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ORIGINAL PAPER

Changes in Lipid Composition During Dry Grind Ethanol Processing of Corn

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Abstract During the dry grind ethanol process, ground corn is fermented and the major co-product is a feed called distillers dried grains with solubles (DDGS). This study investigated the changes that occur in the composition of corn oil that can be extracted from various process fractions during the dry grind ethanol process. In the first part of this study, samples of distillers dried grains, thin stillage, condensed distillers solubles (also known as syrup), and DDGS were obtained from 7 dry grind ethanol plants. The levels of deleterious free fatty acids were high (>7%) and those of valuable total phytosterols were also high in all fractions (>2%). In the second part of this study, changes in the content and composition of the fatty acids, phytosterols, tocopherols and tocotrienols were quantitatively analyzed in crude oil samples extracted from nine dry grind process fractions from three commercial ethanol plants. Fatty acid and phytosterol composition remained nearly constant in all nine fractions, although some significant variations in phytosterol composition existed among the fractions. Examination of the tocopherols and tocotrienols revealed that γ-tocopherol was the most abundant tocol in ground corn but an unknown tocol became the predominant tocol after fermentation and persisted in the remaining processing fractions and in the final DDGS product. Overall, the remaining majority of tocols remained relatively unchanged.

Keywords DDGS · Lipids · Sterols · Tocopherol · Tocotrienol · Fatty acid · Dry grind · Changes

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Introduction

The dry grind ethanol process has been optimized to produce about 2.8 gallons (10.6 L) of ethanol from each bushel (56 lbs, 25.4 kg) of ground corn. Since only the starch portion of the kernel (65–70%) is fermented, the remaining 25–30% of the kernel has traditionally been dried and sold as distillers dried grains with solubles (DDGS), a high protein (25–30%), and high fiber (6–8% crude fiber) ingredient for animal diets. Because of the relative low value of DDGS and the potential of its overproduction leading to lower prices, there is a need to identify higher value co-products for the dry grind ethanol process. One such higher value co-product is corn oil (recovered from corn germ), which has a long history as a co-product of the corn wet milling industry [1]. In recent publications, we have reported the composition of phytosterols and tocopherols in DDGS [2] and the composition of tocopherols and tocotrienols in corn oil obtained post-fermentation from a dry grind ethanol plant via a centrifugation process (sometimes called oil extraction from the

"back end" of a dry grind ethanol plant) [3]. Recently, Han and Liu [4] examined in detail the changes in gross composition and the amino acid profile in ground corn, DDGS, and several intermediate fractions from a dry grind ethanol process. Through a multiple linear regression model of amino acid composition in relative percentages, they estimated that yeast contributes about 20% toward DDGS proteins, and the rest comes from corn.

The objectives of the current study are: (1) to quantitatively analyze and compare the major valuable lipid components in ground corn (the feedstock for a dry grind ethanol plant), in DDGS, the final product after removal of ethanol via distillation, and, (2) to determine changes in lipids during the dry grind ethanol process by analyzing raw corn, DDGS and seven intermediate products that are produced in typical dry grind ethanol plants. Information gained in this study will help us to understand better the effect of dry grind process steps on changes in the lipid profile.

Materials and Methods

Materials

For Experiment I, samples of distillers dried grains (DDG), thin stillage, condensed distillers solubles (CDS, also known as syrup), and DDGS were obtained from seven dry grind ethanol plants (numbered 1–7) located in the Midwestern region of the USA from October 2006 to April 2007. The starting material, ground corn, was obtained from plant #1 and was used as a control, and its composition is reported in the top of Table 1.

For Experiment II, samples of ground corn, intermediate products, and DDGS were obtained from three more dry grind ethanol plants (numbered 8–10) located in the state of Iowa, USA. The samples from the processing streams (intermediate products) included cooked slurry, liquefied mash, fermented mash, whole stillage, thin stillage, distillers solubles, and distillers grains (DG) (Fig. 1). All samples were frozen after collection for transportation and storage, and freeze-dried in our laboratory just before chemical analysis.

Extractions and Chemical Analyses for Experiment I

Samples of DDG, DDGS, and corn kernels were milled to a particle size of ~1 mm (20 mesh) in a Wiley mill and 4 g samples were extracted with hexane in a Dionex ASE 100 Accelerated Solvent Extractor (ASE) at 100 °C and 1,000 psi, as previously described [5]. Samples of thin stillage and distillers solubles were extracted using a modification of a previous method [6] that involved weighing the samples (10 g of each) into 50-ml screw-top tubes. Isopropanol (10 ml) was added and the tubes were shaken for 10 min in a wrist-action shaker (Burrell). Hexane (20 ml) was added and the tubes were inverted 30 times. Water (10 ml) was added, the tubes were shaken for 10 min, and centrifuged (70 g) for 10 min. The top phase was removed, solvent was evaporated in a stream of nitrogen and lipids were weighed and re-dissolved in hexane with 0.01% BHT. Free fatty acids (palmitic, oleic, and linoleic acids), sterol fatty acyl esters and free sterols were measured by a normal phase HPLC method with evaporative light scattering detection, as previously described [5].

Extractions and Chemical Analyses for Experiment II

For analysis of crude fat, the samples were first dried and moisture was measured, then the dried samples were analyzed for crude fat. The two attributes were measured in duplicate. Moisture content was determined according to an official AOAC method [7]. The moisture content was used to convert oil contents into a dry matter basis. The oil content was determined by an AOCS Official Procedure [8], using a fat analyzer (Model XT 10, Ankom Technology, Macedon, NY, USA). However, instead of using petroleum ether, hexane was used as the extracting solvent.

The fatty acid composition was measured using a previously described method [9] which involved preparing fatty acid methyl esters by direct transmethylation and analyzing them with a gas chromatography (GC) instrument (Agilent 6890 N, Agilent Technologies, Santa Clara, CA, USA). Detailed running conditions for the GC were previously described [10]. The percentage of an individual fatty acid relative to the total fatty acids was expressed as area percentage of the total peak area in each sample. Duplicate analyses were performed separately on each sample.

For analysis of phytosterols, tocopherols and tocotrienols, the first step is extraction of oil, based on the following procedure. The lyophilized samples were homogenized in a coffee mill grinder to a particle size of about 1 mm or less. For extraction with a Dionex ASE, duplicate 1–5 g samples were mixed with Ottawa sand and placed in 11-ml cells as previously described [1, 5]. The cells were topped off with additional Ottawa sand (and bottomed off prior to filling (cellulose filter also at bottom). The parameters of the ASE, were: Pressure 1,000 psi, Temperature 100 °C, Preheat time = 0, Heat time = 5 min, Static time = 10 min, Static cycles = 3, Flush volume = 100% (11-ml cell), Purge time = 60 s, with hexane. The hexane extracts were evaporated, BHT was added to stock to create approximately 0.01% BHT in each sample. Following the removal of 0.5 ml samplings for HPLC, the remainder of the hexane extract sample was dried under N_2 and heat to obtain dry weights. The amount lost in the 0.5 ml sampling was accounted for in the dry weights by calculating the amount

Table 1 Summary of results from Experiment I (DDG, thin stillage, CDS and DDGS) samples from seven dry grind ethanol plants

Ethanol Plant	Co-product	Crude oil (% wet sample)	Total free fatty acids (% of extracted oil)	Total sterols[a] (% extracted oil)
Control	Corn Kernels	3.43 ± 0.10	2.28 ± 0.02	1.79 ± 0.14
1	DDGS	10.04 ± 0.08	12.18 ± 0.41	2.11 ± 0.12
2	DDG	9.06 ± 0.01	6.81 ± 0.51	2.21 ± 0.26
	DDGS	11.33 ± 0.04	7.96 ± 0.44	1.69 ± 0.15
3	DDG	11.90 ± 0.04	7.65 ± 0.27	1.91 ± 0.09
	CDS	6.44 ± 0.03	10.10 ± 0.15	2.20 ± 0.07
	DDGS	9.50 ± 0.09	8.06 ± 0.48	1.86 ± 0.21
4	DDG	12.99 ± 0.16	8.63 ± 0.19	1.90 ± 0.12
	Thin stillage	1.64 ± 0.02	8.92 ± 0.12	2.35 ± 0.04
	DDGS	11.41 ± 0.17	7.92 ± 0.45	1.78 ± 0.19
5	DDG	9.34 ± 0.08	6.66 ± 0.51	2.10 ± 0.24
	DDGS	9.57 ± 0.09	9.27 ± 0.59	2.08 ± 0.23
6	DDG	9.02 ± 0.02	7.30 ± 0.62	2.58 ± 0.36
	Thin stillage	2.10 ± 0.03	9.81 ± 0.12	1.98 ± 0.07
	DDGS	11.54 ± 0.03	9.27 ± 0.47	2.34 ± 0.22
7	Standard DDGS	8.67 ± 0.09	9.29 ± 1.55	3.08 ± 0.79
7[b]	Modified DDG	3.48 ± 0.08	20.13 ± 0.93	6.87 ± 0.58

DDG dried distiller grains; *DDGS* dried distiller grains with solubles

[a] Total sterols were estimated as the sum of the masses of sterol fatty acyl esters and free phytosterols

[b] Dry grind ethanol plant was modified and no thin stillage was mixed with DG

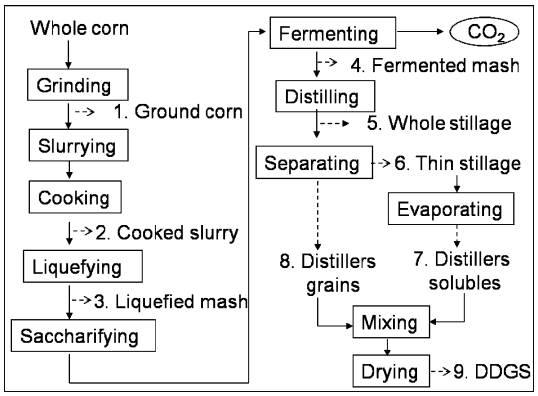

Fig. 1 Process diagram of the dry grind ethanol process. Fractions analyzed in Experiment 2 are numbered 1–9

that would be present if the 0.5 or 1.0 ml samplings had not been removed.

Phytosterols in the extracted oil were identified and quantified by a GC. The procedures for saponification, phytosterol extraction and derivatization are previously described [2]. Phytosterols were injected in triplicate by autosampler onto a Varian (Palo Alto, CA, USA) 3800 GC equipped with an flame ionization detector (FID) and a DB-5 (Agilent, Santa Clara, CA, USA) capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas at 1 ml/min, with a 1:50 injector split. The injector temperature was 270 °C, and the detector temperature was 280 °C. The column oven initial temperature was 250 °C for 0.5 min, increased at 10 °C/min to 270 °C and held for 30 min, then increased at 10 °C/min to 280 °C and held for 3.5 min. Data collection and peak integration were performed using Varian's Galaxie Chromatography Software version 1.9. Phytosterol peaks were identified by comparison of relative retention times (compared to the internal standard, 5α-cholestane) with those of commercially available standards. Quantitation was carried out by the internal standard method developed with available standards. A soybean oil sample was analyzed daily as a control; the coefficient of variation for phytosterol content in the soybean oil sample was 4.4% ($n = 15$). For phytosterols with no available commercial standard, the response factor for β-sitosterol was used for quantitation. The identities of phytosterol peaks were confirmed by gas chromatography-mass spectrometry (GC-MS) analysis performed on an Agilent 6890 GC-MS equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm), a 5973 mass selective detector, and an 7683 autosampler. The transfer line from GC to the mass selective detector (MSD) was set to 280 °C. The injector and oven temperature programs were the same as described for the GC-FID instrument above. MSD parameters were as follows: scan mode 50–600 amu, ionizing voltage 70 eV, and electron multiplier (EM) voltage 1,823 V. Mass spectral identification was performed using the Wiley MS database combined with comparison to literature values for relative RT (compared to β-sitosterol) and mass spectra [11].

Tocopherols and tocotrienols in the extracted oil were analyzed using an high performance liquid chromatography (HPLC) method with fluorescence detection, 294 nm excitation, 326 nm emission, as previously described [12] with a LiChrosorb 5 DIOL (3 × 100 mm), and an isocratic mobile phase of 97/3, hexane/methyl *tert*-butyl ether, v/v,

at a flow rate of 0.5 ml/min. All samples were extracted in duplicate and each extract was analyzed separately.

Statistical Analyses

All results presented are the mean of the duplicate analyses. Data from Experiment II were treated with the JMP software, version 5 (JMP, a business unit of SAS, Cary, NC, USA) for analysis of variance (ANOVA) in order to determine the effects of different processing steps (Fraction No.) and ethanol plants (Plant No.). The Tukey's HSD (honestly significant difference) test was conducted for pair-wise comparisons when ANOVA showed a significant effect at $p < 0.05$.

Results and Discussion

Experiment I, Lipid Content and Profiles in Raw Corn and Some End Products

In the first study, samples of DDGS and other fractions (two samples of thin stillage, one sample of CDS, and six samples of DDG) were obtained from seven ethanol plants and their compositions were compared to that of ground corn (control). Similar to results previously reported for the hexane extraction of ground corn [5], an oil yield of 3.43% (fresh weigh basis) was observed and the levels of free fatty acids and total sterols in the oil were 2.28 and 1.79% in extracted oil, respectively (Table 1). The crude oil yields in the seven DDGS samples ranged from 8.67 to 11.54% (fresh weight basis, and for all types of samples). The crude oil yields from the six samples of DDG ranged from 9.02 to 12.99%. The levels of oil in the two thin stillage samples were 1.64 and 2.20%, and the level of oil in the one CDS sample was 6.44%.

Higher levels of free fatty acids (FFA, 6.81–20.13% in extracted oil) were detected in the oil extracted from all samples, as compared with the ground corn control (2.28%) (Fig. 2; Table 2). The level of the total sterols was fairly constant for the all samples from plants 1–6 (including one control sample), with values ranging from 1.78 to 2.58%, based on extracted oil mass. For plant 7, one sample was obtained when the plant was operating in the standard mode, similar to plants 1–6, and a second sample (called modified DDG) was obtained when the standard dry grind process was modified by removing all of the thin stillage and not mixing it with DDG. For the "standard" plant 7 DDGS sample, the levels of crude oil, FFAs, and total sterols were similar to those reported for samples 1–6, but the modified DDG sample was very different than any other samples. It contained very low levels of crude oil (3.48%, sample mass basis), very high levels of free fatty acids (20.13%, oil mass basis), and total sterols (6.13% sterol ester and 0.74% free sterols, both are oil mass basis).

The major conclusion of Experiment I is that the oil in both the thin stillage fraction and the distillers grains (DG) fraction contain similarly high levels of free fatty acids, so when they are combined to make standard DDGS, the free fatty acids can be considered to be from both fractions. Previously published studies have reported that DDGS [2, 13] and post-fermentation corn oil (removed from the back end of a corn dry grind ethanol plant) [3] contain high levels of free fatty acids, but this is the first report that has shown that the oil obtained in both fractions after centrifugation (DG and thin stillage) contain nearly equally high levels of free fatty acids (Table 1). Also, the high levels of total sterols ($\sim$2%) in the oil from all samples indicate that this valuable phytochemical could potentially be removed from the oil (via deodorization or fractional distillation) if the oil is used for non-food applications such as to make biodiesel.

Experiment II, Change in Lipid Content and Profile During Individual Steps in the Dry Grind Process

Experiment II was designed to identify the specific steps in the dry grind ethanol process that cause changes in the various types of lipids. It should be noted that the oil yields in Experiment I were expressed as % fresh weight, whereas the data in Experiment II were expressed on a dry weight basis.

Figure 2 indicated that all three ethanol plants showed similar trends for oil yields in the nine fractions described in Fig. 1. The fractions included ground corn (#1), cooked slurry (#2), liquefied mash (#3), fermented mash (#4), whole stillage (#5), thin stillage (#6), distillers solubles (#7), DG (#8), and DDGS (#9). The oil yields were low (<5%) in the pre-fermentation fractions (#1–3), in the range expected for whole corn samples. Oil yields increased three–fourfold in the next four fractions (#4–7), due to depletion of starch upon fermentation. Examination of the thin stillage and distillers soluble fractions (#6–7) reveals that a slightly higher percentage of the oil partitions into the liquid thin stillage fraction compared to the solid DG (#8). Final oil yield in the DDGS fraction (#9) is slightly lower than in the fermented mash or whole stillage, indicating there are small oil losses during processing, probably due to a portion of thin stillage being used as a backset for slurrying ground corn.

When the fatty acid composition was expressed in relative % (Table 2), linoleic acid (18:2) was the major fatty acid (53.96–56.53%), followed by oleic acid (25.25–27.15%) and then palmitic acid (13.25–16.41%), with low levels of stearic (1.80–2.34%) and linolenic (1.15–1.40%) acids. These values are consistent with other recently reported results [1, 14]. Although some minor yet

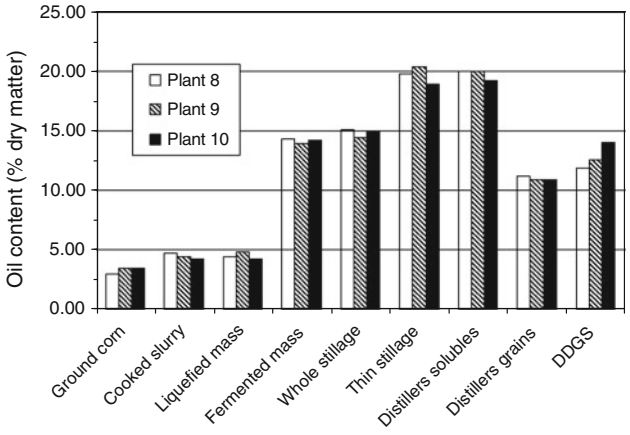

Fig. 2 Oil yields from nine fractions from three ethanol plants. Extractable oil levels were measured by hexane extraction with an Ankom Model XT 10 Fat Extractor

significant difference existed in the mean values of individual fatty acid among steps (fractions), all the major fatty acids generally remained constant (Table 2). This is also true for mean values from among the plants.

In Experiment I, total sterols were analyzed by an HPLC method that separates free sterols and sterol fatty acyl esters into two separate groups (Table 1). For Experiment II, the total content and the composition of phytosterols in each fraction were analyzed by a GC method (Table 3). In both studies, total phytosterol content in oil extracted from DDG and DDGS samples ranged from 1.5 to 2.5% and averaged around 2%. The major phytosterols in ground corn were sitosterol > campesterol > sitostanol > campestanol (Table 3) as noted previously [14]. Ten other minor phytosterols (stigmasterol, avenasterol and others) and squalene were also detected but their total proportions ranged from 12 to 15% (based on total phytosterol mass). Interestingly, ergosterol, the major sterol in yeast [15], was not detected in any of the post-fermentation samples, so the contribution of yeast sterols to the total phytosterol pool was negligible. There were some differences in the sterol levels from the three plants (8–10) and at the nine dry grind processing steps within each plant, but no obvious trends were observed. For plants 9 and 10, phytosterols were approximately 8% lower on average, in the DDGS compared to the ground corn starting material, but in plant 8 phytosterol levels were higher in the DDGS compared to the ground corn. The proportions of the various phytosterols remained relatively constant during the nine dry grind processing steps. These data indicate that phytosterol content and composition remain relatively constant throughout the dry grind process.

Quantitative analysis of the tocopherols (T) and tocotrienols (T3) in the various fractions confirmed our previous reports that γ-tocopherol (γ-T) is the major tocopherol and γ-tocotrienol (γ-T3) is the major tocotrienol in ground corn

Table 2 Fatty acid composition of 9 fractions from 3 dry grind ethanol plants

Plant no.	Fraction no.	Palmitic	Stearic	Oleic	Linoleic	Linolenic
8	1	13.34	1.82	27.21	56.36	1.26
9	1	13.33	1.72	27.57	56.13	1.25
10	1	13.11	1.86	26.68	57.10	1.27
Mean[a]		13.25 c	1.80 d	27.15 a	56.53 a	1.26 bc
8	2	16.85	1.99	26.67	53.16	1.34
9	2	16.65	2.14	25.86	53.53	1.44
10	2	15.24	2.00	25.25	56.21	1.31
Mean[a]		16.24 ab	2.04 bc	25.93 bc	54.30 b	1.36 ab
8	3	17.07	2.09	26.20	53.22	1.44
9	3	16.22	1.76	25.52	55.27	1.23
10	3	15.94	2.06	25.95	54.71	1.34
Mean[a]		16.41 a	1.97 cd	25.89 bc	54.40 b	1.34 ab
8	4	16.48	2.22	26.51	53.45	1.34
9	4	15.93	2.06	25.72	55.04	1.25
10	4	15.20	2.31	25.33	55.88	1.30
Mean[a]		15.87 ab	2.19 ab	25.85 bc	54.79 b	1.29 ab
8	5	16.61	2.28	26.37	53.44	1.30
9	5	15.40	2.10	26.28	54.97	1.26
10	5	15.47	2.33	25.42	55.50	1.29
Mean[a]		15.83 ab	2.24 ab	26.02 bc	54.64 b	1.28 bc
8	6	16.36	2.32	27.11	53.03	1.18
9	6	14.62	2.12	27.40	54.77	1.11
10	6	14.81	2.41	26.80	54.82	1.17
Mean[a]		15.26 ab	2.28 a	27.10 a	54.21 b	1.15 d
8	7	16.97	2.41	26.29	53.10	1.23
9	7	15.05	2.22	27.35	54.26	1.12
10	7	15.35	2.39	26.56	54.52	1.19
Mean[a]		15.79 ab	2.34 a	26.73 ab	53.96 b	1.18 cd
8	8	17.01	2.16	25.60	53.84	1.39
9	8	16.32	2.16	25.54	54.60	1.39
10	8	16.15	2.30	24.61	55.52	1.42
Mean[a]		16.49 a	2.21 ab	25.25 c	54.65 b	1.40 a
8	9	16.84	2.28	25.80	53.56	1.38
9	9	16.10	2.20	25.75	54.62	1.33
10	9	15.77	2.32	25.22	55.36	1.36
Mean[a]		16.24 ab	2.27 a	25.59 c	54.51 b	1.36 ab
8	Mean[b]	16.39 a	2.17 a	26.42 a	53.68 c	1.32 a
9	Mean[b]	15.51 b	2.05 b	26.33 a	54.8 b	1.26 b
10	Mean[b]	15.23 b	2.22 a	25.76 b	55.51 a	1.29 ab

Mean of duplicate results (in relative %)

[a] Column means of 3 plants for each of 9 fractions bearing different letters differ significantly at $P < 0.05$

[b] Column means of 9 fractions for each of 3 plants bearing different letters differ significantly at $P < 0.05$

[12] and in DDGS [2], with small amounts of α- and δ-tocopherols and trace amounts of α- and δ-tocotrienols (Table 4). In our HPLC system, with the ground corn

Table 3 Phytosterol contents of nine fractions from three dry grind ethanol plants

Plant	Fraction no.	Campesterol	Campestanol	Stigmasterol	Sitosterol	Sitostanol	Others[a]	Total
8	1	358.2 (21.4)	129.7 (7.7)	71.6 (4.3)	705.5 (42.2)	197.1 (11.9)	213.5 (12.5)	1,675
9	1	451.0 (19.9)	174.7 (7.5)	93.7 (4.2)	968.2 (42.9)	282.7 (12.4)	290.6 (12.8)	2,261
10	1	453.2 (18.7)	177.1 (7.5)	101.1 (4.1)	1,067 (44.4)	316.8 (13.3)	292.4 (12.0)	2,407
Mean[b]		420.8 (20.0) b	160 (7.5) e	89 (4.2) b	913 (43.2) b	266 (12.5) d	265 (12.4) bc	2,114 bc
8	2	286.6 (20.1)	120.5 (8.3)	53.6 (3.8)	596.7 (41.0)	189.3 (13.2)	189.7 (13.2)	1,436
9	2	318.4 (19.5)	130.3 (7.9)	69.4 (4.3)	696.8 (42.7)	211.7 (12.8)	215.1 (13.4)	1,642
10	2	368.7 (18.5)	157.4 (8.0)	82.9 (4.1)	874.5 (43.5)	277.3 (14.0)	237.3 (12.0)	1,998
Mean[b]		324.5 (19.4) d	136 (8.1) f	69 (4.1) e	723 (42.4) d	226 (13.3) e	214 (12.9) f	1,692 f
8	3	571.9 (20.4)	215.7 (7.9)	119.3 (4.3)	1,179 (42.3)	332.3 (11.8)	374.8 (13.3)	2,793
9	3	409.9 (18.9)	177.6 (8.3)	87.4 (4.0)	927.2 (42.9)	294.4 (13.4)	273.7 (12.4)	2,170
10	3	405.2 (18.6)	170.0 (7.9)	88.0 (4.1)	930.6 (43.2)	289.0 (13.5)	269.9 (12.6)	2,153
Mean[b]		462.3 (19.3) a	188 (8.0) ab	98 (4.1) a	1,012 (42.8) a	305 (12.9) ab	306 (12.8) a	2,372 a
8	4	410.6 (20.0)	182.5 (8.8)	90.3 (4.4)	795.5 (38.5)	286.3 (14.1)	287.9 (14.1)	2,053
9	4	313.9 (17.6)	149.2 (8.5)	70.6 (4.0)	741.8 (42.0)	255.0 (14.2)	233.6 (13.1)	1,764
10	4	363.1 (18.1)	157.8 (8.0)	85.0 (4.3)	854.5 (42.7)	283.4 (14.1)	253.4 (12.6)	1,997
Mean[b]		362.5 (18.6) c	163 (8.4) de	82 (4.2) c	797 (41.1) c	275 (14.1) cd	258 (13.3) c	1,938 de
8	5	518.5 (19.5)	219.6 (8.3)	114.3 (4.1)	1,091 (41.0)	341.8 (12.8)	375.7 (14.3)	2,661
9	5	323.2 (18.2)	156.0 (8.5)	71.0 (4.0)	746.5 (42.6)	224.7 (12.5)	239.6 (13.6)	1,761
10	5	367.3 (18.2)	169.2 (8.4)	84.4 (4.1)	847.6 (41.9)	299.6 (14.8)	265 (12.8)	2,032.9
Mean[b]		403.0 (18.6) b	182 (8.3) abc	90 (4.1) b	895 (41.8) b	289 (13.4) bcd	293 (13.5) ab	2,152 bc
8	6	450.1 (18.4)	231.3 (9.4)	90.3 (3.7)	952.4 (38.8)	362.0 (14.7)	366.9 (15.1)	2,453
9	6	276.7 (17.0)	162.4 (9.7)	56.9 (3.5)	635.0 (38.8)	269.4 (16.4)	247.3 (15.1)	1,648
10	6	346.2 (17.6)	184.6 (9.0)	77.3 (3.9)	795.6 (39.6)	340.9 (17.1)	246.3 (12.6)	1,991
Mean[b]		357.6 (17.7) c	193 (9.4) a	75 (3.7) d	794 (39.1) c	324 (16.1) a	287 (14.3) ab	2,030 cd
8	7	343.2 (18.7)	175.8 (9.3)	69.6 (3.8)	689.2 (37.9)	269.7 (14.8)	272.0 (14.8)	1,819
9	7	306.9 (16.8)	176.9 (9.8)	62.2 (3.4)	721.3 (39.1)	300.2 (16.3)	270.3 (14.7)	1,838
10	7	334.9 (16.8)	187.1 (9.7)	75.9 (3.9)	776.2 (39.8)	322.6 (16.3)	259.5 (13.3)	1,956
Mean[b]		328.3 (17.4) d	180 (9.6) bc	69 (3.7) de	729 (38.9) d	297 (15.8) abc	267 (14.3) bc	1,871 e
8	8	465.0 (21)	142.1 (6.4)	111.3 (5.0)	967.9 (44.3)	217.7 (10.0)	290.3 (13.2)	2,194
9	8	402.5 (19.8)	138.8 (6.9)	99.6 (4.9)	894.0 (44.0)	227.7 (11.4)	257 (12.9)	2,019
10	8	393.0 (19.0)	120.7 (5.9)	100.6 (4.9)	948.9 (46.3)	213.9 (10.2)	269.5 (13.2)	2,046
Mean[b]		420.2 (19.9) b	134 (6.4) f	104 (4.9) a	937 (44.9) b	220 (10.5) e	272 (13.1) bc	2,087 bc
8	9	459.6 (19.9)	182.0 (7.8)	104.6 (4.3)	962.6 (41.6)	277.6 (12.1)	323.3 (13.9)	2,310
9	9	385.3 (18.3)	164.6 (7.7)	88.7 (4.3)	886.0 (42.8)	281.0 (13.5)	272.2 (13.0)	2,078
10	9	409.6 (18.6)	173.9 (7.7)	100.4 (4.5)	944.7 (42.7)	300.6 (13.6)	271 (13.3)	2,200
Mean[b]		418.1 (18.9) b	174 (7.7) cd	98 (4.4) a	931 (42.4) b	286 (13.1) bcd	289 (13.4) ab	2,196 b
8	Mean[c]	429.3 (17.9) a	177.6 (8.2) a	91.6 (4.2)a	882.2 (40.8) b	274.8 (12.8) b	299.3 (13.8) a	2,155 a
9	Mean[c]	354.2 (18.4) c	158.9 (8.3) c	77.7 (4.1) c	801.8 (41.9) c	260.7 (13.7) c	255.4 (13.4) c	1,909 c
10	Mean[c]	382.3 (18.2) b	166.4 (8.0) b	88.4 (4.2) b	893.2 (42.7) a	293.8 (14.1) a	262.7 (12.7) b	2,087 b

Means of duplicate results, expressed as mg/100 g extracted oil. Numbers shown in parenthesis are the weight percentage of total phytosterols

[a] Other peaks identified include: squalene, Δ7-campesterol, clerosterol, Δ5-avenasterol, 24-stigmasterol, gramisterol, cycloartenol, Δ7-avenasterol, 24-methylene cycloartenol, and citrostadienol

[b] Column means of 3 plants for each of 9 fractions bearing different letters differ significantly at $P < 0.05$

[c] Column means of 9 fractions for each of 3 plants bearing different letters differ significantly at $P < 0.05$

extract, α-tocopherol eluted at 5.5 min and α-tocotrienol eluted at 6.9 min and an unknown peak eluted at 5.9 min. In a previous paper [3], we reported the presence of this unknown in ground corn and in "post-fermentation" corn oil samples, and suggested that the unknown peak might be "α-tocomonoenol" because it eluted between α-tocopherol

Table 4 Tocopherols (T) and Tocotrienol (T3) composition of nine fractions from three dry grind ethanol plants

Plant	Fraction no.	α-T	α-T*	γ-T	δ-T	α-T3	γ-T3	δ-T3	Total tocols
8	1	12.74	46.22	109.93	16.19	12.08	24.90	1.35	223.42
9	1	19.80	32.13	84.92	9.96	11.09	19.48	0.98	178.37
10	1	23.16	31.35	85.80	9.65	13.04	25.94	1.48	190.41
Mean[a]		18.57 b	36.56 e	93.55 b	11.94 e	12.07 b	23.44 e	1.27 cde	197.40 f
8	2	9.88	10.01	93.48	11.05	7.23	19.18	1.20	152.04
9	2	16.70	9.47	74.02	10.31	6.84	15.65	0.87	133.85
10	2	22.27	8.88	76.60	6.41	8.55	18.75	0.99	142.45
Mean[a]		16.28 d	9.45 g	81.36 d	9.25 f	7.54 e	17.86 f	1.02 e	142.78 h
8	3	9.95	19.58	89.86	13.74	9.06	21.87	1.23	165.29
9	3	18.86	12.01	80.48	10.85	9.82	20.80	1.05	153.86
10	3	22.27	18.88	73.50	9.84	11.65	24.67	1.28	162.07
Mean[a]		17.03 cd	16.82 f	81.28 d	11.48 e	10.17 cd	22.45 e	1.19 de	160.41 g
8	4	11.79	126.05	95.75	64.82	10.20	25.52	1.89	336.03
9	4	16.50	103.34	72.41	70.23	9.90	22.62	1.17	296.17
10	4	22.47	103.79	73.26	49.72	10.33	23.04	1.59	284.20
Mean[a]		16.92 cd	111.06 d	80.47 d	61.59 a	10.14 cd	23.73 de	1.55 bcd	305.47 c
8	5	13.76	154.29	100.79	69.44	10.90	28.04	2.07	379.29
9	5	18.21	90.28	77.16	61.07	11.08	24.59	1.45	283.83
10	5	18.69	126.79	70.76	48.31	10.67	23.61	1.67	300.49
Mean[a]		16.88 cd	123.79 c	82.90 cd	59.60 b	10.88 c	25.42 c	1.73 bc	321.20 b
8	6	9.33	188.08	91.09	70.66	12.74	33.29	2.48	407.68
9	6	15.98	113.45	77.01	50.41	15.37	32.63	2.64	307.49
10	6	17.80	201.42	67.74	54.01	13.30	30.70	3.16	388.14
Mean[a]		14.37 e	167.65 a	78.61 d	58.36 b	13.80 a	32.21 a	2.76 a	367.77 a
8	7	9.62	93.63	88.05	12.55	12.09	30.72	2.15	248.81
9	7	15.95	123.11	68.84	34.86	13.40	28.28	2.76	287.19
10	7	17.25	155.53	59.94	23.70	13.61	29.12	2.41	301.55
Mean[a]		14.27 e	124.09 c	72.28 e	23.70 d	13.03 a	29.37 b	2.44 a	279.18 d
8	8	14.06	40.29	112.53	27.93	6.71	18.25	1.06	220.82
9	8	21.52	30.79	89.95	28.51	7.31	17.25	0.88	196.21
10	8	28.82	50.84	92.90	27.55	8.01	19.20	0.96	228.29
Mean[a]		21.47 a	40.64 e	98.46 a	28.00 c	7.34 e	18.23 f	0.97 e	215.10 e
8	9	13.21	112.21	104.00	22.14	9.62	25.29	1.91	288.37
9	9	16.18	87.01	73.87	28.32	7.52	20.30	1.41	234.61
10	9	24.05	197.89	84.52	24.39	12.79	29.33	2.35	375.32
Mean[a]		17.81 bc	132.37 b	87.46 c	24.95 d	9.98 d	24.97 cd	1.89 b	299.43 c
8	Mean[b]	11.59 c	87.82 b	98.39 a	34.28 a	10.07 b	25.23 a	1.71 a	269.08 a
9	Mean[b]	17.74 b	66.84 c	77.63 b	33.83 a	10.26 b	22.40 b	1.47 b	230.18 c
10	Mean[b]	21.86 a	99.49 a	76.11 b	28.17 b	11.33 a	24.93 a	1.76 a	263.66 b

Means of duplicate results, expressed as mg/100 g extracted oil

[a] Column means of 3 plants for each of 9 fractions bearing different letters differ significantly at $P < 0.05$

[b] Column means of 9 fractions of 3 plants bearing different letters differ significantly at $P < 0.05$

(no double bonds in the isoprene portion and α-tocotrienol (three double bonds in the isoprene region). This unknown peak which we called α-T* in Table 4, had a peak area that was two–threefold more than the peak area of α-tocopherol. The proportions of α-T* decreased in fractions 2 and 3, increased more than fivefold in fractions 4–6, decreased in fractions 7–8 and again increased in the fraction 9. Some differences in the proportions of all tocopherols and tocotrienols were observed in all samples, with the biggest difference being the much higher levels of α-T* in fraction

9 from plant 10, compared to fraction 9 from the other two plants. Overall, these data indicate that tocol levels and the proportions of the homologues remained relatively stable throughout the dry grind operation. However, there is an exception. Both δ-T and the unknown peak that we named α-T* showed significant increases upon fermentation and remained relatively high thereafter. Since the total tocols included the values of these two attributes, they showed significant higher values for all fractions after the fermentation step. Thus, the dry grind process caused little changes to majority of tocols and some increase of minor rest. The preservation of these important antioxidants may help maintain the oxidative stability of corn oil extracted from DDGS.

Conclusion

In this report, Experiment I demonstrated that the free fatty acids known to occur in DDGS originate in both the particulate (DG) and soluble (thin stillage) centrifugation fractions of the dry grind ethanol process. Although a previous report concluded that yeast proteins significantly contribute to the amino acid profile of DDGS [4], Experiment II in this report indicates that the contribution of yeast to the fatty acid composition and lipid profile (sterols and tocols) was minimal during the dry grind ethanol process. This report also confirms that most of the potentially valuable corn phytosterols, tocopherols, and tocotrienols are retained during the dry grind ethanol process. The presence of a previously reported unknown tocol [3] was confirmed. Further studies are needed to elucidate the chemical structure, properties, and safety of this unknown tocol.

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