

# Isolation of Hemicellulose from Corn Fiber by Alkaline Hydrogen Peroxide Extraction

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## ABSTRACT

Cereal Chem. 74(2):176–181

For the first time, alkaline hydrogen peroxide (AHP) extraction conditions were used to isolate hemicellulose (arabinoxylan) from destarched corn fiber. Yields of the water-soluble hemicellulose B ranged from 35% (24 hr extraction at 25°C) to 42% (2 hr extraction at 60°C). The hemicellulose B resulting from the 2 hr extraction (pH 11.5) was off-white in color, and a very low proportion (1.7%) of water-insoluble hemicellulose A was extracted. AHP treatment caused delignification and facilitated the alkaline extraction of hemicellulose from the lignocellulosic fiber matrix. In the absence of H<sub>2</sub>O<sub>2</sub>, yields were reduced by more than one-third when using otherwise identical extraction conditions of time, temperature and pH. In the standard protocol, corn fiber, NaOH solution, and H<sub>2</sub>O<sub>2</sub> were mixed in a 1:25:0.25 (w/v/w) ratio. Extractions were conducted at pH 11.5 at 25°C or 60°C. The pH was adjusted to 11.5 by addition of

NaOH at ambient and elevated temperatures. The optimum hemicellulose yield (51.3%; dry, starch-free basis) was obtained when the pH was increased to 12.5 for the final one-half of the extraction period. Products obtained after extraction at pH values greater than 11.5 were tan in color, however, and the goal of the research has been to isolate white hemicellulose B and then evaluate its properties. Under most conditions, the yields of hemicellulose B, potentially the most useful form for food and industrial applications, exceeded those of hemicellulose A by more than 10-fold. The hemicellulose B products were lighter in color than those obtained using traditional alkaline extraction conditions of refluxing with calcium or sodium hydroxide. Steps prior to extractions with alkaline H<sub>2</sub>O<sub>2</sub>, such as grinding to 20 mesh and extracting with azeotropic toluene-ethanol, were found to be unnecessary.

Corn fiber is an abundant (4 million tons per year) by-product of the corn wet milling industry, with each bushel generating about 6 lb. of fiber (Carlson 1994). The kernel hull, or pericarp, is the major component of corn fiber. In the corn dry-milling process, the pericarp resides in the bran fraction. Corn fiber is exported primarily to Europe as corn gluten feed after combining with corn steep liquor and pelleting. The price of corn gluten feed fluctuates widely. For example, according to the *Wall Street Journal* (May 28, 1996), between May 1995 and May 1996, the price increased from \$77 to \$142 per ton. Because of uncertain markets for corn gluten feed, there has long been a strong interest in finding more valuable products from corn fiber. Interest has intensified in recent years, with new uses being identified for corn starch, including conversion to about one billion gallons of ethanol per year (Bothast 1994). The cost of ethanol can be significantly lowered if valuable coproducts are developed from product streams of corn milling.

Hemicellulose is the major component of corn fiber-bran, and as an arabinoxylan, is typical of hemicelluloses from graminaceous monocot plants (Fry 1986). It is located in the pericarp (hull) fraction. The quantities of extractable hemicellulose in corn fiber vary, due to variable levels of contaminating nonpericarp materials, mainly starch. A detailed compositional study (Saulnier et al 1995a) of corn bran was conducted after removal of starch and protein from contaminating endosperm. It was reported that arabinoxylan accounted for 67.5%, cellulose 22.5%, and protein 2.4% of the purified bran. A portion of the arabinoxylan is quite inextricably linked in the cell wall matrix and only about 70% is extracted even under exhaustive alkaline conditions (Saulnier et al 1995a). In laboratory-prepared pericarp, hemicellulose levels of

53.1% (Hooper 1942) and 49.5% (Wolf et al 1953) were reported (dry, starch-free basis). In addition, typical corn fiber contains (dry, starch-free basis) cellulose (15%), protein (15%), and other substances, such as lignin and ash (13%) (Carlson 1994). Corn fiber also contains up to 3.7% of an oil with a composition very different from that of normal corn oil (Moreau et al 1996). The actual yields of hemicellulose arabinoxylan from corn fiber and bran, even on a dry and starch-free basis, are quite variable and largely a function of the conditions used for its isolation. When extracted using relatively mild conditions, hemicellulose yields of 38.5–45.2% (Wolf 1953), 35.7% (Rutenberg and Herbst 1957), 30.7% (Watson and Williams 1959), and 42.7% (Antrim and Harris 1977) were obtained. Under more exhaustive conditions of extraction, yields of 57.2% (Sugawara 1994) and 48.1% (Saulnier et al 1995a) were obtained. The composition and properties of corn fiber hemicellulose, commonly referred to as corn fiber gum, have been reviewed (Whistler 1993).

The sugar composition of corn fiber (bran) hemicellulose was determined by several groups. The range reported for each sugar was: D-xylose (48–54%), L-arabinose (33–35%), galactose (5–11%), and D-glucuronic acid (3–6%) (Whistler and BeMiller 1956, Sugawara 1994, Saulnier et al 1995a). The  $\beta$ -(1→4)-D-xylopyranose backbone is heavily substituted at both primary and secondary hydroxyl groups. Side chains consist largely of  $\alpha$ -L-arabinofuranose residues, mainly at O-3 (Whistler and Corbett 1955), but also at O-2 of the xylose moieties (Saulnier et al 1995a). Galactose and xylose residues are linked to arabinofuranosyl branches (Whistler and Corbett 1955). The  $\alpha$ -D-glucuronic acid residues are linked at O-2 of xylose residues in the main chain (Montgomery and Smith 1957).

The hemicellulose fraction is the most unique of the carbohydrate components of fiber, and potentially very useful. Properties of corn fiber hemicellulose worth exploiting are its ability to serve as adhesives, thickeners, and stabilizers (Wolf et al 1953), and as film formers and emulsifiers (Whistler 1993). Corn fiber hemicellulose was prepared (Wolf et al 1953, 1955) by boiling fiber for 1 hr at pH 10.5–11.5 and then extracting with hot water. Several methods for preparing corn fiber hemicellulose were compared in efforts to lighten the color of its solutions (Rutenberg and Herbst 1957). Corn fiber was extracted using sodium or calcium hydrox-

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ide, or combinations of the two, at various temperatures. Extracts using calcium hydroxide were lightest in color, and were further lightened by treatment with activated carbon. The yields in such cases were about 27%, and the hemicellulose B (water soluble) and hemicellulose A (water insoluble) fractions were not separated. The opinion was expressed (Rutenberg and Herbst 1957) that the industrial usefulness of corn fiber gum would be greatest for products with the least color. Hemicellulose B fraction was later isolated after removing hemicellulose A by precipitation, so that clear solutions of the material could be prepared (Watson and Williams 1959, Schweiger 1973). Then a procedure was developed to generate hemicellulose, cellulose, and noncarbohydrate (ferulic acid-rich) fractions from corn fiber (Antrim and Harris 1977).

Hemicelluloses are usually defined as polymers that are solubilized from plant cell walls by alkali (Darvill et al 1980). Most of the hemicellulose fraction is soluble in water after alkaline extraction. Their isolation is actually a two-stage process, involving alkaline hydrolysis of ester linkages to liberate it from the lignocellulosic matrix followed by extraction into aqueous media. It can be inferred that hemicellulose is cross-linked to other cell wall components, and there is ample evidence to support this. It is well known that ferulic and *p*-coumaric acids are esterified to cell wall polysaccharides in various grasses (Mueller-Harvey et al 1986). Partial acid (Yoshida et al 1990, Saulnier et al 1995b) and enzymatic (Ohta et al 1994) hydrolysis of corn fiber-bran hemicellulose yielded oligosaccharide fragments in which arabinosyl units were esterified at primary hydroxyl groups with ferulic acid. Some were esterified with diferulic acids (Saulnier et al 1995b), and acetyl esters were also identified on the arabinoxylan (Saulnier et al 1995a). Diferulate ester bridges in corn bran are probably involved in cross-linking arabinoxylan molecules and in linking them to other cell wall polymers (Saulnier et al 1995a,b). There is evidence to suggest that esterified ferulic and *p*-coumaric acids serve to couple lignin and polysaccharide (Helm and Ralph 1993). From such associations, hemicellulose would be liberated by alkaline extraction. Polyphenolics such as lignin, however, can also form alkali-resistant linkages with the hemicellulose fraction of matrix polysaccharides (Morrison 1974, Fincher and Stone 1986, Jung and Deetz 1993). Ether linkages are present in lignin and there is evidence that ether linkages are also involved in linking lignin to hemicelluloses (Watanabe et al 1989, Hatfield 1991). It is likely that previous preparations of corn fiber hemicellulose samples probably contained residual lignin, contributing off-colors to the products. Protein was also possibly present, since stable linkages between hemicellulose and protein in corn bran (Saulnier et al 1995a) and rye bran (Ebringerova et al 1994) have been identified.

A process was described in which oxidizing agents were used to delignify plant materials and make hemicelluloses more amenable to extraction (Darling 1931). Corn cobs and corn stalks were delignified by treating alternately and repeatedly with dilute hypochlorite and dilute NaOH (0.2%). From the resulting residue, hemicellulose was obtained by extracting with 5–6% NaOH. The hemicellulose preparations became lighter in color with each successive treatment with hypochlorite. This approach was later applied to the isolation of hemicellulose from sunflower hulls (Bazus et al 1993). More recently, both hypochlorite under acidic conditions and H<sub>2</sub>O<sub>2</sub> under alkaline conditions were used to delignify corn bran prior to alkaline extraction and hemicellulose isolation (Hromadkova and Ebringerova 1995). Detailed chemical characterization of the hemicellulose was reported but no mention was made of its physical properties, including color.

In efforts directed at rendering plant cell wall lignocellulosics amenable to saccharification with cellulase, success was achieved after preliminary delignification with alkaline hydrogen peroxide (Gould 1984, 1989). Peroxide served to oxidize lignin to low molecular weight organic acids, thereby removing a barrier to enzyme accessibility. In that work, objectives were to convert

renewable lignocellulosic biomass to a form useful for glucose and ethanol production, and also for use as an energy source in ruminant feeds. To this end, success was achieved in *minimizing* loss of the hemicellulose fraction during alkaline peroxide extraction of various plant sources (Gould 1985a). In this current study, efforts were directed at *maximizing* hemicellulose extraction during and subsequent to delignification, in order to optimize the yield of whitish hemicellulose B preparations from corn fiber.

## MATERIALS AND METHODS

### Corn Fiber Samples

Fiber samples were kindly provided by American Maize Products Co. (Hammond, IN), Cargill Central Research (Minneapolis, MN), and CPC Corn Products Division (Summit-Argo, IL), and a sample of corn hull hemicellulose by Clinton Corn Processing Co. (Clinton, IA). Fiber samples were sometimes ground to 20 mesh using a Wiley mill. Moisture levels were determined after drying samples to constant weight in a vacuum oven at 70°C. In some cases, preliminary to extraction, 20 mesh fiber was first washed by stirring 50 g of sample into 1L of water (80°C for 15 min), and after removal of the water by vacuum filtration, extracting the residue with azeotropic ethanol-toluene (Fry 1988). The washed fiber was then dried in a vacuum oven at 50°C. Corn seed pericarp was isolated from kernels after heating 100 g with 200 mL of distilled, deionized water in a microwave oven for 3 min and then stirring for 15 min. After this process was repeated three times, the kernels became swollen and the pericarp softened. Then, after removing the tip cap, the pericarp was obtained by dissection.

### Procedure for Isolating Hemicelluloses A and B from Corn Fiber

*Standard alkaline hydrogen peroxide extraction procedure.* Corn fiber (3.0 g; 20 mesh destarched, unground not destarched) and aqueous 30% H<sub>2</sub>O<sub>2</sub> (2.5 mL) were added to rapidly stirred water (75 mL, deionized and distilled), to give a 0.25 ratio of H<sub>2</sub>O<sub>2</sub> to fiber, and a 1% aqueous solution of H<sub>2</sub>O<sub>2</sub>. The pH was adjusted to 11.5 by addition of 2*N* NaOH (9 mL). Under these conditions, there were about 15 meq NaOH per gram of fiber. Reactions at 25°C were conducted in Erlenmeyer flasks on magnetic stirrers, while those at 60°C were conducted in a jacketed reaction vessel heated with water from a thermostat-controlled circulating bath. During initial stages of stirring, oxygen evolution was active, and substantial frothing occurred, requiring that extractions be conducted in vessels with volumes two to three times those of extraction mixtures. The pH during extracting were maintained at 11.4–11.6 by dropwise addition of 50% NaOH. After removing residue by centrifugation at 3,000 × *g* for 10 min, the supernatant fluid was adjusted to pH 4.0 with 4*N* HCl (≈2 mL). The hemicellulose A (hemiA) fraction was allowed to precipitate overnight, and then collected after centrifugation at 10,000 × *g* for 10 min. The supernatant fluid containing hemiB was removed by decantation. The hemiA precipitate was washed with isopropanol, collected by filtration, and then dried in a vacuum oven at 50°C. In experiments where the hemiA fraction was not needed, it was removed by filtration through celite. HemiB was precipitated by pouring the supernatant fluid into 2 volumes of stirred isopropanol, from which it settled out as a white flocculent precipitate. HemiB was collected after carefully decanting off the supernatant fluid. It was transferred with the aid of isopropanol to a beaker and the mixture was stirred for 5 min. This converted the material from sticky and rubbery state to a more workable form. Next the hemiB fraction was collected (filtration, centrifugation, or sieve), and air-dried in a fume hood for 1 hr, finely fragmented with a conventional chop-grinder, and dried to constant weight in a vacuum oven at 50°C.

*Other extraction procedures.* In some cases, higher ratios of fiber to liquid were used, and 10 g of corn fiber was mixed with 100 mL of water before addition of H<sub>2</sub>O<sub>2</sub> and NaOH. Extraction

mixtures became quite viscous at these solid-to-liquid ratios, and mechanical stirring was required. Some extractions were conducted at 25°C and pH 11.5 using proportions of fiber-alkali-H<sub>2</sub>O<sub>2</sub> solution of 1:25:0.5, where the proportion of H<sub>2</sub>O<sub>2</sub> was double that of the standard extraction procedure. Extractions in the absence of H<sub>2</sub>O<sub>2</sub> were performed in *N* NaOH and in saturated Ca(OH)<sub>2</sub>, using 1:10 ratios of fiber to alkali solution at elevated temperatures and 1:20 at 25°C. One extraction was conducted in saturated Ca(OH)<sub>2</sub> under reflux conditions, using ratios of 1:10 fiber to alkali solution. HemiA and B were isolated as described above.

### Monitoring Rate of Delignification

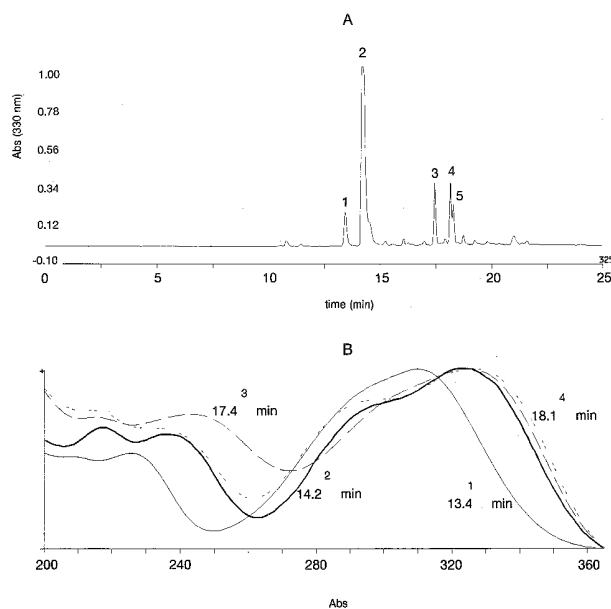
Low molecular weight products that resulted from lignin oxidation by alkaline peroxide were monitored by the increase in absorbance at 280 nm (Gould 1984). Timed aliquots were withdrawn from the extraction medium and diluted with an equal volume of 3-(*N*-morpholino) propane sulfonic acid (MOPS) buffer (pH 7.0, 10 mM) and stored for spectrophotometric analysis. The solutions were diluted with additional MOPS buffer to give absorbance values <2.0.

### Release of Phenolic Acids from Corn Fiber by Alkaline Hydrolysis

A mixture of corn fiber (200 mg, 20 mesh) and *N* NaOH (12 mL) was stirred in a glass vial at 25°C for 3 hr, under a stream of N<sub>2</sub>. After removal of the residue by centrifugation at 3,000 × *g* for 5 min, the supernatant fluid was adjusted to pH 1.0–1.5 by the addition of 4*N* HCl. This solution was extracted twice with *n*-butanol (12 mL). The *n*-butanol layers were combined, filtered through anhydrous MgSO<sub>4</sub> to remove traces of water, and evaporated to dryness on a rotary evaporator. The resulting material was taken up in methanol (10 mL) and filtered (0.45 μM filter) prior to analysis by high-performance liquid chromatography (HPLC).

### HPLC Analysis of Phenolic Acids

A modification of a gradient program developed earlier (Graham 1991) was used to separate phenolic acids released from corn fiber by alkaline hydrolysis. A Rainin (Woburn, MA) C18 reversed-phase column (4.6 × 250 mm) was used, and the gradient consisted of 0–55% acetonitrile in water (adjusted to pH 3.0 with



**Fig. 1.** A, High-performance liquid chromatogram of phenolic acids liberated from corn fiber by alkaline hydrolysis. B, Ultraviolet spectra of major peaks 1 (*p*-coumaric acid), 2 (ferulic acid), and (unknown compounds) 3 and 4.

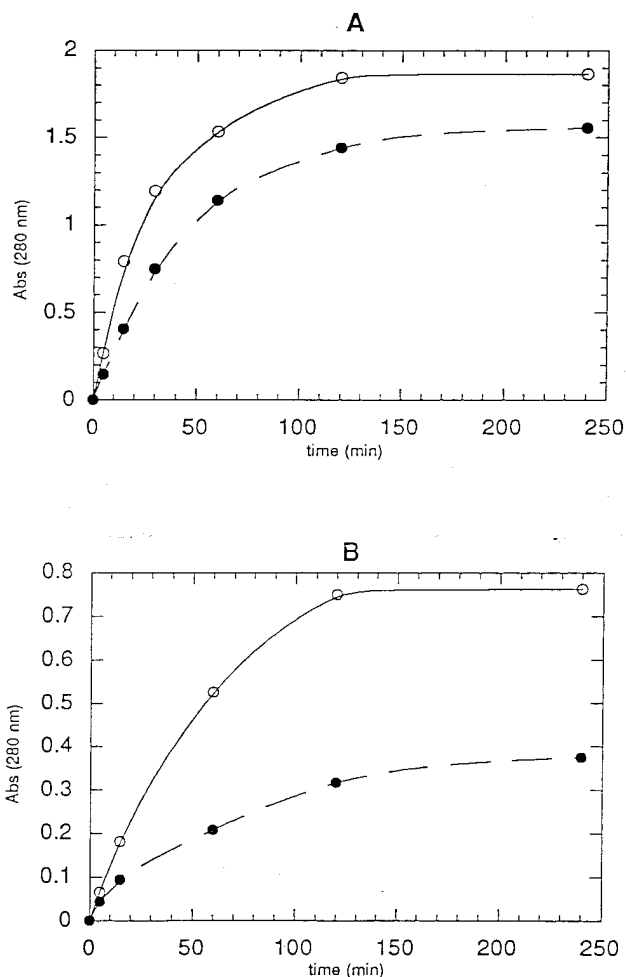
H<sub>3</sub>PO<sub>4</sub>) for 25 min, increasing to 100% acetonitrile in 10 min, and after 2 min returning to pH 3.0 water. A Spectra-Physics (San Jose, CA) system equipped with a scanning Spectra-Focus detector was used, and phenolic acids were detected at 330 nm.

### Starch Removal from 20 mesh Corn Fiber by Treatment with Termamyl

Termamyl is a heat-stable α-amylase which was kindly provided by Novo Nordisk Bioindustrials, Inc. (Danbury, CT). Corn fiber (30 g, 20 mesh, H<sub>2</sub>O washed) and 120 mL of sodium phosphate buffer (pH 6.8, 20 mM) were added to a jacketed reaction vessel heated to 80°C with water from a thermostat controlled circulating bath. Solid Ca(Cl)<sub>2</sub> was added so that the concentration of Ca<sup>++</sup> was 100 ppm, followed by addition of 1.2 mL of Termamyl. After 3 hr, the residue was collected by sintered glass vacuum filtration, and washed with distilled, deionized water (30 mL) to remove remaining maltodextrins. The residue was stirred in 100 mL of isopropanol, the isopropanol removed by vacuum filtration, and the fiber was dried in vacuum oven at 50°C. A yield of 21.4 g (71%) of destarched corn fiber was obtained.

## RESULTS AND DISCUSSION

Preliminary experiments were conducted in which hemiB was isolated from corn fiber according to published procedures, with saturated Ca(OH)<sub>2</sub> solutions as extractant at 70°C for 16 hr (Rutenberg and Herbst 1957) or under reflux conditions for 1 hr



**Fig. 2.** A, Effect of temperature on delignification of washed corn fiber: 25°C (●) and 60°C (○) with an H<sub>2</sub>O<sub>2</sub> to fiber ratio of 0.15. B, Effect of H<sub>2</sub>O<sub>2</sub> to fiber ratios of 0.0625 (●) and 0.25 (○) on delignification of washed corn fiber at 60°C. Conditions: pH 11.5; liquid to fiber ratio, 15:1 (v/w).

(Watson and Williams 1959). Tannish colored products were isolated in yields comparable to the published values (28.2 and 35.1% for the extractions at 70°C and under reflux conditions, respectively). The yields are on a dry, starch-free basis. Solutions of the hemiB preparations were yellowish in color, and at alkaline pH values were intensely yellow. The color could not be removed by dialysis. Preliminary extractions with NaOH were not conducted, since the brownish color of product and its solutions was determined earlier (Rutenberg and Herbst 1957, Watson and Williams 1959).

The pigmentation associated with the hemiB preparations is likely due to the presence of components such as protein or lignin. Protein is (Saulnier et al 1995a) very strongly associated with corn bran arabinoxylans, and lignin may be associated through ether linkages (Jung and Deetz 1993). The phenolic acids released from corn fiber by the alkaline hydrolysis procedure were analyzed by HPLC. As expected, we found ester-bound *p*-coumaric and ferulic acids to be liberated under milder alkaline conditions than those commonly used to extract hemicellulose. Retention times ( $T_r$ ) and ultraviolet spectra were identical to those of pure standards. In addition to *p*-coumaric acid (peak 1,  $T_r = 13.4$  min) and ferulic acid (peak 2,  $T_r = 14.2$  min), other major peaks (3–5) eluted at 17.4, 18.1, and 18.3 min, respectively (Fig. 1A). Peaks 3–5 had ultraviolet spectra nearly identical to that of the major peak, ferulic acid, with  $\lambda_{max}$  at 325–330 nm. The spectra of peaks 3 and 4 are shown in Figure 1B. One of the peaks likely represents diferulic acid, a constituent of corn bran (Saulnier et al 1995b) which is known to crosslink polysaccharides with other macromolecules in plant cell walls (Saulnier et al 1995b, Fincher and Stone 1986, Fry 1988). *p*-Coumaric acid had a  $\lambda_{max}$  at 310 nm and a spectrum unlike the other phenolic acids alkali-extracted from corn fiber. When  $H_2O_2$  was included in the alkaline hydrolysis mixture, phenolic acids were oxidatively destroyed, and none were detected by HPLC.

A mild alkaline  $H_2O_2$  (AHP) process was developed for delignifying agricultural residues (Gould 1984), with minimal concomitant loss of hemicellulose. When the pH rose >11.5, increased solubilization of hemicellulose was noted. The process incorporated  $H_2O_2$  in the extraction medium to convert the lignin portion into soluble, low molecular weight organic acids. (Gould 1985b) showed that delignification was most effective at  $\approx$ pH 11.5, the  $pK_a$  for the dissociation of  $H_2O_2$ , and that the concentration of the species active in delignification,  $\cdot OH$  and  $O_2^{\cdot -}$  were optimal at pH 11.6.

We tested whether lignin fragments that may be contaminating corn fiber hemicellulose preparations could be removed by including

$H_2O_2$  in the extraction medium. Lignin degradation with alkaline peroxide was monitored by the increase in absorbance at 280 nm (Gould 1984). Lignin removal was maximized in  $\approx 4$  hr at both 25 and 60°C, with a 20% higher level of delignification products produced at the higher temperature (Fig. 2A). The effect of  $H_2O_2$  level on delignification is shown in Figure 2B. By quadrupling the ratio of  $H_2O_2$  to fiber ratio from 0.0625 to 0.25 (and the  $H_2O_2$  level from 0.125 to 0.5%), the level of delignification products was about doubled. These and other experiments to optimize extraction were conducted using destarched 20-mesh fiber.

HemiB prepared by AHP extraction was much lighter in color than that obtained under similar conditions but in the absence of peroxide. Efforts were focused on extractions of hemiB using  $H_2O_2$  in NaOH rather than in  $Ca(OH)_2$ , since the pH of corn fiber in saturated  $Ca(OH)_2$  is  $\approx 11.4$ , and drops to 9.8 upon addition of  $H_2O_2$  to the 1% level. At that pH, delignification was extremely slow and little hemiB was extracted.

The results of AHP extractions of corn fiber under different conditions are shown in Table I. Yields (listed on a dry, starch free basis) of the water soluble hemiB generally exceed those of hemiA by more than 10-fold. In two cases (Table I, rows G,L), extractions at 60°C resulted in hemiA yields of >8%. It was essential to remove hemiA by filtration prior to precipitation of hemiB. Otherwise, resulting solutions were turbid, which in some cases would limit the uses of the product. Application of the AHP process to 20-mesh fiber at 25°C indicated a doubling of hemiB yield between an 8- and 24-hr (rows A,B) extraction period. Only a slight gain was realized in extending the extraction period to 72 hr (row C). An extraction in which the proportion of  $H_2O_2$  to fiber was increased from 0.25 to 0.50 resulted in an increase in the yield of hemiB after 72 hr at 25°C from 36% (row C) to 46%. The hemiB was nearly white, and the recovered residue was off-white. In 8 hr, 25°C extractions, the yield more than doubled when the pH was increased from 11.5 to 12.0 (rows A,E). In 24 hr at 25°C, only a modest gain in yield was achieved by increasing the pH from 11.5 to 12.0 (rows B,F). At 60°C, a higher yield of hemiB was obtained at pH 11.5 than at pH 12.0 (rows D,G). This is the trend that might be expected, since pH 11.5 is the optimal pH for lignin oxidation. At 60°C and pH 12.0, 26% of the hemicellulose was hemiA (row G). A maximum hemiB yield of 51% was obtained at 60°C by processing at pH 11.5 for the first hour and then increasing the pH to 12.5 for the final hour (row H). When using unground corn fiber, as with 20-mesh fiber, little gain in yield resulted from extending the 25°C process beyond 24 hr (rows I,J). By comparing results using ground and unground fiber, it is clear that the energy intensive grinding step provides little advantage in

TABLE I  
Yields of Hemicellulose A and B from Alkaline  $H_2O_2$  Extraction of Corn Fiber<sup>a</sup>

Trial	Corn Fiber	Reaction Conditions			Hemicellulose Yield, % <sup>b</sup>	
		Initial pH	Temp., °C	Time, hr	B	A
A	20 Mesh	11.5	25	8	15	3.1
B	20 Mesh	11.5	25	24	35	3.5
C	20 Mesh	11.5	25	72	36	...
D	20 Mesh	11.5	60	2	41	2.8
E	20 Mesh	12.0	25	8	35	4.2
F	20 Mesh	12.0	25	24	40	3.7
G	20 Mesh	12.0	60	2	32	8.3
H	20 Mesh	11.5–12.5 <sup>c</sup>	60	2	51	4.2
I	Unground	11.5	25	24	35	0.4
J	Unground	11.5	25	72	38	...
K	Unground	11.5	60	2	42	1.7
L	Unground	11.5–12.5 <sup>c</sup>	60	2	47	8.5
M	Unground	11.5–12.5 <sup>c</sup>	25	24	46	4.2

<sup>a</sup> Extractions were conducted using fiber-alkaline solution- $H_2O_2$  ratios of 1:25:0.25 (w/v/w); 20 mesh fiber samples were destarched, and the unground fiber samples were unwashed.

<sup>b</sup> Yields for all trials are reported on a dry, starch-free basis.

<sup>c</sup> Initial pH of 11.5 was increased to 12.5 after one-half extraction time had elapsed.

terms of hemiB yield (rows B,I, C,J, D,K, F,M, and H,L).

HemiB yields were reduced by more than one-third when extractions were conducted in the absence of H<sub>2</sub>O<sub>2</sub>. Using conditions such as Trial D (Table I), for example, the yield dropped from 41 to 25%. When hemiB color as well as yield is considered, the AHP process is most effective when conducted at pH 11.5. Possibly at the elevated pH, especially at 60°C, chromophores are generated by alkali-catalyzed modification of reducing end groups on the polysaccharide. The hemiB's generated at pH 11.5 were off-white, while those exposed to pH values of 12.0 and 12.5 were tannish, even when the extractions were done at room temperature. A 2-hr reaction period at pH 11.5 at 60°C with unground fiber (row K) gave a 42% yield of off-white hemiB, with a very low level (1.7%) of hemiA. We found that kernel pericarp, when extracted under these conditions, yielded off-white hemiB in a 24% yield. This yield was lower than expected.

The results in Table I were from experiments using a 1:25:0.25 ratio of fiber-alkali-H<sub>2</sub>O<sub>2</sub> solution. Under these conditions, most of the fluid (and hemicellulose) was isolated after removal of residue by centrifugation. When higher ratios of fiber to alkali solution were used, however, significant quantities of fluid and associated hemicellulose remain with the swelled residue. Then it is necessary to redispense the residue in additional water to extract residual hemicellulose. This is the procedure as commonly practiced (Wolf et al 1953, Rutenberg and Herbst 1957). When AHP extractions are conducted at fiber-to-liquid ratios of 1:10, substantial amounts of hemicellulose-containing fluid remained associated with the residue, since it became very swollen and absorbent. Only ≈70% of the fluid is isolated after the initial centrifugation and decantation, so as much as 30% of product could remain in the fluid associated with the residue.

For hemiB uses where solution clarity and stability are required, it is essential that starch be removed before application of the AHP process. Small quantities of starch were otherwise extracted, which retrograded, resulting in turbidity of hemiB solutions. It was unnecessary to grind corn fiber before hemiB extraction. The fiber was severely fragmented during AHP extractions, and residues appeared the same after initial stages, whether extracting ground or unground fiber. So with both forms of fiber, sufficient surface area was rapidly provided for optimal access of H<sub>2</sub>O<sub>2</sub> and alkali. When hemiB samples isolated from ground or unground fiber were taken up in alkaline peroxide at pH 11.5, no additional 280 nm absorbing material was generated. We concluded that all lignin susceptible to degradation had been removed. It was unnecessary to extract corn fiber with toluene-ethanol before alkaline H<sub>2</sub>O<sub>2</sub> extraction. The unground fiber samples were unwashed and similar yields (Table I) and product color were obtained as when using ground, organic solvent-extracted fiber.

Our goal is the development of a commercial process for the extraction of hemicellulose from corn fiber. Future plans include optimizing the process, to minimize where possible, the use of raw materials such as H<sub>2</sub>O<sub>2</sub> and alkali, and to reduce the time of some processing steps. It will be important to increase the proportion of fiber in the extraction medium and to develop effective means to separate fluid from residue. We will also test the possibility of extracting with alkali, and then separately treating the hemicellulose (and also the cellulosic residue) with alkaline H<sub>2</sub>O<sub>2</sub>. This could help to minimize the use of H<sub>2</sub>O<sub>2</sub>, and possibly allow the use of more extreme extraction conditions to increase hemiB yields. Alternatives to alcohol precipitation and subsequent drying of the hemicellulose will be explored, including ultrafiltration in conjunction with spray-, drum-, or freeze-drying. We will also define the hemiB preparations with regard to monomer composition and molecular weight, to determine the effects of various AHP conditions on hemiB structure. Characterization of the properties of the hemiB will be given priority to help determine whether the product would be marketable. Colors of solid hemiB and its solutions will be measured, as well as

viscosity-concentration relationships at various temperatures. Properties of water-cast films will be determined. Also, the residue remaining after hemicellulose extraction will be characterized, since it could be the source of a unique cellulose material.

## ACKNOWLEDGMENTS

We thank Robert Freidman (American Maize Products Co.), Ting Carlson (Cargill Central Research), and Ronald Hebeda (CPC Corn Products Division) for fiber samples, Edward Kowalski (Novo Nordisk Bioindustrials Inc.) for the α-amylase Termamyl, and Michael Powell for preparing ground fiber. The authors also thank an anonymous reviewer for helpful comments on the manuscript.

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[Received March 11, 1996. Accepted October 9, 1996.]