Phenolic Acids, Lipids, and Proteins Associated with Purified Corn Fiber Arabinoxylans

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Corn fiber gum (CFG) is a hemicellulose (arabinoxylan)-enriched fraction obtained by the extraction of corn bran/fiber using a proprietary alkaline hydrogen peroxide process. When purified CFG prepared by this process was hydrolyzed with more concentrated base (1.5 N methanolic KOH at 70 °C for 1 hour), considerable amounts of hydroxycinnamic acids (up to 0.015% of mainly ferulic acid) and lipids (up to 0.43%) were released. The released phenolic acids and lipids were identified and quantified using high-performance liquid chromatography (HPLC) with detection by both UV and evaporative light-scattering detection (ELSD). During the wet milling of corn, two types of corn fiber are produced: coarse fiber, which is primarily from pericarp, and fine fiber, which is from the endosperm. The total phenolic acid content in CFGs purified from coarse corn fiber (pericarp fiber) is comparatively higher than that purified from fine corn fiber (endosperm fiber). It was also determined that the purified CFG samples contained significant amounts of strongly associated proteins, from 2 to 5% by weight. The presence of these phenolic acids, lipids, and proteins strongly associated or bound to CFG may contribute to its excellent ability to emulsify oil-in-water emulsions.

KEYWORDS: Corn fiber, (CF); coarse corn fiber; fine corn fiber; corn fiber gum, (CFG); p-coumaric acid, (PCA); ferulic acid, (FA)

INTRODUCTION

The most abundant low-valued coproduct of the corn wet-milling process is corn fiber (CF), which contains a large percentage of a valuable arabinoxylan, also called corn fiber gum (CFG) (1). Fibrous byproducts from corn can be prepared either by wet-milling or dry-milling. Corn fiber produced by the corn wet-milling industry is also called white fiber and it contains fiber fractions from the kernel’s pericarp and endosperm. The fibers from pericarp and endosperm are also called “coarse” and “fine” fibers, respectively. The coarse fiber (pericarp fiber) is part of the outer covering of a corn kernel and the fine fiber (cellular fiber) originates from the inner cellular endosperm portion of the corn kernel. The fiber fraction obtained from the corn dry-milling process is called corn bran. Most of the research has been done on CFG from white fiber or corn bran (1–3) except Singh et al. (4), who reported the comparative yield and sugar profile of CFGs isolated from coarse and fine corn fiber. Previous studies had addressed carbohydrate composition and emulsification properties but had not addressed the topics of protein content, phenolic compounds, and lipids in CFGs isolated from coarse and fine corn fiber whose recovery process has already been developed (4, 5).

CFG, a predominantly carbohydrate molecule, has the following sugar composition: D-xylose (50–56%), L-arabinose (31–35%), galactose (7–8%), and glucuronic acid (4–5%) (1, 6). It has a highly branched structure with a β-(1→4)-xylopyranose backbone and α-L-arabinofuranose residues as side chains on both primary and secondary hydroxyl groups (7). Most of the d-glucuronic acid residues are linked to the O-2 position of xylose residues of the main xylan backbone (8). Galactose and some xylose residues are linked to the arabinofuranosyl branches (9).

CFG, an arabinoxylan (AX) polymer (hemicellulose B), can be extracted from CF with alkali and alkaline H2O2 treatment. In the corn kernel, the AX polymer strands are cross-linked to each other or to other cell wall polymers through dehydrodiferulate ester bridges (7, 10, 11) or dehydrodiferulate ester bridges (12, 13). It is well-known that corn bran contains ferulic acid (4-hydroxy-3-methoxycinnamic acid) together with a small amount of p-coumaric (4-hydroxy-cinnamic) and dehydrodiferulic acid and dehydrodiferulic acids covalently bound to cell wall hemicelluloses (10, 12–14). They are believed to be present as esters because of their release from cell walls on treatment with alkali. These hydroxycinnamic acids have been suggested to possess many health-promoting properties. The ester-linked hydroxycinnamic acids can be released from the cell walls into the colon by the action of bacterial esterases and they may be absorbed or converted into other compounds by colonic bacteria. They can also act as antimutagens to reduce the risk of cancer (15). The abilities of ferulic, p-coumaric, and dehydrodiferulic acids to protect against different types of mutations in a bacterial...
model have been determined and compared with the related compound curcumin (16). These phenolic acids (ferulic and p-coumaric acids) are naturally occurring antioxidants (17). The antioxidative activity of corn bran hemicellulose containing hydroxycinnamic acids has also been reported (14, 18). Like gum arabic, CFG is an excellent emulsifier for the use in oil-in-water beverage emulsion systems (1). The importance of lipids on the stabilizing effect of gum arabic in oil-in-water beverage emulsion systems was reported recently by Yadav and co-workers (19, 20). In this paper, we present the first report on the isolation and quantification of phenolic acids (known to be antioxidants and antimutagen agents), lipids, and proteins (both known for emulsion-stabilizing activity) that are strongly associated with CFGs isolated and purified from coarse and fine corn fiber.

MATERIALS AND METHODS

Materials. Coarse and fine corn fiber samples were specially collected and kindly provided by ADM Research (North America). They were oven-dried by the suppliers before shipping. Fiber samples were ground to a 20-mesh particle size using a Wiley mill and were extracted with hexane to remove oil (21). Starch was removed from the 20-mesh deoiled fiber by treating with heat-stable Termamyl α-amylase (Novozymes, Inc., Davis, CA) (3). All the high-performance liquid chromatography (HPLC) standards were obtained from Sigma Chemical Co., St. Louis, MO.

Standard Proximate Analyses. Moisture, protein (N × 6.25), and ash contents of all CFG samples were determined using “AACC Approved Methods” 44-19, 46-30, and 08-01 (22), respectively.

Isolation of Corn Fiber Gum. CFGs were isolated from deoiled and destarched corn fiber according to the alkaline hydrogen peroxide procedure of Yadav et al. (1). In brief, deoiled and destarched corn fiber was mechanically stirred into an alkaline (pH 11.5) solution of 0.1 M NaOH and 0.05 M Ca(OH)2 containing 1 meq of each per gram of fiber in the extraction medium and was boiled for 1 h. The residue obtained after centrifugation was resuspended in water, was boiled for 5 min, and was centrifuged again. The combined supernatant (pH 11.3) was treated with H2O2 (0.1 g/g fiber), which dropped the pH to about 9.6. The pH was readjusted to 11.5 and was stirred at room temperature for 2 h. The pH of the alkaline extract was adjusted to 4.0–4.5 by adding conc HCl to precipitate hemicellulose A (Hemi, A). The supernatant was treated with two volumes of ethanol to precipitate hemicellulose B or Hemi. B (CFG-1) and was collected and dried in a vacuum oven.

The residue left after alkaline extraction was further extracted with alkaline H2O2 (0.1 g/g fiber, pH 11.5) at a boiling temperature for 1.5 h. The residue was removed by centrifugation, and the pH of the supernatant was adjusted to 4.0–4.5 to precipitate Hemi. A. The supernatant was precipitated with 2 volumes of ethanol and was collected and dried in a vacuum oven as CFG-1 above, and this fraction was named CFG-2.

Alkaline Hydrolysis and Extraction of Corn Fiber Gum with Diethyl Ether. CFG (5 mg) was hydrolyzed with 1 mL of 1 M NaOH at 50 °C for 2 h. The alkaline extract was acidified to pH 2–3, with 1 M HCl, and the resulting aqueous solution was extracted with diethyl ether (3 × 1 mL). The combined ether extract was evaporated at 40 °C under a stream of filtered nitrogen. The dry residue was dispersed in 10 mL methanol and its UV spectrum was measured at the wavelength range of 200–400 nm.

After seeing a maximum UV absorbance peak at 320 (characteristic of phenolic acid), CF or CFG was hydrolyzed using a stronger alkaline solution, 1.5 M methanolic KOH (modified technique of Moreau and Hicks (23)) to release all phenolic acids and lipids, and was extracted with chloroform and methanol (24).

Methanol KOH Hydrolysis and Extraction of Corn Fiber and Corn Fiber Gum with Chloroform/Methanol. The deoiled and destarched CF (1 g) was placed in a screw cap glass tube (55 mL, 25 × 150 mm), and 5 mL of 1.5 M methanolic KOH and 250 μL of water were added. The tubes were sealed with Teflon lined screw caps and were boiled by immersing in a water bath at 70 °C with stirring for 1 h. After cooling the reaction mixture to room temperature, 11 mL of methanol and 8 mL of chloroform were added, mixed, and filtered through a Whatman GF/A glass fiber filter (Whatman Laboratory Products, Clifton, NJ) fitted in a Buchner funnel with a gentle vacuum. The filtrate was collected in a 50-mL glass tube with a Teflon lined screw cap viaial and 7.75 mL of water was added. After acidifying the total reaction mixture to pH 2–3, 8 mL of chloroform was added to maintain 2:2.1 (v/v) chloroform, methanol, and water ratio, which helped for the complete phase separation (24). The reaction mixture was mixed well and was centrifuged at 70 g for 10 min for complete phase separation. The lower layer (chloroform layer) was collected in a clean vial and was evaporated at 50 °C under a stream of filtered nitrogen.

The alkaline hydrolysis and extraction of CFG were done in two trials. In the first trial, CFG (0.5 g) was taken in a glass tube, and 10 mL of 1.5 M methanolic KOH and 500 μL water were added to get a complete suspension of the gum sample. The tubes were sealed with Teflon lined screw caps and were boiled by immersing in the water bath at 70 °C for 1 h. After cooling the tubes to room temperature, 6 mL of methanol and 8 mL of chloroform were added to each tube and were mixed well. Then, they were centrifuged at 70 g for 15 min and were filtered through a Whatman GF/A glass filter paper as above. The pellet retained in the glass tube was re suspended in 2 mL of 2:1 methanol and chloroform, was mixed well, and was filtered through the same filtration set up to collect the filtrate in the same tube. The residue on the filter paper was rinsed with 1 mL of 2:1 methanol and chloroform and was collected in the same tube again. To the combined filtrate (total 27 mL containing 18 mL methanol and 9 mL chloroform), 8.5 mL of water was added to make the total volume of water 9 mL. The combined solution was acidified to pH 2–3 with 6 M HCl, and 9 mL chloroform was added to maintain 2:2:1 (v/v) methanol, chloroform, and water ratio for complete phase separation; then, it was centrifuged, and the lower layer was collected and evaporated as above. In the second trial of hydrolysis and extraction, only 50 mg CFG sample was taken in screw cap glass vials (4 mL, 15 × 45 mm). After adding 1.0 mL of 1.5 M methanolic KOH and 50 μL of water to each vial, they were incubated on a heating block (Pearce Reacti-Therm, Heating Module, Pearce Chemical Company, Rockford, IL) at 70 °C for 1 h with stirring. After cooling the reaction mixture to room temperature, the chloroform/methanol extraction was done as in the first trial but at 10 × reduced scale.

Protein Analysis. The total protein content was measured using the Pierce Micro BCA Protein assay reagent kit, which is a colorimetric detection method of protein using bicinchoninic acid (BCA) (25). Bovine serum albumin (BSA) was used as the standard to construct a calibration curve.

Phenolic Acid Analysis. The phenolic acids were analyzed by a binary HPLC system containing both UV and evaporative light-scattering detectors (ELSD). p-Coumaric and ferulic acids were identified by both detection systems, but they were quantified by UV absorption at 320 nm by comparing with the peak area of known amount of standard p-coumaric acid and ferulic acid. HPLC analyses of free ferulic and p-coumaric acids were performed on a Hewlett-Packard Model 1100 HPLC (Agilent Technologies, Palo Alto, CA) with an autosampler, and detection was via two detectors in series, with the effluent first entering a Hewlett-Packard Model 1100 Diode Array UV–vis detector and then entering a Sedex Model 55 Evaporative Light Scatter Detector, ELSD (Richard Scientific, Novato, CA), operated at 30 °C and with nitrogen as a nebulizing gas, at a pressure of 2.0 bar. The HPLC column was a LiChrosorb 7 micron DIOL stationary phase, (3 × 100 mm, packed by Varian/Chrompack, Walnut Creek, CA), and the isocratic mobile phase was hexane/isopropanol/acetic acid, 75/0.2/0.1, v/v, at a flow rate of 0.5 mL/min.

Lipid Analysis. HPLC analysis of nonpolar lipids (including phytosterol fatty acyl esters, hydroxycinnamate phytosterol esters, free sterols, triacylglycerols, and free fatty acids) were performed by a normal-phase HPLC method with evaporative light-scattering detection developed by Moreau et al. (21) with a minor modification in the gradient time table. These analyses were performed on a Hewlett-Packard Model 1050 HPLC, with autosampler, and detection was
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RESULTS AND DISCUSSION

Chemical Extraction of CFGs. From the deoiled and destarched CF, most of the CFG (29%, from coarse and 14.8% from fine fiber, Table 1) was extracted from the fiber by a solution of 0.1 M NaOH and 0.05 M of Ca(OH)₂ (pH 11.5) at boiling temperature and was bleached with 0.1 g H₂O₂/g fiber at room temperature. The percentage yield reported in Table 1 is from one extraction, but a similar result was obtained when the extraction of CFG was repeated. The optimized condition of temperature, pH, and H₂O₂ used for CFG isolation is most effective to get high yield and an off-white product (I-3). This CFG fraction was named CFG-1 and was most likely linked to the insoluble cell wall matrix through phenolic or non-phenolic ester linkages or was crossed-linked by diferulic ester bridges (26). The mild alkali insoluble arabinoxylan, still present on the alkali-treated residue, was solubilized and re-extracted with alkaline H₂O₂ (0.1 g/g fiber, pH 11.5), and the second fraction was named CFG-2. The amounts of CFG-2 isolated from coarse and fine corn fiber were 8.0% (±0.42, one-fourth of respective CFG-1) and 5.2% (±0.48, one-third of the respective CFG-1), respectively, on the basis of the corn fiber taken for gum isolation (Table 1). The considerable amount of CFG which survived the first mild alkali treatment may be linked to the insoluble cell wall matrix through ether linkages or other alkali-resistant linkages. It is also possible that the second fraction (CFG-2) was effective to get high yield and an off-white product (26).

Proximate Analysis. Protein, moisture, and ash contents in all CFG samples are reported in Table 2. In these samples, the amount of protein varies from 1.9 to 5.1% (w/w). The weight percent of protein obtained by both standard AACC method and Micro BCA assay differs slightly. The protein content obtained by standard AACC method is considered more accurate and reliable than the one obtained by micro BCA method using BSA as standard. In general, the protein content obtained by standard AACC method is always slightly higher than the one measured by the colorimetric BCA method. As we reported previously (I), the protein content is comparatively higher in CFG-2 than the corresponding CFG-1 from each fiber source. The combined protein content of CFG-1 and CFG-2 in CFG from fine fiber is slightly higher than the coarse fiber, showing that fine fibers are richer in protein than coarse fiber. Usually, the fibers which originate from the endosperm are richer in protein than the fiber one which comes from the pericarp portion of corn kernels. The moisture and ash content varies from about 4.0-6.6% and 4.0-5.9%, respectively, in these samples.

Extraction of Phenolic Acids and Lipids. The ether extract of the 1 M NaOH hydrolyzate of CFG showed a characteristic UV-spectrum for phenolic compounds, particularly for ferulic acid structure (absorption maxima at 288 and 319 nm) (27). Then, samples of both CF and CFG were hydrolyzed with a stronger alkaline solution in methanol at 70 °C for 1 h to ensure cleavage of all the linked phenolic compounds and lipids. The alkaline hydrolysis of CF and CFG by treating with 1.5 KOH in methanol and about 5% of water at 70 °C for 1 h released a considerable amount of phenolic acids and lipids, which were extracted with chloroform and methanol. In this study, the coarse and fine corn fiber and the CFG-1 and 2 obtained from each of them were used for phenolic acids and lipids isolation.

Analysis of Phenolic Acids and Lipids. Typical HPLC chromatograms (UV detector) of (a) standard p-coumaric acid (PCA), peak 3, and ferulic acid (FA), peak 4, and (b) phenolic acids released from corn fiber gum by methanolic KOH. Peaks numbered correspond to 3, PCA; 4, FA; 1, 2, and 5, unknown compounds; and V, void volume.

![Figure 1](image-url)

Table 1. Percentage Yields° of Corn Fiber Constituents

<table>
<thead>
<tr>
<th>Sample</th>
<th>CFG-1</th>
<th>CFG-2</th>
<th>hemi. A</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>coarse CF</td>
<td>29.0</td>
<td>8.0</td>
<td>5.6</td>
<td>42.6</td>
</tr>
<tr>
<td>fine CF</td>
<td>14.8</td>
<td>5.2</td>
<td>4.8</td>
<td>24.8</td>
</tr>
</tbody>
</table>

° On the basis of the amount of deoiled and destarched corn fiber.

Table 2. Protein, Moisture, and Ash Content of CFG Samples

<table>
<thead>
<tr>
<th>Sample, moisture, and ash content of CFG Samples</th>
<th>Protein content by AACC method, N × 6.25°</th>
<th>Protein content by micro BCA assay</th>
<th>moisture</th>
<th>ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse Corn Fiber</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFG-1</td>
<td>1.9 ± 0.06</td>
<td>2.9 ± 0.42</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>CFG-2</td>
<td>4.8 ± 0.03</td>
<td>4.0 ± 0.66</td>
<td>4.0</td>
<td>5.9</td>
</tr>
<tr>
<td>Fine Corn Fiber</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFG-1</td>
<td>4.6 ± 0.03</td>
<td>4.0 ± 0.48</td>
<td>5.1</td>
<td>4.6</td>
</tr>
<tr>
<td>CFG-2</td>
<td>5.1 ± 0.04</td>
<td>4.1 ± 0.42</td>
<td>6.6</td>
<td>4.5</td>
</tr>
</tbody>
</table>

° Weight percent based on the dry weight of deoiled and destarched CFG samples. Data are average of three runs ± standard deviation.
The UV spectrum looks very similar to the spectrum of acetone extracts of corn kernels reported by Seitz (27), in which he reported peaks 1 and 3 as p-coumarates and peaks 2 and 4 as ferulates. The void volume peak is labeled as V.

The coarse fiber which originates from the pericarp contains higher amount of p-coumaric and ferulic acids (340 and 4200 mg, respectively, per gram of fiber) than the fine fiber, which contains only 60 and 1020 mg, respectively, per gram of fiber (Table 3). This agrees with Seitz (27), who reported that most of the ferulate ester was present on the inner pericarp portion of the fiber. The fine fiber which originates from the endosperm portion of corn kernels also contains lower levels of p-coumaric (PCA) and ferulic acid (FA). It is well-known that p-coumaric, ferulic, and dehydrodiferulic acid are present on corn fiber (7, 10, 21, 27), but to the best of our knowledge, this is the first report on the presence of these phenolic compounds in isolated CFG. In the present study, we have demonstrated that CFG contains a considerable amount of these phenolic acids and lipids which can be released with a strong alkali solution at a high temperature. The yield of p-coumaric and ferulic acids present in CFG-1 and 2 from both coarse and fine fiber is given in Table 3. The total phenolic acids (PCA + FA) in CFG-1 and 2 from coarse corn fiber are 153.7 μg and 11.2 μg (trial 1) and 133.5 μg and 13.7 μg (trial 2), respectively, per gram of CFG. Thus, about 2.9—3.6% of phenolic acids present on coarse corn fiber is resistant to mild alkaline extraction, and the resulting CFG contains up to 0.015% phenolics, by weight. Similarly, the total of these acids present in CFG-1 and 2 from fine corn fiber is 27.5 μg and 5.7 μg (trial 1) and 32.0 μg and 12.6 μg (trial 2), respectively, per gram of CFG, which is about 3.1—4.1% of phenolic acids present in fine corn fiber. The low precision in the phenolic acid content in two trials might be due to slight variation in the extraction procedure. Compared to the CFG from coarse corn fiber, the CFG from fine fiber contains less than 0.003% phenolics on a weight basis. The levels of phenolics in these corn fiber gum samples are relatively low, but they may provide some limited nutraceutical or health-promoting benefits to foods and beverages to which they are added. They may also function as important antioxidants that could help maintain the quality of these food products. In addition to the above-mentioned applications, arabinoxylans may have applications as a prebiotic and potential source of soluble dietary fiber thus increasing the value to the food or drink containing it. It is clear from data in Table 3 that the total amount of phenolic compounds in CFG-1 from each corn fiber source is higher than in CFG-2 from each respective source. In all CF and CFG samples, high amounts of FA and low amounts of PCA were detected, which is a known characteristic of primary plant cell walls (28).

The lipid components in the chloroform/methanol extract of alkaline hydrolyzate of corn fiber gum were analyzed by normal-phase HPLC with evaporative light-scattering detection system and were quantified using the technique developed in our laboratory (21). The quantitative lipid analysis results of CFG-1 and 2 extracted from both coarse and fine corn fiber is presented in Table 4. The standard deviation of lipid analyses from three to four extractions is high, but they show a similar trend of lipid content in CFG samples from each extraction. In our previous publication, we reported that CF contained ~2% total lipid using a similar quantitative methods (21). To the best of our knowledge, this is the first quantitative analysis of lipids in purified CFGs. All four CFG samples contained sterol-FA esters, triacylglycerols, and free fatty acids (palmitic and oleic acids). The total amount of lipids in these samples varies from 2444 to 4289 μg per gram (0.24—0.43%) of CFG sample. CFG-2 from coarse corn fiber has a higher total lipid content (3843 μg/g) than the CFG-1 (2444 μg/g) from the same fiber source. This CFG-2, which was reported as CFG-2 from wet pericarp fiber (WPF) in our previous publication (1), was a better emulsifier than CFG-1 from the same source for the oil-in-water emulsion system. A similar superior emulsion stabilizing capacity of CFG-2 than the corresponding CFG-1 from WPF and WEF (wet endosperm fiber) was observed when their emulsifying properties were investigated with 10:1 oil-to-gum ratio (29). These data, in agreement with our previous result (19, 20), suggest that lipid components present on an emulsifier (CFG or gum arabic) may play an important role in their great emulsification properties for oil-in-water emulsions. Hydrophobic lipid molecules on CFG might play an important role in associating the CFG at the oil droplets in oil-in-water emulsion systems, stabilizing the emulsion. The hydrophobic lipid groups in combination with other hydrophobic moieties on CFG molecules might adsorb at the oil droplets and act as an anchor. The large hydrophilic carbohydrate portion of CFG may extend into the aqueous phase and may stabilize the tiny oil droplets by steric repulsion. Indeed, more work is needed to elucidate the role of the lipids present on CFG in stabilizing oil-in-water beverage emulsion system. The fact that some fatty acids are not extracted from the original hexane wash but are extracted after strong alkaline hydrolysis suggests that they may be esterified to the arabinobioxyan or some other component in CFG. The presence of only palmitic and stearic acids suggests that the fatty acids in CFG are not simply due to contamination by corn oil, since the triacylglycerols in corn oil contain high levels of polyunsaturated fatty acids and low levels of palmitic acid (30). Palmitic and myristic acids are selectively esterified to some fatty acylated proteins (31). It is possible that some of the
palmitic acid in CFG may be esterified to proteins. The fact that purified corn fiber gums can contain over 4% protein and up to 0.5% of various phenolics, lipids, and nutraceuticals such as phytosterols may shed light on the functional properties of this valuable hydrocolloid. In conclusion, it is possible that the presence of these phenolic acids, lipids, and protein in CFG may contribute to its excellent emulsification and other functional properties. This possibility is now being investigated.

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