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Phytosterol and Tocopherol Components in Extracts of Corn Distiller's Dried Grain

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As the ethanol industry continues to grow, it will become very important to develop value-added markets for its coproducts in order for the industry to remain profitable. Corn distiller's dried grain (DDG) is a major coproduct of ethanol fermentation from corn processed by dry-milling and is primarily sold as livestock feed. The objective of this research was to determine if valuable phytochemicals found in corn oil and corn fiber oil, such as phytosterols and their saturated equivalents, phytostanols, ferulate phytosterol esters (FPE), tocopherols, and tocotrienols, are retained in DDG. Hexane and supercritical carbon dioxide (CO_2) extracts of DDG were similar in their concentrations of total phytosterols (15.8−17.3 mg/g of extract), FPE (3.75−3.99 mg/g of extract), and tocols (1.7−1.8 mg/g of extract). Ethanol extracts were slightly lower in concentration of phytosterols (8.9−11.4 mg/g of extract), FPE (1.62−1.98 mg/g of extract), and tocols (0.73−0.76 mg/g of extract).

KEYWORDS: Distiller's dried grains; phytosterols; ferulate phytosterol esters; corn oil; corn fiber oil; tocopherols; tocotrienols

INTRODUCTION

In the United States, the ethanol industry is already producing >3.9 billion gallons of fuel ethanol, 95% of this from corn (*1*). Ethanol production is expected to grow tremendously as demand increases for alternative fuel oxygenators, alternative fuels, and a decreased dependency on foreign oil. Ethanol can be produced from corn using either a wet- or dry-milling process. In 2005, 79% of ethanol was produced in dry-milling facilities, whereas 21% was produced in wet-milling facilities (*1*). Farmers and farmer cooperatives favor dry-milling facilities for ethanol production because the capital costs are lower than for wet-milling production facilities. Therefore, much of the growth in ethanol production is expected to be from dry-milling facilities.

There are few coproducts from dry-milling ethanol operations, including distiller's dried grain (DDG), distiller's solubles, and CO_2 (*2*). In the dry-grinding process for ethanol production, corn is ground, cooked, steeped with enzymes, and fermented. Following distillation, the stillage is centrifuged or screened to produce wet distiller's grain and thin stillage. The wet distiller's grain can be sold only locally due to shelf-life issues, so it is usually dried to increase shelf life and is thus called distiller's dried grain (DDG). Thin stillage is evaporated to a syrup and added back to the DDG to produce distiller's dried grain with solubles (DDGS). DDGS is sold primarily as a livestock feed at market prices ranging from $85 to $140/ton.

For the growing ethanol industry to succeed, it will be important to develop new value-added uses for its byproducts (*2*). Because only the starch and sugars are utilized for fermentation, most of the oil, protein, and fiber are retained in DDG. Several high-value components have been isolated from corn oil and corn fiber oil extracted during the wet-milling process or from alternative dry-milling processes that fractionate the corn fiber before fermentation (*3*, *4*). Corn oil, which is oil extracted from corn germ separated during the wet-milling process (*5*), is rich in tocopherols, phytosterols, and phytosterol fatty acid esters (**Figure 1**). Corn fiber oil also has high tocopherol, tocotrienol, and phytosterol contents, in addition to having ferulate phytosterol esters (FPE), which are typical in nutraceutical oils such as rice bran oil (**Figure 1**). Tocopherols and tocotrienols, collectively known as tocols, or vitamin E, are important antioxidants (*6*), and phytosterols, when consumed, are well-known for their ability to lower blood cholesterol by blocking re-adsorption of cholesterol from the gut (*7*). The demand for phytosterols as nutraceutical ingredients in the growing "functional food" and "heart healthy" categories has skyrocketed since the FDA approved a health claim for foods containing at least 0.65 g or 1.7 g/serving of plant sterol or plant stanol esters, respectively (*8*). In addition, ferulate phytosterol esters have been shown to have antioxidant activity (*9*) and are believed to contribute to the high stability of rice bran oil.

One study (*10*) has shown that oils extracted from corn kernels, using either hexane or ethanol, were higher in phytosterols, tocopherols, and tocotrienols than hexane-extracted corn germ (commercial corn oil). Extracts of DDG might be expected to have a composition similar to that of corn kernel

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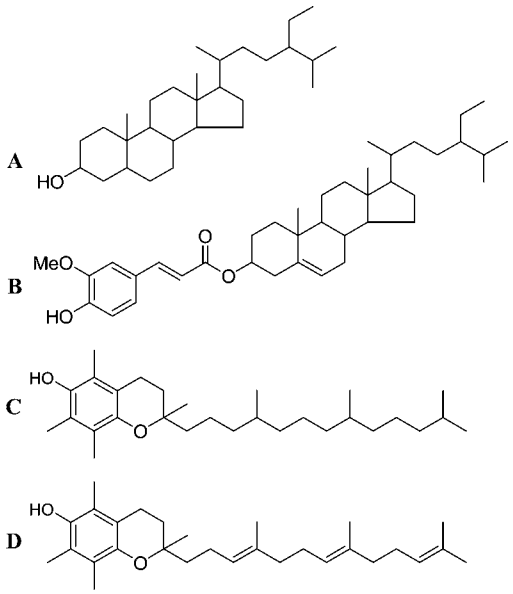

Figure 1. Structures of (**A**) a phytostanol, sitostanol; (**B**) a ferulate phytosterol ester, sitosteryl ferulate; (**C**) α-tocopherol; and (**D**) α-tocotrienol.

extracts, but the processing conditions that the corn is subjected to during and after fermentation may affect the final composition. The purpose of this study was to determine if phytosterols, tocopherols, and ferulate phytosterol esters are retained in DDG, which would thus provide an abundant source for these valuable nutraceuticals while at the same time providing another value-added market for this coproduct. Using several different extraction methods, hexane, supercritical carbon dioxide (CO_2), and ethanol extracts of DDG were characterized for their contents of phytosterols, ferulate phytosterol esters, tocopherols, and tocotrienols.

MATERIALS AND METHODS

Chemicals and Materials. Tocopherol standards (95% purity) were purchased from Matreya, Inc. (Pleasant Gap, PA). Tocotrienol standards (≥95% purity) were purchased from EM Science (Gibbstown, N.J.). γ-Oryzanol (≥98% purity) was purchased from CTC Organics (Atlanta, GA). Stigmasterol and 5α-cholestane were purchased from Matreya, Inc. Campesterol was purchased from Steraloids (Newport, RI). Sitosterol and sitostanol were from Sigma-Aldrich, Inc. (St. Louis, MO). Each phytosterol standard was ≥97% pure. *N*,*O*-Bis(trimethylsilyl)-fluoroacetamide with 1% trimethylchlorosilane (BSTFA+1% TMCS) was purchased from Regis (Morton Grove, IL). All solvents used for extractions and for high-performance liquid chromatography (HPLC) were high-purity, HPLC grade solvents purchased from Sigma-Aldrich. Corn distiller's dried grains were obtained from Big River Resources, LLC (West Burlington, IA). The moisture content of the DDG was 9.2 ± 0.6% (w/w) as determined in triplicate using the official AOAC method (*11*).

Preparation of Distiller's Dried Grain Extracts. Triplicate 50 g samples of DDG were extracted for 24 h using a Soxhlet apparatus with hexane or anhydrous ethanol. The organic solvents were removed under reduced pressure by rotoevaporation, and gravimetric weights were determined after a constant weight was achieved. Accelerated solvent extractions (ASEs) were performed with an ASE 200 accelerated solvent extractor (Dionex Corp., Sunnyvale, CA) using absolute ethanol and hexane delivered by an ASE 200 solvent controller. An 11 mL ASE extraction cell was used for all experiments. A cellulose fiber was pressed into the extraction cell before the cell was filled with distillers dried grain (ca. 4 g). All extractions were performed at 80 °C and 1500 psi without a preheat step, a 5 min heating period, three 5 min static extraction cycles, a 50% flush volume, and a 1 min purge time. Gravimetric weights were determined after the solvent was removed under a gentle stream of nitrogen to a constant weight. Three replications were performed for each solvent.

Supercritical carbon dioxide extractions of the DDG were performed with a TFE 2000 total fat extractor (Leco Corp., St. Joseph, MI) using SFC/SFE grade carbon dioxide (Airgas Specialty Gases, Chicago, IL). The DDG (ca. 2.5 g) was placed in the extraction thimble between two glass-fiber filter disks (Leco Corp.). The supercritical extractions were performed at 80 °C and 8000 psi at a flow rate of 2 L/min (measured at NSTP) for 60 min after an initial static hold of 1 min. The variable restrictor was heated to 80 °C. Gravimetric weights were determined ca. 5 min after the end of the collection to allow the carbon dioxide to outgas from the sample. Three replications were performed.

Phytosterol Analysis. Each extract was sampled in duplicate for quantitation of phytosterols (*12*). Approximately 25 mg of extract with added 5α-cholestane (internal standard) was saponified in 2 N ethanolic KOH. Nonsaponifiable material was extracted twice with hexane, and the combined hexane fractions were dried under N_2. Trimethylsilyl (TMS) derivatives of the phytosterols were made by adding 100 μL of each pyridine and BSTFA+1%TMCS and heating at 60 °C for 1 h on a heating block. Samples were injected twice by autosampler onto a Varian (Palo Alto, CA) 3800 GC equipped with an FID and a Supelco (Bellefonte, PA) SPB-1701 30 m × 0.25 mm × 0.25 μm capillary column. Helium was used as a carrier gas, with a 1:50 injector split. The injector temperature was 270 °C, and the detector temperature was 290 °C. The column oven initial temperature was 250 °C for 0.5 min, increased at 10 °C/min to 270 °C, and held for 27 min, then increased at 10 °C/min to 280 °C and held for 3.5 min. GC control, data collection, and integration were performed using Varian Galaxie Chromatography Software ver. 1.9.3.2. Phytosterols were identified by comparison of their retention times (relative to 5α-cholestane) with those of commercially available standards. Phytosterols without commercially available standards such as Δ5-avenasterol and Δ7-stigmastenol were identified by their relative retention time compared to literature, and by comparison with samples known to contain those phytosterols (*12*, *13*). Quantitation was carried out using the internal standard method.

Analysis of Ferulate Phytosterol Esters and Tocopherols. Extracts (ca. 50 mg) were weighed into test tubes, and 1 mL of hexane was added. The tubes were mixed by vortex for 30−60 s and then filtered through a 0.45 μm filter (Chrom Tech, Inc., Apple Valley, MN), and these diluted extracts were immediately analyzed in triplicate by HPLC. Tocols and ferulate phytosterol esters were separated and quantitated using a procedure (*14*) that was modified to employ dual UV and fluorescence detection rather than UV alone. All eight tocols (α-, β-, γ-, and δ-tocopherols and -tocotrienols) were separated except for β-tocopherol, which coelutes with γ-tocopherol. However, because β-tocopherol is usually either not detected or present only in small quantities in corn (*10*, *22*), we determined that the separation was sufficient for the analysis of DDG extracts. Analyses were carried out with a Thermo Separation Products (San Jose, CA) SpectraSYSTEM pump and autosampler connected by a Starrett column splitter (40:60) to a SpectraSYSTEM FL fluorescence detector and a SpectraSYSTEM UV2000 in parallel. Samples (50 μL) were injected onto a YMC-Pack-Diol-NP, 5 μm, 4.6 × 250 mm, column (YMC, Wilmington, NC). The mobile phase consisted of 98:2 v/v hexane/2-propanol with a continuous flow rate of 1.5 mL/min. The UV detector, set at 325 nm, was used for the detection and quantitation of FPE. FPE were quantitated by external calibration with pure oryzanol injected at concentrations between 10 and 250 μg/mL. Tocopherols and tocotrienols were identified on the basis of retention time similarity with known standards using fluorescence detection (excitation, 292 nm; emission, 344 nm). Tocopherols and tocotrienols were quantified by external calibration with pure standards injected at concentrations between 5 and 50 μg/mL for α- and γ-tocopherols and -tocotrienols, between 0.5 and 20 μg/mL for δ-tocopherol, and between 0.5 and 10 μg/mL for δ-tocotrienol. To ensure that there were no other UV-absorbing contaminants that would bias quantitation of FPE and to ensure the peak identity of tocopherols and tocotrienols, samples were also run, under the same conditions, with a SpectraFocus scanning UV detector. FPE in DDG extracts had the same spectral scan as oryzanol, with two peak λ maxima at 285 and 310 nm, and there was no evidence of interfering peaks in the DDG extracts. The identity and purity of tocols were also verified

Table 1. Oil, Phytosterol, and Ferulate Phytosterol Ester Contents in Extracts of Corn Distiller's Dried Grain[a]

		phytosterol composition[b] (mg/g of extract)									ferulate phytosterol esters	
solvent, method	% oil[c] (w/w)	Camp	Campst	Stigm	Sito	Sitost	Aven	Stigsten	total[d] (mg/g of extract)	total (mg/g of DDGS)	mg/g of extract[e]	mg/g of DDG[e]
hexane, Soxhlet	12.67 ± 0.16 c	2.53 (15.6)	1.10 (6.8)	0.82 (5.0)	8.05 (49.6)	2.70 (16.6)	0.73 (4.5)	0.31 (1.9)	16.2 ± 0.65 b	2.05 ± 0.09 b	3.99 ± 0.04 a	0.51 ± 0.06 b
hexane, ASE	11.15 ± 2.19 c	2.69 (15.4)	1.19 (6.8)	0.89 (5.1)	8.58 (49.1)	2.94 (16.9)	0.81 (4.6)	0.34 (2.0)	17.3 ± 0.26 a	1.92 ± 0.33 d	3.97 ± 0.39 a	0.44 ± 0.04 d
ethanol, Soxhlet	32.73 ± 1.90 a	1.36 (15.4)	0.59 (6.6)	0.50 (5.6)	4.30 (48.6)	1.44 (16.3)	0.45 (5.1)	0.22 (2.5)	8.87 ± 0.30 e	2.91 ± 0.22 a	1.62 ± 0.06 d	0.53 ± 0.02 a
ethanol, ASE	17.55 ± 0.09 b	1.75 (15.3)	0.82 (7.2)	0.62 (5.4)	5.50 (48.3)	2.03 (17.8)	0.49 (4.3)	0.19 (1.6)	11.4 ± 0.78 d	2.00 ± 0.14 c	1.98 ± 0.09 c	0.35 ± 0.01 e
CO_2	12.50 ± 0.26 c	2.46 (15.6)	1.06 (6.7)	0.83 (5.2)	7.85 (49.7)	2.63 (16.6)	0.72 (4.6)	0.25 (1.6)	15.8 ± 0.67 c	1.97 ± 0.11 c	3.75 ± 0.47 b	0.47 ± 0.07 c

[a] Abbreviations: Camp, campesterol; Campst, campestanol; Stig, stigmasterol; Sito, β-sitosterol; Sitost, sitostanol; Aven, Δ5-avenasterol; Stigsten, Δ7-stigmastenol. See text for other abbreviations. [b] Values in parantheses are the relative percent for each phytosterol. [c] Values are the average ± standard deviation for three extracts. [d] Values are the average ± standard deviation for three extracts, each analyzed in duplicate ($n = 6$). Within each column, means followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple-range test. [e] Values are the average ± standard deviation for three extracts, each analyzed in triplicate ($n = 9$). Within each column, means followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple-range test.

using the scanning UV detector as above, by comparing the spectra of peaks with the spectra of standards. In some cases, higher concentrations of sample were injected to verify the identification and purity of some of the less prominent peaks, due to the lower sensitivity of the scanning UV detector as compared to the fluorescence detector.

Statistical Analysis. All data were imported into SAS for Windows version 9.1 for statistical analysis. The general linear models procedure (GLM) was used to conduct analysis of variance for the effect of treatment (extraction method) on oil yield and phytosterol, FPE, tocopherol, and tocotrienol contents. Means were compared using Duncan's multiple-range test, with $p < 0.05$ used for determining statistical significance.

RESULTS AND DISCUSSION

Oil Extracted from DDG. The DDG samples were extracted three times using five different solvent method combinations: hexane Soxhlet extraction, hexane accelerated solvent extraction (ASE), supercritical carbon dioxide (CO_2), ethanol Soxhlet extraction, and ethanol ASE. The hexane and supercritical CO_2 extracts, after removal of solvents, were transparent oils of a bright yellow color. Oil yields (**Table 1**) were similar using either hexane Soxhlet (12.67%) or supercritical CO_2 extractions (12.5%). The yields from ASE with hexane were slightly lower (11.15%), but due to higher variability in extraction yield, these differences were not significant ($p < 0.05$). Ethanol extracts of DDG were dark brown in color and became viscous and nonhomogeneous upon solvent removal. The yield after ASE with ethanol was 17.55%, but was much higher from ethanol Soxhlet extractions (32.73%). The longer extraction time used for the Soxhlet extraction versus the accelerated solvent extraction likely explains the difference in yield. The more highly polar ethanol likely extracted some non-lipid components as well as lipid components, resulting in the higher yield. Singh and Cheryan (*15*) extracted DDGS with various ratios of ethanol to DDGS and obtained total solid yields of 7.57−12.8% and fat yields of 4.2−6.9% and also reported glycerol and small amounts of protein in the extracts. However, it is important to note that their extractions were carried out for only 30 min at 50 °C, which may explain their lower total yield compared to this study. Oil yield may also be dependent on the source of DDG. The current analyses were performed on just one source of corn DDG from a fuel ethanol fermentation plant. However, Singh et al. (*16*) reported oil yields (using hexane ASE) ranging from 8 to 15% in distillers dried grain with solubles (DDGS) samples from six different fuel and beverage ethanol plants, some of which utilized 100% corn and others that utilized corn with a mixture of other grains.

Phytosterols in DDG. Phytosterols are most commonly present in oils either in the free form, esterified to a fatty acid (phytosterol fatty acid ester), or, in some cases, esterified to a ferulic acid group (ferulate phytosterol esters). Total phytosterol content was measured by saponification of a portion of each extract, extraction of nonsaponified material, and GC separation and analysis of phytosterols. The saponification step hydrolyzes phytosterol fatty acid esters as well as ferulate phytosterol esters, releasing the free phytosterols for subsequent derivitization and analysis. Phytosterol content was significantly ($p < 0.05$) different among all of the extracts and was higher in the hexane and supercritical CO_2 extracts of DDG (15.8−17.3 mg/g of oil, **Table 1**) compared to the ethanol extracts (8.9−11.4 mg/g of oil). However, taking into account the yield of oil, Soxhlet extraction with ethanol actually extracted the highest amount of phytosterols from the DDG. Corn fiber extracts contain approximately 10-fold higher phytosterol content (*3*), but the amount of extractable oil from corn fiber is much lower than from DDG. The phytosterol content of all of the DDG extracts, except for the ethanol Soxhlet extract, are higher than what was reported (*10*) for unrefined, hexane-extracted wet-milled corn germ (9.7 mg/g of oil, 0.97 wt %), as well as for commercial corn oils (*17*, *18*), although values can vary somewhat depending on factors such as corn variety and growth and processing conditions. In addition, the phytosterol content in the DDG extracts surpasses the content of most commercial vegetable oils such as soybean (1−3.5 mg/g), sunflower (3.5−4 mg/g), and canola (5−7 mg/g) (*5*). Singh et al. (*16*) reported a slightly higher phytosterol content in hexane extracts of DDGS (2.4−3.4 mg/g of DDGS), but this may be explained by varietal differences, grain source, additional phytosterols in the soluble portion of DDGS, or differences in their extraction protocol (they used a higher temperature and pressure in ASE).

The composition of phytosterols (relative percent, **Table 1**) was similar in all of the different extracts of DDG. However, the phytosterol composition is quite different from that of regular corn oil in that there is a much higher ratio of the saturated phytostanols, campestanol (7%) and sitostanol (16.7%), compared to commercial corn oil (*17*). The phytosterol composition of DDG extracts resembles that of corn fiber oil (*19*), which also has higher campestanol and sitostanol ratios. These saturated phytostanols have been found to be preferentially

Table 2. Tocopherol and Tocotrienol Content (Milligrams per Gram) in Extracts of Corn Distiller's Dried Grain[a]

	tocopherols			tocotrienols			
solvent, method	α	γ[b]	δ	α	γ	δ	total[c] (T + T3)
hexane, Soxhlet	0.19 ± 0.00 c	0.95 ± 0.02 a	0.051 ± 0.002 b	0.16 ± 0.00 b	0.46 ± 0.00 a	0.015 ± 0.001 a	1.82 ± 0.01 a
hexane, ASE	0.20 ± 0.02 b	0.72 ± 0.08 c	0.049 ± 0.005 c	0.17 ± 0.02 a	0.45 ± 0.04 b	0.018 ± 0.006 a	1.60 ± 0.16 c
ethanol, Soxhlet	0.09 ± 0.00 e	0.32 ± 0.00 e	0.021 ± 0.000 e	0.08 ± 0.00 d	0.21 ± 0.00 c	0.005 ± 0.000 b	0.73 ± 0.04 e
ethanol, ASE	0.10 ± 0.01 d	0.35 ± 0.02 d	0.024 ± 0.001 d	0.07 ± 0.00 e	0.21 ± 0.01 c	0.006 ± 0.000 b	0.76 ± 0.04 d
CO_2	0.26 ± 0.05 a	0.83 ± 0.13 b	0.058 ± 0.004 a	0.13 ± 0.03 c	0.45 ± 0.03 b	ND[d]	1.71 ± 0.18 b

[a] Values are average ± standard deviation for three extracts, each analyzed in triplicate ($n = 9$). Within each column, means followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple-range test. [b] There may also be small quantities of β-tocopherol present within the γ-tocopherol peak. [c] Total (mg/g) of tocopherols and tocotrienols. [d] ND, not detected.

esterified with ferulic acid to form ferulate phytosterol esters. FPE are primarily found in the inner pericarp tissue of corn kernels (*20*); thus, the increased phytostanol content, along with the FPE content (discussed below), in DDG extracts indicates that the some or all of the corn fiber oil is being extracted along with the corn germ oil.

Ferulate Phytosterol Esters in DDG. At least five forms of FPE, varying in the sterol or stanol head group, are present in corn (*19*), but under the HPLC conditions we used, they elute as a single peak (**Figure 2A**). The content of FPE in DDG extracts ranged from 1.6 to 4.0 mg/g of oil (**Table 1**). These values are high in comparison to corn oil extracted from corn germ, which has negligible FPE content (*10*), because FPE are mainly found in the inner pericarp of the corn kernel (*20*). FPE content in the ethanol Soxhlet extract of DDG (1.62 mg/g of oil) was similar to FPE in ethanol-extracted ground corn kernels (*10*), whereas the contents of FPE in ethanol ASE, CO_2, and hexane (Soxhlet and ASE) extracts of DDG were similar to FPE in hexane extracts of ground corn kernels and corn bran (*10*). It is also useful to determine the total amount of FPE that is extracted from the DDG in relation to the total content of FPE in corn. The most FPE were extracted using ethanol Soxhlet (0.53 mg of FPE/g of DDG), whereas hexane Soxhlet, hexane accelerated solvent extraction, and supercritical CO_2 extracted 0.51, 0.44, and 0.47 mg of FPE/g of DDG, respectively. These amounts are similar to the FPE found in DDGS hexane extracts, as well as to content in extracts of fiber aspirated from DDGS (*16*). It is estimated that 1 kg of DDGS is produced from every 3.29 kg of corn (*2*). Therefore, by interpolation, ethanol extracted approximately 0.16 mg of FPE/g of corn, whereas hexane and supercritical CO_2 extracted 0.13−0.16 mg of FPE/g of corn. In comparison, Moreau et al. (*21*) measured approximately 0.11 mg of FPE/g of corn kernels in hexane extracts. Thus, it seems that practically all of the FPE that are extractable in corn kernels are retained, and are extractable, in DDG using a variety of solvents.

Tocopherols and Tocotrienols in DDG. Tocopherols and tocotrienols each occur in four different forms, known as α, β, γ, and δ, which differ in the number and positions of the methyl side groups on the chromanol ring. Corn kernels contain α- and γ-tocopherols and -tocotrienols, as well as low amounts of δ-tocopherol and δ-tocotrienol (*10*). The most prominent tocopherol is γ-tocopherol, followed by α-tocopherol and γ-tocotrienol. The amount of tocopherols and tocotrienols in extracted oils depends on the extraction solvent as well as the source (whole kernel, bran, or germ) and milling technique. We were able to identify and quantify tocopherols and tocotrienols in DDG extracts using HPLC with fluorescence detection (**Figure 2B**). All of the extracts contained, in order of quantity, γ-tocopherol, γ-tocotrienol, α-tocopherol, α-tocotrienol, and small quantities of δ-tocopherol (**Table 2**). There were also low quantities of δ-tocotrienol found in all of the extracts with the exception of the supercritical CO_2 extracts. Supercritical CO_2 and hexane extracts of DDG had the highest quantity of tocopherols and tocotrienols, at 1.6−1.8 mg/g of oil. These levels are similar to levels reported in alcohol and hexane extracts of ground corn kernels and higher than levels in hexane and ethanol extracts of corn bran and both wet- and dry-milled corn germ (*10*). Ethanol Soxhlet and ASE extracts had 0.73

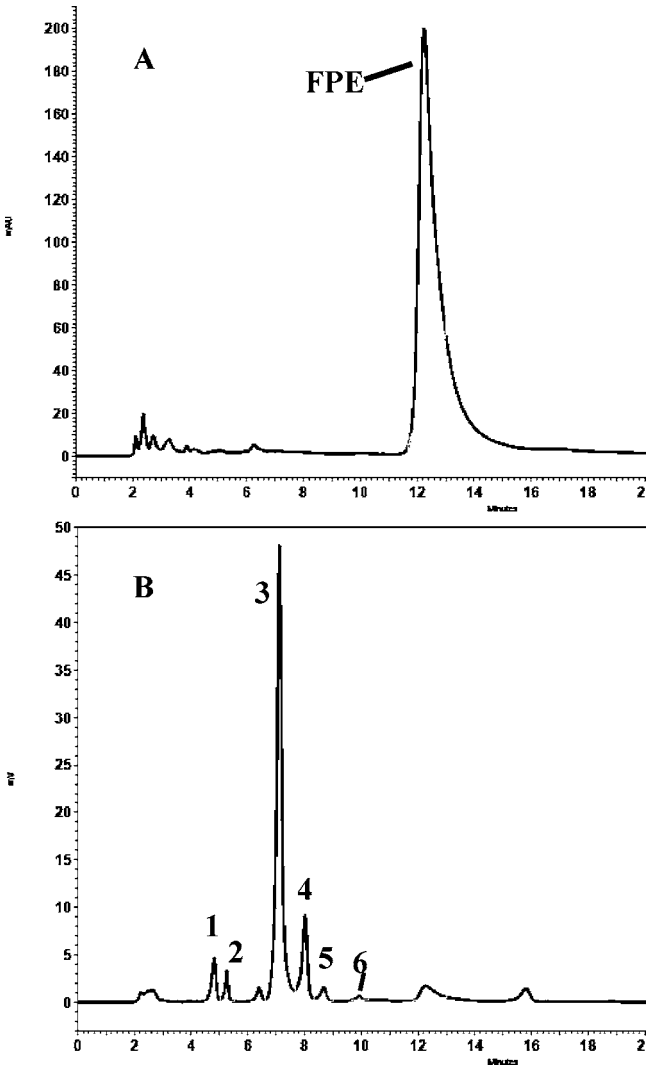

Figure 2. HPLC chromatograms of a hexane Soxhlet extract of DDG: (**A**) UV 325 nm for ferulate phytosterol ester (FPE) detection; (**B**) fluorescence (ex, 292 nm; em, 344 nm) for detection of tocopherols and tocotrienols. Peaks: 1, α-tocopherol; 2, α-tocotrienol; 3, γ-tocopherol; 4, γ-tocotrienol; 5, δ-tocopherol; 6, δ-tocotrienol. See Materials and Methods for chromatography conditions.

and 0.76 mg/g total tocopherols, respectively. However, taking into account the yield of oil, on average, Soxhlet extraction using either hexane or ethanol yielded the most tocopherols and tocotrienols from the DDG (0.23−0.24 mg/g of DDG, respectively, data not shown) followed by supercritical CO_2 (0.21 mg/g of DDG), hexane ASE (0.18 mg/g of DDG), and ethanol ASE (0.13 mg/g of DDG). Most notably, the tocotrienol contents in all extracts of DDG are higher than what is reported for oils extracted from corn germ or commercial corn oil (*10*, *22*). Therefore, tocotrienols, like FPE, appear to be more highly associated with the fiber-rich portions of the corn kernel and appear to be readily extracted from the DDG.

Some of the previous research on coproducts from ethanol dry-milling processes has focused on alternative milling techniques to remove corn fiber before the fermentation process or on separating the fiber from DDG after fermentation to extract the corn fiber oil and its valuable phytosterols, tocopherols, and ferulate phytosterol esters (*4*, *16*). Extracting oil from DDG has several possible advantages over the previous tactics. As this study has demonstrated, the phytosterols, ferulate phytosterol esters, tocopherols, and tocotrienols found in corn fiber oil are retained, yet there is no need for separation of the fiber from the other corn kernel components before extraction. Thus, there is no need to change the milling process or to add additional chemical or equipment costs for ethanol production. In addition, DDG has a higher oil content than corn fiber, which may make the oil extraction more profitable. The oil from DDG has several potential markets: as feedstock for the extraction of phytosterol components for use as nutraceutical ingredients and/or as feedstock for biodiesel production. There is still a need for further characterization of the oil from DDG for its physical characteristics, as well as its nutritional and sensory quality, but with its high phytosterol and tocopherol contents, it may also have a potential market as a nutritional oil for human consumption. In addition, the residual grain following lipid extraction would retain its protein content and would still be useful as an animal feed. Slightly higher yields of phytosterols and FPE were obtained using ethanol as a solvent compared to hexane or supercritical CO_2. However, the ethanol may also extract other non-lipid components (such as protein), which might detract from the quality of the residual as an animal feed. Supercritical CO_2 extracted phytosterols, FPE, and tocopherols almost as efficiently as hexane and may be a suitable "green" alternative to traditional hexane extractions.

ABBREVIATIONS USED

ASE, accelerated solvent extraction; DDG, distiller's dried grain; DDGS, distiller's dried grain with solubles; FPE, ferulate phytosterol esters.

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