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ABSTRACT

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Grain sorghum can be a major source of policosanols, long-chained alcohols, that have beneficial physiological activities. Sorghum dried distillers grains (DDG), a by-product of ethanol production from grain sorghum, contain a large amount of policosanols. Content and composition of policosanols in long-chained lipids extracted from grain sorghum kernels and DDG were determined. Long-chained lipids were extracted using hot hexane or hot ethanol. The major components of the long-chained lipids extracted from grain sorghum kernels, as determined using HPLC, were policosanols (37–44%), aldehydes (44–55%), and acids (4–5%). Long-chained lipids from DDG contained 52% policosanols, 23% aldehydes, 6.4% acids, and 17% wax esters/steryl esters. Composition of policosanols in DDG matched the composition in grain sorghum kernels, as determined by gas chromatography, even though the content of policosanols in DDG was greater than the content in grain sorghum kernels. Policosonal composition ranges were 0–1% C22:0, 0–3% C24:0, 6–8% C26:0, 1% C27:0, 43–47% C28:0, 1–2% C29:0, 40–43% C30:0, and 1–4% C32:0.

A considerable amount of wax-like material (≈0.2%, w/w, db) can be extracted from whole kernels of grain sorghum (Weller et al 2000). The chemical composition of this material has been reported sporadically and without consensus over the last half century (Bunger and Kummerow 1951; Cannon and Kummerow 1957; Dalton and Mitchell 1959; Bianchi et al 1979; Avato et al 1990; Seitz 1997). Recently, Hwang et al (2002a) confirmed that the major components of the wax-like material were long-chained aldehydes, alcohols, and acids. Bianchi et al (1979) and Avato et al (1990) previously reported that these components were mainly saturated 28- and 30-carbon compounds. Only a small amount of wax esters, a traditional wax component, was contained in the wax-like material. Therefore, long-chained lipids may describe the material more appropriately.

Annual production of grain sorghum in the United States is 10–20 million metric tons. About 12% of the grain sorghum produced in the United States is used for ethanol production (Stan Fury, National Grain Sorghum Producers Director, *personal communication*). One of the by-products from the ethanol production is dried distillers grains (DDG). Sorghum DDG, in which little, if any, starch remains after ethanol production, still contains lipid and lipid-related materials. It is assumed long-chained lipids are a part of the lipid materials; however, their presence has never been reported.

Policosanols are a mixture of primary long-chained alcohols and are commercially available as nutritional supplements containing mostly octacosanol (28:0), triacontanol (30:0), hexacosanol (26:0), and dotriacontanol (32:0). They have been produced from sugar cane, beeswax, and cereal germs. The alcohols in the long-chained lipids extracted from grain sorghum discussed above are also policosanols. Policosanols reportedly have physiological activities such as improving lipid levels in the blood (Aruzazabala et al 1994; Kato et al 1995; Gouni-Berthold and Berthold 2002), reducing platelet aggregation (Aruzazabala et al 1996), improving exercise performance of coronary heart disease patients (Stüsser et al 1998), and increasing muscle endurance (Kabir and Kimura 1995).

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Stewart and Downing (1981) pioneered an HPLC method for the separation of wax esters and steryl esters in human skin surface wax. Nordbäck and Lundberg (1999) also successfully separated steryl esters from wax esters in wax from a zooplankton sample using an HPLC system. To our knowledge, the entire composition of a wax using HPLC had not been reported before Hwang et al (2002b) developed an HPLC method to analyze the composition of the major components in carnauba and sorghum waxes. Components of the long-chained lipids extracted from grain sorghum were analyzed by this HPLC method and ≈40% of the long-chained lipids were policosanols (Hwang et al 2002b).

The objectives of this study were to determine and compare yield and composition of long-chained lipids extracted from grain sorghum kernels and DDG, and to determine the content and composition of policosanols in the long-chained lipids.

MATERIALS AND METHODS

Grain Sorghum Kernels and DDG

Grain sorghum kernels were Golden Harvest H512 (harvested in 1999), Warner W902W FG (harvested in 1999 and 2000), Producers (harvested in 1999), and PH77FG (harvested in 2000). All were grown in Nebraska. Sorghum DDG from ethanol production using mixed commercial grain sorghum hybrids was obtained from a production run at U.S. Energy Partners (Russell, KS) in April 2002.

Extraction of Long-Chained Lipids

Extraction of long-chained lipids from whole kernels of grain sorghum followed the method of Hwang et al (2002a). Grain sorghum kernels were washed with tap water and dried at 45°C over 24 hr. The grain sorghum (800 g at ≈10%, wb, moisture) with 800 mL of hexane or ethanol was refluxed for 30 min. The mixture was filtered through a coffee filter paper lying on top of a Whatman No. 2 filter paper (Whatman, Maidstone, Kent, UK). The filtrate was placed in a freezer at −18°C for at least 8 hr. Filtering the cold miscella onto Whatman No. 42 filter paper, which was then desolvented under vacuum, collected the precipitate of the long-chained lipids.

Extraction of long-chained lipids from DDG was done in the same way as for grain sorghum kernels except that the DDG sample was not washed before extraction and that 400 g of DDG (at ≈10%, wb, moisture) and 600 mL of hexane were used for the extraction. For half of the DDG sample, the precipitate of long-chained lipids, collected on Whatman No. 42 filter paper, received an additional rinse with ≈30 mL of hexane at room temperature to remove any compounds soluble at room temperature. Yield of

long-chained lipids % db) was then calculated as $W/S/(100 - M) \times 100$, where W is total desolvented long-chained lipid mass (g) collected on filter papers, and S is sorghum or DDG mass used for extraction (g); M is moisture content of sorghum or DDG (% wb).

Policosanol Content in Long-Chained Lipids

Policosanol content along with contents of major components in the long-chained lipids extracted from grain sorghum kernels and DDG were determined using HPLC as in Hwang et al (2002b). Two 510 HPLC pumps (Waters Corp., Milford, MA) were operated in gradient modes as in Table I (gradients I and II). Flow rate of mobile phase was 1 mL/min. Column was a Luna 5-μm silica column (250 mm L × 4.6 mm i.d.) (Phenomenex, Torrance, CA) connected to a guard column (4 mm L × 3 mm i.d. silica cartridge in a SecurityGuard cartridge system (Phenomenex). The column and guard column were heated at 40°C using a Waters column heater module. Exposed lines from injection loop to detector connection were maintained at ≈38–40°C using a wrapped heating tape. Detector was a Varex ELSD II (Rockville, MD) operated at 50°C with nitrogen pressure of 930 kPa. Samples were dissolved in hexane at 20 μg/100 μL and 100 μL of each was injected. By injecting standards (aldehyde fraction for aldehydes; lignoceryl alcohol for alcohols; lignoceric acid for acids; *n*-octacosane for hydrocarbons; trilinolenin for triacylglycerols; and behenyl behenate for wax esters) with different concentrations covering the levels of sample components for each HPLC system, relationships between concentration and area were plotted for calibration, which was then used for calculation of the component levels in the long-chained lipids.

Policosanol Composition in Long-Chained Lipids

Policosanol fractions (2 mL) from 20 μg of long-chained lipids, which were dissolved in 100 μL of hexane and injected into HPLC operated with gradient II system, were collected by disconnecting the tubing to the detector at the appropriate time and eluting directly from the HPLC column into a vial. The solvent was evaporated from the fraction in the vial under a nitrogen stream using a temperature <40°C. Compositions of policosanols were determined using gas chromatography (GC) with a minor modification of the González-Canavaciolo and Magraner-Hernández (1999) method. To the desolvented policosanol fraction, 0.2 mL of chloroform and 0.05 mL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (Sigma Chemical, St. Louis, MO) were added. The contents were heated at 60°C for 10 min to make trimethylsilyl (TMS) ether derivatives. A standard mixture of palmityl alcohol, stearyl alcohol, eicosanol, behenyl alcohol, tricosanol, lignoceryl alcohol, hexacosanol, heptacosanol, octacosanol, and triacontanol (purchased from Nu-Chek Prep, Inc., Elysian, MN) containing 1–8 μg of each in 0.2 mL of chloroform was prepared and derivatized in the same manner as above for identifying the retention times of the alcohols and for calculating their response factors. The TMS ether derivative solution (2 μL) was injected into a gas chromatograph (HP 6890, Hewlett-Packard, Wilmington, DE) equipped with a DB-5 column (30 m, 0.25 mm, i.d. and 0.25 μm, film thickness) (J&W Scientific, Folsom, CA) and using helium as the carrier gas. The injector was initially in a pulsed splitless mode, which shifted to a split mode (ratio 100:1) after 1.5 min; the detector was a flame-ionization detector. Injector and detector temperatures were both set at 315°C. Oven temperature was programmed to start and hold at 150°C for 1 min before increasing to 210°C at 20°C/min, increasing to 310°C at 4°C/min, holding at 310°C for 1 min, increasing to 315°C at 25°C/min, and finally holding for 5 min.

RESULTS AND DISCUSSION

Yields of long-chained lipids from the grain sorghum kernels were 0.2–0.3% (w/w, db) (Table II). This range of yields is consistent with the 0.16–0.31% range of yields observed by Weller et al (2000) for 86 commercial hybrids grown in Nebraska over five years. The yields from Golden Harvest H512 showed no difference between hexane and ethanol extractions, although appearances of the hexane- and ethanol-extracted long-chained lipids were apparently different. Differences between hexane- and ethanol-extracted long-chained lipids were discussed in detail in previous reports (Weller et al 2000; Hwang et al 2002a). PH77FG and Warner W902W FG, harvested in 2000, had greater yields of the long-chained lipids (0.30 and 0.28%, respectively) than the other sorghum kernels evaluated. Lipid yields from DDG, using the same method as that for grain sorghum kernels, were ≈0.6%. Yields for lipids recovered from DDG, including rinsing of the cold-filtered precipitate with hexane at room temperature were ≈0.5%.

The high yield of long-chained lipids from DDG implies that a large amount of long-chained lipids remains intact during the ethanol production process, and indicated that DDG may be a good source for production of long-chained lipids and that the lipids are concentrated in the process of ethanol production.

Conversion of starch to ethanol and carbon dioxide during ethanol production theoretically concentrates nonstarch components three times. For example, for every 3 kg of lipid and 9 kg of protein in 100 kg of whole kernels entering an ethanol production process, ≈9 kg of lipid and 27 kg of protein would be expected in every 100 kg of DDG.

The extent of concentration may not have reached three times due to minor losses during the various processes used in ethanol production or due to differences in the initial contents of long-chained lipids in the known commercial hybrids of this study and the unspecified commercial hybrids used in Russell, KS. Further study tracking levels of grain sorghum kernel components, specifically lipid components, during ethanol production processes is warranted.

TABLE I
HPLC Gradient Mobile Systems for Analysis of Grain Sorghum Wax[a,b]

Gradient I			Gradient II		
	Solvent			Solvent	
Time (min)	A (%)	B (%)	Time (min)	C (%)	B (%)
0	100	0	0	100	0
7	100	0	2	100	0
9	95	5	3	95	5
14	95	5	10	95	5
16	75	25	14	55	45
20	75	25	15	45	55
24	40	60	23	0	100
28	40	60	26	0	100
29	100	0	27	100	0
85	100	0	40	100	0

[a] Solvents: A, 0.2% (v/v) acetic acid and 0.02% methyl *tert*-butyl ether (MTBE) in hexane; B, 0.2% (v/v) acetic acid in MTBE; C, hexane.
[b] Flow rate was 1 mL/min. Ratio changes of the two solvents were linear.

Heating the whole kernels in hexane extracted the long-chained lipids, which were mainly on the surface of the kernels. However, other lipid materials in DDG were likely extracted into the hexane along with long-chained lipids because inner parts of kernel were exposed to the solvent. Accordingly, some of the lipids in DDG precipitated in the hexane solution at a freezing temperature and were retained on the filter paper with the long-chained lipids. These lipids were rinsed off readily with hexane at room temperature.

The major components of the long-chained lipids extracted from grain sorghum kernels were policosanols (≈40%), aldehydes (≈50%), and acids (≈4%) (Fig. 1, Table II). That is, policosanols comprised ≈0.08% (w/w, db) of grain sorghum kernels. Hydrocarbons and triacylglycerols were ≤1% in the long-chained lipids from grain sorghum kernels.

The Bianchi group studied plant waxes extensively and prepared two reports regarding the composition of wax from grain sorghum (Bianchi et al 1979; Avato et al 1990). The first reported that surface wax of dried grain sorghum contained 34% alcohols, 32% aldehydes, 24% acids, 4% esters (contaminated with unidentified materials), and 1% alkanes. The second reported that wax contained 32% alcohols, 21% aldehydes, 27% acids, 13% esters, and 7% hydrocarbons. To our knowledge, only those manuscripts and the two by Hwang et al (2002a,b) reported that sorghum wax contained a large amount of aldehydes. Furthermore, both studies by the Bianchi group and this study found the major components of long-chained lipids extracted from grain sorghum were alcohols, aldehydes, and acids. The differences in levels of long-chained lipid components in the studies of the Bianchi group from this study may have resulted from the Bianchi group using different sorghum types (*Sorghum bicolor* SD-102 and *Sorghum bicolor* L. cv. Martin B grown in Italy), a different wax extraction method (using benzene as extraction solvent), and a different analytical method (separating the wax components using column chromatography, evaporating the solvent, and weighing the dried materials).

Policosanol levels in the long-chained lipids extracted from sorghum DDG were more than twice as great as aldehyde levels (Fig. 1, Table II). Comparing similar levels in grain sorghum kernels, aldehydes were more plentiful than policosanols. The long-chained lipids extracted from sorghum DDG contained a large amount of triacylglycerols (13%) and wax esters and steryl esters (14%) compared with grain sorghum kernels (0.3–0.4% and 0%, respectively). Triacylglycerols and wax esters and steryl esters likely reside in the interior of whole kernels and are released during ethanol production processes. Therefore, the glycerols and esters can be extracted more easily from DDG than from intact kernels.

The yield difference between rinsed and nonrinsed DDG lipid samples discussed above was mainly due to the presence or lack of triacylglycerols, which decreased from 13% in the nonrinsed sample to 0.7% in the rinsed sample. It also implies that the triacylglycerols extracted from DDG were precipitated in hexane at a freezing temperature and dissolved in hexane at room temperature. Hwang et al (2002b) were not able to distinguish between wax esters and steryl esters using HPLC.

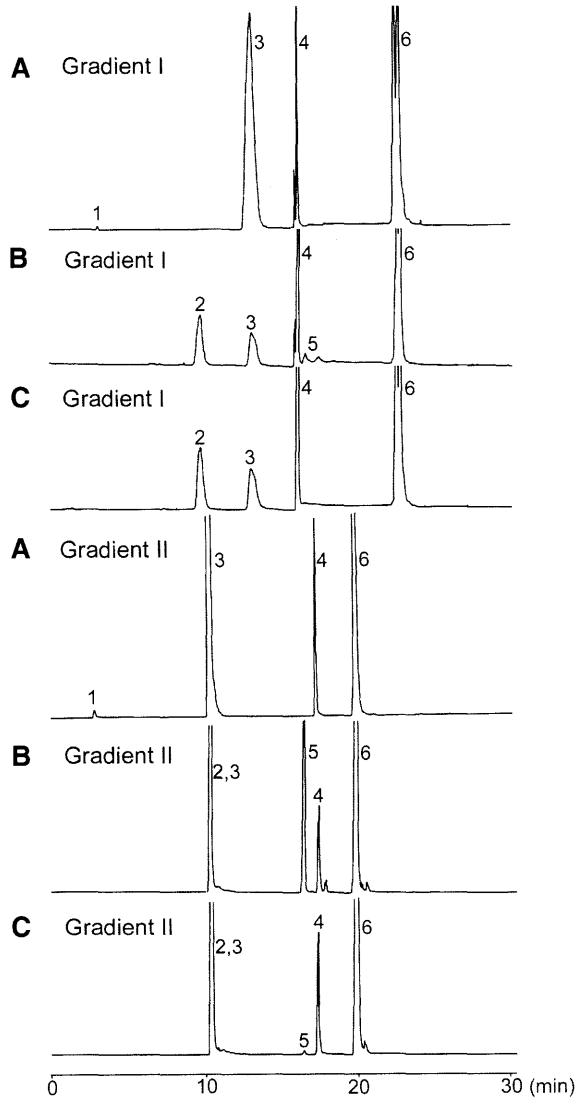

Fig. 1. HPLC of long-chained lipids extracted from grain sorghum Golden Harvest H512 1999 (**A**); dried distillers grains (DDG), filtered precipitate not rinsed (**B**); and DDG, filtered precipitate rinsed (**C**). (1) Hydrocarbons; (2) wax esters and steryl esters; (3) aldehydes; (4) acids; (5) triacylglycerols; and (6) policosanols.

TABLE II
Long-Chained Lipids Extracted from Grain Sorghum Kernels and Sorghum Dried Distillers Grains (DDG)[a,b]

	Yield	Composition of Long-Chained Lipids[c] (%, w/w)					
	(%, w/w, db)	Policosanols	Aldehydes	Acids	HC	TG	WE & SE
Sorghum kernels							
Golden Harvest H512 (1999)	0.23 (0.03)	43.9 (0.6)	48.4 (0.9)	4.8 (0.2)	0.6 (0.1)	0.4 (0.0)	···
Ethanol-extracted	0.24 (0.02)	37.1 (1.2)	55.0 (1.5)	4.4 (0.6)	1.1 (0.1)	0.3 (0.1)	···
Warner W902W FG (1999)	0.20 (0.00)	38.2 (1.0)	54.6 (1.3)	4.1 (0.4)	0.6 (0.1)	0.4 (0.0)	···
Producers (1999)	0.20 (0.01)	39.7 (5.0)	53.5 (5.5)	3.9 (0.5)	0.5 (0.0)	0.3 (0.0)	···
PH77FG (2000)	0.30 (0.00)	40.0 (0.6)	53.3 (0.9)	3.9 (0.2)	0.5 (0.1)	0.3 (0.0)	···
Warner W902W FG (2000)	0.28 (0.00)	39.6 (2.5)	53.1 (2.8)	4.4 (0.2)	0.6 (0.0)	0.3 (0.0)	···
DDG							
Filtered precipitate not rinsed	0.59 (0.04)	46.3 (0.8)	18.4 (0.5)	6.3 (0.3)	···	12.6 (0.8)	14.4 (0.9)
Filtered precipitate rinsed with hexane	0.48 (0.06)	52.2 (0.9)	22.8 (0.5)	6.4 (0.1)	···	0.7 (0.2)	17.4 (1.0)

[a] Extraction solvent was hexane, unless otherwise specified.
[b] Mean values with standard deviations (in parentheses) of three replicates.
[c] HC, hydrocarbons; TG, triacylglycerols; WE & SE, wax esters and steryl esters.

TABLE III
Policosanols in Grain Sorghum Kernels and Sorghum Dried Distillers Grains (DDG) (%, w/w)[a]

	Alcohols								
	22:0	24:0	25:0	26:0	27:0	28:0	29:0	30:0	32:0
Sorghum kernels									
Golden Harvest H512 (1999)	0.4 (0.1)	3.1 (0.2)	0.1 (0.1)	8.2 (0.3)	0.8 (0.1)	43.7 (0.4)	1.3 (0.0)	40.9 (0.7)	1.5 (0.1)
Ethanol-extracted	···	0.4 (0.1)	···	6.1 (0.5)	0.8 (0.0)	47.2 (2.1)	1.4 (0.0)	42.8 (2.3)	1.4 (0.3)
Warner W902W FG (1999)	0.5 (0.1)	2.9 (0.2)	···	7.3 (0.2)	0.8 (0.0)	43.0 (0.4)	1.5 (0.0)	40.7 (0.6)	3.5 (0.2)
DDG									
Filtered precipitate not rinsed	···	1.0 (0.3)	···	5.2 (0.4)	1.0 (0.2)	45.7 (0.8)	0.7 (0.1)	44.8 (0.3)	1.7 (0.5)
Rinsed with hexane	0.3 (0.1)	2.3 (0.3)	···	7.5 (0.6)	1.0 (0.1)	44.6 (1.2)	1.8 (0.0)	40.3 (1.8)	2.2 (0.4)

[a] Mean values with standard deviations (in parentheses) of three replicates.

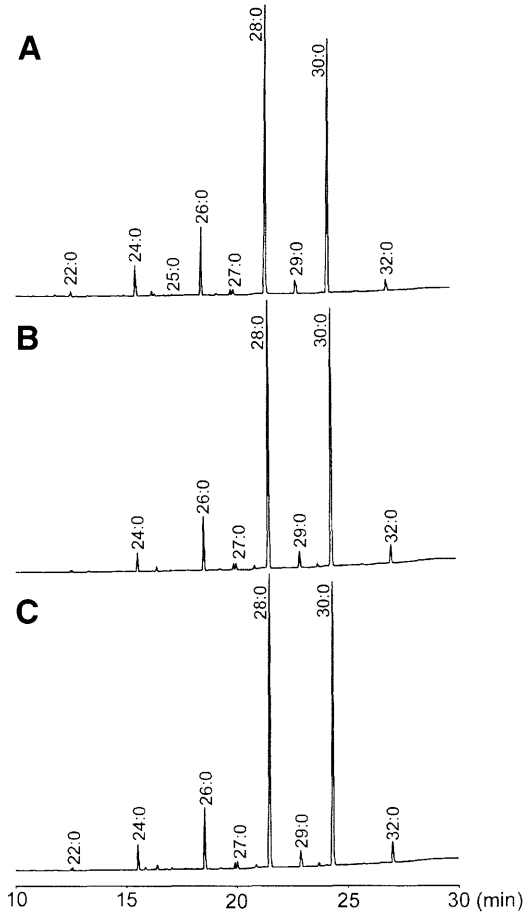

Fig. 2. Gas chromatography of policosanols in grain sorghum (Golden Harvest H512 1999) (**A**); DDG-filtered precipitate not rinsed (**B**); and DDG-filtered precipitate rinsed (**C**).

Pink coloration (steryl esters, if not stanyl esters, turn pink on TLC plates during charring when 10% cupric sulfate in 8% phosphoric acid is used as a visualization solution [Bitman and Wood 1982]) was barely noticeable on TLC bands corresponding to wax esters and steryl esters of the long-chained lipids extracted from sorghum DDG. Therefore, existence of a detectable amount of steryl esters may have been remotely possible, but the presence of stanyl esters could not be excluded.

Further analyses are needed to identify and quantify wax esters and steryl esters in long-chained lipids extracted from sorghum DDG.

Octacosanol (28:0) and triacontanol (30:0) comprised >80% of policosanols in grain sorghum kernels and DDG (Fig. 2, Table III). Hexacosanol (26:0), dotriacontanol (32:0), lignoceryl alcohol (24:0), and nonacosanol (29:0) followed in descending order of concentration. These results were consistent with the reports by Bianchi et al (1979) and Avato et al (1990).

Rice germ, wheat germ, beeswax, and sugar cane are major sources of commercial policosanols. Triacontanol, octacosanol, hexacosanol, and lignoceryl alcohol are major components in commercially available policosanols. Kawanishi et al (1991) reported the compositions of policosanols in oils and waxes of various nuts, seeds, fruits, and cereals. They reported policosanols extracted from rice germ were composed of 51% triacontanol, 35% octacosanol, and 14% hexacosanol. Policosanols from wheat germ contained 62% triacontanol, 21% octacosanol, and 18% hexacosanol.

Comparing the contents of octacosanol in rice germ (5.2 ppm) and wheat germ (2.6 ppm) reported by Kawanishi et al (1991), grain sorghum kernels (≈800 ppm of policosanols) and DDG (≈2,500 ppm) are sources of abundant policosanols. Commercial policosanols are produced mostly through saponification of wax esters in cereal germ waxes. No saponification process is needed for policosanols recovered from grain sorghum kernels and DDG due to the presence of free forms of the policosanols. Simple comparison of commercial policosanols to the free policosanol contents of grain sorghum kernels and DDG may not be proper. Additionally, no cereal grain is comparable with grain sorghum in terms of wax (or long-chained lipid) yield.

CONCLUSIONS

Two major benefits of utilizing policosanols present in grain sorghum as a commercial source of policosanols can be envisioned. One benefit relates to the abundance of policosanols in grain sorghum. The other benefit comes from not needing a saponification process, which is a method for producing policosanols from wax esters. Moreover, obtaining policosanols from sorghum DDG adds value to an underutilized by-product. Lipids in DDG are readily extractable due to greater exposure of the inner part of the grain sorghum kernels to solvent because of grinding, fermentation, and distillation processes. High yield of long-chained lipids and high level of policosanols in sorghum DDG compared with grain sorghum kernels and the similarity of policosanol composition between DDG and grain sorghum kernels can be additional benefits of the using of sorghum DDG for policosanol production.

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