

Isolation and Characterization of Cellulose/Arabinoxylan Residual Mixtures from Corn Fiber Gum Processes

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ABSTRACT

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White, fluffy cellulose/arabinoxylan mixtures (CAX) were generated from the solid residues remaining after corn fiber gum (CFG) production. Most CAX were produced using variations of a process in which a single alkaline hydrogen peroxide (AHP) step was used for delignification and for CFG (arabinoxylan) extraction. The optimal ratio of H₂O₂ to corn fiber to water was 0.1:1:20. Holding this ratio constant, time and temperature conditions were systematically varied, and yields of CAX and CFG determined. Parallel processes were conducted without H₂O₂ to determine its effect on CAX and CFG yield. CAX prepared under identical conditions but without H₂O₂ retained nearly twice the levels of CFG sugars, as

revealed from L-arabinose, D-xylose, and D,L-galactose levels. Even the CAX prepared under extreme AHP conditions (1 hr, 100°C), however, contained 32.9% of these CFG sugars. This CAX was obtained in a 25.1% yield, whereas those produced under less vigorous conditions were obtained in higher yields, because they retained more CFG. CAX prepared in the presence of H₂O₂ hydrated very effectively, as indicated by their high swollen volumes and water absorbance values. This suggests potential food applications for CAX as a bulking agent. In addition, the open structure of the CAX matrix would render these residues suitable for chemical derivatization and enzymatic saccharification.

Corn fiber (CF) is a low value product of corn wet-milling and is a mixture of coarse fiber (from kernel pericarp or hull) and fine fiber (from endosperm cellular material). Variable levels (11–23%) of adherent starch also are present in CF (Leathers 1998). A mixture of CF with spent flake (corn germ meal) and steep water is concentrated and mostly exported to Europe as corn gluten feed (Gulati et al 1996). Over 8 million tons of corn gluten feed per year are produced in the United States, with a value of just \$0.03/lb (as of August 1999).

Searches for uses of CF more highly valued than animal feed have long focused on the hemicellulose fraction, which accounts for over 50% of dry, starch-free fiber (Watson 1987). CF hemicellulose B is a glucuronoarabinoxylan, commonly referred to as corn fiber gum (CFG). Low levels of both D- and L-galactose are also present in the polysaccharide (Whistler and BeMiller 1956). Several processes for producing CFG using various alkaline extraction conditions have been described in the patent literature (Wolf et al 1955; Rutenberg and Herbst 1957; Watson and Williams 1959; Schweiger 1973; Antrim and Harris 1977). No food or industrial applications for CFG resulted, in part because of the color and flavor associated with the materials. Nonetheless, several useful properties were demonstrated, suggesting food uses as emulsifiers, stabilizers, and extenders, and industrial uses as adhesives (Whistler 1993; Voragen 1998). Our patented (Doner et al 2000) processes overcame the problems noted above and resulted in off-white CFG in yields $\geq 42\%$. In one process (Doner and Hicks 1997), alkaline extraction and alkaline hydrogen peroxide (AHP) treatments were conducted simultaneously. In the other (Doner et al 1998), hemicellulose was extracted and then its solution bleached separately under AHP conditions. CFG yield was somewhat reduced using the latter process, but H₂O₂ consumption was minimized and the product was whiter in color.

Our approaches for CFG production were derived from AHP processes previously developed, wherein agricultural residues such as corn stover and wheat straw were delignified. That research was

aimed at rendering the cellulose in these residues amenable to derivatization (Dreyfus 1949), for saccharification and fermentation to ethanol, and for use as ruminant feed (Gould 1984; Gould and Jasberg 1991). In that work, maximum delignification occurred at pH 11.5 and conditions were optimized to conserve hemicellulose and thereby retain the feed value of the materials. Our goal, on the other hand, had been to maximize hemicellulose extraction (and CFG yield); this was achieved by modifying variables such as alkali concentration and temperature.

Our CFG isolation processes leaves a solid residue that can be processed to cellulose/arabinoxylan mixtures (CAX). Its isolation and characterization is the subject of this article. We will focus on CAX prepared by our process (Doner and Hicks 1997), wherein AHP delignification and bleaching occur in the same step as CFG extraction. Earlier work has described related efforts to isolate cellulose suitable for human consumption from other agricultural residues. In one process (Thompson 1984), a short fiber cellulose was isolated from soybean hulls, sugarbeet pulp, and corn bran after applying two chlorine bleaching steps, an alkaline extraction step, and extensive rinsing. From corn bran, a slightly cream-colored product (98.3% cellulose) was obtained with a 19.4% yield. In an extension of that work (Vail 1991), the focus was again on cellulose from soybean hulls, and the problems associated with chlorine in the waste water were eliminated. Instead of using chlorine bleaching, hydrogen peroxide was used in combination with alkaline extraction steps. Both of these processes contained time-consuming steps, but did generate cellulose with fine, short-fiber character. Such cellulose, although inferior for paper manufacture, is superior for use in foods (Thompson 1984). An early patent described the application of an AHP to produce α -cellulose from wheat straw, suitable for further derivatization (Dreyfus 1949). More recently, AHP bleaching has been applied to a variety of plant materials (Devic 1996) including corn bran to produce edible, low-calorie food products.

MATERIALS AND METHODS

Corn Fiber Samples

CF was a gift from Cargill Central Research (Minneapolis, MN) and was ground to 20-mesh particle size using a Wiley mill. Moisture levels of fiber and products derived were determined after drying samples to constant weight in a vacuum oven at 70°C. After extracting CF oil with hexane (Moreau et al 1996), CF was destarched using Termamyl α -amylase (a gift of Novo Nordisk Bioindustrials, Inc., Danbury, CT). CF (400 g) was stirred at 90–95°C in 4 L of H₂O, and adjusted to pH 5.6 by the addition of 50% NaOH solution. Termamyl (25 mL) was added and the mixture was

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stirred for 1 hr. The destarched fiber was isolated by sieving, rinsed with hot water and ethanol, and dried in a 60°C vacuum oven, yielding 316 g (79%).

Isolation of CAX from CF

CAX preparation entailed extracting CFG and then processing the residues to fine powders. In preliminary experiments, CAX were prepared to establish optimal ratios of H₂O₂ to CF to liquid and to gain information regarding the effects of process time and temperature on CAX yield and whiteness (WI). From the results of the preliminary experiments, a range of CAX was systematically prepared, using various time-temperature conditions. In some cases, CF was extracted using AHP conditions and, in others, H₂O₂ was absent. For the process for the CAX prepared using AHP conditions for 60 min at 100°C, destarched, deoiled CF (20 g, unground) was mechanically stirred into water (400 mL) at 100°C. H₂O₂ (2.0 g; 6.7 mL of 30% H₂O₂) was added and the pH was adjusted to 11.5 with 50% NaOH. After 60 min, the reaction mixture was cooled in an ice bath to ≈20°C, then centrifuged at 6,000 × g for 10 min. The CFG solution was decanted from the residual pellet. The decantate was saved and the residue stirred in water (300 mL) for 5 min, then centrifuged again. The decantates were combined and processed to CFG as described earlier (Doner et al 1998). The residue was stirred into water (250 mL) and, after adjusting to pH 5.5–6.0, stirring was continued for 15 min. The CAX was isolated by vacuum filtration, applying an isopropanol rinse after most of the water had been removed. The filter cake was broken up using a conventional chopper-grinder, then dried at 40°C in a vacuum oven.

A range of other CAX were similarly produced, but at 50 and 70°C, with and without H₂O₂. Twelve CAX (Table I, trials A through L) were thereby generated, (50, 70, and 100°C; 20 and 60 min; + or –H₂O₂). Two additional CAX were prepared. CAX-M was produced by further extraction of the CAX-L prepared above (60 min, 100°C, +H₂O₂), using the vigorous alkaline extraction conditions previously used for isolating hemicellulose from corn bran (Saulnier et al 1995). CAX-L was extracted with 1.5N KOH for 2 hr at 100°C and worked up to CAX-M as described above. Finally, CAX-N was prepared by AHP processing of CAX-J prepared by alkaline extraction (–H₂O₂) of CF. This was accomplished by stirring CAX-J (10 g) in H₂O (400 mL) and H₂O₂ (1.0 g; 3.3 mL of 30% H₂O₂) at 25°C after adjusting to pH 11.5 with 50% NaOH. After 30 min, CAX-N was worked up (as described above) to a dry powder; the yield was 7.7 g (77%).

HPLC Determination of Sugar Composition of CAX

The neutral sugar composition of the CAX was determined by HPLC after acid hydrolysis of the polysaccharides to constituent sugars. CAX and the starting CF sample (20 mg) were weighed into a screw-cap vial and mixed with 150 µL of 12N H₂SO₄, vortexing periodically over 45 min at room temperature. The acid was then diluted to 1N H₂SO₄ by adding water (1.65 mL) and the mixture was heated in an oven at 100°C for 1.5 hr. After cooling to room temperature, BaCO₃ was gradually added until solution pH was neutral by filter paper. The BaSO₄ was removed by vacuum filtration and the sugar-containing filtrate was dried under a stream of nitrogen. The syrups were dissolved in water (500 µL) and the solution was filtered through a 0.2-µm Anotop 10 plus membrane filter (Whatman Cat. No. 6809 3022).

Hydrolyzed CAX were then analyzed for sugar composition on a BioRad Aminex HPX-87P column (300 × 7.8 mm) with deashing guard cartridges. Column temperature was controlled at 60°C using a BioRad column heater. Degassed deionized water was used as the eluent at a flow rate of 0.6 mL/min. Samples (10 µL) were injected using an Alcott autoinjector. An HP refractive index detector (HP1047A) was used to measure eluted sugars.

Data was collected and analyzed using ChromPerfect LE data software. Standard sugars solutions were injected and analyzed to construct calibration curves for the RI response of each sugar. Quantitation was based on integrated peak area relative to that of known quantity of standard sugar. Retention times of sugars of interest were D-glucose (11.3 min), D-xylose (12.3 min), D,L-galactose (13.0 min), and L-arabinose (14.5 min).

Water Absorbency and Swollen Volume

Earlier procedures (Gould et al 1989; Renard et al 1997) for determining water absorbency and swollen volume were somewhat modified. CAX samples were passed through a no. 20 (850 µm) sieve using preliminary grinding (convention chopper-grinder) when necessary. Samples (1.0 g) were then stirred for 4 hr in deionized water (100 mL) in a graduated cylinder. The CAX was then allowed to settle out overnight. The bed volume occupied by the CAX was the swollen volume, recorded as mL/g of CAX. The CAX was resuspended in the graduated cylinder and gently poured onto a tarred no. 60 fine-meshed (250 µm) sieve. Water was allowed to drain from the hydrated CAX for 1 hr, after which the sieve was weighed again. The water absorbency (g of water absorbed/g of CAX) was calculated from (wet CAX – dry CAX)/dry CAX.

TABLE I
Yields (db) of Cellulose/Arabinoxylan Mixtures (CAX) and Corn Fiber Gum (CFG)
Under Various Conditions of Corn Fiber Processing
and Sugar Composition of CAX Polysaccharides

Trial	Time (min)	Temp. (°C)	H ₂ O ₂	CAX	Glu ^a	Ara ^a	Xyl ^a	Ara/Xyl	Gal ^a	CFG	CAX + CFG
A	20	50	No	75.0	20.3	27.9	45.8	0.61	6.0	3.3	78.3
B	60	50	No	74.2	19.1	28.9	46.0	0.63	5.9	5.7	79.9
C	20	50	Yes	52.1	23.8	26.5	43.7	0.61	5.9	15.0	67.1
D	60	50	Yes	46.1	28.3	25.2	41.4	0.61	5.1	29.3	75.4
E	20	70	No	61.5	14.3	31.5	47.9	0.66	6.3	11.3	72.8
F	60	70	No	50.0	21.3	28.3	44.0	0.64	6.3	22.7	72.7
G	20	70	Yes	38.5	47.3	19.0	28.7	0.66	5.1	29.4	67.9
H	60	70	Yes	34.5	45.6	19.5	29.5	0.66	5.3	33.6	68.1
I	20	100	No	42.2	36.5	22.6	35.2	0.64	5.7	25.3	67.5
J	60	100	No	35.1	42.1	20.6	32.0	0.64	5.2	28.2	63.3
K	20	100	Yes	28.5	63.0	13.2	20.1	0.66	3.7	35.4	63.9
L	60	100	Yes	25.1	67.1	11.7	17.9	0.65	3.3	37.0	62.1
M ^b	24.8	71.0	10.0	16.2	0.62	2.7
N ^c	23.2	28.4	43.1	0.66	5.3
Corn fiber ^d	33.3	25.5	35.3	0.72	5.8

^a Relative percentages.

^b From further extraction of CAX-L with 1.5N KOH for 2 hr at 100°C.

^c From alkaline hydrogen peroxide processing of the CAX-J.

^d Destarched and deoiled.

Molecular Weight, WI, and Nitrogen and Ash Levels

Molecular weights were determined using the 2,2-bicinchonate end-group assay (Johnston et al 1998). Color values were measured by a handi-color colorimeter (BYK Gardner USA, Silver Spring, MD). Nitrogen and ash determinations were performed by Galbraith Laboratories, Inc. (Knoxville, TN).

RESULTS AND DISCUSSION

The CAX were produced from the solid residues remaining from corn fiber gum production using alkaline hydrogen peroxide. CAX containing the highest proportions of cellulosic β -glucan resulted from AHP processes providing maximal yields of CFG.

A series of experiments was conducted, mainly at 70°C, to determine conditions for optimizing CFG yields and thereby yielding CAX maximally depleted in arabinoxylan hemicellulose. The dramatic effect of H₂O₂ addition on CFG yield at 25 and 70°C is shown in Fig. 1. At 70°C, the yield of CFG more than tripled when going from -H₂O₂ (12.4% yield) to a 0.1:1 ratio H₂O₂ to corn fiber (43.4% yield). The CFG yield further increased only marginally to 44.2% when the proportion of H₂O₂ was further doubled. CAX yields were correspondingly nearly minimized at the 0.1:1 ratio, dropping to 31.6%. At 25°C and with 60 min of extraction, CFG yields gradually increased with added H₂O₂, but only reached 24.0% even at a 1:1 ratio for H₂O₂ to corn fiber. Clearly, significant quantities of arabinoxylan (CFG) resided in the CAX when corn fiber was extracted at this lower temperature. A 24-hr extraction time was required for CFG yields to exceed 30% at 25°C. From the experiments summarized in Fig. 1, it was clear that a 0.1:1 ratio of H₂O₂ to corn fiber at elevated temperatures would be recommended for economically generating maximal CFG yield and highest quality CAX.

Further preliminary experiments at 70°C had established 20:1 as the optimal liquid to CF ratio for CAX production using AHP conditions. Lower levels of liquid resulted in highly viscous solutions due to the extraction of CFG and the concomitant generation of highly water-absorbent CAX. Using 0.1:1:20 ratios of H₂O₂ to fiber to liquid at 70°C, it was further shown that the CAX produced at 70°C in 30 min was whiter (WI = 39.4) than that produced in 8 hr at ambient temperature (WI = 26.0). Also using these conditions, yield of CAX diminished with time up to 1 hr, after which the yield was unchanged. Again, the proportion of CAX was higher when CFG and other materials such as delignification products, protein, and low molecular weight compounds were most efficiently removed. Having optimized some key process variables, we prepared a range of CAX and CFG from destarched

and deoiled CF to determine effects of time and temperature on yields. For these experiments (Table I, trials A through L), the ratio of H₂O₂ to fiber to liquid ratios was held constant at 0.1:1:20. The CAX with maximal cellulose level had the greatest quantity of CFG (arabinoxylan) extracted at 100°C with H₂O₂ (trials K and L). These CAX also appeared extremely white, with WI = 48.0 for CAX-L. Without H₂O₂ at 100°C (trials I and J) and at lower temperatures (trials A, B, E, and F), CAX yields were higher, because they contained elevated levels of arabinoxylan. The dramatic effect of H₂O₂ on removing arabinoxylan is noted by comparing CFG and CAX yields under otherwise identical conditions (trials A-C, B-D, E, G, F-H, I-K, and J-L). The total yields of CAX and CFG were also lower in the trials with H₂O₂. The total yields also diminished with increased time and temperature, suggesting that materials in addition to arabinoxylan were extracted from the fiber. Nitrogen analyses suggested that protein removal may partially account for this difference. CF had been destarched and CFG hemicellulose B contains no D-glucose; therefore, the D-glucose levels in CAX reflect its level of cellulose. Under conditions of trial L, the CAX still contained only 67.1% cellulose. The extreme difficulty of removing all arabinoxylan from CAX can be seen when comparing trials L and M. The CAX-L was further extracted under extremely vigorous alkaline conditions (1.5N KOH at 100°C for 2 hr), yielding CAX-M. Based on starting CF, the yield of CAX dropped very little, however, from 25.1% (CAX-L) to 24.8% (CAX-M) and the D-glucose (cellulose) level increased from 67.1% to just 71.0%, showing that little additional arabinoxylan had been removed. In earlier work on generating cellulose from soybean hulls using an AHP process (Vail 1991), the product had a cellulose level of 77.6%. It is clear that substantial quantities of arabinoxylan remain inextricable under vigorous AHP processing conditions.

The degree of contamination of CAX with arabinoxylan can be estimated from the total of D-xylose, L-arabinose, and D,L-galactose (Table I). This total was 32.9% in trial L and under the mildest conditions (trial A) it was 79.7%. The L-arabinose to D-xylose ratio was 0.722 in the starting CF, higher than the ratio in any of the CAX. This ratio in the arabinoxylan polymer present in CAX-A through CAX-N is lower in all cases. It is well known that L-arabinose residues are labile under mild acid conditions, but its removal even under the vigorous alkaline conditions used here would be unexpected. It is likely that changes in L-arabinose to D-arabinose ratios were due to changes in proportions of molecular

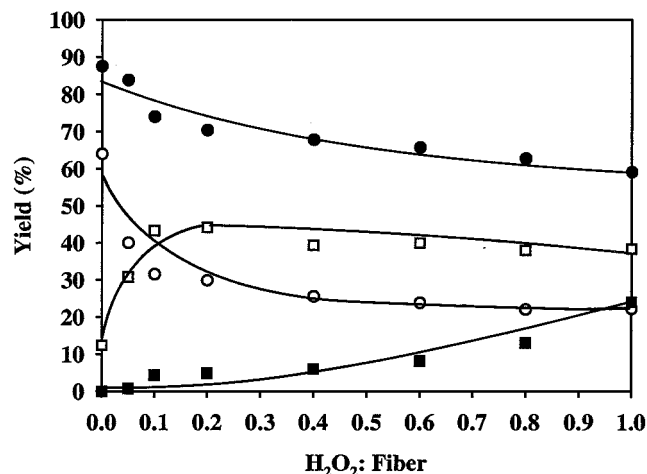


Fig. 1. Effect of H₂O₂ addition on yields in 60 min of cellulose/arabinoxylan mixtures (● = 25°C, ○ = 70°C) and corn fiber gum (■ = 25°C, □ = 70°C).

TABLE II
Effect of Corn Fiber Processing Conditions
on Hydration Properties and Nitrogen and Ash Levels
of Cellulose/Arabinoxylan Mixtures (CAX)

CAX	Time (min)	T (°C)	H ₂ O ₂	Swollen Volume ^a	Water Absorb. ^b	N (%)	Ash (%)
A	20	50	No	0.98	1.12
B	60	50	No	0.88	0.80
C	20	50	Yes	13	4.4	<0.5	0.81
D	60	50	Yes	31	28.2	<0.5	0.86
E	20	70	No	0.8	0.90
F	60	70	No	0.61	0.87
G	20	70	Yes	62	33.0	<0.5	0.67
H	60	70	Yes	34	19.5	<0.5	0.68
I	20	100	No	0.51	0.66
J	60	100	No	29	14.0	<0.5	0.48
K	20	100	Yes	47	30.2	<0.5	0.66
L	60	100	Yes	93	39.9	<0.5	0.71
M ^c	44	22.7	<0.5	13.44
N ^d	106	40.0	<0.5	1.53
Corn fiber ^e	1.26	0.75

^a Expressed as mL/g of CAX.

^b Water absorbency (g of water absorbed/g of CAX).

^c From further extraction of CAX-L with 1.5N KOH for 2 hr at 100°C.

^d From alkaline hydrogen peroxide processing of the CAX-J.

^e Destarched and deoiled.

species of arabinoxylans. Note, for example, the reduced L-arabinose to D-xylose (0.617) in CAX-M compared to its precursor CAX-L (0.653). The L-arabinose to D-xylose ratios were very consistent in CAX prepared under conditions where only process time was varied from 20 to 60 min (compare, for example, CAX-G with CAX-H and CAX-I with CAX-J). Although CFG contains ≈4% D-glucuronic acid (Doner et al 1998), we did not detect this sugar in CAX by using a sensitive spectrophotometric procedure (Blumenkrantz and Asboe-Hansen 1973). Although it may have been present in low levels, background chromophores generated during acid hydrolysis of CAX were too dominant to allow detection.

AHP processing of agricultural residues such as wheat straw (Gould et al 1989) and apple pomace (Renard et al 1997) has pronounced effects on the hydration properties of these materials. These changes may be due to the generation of open amorphous structures in the resulting cellulosic matrix through AHP-induced delignification and removal of hemicellulose (Gould et al 1989). Changes in functional properties such as hydration suggest potential applications, such as for bulking agents in foods. The hydration properties of some of the CAX were characterized by measuring swollen volumes and water absorbencies. These values were determined for all CAX produced using AHP conditions, and generally increased with temperature and process time. The effect of H₂O₂ can be seen by comparing values of CAX-J (produced without using H₂O₂) with CAX-L. These were produced under otherwise identical conditions, and H₂O₂ presence resulted in tripling of both swollen volume and water absorbency values. When CAX-J was AHP processed to produce CAX-N, the swollen volume increased from 29 to 106 mL/g (Table II), the highest value observed. The delignification and additional arabinoxylan removal resulting from AHP processing resulted in CAX with more open cellulosic structures, allowing greater water penetration. These swollen volume and water absorbency values are higher than those found for AHP-processed wheat straw (Gould et al 1989) and similar to those found for AHP-processed apple pomace (Renard 1997). The CAX generated at elevated temperatures are likely cellulose II allomorphs, the same form produced by cellulose mercerization (15% NaOH treatment) at ambient temperature (Attala 1983). Mercerization enhances the accessibility of cellulose, making it more amenable to hydrolysis and derivatization.

The starting material in our work, destarched and deoiled CF, contained 1.26% nitrogen. Using a nitrogen-to-protein conversion factor of 6.25, that would translate to 7.9% protein. All of the CAX contained less protein than this, and nitrogen was present at <0.5% levels in all AHP-processed CAX (Table II). The CAX generated without H₂O₂ contained less nitrogen as process time and temperature increased. Ash levels were quite low in the CAX (Table II), and these also generally decreased somewhat with process time and temperature. The presence of H₂O₂ did not appear to have much effect on ash levels. The elevated ash level in CAX-M may be due to the high concentration of KOH used to produce it from CAX-L.

Molecular weight determinations were conducted on selected CAX by a sensitive reducing end-group determination, based on Cu⁺-bicinchoninate formation. The higher molecular weight CAX were generally those that contained the lowest levels of residual extractable arabinoxylan; using this assay, we found they had molecular weights <20,000. The highest molecular weight was 95,000 for CAX-L. CAX-J had a molecular weight of just 44,000. It was prepared under time-temperature conditions identical to CAX-L but without H₂O₂, so that it retained some arabinoxylan. CAX-K was preparation was identical to CAX-L, except for a shorter time period. Its molecular weight was 61,000, again lower than CAX-L because of residual arabinoxylan. When CAX-J was treated with H₂O₂, the molecular weight increased from 44,000 to 57,000 as additional arabinoxylan was removed. CAX-M was generated under exhaustive extraction conditions and contained the

highest cellulose level of the five CAX evaluated. Its molecular weight was 71,000, less than that of CAX-L, possibly because of cellulose depolymerization. For all CAX, the molecular weight measurements were the average of all polysaccharides that contained reducing end groups.

CONCLUSIONS

The residues remaining from AHP processes to produce CFG were further processed to yield white, fluffy CAX. In the optimal process, delignification of CF and CFG extraction were performed in a single step. In that process, H₂O₂, CF, and water were mixed in a ratio of 0.1:1:20. Following adjustment to pH 11.5, the mixture was stirred at 100°C for 1 hr. The solubilized CFG was removed and processed to a white powder, leaving the insoluble CAX. It was slurried in water, neutralized, filtered, washed with isopropanol, and dried. This CAX, generated after solubilizing most arabinoxylan and other materials, was obtained in a yield of 25.1%. Even under these vigorous AHP extraction conditions, the CAX was not pure β-glucan because it contained 32.9% of the CFG hemicellulose sugars D-xylose, L-arabinose, and D,L-galactose. CAX prepared under identical conditions but without H₂O₂ retained nearly twice the levels of these sugars. CAX prepared with H₂O₂ bound water very effectively, as indicated by swollen volumes and water absorbance values. This suggests potential food application as a bulking agent. In addition, the open structure of the matrix would render these residues suitable for chemical derivatization and for enzymatic saccharification.

LITERATURE CITED

- Antrim, R. L., and Harris, D. W. 1977. Method for treatment of corn hulls. U.S. patent 4,038,481.
- Attala, R. H. 1983. The structure of cellulose: Recent developments. Pages 59-77 in: Wood and Agricultural Residues. E. J. Soltes, ed. Academic Press: New York.
- Blumenkrantz, N., and Asbo-Hansen, G. 1973. New method for quantitative determination of uronic acids. *Anal. Biochem.* 54:484-489.
- Devic, M. 1996. Bleaching of plant materials. U.S. patent 5,480,788.
- Doner, L. W., and Hicks, K. B. 1997. Isolation of hemicellulose from corn fiber by alkaline extraction. *Cereal Chem.* 74:176-181.
- Doner, L. W., Chau, H. K., Fishman, M. L., and Hicks, K. B. 1998. An improved process for isolation of corn fiber gum. *Cereal Chem.* 75:408-411.
- Doner, L. W., Sweeney, G. A., and Hicks, K. B. 2000. Isolation of hemicellulose from corn fiber. U.S. patent 6,147,206.
- Dreyfus, H. 1949. Process for producing acetyltable cellulose from straw. U.S. patent 2,487,114.
- Gould, J. M. 1984. Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. *Biotechnol. Bioeng.* 26:46-52.
- Gould, M. J., Jasberg, B. K., and Cote, G. L. 1989. Structure-function relationships of alkaline peroxide-treated lignocellulose from wheat straw. *Cereal Chem.* 66:213-217.
- Gould, M. J., and Jasberg, B. K. 1991. Combined physical and chemical treatment to improve lignocellulose digestibility. U.S. patent 4,997,448.
- Gulati, M., Kohlmann, K., Ladisch, M. R., Hespell, R., and Bothast, R. J. 1996. Assessment of ethanol production options for corn products. *Bioresour. Technol.* 58:253-264.
- Johnston, D. B., Shoemaker, S. P., Smith, G. M., and Whitaker, J. R. 1998. Kinetic measurements of cellulase activity on insoluble substrates using disodium 2,2'-bicinchoninate. *J. Food Biochem.* 22:301-319.
- Leathers, T. D. 1998. Upgrading fuel ethanol components. *SIM Ind. Microbiol. News* 48:210-217.
- Moreau, R. A., Powell, M. J., and Hicks, K. B. 1996. Extraction and quantitative analysis of oil from commercial corn fiber. *J. Agric. Food Chem.* 44:2149-2154.
- Renard, G. M. G. C., Rohou, Y., Hubert, C., Della Valle, G., Thibault, J.-F., and Savina, P.-P. 1997. Bleaching of apple pomace by hydrogen peroxide in alkaline conditions: Optimization and characterization of the products. *Lebensm. Wiss. Technol.* 30:398-405.
- Rutenberg, M. W., and Herbst, W. 1957. Process for extraction of hemicellulose. U.S. patent 2,801,955.
- Saulnier, L., Marot, C., Chanliaud, E., and Thibault, J.-F. 1995. Cell wall

- polysaccharide interactions in maize bran. *Carbohydr. Polym.* 26:279-287.
- Schweiger, R. G. 1973. Refining of hemicelluloses. U.S. patent 3,716,526.
- Thompson, J. B. 1984. Process for preparing cellulose. U.S. patent 4,486,459.
- Vail, W. J. 1991. Process for recovery of cellulose. U.S. patent 5,057,334.
- Voragen, A. G. 1998. Technological aspects of functional food-related carbohydrates. *Trends Food Sci. Technol.* 9:328-335.
- Watson, S. A. 1987. Structure and composition. Pages 53-82 in: *Corn: Chemistry and Technology*. S. A. Watson and P. E. Ramstad, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Watson, S. A., and Williams, C. B. 1959. Process for extracting hemicellulose from corn coarse fiber. U.S. patent 2,868,778.
- Whistler, R. L. 1993. Hemicelluloses. Pages 298-308 in: *Industrial Gums*. R. L. Whistler and J. N. BeMiller, eds. Academic Press: New York.
- Whistler, R. L., and BeMiller, J. N. 1956. Hydrolysis components from methylated corn fiber gum. *J. Am. Chem. Soc.* 78:1163-1165.
- Wolf, M. J., Cannon, J. A., and MacMasters, M. M. 1955. Extracting hemicelluloses. U.S. patent 2,709,699.

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