Extraction and Quantitative Analysis of Oil from Commercial Corn Fiber

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Extraction of commercial corn fiber with hexane or supercritical CO_2 yielded an oil that comprised from 0.54 to 3.68 wt % of the fiber. An HPLC method with sensitive evaporative light-scattering detection (ELSD) was developed to analyze the lipid classes in corn fiber oil. Triacylglycerols were the most abundant lipid class, but the oil also contained sterol esters, free fatty acids, phytosterols, and very low levels of tocopherols. All fiber samples contained ferulate esters, similar in structure to "oryzanol", a cholesterol-lowering substance found in rice bran and rice bran oil. Much more oil (up to 10-fold) and more ferulate esters (up to 2-fold) could be obtained from the fiber by grinding it before hexane extraction. The finer the fiber was ground, the more oil and ferulate esters were removed. Essentially all of the extractable oil and all of the ferulate esters were removed by extraction with hexane for 1 h at 25 °C.

Keywords: *Corn fiber; ferulate esters; ferulate-phytosterol esters; corn; Zea mays*

INTRODUCTION

Corn fiber is obtained from the processing technique called "wet milling", which involves an initial steeping of corn kernels in 0.12−0.20% aqueous SO_2 at 50−55 °C for 24−48 h, followed by a gentle grinding and physical separation of the outer fiber layers from starch, protein, and other components. Wet milling of corn is used by all companies that produce corn starch and corn sweeteners and by many companies that produce fuel ethanol from corn. In contrast, "dry milling" of corn involves the grinding of corn kernels without steeping and separation of the outer layers into a fraction called "corn bran".

Recently, Norton (1994, 1995) and Seitz (1989) have reported that a hexane extract from corn bran contains high levels of ferulate esters, similar in composition to "oryzanol" found in rice bran and rice bran oil. Oryzanol has been shown to lower the levels of serum cholesterol in laboratory animals and man (Kahlon et al., 1992; Nicolosi et al., 1991). The present study was undertaken to investigate the quantities of oil obtained from several corn fiber samples and to quantitatively analyze the levels of ferulate esters and other lipids in this oil using a sensitive HPLC technique. It was not known whether the different processing steps involved in the production of corn fiber vs corn bran would result in products with oils of different yields and chemical compositions.

MATERIALS AND METHODS

Samples of fiber and other materials were obtained from the following sources: Samples of corn gluten feed, yellow waxy corn kernels, and corn fiber (from common, yellow waxy, white waxy, and high amylose hybrids) were obtained from American Maize, Hammond, IN. Samples of common fiber and corn gluten feed were obtained from Cargill, Inc., Dayton, OH. Samples of hull fiber, fine fiber, corn cleanings, spent flake, and purified fiber were obtained from Corn Products, CPC International, Summit-Argo, IL. A sample of corn bran was obtained from Lauhoff Grain Co., Danville, IL. A sample of distillers grains and solubles was purchased from Sigma Chemical Co., St. Louis, MO. Samples of corn cobs, wheat bran, and oat bran were purchased locally. All samples except the corn gluten feed were dried at 65 °C in a convection tray dryer. The oryzanol standard was obtained from CTC Organics, Atlanta, GA. Tocopherol standards were obtained from Matreya, Inc., Pleasant Gap, PA. All other HPLC standards were obtained from Sigma Chemical Co., St. Louis, MO.

Sample Preparation and Oil Extraction. Samples were ground to a particle size of 20 mesh with a Wiley mill (Thomas Scientific, Philadelphia, PA) for most studies. Unground fiber and fibers of smaller particle sizes were prepared for other studies with the Wiley mill using the appropriate screens. Fiber samples (4 g) were placed in screw-top vials (55 mL, 25 × 150 mm), and 40 mL of hexane containing 0.01% butylated hydroxytoluene (BHT) was added. The tubes were sealed with Teflon-lined caps and shaken for 1 or 18 h in a Burrell Model 75 wrist action shaker (Burrell Inc., Pittsburgh, PA) at 25 °C. Tubes were shaken horizontally for 1 h in a water bath shaker (New Brunswick Scientific, Edison, NJ) for the 65 °C extractions. After extraction, the hexane extracts were filtered through a Whatman GF/A glass fiber filter (Whatman Laboratory Products, Clifton, NJ) fitted in a Buchner funnel, with gentle vacuum. Separate samples (8 g) of unground and ground corn fiber were extracted with supercritical CO_2 (25 min at 2.5 L/min, 10 000 psi, and 40 °C) in an Applied Separations (Allentown, PA) Spe-ed supercritical fluid extractor. The supercritical fluid-extracted oil was dissolved in hexane and injected into the HPLC. BHT (0.01%) was added to all samples to prevent oxidation.

High Performance Liquid Chromatography. The lipid classes in crude filtered hexane extracts were separated and quantified by a modified version of an HPLC technique developed in our laboratory (Moreau et al., 1990). The ternary gradient HPLC system used was a Hewlett Packard Model 1050 modular system (Hewlett Packard, Avondale, PA). Two detectors were connected in series. The first was a Hewlett Packard Model 1050 fixed wavelength UV−visible detector set at 295 nm. The second was an Alltech-Varex Mark III evaporative light scattering Detector (Alltech Associates, Deerfield, IL) operated at a temperature of 40 °C, with nitrogen as a nebulizing gas at a flow rate of 1.60 L (STP)/min. The column was a Chromsep Cartridge, LiChrosorb DIOL, 5 μm, 3 × 100 mm (Chrompack, Raritan, NJ). The mobile phase gradient of hexane/2-propanol/acetic acid is described in Table 1, and the flow rate was constant at 0.5 mL/min.

Each fiber sample was extracted at least twice, and each of these extracts was analyzed via HPLC at least twice. Results presented are the means and standard deviations combined from multiple analyses.

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Table 1. Linear Gradient Program Used for Normal-Phase Separation of Lipid Classes in Corn Fiber Oil[a]

time (min)	% A	% B
0	100	0
8	100	0
10	99.1	0.9
30	99.1	0.9
31	100	0

[a] Column was LiChrosorb DIOL, 5 μm (3 × 100 mm) and the flow rate was 0.5 mL/min; A, hexane/acetic acid, 1000/1, v/v; B, 2-propanol. (Both were added fresh daily to eliminate errors caused by evaporation or adsorption of moisture.)

RESULTS AND DISCUSSION

HPLC Analysis of Lipid Classes in Corn Fiber Oil. A binary HPLC system was developed to separate (Figure 1) and quantify (using the standard curves in Figure 2) the various lipid components in the hexane extracts of corn fiber. A sample (100 μL) of the crude filtered hexane extract was injected directly into the HPLC without further preparation. By using two detectors in series, it was possible to identify the peaks on the ELSD that were also UV_{295nm}-absorbing peaks. The wavelength of 295 nm was chosen because both tocopherols and ferulate esters could be detected (Diak and Saska, 1994). Analysis of the extract in a second HPLC system (data not shown) designed to examine more polar lipids (Moreau et al., 1990) revealed that there were no lipids more polar than those separated in the current HPLC system. The first step of the binary HPLC system (which consisted of 8 min isocratic at 99.9/0.1, hexane/acetic acid, v/v) separated sterol esters, triacylglycerols and four free fatty acids (stearic, palmitic, oleic, and linoleic). The second step of the gradient (which was essentially a step to isocratic 99.1 A/0.9 B = 99.0/0.9/0.1 hexane/2-propanol/acetic acid, v/v, held from 10 to 30 min after injection) separated phytosterols, ferulate esters, and several minor unknown peaks. An unknown lipid with a large UV_{295nm} absorbance was detected at 12.2 min, but the mass of this compound (as measured by the ELSD) was very

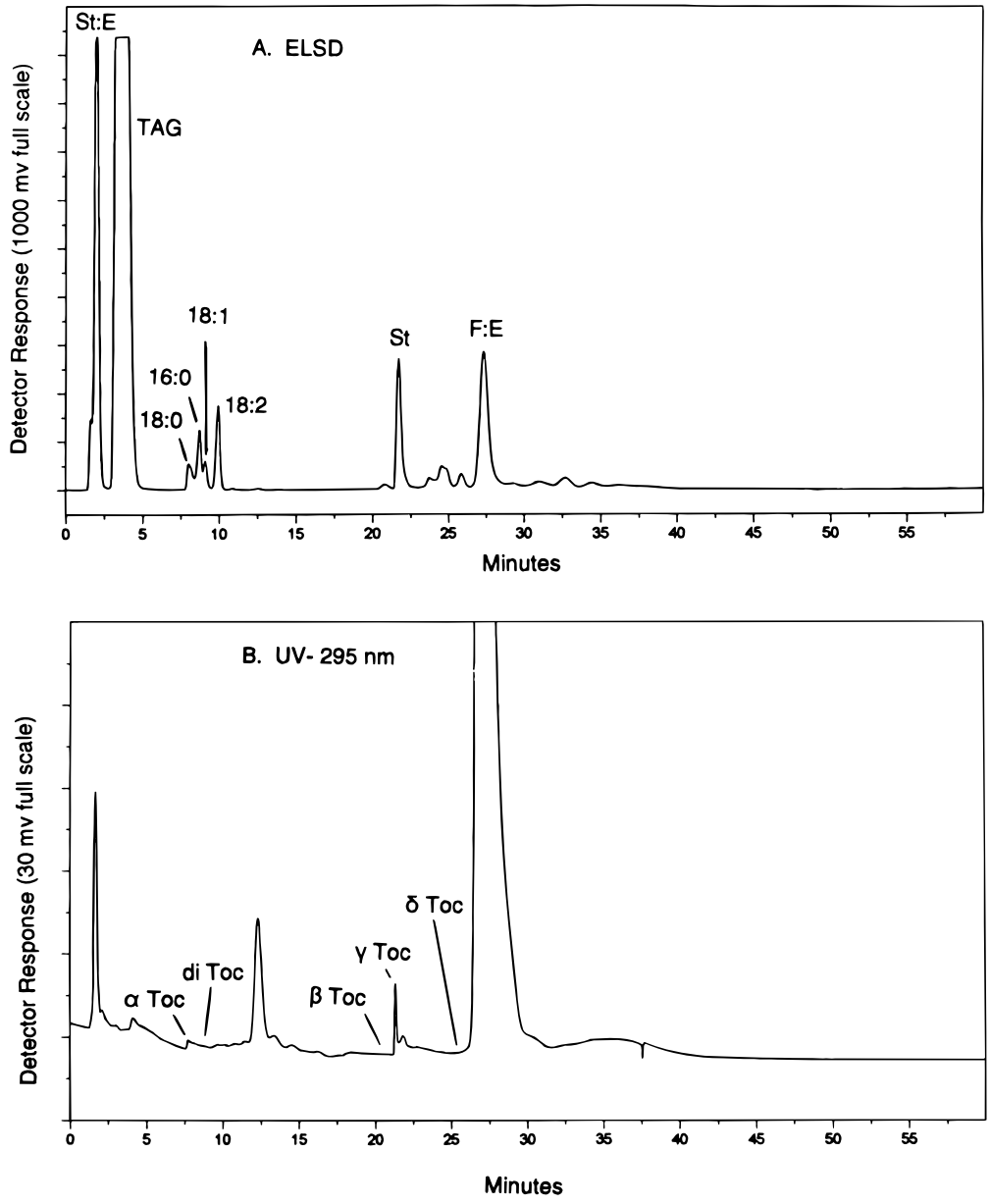

Figure 1. Normal phase HPLC chromatogram of the lipids in corn fiber oil. (A) Detection with evaporative light scattering detector. (B) Detection at UV 295 nm. See Table 2 for abbreviations.

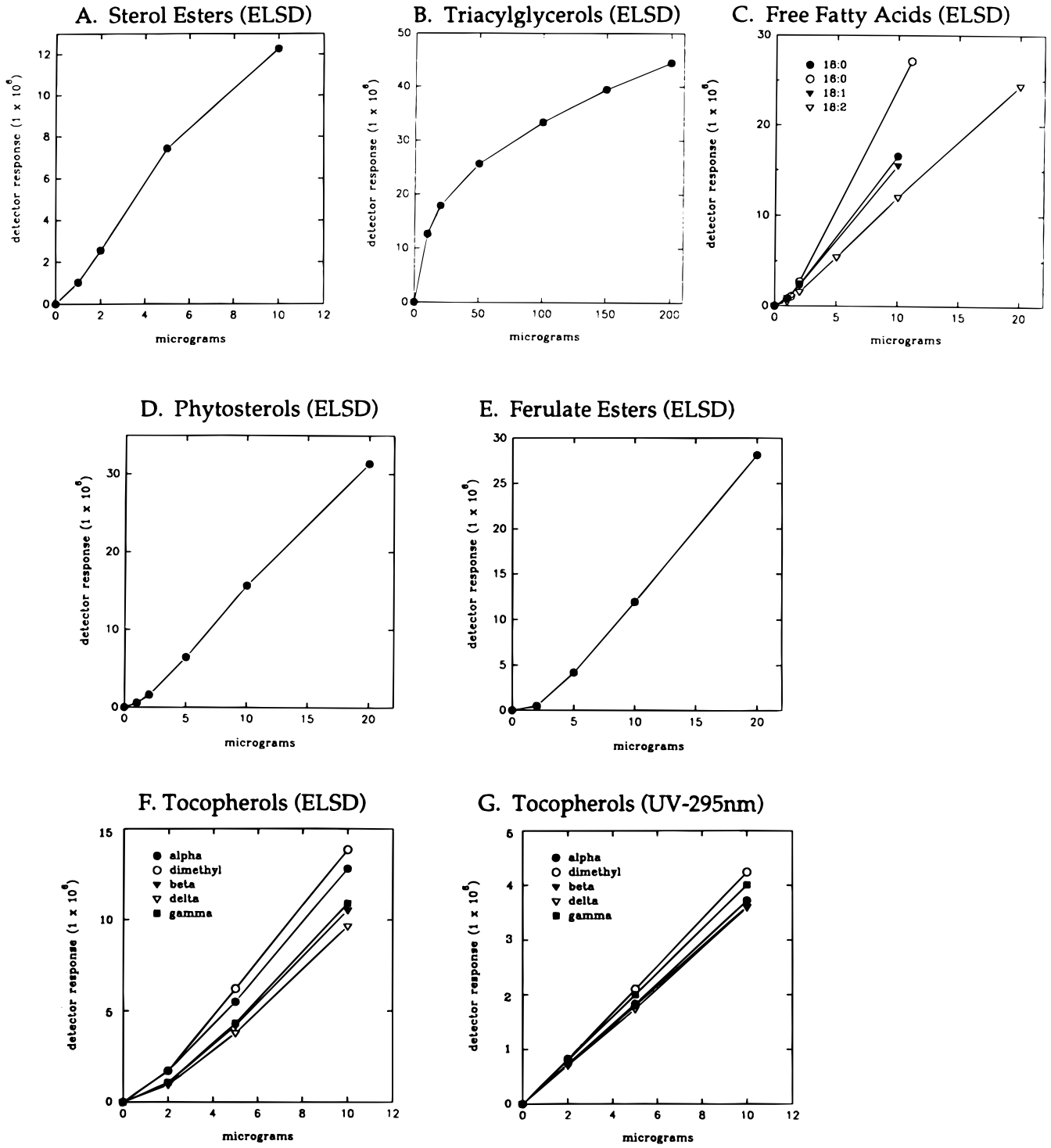

Figure 2. Calibration of detector response for selected lipid classes in corn fiber oil: (A) sterol esters, (B) triaclyglycerols, (C) free fatty acids, (D) phytosterols, (E) ferulate esters, (F) tocopherols (ELSD), and (G) tocopherols (UV 295 nm).

low. The oil also contained sterol esters, free fatty acids, phytosterols, and very low levels of tocopherols. All fiber samples also contained ferulate esters, which co-chromatographed with a standard of "oryzanol". Oryzanol is a cholesterol-lowering substance found in rice bran and rice bran oil. Norton (1994, 1995) has shown that the ferulate esters in oil obtained from corn bran are similar in structure to those in oryzanol, but the most common phytosterol (including both sterols and stanols) of corn bran oil is sitostanol, a saturated phytosterol that also has hypocholesterolemic properties when administered in its free sterol form (Vanhanen et al., 1993).

Curves of standards of the major lipid components were constructed (Figure 2). The relationship between sample mass and ELSD response was not always linear, but was reproducible, and the data could easily be entered into our HPLC data acquisition software to enable it to calculate the mass (in units of micrograms) of each lipid component. The range of masses for each of the standards was calculated to approximate the ranges encountered in the analyses of the corn fiber oil samples. Because triacylglycerols were present in much higher concentrations than any of the other lipid classes, the upper mass range of triacylglycerols was 200 μg, while the upper mass range of all other lipid classes was

Table 2. HPLC-ELSD Analysis of Corn Fiber Oil Extracted with Hexane from Ground (20 Mesh) American Maize Common Corn (Yellow Dent No. 2) Fiber[a]

lipid class	abbrev	rt (min)	wt % of fiber	wt % of oil
sterol-FA esters	St:E	2.0	0.19 ± 0.01[b]	9.05 ± 0.54
triacylglycerols	TAG	4.1	1.321 ± 0.23	79.43 ± 2.01
free fatty acids				
	18:0	8.05	0.011 ± 0.000	0.55 ± 0.00
	16:0	8.78	0.019 ± 0.001	0.93 ± 0.04
	18:1	9.16	0.011 ± 0.001	0.51 ± 0.01
	18:2	10.01	0.018 ± 0.000	0.87 ± 0.01
tocopherols				
a	α-Toc	7.4	tr[c]	tr
dimethyl	di-Toc	8.6	nd	nd
b	β-Toc	19.6	nd	nd
g	γ-Toc	20.7	0.001 ± 0.000	0.04 ± 0.1
d	δ-Toc	26.3	nd	nd
phytosterols	St	21.7	0.039 ± 0.017	1.92 ± 0.83
ferulate esters	F:E	27.5	0.11 ± 0.01	6.70 ± 0.25

[a] 4 g of fiber extracted in 40 mL of hexane for 1 h at 25 °C. [b] Data presented are the mean of two extractions with two injections each ± standard deviation. [c] tr, trace; nd, not detectable.

10−20 μg. The standard curves reported here were similar in shape to those recently reported by Christie and Urwin (1995), using the same model of ELSD. Standard curves for the tocopherols were constructed using both the ELSD and UV detectors (Figure 2F. 2G). For quantification of the levels of the only measurable tocopherol, γ-tocopherol, the UV standard curve (Figure 2G) was used because the retention time of this component was very close to that of the phytosterol and it would have been difficult to distinguish between phytosterol and γ-tocopherol with the ELSD. However, since phytosterols do not absorb at 295 nm, the UV chromatogram could be used for accurate quantification of γ-tocopherol.

The standard curves (Figure 2) were used to provide a quantitative analysis of the lipid classes in corn fiber oil (Table 2). Triacylglycerols were the major component in all types of corn fiber oil. The other major components in a representative sample of corn fiber oil (American Maize, common corn fiber ground to 20 mesh) were ferulate esters (6.70 wt %), phytosterols (1.92 wt %), free fatty acids (2.86 wt %), and sterol esters (9.05 wt % of oil) (Table 2). Among the tocopherols, only two were detected; low levels (0.04 wt %) of γ-tocopherol and trace levels of α-tocopherol.

Extraction of Oil and Ferulate Esters from Corn Fiber. As obtained from the corn processors, the physical structure of dry corn fiber is comprised mainly of large pieces (>5 mm) or "shreds". When unground fiber was extracted with hexane, very little oil (0.31 wt %) was extracted (Table 3). Grinding the fiber to a particle size of 20 mesh before hexane extraction increased the levels of extractable oil by 6-fold as compared to the unground material. The finer the fiber was ground (30, 40, 60, and 80 mesh), the more oil was extracted (the finest grind of 80 mesh resulted in an extractable oil content of 3.33 wt %). Attempts to "shatter" the 60 mesh ground fiber into even smaller pieces in a mortar and pestle with liquid nitrogen did not further increase the levels of extractable lipids. Extending the time of extraction from 1 h to 18 h or increasing the temperature of extraction from 25 to 65 °C did not significantly increase the levels of extractable oil (Table 3). Supercritical fluid extraction of unground corn fiber yielded about 50% more extractable oil than hexane extraction of unground fiber. However, supercritical fluid extraction of 20 and 80 mesh ground corn fiber yielded slightly less oil than hexane extraction.

In addition to measuring the levels of extractable oil in unground vs ground fibers, the levels of ferulate esters were also quantitatively analyzed (Table 3). Grinding increased the levels of hexane-extractable ferulate esters by 33% and 89% in fiber ground to 20 and 80 mesh, respectively. Although the concentrations of ferulate esters (wt % ferulate esters) in the oils obtained in fiber ground to various particle sizes was highest in unground fiber, the yields of oil from unground fiber were very low. Soxhlet extraction of the 20 mesh fiber increased the amount of oil extracted by 21% and decreased the concentration of ferulate esters in the oil by 24% (compared to the 1 h 25 °C extraction.) Grinding also increased the levels of supercritical fluid-extractable ferulate esters by 33% and 300% in fiber ground to 20 and 80 mesh, respectively. For the remainder of the extractions in this study, we chose to grind all fibers to a particle size of 20 mesh and extract with hexane for 1 h at 25 °C.

Comparison of the Levels of Oil and Ferulate Esters in Various Fiber Samples. Samples of corn fiber and fiber-related products were obtained from several sources and extracted, and the levels of ferulate esters in the oils were analyzed (Table 4). Fiber samples derived from the milling of four different corn hybrid varieties were obtained from American Maize. Among the four, the fiber from high amylose corn had the

Table 3. Effect of Particle Size and Temperature on Levels of Oil and Ferulate Esters Extracted from Corn Fiber

particle size	extraction solvent	extraction time and temp	extractable oil from fiber (wt %)	ferulate esters in fiber (wt %)	ferulate esters in oil (wt %)
unground	hexane	1 h, 25 °C	0.31	0.09 ± 0.00[a]	29.00 ± 0.74
1.5 mm	hexane	1 h, 25 °C	1.10	1.10	0.10 ± 0.00
20 mesh	hexane	1 h, 25 °C	1.69	0.12 ± 0.10	6.77 ± 0.72
30 mesh	hexane	1 h, 25 °C	2.23	0.14 ± 0.01	6.28 ± 0.74
40 mesh	hexane	1 h, 25 °C	2.45	0.15 ± 0.02	6.01 ± 0.81
60 mesh	hexane	1 h, 25 °C	3.24	0.18 ± 0.02	4.95 ± 0.45
80 mesh	hexane	1 h, 25 °C	3.33	0.17 ± 0.02	4.95 ± 0.45
60 mesh	hexane	1 h, 25 °C & ln[b]	2.96	0.14 ± 0.00	4.68 ± 0.04
20 mesh	hexane	18 h, 25 °C	1.78	0.11 ± 0.01	6.06 ± 0.90
20 mesh	hexane	1 h, 65 °C	1.67	0.11 ± 0.01	6.42 ± 1.30
20 mesh	hexane	18 h Soxhlet	2.05	0.11 ± 0.01	5.17 ± 0.25
unground	SC-CO_2[c]	25 m, 40 °C	0.48	0.06 ± 0.00	12.28 ± 0.83
20 mesh	SC-CO_2	25 m, 40 °C	1.47	0.08 ± 0.00	5.38 ± 0.06
80 mesh	SC-CO_2	25 m, 40 °C	2.91	0.18 ± 0.00	4.37 ± 0.02

[a] Data presented are the mean of two extractions with two injections each ± standard deviation. [b] ln, = ground fiber (60 mesh) was further pulverized by grinding for 5 min in a mortar and pestle with liquid nitrogen before hexane extraction. [c] SC-CO_2, supercritical CO_2.

Table 4. Extractable Oil and Ferulate Esters in Corn Fiber and Other Materials Obtained from Various Sources

source	sample[a]	extractable oil (wt %)	ferulate esters in oil (wt %)
American Maize	common[b] fiber	1.72[c]	6.75
	yellow waxy fiber	2.12	5.05
	white waxy fiber	2.82	4.54
	high amylose fiber	3.68	2.00
	gluten feed	1.29	3.64
	yellow waxy kernels	2.81	0.23
Cargill	common[b] fiber	2.26	2.70
	gluten feed	2.37	1.08
CPC	hull fiber	1.09	5.65
	fine fiber	0.54	3.37
	corn cleanings	1.24	1.62
	spent flake	1.75	0.25
	purified fiber	1.59	1.83
Lauhoff	corn bran	1.32	1.50
Sigma Chemical purchased locally	distillers grains & sols	2.53	0.79
	corn cobs	0.20	0
	wheat bran	3.36	0.80
	oat bran	6.75	0.40

[a] All samples were ground to 20 mesh, and 4 g of sample was extracted with 40 mL of hexane for 1 h at 25 °C. [b] Common, yellow dent no. 2 corn. [c] Data presented are the mean of two extractions and two injections of each extraction.

highest levels of extractable oil, and the oil extracted from the fiber of common (yellow dent no. 2) corn had the highest levels of ferulate esters. Corn gluten feed, which is a blend of corn fiber and corn steep liquor, had 1.29% extractable oil, and its oil contained 3.64% ferulate esters. Yellow waxy kernels also were ground and extracted and found to contain 2.81% extractable oil, and their oil contained 0.23% ferulate esters. Seitz (1989) reported that most of the ferulate esters in corn kernels are in the pericarp, which is the major part of the fiber fraction. If we assume that a corn kernel is composed of 5% pericarp (Watson, 1987), that the fiber is composed primarily of pericarp, and that all of the ferulate esters are in the fiber, then we could estimate (using the measurement of 5.05 wt % of ferulate esters in the fiber of yellow waxy kernels) that yellow waxy kernels should contain (0.05) (5.05 wt %) = 0.25 wt % ferulate esters, which is close to the value of 0.23 wt % actually measured in the yellow waxy kernels (Table 4).

Samples of common fiber and corn gluten feed were obtained from Cargill. Each of these samples contained more oil, and their oil contained less ferulate esters than samples of the analogous materials from American Maize.

The first three fiber samples from CPC were obtained from different parts of the wet milling process. Among these, the hull fiber is produced in the largest volumes during processing, and the oil obtained from it contained the highest levels of ferulate esters. Fine fiber and corn cleanings contained relatively low levels of oil, and their oil contained relatively low levels of ferulate esters. Spent flake is the "cake" left over when corn germ is hexane extracted; it contained 1.75% extractable oil, and this oil contained only 0.25% ferulate esters. The purified fiber, a finely ground food-grade fiber, contained 1.59% extractable oil, and this oil contained 1.83% ferulate esters.

The Lauhoff bran contained relatively low levels of extractable oil, and its oil contained low levels of ferulate esters. Distillers grains and solubles is the "mash" remaining after the fermentation of dry-milled corn. It contained 2.53% extractable oil, and this oil contained 0.79% ferulate esters. It is not clear whether the lower values of ferulate esters in these samples are due to differences in wet milling (fiber) vs dry milling (bran) or due to different corn hybrid varieties used by the various processors.

The last three samples were not corn fiber, but were related materials that were included mainly to investigate the utility of our HPLC method for measuring the levels of ferulate esters in these materials. Among these three samples, low levels of ferulate esters were detected in wheat bran and oat bran, and none were detected in ground corn cobs.

In addition to containing ferulate esters, rice bran oil was also reported to contain nearly equal levels of a second type of hypocholesterolemic agent, tocotrienols (Diak and Saska, 1994; Rogers et al., 1993; Shin and Godber, 1994). In the current study, tocotrienols were not detected in corn fiber oil (as evidenced by the absence of major UV-absorbing peaks other than those of ferulate esters and tocopherols). However, since the identity of the 12.2 min UV-absorbing peak is not yet known, we are now determining its structure to learn whether it may be a tocotrienol or a related compound.

Although this study revealed that commercial corn fibers contain relatively low levels of oil (0.54−3.5 wt %) as compared to rice bran (≈18 wt %) (Kahlon et al., 1992), the oil obtained from corn fiber is richer in ferulate esters (up to 6.75 wt %) than rice bran oil (0.1 to 0.8 wt %) (Rogers et al., 1993). However, if you calculate the levels of ferulate esters in corn fiber itself, corn fiber may actually contain more ferulate esters than rice bran; a typical sample of commercial corn bran contains 0.12% ferulate esters (Table 3), whereas rice bran contains 0.018−0.14 wt % ferulate esters (estimated by 0.18 × 0.1 wt % and 0.18 × 0.8 wt %). The phytosterol component of corn fiber ferulate esters is mainly sitostanol (Norton, 1994, 1995), while the predominant triterpene alcohols and phytosterol in rice bran ferulate esters (oryzanols) are cycloartenol, 24-methylene cycloartenol, and campesterol (Rogers et al., 1993). These compositional differences may make corn fiber oil a more potent hypocholesterolemic substance than rice bran oil. We are currently planning collaborative studies to investigate the hypocholesterolemic properties of corn fiber oil.

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