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Corn Stover Fractions and Bioenergy

Chemical Composition, Structure, and Response to Enzyme Pretreatment

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Abstract

Information is presented on structure, composition, and response to enzymes of corn stover related to barriers for bioconversion to ethanol. Aromatic compounds occurred in most tissue cell walls. Ferulic acid esterase treatment before cellulase treatment significantly improved dry weight loss and release of phenolic acids and sugars in most fractions over cellulase alone. Leaf fractions were considerably higher in dry weight loss and released sugars with esterase treatment, but stem pith cells gave up the most phenolic acids. Results help identify plant fractions more appropriate for coproducts and bioconversion and those more suitable as residues for soil erosion control.

Index Entries: *Zea mays* L.; lignin; phenolic acids; esterase; cellulase.

Introduction

The US ethanol industry produced more than 3.4 billion gal in 2004, up from 2.8 billion gal in 2003 and 2.1 billion gal in 2002. (from the Renewable Fuels Assn. in http://www.eere.doe.gov/biomass/ethanol.html). New ethanol plants with increased production capacity signify further increases in future years. Fuel ethanol, which is generally added at about a 1:9 ratio to gasoline, has potential to be used at considerably higher ratios, such as a blend of 85:15 ethanol to gasoline *(1,2)*. Approximately 95% of this ethanol is produced from corn starch, with the remaining from biomass such as wheat, barley, sugarcane, and wastes from a variety of crop/fiber industries *(1)*. A substantial increase in ethanol production based on corn grain may conflict with use of corn for food and feed and therefore be impractical. Further, the cost of corn is a major factor in the economics of bioethanol.

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Lignocellulosic materials potentially offer a low-cost resource to expand ethanol production. Considerable research has been conducted to develop a conversion process and recently the first commercial production of ethanol for biofuel from a lignocellulosic resource was reported by the Iogen Corporation. Corn (*Zea mays* L.) stover, the above ground plant material left after grain harvest, has been identified by the US Department of Energy as a near- to mid-term agricultural residue for lignocellulose-to-ethanol production, and considerable interest and study have been devoted to this purpose *(3)*. Annual production of corn stover in the United States is reported to be well over 200 millon t *(4)*, but use of this large resource requires investigation. Wilhelm et al. *(5)* assessed the complex agronomic issues of corn stover removal for bioenergy as related to soil conditions and sustainable crop production; they concluded that a portion of the corn stover could be harvested within limits for ethanol production. Removal of approx 30% of the stover from the field is often mentioned as an acceptable practice for both biomass conversion and for soil erosion protection, but the amount that should be removed is dependent on several factors *(5)*. Selective use of portions of the corn stover for bioenergy, while leaving enough material for sustainable soil productivity, is a potential strategy, but information on the most advantageous fractions for bioconversion and for soil conservation is required.

Corn stover lignocellulose is recalcitrant to biodegradation, and the sugars comprising plant tissues are not readily available for conversion. Lignin and other aromatics associated with plant cell walls are known generally to be major detriments to biodegradation in plants *(6)*. Pretreatment of the lignocellulose is required before corn stover can be efficiently converted to ethanol *(7)*. Galbe and Zacchi *(8)* reported that the most investigated pretreatment for lignocellulosic conversion is steam explosion, with or without acid. Numerous studies have been undertaken to optimize use of dilute acid pretreatment to improve the enzymatic saccharification of corn stover *(9,10)*. Biological methods, including the use of the delignifying white rot fungi *Cyathus stercoreus* and *Phanerochaete chrysosporium*, have been used to improve enzymatic degradation of corn stover *(11)*.

It is clear from general knowledge of plants and biodegradation *(6)*, and from studies of corn stover particularly *(7)*, that fundamental knowledge on the cell-wall structure and location of particular aromatics is needed to optimize methods to improve fermentation of corn stover. The objective of work presented herein is to identify the location and types of aromatics within particular corn stover fractions and to rank their degradation by enzymes as a basis to assess potential and develop strategies for improved bioconversion. Particularly, in light of the need to leave some of the corn stover to benefit the soil, results should help identify the plant fractions most appropriate for bioconversion and the fractions most suited to leave as residues.

Materials and Methods

Samples

Mature, standing plants of Pioneer 3085 were cut approx 5–10 cm from the soil after grain harvest. The seeds were planted on 28 March 2002, and the corn harvested on 17 July 2002, from an experimental plot at the Pee Dee Research and Education Center, Florence, SC. Five intact, standing plants were harvested in October, 2002, and the various fractions separated and used are described for individual studies.

For baled corn stover, a commercial product was purchased. The corn stover (unknown cultivar) was harvested and shredded after grain harvest, wrapped in plastic, and boxed. Random samples of mixed fractions and pure fractions were selected from one bale.

Separation of Plant Fractions

For fractions of standing plants, the five intact plants of Pioneer 3085 were partitioned into the leaf blades (laminae and midveins), sheaths (laminae and midveins), and stems. The husk and cobs were not considered in this sample. For the shredded and baled corn stover, three representative lots of about 500 g were separated into the three parts listed above and also into husks and cobs. The percentage of each fraction was determined based on the total weight of the sample as is (without further drying). For the baled sample, the percentage of all five fractions was determined; the percentage of the leaf blades, sheaths, and stems was also calculated based on the sum of only these fractions.

Histochemistry

The following fractions were collected from both the third and eight internodes regions from the flower for each of three standing plants: leaf blade lamina, blade midvein, leaf sheath, sheath midvein, and intact stem. The rigid midveins were excised using a razor blade from the lamina of blades and sheaths. Fractions were soaked in water for 1 h under vacuum, and free-hand sections (approx 100-μm thick) were cut with a razor blade. Sections were then treated with a series of histochemical stains emphasizing particular aromatic constituents as follows: coniferyl lignin by acid phloroglucinol *(12)*, syringyl lignin by chlorine-sulfite *(12)*, and phenolic constituents by diazotized sulfanilic acid *(13)*. No additional preparation was done to minimize staining of aromatic amino acids. Stained sections were observed through an Olympus BH-2 light microscope under bright field illumination and scored for intensity as follows: 3 = intense, 2 = definite, 1 = slight, and 0 = none.

Chemical Analyses

Samples of untreated fractions as well as pretreated residues were freeze-dried and ground in a SPEX 5100 Mixer Mill (SPEX Industries, Inc.,

Metuchen, NJ) before chemical analysis. Fractions of the standing plants were treated with 4 *M* NaOH at 170°C for 2 h to release a variety of mono- or dimethoxylated aromatic compounds. The aromatic compounds, including phenolic acids, were measured as their silyl ethers using *N,O-bis*(trimethylsilys)trifloroacetamide (BSTFA) using gas-liquid chromatography as previously described *(14)*. The amounts of compounds of similar structure were summed to provide estimates of coniferyl lignin and syringyl lignin, and these compounds were summed with and total *p*-coumaric and ferulic acids to provide concentrations of aromatics with plant fractions. Ester- and ether-linked phenolic acids were determined by treatment of freeze-dried ground material with (a) 2 *M* NaOH at room temperature for 24 h or (b) 4 *M* NaOH at 170°C for 2 h, respectively. A follow-up study using sequential treatments of 2 *M* NaOH and 4 *M* NaOH under conditions described earlier was carried out to confirm the high amount of ester-linked phenolic acids.

Cellulase Degradation of Corn Fractions

Cellulase (EC 3.2.1.4, catalog No. C-8546, Sigma Chemical Co, St. Louis, MO), which had shown high activity against cellulosic materials in our laboratory, was used to evaluate and rank the biodegradation of various nonpretreated corn fractions. The general potency of the enzyme was verified with 100 × 12 mm filter-paper strips (Whatman No. 541), which broke in 5 h when incubated in 50 m*M* sodium acetate buffer at pH 5.0. This enzyme was then tested against plant fractions from both the standing plants and the baled corn stover. The cellulase was added to 50 m*M* sodium acetate buffer, pH 5.0, to give an activity of 20 U/mL (192.3 g/100 mL buffer). Twenty milliliters of enzyme solution were added to 1 g of freeze-dried corn stover material, which had been ground in a Wiley Mill to pass through a 10 mesh and then a 20 mesh screen. Samples were incubated at 37°C for 72 h unless otherwise indicated. Afterward, the tubes were centrifuged, the liquid decanted, and the residue freeze-dried and weighed. Dry weight loss was calculated as follows: 1 – (residual weight/starting weight) × 100.

Pretreatment of Corn Stover Fractions With Esterases Before Cellulase Hydrolysis

Separated fractions of the corn stover were ground as described and freeze-dried, and 0.5 g samples were used in triplicate for each test. For this study, the rind was manually separated from pith tissue for stems, and these stem parts were tested individually. Samples were treated with the commercial products Depol 740 L, which is one of a range of ferulic acid esterase-containing enzymes, or with TP 692 L from Biocatalysts Ltd. (Pontypridd, UK). 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 U/g and 800 U/g, respectively (product information from Biocatalysts Ltd., UK). These commercial enzymes were used as supplied and in high amounts to ensure maximal loss of ester-linked aromatic acids. Previous work on other lignocellulosic material had indicated that levels of Depol 740 L at 1 g/1 g sample was as sufficient as higher levels in releasing ferulic acid; therefore, 1 g was used. Each sample was mixed with 20 mL of 50 m*M* sodium acetate buffer, pH 5.0, containing 1.0 g of Depol 740 L or 1.0 g TP 692L, mixed for 5 s, and incubated at 37°C for 24 h in a reciprocal water bath at about 100 back and forth strokes/min. After incubation with esterases, tubes were centrifuged at 730 × *g* for 2 min and the liquid decanted. The samples were washed by adding the pellet to 20 mL distilled water, mixing for 5 s, centrifuging, and decanting the liquid. Decanted liquids, enzyme mixture and washing, were analyzed for phenolic acids and for sugars. The esterase-treated, washed residues were subsequently incubated as above with cellulase (as described earlier) or freeze-dried and weight loss calculated before subsequent incubation with cellulase. Dry weight loss was calculated as described earlier.

Ferulic and *p*-coumaric acids and 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 mg/g amounts of the starting weights of freeze-dried, ground fractions.

Results and Discussion

Plant Fractions

Proportions of the various fractions of standing plants of Pioneer 3085 and of commercially baled corn stover are shown in Table 1. Husk and cob fractions contributed 15% and less than 1%, respectively, of the total weight in the baled stover (not shown). Considering only the leaf blade, sheath, and stem portions, the standing plants had roughly similar amounts of leaf blade and stems but lesser amounts of leaf sheath. In the baled stover, amounts of stem fractions were considerably higher than either of the leaf fractions, which were lower than in the standing plants. Other work has indicated a stem content of near 50%, when all fractions were considered in mature corn *(4,16)*. The shattering of the dry and brittle leaf blade and sheath during harvesting and baling likely increased stem proportions in stored corn stover.

Lignin and Aromatic Content

Lignins in higher plants vary in type based on the functional groups of the aromatic ring *(12)*. We tested for mono and dimethoxylated lignins as well as phenolic acids. Total aromatics of a single sample of mature Pioneer 3085 as determined for coniferyl and syringyl lignin groups plus

Table 1
Proportion of Plant Fractions in Corn

	Weight[a] (%)	
Plant part[a]	Standing plant[b]	Baled stover[c]
Blade	36.1 ± 3.6	30.9 ± 6.7
Sheath	24.5 ± 1.9	16.9 ± 1.23
Stem	39.4 ± 2.8	52.2 ± 8.0

[a]Average and standard deviation of weight as is.
[b]From five plants of Pioneer 3085 planted 28 March 2002, and harvested 17 July 2002.
[c]From a commercial source of harvested, shredded, and baled stover (unknown cultivar) delivered to the laboratory. Data derived from three replicates (approx 500 g) from one bale.

p-coumaric and ferulic acids gave the following amounts (mg/g dry weight): leaf blade, 7.5; leaf sheath 16; stem pith, 21.7; and stem rind, 45.4. The plant fractions varied in aromatic types and linkages, with the rind containing higher levels of all components. The ratio of coniferyl lignin to syringyl lignin varied for the different plant components, and *p*-coumaric acid was higher than ferulic acid in all components except the leaf blade.

Warm-season grasses like corn often are particularly high in phenolic acids, with high proportions of *p*-coumaric acid *(17)*. Ester-linked *p*-coumaric and ferulic acids, as determined by susceptibility to 2 *M* NaOH, and total phenolic acids (i.e., both ester- and ether-linked acids), as determined by a more rigorous extraction with 4 *M* NaOH under high temperature and pressure, indicated variable amounts of phenolic acids in the fractions (Table 2). The amounts of *p*-coumaric and ferulic acids were similar in blade laminae, whereas the stem tissues had considerably more *p*-coumaric acid. Although the milder 2 *M* NaOH extraction removes more ester-linked phenolic acids and the more rigorous 4 *M* treatment releases both ester-and ether-linked acids, little difference occurred between the two treatments. In fact, levels after 2 *M* treatment tended to be greater, although not significant ($p < 0.05$), except for leaf-sheath midveins. A separate evaluation using sequential treatments of 2 *M* (room temperature, 24 h) followed by 4 *M* NaOH (170°C, 2 h) confirmed that most of these phenolics acids were ester-linked; the more rigorous 4 *M* NaOH treatment destroyed some of the acids based on tests with known amounts of standards. These data provide further evidence of high levels of ester-linked *p*-coumaric and ferulic acids in the various fractions of corn lignocellulose.

Histochemical Staining and Aromatic Content of Specific Cell Walls

The anatomy of leaf blade and stem is well known *(18,19)*. Leaf laminae have a high amount of thin-walled, living cells that do not stain for aromatics; most of the thickened cell walls are associated with the

Table 2
Ester- and Ether-Linked Phenolic Acids in Corn Fractions[a]

	p-Coumaric acid (mg/g)				Ferulic acid (mg/g)			
	2 *M* NaOH		4 *M* NaOH		2 *M* NaOH		4 *M* NaOH	
Leaf-blade lamina	1.5	0.5	1.7	0.2	2.5	0.8	2.8	0.5
Leaf-blade midvein	9.3	1.6	6.4	1.1	4.4	1.5	5.0	1.1
Leaf sheath	8.5	2.4	5.4	0.6	8.4	5.0	6.1	1.2
Leaf-sheath midvein	12.3	2.2	7.2	0.6	8.6	1.6	7.2	0.7
Stem pith	28.1	7.8	18.8	2.9	5.7	1.5	7.4	1.4
Stem rind	32.1	11.1	15.4	1.4	6.6	3.2	6.6	0.8

[a]Average ± standard deviation of three plants from Pioneer 3085. Extractions at 2 *M* were at room temperature for 24 h and extractions at 4 *M* were at 170°C for 2 h, with samples analyzed as in Table 3. For 2 *M* extractions, each plant part was run a second time, and the mean value of the two runs was taken as the average for that plant. No differences occurred ($p > 0.05$) between 2 *M* and 4 *M* extractions for any part for either acid, with the exception of pCA for leaf-sheath midvein.

lignified vascular tissue and the epidermis *(6)*. Sheaths show a considerable amount of thin-walled cells but a higher proportion of thick-walled cells than leaf blades. Sheaths contain more lignin overall than blades. In stems, an outermost rind consists of thick-walled, subepidermal sclerenchyma and parenchyma cells with a concentration of lignified vascular bundles *(18)*. Pith consists of thin-walled parenchyma cells within which are embedded lignified vascular bundles. The rind, which consists of thickened cell walls in epidermal and subepidermal layers, is considerably thicker in lower internodes; pith cell walls are relatively thin in both upper and lower internodes.

Just as the various fractions of the corn plant differed in aromatics, individual tissues within these fractions varied with the presence of aromatics as determined by histochemical stains. Sarkanen and Ludwig *(12)* reported that acid phloroglucinol "had universal application to all lignins, although the reaction may be weak or even absent in lignins containing high amounts of syringyl propane units." The chlorine-sulfite stain, however, gives a red color with syringyl units of lignin *(12)*. The application of these two stains, therefore, can identify the location of lignin types within tissues. In addition to stains for coniferyl (acid phloroglucinol) and syringyl (chlorine-sulfite) lignin, diazonium salts react with phenolic compounds in general *(13)* and are useful to show phenolic acids (presumably ester-linked) in nonlignified, "living" tissues *(20)*. Although these methods stain for aromatic compounds in cell walls, a role for aromatic amino acids was not addressed. The occurrence of aromatic compounds in unlignified cell walls is a major feature of grasses *(21)*. The linkages and compounds of aromatics in unlignified and lignified cell walls of bermudagrass (*Cynodon dactylon*), another C-4 grass, have been verified using ultraviolet

absorption microspectroscopy *(22,23)*. Because maturity and plant position have been shown to affect lignification in plant parts *(24)*, fractions from the third and eighth internodes from the top of the plant were analyzed.

The salient histochemical results are summarized from three replicates of sections from each fraction of standing plants of the third and eighth internodes. Some differences in histochemistry between the two lignin stains occurred among tissues, both in positive reactions and in intensities. In sheath midveins and stem tissues, eighth internodes tissues often gave more intense reactions, especially with chlorine-sulfite staining. Diazotized sulfanilic acid indicated that most of the cell walls contained aromatic compounds. Mesophyll cell walls of leaf-blade laminae, however, lacked a positive reaction for aromatics. In stems, even the thin-walled parenchyma cells gave a strong reaction (score of 2.7 out of a possible 3.0). Eighth internode fractions did not have consistently more intense reactions than the third internodes. These data complement that for total aromatics in indicating that considerable lignification exists in most cell walls of corn stover, but variations are evident in different fractions. Phenolic acids, presumably ester-linked to carbohydrates, are prevalent in most of the tissues.

Incubation With Esterase and Cellulase

A preliminary test was carried out to assess dry weight loss after cellulase treatment of fractions from standing plants and corn stover. Material lost in buffer solutions (without cellulase) was greater in standing plants at approx 30% in all fractions in comparison with 7–16% loss in baled stover. This difference suggested more drying or loss of soluble components from corn stover that was baled and stored. After incubation with cellulase, dry weight loss was low in all samples. Dry weight loss for leaves (blades and sheaths) was 7–16% over that by buffer alone, whereas that for stems was approx 5%. This low biodegradabililty with cellulase was consistent with the recalcitrance owing to high levels of aromatics in cell walls, particularly for stems.

Pretreatment with Depol 740 L was equal to or better than TP 692 L in increasing dry weight loss (not shown). Pretreatment with esterase resulted in significantly greater dry weight loss with cellulase than without esterase pretreatment for all fractions (Table 3). Improvement was greater in leaf blades and sheaths (near 62% dry weight loss) compared with that in stem rind or pith (21–29%). Incubation filtrates (plus washings) from stem fractions contained considerably more phenolic acids than those from leaf fractions (Table 3). The ferulic acid esterase was significantly more effective in releasing both *p*-coumaric and ferulic acids into the liquid than treatment with cellulase alone. Levels of ferulic acid released were about twofold higher than those of *p*-coumaric acid in leaf fractions but similar in amounts in stem fractions. These data suggest that some *p*-coumaryl esterase exists in Depol 740 or the enzyme has crossreactivity with both ester-linked phenolic acids. On subsequent treatment

Table 3
Dry Weight Loss and Phenolic Acids in Filtrate and Residue of Corn Fractions Treated With Enzymes

			Phenolic acids (mg/g)			
			Filtrate		Residue	
Fraction	Treatment[a]	Dry weight loss (%)	*p*-Coumaric	Ferulic	*p*-Coumaric	Ferulic
Leaf blade	Buffer 72 h	32.0 ± 0.7^{b}	$0.12 \pm 0^{b,c}$	0.32 ± 0.10^{b}	0.92 ± 0.26^{b}	0.63 ± 0.22^{b}
	Esterase 24 h		0.24 ± 0.02^{d}	0.50 ± 0.06^{c}		
	then cellulase 72h	61.8 ± 1.0^{d}	0.08 ± 0.06^{c}	0.08 ± 0.06^{d}	2.86 ± 0.26^{c}	1.20 ± 0.10^{c}
	Cellulase 72 h	40.9 ± 0.4^{c}	0.14 ± 0.02^{b}	0.33 ± 0.07^{b}	1.17 ± 0.48^{b}	1.22 ± 0.36^{c}
Leaf sheath	Buffer 72 h	30.8 ± 1.5^{b}	0.07 ± 0.02^{b}	0.13 ± 0.06^{b}	1.94 ± 0.51^{b}	1.23 ± 0.36^{b}
	Esterase 24 h		0.36 ± 0^{c}	0.69 ± 0.03^{c}		
	then cellulase 72h	62.7 ± 1.8^{d}	0.16 ± 0.01^{d}	0.13 ± 0.01^{b}	6.16 ± 0.47^{c}	1.82 ± 0.10^{c}
	Cellulase 72 h	47.1 ± 1.3^{c}	0.09 ± 0.01^{b}	0.35 ± 0.02^{d}	7.44 ± 0.10^{d}	3.52 ± 0.16^{d}
Rind	Buffer 72 h	12.8 ± 0.9^{b}	0.38 ± 0.05	0.14 ± 0.02^{b}	6.81 ± 2.12^{b}	0.99 ± 0.29^{b}
	Esterase 24 h		0.44 ± 0.02	0.25 ± 0.01^{c}		
	then cellulase 72h	20.5 ± 1.1^{d}	0.31 ± 0.03	0.14 ± 0.02^{b}	12.12 ± 32.78^{c}	1.58 ± 0.40^{c}
	Cellulase 72 h	16.4 ± 1.0^{c}	0.23 ± 0.14	0.10 ± 0.05^{b}	28.07 ± 0.75^{d}	4.56 ± 0.15^{d}
Pith	Buffer 72 h	14.1 ± 2.6^{b}	0.44 ± 0.04	0.37 ± 0.04	25.55 ± 1.91^{b}	5.91 ± 0.38^{b}
	Esterase 24 h		0.48 ± 0.01	0.57 ± 0.29		
	then cellulase 72h	28.8 ± 0.7^{c}	0.46 ± 0.03	0.30 ± 0.03	8.61 ± 2.06^{c}	1.43 ± 0.34^{c}
	Cellulase 72 h	16.7 ± 0.7^{b}	0.26 ± 0.15	0.22 ± 0.11	21.67 ± 0.85^{d}	4.71 ± 0.15^{d}

[a]Cellulase, Cat. No. C8546, Sigma-Aldrich; Esterase, ferulic acid esterase (Depol 740L, Biocatalysts Ltd.).
[b-d]Values within columns with different values differ at $p \leq 0.05$.

with cellulase, the esterase-treated residues often gave up more phenolic acids than cellulase treatment alone; this difference was reflected in the higher dry weight losses (Table 3). With the exception of leaf blades, the levels of phenolic acids remaining in the treated residues were lower after esterase plus cellulase compared with cellulase only, based on dry weight of starting material (Table 3). Although the results indicate preferential loss of phenolics with esterase pretreatment, not all phenolic acids were released. Additional physical treatments, such as homogenization or grinding (Akin, unpublished data; *25*) may improve the interaction of enzyme and substrate and increase the amount of phenolic acids released.

The release of soluble sugars by esterase and cellulase treatments is shown in Table 4. Release of soluble sugars by esterase pretreatment, either directly or in subsequent cellulase treatments, was particularly effective in removing glucose and xylose from leaves. The additional available glucose from esterase and subsequent cellulase treatments included an increase of 2.53 and 1.44 times in blades and sheaths, respectively. Sugar release from stem fractions was not, or was less, improved with esterase pretreatment. Stem rinds were particularly recalcitrant to esterase pretreatment.

Cell walls of grasses, such as corn, vary from other plant types, for example, woody tissue, dicotyledonous plants, in having a high level of phenolic acids, particularly *p*-coumaric and ferulic acids *(21,26)*. These phenolic acids are often esterified to arabinose and then linked to xylose moieties *(27,28)*; dimers of these compounds commonly occur as recalcitrant bridges in grasses also. Ester-linked phenolic acids appear to be the main aromatic compound in certain unlignified "living" cell walls of grasses *(20,22)*. The present data indicates that a high proportion of the aromatics in all corn stover fractions are ester-linked phenolic acids. These esters, which can inhibit utilization of linked sugars *(29)*, are more readily susceptible to pretreatment than highly lignified (e.g., ether-linked) tissues and can be disrupted by milder treatments that break ester bonds and release phenolic acids. Other recent research has similarly shown that ferulic acid esterases, particularly with xylanases, release phenolic acids from plant cell walls *(30)*. The present research indicated that Depol 740 L was as effective as products with esterase plus other cell wall degrading enzymes (based on information from the supplier); the exact enzyme composition of these products is not available. Further research on specific combinations of esterases and xylanases for particular plant fractions could improve removal of phenolic acids.

The release of phenolic acids from corn stover may provide a high-value coproduct and improve the cost efficiency of bioconversion. Graf *(31)* reviewed several actual or potential uses of ferulic acid:

1. Substrate for conversion to vanillin used as a flavor constituent;
2. UV protection in suntan lotions and cosmetic preparations;
3. allelopathic functions and regulation of plants, and;
4. protection against pests.

Table 4
Carbohydrates in Filtrates of Enzyme-Treated Corn Fractions

Sample	Enzyme treatment	Arabinose (mg/g)	Xylose (mg/g)	Mannose (mg/g)	Galactose (mg/g)	Glucose (mg/g)
Leaf blade	Buffer 72 h	0.8 ± 0.1^{a}	1 ± 0.6^{a}	7.6 ± 1.7^{a}	1.4 ± 0.4^{a}	4.4 ± 1.4^{a}
	Esterase 24 h	2.2 ± 0.5^{a}	20.6 ± 3.9^{b}	0.4 ± 0.6^{b}	0.9 ± 0.2^{a}	35 ± 9.9^{b}
	Esterase + Cellulase 72 h	5.6 ± 3.1^{b}	16.1 ± 0.6^{c}	7.3 ± 0.6^{a}	7.3 ± 0.6^{b}	90 ± 3.6^{c}
	Cellulase 72 h	$3.3 \pm 0.7^{a,b}$	8 ± 0.7^{d}	5.6 ± 2^{a}	2.8 ± 0.1^{c}	53 ± 20.1^{b}
Leaf sheath	Buffer 72 h	0.8 ± 1.3^{a}	0^{a}	27.2 ± 14.5^{a}	0.2 ± 0.4^{a}	54.4 ± 31.7^{a}
	Esterase 24 h	2.5 ± 0.3^{a}	13.3 ± 2.1^{b}	0.4 ± 0.4^{b}	0.4 ± 0.4^{a}	30.9 ± 3.3^{a}
	Esterase then Cellulase 72 h	7.8 ± 1.7^{b}	25.7 ± 3.8^{c}	$14.1 \pm 3.1^{a,b}$	2.3 ± 0.2^{b}	150 ± 8.9^{a}
	Cellulase 72 h	6 ± 0.3^{b}	11.1 ± 0.2^{b}	9.4 ± 1.5^{b}	1.4 ± 0.2^{c}	125.3 ± 99.1^{a}
Stem rind	Buffer 72 h	1 ± 0.9^{a}	3.5 ± 3.2^{a}	0.7 ± 0.6^{a}	0.5 ± 0.4^{a}	8.6 ± 8.1^{a}
	Esterase 24 h	2.1 ± 1.9^{a}	7.5 ± 1.0^{b}	0^{b}	0^{b}	11.9 ± 1.6^{a}
	Esterase then Cellulase 72 h	1.7 ± 1.4^{a}	7.3 ± 1.8^{b}	1.5 ± 0.1^{c}	0.8 ± 0.1^{a}	22.7 ± 3.1^{b}
	Cellulase 72 h	2.7 ± 0.3^{a}	10.4 ± 0.6^{b}	$1 \pm 0.3^{a,c}$	1.4 ± 0.1^{c}	39.2 ± 2.8^{c}
Stem pith	Buffer 72 h	5 ± 0.7^{a}	17.8 ± 1.8^{a}	0.6 ± 0.1^{a}	1.6 ± 0.2^{a}	27.3 ± 2.5^{a}
	Esterase 24 h	3.2 ± 0.6^{b}	16.2 ± 2.3^{a}	0.3 ± 0.2^{a}	3.9 ± 4.4^{a}	27.5 ± 11.1^{a}
	Esterase then Cellulase 72 h	$4.3 \pm 0.6^{a,b}$	17.8 ± 1.4^{a}	0.5 ± 0.5^{a}	1.5 ± 0.1^{a}	56.3 ± 4.5^{b}
	Cellulase 72 h	6.4 ± 0.4^{c}	23 ± 0.7^{b}	1.1 ± 0.1^{b}	2.4 ± 0.3^{a}	68.6 ± 1.5^{c}

[a-d]Values within columns with different values differ at $p \leq 0.05$.

Much of the usefulness of ferulic acid and other plant phenolics is owing to their antioxidant and antimicrobial properties *(32)*. These phenolic monomers, when released from cell walls, can be toxic to biological systems *(33)* and become a detriment to bioconversion unless they are removed.

Leaves of corn stover showed the greatest improvement in dry weight loss with esterase pretreatment. Stem rind cells are the most heavily lignified and recalcitrant and may be of little value without harsh, chemical pretreatment. A physical processing method (e.g., shredding and rubbing) on stems could separate rind from pith cells, which differ in lignification and release of ferulic acid and are more amenable to pretreatments. The more recalcitrant fractions could be left in the field for soil erosion control and soil organic carbon *(5)*, thereby reducing transport weight and concentrating the most useful resource for bioconversion.

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Mention of trade names does not constitute an endorsement of one commercial product over another but is used only for identification purposes.

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