Analysis and Properties of Arabinoxylans from Discrete Corn Wet-Milling Fiber Fractions

Landis W. Doner,*,† David B. Johnston,† and Vijay Singh‡

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, and Department of Agricultural Engineering, University of Illinois, Urbana, Illinois 61801

Three fibrous corn wet-milling fractions, coarse fiber, fine fiber, and spent flake, were isolated. More highly valued uses are sought for these milling products, which are generally directed into the corn gluten feed product stream. Coarse fiber was further dissected into pericarp and aleurone layers. An alkaline hydrogen peroxide process was used to efficiently extract corn fiber gum (CFG) from each of the materials. CFG is a hemicellulose B arabinoxylan which also contains low levels of D,L-galactose and D-glucuronic acid. CFG yield information was obtained from each source, as well as structural information in terms of degrees of branching of the β-D-xylopyranose backbone with α-L-arabinofuranosyl moieties. There were significant differences in degree of branching among the CFGs from the various fractions. A novel capillary electrophoresis procedure was developed to measure these differences. Solution viscosity differences among the CFGs were also observed.

Keywords: *Corn fiber; corn wet-milling; coarse fiber; fine fiber; hemicellulose; arabinoxylan; sugar analysis; capillary electrophoresis*

INTRODUCTION

Corn wet-milling involves the steeping, grinding, and fractionating of corn to produce valuable starch, oil, and feed fractions (corn gluten meal and corn gluten feed). Corn gluten feeds contain 21% protein, but also have significant levels of complex-carbohydrate-containing fibrous materials. The fiber in corn gluten feed fractions is commonly known as white fiber, which is a low-valued material. There exists a need to find more valuable uses for the 4−5 million tons/year of white fiber that is produced in the United States (*1*). White fiber is a mixture of coarse and fine fibers. Coarse fiber from wet milling includes the kernel pericarp (hull), and the aleurone, which is the outer layer of the endosperm. From an anatomical perspective, the aleurone layer is considered to be the cell layer surrounding the endosperm. Fine fiber is cellular fiber from the seed endosperm. A third commercial fiber fraction is spent flake, which results from corn oil production and is often added to the corn gluten feed product stream (*2*). The major components of corn fiber, calculated after removal of the 11−23% (*3*) adherent starch, is typically hemicellulose, 50%; cellulose, 20%; and protein, 15% (*3*). The remainder consists of lignin and ash (13%) (*4*), and corn fiber oil (2−3%) (*5*).

The hemicellulose isolated from corn fiber is over 90% hemicellulose B (*6*), the water-soluble form which can be isolated in yields exceeding 40%. This material was long ago termed corn fiber gum (CFG), because it possessed solution properties similar to some valuable plant exudate or seed gums. These properties suggested uses as adhesives, thickeners, and stabilizers (*7*), and as film formers and emulsifiers (*8*). For more than 50 years, efforts at various locations have been directed to producing CFG suitable for some of these applications. Most of the products were unacceptable because of dark colors associated with the powders and their solutions (*9*, *10*). We developed two processes (*6*, *11*) for preparing white CFG, utilizing various conditions of alkaline hydrogen peroxide extraction, and this gum is being commercially developed.

CFG is an arabinoxylan, typical of hemicelluloses from graminaceous monocot plants (*12*). The β-D-xylopyranose backbone is heavily substituted with α-L-arabinofuranosyl units at both primary and secondary hydroxyl groups (*13*). Lower levels of xylose, D,L-galactose (*14*), and D-glucuronic acid (*15*) are also present in the branches. The sugar composition of CFGs isolated using various processes have been reported to be xylose (48−58%), arabinose (32−38%), galactose (5−11%), and glucuronic acid (3−7%) (*8*, *9*, *11*, *13*, *16*).

Others researchers have reported variable arabinose/xylose ratios among arabinoxylans from various tissues from a variety of grains (*17*, *18*). Earlier we reported higher arabinose/xylose ratios in CFG (arabinoxylan) from fine fiber when compared to that from coarse fiber (*19*). In the present study we also examined CFGs from spent flake and from the pericarp and aleurone layers of dissected kernels. Also, we compare solution viscosities of gums from the various sources. Because solution properties of arabinoxylans vary with arabinose/xylose ratio (*20*), it is possible that one could exploit these differences for unique applications.

MATERIALS AND METHODS

Materials. Coarse and fine fiber fractions were obtained using the 100-g corn wet-milling laboratory procedure described earlier (*21*). Pericarp and aleurone samples were

* To whom correspondence should be addressed (telephone 215-233-6422; fax 215-233-6406; e-mail ldoner@arserrc.gov).
† Eastern Regional Research Center.
‡ Visiting Assistant Professor, Department of Agricultural Engineering.

10.1021/jf001105o
Published on Web 02/01/2001

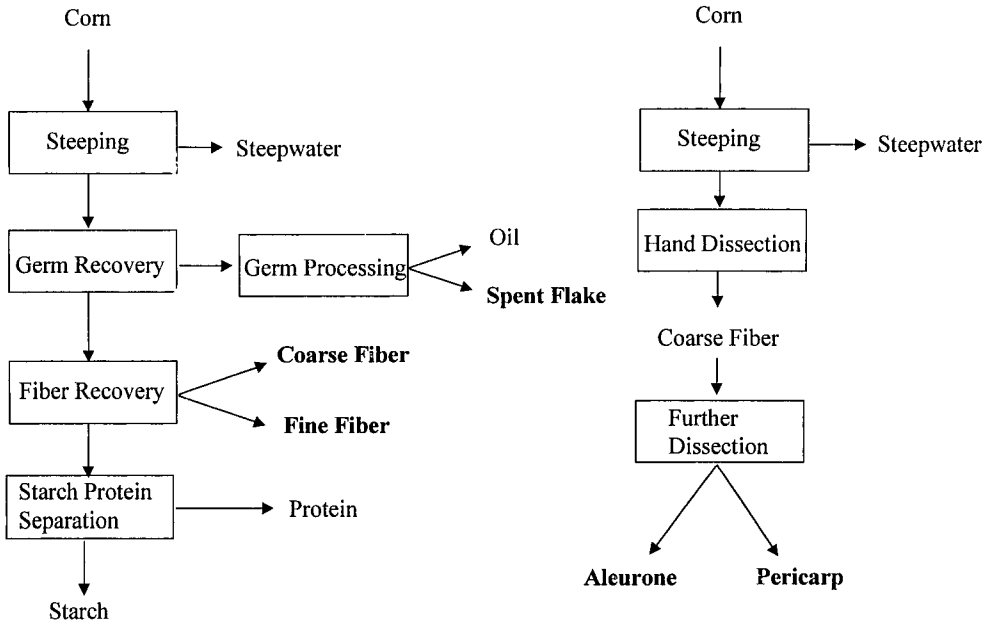

Figure 1. The corn wet-milling process, with fiber sources of corn fiber gum indicated in bold. Coarse fiber was obtained from 100-g scale wet-milling and by hand dissection from steeped kernels.

obtained by hand dissecting (using a scalpel) steeped corn kernels to remove pericarp and aleurone together and then separating the pericarp and aleurone using forceps. The aleurone layer was then removed (peeled) from the inner surface using a forceps, leaving the intact outer pericarp layer. Spent flake was a gift from CPC International (Summit-Argo, IL). Termamyl α-amylase was a gift of Novo Nordisk Bioindustrials, Inc. (Franklinton, NC). Before processing to CFG, the materials were ground to 20 mesh using a Wiley mill.

Starch Analysis and Removal. The starch content of fiber samples was determined by Silliker Laboratories Group (Cedar Rapids, IA). Starch was removed from coarse, fine, and spent flake fiber by stirring into 10 vol H_2O at 90−95 °C and adding 1 mL of termamyl/20 g fiber. After 1 h, the fibers were isolated by sieving, rinsed with hot water and ethanol, and then dried to constant weight in a vacuum oven at 70 °C.

Corn Fiber Gum Isolation. CFG was extracted from destarched fiber samples according to the following alkaline hydrogen peroxide process (*6, 11*) variation. Fiber (10 g) was added to 200 mL of H_2O at 90−95 °C and 1 g of H_2O_2 [3.33 mL of 30% solution (Sigma)] was added. After the pH of the mixture was adjusted to 11.5 with 50% NaOH, it was stirred for 1 h. Residue was removed by centrifuging at 6000*g*, the supernatant was decanted, and the residue was then washed with 150 mL of hot water to remove additional hemicellulose. After the CFG-containing supernatants were recentrifuged, they were combined, and the pH was reduced to 4.5 by addition of 4N HCl. The insoluble hemicellulose A fraction was allowed to settle over 1 h, and then removed by vacuum filtration through Celite filter aid. To the resulting solution was added with stirring 2 vol 95% ethanol. A white flocculant precipitate of CFG was allowed to settle out, and the ethanol/H_2O mixture was removed by decantation. The CFG was rinsed with a small volume of ethanol, collected by vacuum filtration, and dried in a vacuum oven at 50 °C. The centrifugation precipitates were cellulosic residues; these were washed with water and 2-propanol, dried in a vacuum oven at 50 °C, and their yields were determined.

CFG Hydrolysis. The neutral sugar composition of the CFG samples was determined after hydrolyzing 10−20 mg of polysaccharides with N H_2SO_4 (1 mL) for 1.5 h at 100 °C. After the solutions were cooled, they were neutralized by gradual addition of $BaCO_3$. After 1 h, $BaSO_4$ was removed by vacuum filtration, and the aqueous sugar solutions were evaporated to dryness under a stream of N_2. The residues were dissolved in 200 μL of H_2O and filtered through a 0.2-μ Anotop 10 plus membrane filter (Whatman Cat. No. 6809 3022), and an appropriate quantity was transferred to a 0.5-mL microcentrifuge tube and evaporated to dryness under a stream of N_2.

Sugar Analysis by Capillary Electrophoresis (*22, 23*). To the dried sample of 10−50 nmol of reducing sugar in a 0.5-mL microcentrifuge tube was added 10 μL of 8-aminonaphthyl-1,3,6-trisulfonic acid (ANTS, Molecular Probes, Eugene, OR) reagent. This was a 200 mM solution prepared by dissolving 0.086 g ANTS in 5 mL of acetic acid/water (3:17; v/v). The mixture was vortexed for 10 s and briefly centrifuged to bring reagents to the bottom of the tube. To this mixture was added 10 μL of reducing agent (1.0 M solution of $NaCNBH_4$ prepared in HPLC grade dimethyl sulfoxide (DMSO) by adding 2.5 mL of DMSO to 0.25 g $NaCNBH_4$). The tube was again mixed by vortexing and centrifuged to bring reagents together. The reaction mixture was incubated at 40 °C for 15 h, protected from light. Undiluted labeled samples could be stored for several weeks at 4 °C if kept in the dark.

Capillary electrophoresis of ANTS-labeled reducing sugars (*22, 24*) was carried out using a Beckman P/ACE instrument. The bare silica capillary (67 cm × 50 μm) was rinsed before each separation with 0.5 M NaOH, followed by extensive water rinsing and buffer reconditioning. Running buffer was prepared by titrating 250 mM phosphoric acid to pH 4.5 with triethylamine. ANTS-labeled samples were diluted 1:20 with water before loading. Injections were done using pressure for 4 s. Samples were run at 20 kV with the polarity (−) to (+) and monitored at 235 nm. Capillary temperature was set at 30 °C and run times were 15 min.

Viscosity Measurement. Viscosities of solutions were determined at 28 °C using a rotary viscometer (Cannon 2000, State College, PA) with a low centipoise adapter equipped for temperature control.

RESULTS AND DISCUSSION

The fiber-containing processing streams of corn wet-milling are indicated in Figure 1. Variable but considerable quantities of starch are typically associated with these fiber fractions. Fine fiber originates in the seed endosperm and contained 49% starch; coarse fiber originates from the pericarp and contained 20% starch (the results of the starch analysis are given in Table 1). Even germ-derived spent flake fiber contained 17% starch. It was necessary to remove starch before processing fiber to corn fiber gum (CFG), otherwise some

Table 1. Analysis and Properties of Polysaccharides from Corn Wet-Milling Fiber Fractions

	coarse fiber	pericarp layer	aleurone layer	fine fiber	spent flake
starch (%)	20.0			49.0	17.0
yield fiber after destarching (%)	74.0			42.0	60.0
yield CFG (%)	39.0	44.0	29.0[a]	33.0	37.0
CFG sugar composition:					
xylose (%)	55.8	58.0	10.3	50.1	43.1
arabinose (%)	35.2	31.8	15.9	41.2	41.8
galactose (%)	7.8	7.4	1.6	7.5	7.5
glucose (%)	1.2	2.7	72.1	1.2	7.6
arabinose/xylose	0.631	0.548	1.54	0.822	0.970
yield residue (%)	26.0	23.0	20.0	24.0	27.1
viscosity (cP)[b]	22.9			29.7	36.5

[a] Calculated yield of CFG, accounting for major contribution of glucan to this fraction. [b] 10% CFG solution was analyzed at 28 °C

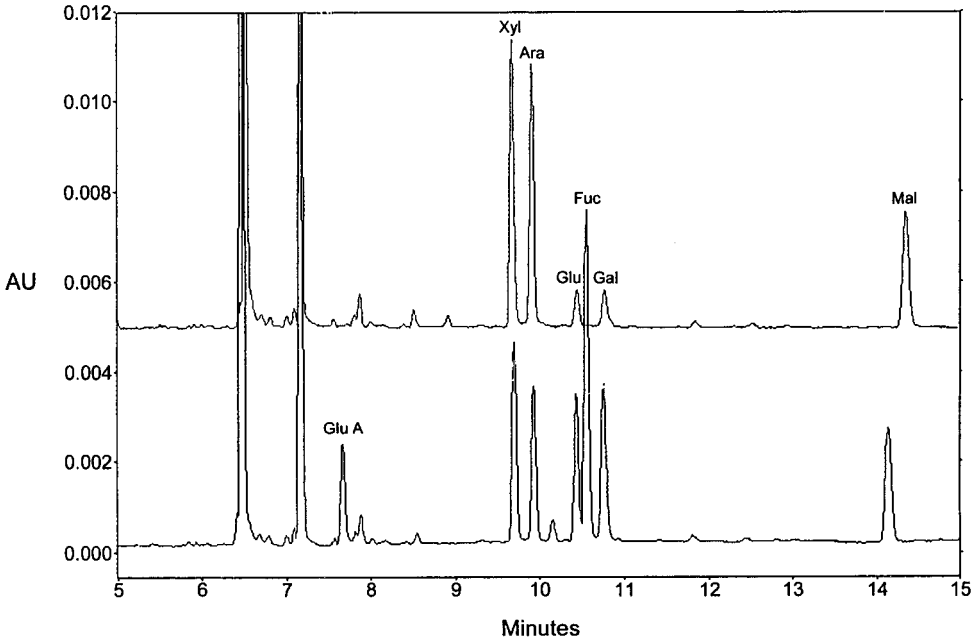

Figure 2. Electropherograms: Top panel represents separation of CFG sugars from coarse corn fiber, along with internal standard maltose; lower panel represents separation of standard sugar mixture glucuronic acid, xylose, arabinose, glucose, fucose, galactose, and maltose.

alkali-soluble starch makes its way into the CFG. Destarching was accomplished using Termamyl α-amylase. From the yields of destarched fiber obtained (Table 1), it is clear that materials in addition to starch were removed during the α-amylase processing step in 90−95 °C water. This material is likely a combination of protein extracted in the 90−95 °C water, and corn fiber oil, extracted during the ethanol rinse step. These materials accounted for about 23% in spent flake. Insufficient quantities of pericarp and aleurone preparations from the dissected kernels were available to allow destarching and processing to corn fiber gum. A sizable quantity of soluble starch glucan came through in the CFG extracted from aleurone layer, as it contained 72.1% glucose. CFGs from the other fractions contained only low levels of glucose, and this represents the typical inclusion of small quantities of soluble glucan along with CFG.

Yields of CFG from coarse fiber, fine fiber, and spent flake were 39%, 33%, and 37%, respectively (Table 1). Coarse and fine fiber account for the wet-milling fraction known as "white fiber", and are present in similar quantities (*19*). As a result, CFG yields from commercial white fiber are generally about 35% (*6*). Our present results indicate that about 80% of CFG from coarse fiber is derived from its pericarp layer. The pericarp layer from coarse fiber gave a 44% yield of CFG. A 29% yield of material came through processing from the aleurone layer, but most of this was glucan, not arabinoxylan. Subtracting out this glucan contribution, a yield of just 11% arabinoxylan was obtained from the aleurone layer. Yields of residues, which consist of cellulosic cell wall materials containing about 30% of inextricable arabinoxylan (*25*), ranged from 20 to 27%.

The neutral sugar composition of the CFG arabinoxylans was determined after hydrolysis using a novel capillary electrophoresis method, which efficiently resolved the neutral sugars of interest. Electropherograms show the separation of a mixture of sugar standards along with the hydrolyzate of CFG from coarse fiber (Figure 2). Of special interest is the variation in levels of the predominant CFG sugars, xylose and arabinose, among the five fiber fractions examined. The levels of these sugars and galactose and glucose from the five fiber fractions are given in Table 1, along with arabinose/xylose (A/X) ratios. Coarse fiber and fine fiber fractions had A/X ratios of 0.631 and 0.822, respectively. These results indicate a much higher degree of branching of the β-D-xylopyranosyl backbone of CFG with L-arabinofuranosyl units, and agree closely with our earlier analysis (*19*) of CFGs from three corn hybrids, where coarse and fine fiber had values of 0.627 and

0.836. Those analyses were conducted by HPLC, providing support for the precision of our CE analytical method.

The CFG extracted from spent flake contained nearly as much arabinose as xylose, giving an A/X ratio of 0.970 (Table 1). The hand-dissected coarse fiber from steeped corn kernels was further dissected into its component pericarp and aleurone layers. Their yields on a dry basis were 62% and 38%, respectively. The CFGs from these materials had widely different A/X ratios of 0.548 (pericarp) and 1.54 (aleurone). We are confident in the value for A/X ratio in the pericarp CFG, but will reexamine the aleurone CFG when we obtain a sufficient quantity of starch-free material.

Properties of arabinoxylans are closely associated with their molecular weights and their degrees of branching (*20*). Solution viscosity is an important property when considering use of polysaccharides such as CFG for replacing gum arabic, whose solutions have low viscosity. We found that viscosities correlated quite well with degree of arabinose branching, with spent flake having a viscosity about 60% greater than that of coarse fiber (Table 1). Fine fiber had an intermediate value. So, for use as a source of CFG for use as a gum arabic substitute, it would be preferable if spent flake was excluded from the fiber mixture, and the contribution of fine fiber was minimized.

ACKNOWLEDGMENT

We thank Karen Kohout for dissecting corn kernels into pericarp and aleurone layers.

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Received for review September 7, 2000. Revised manuscript received December 19, 2000. Accepted December 19, 2000. Mention of brand or firm name does not constitute an endorsement by the U. S. Department of Agriculture above others of a similar nature not mentioned.

JF001105O