typically, 2 g water/g corn) to save energy consumed in this step. In the work of Krochta et al. (1981), the yield of mill-starch obtained from corn steeped with reduced steep solution (1 g water/2 g corn) was comparable to normal operation so long as the amount of SO_2 per weight of corn remained the same. Increasing the SO_2 concentration or removing the mass transfer barrier between the endosperm and the steep solution (by

degerminating) between two steeping periods improved mill-starch yield. Roushdi et al. (1981) also showed that using increased concentration of SO_2 solution increased the yield of starch and reduced the protein content of the extracted starch, although SO_2 inhibited lactic acid formation. Use of Alcalase and Neutrase, proteolytic enzymes, during steeping of broken and scratched corn decreased steeping time by 50% (Roushdi et al., 1981). It was also found that proteolytic enzyme activity in the germ and endosperm played an important role in softening corn kernels and in starch-protein separation (Wahl and Barby/Elble, 1971; Wall and Pairlis, 1978). More recently, Steinke (1988) found that addition of enzymes to the corn steep solution increased starch yield and purity.

Processing high-moisture corn could be a way to reduce steeping time. High-moisture corn needs less time to reach moisture saturation than dried corn. Furthermore, eliminating drying will avoid heat damage.

Degermination

The steeped corn is ground in an attrition mill. The common attrition mill has one stationary and one rotating surface. The gap between them can be adjusted in order to maximize free germ and minimize germ breakage. The ground wet mass is transferred to hydroclones to separate the germ from

the rest of the slurry. Germs have lower specific gravity than endosperm particles because of the higher oil content and germs exit in the overflow from the hydroclones. Recovered germ is washed and dried for oil extraction. Over half of the starch and gluten (mill-starch) is also freed in this first milling step and can be separated from fiber by screening. The starch recovered from this slurry is called prime starch which is mainly from floury endosperm. The prime starch has higher purity than the whole starch (Smith et al., 1966).

Degermination is a crucial step in wet milling, high yield and purity of germ lead to high oil yield and more importantly to high quality of starch. Oil contamination of starch alters physical and chemical properties of starch, and contributes off-flavors due to rancidity. Poor steeping results in poor degermination, since insufficient solids in the germ diffuse into the steep solution to reduce the specific gravity of germ (Watson, 1967). In present industrial practice, starch is added to increase the specific gravity (7-8 Baumé) of the separation medium to improve the degermination efficiency (Watson, 1984).

Second Milling and Fiber Separation

The underflow from germ cyclones, containing fiber and pieces of horny endosperm, is more thoroughly milled. The objective of this second milling step is to obtain maximum

starch release with minimum fiber attrition. Starch and gluten are better separated with washing when the fiber is not too fine.

The current wet-milling practice uses screen bends, also called DSM screens, over which the slurry is pumped with considerable force, and the fiber and fine slurry are separated by particle size. Fiber is usually washed off the screen bends by water and then dewatered by centrifugal screens and mechanical squeezers or continuous-discharge centrifuges (Bier et al., 1974). Fiber can be either sold directly as an industrial raw material after drying or be blended with other wet-milling by-products to make gluten feed.

Starch and Gluten Separation

The defibered slurry, mixture of starch and gluten, is known as mill-starch. The low density of gluten particles (1.2 g/cc) compared to starch (1.5 g/cc) permits their ready separation by settling or centrifugation (Watson, 1984). In old wet-milling plants, starch tables were used to accomplish this separation. A long table was positioned at an incline, the slurry starts at the top end and flows slowly down the table. Eventually the starch settles down on the table and the gluten flows off the table, additional gluten is washed from the starch and off the table. This old technique was

labor intensive and was given up many years ago. Starch tables were replaced with disc-nozzle type centrifuges (May, 1987) where the heavier starch granules are thrown to the periphery of the centrifuge bowl and are ejected through nozzles. The lighter gluten particles are carried up between the discs by a stream of water and are ejected at a low solids concentration. Centrifuges for starch separation have advantages over starch tables due to purity and efficiency that are achieved, but centrifuging is energy intensive and requires frequent cleaning and adjustment. Chwalek and Schwartz (1979) suggested that the entire starch-protein separation could be completed by using hydroclones without centrifugation. This system produces gluten with >70% protein and starch with <0.33% protein (dry weight basis) at much lower cost.

The gluten discharge from the centrifuge is concentrated by filtration or centrifugation and blended with fiber and steep water concentrate to form gluten meal. The starch discharge from the centrifuge usually needs to be diluted and goes through one more centrifugation to get rid of residual gluten. After this, the starch still requires 8-14 stages of hydroclones to wash away solubles and further purify it (Vegter, 1954; 1957).

Water in Wet Milling

With about 1420-1830 L of water needed to wet mill one metric ton of corn, water is essential in all of the steps in wet milling. Proper use and reuse of water are critical to reducing cost and minimizing pollution.

Water is reused several times. The reuse process is countercurrent to the flow of corn. Fresh demineralized water is introduced only at the starch washing step. Fresh water first enters the last starch washing cyclone, passes through the next hydroclone, and finally exits the starch washing step in the first starch washing hydroclone. Then the water goes to the primary centrifuge where it is used to adjust the specific gravity of the starch-gluten slurry and ends up in the separated gluten. Water is recovered when the gluten is concentrated, and then sent to fiber washing and to germ washing. After these washing steps, water, which contains 5-6% solids, is used for steeping after addition of SO_2. The movement of water in the steeping system itself is also countercurrent to the movement of corn. Water finally exits the wet milling system after steeping the newest corn in the form of steep solution which contains about 7% solids. The steep solution solids can be concentrated to steep liquor (40-50% solids) in an evaporator. The vapor is condensed and recycled for processing water.

MATERIALS AND METHODS

Corn Treatment

Corn

Yellow dent Pioneer 3475 corn with 25% moisture was harvested by combine from agronomy test plots of the Iowa Agricultural and Home Economics Experiment Station at Ames, Iowa. The harvested corn was cleaned the same day using the Carter dockage tester equipped with a 12/64R screen to remove the broken corn-foreign material (BCFM). The moisture content was determined using AOAC method 14.003 (AOAC, 1980) after the corn was cleaned. Then the corn was stored at 4°C overnight and treated the following day.

Chemicals and Application Dose

Propionic acid and acetic acid are the two major products from *Propionibacteria* fermentation. The ratio of the two acids produced is 5 moles propionic acid to 1 mole acetic acid. The usual products from the fermentation process are the sodium salts of these acids. Therefore, in addition to pure commercial propionic acid, the mixture of sodium propionate and sodium acetate was used as a treatment. The weight ratio of sodium propionate to sodium acetate was 4.86:1 in order to

make the ratio of the two acids 5:1. The salts were applied in solution form by adjusting the pH with HCl to 9.6 (pH of the mixed salt solution), 4.8 (the pKa of propionic acid), and 1.8 (the pH of pure propionic acid). Thus, as in Table 1, there were four treatments: pure propionic acid, salt solution of propionate and acetate, semi-acidified salt solution of propionate and acetate, and acidified salt solution of propionate and acetate. The salt solution treatment (pH 9.6) failed to keep the corn mold-free for two weeks of storage, so this treatment was dropped. The application dose of each treatment was 1% propionate based on the weight of the corn (as-is basis). The same untreated corn was dried to 12% moisture content using a forced-air drier at room temperature for about 24 hr. The dried corn with 12% moisture content was used as a control for the study.

Solution Preparation

Each treatment was designed to: minimize the amount of moisture added to the corn; provide sufficient water to dissolve all of the salt solids at 25°C; and achieve equivalent propionate ion levels (1% propionate based on the weight of corn).

Pure propionic acid Treatment of 50 kg corn required 511.97 g of commercial propionic acid (99% purity).

Salt solution A solution was prepared using 664.18 g of

sodium propionate, 137.22 g sodium acetate, and 801.40 g of

Table 1. Corn treatments and solutions

Treatments	Propionate Solution				
	pH	Propionate Content (%)	Water[a] Content (%)	Corn Moisture (%)	Propinate Dose (g/100 corn)
Pure acid	1.80	98.00	0.00	25.78	1.00
Salt solution	9.60	31.54	50.00	26.00	1.00
Semi-acidified	4.80	21.19	59.28	26.59	1.00
Acidified	1.80	18.32	61.27	29.40	1.00
Control	---	---	---	12.00	0.00

[a]Moisture content after treatment, initial moisture content of the corn was 25.0%.

water to dissolve the salts. The solution had 31.54% propionate and 50% water. This was sufficient to treat 50 kg of corn.

Acidified salt solution Salt solution was prepared using the same formula as above. Then 730.4 ml of hydrochloric acid was slowly added to the solution. With the addition of HCl, NaCl crystallized out and additional water (395.94g) was necessary to dissolve NaCl. Minor pH adjustment was necessary to bring the pH of the solution to exactly 1.80. The solution had 18.32% propionate and 61.27% water. This was sufficient to treat 50 kg of corn.

Semi-acidified solution The procedure was the same as the preparation of the acidified salt solution, except that less HCl (68.61 ml) and less additional water (302.57 ml) were used. The final solution pH was adjusted to 4.8. The solution had 21.19% propionate and 59.28% water. This was sufficient to treat 50 kg of corn.

Propionate Application

About 50 kg of cleaned corn was weighed and transferred to a ribbon mixer.[1] The mixing speed was controlled so that breaking of corn kernels was avoided while still attaining adequate mixing efficiency. The appropriate solution was applied to the corn with a 1/2-gal garden sprayer as the corn was being mixed.

Two batches of 50 kg each were treated with each treatment. The treated high-moisture corn was transferred to a 30-gal plastic garbage can with double-lined polyethylene bags, sealed, and placed in storage. The corn samples were stored at 25°C for about six months before they were subjected to wet milling.

[1]Manufactured by Rapid Machinery Company, Marion, IA.

Experimental Design

Eight samples from each treatment were wet milled. Four of them were steeped for 48 hr. and the other four were steeped for 24 hr. The samples for wet milling were obtained from mixing top, middle, and bottom of a basket, so that the samples were representive. Analysis of variance for the results were done using the Statistical Analysis System (SAS). The means for each combination of propionate treatment and steeping time were compared using Duncan's multiple range test. As examples, the ANOVA's for analysis of starch yield and protein contents of starch are given in Appedix.

Laboratory Steeping System

A laboratory steeping system was developed to closely simulate countercurrent steeping practices currently used in the wet-milling industry. Fresh steeping solution feed, solution advancement to the next tank, solution circulation within each vessel, and steep liquor collection were all continuous.

Steeping Vessels

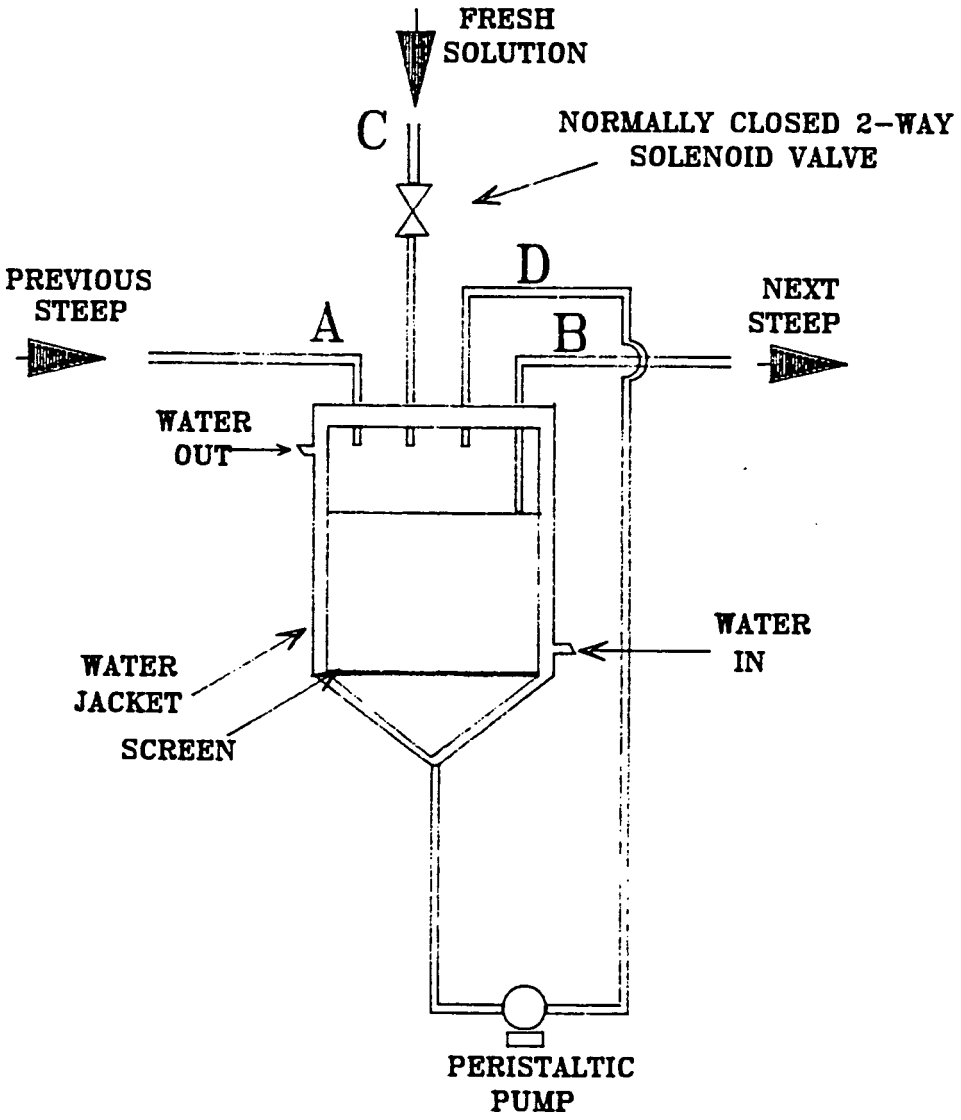
Seven 2-L glass vessels were used to simulate the steeping tanks. Six vessels composed a complete system. The

spare spare was for operational purposes, such as draining, emptying and refilling with fresh corn to be brought on line next. The vessels were jacketed for circulating water from a water bath to maintain the temperature at 50±1°C. A rubber stopper with four tubes was fitted in each vessel. As shown in Figure 2, Each vessel had two inlets, inlet A connected the vessel with the previous vessel of the system, inlet C was for addition of fresh steeping solution. Outlet B connected to the next vessel for solution overflow or steep liquor collection. The depth of tube B was adjusted so that the desired level of solution was maintained in each vessel. Inlet D at the top and outlet D at the bottom of the vessel were used for recirculating steeping solution within the jar. The vessels were secured at the same height on a rack with chain clamps facilitate continuous advancing of steeping solution.

Temperature Control

The temperature control system consisted of a water bath with hot water (51-52°C), a thermally-protected pump (model DC 3C MD), and the tubing for the connections. The hot water was pumped out of the water bath to enter the bottom of each vessel's jacket and exit at the top and flow back to the water bath to be reheated. The lengths of plastic tubes for connecting the pump to the vessels were adjusted so that the

Figure 2. The steeping vessel setup


FRESH
SOLUTION
C
NORMALLY CLOSED 2-WAY
SOLENOID VALVE
D
PREVIOUS
STEEP
A
B
NEXT
STEEP
WATER
OUT
WATER
IN
WATER
JACKET
SCREEN
PERISTALTIC
PUMP

circulation rate to each vessel was equal, thus keeping the same temperature among different vessels. The pumping rate was adjusted by using a voltage controller (Cole-Parmer Inc. model 2603) in order to keep temperature of the steeping at 50°C and not over-pressure the tube connections.

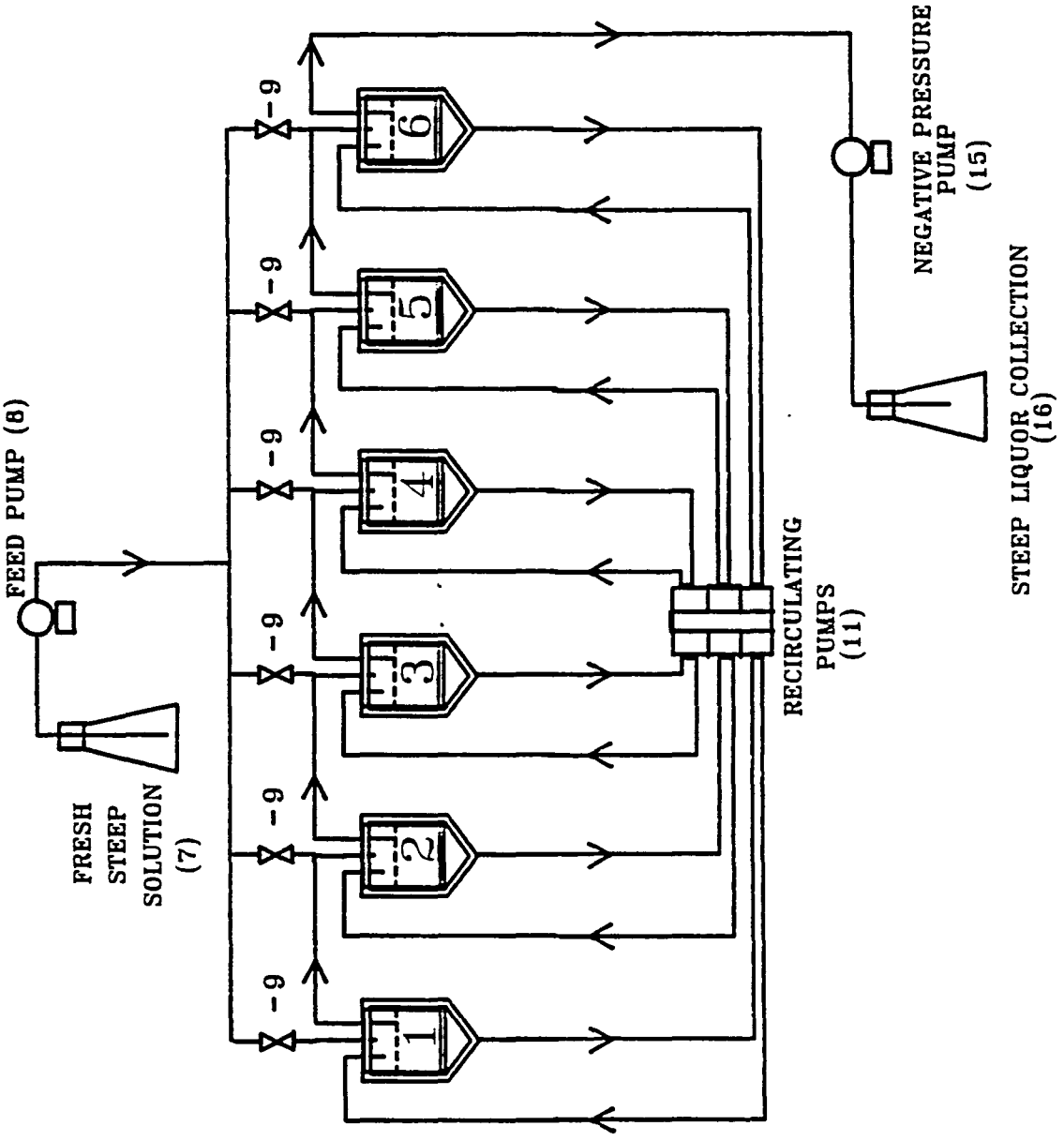
Flow of Steeping solution

Figure 3 shows the continuous counter-current steeping (CCS) system. Feeding of the fresh steeping solution was accomplished by using a small peristaltic pump (Cole-Parmer Inc. Model 70:14) (8). The fresh steeping solution was fed from a 6-L flask (7) and pumped into the first vessel. The feeding rate was controlled at 100 and 200 ml/hr for 48-hr and 24-hr steeping times, respectively.

Advancing steeping solution was accomplished in two ways. Firstly, a peristaltic pump (15) was used to create a positive pressure at the first vessel receiving fresh steeping solution. When the level of the solution reached outlet B, the solution overflowed to the next vessel. Secondly, a negative pressure was achieved with a second peristaltic pump at the end of the system (No. 6 vessel which contained the oldest steep solution).

Recirculation of steep water within the vessels was accomplished by peristaltic pumps (Cole-Parmer Inc. model 70:18) (11). The recirculation rate was adjusted with

Figure 3. Continuous counter-current steeping system


FEED PUMP (8)
FRESH STEEP SOLUTION (7)
1
2
3
4
5
6
-9
RECIRCULATING PUMPS (11)
NEGATIVE PRESSURE PUMP (15)
STEEP LIQUOR COLLECTION (16)

changes in the feeding rate; the ratio of overflow to recirculation was 1:7. The seven pumps for recirculation were installed in one variable-speed driver (Cole-Parmer Inc. model 756800).

Automatic Shifting System for Fresh Steep Solution

The auto-shifting system for fresh steep solution was composed of a timer with a recycler, a step switch, and seven solenoid valves (Dayton model 3A 425) (9). The timer was set to 4-hr time interval for 24-hr steeping and 8-hr time interval for 48-hr steeping. When the timer completed the set time interval, it drove the step switch one step forward. The timer was recycled by the recycler. The step switch was programed so that the switch activated the appropriate solenoid valve allowing fresh solution to fill the steep vessel.

Example

For 24-hr steeping, fresh steeping solution was fed into the No. 1 vessel for 4 hr (timer was set for 4 hr). The solution was recirculated, overflowed along the system, and exited the system from vessel No. 6 as steep liquor, which was collected in a container (16). After 4 hr, the timer activated the solenoid valve connected with vessel No. 2, and fresh solution was fed into No. 2 vessel and the steep liquor

was collected from vessel No. 7 (not shown in Figure 3). Every 4 hr the sequence was repeated with the feed and collection system advancing one vessel. The feed pump (8) was set at a rate so that one vessel became full and overflowed 250 ml of steep liquor in 4 hr.

Before collecting samples, the system needed to run through at least 8 cycles to achieve steady-state. Four samples were then collected and wet milled for each treatment.

In 48-hr steeping, everything was the same as described for 24-hr steeping, except that the timer was set for 8-hr cycletime, and the pumping rate was one-half of that used in 24-hr steeping.

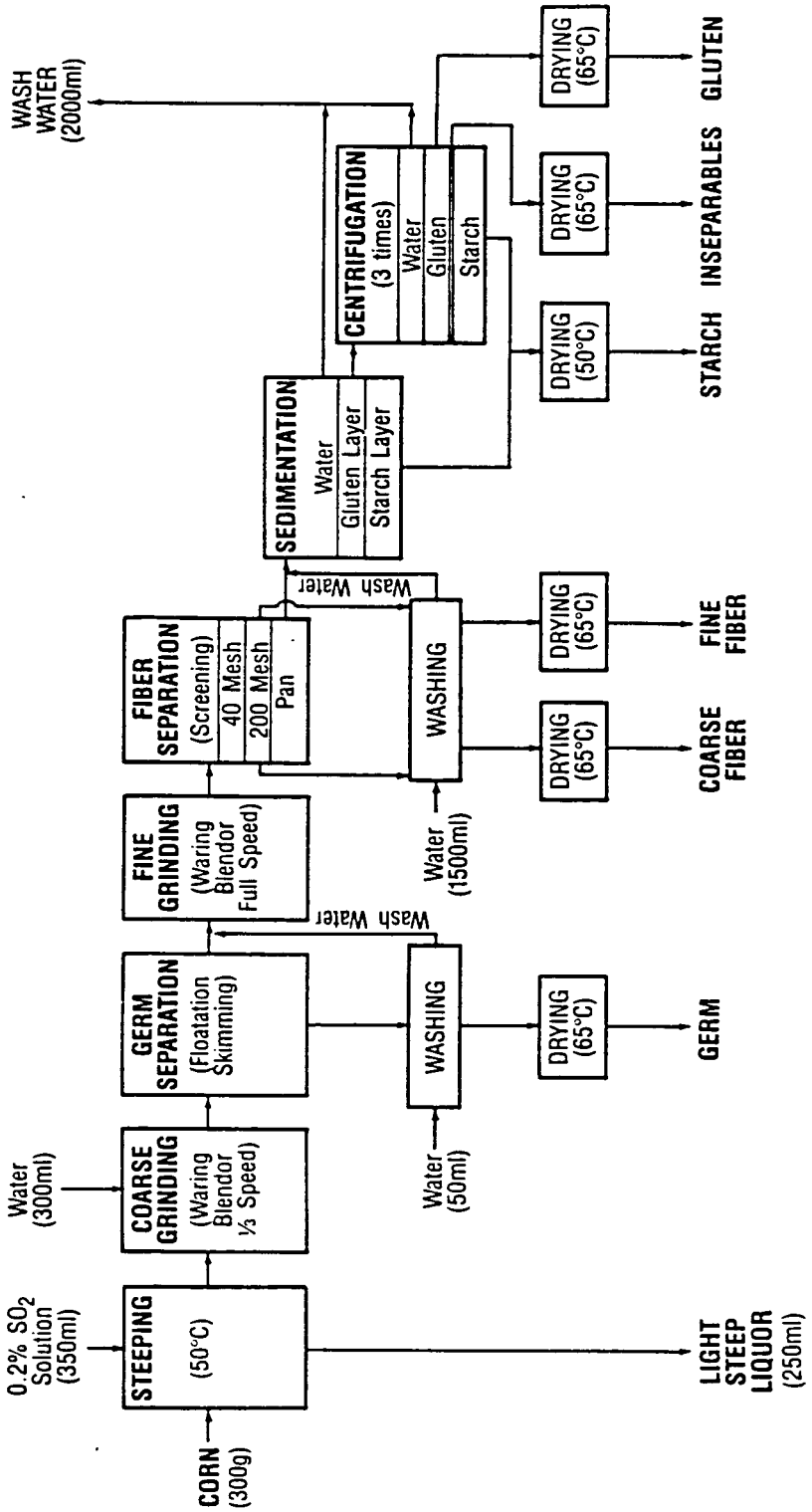
Laboratory Corn Wet Milling

Laboratory wet milling was conducted using a modified Watson Laboratory Method (Watson et al., 1955). Just as industrial wet milling, the procedure includes: steeping, germ separation, fiber separation, and gluten and starch separation. The procedures are summarized in Figure 4.

Steeping

About 300 g of corn was steeped in the continious countercurrent steeping (CCS) system for 24 or 48 hr as previously described. The initial concentration of SO_2 was

Figure 4. Laboratory corn wet milling procedures


CORN (300g)
STEEPING (50°C)
0.2% SO_2 Solution (350ml)
LIGHT STEEP LIQUOR (250ml)
COARSE GRINDING (Waring Blendor ⅓ Speed)
Water (300ml)
GERM SEPARATION (Floatation Skimming)
WASHING
Water (50ml)
DRYING (65°C)
GERM
FINE GRINDING (Waring Blendor Full Speed)
Wash Water
FIBER SEPARATION (Screening)
40 Mesh
200 Mesh
Pan
WASHING
Water (1500ml)
DRYING (65°C)
FINE FIBER
DRYING (65°C)
COARSE FIBER
Wash Water
SEDIMENTATION
Water
Gluten Layer
Starch Layer
WASH WATER (2000ml)
CENTRIFUGATION (3 times)
Water
Gluten
Starch
DRYING (65°C)
GLUTEN
DRYING (65°C)
INSEPARABLES
DRYING (50°C)
STARCH

0.2%.

Degermination

The steeped corn was ground in the first step in a Waring Blendor at 1/3 speed for 2.0 min to release germs from endosperm and pericarp. An equal amount of water was added before grinding. About 100 ml of additional water was added to adjust the slurry density and allow the germs to float to the top of the slurry. Germs floating on the top were skimmed using a piece of steel screen (20 mesh), then the slurry was agitated, and germs were skimmed again. The process was repeated until no more germs separated. The recovered germs were washed twice with distilled water. The wash water was returned to the ground slurry.

Fiber Separation

The slurry was reground a second time in the same blender but at full speed for 2.0 min. The reground slurry was sieved using U.S. No. 40 (35 mesh) and No. 200 (200 mesh) sieves on Tyler Ro-Tap testing sieve shaker.[1] The coarse material remaining on the No. 40 and No. 200 screens were collected separately and washed in three different beakers using nylon porous cloths with 52-μm openings. The washed materials that

[1]W. S. Tyler Inc., Mentor, OH.

remained on the No. 40 and No. 200 sieves were collected and termed 'coarse' and 'fine' fiber, respectively. The wash water and the fine slurry passing through the No. 200 screen were combined as the starch-gluten slurry (mill-starch).

Starch and Gluten Separation

The gluten-starch slurry obtained from the previous step was allowed to settle overnight at 4°C in a 3000-ml beaker. The next day, the clear water was decanted and collected as wash water for later analysis. The solids at the bottom of the beaker were reslurried and transferred to a 1000-ml beaker. Wash water was used to wash the 3-L beaker. The volume of the new slurry was about 800 ml and the slurry was stored at 4°C overnight. During refrigerated storage, starch settled to the bottom of the beaker forming a white layer and a yellow gluten layer settled on the top of the starch layer. The upper volume of clear water was collected and used for washing in later centrifugation steps. The yellow layer was washed out and collected as a gluten slurry. The bottom layer was washed and collected as a starch slurry. The gluten slurry and water from washing the starch layer were combined and centrifuged (Sorvall RC2-B) at 6000 rpm (5860 xg) for 30 min. The top yellow layer was scooped out and collected as gluten. The mixed layer between the gluten and starch layers was washed out and centrifuged again. Starch at the bottom of

the bottle was combined with the starch slurry. The mixed layer slurry generally was centrifuged three times and the unseparated portion after three separations was collected as 'inseparables'.

Drying

The wet-milling products (germ, coarse fiber, fine fiber, gluten and inseparables) were dried in a forced air oven at 60°C for at least 24 hr and in a vacuum oven at 65°C for an additional 8 hr. Starch was dried in a forced-air oven at a lower temperature (50°C) for a longer time (48 hr). Moisture contents were determined in duplicate by AOAC method 14.003 (AOAC, 1981) for each fraction so that the recovery or yield could be calculated on a moisture-free basis.

Product Evaluation or Analysis

Solids Content Determination

About 10 ml of solution was placed in an aluminum weighing dish which was previously dried and tared. The samples were placed in an oven at 90°C for 12 hr and then transferred to vacuum oven and dried for an additional 6 hr at 110°C. The samples were removed from the oven and put in a desiccator for about 30 min for cooling prior to weighing. The solids content was calculated by weight of dry solids

expressed as grams per 100 ml solution. Duplicate solids determination were done on each solution sample.

Protein Determination

Protein content of each wet-milling product was determined using the macro-Kjeldahl method. A Tector Kjeltec System comprised of a model 1007 digester, a model 1002 distilling unit and a titration unit was used.

Different sample sizes were used for different products because of widely different ranges of protein content: 2 g of starch, 1 g of fiber fractions, 0.5 g of gluten, 5 ml of steep liquor, and 10-20 ml of starch wash water.

Starch Quality Evaluation

In addition to protein content, thermal and pasting properties of starch were studied using a visco-amylograph and a differential scanning calorimeter (DSC).

A VISCO/amylo/GRAPH Temperature programmed viscometer (C.W. Brabender Inc.) was used to measure viscosity of the starch slurry at different temperatures. Five hundred milliliters of an 8% starch slurry was prepared for the viscosity measurement. The temperature of starch slurry was programed as follows: starting at 26°C, temperature increased at the rate of 1.5°C/min. for 46 min to reach the maximum temperature of 95°C, then the temperature was held for 15 min

at 95°C, and finally the slurry was cooled at the same rate at which the temperature increased. The viscosity was recorded on amylogragh paper in Brabender Units (BU).

The DSC properties of the starch from air-dried, pure propionic acid-treated, and salt solution-treated corn were determined using a Perkin-Elmer DSC7 analyzer equipped with a thermal analysis data station. Approximately 4 mg of starch was weighed into an aluminum pan with 8 mg of water. The pan was hermetically sealed. The sample was heated from 30°C to 120°C at the rate of 10°C/min. The enthalpy of gelatinization and onset, peak, and conclusion temperatures were calculated by the data system after the peak was defined.

Color Evaluation

Colors of whole kernels and wet and dried wet-milling products (starch, gluten, coarse and fine fiber, and germ) were measured using a Hunter colorimeter. The 'Lab' color unit system was used. In 'Lab', 'L' measured lightness, 'a' measured red (+) and green (-), and 'b' measured yellow (+) and blue (-). The larger the 'a' number, the redder the sample. When 'a' was negative, the sample was green. The larger the 'b', the more yellow the sample. When 'b' was negative, the sample was blue.

After the colorimeter was turned on and calibrated, daylight was chosen as the light source. A container full of

sample was placed in the sensing position and the readings were recorded in 'Lab' values.

Enhancing Starch-Gluten Separation

Starch-gluten separation after the sedimentation step was very poor for the mill-starch from propionate-treated high-moisture corn. Several approaches were explored to enhance the starch-gluten separation.

The pH of the mill-starch slurry (about 16% solids content) was adjusted in 0.5 pH units over the range of 2-10. The starch-gluten separation during sedimentation was evaluated visually.

Potassium bromide, an oxidizing agent, was gradually added (from 0.1 g to 4 g) to 1000 ml of mill-starch slurry (about 16% solids content), visual evaluation of starch-gluten separation was made.

Sodium chloride was gradually added (from 0.1 g to 4 g) to 1000 ml of mill-starch slurry (about 16% solids content). The starch-gluten separation was evaluated for each level of sodium chloride added.

About 2 g of NaCl was added to 1000 ml of mill-starch (about 16% solids content) from air-dried corn and pure propionic acid treated high-moisture corn. A sample with no addition of NaCl was used as control. Three replicates were

done for each treatment. The mill-starch samples were separated into starch and gluten by sedimentation and centrifugation (5860 xg for 30 min.) as previously described (Page 37). Protein contents in starch were determined by macro-Kjeldahl as previously described.

RESULTS AND DISCUSSION

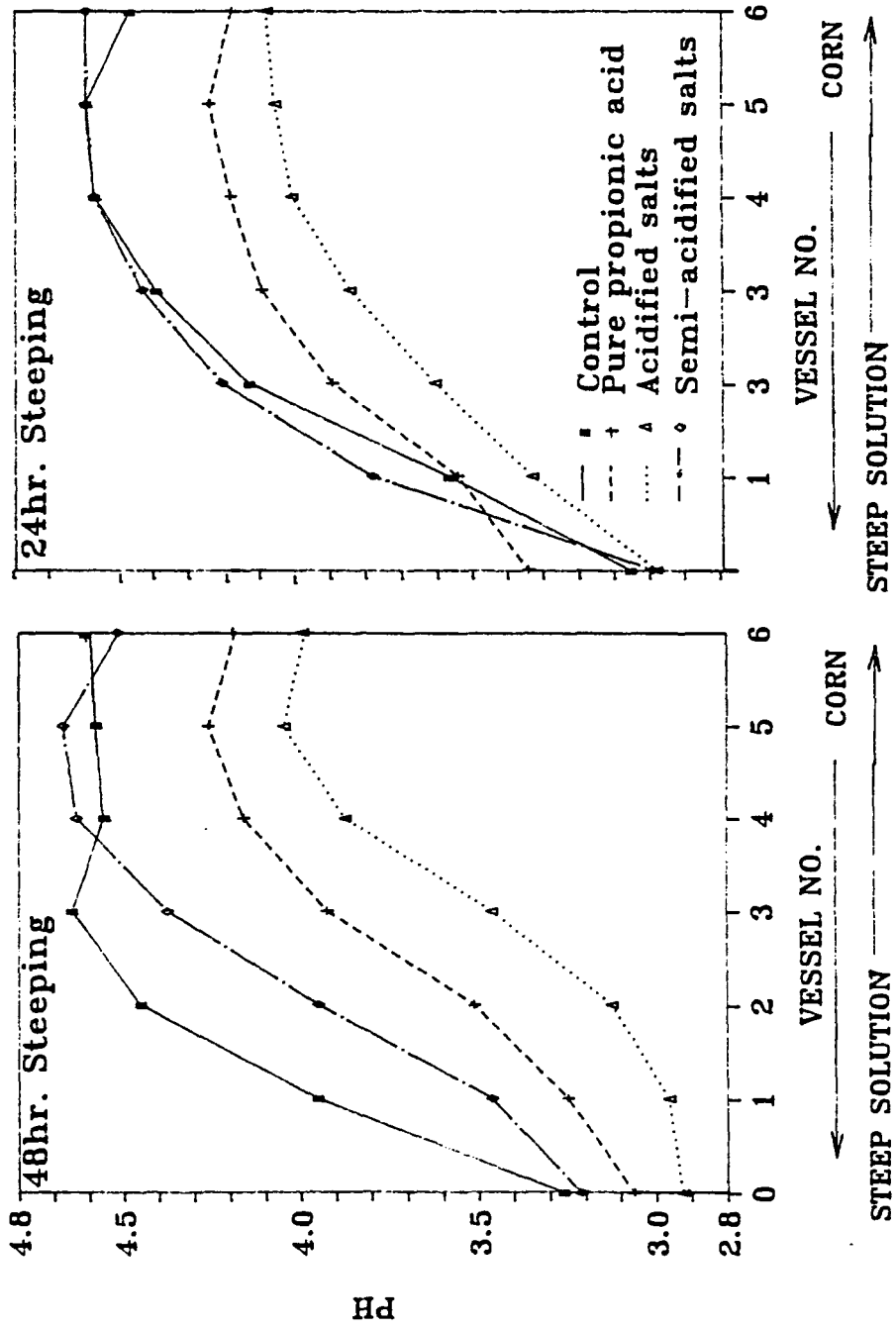
CCS System Operation

The operation of the CCS system was closely monitored. The most important factors were acidity changes and solids leaching in steeping.

PH Profile of Steeping Solutions

Differences were found between the acidity profiles of control corn (air-dried) at both 48-hr steeping and 24-hr steeping and that of commercial steeping. The pH profile in commercial steeping is relatively constant (pH 3.5-3.9) from one steeping stage to another (Watson, 1984). The pH profile in the CCS system ranged from 4.5 to 3.1 as the corn approached the freshest steeping solution. As shown in Figure 5, the pH of the solution steeping the oldest corn was about 3. The pH increased as the solution steeped more corn and reduced the SO_2 concentration. At the final stage where the freshest corn was steeped, the pH increased to 4.6 in both 48- and 24-hr steeping. The cause for this difference was probably due to the fact that the wash water used in commercial steeping comprised the steeping solution which had more buffering capacity. The SO_2 solution used in the CCS

Figure 5. Effects of propionate treatments on the acidity of steeping solutions (LSD=0.31 at 0.05 level)


24hr. Steeping
48hr. Steeping
Control
Pure propionic acid
Acidified salts
Semi-acidified salts
PH
4.8
4.5
4.0
3.5
3.0
2.8
0
1
2
3
4
5
6
VESSEL NO.
STEEP SOLUTION
CORN

system was made from distilled water.

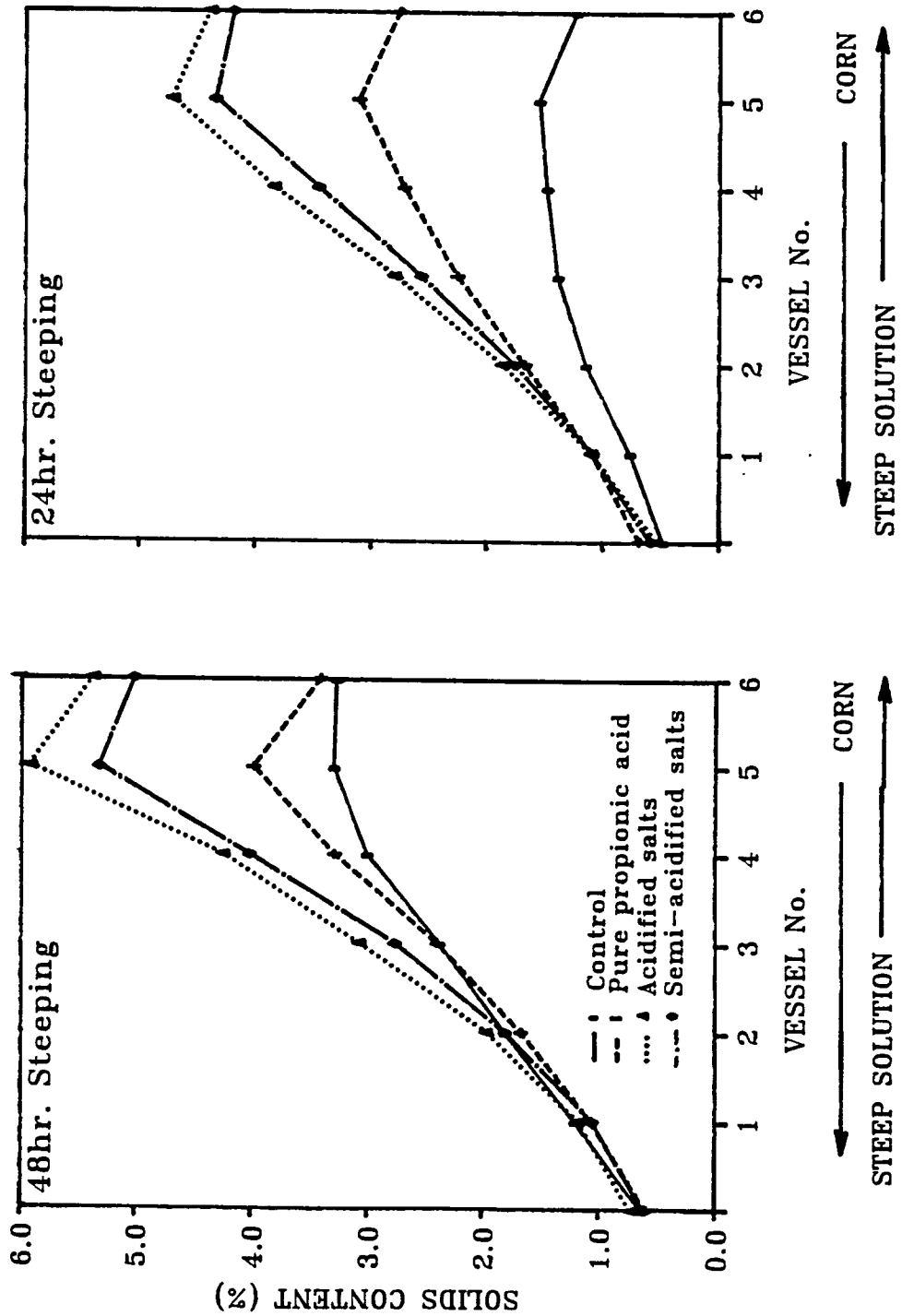
Differences in pH profiles were also found among the propionate treatments. Corn treated with acidified salts solution had the lowest pH at each stage, followed by pure propionic acid, semi-acidified salts, and the air-dried corn. The profiles of air-dried and the semi-acidified solution were very similar. The cause for the differences was the hydrogen ion concentration present in the corn preservation treatment.

There were also differences between the two steeping times. The pH profile of 48-hr steeping period was higher than that for the 24-hr steeping. This was attributed to more solubles being leached out from corn kernel in longer steeping, which increased the buffering capacity of the steeping solution.

Solid Content Profile

The profile of solids content of the steeping solution for each stage in the CCS system was very similar to that of industrial steeping, where the solids content of the steeping solution increases from 1.5% to 6.5% during 48-hr steeping. However, in CCS system 48-hr steeping, as shown in Figure 6, the solids content increased from 0.5% to 3.2%. This was because industrial steeping solution is made of water from starch washing while the steeping solution in CCS system was made of distilled water.

Figure 6. Effects of propionate treatments on solids content profile of steeping solutions in the CCS system (LSD=0.78 at 0.05 level)


48hr. Steeping
24hr. Steeping
SOLIDS CONTENT (%)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1
2
3
4
5
6
VESSEL No.
STEEP SOLUTION
CORN
Control
Pure propionic acid
Acidified salts
Semi-acidified salts

Differences among treatments were also significant. At almost every steeping stage, the solids contents of steep solutions from acidified salts-treated corn were the highest; followed by semi-acidified salts-treated; pure propionic acid-treated; and air-dried corn. It was apparent that propionate-treated high-moisture corn released more solids into the steeping solution.

The solids profiles for the two steeping periods were also different. At each steeping stage, the 48-hr steeping treatment had higher solids content than 24-hr steeping treatment. It was no surprise that longer steeping increased solids leaching.

Observations on Wet-Milling Performance

Germ Separation

Qualitative differences were observed in the ease of germ separation among the treatments and between the two steeping times. All of the propionate treatments at both steeping times had much easier germ separation than the air-dried sample. In propionate-treated samples, more germs floated on the top, and there were less tip caps and endosperm chunks floating with the germs. For the air-dried corn, the germ separation of samples steeped for 48 hr was much easier than for those steeped for 24 hr.

Starch and Gluten Separation By Sedimentation

Starch-gluten separation is crucial to the yield and the purity of starch. When the starch-gluten slurry was allowed to settle, the starch settled quickly to the bottom of the beaker while the gluten settled more slowly on top of the starch layer. The two layers should be very distinct, gluten being yellow, and starch being white. The separation of the air-dried corn appeared normal when steeped for either 24 or 48 hr. However, the starch-gluten separation of propionate-treated high-moisture corn was not as good as for air-dried corn. Figure 7 compares the sedimentation characteristics of the starch-gluten slurry from air-dried corn and pure propionic acid treated corn. The layers in the air-dried sample was distinct, while there was little separation in corn treated with propionic acid.

Yield of Products

Starch Yield

Starch yield and its protein content for each treatment are shown in Table 2. Both yields and protein contents are reported on a moisture-free basis.

In both steeping times, chemically-treated high-moisture corn gave higher starch yield than air-dried corn. Acidified

Figure 7. Sedimentation separation of mill-starch from air-dried corn (Control) and corn treated with pure propionic acid (P.A. Treated)
(Sample allowed to settle for 10 min)

Figure 7. Sedimentation separation of mill-starch from air-dried corn (Control) and corn treated with pure propionic acid (P.A. Treated)
(Sample allowed to settle for 10 min)

salts treated corn gave the highest starch yield. For pure propionic acid treated corn and air-dried corn, there were differences between the two steeping times where longer steeping gave higher starch yield. Corn treated with acidified salts and semi-acidified salts did not give higher yields when steeping for 48 hr instead of 24 hr (ANOVA in Appendix A-1).

Table 2. Effects of propionate treatments on yield and protein content of starch[1]

Treatments	Steeping Time (hr)	Starch Yield[2] (%)	Protein Content[3] (%)
Air-dried	24	58.4^{d}	0.61^{d}
	48	60.2^{c}	0.51^{d}
Pure propionic acid	24	61.6^{bc}	0.95^{c}
	48	63.6^{a}	1.05^{bc}
Semi-acidified salts	24	62.9^{ab}	1.24^{a}
	48	60.9^{c}	1.19^{ab}
Acidified salts	24	64.3^{a}	1.19^{ab}
	48	63.7^{a}	1.06^{bc}

[1]Means with common superscripts are not significantly different at 5% level. LSD for starch yield and its protein content were 1.6% and 0.18%, repectively.
[2]Percent of total corn solids on moisture-free basis.
[3]Calculated on moisture-free basis.

Higher starch yields of the treated samples were partly due to lower amounts of inseparables and poor separation of starch and gluten for the treated high-moisture corn as indicated by higher protein content in the starch fractions (ANOVA in Appendix A-2).

Gluten Yield and Protein Content

Means for the yields and protein contents of the gluten fractionate are listed in Table 3. Both yields and protein contents were calculated on moisture-free basis.

For both steeping times, the air-dried corn had the highest gluten yield followed by corn treated with pure propionic acid, semi-acidified salts, and acidified salts. There was no significant difference between the two steeping times. Gluten protein contents had a similar trend. The gluten from air-dried corn had the highest protein content. Gluten from air-dried corn steeped for 24 hr had higher protein content than the same corn steeped for 48 hr.

Acid treatment of high-moisture corn or longer steeping time decreased the gluten yield and its protein content. These were attributed to two reasons. Firstly, the endosperm gluten was solubilized more completely during steeping of propionate-treated high-moisture corn. Secondly, low yield and low protein content of gluten from the treated samples result from the poor separation of starch and gluten.

Table 3. Effects of propionate treatments on yield and protein content of gluten[1]

Treatments	Steeping Time (hr)	Gluten Yield[2] (%)	Protein Content[3] (%)
Air-dried	24	5.65[a]	50.2[a]
	48	5.31[a]	46.6[b]
Pure propionic acid	24	4.70[b]	45.4[cd]
	48	4.85[b]	43.3[cd]
Semi-acidified salts	24	4.73[bc]	43.0[cd]
	48	4.40[bc]	40.4[d]
Acidified salts	24	4.63[bc]	40.2[d]
	48	4.27[c]	41.3[d]

[1]Means with common superscripts are not significantly different at the 5% level. LSD for gluten yield and its protein content were 0.44% and 2.9%, repectively.
[2]Percent of total corn solids.
[3]Calculated on moisture-free basis.

Fiber and Mill-starch Yield

Table 4 compares the total fiber yield (sum of coarse and fine fiber) for each treatment at two steeping times. For 48-hr steeping, the air-dried corn had the lowest fiber yield, significantly lower than the fiber yield for either acidified salts or semi-acidified salts treated corn. Corn treated with acidified salts had the highest yield of fiber. However, for 24 hr of steeping, air-dried and pure propionic acid had the

highest fiber yield.

Table 4. Effects of propionate treatments on yields of fiber and mill-starch[1]

Treatments	Steeping Time (hr)	Fiber Yield[2] (%)	Mill Starch[2] (%)
Air-dried	24	12.7^{bc}	69.8^{b}
	48	10.9^{e}	69.9^{b}
Pure propionic acid	24	13.1^{a}	70.6^{ab}
	48	11.6^{de}	71.8^{a}
Semi-acidified salts	24	12.1^{dc}	69.7^{b}
	48	12.9^{ab}	68.1^{c}
Acidified salts	24	12.2^{bdc}	70.3^{b}
	48	12.0^{cd}	69.7^{b}

[1]Means with common superscripts are not significantly different at 5% level. LSD for fiber and mill-starch yield were 0.73% and 0.92% respectively.
[2]Percent of total corn solids on moisture-free basis.

Mill-starch is the starch-gluten solids after germ and fiber separation. Mill-starch includes starch, inseparables, gluten, and solids from wash water. Table 4 also shows the yield of mill-starch for each treatment. For 48-hr steeping, pure propionic acid-treated corn gave the highest yield of mill-starch, followed by air-dried corn, acidified salt

treated-corn and semi-acidified salt-treated corn. For 24-hr
steeping, there were no differences among the treatments. No differences in mill-starch yield were observed between the two steeping periods for any of the treatment. The results suggest that the propionate treatment of high-moisture corn did not affect the yield of mill-starch.

Yield of Inseparables

Table 5 compares the amount and protein content of the inseparable fraction. For both steeping periods, the air-dried corn had the highest amount of inseparables followed by pure propionic acid-treated corn, semi-acidified salt-treated corn, and acidified salt-treated corn. Longer steeping time reduced the amount of inseparables. These results suggested that propionate-treated high-moisture corn and longer steeping time enhance the separation between starch and protein.

The separation of the inseparable fraction is very subjective. No differences in protein contents of the inseparables fraction were observed among different treatments. This indicates that the separations were quite consistent.

Table 5. Effects of propionate treatments on amount and protein content of inseparables[1]

Treatments	Steeping Time (hr)	Inseparables Yield[2] (%)	Protein Content[3] (%)
Air-dried	24	5.66[a]	4.01[a]
	48	4.39[ab]	4.10[a]
Pure propionic acid	24	4.24[ab]	4.10[a]
	48	3.31[bc]	3.71[a]
Semi-acidified salts	24	2.05[cd]	4.25[a]
	48	2.73[cd]	3.33[a]
Acidified salts	24	1.43[d]	3.61[a]
	48	1.71[d]	4.09[a]

[1]Means with common superscripts are not significantly different at the 5% level. LSD for inseparables yield and protein content were 1.4% and 1.7%, respectively.
[2]Percent of total corn solids on moisture-free basis.
[3]Calculated on moisture-free basis.

Wash-Water Solids Recovery

Results for the solids yield from wash water and protein content of the wash water (as-is basis) are compared in Table 6. For both steeping periods, the yield of wash-water solids from the air-dried sample was significantly higher than solids yield from the chemically-treated high-moisture samples. There were no differences among the three chemical treatments. For each treatment, corn steeped for 24 hr had higher solids yield in wash water than for corn steeped for 48 hr. Less

solids in wash water would be an advantage to industry because higher yields of starch and gluten should result.

Table 6. Comparison of solids recovery and protein content of wash water[1]

Treatments	Steeping Time (hr)	Solids Yield[2] (%)	Protein Content[3] (%)
Air-dried	24	2.35[a]	0.11[a]
	48	2.15[b]	0.10[b]
Pure propionic acid	24	1.78[c]	0.10[ab]
	48	1.43[de]	0.06[d]
Semi-acidified salts	24	1.63[cd]	0.08[c]
	48	1.31[e]	0.06[d]
Acidified salts	24	1.74[c]	0.08[c]
	48	1.27[e]	0.05[d]

[1]Means with common superscripts are not significantly different at 5% level. LSD for wash solid content and protein content were 0.19% and 0.005%, repectively.
[2]Percent of total corn solids on moisture-free basis
[3]Calculated on as-is basis.

One possible reason for higher solids in wash water from air-dried corn is that the air-dried corn released less water or salt soluble solids during steeping. Another possible reason is that chemical treatment denatured protein which precipitated when the mill-starch was allowed to settle. Protein contents of in wash-water samples had the same trend

as total wash-water solids yield. However, the differences in solids contents were not caused by protein content alone.

Solids and Protein Yield in Steep Liquor

Steep liquor is the steeping solution exiting the steeping system. Solids content of the steep liquor was a criteria used to measure how well the corn was steeped.

As shown in Table 7, corn treated with acidified salts

Table 7. Effects of propionate treatments on the solids content recovery and protein content of steep liquor[1]

Treatments	Steeping Time (hr)	Solids Yield[2] (%)	Protein Yield[2] (%)
Air-dried	48	3.07[c]	1.11[c]
	24	1.16[e]	0.39[d]
Pure propionic acid	48	3.20[c]	1.53[b]
	24	2.60[d]	1.14[c]
Semi-acidified salts	48	4.71[a]	1.65[b]
	24	3.94[b]	1.25[c]
Acidified Salts	48	5.05[a]	2.08[a]
	24	4.13[b]	1.71[b]

[1]Numbers with one or more common superscripts are not significantly different at the 5% level. LSD for solids yield and for protein yield were 0.62% and 0.35%, respectively.

[2]Calculated on moisture-free basis (g solids or protein in steep liquor/g corn solids). There was 9.5 g protein/100 gr corn solids.

had higher solids yield, followed by corn treated with semi-acidified salts, corn treated with pure propionic acid, and air-dried corn. For each treatment, longer steeping caused higher steep liquor solids yield. The protein yield had a similar trend. These results suggest that propionate-treated high-moisture corn or corn steeped for a longer time release more solids and protein during steeping.

Some solids leaching during steeping is crucial for germ separation. With the increase of solids loss from germs, the density of germs decreases. This increases germ separation efficiency.

Germ Composition and Separation

Germ separation is the first milling operation for steeped corn. If the germ cannot be separated from the endosperm, the starch will contain high fat levels making it prone to development of oxidized flavors during storage. Also, corn germ is a major source of valuable corn oil and reduced yields will adversely impact returns because oil is the most valuable product (per unit weight basis) from milling.

Table 8 shows the yields and the compositions of germ fractions from corn with different treatments and steeping times. In 24-hr steeping, the air-dried corn had the highest

germ yield, followed by pure propionic acid-treated corn, semi-acidified salts-treated corn, and acidified salts-treated corn. At longer steeping (48 hr), air-dried corn also had significantly higher germ yield than the other three

Table 8. Effects of propionate treatments on the yield of germ and its constituents[1]

Treatments	Steeping Time (hr)	Germ Yield (%)[2,3]	Protein Content (%)[3]	Oil Content (%)[3]
Air-dried	24	7.24^{a}	17.3^{a}	39.0^{e}
	48	6.56^{bc}	15.6^{b}	43.8^{bcd}
Pure propionic acid	24	6.74^{b}	8.4^{d}	42.2^{d}
	48	6.40^{cd}	8.3^{d}	43.5^{cd}
Semi-acidified salts	24	6.43^{cd}	9.2^{c}	43.4^{cd}
	48	6.30^{cd}	8.4^{d}	44.3^{bc}
Acidified salts	24	6.35^{cd}	7.2^{e}	46.4^{a}
	48	6.21^{d}	7.0^{e}	45.7^{ab}

[1]Means with common superscripts are not significantly different at the 5% level. LSD for germ yield, protein content, and oil content were 0.24%, 0.54%, 1.67%, respectively.
[2]Percent of total corn solids.
[3]Calculated on moisture-free basis.

treatments, but there were no significant differences among the three propionate treatments. The same order of yield was

observed at 24 hr of steeping and 48 hr of steeping. For all the treatments, those steeped for 24 hr tended towards lower yield of germ, but differences were significant for only air-dried corn and corn treated pure propionic acid.

As shown in Table 8, protein contents of germ fractions from air-dried corn were much higher than those from treated samples. For both steeping periods, the order (high to low) of protein contents of the germ fractions were: air-dried corn, semi-acidified salts-treated corn, pure propionic acid-treated corn, and acidified salts-treated corn. Steeping time had a significant effect on germ protein content for both air-dried corn and pure propionic acid treated corn. Corn steeped for shorter time gave germ with higher in protein. The composition differences indicated that protein was more extensively leached from germ during steeping in high-moisture corn treated with propionate. Whether this was due to increased solubility of protein because of propionate treatment of high-moisture corn or reduced solubility in air-dried corn due to drying is not known.

The oil contents of germ fractions from different treatments are also shown in Table 8. Oil contents were higher in germ fractions from propionate-treated corn. This reflects diffences in leaching of protein from germ. For 24-hr steeping, the acidified salt-treated corn gave germs with the highest oil content, followed by semi-acidified salt-

treated corn and pure propionic acid-treated corn. No differences were observed among the treatments steeped for 48 hr. For air-dried corn, germs from 48-hr steeping had significantly higher oil contents than those steeped for 24 hr. This was due to greater protein leaching at longer steeping times.

It was easier to separate germs from propionate-treated high-moisture corn and from corn steeped for longer periods. It was especially difficult to separate germs from air-dried corn steeped for 24 hr. This was attributed to differences in density of the germs. Germ density would be expected to decrease when germs had higher oil content because protein is more dense than oil.

Improved germ separation due to propionate treatment is desirable, but increased protein leaching into steep liquor may not be desirable even though steep liquor is eventually added back to corn gluten feed. Increased solids in steep liquor may cause handling problems of the steep liquor.

Starch Quality

Protein Content

Protein content is a very important quality factor for industrial starch. As low protein content as possible is desirable; protein content must be less than 0.4%. Starch

with higher content of protein causes scumming and fouling of equipment, and discolors many finished products. The differences in protein contents among the treatments are very significant as shown in Table 2. For 24 hr of steeping, starch from the air-dried corn contained the lowest protein, about a half of that of starch from propionate-treated high-moisture corn. There were no significant differences among the three propionate treatments. For 48 hr of steeping, air-dried corn also had the purest starch. The starch from pure propionic acid-treated corn was the second purest. There was no significant difference between protein contents of starch from corn treated with semi-acidified salts and starch from corn treated with acidified salt. The chemically-treated high-moisture corn produced starch with poorer purity than air-dried corn because of the poor starch-gluten separation in the former.

Thermal Properties

Table 9 shows thermal properties of starch from the various treatments. There were no significant differences among treatments for gelatinization onset, peak, and conclusion temperatures and enthalpy. No significant difference for enthalpy suggested that the starch granules were still intact.

Pasting Properties

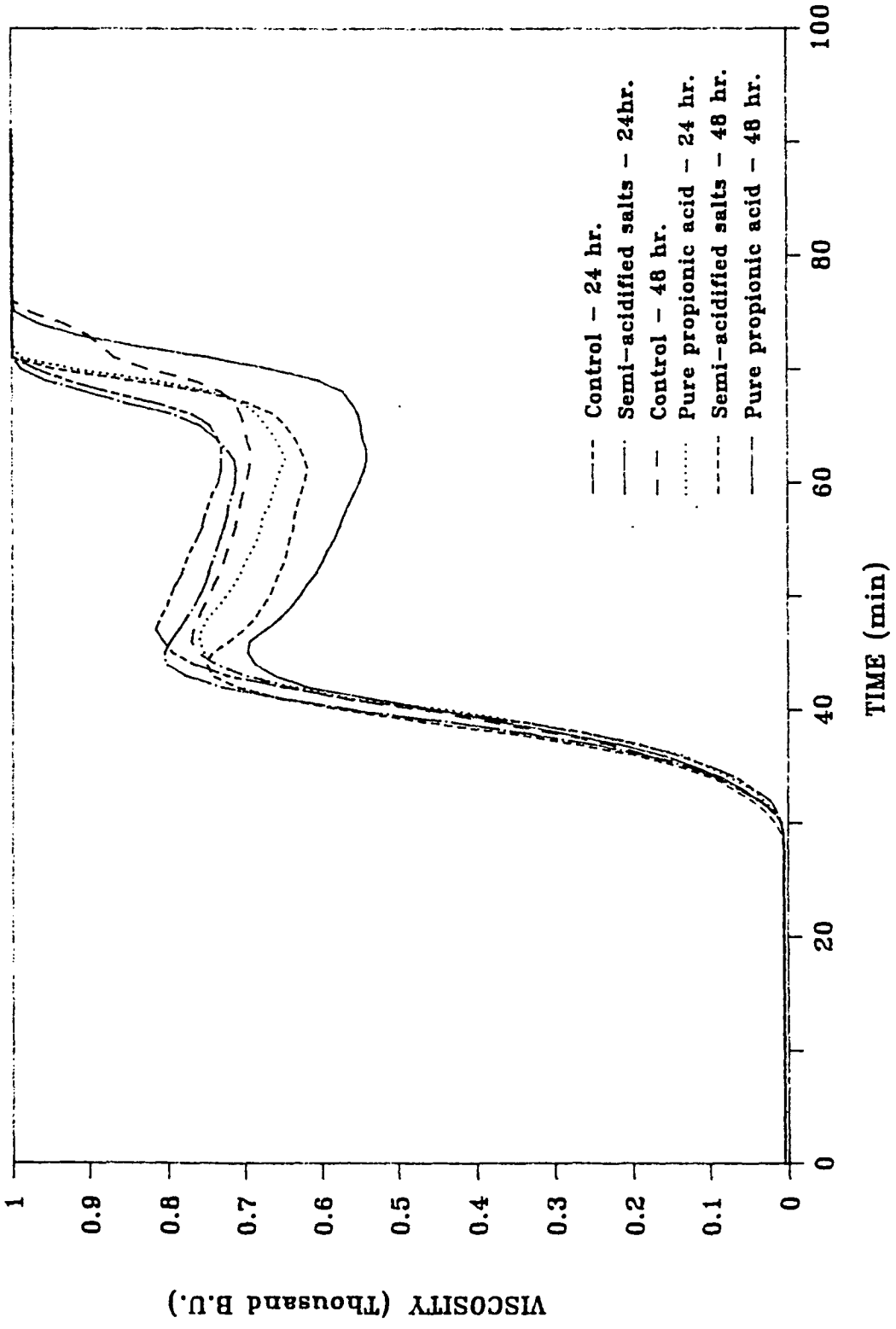
Figure 8 shows the viscoamylograph patterns of starch samples from air-dried corn, pure propionic acid-treated corn, and acidified salt-treated corn. The patterns were different among treatments and between the two steeping times. Differences were especially apparent for maximum viscosity (when temperature was 96°C) and the minimum viscosity (before the slurry began to cool).

Table 9. Effects of propionate treatments thermal properties[1]

Treatments	Steeping Time (hr)	Onset Temp. (°C)	Enthalpy (Kcal/g)
Air-dried	24	66.4[a]	12.4[a]
	48	65.0[a]	12.7[a]
Pure propionic acid	24	66.8[a]	12.4[a]
	48	65.9[a]	13.2[a]
Semi-acidified salts	24	66.4[a]	11.5[a]
	48	65.6[a]	11.8[a]

[1]Means with common superscripts are not significantly different at the 5% level. LSD for onset temp. and enthalpy were 2.2 °C and 1.61 kcal/g, respectively.

Figure 8. Effects of propionate treatment on pasting properties of starch


VISCOSITY (Thousand B.U.)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
TIME (min)
Control – 24 hr.
Semi-acidified salts – 24hr.
Control – 48 hr.
Pure propionic acid – 24 hr.
Semi-acidified salts – 48 hr.
Pure propionic acid – 48 hr.

The maximum and the minimum Brabender viscosity values are listed in Table 10. In both 48-hr and 24-hr steeping, the starch from the air-dried corn had the highest maximum and minimum viscosities, followed by starch from semi-acidified

Table 10. Effects of propionate treatments and steeping time on starch pasting properties[1]

Treatments	Steeping Time (hr)	Pasting Viscosity	
		Maximum (B.U.)	Minimum (B.U.)
Air-dried	48	773^{bc}	691^{ab}
	24	823^{a}	725^{a}
Pure propionic acid	48	697^{d}	540^{d}
	24	772^{bc}	646^{bc}
Semi-acidified salts	48	752^{c}	616^{c}
	24	810^{ab}	708^{ab}

[1]Means with common superscripts are not significantly different at 5% level. LSD for maximum and minimum were 43.2 and 59.2 B.U., respectively.

salts-treated corn. The starch from pure propionic acid treated corn had the lowest viscosities. There were also significant differences between the two steeping times for starch samples from corn treated with pure propionic acid and semi-acidified salts. The starch from 24-hr steeping had significantly higher viscosities than the starch from 48-hr

steeping.

From the viscosity data, chemical treatments, especially low pH treatment, decreased starch pasting viscosity. Longer steeping time also produced lower pasting viscosity. Starch is probably acid modified during steeping, because acid modification of starch can decrease the pasting viscosity without destroying the granular structure. The acid hydrolysis probably only occurred in amorphous areas of granules.

Color Evaluation

Color of Corn After Storage

Values of 'Lab' from Hunter colorimeter are compared in Table 11. The propionate treatments bleached the grain, with bleaching becoming more extensive the longer the storage. Air-dried corn had the highest 'L' value, followed by pure propionic acid-treated corn, and the other propionate-treated high-moisture corn. Corn stored for a longer storage time had lower 'L' values than corn stored for shorter time. The air-dried corn also had the highest 'a' value. Hunter values indicated more intense red color in air-dried corn, but differences among propionate-treated high-moisture corn were not significant. Air-dried corn also had the highest 'b' value indicating more intense yellow color, followed by pure

propionic acid-treated corn, semi-acidified salts-treated corn, acidified salts-treated corn, and salts-treated corn. There were also differences in 'b' values between the two storage times. Corn stored for a longer time had less intense yellow color than samples stored for shorter times.

Air-dried corn had better color quality being brighter, slightly more red, and more yellow than propionate-treated high-moisture corn. Among the four chemical treatments, corn treated with pure propionic acid had more desirable color, followed by corn treated with acidified salts or semi-acidified salts; corn treated with salts was the worst. The color quality differences can be also observed in Figure 9. The change in yellow color was attributed to degradation of carotinoid.

Color of Starch and Gluten

Tables 12 and 13 show the colors of gluten and starch as measured by the Hunter colorimeter, respectively. Differences of 'L' and 'a' values were not as significant as those of 'b' values among the treatments. The propionate treatments had significant effects on yellow color of both starch and gluten. Gluten from propionate-treated high-moisture corn was less yellow than gluten from air-dried corn because of the rapid degradation of carotinoids under higher moisture conditions. Starch from the propionate-treated high-moisture corn was more

Table 11. Effects of propionate treatments on kernel color at two different storage times

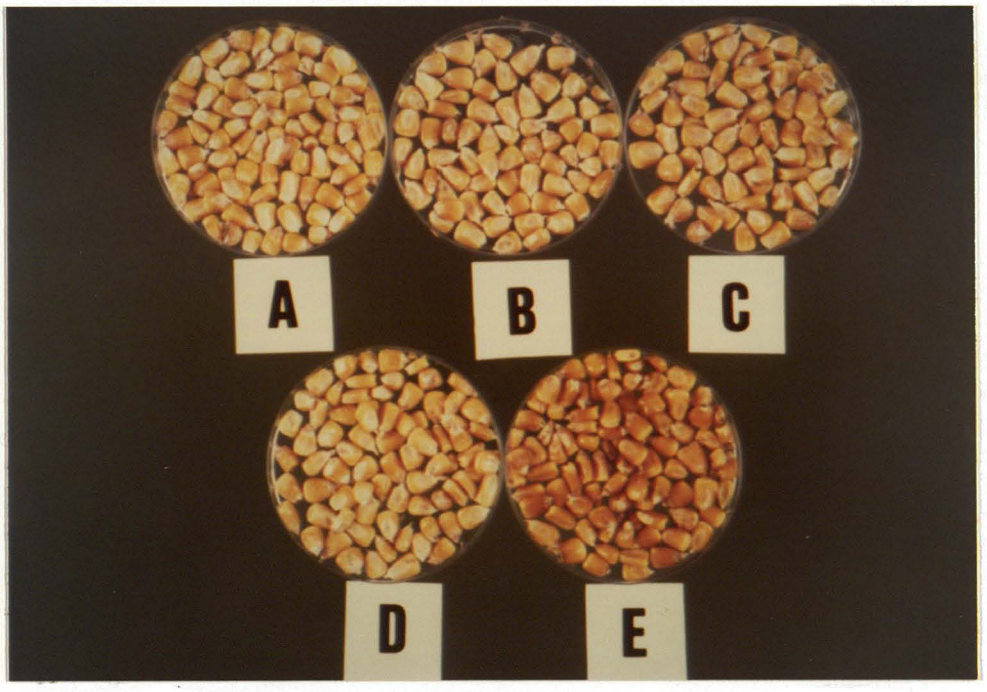
Treatments	Storage time (months)	'L' value	'a' value	'b' value
Air-dried	4	61.0[a]	15.5[a]	28.3[a]
Pure propionic acid	4	58.8[ab]	13.5[ab]	26.4[b]
	8	57.3[bc]	12.8[b]	24.9[c]
Salts solution	4	48.2[e]	13.6[ab]	24.7[c]
	8	46.7[e]	13.3[b]	23.1[d]
Semi-acidified salts	4	55.7[cd]	12.3[b]	26.2[b]
	8	54.0[d]	12.0[b]	24.9[c]
Acidified salts	4	55.1[cd]	11.8[b]	26.4[b]
	8	53.5[d]	12.5[b]	25.3[cb]

[1]Means with common superscripts are not significantly different at the 5% level. LSD for L, a, and b were 2.2, 1.9, and 1.2, respectively.

yellow than starch from air-dried corn due to higher gluten contamination in starch.

Color of Moist Products

Measuring the color of wet-milling products before they were dried avoided color changes during high-temperature drying and detected color changes caused only by storage of grain and steeping. Table 14 compares the colors of products from air-dried corn with those of pure propionate-treated



A: Air-dried corn
B: Pure propionic acid treated corn
C: Semi-acidified salts treated corn
D: Acidified salts treated corn
E: Salt solution treated corn

Figure 9. Color of corn samples after storage

Table 12. Effects of propionate treatments on color of gluten[1]

Treatments	Steeping Time (hr)	'L' Value	'a' Value	'b' Value
Air-dried	24	49.8^{abc}	3.30^{a}	15.1^{a}
	48	53.0^{a}	3.10^{a}	15.4^{a}
Pure propionic acid	24	46.1^{c}	1.72^{c}	10.5^{c}
	48	52.7^{ab}	2.21^{bc}	12.5^{b}
Semi-acidified salts	24	47.7^{bc}	2.22^{bc}	12.5^{b}
	48	49.2^{abc}	2.18^{bc}	12.1^{b}
Acidified salts	24	50.0^{abc}	2.29^{bc}	12.7^{b}
	48	45.8^{c}	2.55^{b}	11.9^{b}

[1]Means with common are not significantly different at the 5% level. LSD for L, a, and b were 4.7, 0.52, and 1.27, respectively.

corn. Almost all by-products from wet milling of air-dried corn had higher 'L' and 'b' values than those from the pure propionic acid-treated corn. Products from the pure propionic acid-treated corn had been bleached and lost brightness.

Table 13. Effects of propionate treatments on the color of starch[1]

Treatments	Steeping time (hr)	'L' value	'a' value	'b' value
Air-dried	24	74.2[a]	-0.3[ab]	3.87[c]
	48	75.9[a]	-0.3[ab]	3.79[c]
Pure propionic acid	24	75.8[a]	-0.3[a]	3.96[bc]
	48	72.2[a]	-0.3[b]	4.12[bc]
Semi-acidified salts	24	73.3[a]	-0.1[c]	4.46[ab]
	48	73.7[a]	-0.3[ab]	4.82[a]
Acidified salts	24	71.6[a]	-0.2[b]	4.82[a]
	48	74.7[a]	-0.3[b]	4.81[a]

[1]Means with common superscript are not significantly different at the 5% level. LSD for L, a, and b were 4.1, 0.06, and 0.51, respectively.

Table 14. Effects of propionate treatments on color of wet-milling by-products before drying

By-Products	Values	Air-dried corn	Pure propionic acid treated corn
Germ	L	81.6	73.8**
	a	3.21	4.51*
	b	24.4	24.16
Coarse Fiber	L	87.2	85.2**
	a	7.90	6.07**
	b	34.2	26.8**
Fine Fiber	L	70.3	65.1**
	a	8.44	8.99*
	b	27.1	24.7**
Gluten	L	85.4	83.15**
	a	13.3	9.57**
	b	42.3	31.5**

*Significant at 5% level;
**Significant at 1% level.

Starch and Gluten Separation

Mechanism of Separation Effects

The separation of starch and gluten is facilitated by their differences in specific gravities. The propionate-

treated high-moisture corn did not exhibit good starch-gluten separation. The causes for reduced separation efficiency may be one or the combination of the following causes.

The first hypothesis is the density changes in starch granules due to propionate treatment. After low-pH steeping due to propionate treatment, starch is partially acid-modified. Being more hydrophilic, the acidified starch takes up more water decreasing the density of starch. Lower density starch granules do not separate well from gluten particles because of the reduced density difference between the starch granule and gluten particles.

Secondly (and less likely) gluten particles could absorb more water and become more completely hydrated due to storage at high moisture and steeping at low pH. This would lead to increased density and the gluten particles would tend to settle at similar rates as starch granules. Because the density of gluten is 1.2 g/cc and water is 1.0 g/cc, the density increases when the water absorbed fills void space in the gluten particles without increasing the overall volume. If there is no void space, the density would decrease and moisture absorption could not explain the poor separation that was observed.

Methods to Enhance the Separation

Considering the two hypotheses for poor starch-gluten separation, a number of tests on starch-gluten slurry were attempted to enhance the separation.

The pH of the slurry was adjusted over the pH range of 2-10. No improvement in starch-gluten was observed. This implied that the poor separation was not due to electrostatic reasons.

Addition of potassium bromide, an oxidizing agent, to the starch-gluten slurry did not improve the separation. The basis for this test was that the gluten particles were destroyed by long-term high-moisture storage. Addition of an oxidizing agent would promote more disulfide bonds increasing gluten-gluten interaction and thus increasing gluten particle size.

Addition of NaCl to the starch-gluten slurry improved the starch-gluten separation. The intent was to increase the slurry density so that the gluten particles could separate more efficiently from starch. The density was checked by using a hydrometer, but no significant change in density was observed over the range of NaCl evaluated. The separation of starch and gluten was observed at each level of NaCl after 20 min of settling. The results are shown in Table 15.

Table 15. NaCl addition on starch-gluten separation

Amount of NaCl (g/300 gr corn)	Concentration (%)	Density (g/cc)	Separation Observed
0.2	0.02	1.049	extremely poor
0.5	0.05	1.050	extremely poor
1.0	0.10	1.050	little visible
1.5	0.15	1.050	medium
2.0	0.20	1.050	good
2.5	0.25	1.049	good
3.0	0.30	1.050	good

Protein contents of starch from air-dried corn and pure propionic acid-treated high-moisture corn with and without addition of 2 gr of NaCl are shown in Table 16. For both corn

Table 16. Effects of addition of NaCl on satrch protein content

Addition of NaCl (g)	Protein Content of Starch	
	Air-dried corn	Treated high-moisture corn
0	0.59[b]	0.84[a]
2.0	0.53[c]	0.44[d]

[1]Means with the same superscript are not significantly different. LSD was 0.02%.

treatments, addition of NaCl reduced protein content significantly. The addition of NaCl reduced the protein content of starch from pure propionic acid-treated high-moisture corn by almost 50%.

The mechanism for the improved separation is not understood. However, the minor change of slurry density may have improved the separation. Also, with increased ionic strength due to the addition of NaCl in the slurry, the water within the starch granules and gluten particles tends to be desorbed causing the density of starch granules to increase and/or gluten particles to decrease.

CONCLUSIONS

Propionate-treated high-moisture corn had significantly different wet-miiling properties than air-dried corn. The extent of this effect varied by type of propionate treatment (pure propionic acid, semi-acidified salts or acidified salts). Such treatments did not enhance starch recovery and did not facilitate reduced steeping time.

Propionate treatments of high-moisture corn cause the steeping solution to be more acidic than those for air-dried corn because of residual acids. Losses of solids during steeping were greater for propionate-treated high-moisture corn than for air-dried corn. Corn treated with propionate required less steeping time than air-dried corn to obtain acceptable germ separation.

The propionate-treated high-moisture corn had 0.7-3.0% higher starch yields than air-dried corn when steeped for 48 hr and 3.0-6.0% higher yields when steeped for 24 hr. The higher yield was due, in part, to unseparated gluten in the starch fraction, a result of poor starch-gluten separation. Starch from propionate-treated high-moisture corn had as much as 1.0% protein while that from air-dried corn had only 0.5-0.6% protein. Poor starch-gluten separation was responsible for the high protein content in starch from propionate-treated high-moisture corn. Starch from corn treated with pure

propionic acid and acidified salts had lower viscosity due to partial acid hydrolysis of starch. There were no significant differences in the thermal properties of starch among treatments.

Gluten from the propionate-treated high-moisture corn was less yellow than that from the air-dried corn. Starch from the propionate-treated high-moisture corn had more intense yellow color than starch from air-dried corn due to higher gluten contamination of the starch.

Propionate-treated high-moisture corn had lower recoveries of germ than air-dried corn due to germs from propionate-treated high-moisture corn leaching more protein during steeping. Lower protein contents and higher oil contents decreased germ density, which enhanced germ flotation and separation for propionate-treated high-moisture corn.

Addition of 2.0 g sodium chloride per 300 g corn to the starch-gluten slurry enhanced starch-gluten separation reducing the protein content of the starch to 0.4% for propionate-treated high-moisture corn. The mechanism for improved starch-gluten separation of propionate-treated high-moisture corn due to salt is not well understood.

In general, propionate-treated high-moisture corn had much poorer wet-milling properties than air-dried corn. Further studies on mechanisms accounting for poor starch-

gluten separation for propionate-treated high-moisture corn and improved starch-gluten separation due to salt are needed.

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APPENDIX

Appendix 1

Analysis of variance for starch yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatments	7	114.49	16.35	14.24	0.0001
Error	24	27.56	1.14		
Total	31	142.05			

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: Starch Yield

Alpha= 0.05 df= 24 MSE= 1.148567

Number of Means	2	3	4	5
Critical Range	1.5625044	1.6416008	1.6961129	1.7292867

Number of Means	6	7	8
Critical Range	1.7559688	1.7769848	1.7936101

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatments
A	64.268	4	APN24
A			
A	63.727	4	APN48
A			
A	63.575	4	PA 48
A			
B A	62.878	4	NPA24
B			
B C	61.638	4	PA 24
C			
C	60.930	4	NPA48
C			
C	60.220	4	CK 48
D	58.420	4	CK 24

Appendix 2

Analysis of variance for protein content of starch

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatments	7	2.10	0.300	18.1	0.0001
Error	24	0.398	0.017		
Total	31	2.50			

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: Protein

Alpha= 0.05 df= 24 MSE= .0165792

Number of Means	2	3	4	5
Critical Range	.18772603	.19722901	.20377833	.20776397

Number of Means	6	7	8
Critical Range	.21096968	.21349464	.21549207

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	1.2425	4	NPA24
A			
A	1.1900	4	NPA48
A			
A	1.1900	4	APN24
A			
B A	1.0575	4	APN48
B A			
B A	1.0500	4	PA48
B			
B	0.9500	4	PA24
C	0.6075	4	CK24
C			
C	0.5125	4	CK48