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Corn Fiber: Structure, Composition, and Response to Enzymes for Fermentable Sugars and Coproducts

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Abstract Corn (*Zea mays* L.) fiber, which is the seed coat and residual endosperm left after grain processing, is a low-value residue that contains carbohydrates and aromatic compounds that could provide value-added coproducts. Treatment of corn fiber with NaOH and assessment by gas chromatography indicated a prevalence of ferulic acid, with about 90% ester-linked in the cell walls. *p*-Coumaric acid was much lower at about 10% of the amount of ferulic acid. Histochemical reactions employing acid phloroglucinol and diazotized sulfanilic acid indicated the presence of phenolic acids in cell walls of the pericarp and aleurone layer. Various protocols were tested using milled corn fiber and pretreatment with commercial ferulic acid esterases before cellulase treatment, and dry weight loss and sugars and phenolic acids released into the filtrate were evaluated. Ferulic acid esterases effectively degraded corn fiber and released substantial amounts of ferulic acid and sugars (e.g., glucose and xylose) in the incubation medium. Light microscopy showed that ferulic acid esterase substantially disrupted the aleurone layer but caused little visible damage to the lignified pericarp cell walls. Amounts of compounds released varied with protocols, and one study with various milling methods showed that esterase pretreatment followed by cellulase released about 2.8 to 4.4 and 1.5 to 2.9 times more ferulic acid and glucose, respectively, than cellulase alone. The highest levels for one lot of corn fiber with esterase pretreatment followed by cellulase were 3.9 and 218 mg/g of ferulic acid and glucose, respectively.

Keywords *Zea mays* L. · Ferulic acid · Glucose · Esterase · Cellulase

Introduction

The rapid rise in ethanol production from corn grain is predictably increasing the cost of corn for other uses, such as feed and food. Furthermore, as predicted, cellulose-to-ethanol protocols are increasing in importance to meet mandated levels of ethanol for liquid fuels without jeopardizing food and feed supplies. Progress in this area is shown by recently

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awarded Department of Energy grants to several companies who will use a variety of lignocellulosic materials, e.g., yard trash, trees, and grasses, for ethanol (http://www.ethanolrfa.org [March 14, 2007]).

In the US the mature fuel ethanol industry currently relies on corn grain. Corn fiber is a residue of the grain wet-milling process and consists of the hulls and residual starch not extracted by the milling process [1]. While used as a low-grade animal feed ingredient, corn fiber is a potential source of additional sugars for fermentation to ethanol [2, 3]. Many of the potentially fermentable carbohydrates are bound in the cell walls of corn fiber, however, and require pretreatment to disrupt the chemical bonds and integrity of the lignocellulose [1–3]. Indeed, much research has taken place on a variety of pretreatments for subsequent use of corn fiber for bioenergy.

In addition to potential fermentable carbohydrates in corn fiber, research has focused on other high-value coproducts, such as corn fiber oil to lower cholesterol [4, 5]. In addition, it is well established that grasses, and particularly warm-season species such as corn, have high levels of aromatic compounds such as ester-linked *p*-coumaric (*p*-CA) and ferulic (FA) acids within the cell walls [6, 7] that also could serve as value-added coproducts. These phenolic acids, as well as lignin, prevent the utilization of the cell wall carbohydrates, thus presenting a formidable barrier to fermentation. This work was carried out to provide information on the types and locations of phenolic acids in corn fiber and to assess various milling methods and the use of commercial esterases as a pretreatment to release sugars and phenolic acids for potential coproducts from corn fiber.

Materials and Methods

Corn Samples

Corn (*Zea mays* L.) fiber was acquired from two commercial wet-milling operations. Samples were ground through a Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ, USA), a SPEX Mixer/Mill (catalog no. 8000), which is a high-energy ball shaker mill (SPEX Industries, Inc., Metuchen, NJ, USA), or a Retsch ZMI Centrifugal Grinding Mill (Glen Mills, Inc., Clifton, NJ, USA). For some samples, ground corn fiber was partitioned by shaking through a US Standard Sieve Series (W.S. Tyler Co., Mentor, OH, USA). Intact corn kernels from Pioneer 3167 (yellow dent corn) and corn fiber material were the sources for free-hand sections for histochemical study and for enzyme incubations.

Histochemistry

Ground corn fiber and free-hand sections of kernels were examined using histochemistry by the following staining methods: acid phloroglucinol and chlorine-sulfite for lignin [8–10], diazotized sulfanilic acid for aromatics and phenolic compounds [11, 12], I_2/KI for starch [8], oil red for wax [13], and congo red for cellulose [8].

Chemical Analyses

Samples of untreated corn fiber and pretreated residues were freeze-dried and ground in a SPEX Mixer/Mill before chemical analyses. Ester-linked and total phenolic acids were determined by treatment of material with (1) 2 M NaOH at room temperature for 24 h or (2) 4 M NaOH at 170 °C for 2 h, respectively. These acids were measured as their silyl ethers using

N,*O*,*bis*(trimethylsilyl) trifloroacetamide by gas–liquid chromatography as previously described [14]. In addition to FA and *p*-CA, sugars in the decanted liquids from the pretreatments were analyzed as their silyl ethers and measured by gas–liquid chromatography as described [15]. Data are presented as milligram per gram of the starting weights of freeze-dried, ground fractions.

Enzymes for Treatment of Corn Fiber

A series of enzymes was tested for efficiencies of activity against corn fiber based on dry weight loss. Samples were treated with the commercial products Depol 740 L or with TP 692 L, which are from a range of ferulic acid esterase-containing enzymes from Biocatalysts Ltd. (Cardiff, United Kingdom). Depol 740 L reportedly removes free phenolic acids from plant material and increases amounts of fermentable sugars. Depol 692 L is also used as a macerating agent for plant material and consists of a more complex mixture of ferulic acid esterases, cellulases, and hemicellulases. Typical ferulic acid esterase activities for Depol 740 L and Depol 692 L are 36 and 800 U/g, respectively (product information from Biocatalysts Ltd.). These commercial enzymes were used as supplied and in high amounts (about 1.0 g/0.05 g corn fiber) to ensure maximal release of ester-linked aromatic acids. Cellulase (EC 3.2.1.4, catalog no. C-8546, Sigma Chemical Co, St. Louis, MO, USA) was used at 20 U/ml in studies with or without esterase pretreatment. The general potency of the enzyme had been verified with 100×12-mm filter paper strips (Whatman no. 541), which broke in 5 h when incubated in 50 mM sodium acetate buffer at pH 5.0.

Pretreatment of Corn Fiber with Esterases and Cellulase Treatment

Corn fiber samples were ground in a Wiley Mill to pass through a 1-mm screen and freeze-dried, and 0.5 g samples were used in triplicate for each test. Samples were treated with the commercial products Depol 740 L at 1.0 g/0.05 g corn fiber or TP 692 L at various levels indicated in specific experiments. Generally, the incubations were carried out at pH 5.0 (sodium acetate buffer 50 mM), and 37°C for 24 h in a reciprocal water bath at about 100 back and forth strokes per minute. After incubation with esterases, tubes were centrifuged at 730×*g* for 2 min and the liquid decanted. The samples were washed by addition of 20 ml of distilled water to the pellet, vigorously mixed for 5 s, centrifuged, and the liquid decanted and added to the enzyme filtrate. Decanted liquids, both enzyme mixture and washings, were analyzed for phenolic acids and for sugars as described above. The esterase-treated, washed residues were subsequently incubated as above with cellulase or freeze-dried and weight loss calculated before subsequent incubation with cellulase. For the cellulase treatment, 20 ml of enzyme solution was added to freeze-dried corn fiber. Samples were incubated at pH 5.0 and 37°C for 72 h unless otherwise indicated. After incubation, the tubes were centrifuged, the liquid decanted, fiber residue washed and centrifuged, the liquid decanted, and the residue freeze-dried and weighed. The decanted liquid (filtrate and washing) was analyzed for phenolic acids and sugars as described above. Dry weight loss was calculated as follows: $1 - (\text{residual weight}/\text{starting weight}) \times 100$.

Results and Discussion

The structure of corn kernels is shown in free-hand sections (Fig. 1a). Sections similar to those in Fig. 1 were stained with histochemical stains for a variety of compounds, notably

aromatics, waxes, cellulose, and starch. Staining with acid phloroglucinol produced little to no color, indicating the lack of coniferyl lignin or aromatic aldehydes [9]. In contrast, the pericarp and aleurone cell walls gave a red color with diazotized sulfanilic acid and chlorine-sulfite staining, suggesting the presence of phenolic acids and aromatics [11, 12]. Oil red for wax [13] showed a red colorization just in the cuticle outside the kernel and a reaction, but less intense, in parts of the aleurone layer. Subaleurone cell walls stained with congo red, which is indicative of cellulose [8], but did not stain for aromatics. Therefore, results indicate the presence of phenolic acids and other aromatics in multiple tissues in the pericarp and the aleurone layer. Examination of fragments of corn fiber often showed an intact aleurone layer of cells attached to portions of endosperm with starch grains (Fig. 1b). In these fragments, histochemical staining for phenolic acids was also observed in the aleurone layer.

Phenolic acids and their linkages were assessed chemically in one lot of corn fiber. Amounts of ester-linked *p*-CA and FA were 1.56 and 14.84 mg/g, respectively (average for two replicates). Total phenolic acids, which were released after treatment with 4 M NaOH at 170°C, were 1.97 and 16.65 mg/g of *p*-CA and FA, respectively. These data showed that most of these phenolic acids (80% for *p*-CA and 90% for FA) were ester-linked in this corn fiber sample.

With the high level of ester-linked phenolic acids, a series of tests was conducted to determine the efficiency of commercial phenolic acid esterases, which are mixtures and not

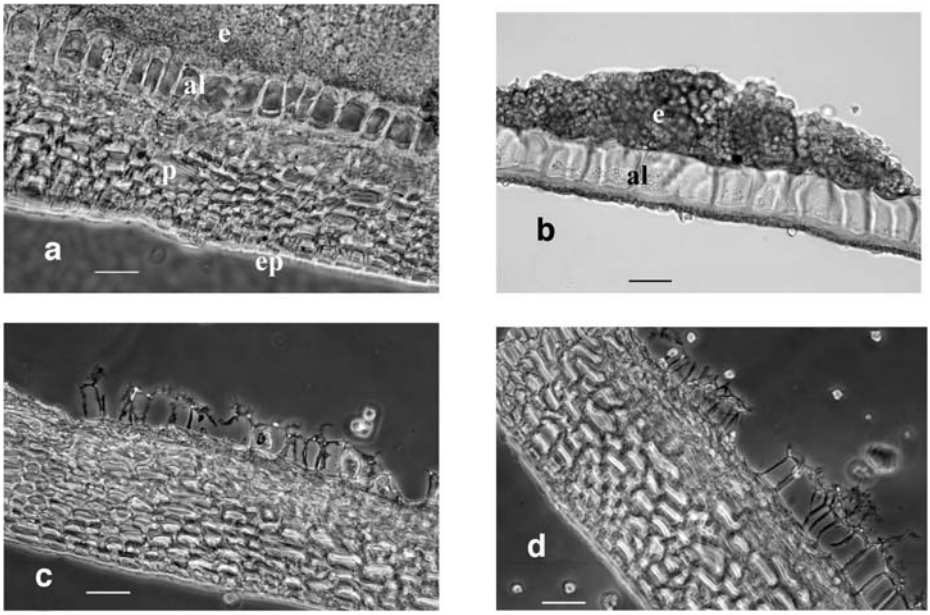

Fig. 1 **a** Free-hand section of corn kernel under transmitted light showing following tissues: epidermal cell (ep) covered with cuticle, pericarp (p), aleurone layer (al), and endosperm (e) with starch grains. **b** Fraction of corn fiber sample showing aleurone layer (al) and attached endosperm (e) filled with starch grains. **c** Free-hand section as in subpanel **a** but treated with Depol 740 L ferulic acid esterase. The pericarp is mostly intact, but the aleurone layer has been degraded to only remnants of the cell walls. Some starch grains are near the fragment. **d** Free-hand section as in subpanel **a** but treated with Depol 740 L ferulic acid esterase and subsequently with cellulase showing intact pericarp and highly degraded aleurone layer. Bar=50 μm

Table 1 Effect of physical and enzyme treatments on dry weight loss and release of phenolic acids from corn fiber.

Grind method	Incubation vessel	Enzyme treatment	DW loss	*p*-Coumaric acid	Ferulic acid
			%	mg/g	
W	T	B	7.1±0.1f	0.03±0.01c	0.03±0.01c
W	T	C	26.5±0.7e	0.04±0.01c	0.05±0.02e
W	T	E + C	48.2±0.9d	0.23±0.03b	2.7±0.2c
S	T	E + C	56.9±0.4b	0.28±0.01a	3.5±0.1b
W	F	E + C	53.2±2.7c	0.22±0.03b	2.3±0.01d
S	F	E + C	60.0±0.4a	0.30±0.03a	3.9±0.3a

Corn fiber comes from a commercial source (lot 1).

abcd values within columns with different numbers differ at $P \leq 0.05$.

W = Wiley mill through a 1.0 mm screen, S = SPEX mill for 5 min, T = centrifuge tube, F = 50 ml Erlenmeyer flask, B = buffer only for 72 h, C = cellulase only for 72 h, E + C = Depol 740 L (ferulic acid esterase) 24 h, washed, and residue-incubated with cellulase 72 h (summed values)

pure enzymes, to biodegrade corn fiber using dry weight loss as the criterion for biodegradation. Of those tested, two enzymes mixtures with ferulic acid esterase, namely, Depol 740 L and TP 692 (Biocatalysts), were very effective and, therefore, used in further studies. Depol 740 L was as effective in causing dry weight loss of corn fiber as was cellulase alone in the first series of tests. A subsequent incubation of the esterase-treated and washed residue with cellulase gave a further increase in dry weight loss. Increases in dry weight loss occurred with Depol 740 as high as 1.25 g esterase/tube of corn fiber, which was the limit of this test. TP 692 L, an enzyme mixture reportedly with esterase, hemicellulases, and cellulase, was also tested with subsequent cellulase incubation and showed increasing dry weight losses up to about 1 g esterase/tube.

Free-hand sections of corn kernels (Fig. 1) indicated that the aleurone layer was particularly degraded by enzymes with ferulic acid esterase. Little physical destruction appeared in the lignified pericarp tissues, although some phenolic acids could have arisen from these cell walls. The physical changes in the aleurone layer were similar with or without a subsequent cellulase treatment (Fig. 1c,d).

Table 2 Effect of physical and enzyme treatments on release of sugars from corn fiber.

Grind method	Incubation vessel	Enzyme treatment	Ara	Xyl	Glu
			mg/g		
W	T	B	0d	0c	0d
W	T	C	2.9±0.9c	2.6±0.4c	36.7±29.0c
W	T	E+C	7.0±0.5b	8.2±1.6b	159.2±3.0b
S	T	E+C	10.1±0.9a	12.1±0.9a	187.4±24.9ab
W	F	E+C	7.4±1.9b	9.2±1.6b	169.5±21.5b
S	F	E+C	9.5±1.0a	12.6±0.5a	218.1±5.2a

Corn fiber comes from a commercial source (lot 1).

abc values within columns with different numbers differ at $P \leq 0.05$.

W = Wiley mill through a 1.0 mm screen, S = SPEX mill for 5 min, T = centrifuge tube, F = 50 ml Erlenmeyer flask, B = buffer only for 72 h, C = cellulase only for 72 h, E + C = ferulic acid esterase (Depol 740 L) 24 h, washed, and residue-incubated with cellulase 72 h (summed values)

Table 3 Effect of enzymes on dry weight loss and phenolic acids released from corn fiber.

Treatment	Dry weight loss	Phenolic acids	
		p-Coumaric	Ferulic
	%	mg/g	
Buffer	8.9±0.0e	0.02±0.01d	0.02±0.01c
Cellulase only	35.3±0.3d	0.07±0.01c	0.03±0.02c
E/H (0.5 g) + C[a]	55.0±0.6c	0.19±0.02b	1.57±0.21b
E/H (1.0 g) + C[a]	58.0±1.6b	0.18±0.01b	1.76±0.07b
E/H (2.0 g) + C[a]	60.7±0.1a	0.22±0.03a	2.20±0.22a

Corn fiber comes from a commercial source (lot 1); Wiley-milled and then reground in SPEX mill for 5 min.

abc values within columns with different numbers differ at $P \leq 0.05$.

[a] Summed values from incubation in TP 692L (E/H) 24 h, washed, and residue-incubated with cellulase (C) 72 h

Dry weight loss and the release of fermentable sugars and phenolic acids by esterase followed by cellulase, compared to buffer and cellulase alone, are shown for various conditions (Tables 1 and 2). In this study, SPEX milling and flask incubation resulted in the highest dry weight loss and levels of released phenolic acids, xylose, and glucose. Rhamnose, mannose, and galactose were also detected, but levels were low and generally not different among treatments. SPEX milling and tube incubation resulted in the next highest values, many of which were not different ($P>0.05$) from flask incubation. Whereas dry weight loss was higher for flask- over tube-incubated corn fiber that had been Wiley-milled, compounds released by the enzymes were similar for the two incubation methods. Dry weight loss and released products from cellulase-only treated corn fiber were extremely low, showing the efficacy of esterase pretreatment.

Corn fiber ground in the SPEX mill was incubated in flasks with varying levels of TP 692 L. The greatest dry weight loss was 60%, with 2 g of TP 692 L per tube. A second test was conducted with SPEX-milled corn fiber incubated with TP 692 L followed by cellulase (Tables 3 and 4). Levels of phenolic acids and sugars were released at greater levels than

Table 4 Effect of enzymes on sugars released from corn fiber.

Treatment	Arabinose	Xylose	Glucose
	mg/g		
Buffer	0d	0d	1.6±2.8d
Cellulase	3.9±0.3c	2.3±0.2c	143.9±3.3b
E/H (0.5 g) + C[a]	4.3±0.3c	3.3±0.2c	103.0±7.1c
E/H (1.0 g) + C[a]	11.6±1.9a	10.7±1.8a	215.1±33.7a
E/H (2.0 g) + C[a]	6.2±0.7b	6.4±0.7b	118.0±8.6c

Corn fiber comes from a commercial source (lot 1); Wiley-milled and then reground in SPEX mill for 5 min.

abc values within columns with different numbers differ at $P \leq 0.05$.

[a] Summed values from incubation in TP 692L (E/H) 24 h, washed, and residue-incubated with cellulase (C) 72 h

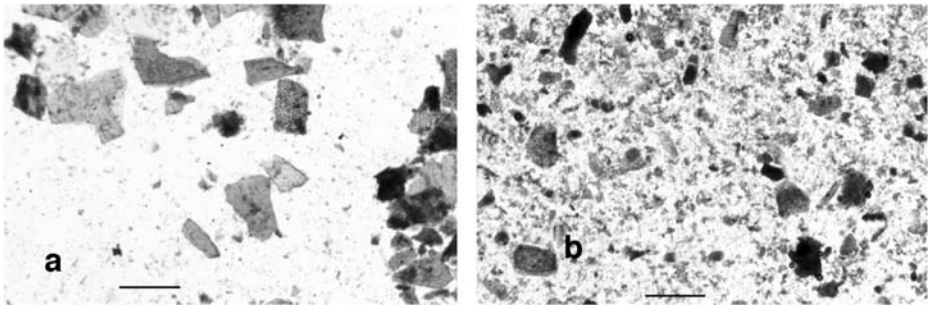

Fig. 2 Corn fiber sample **a** ground through the Wiley mill with a 1-mm screen and **b** ground through a Wiley mill with a 0.5-mm mesh and viewed under a stereomicroscope. In subpanel **a**, large fragments resembling pericarp are present, whereas in subpanel **b** finer materials of unknown origin are present. Bar= 1 mm

with cellulase alone. Depol 740 L released more sugars and phenolic acids than TP 692 L at similar dry weight losses. In both tests, FA and glucose were released in greater amounts than other compounds within similar categories.

Grinding influences particle size. Examination by stereomicroscopy (Fig. 2) showed that Wiley milling through a 1-mm screen resulted in relatively large fragments of pericarp. Grinding to a screen size of 0.5 mm resulted in breakup of these larger fragments, with substantial amounts of material from unidentified sources.

Because different grinding methods influenced the release of phenolic acids and sugars by esterase and cellulase, additional tests were carried out. Grinding through a Wiley mill

Table 5 Dry weight loss and phenolic acids in residue of corn fiber treated with enzymes.

Grind treatment	Enzyme treatment	Dry weight loss (%)	Phenolic acids			
			2 M NaOH		4 M NaOH	
			p-Coumaric acid	Ferulic acid	*p*-Coumaric acid	Ferulic acid
			mg/g			
Wiley	B	11.4±2.4a	1.14±0.06a	12.77±0.76a	1.06±0.09a	8.07±0.66a
	E	29.6±3.1b	0.45±0.07b	3.56±0.51d	1.08±0.06a	7.21±1.48a
	+C	46.4±2.5c	0.73±0.41ab	5.31±3.40cd	0.78±0.08a	4.34±1.05a
	C	22.4±1.5d	0.98±0.11a	8.47±1.42b	1.09±0.14a	7.60±1.05a
SPEX	B	9.4±2.1a	0.95±0.10a	10.86±0.86a	1.27±0.06a	10.90±1.55a
	E	32.3±2.1b	0.53±0.06b	3.79±0.73b	1.02±0.21a	7.02±0.57a
	+C	50.3±2.3c	0.93±0.09a	6.47±1.94c	0.93±0.16a	5.74±0.06a
	C	25.9±1.7d	1.15	10.7	0.91±0.47a	6.72±2.17a
Retsch	B	8.8±0.3a	1.12±0.08a	12.73±0.80a	1.42±0.13a	10.80±3.40a
	E	34.9±2.4b	0.53±0.07b	3.36±0.79b	0.89±0.13b	6.03±1.36a
	+C	51.7±3.1c	0.80±0.08c	3.98±0.61b	0.97±0.07b	4.01±0.74a
	C	22.5±10.6d	1.29±0.13a	13.47±1.00a	1.32±0.02a	8.07±2.50a

Corn fiber comes from a commercial source (lot 2).

abc values within columns with different numbers differ at $P \leq 0.05$.

B = Buffer only control 72 h, E = Depol 740 L (ferulic acid esterase) for 24 h, + C = after E, cellulase treatment of residue for 72 h, C = cellulase only for 72 h

Table 6 Sugars and phenolic acids released by enzymes from corn fiber.

Grind treatment	Enzyme treatment [b]	ρ-Coumaric acid	Ferulic acid	Arabinose	Xylose	Glucose
Wiley mill	B	0.04±0.02a	0.05±0.03a	0.75±0.78a	0.5	1.0±0a
	E	0.08±0.01b	0.53±0.06b	7.82±0.99b	7.58±0.79a	89.3±12.0c
	+C	0.2	0.15	0.81±0.22a	1.09±0.45b	16.3±6.1ab
	C	0.04±0.01a	0.24±0.06c	4.20±0.26c	2.23±0.06b	37.3±37.6b
SPEX mill	B	0.03±0.01ab	0.06±0.07a	1.9	0.5	9.2±0.7a
	E	0.09±0.02c	0.86±0.23b	7.82±0.90a	7.72±0.77a	106.2±17.6b
	+C	0.15±0.01b	0.19±0.05a	1.70±0.14b	1.97±0.06b	49.3±9.1c
	C	0.05±0.01a	0.24±0.05a	3.13±0.76b	1.90±0.60b	68.4±27.9c
Retsch mill	B	0.02±0a	0.02±0.01a	0.4	0.7	8.4±3.3a
	E	0.13±0.04b	1.42±0.41b	7.93±0.98a	8.03±1.15a	114.2±19.8b
	+C	0.05±0.02a	0.48±0.23a	2.03±0.75b	2.30±0.72b	60.8±16.9c
	C	0.03±0.01a	0.04±0.02a	2.65±0.49b	1.30±0.28b	112.5±9.2b

Corn fiber comes from a commercial source (lot 2).

abc values within columns within a grind treatment with different numbers differ at $P \leq 0.05$.

B = Buffer only control 72 h, E = Depol 740 L (ferulic acid esterase) for 24 h, +C = after E, cellulase treatment of residue 72 h, C = cellulase only for 72 h

(1.0 mm screen) and then SPEX milling for 5 min or milling through a centrifugal (Retsch) mill with a 0.08-mm screen were compared for dry weight losses, residual phenolic acids, and sugars and phenolic acids released into the incubation medium (Tables 5 and 6). SPEX and Retsch milling appeared to produce a finer grind and both resulted in greater dry weight losses compared with Wiley milling after esterase treatments (Table 5). Phenolic acids remained in the residues after enzymatic treatments, but lower amounts of ester-linked phenolic acids were generally present compared with buffer- or cellulase-only treatments. The prevalence of FA in residues occurred for both ester-linked and total phenolic acids. Amounts of FA and glucose released into the filtrate by enzyme treatments tended to be

Table 7 Effect of particle size on dry weight loss of corn fiber.

Sieve sizes (μm)	Enzyme treatment	Dry weight loss (%)
250	Buffer only	7.1±1.0 g
	TP 692 L 24 h	28.6±01.4 e
	Then cellulase 72 h	35.5±2.1 d
150	Buffer only	8.9±0.5 g
	TP 692 L 24 h	49.1±1.3 c
	Then cellulase 72 h	58.1±0.2 b
88	Buffer only	14.5±9.0 f
	TP 692 L 24 h	56.2±2.9 b
	Then cellulase 72 h	71.1±6.4 a

Corn fiber was ground through Wiley mill then reground through the SPEX mill for 5 min. This sample was then agitated through a US Standard Sieve Series and triplicate samples taken from each of the three sizes listed above. Samples were incubated in flasks in a reciprocal water bath at 100 strokes per minute for periods indicated. All samples were in triplicate except the cellulase, which was in duplicate.

abcdefg values within column with different numbers differ at $P \leq 0.05$.

higher and similar for SPEX and Retsch milling compared with Wiley milling (Table 6). Levels of FA were significantly higher for Retsch-milled, esterase-treated samples.

The effect of particle size from SPEX-milled corn fiber was further evaluated with enzyme treatments. Fractions at 250, 150, and 88 μm sieve sizes were evaluated for dry weight loss with enzymes (Table 7). Smaller particle size fractions were significantly more degraded, with further increases with the finer particles up to 71% dry weight loss with TP 692 L plus cellulase. These various fractions likely do not contain similar tissue types but tissues more degradable and/or more amenable to reduction in particle size, but further research is needed to fully define this issue.

Data presented in this study (Fig. 1) support the view that the aleurone layer is a rich source of phenolic acids in corn fiber. Histochemistry and response to ferulic acid esterase both indicate a prevalence of FA that can be partially released through enzyme activity. Considerable amounts still remain after enzyme treatment, and further work is required to distinguish between resistant bonds or reduced surface area as a limitation to more release.

Corn, which as a warm-season grass, is rich in phenolic acids, notably *p*-CA and FA, which are an integral part of the lignified and nonlignified cell walls [6]. Analysis of corn kernel tissues by synchrotron Fourier transform infrared microspectroscopy [16] provided chemical information on the different structures and indicated that pericarp, but not endosperm, has high levels of aromatics, based on absorbances for specific functional groups. Other work using ultraviolet absorption microspectrometry for a series of wheat brans clearly indicated aromatic constituents in the pericarp and aleurone layers [17]. Absorption at a λ_{max} of about 326 nm, which is indicative of ester-linked ferulic acid [18], occurred in most layers of the pericarp and was almost exclusive for aromatics in the aleurone layer. In studies of corn bran using gel permeation chromatrography, high-performance liquid chromatography, and mass spectrometry, the complexity of the linkages of ferulic acid and various sugars was reported [19]. This work also reported the presence of a *p*-coumaroylated heteroxylan side chain isolated from cereal bran. These data show the complexity of ester-linked FA in linkages to cell wall sugars in cereal brans. That this linkage limits enzymatic biodegradation is also well established [18].

Considerable data is available to show the ability of phenolic acid esterases and other cell wall-degrading enzymes to release phenolic acids from cereal brans such as corn [20], wheat [21], and oat [22]. Different types of ferulic acid esterases, designated for example as type A or B, are defined by their substrate specificity [23] and may therefore vary in activity against particular cell wall constituents and linkages. Further, the type of xylanase can affect esterase activity [24]. In some studies, the specificity of an esterase to release FA but not *p*-CA from cell walls exists [22]. The release of *p*-CA acid in addition to FA, however, as reported in this current work, may likely be advantageous to degrade structurally intact plant cell walls and release more constituents. Use of specific esterases, and mixes, in conjunction with cell wall carbohydrases could maximize sugars and various phenolic acids released from corn fiber. These enzymatic studies, when coupled with further work to optimize grinding, could substantially increase the release of sugars for ethanol and FA as a coproduct from corn fiber.

FA is abundant in plants [20]. Work in our laboratory has shown the use of esterases, with cellulases, in releasing FA and *p*-CA from a host of potential biofuels [15, 18, 25]. FA would require separation from the filtrate after release by enzymes, because this phenolic acid is inhibitory to enzymes and microbes [18]. The uses of FA are numerous and potentially of high value, such as antioxidants, antimicrobial agents, and substrate for vanillin production [26]. Corn fiber would appear to be a particularly good source of phenolic acids, particularly FA.

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Mention of product names are for identification only and do not constitute a recommendation from ARS-USDA.

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