Yield and Phytosterol Composition of Oil Extracted from Grain Sorghum and Its Wet-Milled Fractions

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ABSTRACT

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Corn fiber contains an oil with high levels of three potential cholesterol-lowering phytosterol compounds. Little information is available about the levels and types of phytosterols in sorghum. In this study, phytosterols were evaluated in grain sorhgum and its wet-milled fractions and were compared with the phytosterols in corn. The study showed that sorghum kernels can provide a significant source of two phytosterol classes, free phytosterols (St) and fatty acyl phytosterol esters (St:E). Most of these phytosterols are concentrated in the wet-milled fiber fraction followed by the germ fraction. In addition to phytosterols, other lipid classes such as wax esters and an aldehyde (50% C28 and 50% C30) are also present in the sorghum oil. Comparison of sorghum and corn kernels show that corn has 72–93% more phytosterols than sorghum.

Ethanol can be produced from cereal grains using the dry grind process. Presently in the United States, more than 50% of fuel ethanol is produced by the dry grind process using corn as the main feedstock. Several dry grind plants also use sorghum in addition to corn for ethanol production. Dry grind ethanol production suffers from low coproduct value. In a dry grind ethanol process, cereal grain is ground and mixed with water to make a mash. The mash is cooked, liquefied, saccharified, fermented, and distilled to produce ethanol and a feed product called distillers dried grains with solubles (DDGS). Efforts have been made to recover other valuable coproducts such as germ (Singh and Eckhoff 1996) and fiber (Singh et al 1999; Wahjudi et al 2000) from the corn dry grind ethanol process. Recovery of valuable coproducts lowers the net cost and makes ethanol production more profitable.

Recent research on corn fiber shows that valuable corn fiber oil can be recovered (Moreau et al 1998; Hicks and Moreau 2001). This oil contains unique compounds that lower serum LDL-cholesterol levels (Hicks and Moreau 2001). These compounds are called phytosterols (plant sterols). The three main classes of phytosterols in corn fiber oil are 1) ferulate phytosterol esters (FPE); 2) free phytosterols (St); and 3) fatty acyl phytosterol esters (St:E). Phytosterols have recently received much attention because of their valuable nutraceutical properties. Recent health concerns with the use of statin (cholesterol-lowering) drugs and the allowance by the FDA of a health claim for phytosterols and phytostanols has created much consumer interest. The demand for these natural cholesterol-lowering products is growing considerably. The demand for phytosterol products could exceed the supply in near future (Deke Bladgon, ACHumko Corporation, *personal communication*).

There is a need to find new sources of phytosterols. Recovery of phytosterols from grain sorghum is possible. Although phytosterols have been studied in several types of grains, there is little information available about the levels and types of phytosterols in sorghum. Fractionation of cereal grains using the wet-milling process allows recovery of individual components (steepwater, germ, fiber, starch, and protein) in relatively pure form and, therefore, allows analysis of phytosterols from each individual component. The objectives of this study were 1) determine the types and levels of phytosterols in sorghum kernels and wet-milling fractions, and 2) compare the wet-milling fraction yields and total phytosterols in sorghum with those in corn.

MATERIALS AND METHODS

Two sorghum hybrids, hybrid A (Cargill 737) and hybrid B (Cargill 888Y) with different genetics were obtained from a commercial seed company grown during the 2000 crop season. Sorghum samples were hand-cleaned to remove broken kernels and foreign material, packaged in plastic bags, and stored at 4°C until wet-milled. The whole kernel moisture content of the samples was measured using the 103°C convection oven method (Approved Method 44-15A, AACC 2000). The fatty aldehyde standard was provided by the Department of Food Science and Technology, University of Nebraska-Linclon, NE (Hwang et al 2002a,b).

The wet-milling process for sorghum is basically the same as the corn wet-milling process (Watson 1984). The wet-milling of sorghum samples was performed using the 1-kg laboratory corn wet-milling procedure as outlined by Eckhoff et al (1993) with slight modifications. The modifications included reducing the batch size to 500 g and using a Doxie A hydrocyclone (Singh and Eckhoff 1995) instead of starch tables to separate starch and protein fractions. The moisture content of all the fractions was determined using the two-stage convection oven method (Approved Method 44-18, AACC 2000).

All the dried wet-milled fractions except the germ were ground (2–4 g) in a Wiley mill to 20-mesh size. The dried germ (1–2 g) was placed in a 55-mL screw-top vial, 40 mL of hexane was added, and the mixture was homogenized (Brinkman Polytron). The tube was shaken horizontally for 1 hr in a wrist-action shaker at room temperature. After extraction, the hexane extracts were filtered through a glass fiber filter (Whatman GF/A) fitted with a Buchner funnel under vacuum. A part of the sample was removed for HPLC analysis as previously outlined by Moreau et al (1996). Oil and phytosterols were extracted from the other dried wet-milled samples with hexane using an accelerated solvent extractor (Dionex ASE200). Ground samples (1–2 g) were placed in 11-mL sample extraction cells. The extraction conditions in the cells were pressure of 1,000 psi, temperature of 100°C, heat time of 5 min, start time of 10 min, three static cycles, 100% flush volume, and a purge time of 60 sec.

For HPLC analysis, part of the sample was removed from the extracted solvent as outlined by Moreau et al (1996). The lipid classes, triacylglycerols (TAG), free fatty acids (FFA), free phyto-

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sterols (St), and ferulate phytosterols esters (FPE) in the samples were separated and quantified by a modified version of an HPLC technique developed by Moreau et al (1996). A ternary gradient HPLC modular system was used (HP1050, Hewlett Packard). Two detectors were connected in series. The first was a HP1050 fixed wavelength UV-visible detector set at 295 nm. The second was an evaporative light scattering detector (Alltec-Varex Mark III) operated at 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 was the same as used by Moreau et al (1996) and the flow rate was constant at 0.5 mL/min.

Another HPLC with a different column was used to separate wax esters, fatty aldehyde (C28), and fatty acyl phytosterol esters (St:E). The HPLC method employing an alumina column had previously reported to separate steryl and wax esters (Nordbäck and Lundberg 1999). A part of the sample was removed from the extracted solvent for analysis. These HPLC analyses were performed with an HP1100 HPLC with autosampler, column heater, and detection by both an HP1100 diode-array UV-visible detector (Agilent Technologies, Collegeville, PA), and an evaporative light scattering detector (Sedex 55, Richard Scientific, Modesto, CA), operated at 25°C and a nitrogen gas pressure of 2 bars. The column was an Aluspher Al 100, 5 µm column (125 × 4 mm) packed in a LiChroCART cartridge (Merck KgaA, Darmstadt, Germany). The binary gradient had a constant flow rate of 0.6 mL/min with Solvent A (hexane and tetrahydrofuran 1,000:1) and Solvent B (isopropanol). Gradient timetable at 0 min, 100% A/0% B; at 10 min, 100% A/0% B; at 20 min, 95% A/5% B; at 21 min, 100% A/0% B; at 60 min, 100 % A/0% B.

The remainder of the solvent sample was dried under nitrogen and heat using an N-EVAP analytical evaporator (Organomation, Berlin, MA) to determine the total extractable oil.

All wet-milling experiments were done in duplicate. Ground samples from both replicates were analyzed using HPLC at least twice. Results presented are the means of the multiple analyses.

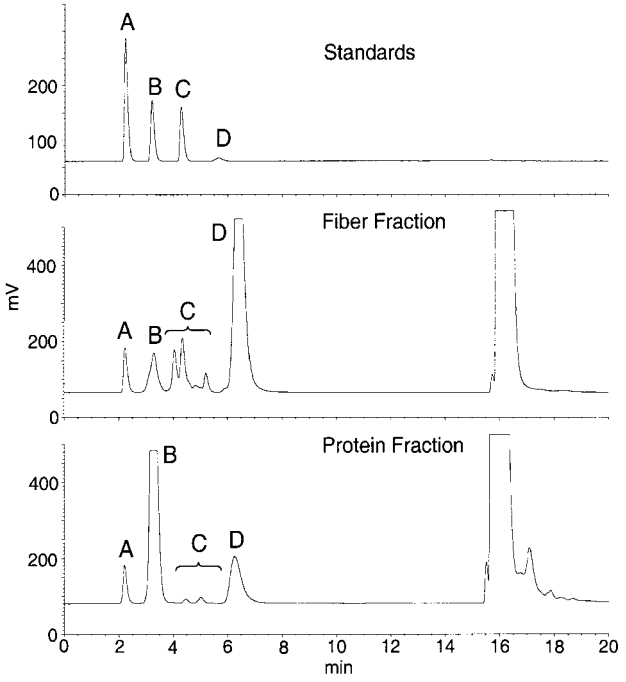

Fig. 1. HPLC chromatogram of very nonpolar lipids in extracts of wet-milled fractions of sorghum kernels. A, hydrocarbons; B, wax esters (stearyl-stearate as standard); C, sterol esters (cholesterol stearate as standard); D, fatty aldehyde (C28 standard).

RESULTS AND DISCUSSION

Initial analysis of oil extracted from sorghum kernels and their wet-milled fractions using the HPLC method (with a DioL column) indicated high concentration of phytosterol fatty acyl esters (St:E), however, previous reports had suggested that the high concentration of St:E may be due to other sorghum lipid classes coeluting. Bianchi et al (1979) reported that sorghum lipids are composed of fatty aldehydes, fatty alcohols, fatty acids, hydrocarbons, wax esters, and steryl esters. Hwang et al (2002a) reported that the sorghum fatty aldehyde is 50% C28 and 50% C30. Injection (in a DioL column) of wax esters (stearyl-stearate as standard), sterol esters (cholesterol stearate as standard), fatty aldehyde (C28 and C30 standard), and hydrocarbons on a DioL column showed very similar retention times. Nordbäck and Lundberg (1999) reported a new method to successfully separate steryl ester and wax esters in animal lipid extracts using an HPLC with an alumina column. Use of an alumina column for analysis of oil from sorghum kernels and its wet-milled fractions resulted in separation of hydrocarbons, wax esters, sterol esters, and fatty aldehyde (Fig. 1). All further analysis of oil from sorghum and its wet-milled fractions for quantitative analysis of hydrocarbons, wax esters, fatty acyl phytosterol esters, and fatty aldehyde were done using the HPLC alumina method. For quantitative analysis of free phytosterols, ferulate phytosterol esters, free fatty acids and triacylglycerols, the HPLC DioL method was used.

Significant amounts of free phytosterols (St) and phytosterol fatty acyl esters (St:E) were observed in sorghum kernels and their wet-milled fractions for both the hybrids (Tables I and II). Both these phytosterol classes were mainly recovered from the fiber, germ, and protein fractions of the sorghum kernel. High concentrations of these two phytosterols were observed in the oil recovered from the fiber and the protein fraction, compared with the other wet-milled fractions. For hybrid A, sorghum fiber had ≈2.5% of oil and ≈1.3% of that oil was St fraction and ≈1.4% was the St:E fraction (Table I). Compared with St and St:E, significantly low amounts of ferulate phytosterol esters (FPE) were also present in the sorghum fiber oil. The sorghum germ fraction (for hybrid A) had the maximum amount of oil (13.6%) compared with the other fractions, however, the concentration of the St (≈0.5%) and St:E (≈0.3%) are low compared with the oil recovered from fiber and protein. The higher concentration of phytosterols in the fiber oil compared with the germ oil was also observed by Moreau et al (1996, 1999) for corn kernels. Analysis of other lipid classes showed that TAG were the most abundant component. TAG constituted >91.0% of the oil recovered from sorghum kernels or their wet-milled fractions. A low concentration of FFA (<0.05%) was observed in all the oils. Significant amounts of wax esters (0.83–2.86%) and fatty aldehydes (2.93–3.17%) were observed in the oil from the fiber and protein fractions. However, negligible or very low (0.08%) concentrations of wax esters and fatty aldehyde were observed in the oil recovered from the germ fraction. A negligible amount of oil was recovered in the starch and steep-water fraction of the grain sorghum.

The trends in results as observed for hybrid A (Table I) were similar to those observed for hybrid B (Table II). Sorghum hybrid B had significantly higher amount of oil in fiber, germ, and protein fractions compared with hybrid A. The amount of oil recovered from the fiber was ≈3.5%, and ≈1.1% of that oil was St and ≈1.08% was St:E. The amount of oil in the germ was ≈18.9% and ≈0.6% of that oil was St and ≈0.27% was St:E. Although the amounts of oil in the germ were significantly lower than in corn germ, wax esters and fatty aldehydes were essentially absent in the germ.

Total Phytosterol Recovery

Previously, we reported the levels of phytosterols in different wet-milled fractions of a yellow dent corn (Moreau et al 1999).

Comparison of the sorghum and corn wet-milling fractions show comparable yields for germ and protein fractions (Table III). For sorhgum kernels, depending on the hybrid, the amount of fiber (≈1.8–3.9%) and steepwater solids (≈2.1–2.4%) were high and the amount of starch was low (≈3.8–4.9%) compared with the corn kernels. Although the germ yields for the sorghum and corn kernels were comparable, the amount of oil in the germ was ≈1.8 to 2.5 times higher in the corn kernels. The germ oil content in sorghum kernels is lower than the oil content in the corn kernels (Watson 1984). The protein fraction yield for the sorghum and corn kernels was also comparable, however, the amount of oil in the sorghum protein fraction was ≈4 to 7 times higher compared with the oil in the corn protein fraction (Table III). This lipid fraction in sorghum protein consists of some phytosterols but mainly wax esters and an aldehyde. Clean separation between starch and protein is a problem during the sorghum wet-milling process because small pieces of pericarp interfere in the separation (Watson 1984) or there is a highly cross-linked protein matrix in sorghum endosperm (Hamaker et al 1992). It is possible that these lipid compounds (wax esters and fatty aldehydes) found mainly in protein fraction and small amounts in the starch fraction could also have an effect on the starch protein separation during sorghum wet-milling process.

Comparison of total phytosterol recovery shows that more phytosterols can be recovered from corn than from sorghum. From the wet-milled fractions most of the phytosterols (for both corn and sorghum) were recovered from the fiber fraction followed by the germ fraction. Depending on the sorghum hybrid, the total recovery of phytosterols from the wet-milled fractions is considerably lower (27–47%) compared with the total amount of phytosterols present in the whole sorghum kernels. The total recovery of phytosterols from wet-milled corn fractions is also lower (≈53%) compared with the amount of phytosterols present in the whole corn kernels. The reasons for lower recovery in the wet-milled fractions compared with the whole kernels are difficult to ascertain. It is likely that there are some losses during the milling of grain into their individual components.

The results from this study indicate that sorghum kernels are a relatively significant source for two phytosterols classes (St and St:E). Removal of fiber in a dry grind ethanol plant using sorghum (in addition to corn) can provide a source for these phytosterol compounds. New value-added dry grind ethanol technologies such as the Quick Fiber (Singh et al 1999; Wahjudi et al 2000) would be applicable to the dry grind ethanol plants that use corn as well as sorghum as their feedstock.

CONCLUSIONS

Sorghum kernels can provide a significant source of two phytosterol classes: St and St:E. Most of these phytosterols were concentrated in the fiber fraction. In addition to phytosterols, other lipid classes such as wax esters and fatty aldehyde are also present in the sorghum oil. The presence of wax esters and fatty aldehydes in sorghum could make recovery of phytosterols difficult. Comparison of sorghum and corn kernels showed that corn has higher

TABLE I
Oil Yield and Its Phytosterol and Other Lipid Classes[a] in Sorghum Kernels: Hybrid A

		Phytosterols and Other Lipid Classes in Oil (%)[b]							
Sample	**Oil %**	**FPE**	**St**	**St:E**	**FFA**	**TAG**	**Hydrocarbons**	**Wax Esters**	**Fatty Aldehyde**
Kernels	3.22 ± 0.08	0.03 ± 0.00	0.96 ± 0.07	0.61 ± 0.05	0.03 ± 0.00	96.64 ± 0.19	0.16 ± 0.01	0.12 ± 0.01	1.47 ± 0.07
Fiber	2.53 ± 0.18	0.15 ± 0.00	1.32 ± 0.10	1.36 ± 0.37	0.04 ± 0.01	92.77 ± 0.83	0.60 ± 0.03	0.83 ± 0.44	2.93 ± 0.81
Germ	13.67 ± 1.39	0.01 ± 0.00	0.52 ± 0.09	0.32 ± 0.03	0.04 ± 0.01	91.39 ± 0.40	0.72 ± 0.20	0.00 ± 0.00	0.08 ± 0.01
Protein	3.53 ± 0.55	0.01 ± 0.00	1.99 ± 0.66	0.83 ± 0.42	0.04 ± 0.00	91.28 ± 0.05	0.68 ± 0.09	2.86 ± 0.35	3.17 ± 0.58
Starch	0.10 ± 0.00	0.00 ± 0.00	2.63 ± 0.52	0.00 ± 0.00	0.03 ± 0.00	99.03 ± 0.14	0.03 ± 0.09	2.01 ± 0.96	3.35 ± 0.77

[a] Average ± standard deviation.
[b] All yields are averages of two values. FPE = ferulate phytosterol esters; St = free phytosterols; St:E = phytosterol fatty acyl esters; FFA = free fatty acids; TAG = triacylglycerols. Steepwater did not contain any of the phytosterols analyzed.

TABLE II
Oil Yield and Its Phytosterol and Other Lipid Classes[a] in Sorghum Kernels: Hybrid B

		Phytosterols and Other Lipid Classes in Oil (%)[b]							
Sample	**Oil %**	**FPE**	**St**	**St:E**	**FFA**	**TAG**	**Hydrocarbons**	**Wax Esters**	**Fatty Aldehyde**
Kernels	3.66 ± 0.30	0.03 ± 0.00	0.59 ± 0.07	0.63 ± 0.08	0.05 ± 0.00	96.92 ± 0.41	0.17 ± 0.03	0.28 ± 0.03	1.34 ± 0.20
Fiber	3.46 ± 0.56	0.08 ± 0.00	1.11 ± 0.29	1.08 ± 0.18	0.05 ± 0.00	93.15 ± 0.15	0.44 ± 0.02	1.55 ± 0.94	2.54 ± 0.63
Germ	18.93 ± 1.67	0.01 ± 0.00	0.56 ± 0.00	0.27 ± 0.02	0.04 ± 0.00	91.47 ± 0.05	0.46 ± 0.17	0.06 ± 0.01	0.13 ± 0.02
Protein	6.17 ± 0.01	0.00 ± 0.00	1.50 ± 0.03	0.62 ± 0.11	0.03 ± 0.01	92.38 ± 0.05	0.44 ± 0.08	3.94 ± 0.41	1.98 ± 0.31
Starch	0.15 ± 0.02	0.00 ± 0.00	1.67 ± 0.33	0.00 ± 0.00	0.03 ± 0.01	98.93 ± 0.05	0.03 ± 0.00	4.63 ± 0.30	0.84±0.11

[a] Average ± standard deviation.
[b] All yields are averages of two values. FPE = ferulate phytosterol esters; St = free phytosterols; St:E = phytosterol fatty acyl esters; FFA = free fatty acids; TAG = triacylglycerols. Steepwater did not contain any of the phytosterols analyzed.

TABLE III
Comparison Between Sorghum (Hybrids A and B) and Corn for Yield,[a] Oil Content, and Total Phytosterols in Different Wet-Milling Fractions

	Hybrid A (%)		Hybrid B (%)		Corn (%)[b]		Total Phytosterols (mg/100 g)[b]		
Sample	**Yield**	**Oil**	**Yield**	**Oil**	**Yield**	**Oil**	**Hybrid A**	**Hybrid B**	**Corn**
Kernels	100.0	3.22 ± 0.08[c]	100.0	3.66±0.30	100	2.82 ± 0.04	51.10	45.68	88.01
Fiber	15.9	2.53 ± 0.18	18.05	3.46 ± 0.56	14.1	2.16 ± 0.06	11.43	14.18	19.3
Germ	4.9	13.67 ± 1.39	4.73	18.93 ± 1.67	4.9	35.56 ± 0.41	8.62	10.08	16.8
Protein	8.91	3.53 ± 0.55	7.69	6.17 ± 0.01	8.34	0.89 ± 0.00	1.74	1.56	4.8
Starch	64.77	0.10 ± 0.00	63.62	0.15 ± 0.02	68.55	0.02 ± 0.01	5.74	7.52	0.6
Steepwater	5.47	0.00 ± 0.00	5.76	0.00 ± 0.00	3.33	0.06 ± 0.08	0.00	0.00	0.00

[a] All yields are averages of two values.
[b] Data previously reported by Moreau et al (1999).
[c] Average ± standard deviation.

concentration of phytosterol compounds (St and St:E) and an additional class of phytosterol compounds, ferulate phytosterol esters (FPE) can also be recovered from corn kernels.

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LITERATURE CITED

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Methods 44-15A and 44-18. The Association: St. Paul, MN.

Bianchi, G., Avato, P., and Mariani, G. 1979. Communication to the editor: Composition of surface wax from sorghum grain. Cereal Chem. 56:491-492.

Eckhoff, S. R., Rausch, K. D., Fox, E. J., Tso, C. C., Wu, X., Pan, Z., and Buriak, P. 1993. A laboratory wet-milling procedure to increase reproducibility and accuracy of product yields. Cereal Chem. 70:723-727.

Hamaker, B. R., Mertz, E. T., and Axtell, J. D. 1992. Effect of extrusion on sorghum kafirin solubility. Cereal Chem. 71:515-517.

Hwang, K. T., Cuppett, S. L., Weller, C. L., and Hanna, M. A. 2002a. Properties, composition, and analysis of grain sorghum wax. J. Am. Oil Chem. Soc. 79:521-527.

Hwang, K. T., Cuppett, S. L., Weller, C. L., Hanna, M. A., and Shoemaker, R. K. 2002b. Aldehyde in grain sorghum wax. J. Am. Oil Chem. Soc. 79:529-533.

Hicks, K. B., and Moreau, R. A. 2001. Phytosterols and phytostanols: Functional food cholesterol busters. Food Technol. 55:63-67.

Moreau, R. A., Hicks, K. B., Nicolosi, R. J., and Norton, R. A. 1998. Corn fiber oil its preparation and use. U.S. patent 5,843,499.

Moreau, R. A., Powell, M. J., and Hicks, K. B. 1996. Extraction and quantitative analysis of oil from commercial corn. J. Agric. Food Chem. 44:2149-2154.

Nordbäck, J., and Lundberg, E. 1999. High resolution separation of non-polar lipid classes by HPLC-ELSD using alumina as stationary phase. J. High Resol. Chromatogr. 22:483-486.

Singh, V., and Eckhoff, S. R. 1996. Effect of soak time, soak temperature, and lactic acid on germ recovery parameters. Cereal Chem. 73:716-720.

Singh, V., Moreau, R. A., Doner, L. W., Eckhoff, S. R., and Hicks, K. B. 1999. Recovery of fiber in the corn dry-grind ethanol process: A feedstock for valuable coproducts. Cereal Chem. 76:868-872.

Wahjudi, J., Xu, L., Wang, P., Singh, V., Buriak, P., Rausch, K. D., McAloon, A. J., Tumbleson, M. E., and Eckhoff, S. R. 2000. Quick fiber process: Effect of mash temperature, dry solids and residual germ on fiber yield and purity. Cereal Chem. 77:640-644.

Watson, S. A. 1984. Corn and sorghum starches: Production. Pages 417-468 in: Starch: Chemistry and Technology. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: Orlando, FL.

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