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Characterization of Oil Precipitate and Oil Extracted from Condensed Corn Distillers Solubles

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Abstract Oil extracted from condensed corn distillers solubles (CCDS) can form a semi-solid and waxy precipitate at the bottom of containers during storage. CCDS is a good source to recover oil, and such oil can be converted to biodiesel. Precipitate formation in the extracted oil is mainly a physical stability problem, but it may become a performance problem for biodiesel. The objective of the present work was to determine the composition of the CCDS oil precipitate and also determine if valuable phytosterols were present in high concentration. The free fatty acid (FFA) content was very high, 35.7%, and fatty acid composition of the FFA fraction was predominantly palmitic acid, 70.3%. The solid appearance was mainly due to a high percentage of high-melting point free saturated fatty acid. The total unsaponifiable matter was 2.0%, and total phytosterol content was 8.6 mg/g of CCDS oil precipitate. Therefore, CCDS oil precipitate is a not an enriched source of phytosterols compared to total sterols present in crude corn oil (15.6 mg/g oil). The wax content was high, 2.5 mg/g of CCDS oil precipitate compared to 0.5 mg/g of crude corn oil. CCDS oil that is uncentrifugable but polar solvent extractable (trapped oil fraction) was also characterized and found to contain more polar lipids than those in the free oil fraction (centrifugable oil).

Keywords CCDS oil · CCDS oil precipitate · Fatty acid composition · Phytosterols · Wax

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Introduction

After ethanol fermentation of corn, oil from the corn is distributed relatively equally between the stillage and the solid after the centrifugation (from our own calculation according to the typical oil contents in the fermentation solids and in the original corn). The liquid fraction is relatively more oil rich than the solids fraction on the dry weight basis, so oil can be extracted from the liquid for biofuel applications. Corn oil recovered from the condensed corn distillers solubles (CCDS, a fraction also referred to in the industry as thick stillage or concentrated thin stillage), a corn fermentation co-product, contains lipids that solidify and settle at ambient (25 °C) temperature. The solidified lipids have been termed "CCDS oil precipitate" and it appears sticky and waxy, and is bright orange in color. Such a precipitate may be a problem when the oil is used to make biodiesel and if the solid fraction cannot be converted to the low-melting point methyl esters, because it may solidify and cause engine failure. Therefore, it is important to characterize the oil precipitate and determine why the precipitate is semi-solid at room temperatures and to find other potential uses for the precipitate. The possible presence of high-concentration phytosterols in CCDS oil precipitate could provide additional revenue for the dry-grind ethanol processing industry.

The oil extracted from co-products of dry-grind corn ethanol production should have the lipid constituents of germ, endosperm, bran, fiber and yeasts (*Saccharomyces cerevisiae*). The crude corn oil composition from germ has been reported [1] and also that of whole corn kernel [2], and they are shown in Table 1. Corn kernels contain 3–5% oil [3]. The corn germ contains about 85% of the total oil of the kernel [4] whereas the remainder of the oil is found in the endosperm and hull fractions. The corn germ contains 45–50% oil [4].

Table 1 Comparison of lipid class composition (%) of corn germ oil, corn kernel oil and yeast lipids

Lipid component	Corn germ oil[a]	Corn kernel oil[b]	Yeast lipids[c]
Triacylglycerols	95.6	75.8	40
Free fatty acids	1.7	1.1	6
Waxes	0.05	NR	NR
Hydrocarbons and steryl esters	NR	3.4	NR
Phospholipids	1.5	NR	30
Phospholipids and glycolipids	NR	13.0	NR
Diglycerides and monoglycerides	NR	2.1	NR
Phytosterols	1.2	4.6	20 (esterified sterols)
Tocopherols	0.06	NR	NR

NR not reported

[a] Corn germ has 45–50% crude oil on a dry basis [4]

[b] Corn kernel has 3–5% crude oil on a dry basis [3]

[c] Yeast has 9.0% oil on a dry basis [5]

The yeast cells may partially contribute to the fatty acid composition of the corn oil derived from CCDS, especially if the high temperature alcohol distillation damages the cells. The yeast species *S. cerevisiae* contains about 9% (d.b.) total lipids [5], and its lipid class composition is also shown in Table 1. The fatty acid composition of this yeast is typically 3% myristic (14:0), 16% palmitic (16:0), 42% palmitoleic (16:1), and 27% oleic (18:1) [5]. However, the growth conditions of the yeast and nutrients have an impact on the fatty acid composition of the yeast. For example, yeast growing in a medium containing palmitoleic acid (16:1) would result in the 16:1 becoming 91% of the total fatty acid composition and supplementation with oleic acid (18:1) resulted in 18:1 becoming 90% of the total fatty acid [6]. However, for the corn dry-grind ethanol production process, the amount of yeast accumulated at the end of fermentation has not been reported and the degree of cell damage is not clearly documented, so the contribution of the yeast lipid to oil content and composition is unknown.

The composition of the CCDS oil precipitate needs to be characterized so potential uses for this oil fraction can be explored. If these components do not settle during a short storage time and remain in the bulk oil, we need to know whether they will potentially affect biodiesel quality. Since the CCDS oil precipitate separates from the oil at ambient temperature, the presence of an elevated level of saturated fatty acids in the precipitate is expected. The precipitate may also contain a greater proportion of high-melting point waxes and phytosterols.

CCDS oil may be a good source of phytosterol ferulate esters because ferulate ester is rich in corn fiber oil [7], and during the fermentation of whole corn, the ethanol produced may help solubilize or extract such components from the aleurone layer of the kernel or fiber. Sitostanol ferulate is present in high levels in corn fiber oil [8] and has been found to be very effective in lowering cholesterol in hamsters [9]. Corn fiber oil extracted using hexane gave 3.3% extractable oil of which 5.0 wt% was ferulate esters, 9.1% was phytosterol esters, 1.0% was free phytosterols [7]. The ferulate esters are similar in structure to those in "gamma oryzanol" found in rice bran oil [10].

In our previous research on oil extraction from CCDS, Majoni et al. (unpublished data) showed that some oil remains trapped in the CCDS solid residue and cannot be separated by centrifugation. The oil is termed trapped oil and characterization of this trapped oil fraction may provide some additional information on why this fraction cannot be separated by centrifugation.

Our research hypothesis is that the CCDS oil precipitate contains a high level of saturated fatty acids and high wax content that contributes to the physical appearance at room temperatures. In addition, the CCDS oil precipitate may be a good source of phytosterols. The objective of the present study was to determine the composition of the CCDS oil precipitate by quantifying the free and total phytosterols, phytosterol ferulate ester, wax content and fatty acid composition. The CCDS oil, free and trapped oil fractions were also characterized.

Experimental Procedures

All reagents used were of analytical grade. Sitostanol and campestanol were obtained from Supelco (Bellefonte, PA, USA), 5α-cholestane standard was obtained from Sigma-Aldrich (St Louis, MO, USA). A mixture of soy sterols with 95% purity was obtained from Archer Daniels Midlands (Decatur, IL, USA), and it contained β-sitosterol (45.7%), campesterol (27.3%), stigmasterol (15.3%), and brassicasterol (4.4%). The CCDS oil precipitate was obtained from LincolnWay Energy (Nevada, IA, USA) and that was collected from the bottom of a large storage tank for the commercially separated CCDS oil. Our own CCDS was obtained from LincolnWay Energy (Nevada, IA, USA) and stored in the refrigerator at 4 °C until used. To prevent mold growth CCDS, sodium azide at about 100 ppm was added.

Free Fatty Acid Content Quantification

The free fatty acid content of the corn oil precipitate was determined by using the AOCS official method Ca 5a-40 [11]. The CCDS oil precipitate (300 g) was washed once or ten times with hot water (300-mL each time) to remove lactic and acetic acids produced during fermentation that may interfere with the determination of the free fatty acids. These samples were compared to unwashed samples. The percentage of free fatty acids was calculated as oleic acid.

Thin Layer Chromatography (TLC) Separation of the Neutral Lipids and Fatty Acid Composition Determination

To separate the CCDS oil precipitate into two lipid fractions of free fatty acid (FFA) and triacyglycerol (TAG), preparative TLC, 20 × 20 cm, 500 μm thickness Adsorbosil Plus 1 Silica Gel (Alltech Associates Inc., Deerfield, IL, USA) and developing solvent of hexane/diethyl ether/acetic acid (90:10:2 v/v/v) were used. The plate was developed twice to ensure complete separation and then sprayed with 2′,7′dichlorofluorescein and viewed under UV light. Following identification by comparing with standards, the bands were scraped and placed in vials. The lipid fraction were extracted from silica with 10 mL chloroform/methanol (1:1 v/v), three times. The solvent was then removed.

To produce fatty acid methyl esters (FAMEs) of the lipid fractions, FFA was esterified by using 3% sulfuric acid in methanol for 24 h at 60 °C whereas the TAG fraction was transesterified using 1 M sodium methoxide in methanol for 1.5 h at 60 °C. Reactions were terminated with water and FAME was extracted twice using 2 mL of hexane. The CCDS oil precipitate, and CCDS oil fatty acid composition were also determined by first transforming the fatty acids into FAMEs with 3% sulfuric acid in methanol for 24 h at 60 °C followed by base catalyzed transesterification with 1 M sodium methoxide in methanol for 1.5 h at 60 °C.

The FAMEs were analyzed with the Hewlett-Packard 5890 series II GC equipped with a flame ionization detector and Supelco™ 2330 capillary column (15 m length × 0.25 mm i.d × 0.2 μm film thickness) (Bellefonte, PA, USA). Initial oven temperature was 150 °C, oven temperature program was 150–180 °C at a rate of 5 °C/min, inlet and detector temperatures were 230 °C and the split ratio was 10:1. Sample injection volume was 1 μL.

Total Unsaponifiable Matter Content

The CCDS oil precipitate was saponified to free the esterified phytosterols and remove the glycerol lipids. Saponification was done according to the AOCS official method Ca 6b-53 [12]. The saponification was performed for 1 h using 5 mL of 50% KOH, 25 mL 95% ethanol with about 2.5 g CCDS oil precipitate under reflux. The total unsaponifiable extract was saponified again with 5 mL of 50% KOH for 1 h. The procedure was repeated once more to ensure full hydrolysis of the ester bonds.

Sample Preparation for Total Phytosterol Quantification by GC

The same saponification procedure as described above was used for 2.5 g CCDS oil precipitate. The saponification was carried out for 30 min. The unsaponifiable matter was then dissolved in 1 mL of ethyl acetate and streaked on a preparative TLC plate, 20 × 20 cm, 500 μm thickness Adsorbosil Plus 1 Silica Gel (Alltech Associates Inc., Deerfield, IL, USA). The plate was developed and analyzed as before. The free phytosterol band was collected, 5α-cholestane internal standard was added to the silica, and the silica was extracted with 3 × 10 mL ethanol/diethyl ether/hexane (50:25:25 v/v/v) [13]. The solvent was evaporated and the free sterols were dissolved in ethyl acetate for GC analysis. The free phytosterols were separated on a SAC-5 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Supelco, Bellefonte, PA, USA). The following temperature program was used: 250 °C for 5 min, temperature was then increased to 265 °C at a rate of 1 °C/min, and then held at 265 °C for 25 min. The injector and detector temperatures were 280 °C. The flow rate of the carrier gas was 1 mL/min. Phytosterols were identified by comparing with commercial sterols standards, and quantified by internal standard method.

Ferulate Phytosterol Separation and GC Quantification

Solid-phase extraction (SPE) was performed to separate the phytosterol fraction from neutral lipids. About 0.2 g of the total lipids was dissolved in 2 mL of ethyl acetate and loaded on a 900-mg silica SPE column (Alltech Associates Inc., Deerfield, IL, USA). The neutral lipids were eluted by 15 mL of 5% diethyl ether in hexane [13]. The phytosterols were eluted by a solvent mixture of 15 mL ethanol/diethyl ether/hexane (50:25:25 v/v/v) [13]. The solvent was evaporated under nitrogen to obtain the phytosterol fraction. Qualitative TLC analysis showed that there was no loss of phytosterols in the neutral lipid fraction and that all the phytosterols were eluted with the polar solvent. The phytosterols fraction was redissolved in 0.2 mL ethyl acetate and streaked on preparative TLC plate (20 × 20 cm, 500 μm thickness). The plate was developed using hexane/diethyl ether/acetic acid (60:40:2 v/v/v). Identification of the band was done with the 2′,7′-dichlorofluorescein spray.

Dark blue bands compared to yellow fluorescent sterols above the free phytosterols were identified as phytosterol ferulate ester [13]. The ferulate phytosterol ester band was collected, internal standard added (5α-cholestane) and extracted three times using 10 mL of ethanol/diethyl ether/hexane (50:25:25 v/v/v). The extracts were saponified as previously described. The freed sterols were then quantified by GC as previously described. The total ferulate ester content was calculated based on the free sterols obtained and using the weighed average molecular weight of sterols identified.

Wax Quantification by GC

The CCDS oil precipitate was subjected to partial hydrolysis for 30 min in order to obtain the unsaponified wax esters using 2.0 g of the CCDS oil precipitate with 5 mL 50% KOH and 25 mL 95% ethanol under reflux conditions (AOCS official method Ca 6b-53) [12]. Qualitative TLC was performed to determine degree of hydrolysis of the glycerol lipids and presence of wax esters. TLC analysis confirmed that the wax esters were present by comparing with standards. Beeswax was used as a control to confirm that partial hydrolysis of the wax esters did not occur under the same saponification reaction.

To separate the wax esters, preparative TLC was performed with hexane/diethyl ether/acetic acid (90:10:2 v/v/v) as developing solvent. The wax ester band was scraped off and extracted three times with 10 mL ethanol/diethyl ether/hexane (50:25:25 v/v/v) [13]. The wax esters were then subjected to alkaline hydrolysis with 2 mL of 50% KOH and 10 mL 95% ethanol for 6 h to fully hydrolyze the ester bond and to produce potassium soap and fatty alcohols. Following saponification of the wax esters, the sample was acidified (about pH 2) with concentrated sulfuric acid to liberate the free fatty acids. The free fatty acids were extracted using 10 mL diethyl ether four times.

The free fatty acids were esterified into FAMEs using 3% (v/v) sulfuric acid in methanol for 24 h at 60 °C after adding the internal standard methyl heptadecanoate (C17:0). Quantification of the FAME by GC was done as previously described, however, oven temperature was held for 25 min at 180 °C for longer chain fatty acids detection. For calculating the wax ester content in the CCDS oil precipitate, the wax ester content in the unsaponifiable fraction was calculated using the internal standard method and weighed average molecular weight of fatty acids and fatty alcohols as reported in the literature [14]. The wax ester content in the CCDS oil precipitate was calculated knowing the total unsaponifiable matter content in the CCDS oil precipitate. Since corn kernel wax contains 76% wax esters [14], the wax ester content was then multiplied by 1.32 to give the total wax content of the CCDS oil precipitate, so it can be compared to the literature value.

Thermal Transition Profiles by DSC

The crystallization and melting thermograms of the CCDS oil precipitate and corn oil from CCDS were measured using differential scanning calorimetry. The lipid fractions were transferred to aluminum DSC pans and hermetically sealed. The indium test was used for calibration and the onset temperature as instructed. Nitrogen gas was used as purging gas at a flow rate of 20.0 mL/min. The sample weight ranged from 3.9 to 10.5 mg. An empty pan was used as a reference. The DSC program was 1.0 min hold at −50 °C followed by heating from −50 to 80 °C at 5 °C/min and cooling from 80 to −50 °C at the same rate.

Characterization of Neutral and Polar Lipids of CCDS Oil

Free oil from CCDS was obtained by centrifugation of the CCDS (30% solid content) using a Centra MP4 centrifuge fitted with an 854 rotor, fixed angle 20°, 7.6 cm radius at 10,000 rpm (8,500×g) for 10 min in 50-mL centrifuge tubes. The separated oil was transferred using hexane at least five times (10 mL each time). The trapped oil was obtained from the CCDS residue after free oil extraction. It was extracted with chloroform: methanol (2:1 v/v) followed by Folch wash [15]. The solvent and oil mixture was collected and solvent was removed by using the lab scale rotavapor evaporation system at 60 °C. Residual solvent was removed by using a vacuum oven at 25 °C.

Neutral and polar lipid class separation of the free and trapped oils was achieved by solid-phase extraction using a 900-mg silica cartridge (Alltech Associates Inc., Deerfield, IL, USA). Neutral lipid was eluted with 15 mL of chloroform, and polar lipids were eluted sequentially with 5 mL of chloroform: methanol (1:1 v/v), and 10 mL of methanol, and then eluents combined [16]. Solvent was removed by using nitrogen at room temperature and the weight of the fractions was recorded.

Qualitative TLC using 20 × 20 cm, 250 μm thickness plates was done on the neutral and polar lipid fractions of the free and trapped oil in order to examine the different lipid classes present in each fraction. For the neutral lipids, hexane/diethyl ether/acetic acid (90:10:2 v/v/v) was used as developing solvent. For the polar lipids, chloroform/methanol/acetic acid (100:45:5 v/v/v) was used as developing solvent. Identification of the lipid classes was done by using commercial standards.

The polar lipid fraction of the trapped oil was further separated into its major phospholipid classes using preparative TLC with a 20 × 20 cm, 500-μm thickness plate

with chloroform/methanol/acetic (100:45:5 v/v/v) as developing solvent. The polar lipid fraction was initially dissolved in 0.25 mL of chloroform, streaked on the TLC plate, sprayed and viewed as previously described. The lipids classes were identified by using commercial standards. The bands of the phospholipid classes were collected and fatty acid determination was carried out by converting the fatty acids into FAMEs with 1 M sodium methoxide in methanol for 1.5 h at 60 °C. FAMEs were analyzed by GC as previously described.

Statistical Analysis

Statistical analysis to determine significant differences among the different treatments was performed using the statistical analysis software SAS 9.1 (Cary, NC, USA), and one-way analysis of variance (ANOVA). Least Significant Differences (LSD) were calculated at $p = 0.05$. All treatments were carried out in duplicate and results are shown as the means of two replicates ±SD.

Results and Discussion

Free Fatty Acid (FFA) Content of the CCDS Oil Precipitate

The FFA content of the corn oil precipitate is shown in Table 2. Lactic and acetic acids are formed during corn fermentation and they can become dissolved in the oil especially when the FFA content is high [17]. The FFA content after washing one time using 1:1 oil: water ratio was not significantly different from washing ten times, indicating that one wash was sufficient to remove the lactic and acetic acids dissolved in the sample. The unwashed corn oil precipitate had a FFA value of 38.3%, which was significantly greater than the washed corn oil precipitate. The elevated level of FFA in the corn oil precipitate may have partially contributed to the physical state of the oil at room temperature. The presence of a large quantity of FFA allows strong molecular interaction [18] and the tendency of tight molecular packing increases when the FFAs are mostly saturated because they can align themselves better without the double bond "kinks" [18].

Fatty Acid Composition of FFA and TAG Fractions of the Precipitate

The fatty acid compositions of the CCDS oil precipitate, CCDS oil, FFA and TAG fractions are shown in Table 3. Palmitic acid compositions in the TAG fraction and CCDS oil were not significantly different. However, the FFA fraction was characterized by unusually high palmitic acid content (70.3%) compared to that present in the CCDS oil precipitate (34.6%), TAG fraction (18.2%) and CCDS oil (13.8%). The fatty acid composition of CCDS oil was similar to that of refined corn germ oil.

Table 2 Free fatty acid content of CCDS oil precipitate

Sample description	Free fatty acids (%) ± SD
Unwashed	38.3 ± 0.4a
Washed one time	35.7 ± 0.1b
Washed ten times	36.6 ± 0.4b

Means followed by different letters are significantly different ($p < 0.05$)

The elevated level of palmitic acid may be attributed to the hydrolysis of the ester bonds in TAG *sn*-positions 1 and 3 by lipase that is typically specific for *sn*-1,3 positions. Positions 1 and 3 tend to be occupied by saturated fatty acids. Since palmitic acid has a high melting point (64–65 °C), it tends to solidify and precipitate under ambient conditions. Therefore, the precipitate formed in CCDS oil is enriched with palmitic acid. It should be noted that the precipitate sample was collected from a large CCDS oil storage vessel. Therefore, the solidified fraction is highly enriched with saturated FFA. Stearic acid is also enriched in the FFA and TAG fractions. The TAG fraction of the precipitate tends to be more saturated than the TAG of the CCDS oil.

In addition to the high-melting point palmitic acid in the CCDS oil precipitate, the high-melting point phytosterols (138–145 °C) [19], waxes (40–120 °C) [20] may also be enriched in the CCDS oil precipitate.

Total Unsaponifiable Matter Content in the CCDS Oil Precipitate

The total unsaponifiable matter content of the CCDS oil precipitate was 2.0% as shown in Table 4. Crude corn oil contains 1.3–2.3% of unsaponifiable matter [3], therefore, CCDS oil precipitate does not have exceptionally high unsaponifiable matter content. Saponification was carried out three consecutive times for a total of 3 h in order to ensure complete hydrolysis, especially if the sample had a high wax content. It was observed that for the three consecutive hydrolyses of the CCDS oil precipitate, the total unsaponifiable matter did not to change suggesting that 1 h of hydrolysis was probably sufficient.

Total Phytosterols in the CCDS Oil Precipitate

Quantification of phytosterols in the CCDS oil precipitate is shown in Table 5. The total phytosterol content in the CCDS oil precipitate was 8.6 mg/g of CCDS oil

Table 3 Fatty acid composition (%) of the CCDS oil precipitate, CCDS oil, free fatty acid (FFA) and triacylglycerol (TAG) fractions of the precipitate

Fatty acid	FFA fraction of precipitate	TAG fraction of precipitate	CCDS oil precipitate	CCDS oil	Refined corn germ oil[a]
Palmitic (16:0)	70.3 ± 2.4a	18.2 ± 5.5c	34.4 ± 0.03b	13.8 ± 0.4c	12.2
Stearic (18:0)	5.9 ± 0.1b	16.2 ± 5.8a	4.1 ± 0.0b	2.3 ± 0.07b	2.2
Oleic (18:1)	6.3 ± 0.7b	20.0 ± 5.9a	20.2 ± 0.3a	27.8 ± 0.6a	27.5
Linoleic (18:2)	16.2 ± 1.5c	45.6 ± 5.4b	39.4 ± 0.2b	54.1 ± 0.9a	57.0
Linolenic (18:3)	1.2 ± 0.1b	–	1.7 ± 0.1b	1.9 ± 0.2a	0.9

Means in each row followed by different letters are significantly different ($p < 0.05$)

CCDS-condensed corn distillers solubles

[a] Durkee Foods [27]

Table 4 Unsaponifiable matter (%) of the CCDS oil precipitate following triple saponification

CCDS oil precipitate	Number of consecutive hydrolysis	Unsaponifiable content (%)	Average unsaponifiable content (%) after triple hydrolysis ±SD	Overall average (%) ± SD
1	1	2.2	2.1 ± 0.1	2.0 ± 0.1
	2	2.1		
	3	2.0		
2	1	2.1	2.0 ± 0.1	
	2	2.0		
	3	2.0		
3	1	1.9	2.0 ± 0.1	
	2	2.0		
	3	2.0		

Table 5 Total phytosterol contents (mg sterols/g) in CCDS oil precipitate

Phytosterol	Unsaponifiable (mg/g)	CCDS oil precipitate (mg/g)	Relative (%)	Average corn germ oil phytosterol[a] (%)
Campestanol	58.9 ± 0.8	1.3 ± 0.0	15.4	NR
Campesterol	27.0 ± 5.3	0.6 ± 0.1	7.1	21.4
Stigmasterol	18.9 ± 0.8	0.4 ± 0.0	5.0	6.1
β-Sitosterol	193.9 ± 7.7	4.4 ± 0.3	50.9	69.5
Unknown	68.6 ± 14.4	1.5 ± 0.3	17.9	NR
Sitostanol	14.2 ± 3.2	0.3 ± 0.1	3.7	NR
δ^5-Avenasterol	NR	NR	NR	2.4
Total	381.4 ± 6.4	8.6 ± 0.1	100	99.4
Ferulate esters	NR	0.9 ± 0.3	NR	NR

NR not reported

[a] Worthington and Hitchcock [28]

precipitate. Commercially prepared crude corn oil contains about 15.6 mg phytosterols/g oil [21]. Therefore, CCDS oil precipitate is a less concentrated source of beneficial phytosterols since it had about 55% of the concentration in crude corn oil. The corn oil precipitate contained sitosterol (50.9%) as the most abundant unsaturated phytosterol, followed by campestanol (15.4%), campesterol (7.1%), stigmasterol (5.0%), and sitostanol (3.7%). There was an unidentified component that was quite high in concentration (17.9%). The phytosterol composition was somewhat similar to that of hexane-extracted DDG phytosterols, which contained higher levels of the unsaturated phytosterols, sitosterol (49.6%), campesterol (15.6%) and stigmasterol (5.0%) [22]. The content of saturated phytosterols, campestanol (15.4%) in the CCDS oil precipitate was higher than sitostanol (3.7%). The saturated phytosterol composition of the corn oil precipitate is similar to that of corn fiber oil, which has relatively high levels of

campestanol (10.1%) and sitostanol (8.6%) [13]. These saturated phytostanols are mostly found in corn fiber oil and have been shown to be preferentially esterified with ferulic acid to form ferulate phytosterol esters [22].

Ferulate phytosterol esters are mostly concentrated in the inner pericarp [23]. If the corn kernel is composed of 5–6% pericarp [24] and that the corn fiber (2% extractable oil) is composed primarily of the pericarp, then we can estimate that the concentration of ferulate esters (4–5% in corn fiber oil) in crude corn kernel oil will be 0.12–0.15 wt%. The total ferulate phytosterol content in the CCDS oil precipitate was 0.9 mg/g (0.09%) which is less than the estimated ferulate phytosterol content in crude corn kernel oil. Therefore, we conclude that CCDS oil precipitate is a less concentrated source of these beneficial ferulate phytosterol esters.

Wax Quantification by GC

The partial hydrolysis of CCDS oil precipitate was done for 30 min in order to remove glycerol lipids and leave the wax esters for TLC separation and quantification. Generally, wax esters require a 2-h hydrolysis time with 5 N KOH under reflux for complete saponification [25]. As an example, rice bran wax esters were fully hydrolyzed for 4 h under reflux conditions using 30% KOH in isopropanol [26]. The chemical composition of surface wax of maize inbred WF9 was comprised mainly of 6% alkanes, 2% alcohols, 11% acids, 76% esters and 5% sterols [14] suggesting the maize kernel wax comprises mostly wax esters. The composition of the wax esters from maize kernel wax comprised mainly 46, 48, 52 and 54 carbon chain lengths and the predominant esterified fatty acids were C22 and C24, whereas, the esterified alcohols were C22, C24, C26 and C32 [14]. These data were used in our wax quantification and calculation.

The total wax ester content of the CCDS oil precipitate was calculated based on the wax ester content in the unsaponifiable fraction and then converted to the total wax content in the CCDS oil precipitate. Since the wax ester content of corn kernel wax is 76%, the wax ester of the CCDS oil precipitate was multiplied by 1.32 to give the total wax content. The total wax content in the CCDS oil precipitate was 2.5 mg/g as shown in Table 6. The predominant esterified fatty acids were C16 and C18 for the CCDS oil precipitate wax ester fraction. Under the experimental conditions for this study, the C22 and C24 esterified acids were not observed. The wax content in CCDS oil precipitate is five times greater than that present in crude corn oil which is about 0.5 mg/g.

The presence of high-melting point wax in the CCDS oil precipitate may have partially contributed to the physical appearance of waxiness at room temperature. Melting point of waxes usually ranges from 40 to 120 °C [20].

Neutral Lipid Phase Transitions

The phase transitions temperatures are shown in Table 7. The CCDS oil precipitate had higher endothermic peak temperatures than the oil from CCDS. The high melting peak temperatures in the CCDS oil precipitate may be attributed to the high-melting point fractions in the oil compared to oil from CCDS. The lower-melting point phase transitions of the oil from CCDS are consistent with the literature [16].

Neutral and Polar Lipid Composition of CCDS Oil

The percentage free oil and trapped oil recovered from CCDS was 70 and 30%, respectively, as shown in Table 8. The total (polar + neutral lipids) of each fraction did not add up to 100% because very polar lipids or non-lipid

Table 6 Wax content of CCDS oil precipitate

Wax ester content in CCDS oil precipitate (mg/g)	Total wax content in CCDS oil precipitate (mg/g)	Total wax content in commodity crude corn germ oil (mg/g)
1.9 ± 0.4	2.5 ± 0.6	0.5

Table 7 Lipid phase transitions of the CCDS oil precipitate and oil from CCDS

Lipid	Heating curve			Cooling curve		
	Onset (T^a)	Peak (T^b)	Terminal (T^c)	Onset (T^a)	Peak (T^b)	Terminal (T^c)
CCDS oil precipitate	43.6 ± 4.0	47.6 ± 0.1	49.5 ± 0.5	40.6 ± 0.6	39.1 ± 0.7	34.5 ± 3.3
CCDS oil	−15.7 ± 6.0	−14.3 ± 4.7	−13.4 ± 4.0	−2.2 ± 14.6	−3.5 ± 13.4	−4.8 ± 12.4

[a] Temperature at the start of the transition peak

[b] Temperature of the peak

[c] Temperature of the end of the transition peak

Table 8 Neutral and polar lipid fraction in the free and trapped oil extracted from CCDS

Oil extracted (as % recovered)	Oil (%) ± SD	
	Neutral lipid	Polar lipid
Free oil (70)	88.9 ± 0.4a	1.4 ± 0.1b
Trapped oil (30)	73.2 ± 1.9b	16.3 ± 0.0a

Means in each column followed by different letters are significantly different ($p < 0.05$)

Table 9 Fatty acid composition of the polar lipid fraction of trapped oil

Fatty acid	Fatty acid composition (%)		
	PC	PI	PE
Palmitic (16:0)	19.7	32.6	18.9
Stearic (18:0)	2.9	20.3	6.7
Oleic (18:1)	34.0	1.4	24.5
Linoleic (18:2)	42.4	44.3	48.8
Linolenic (18:3)	0.9	0.3	1.1

PC phosphatidylcholine, *PI* phosphatidylinositol, *PE* phosphatidylethanolamine

material may have been present and these could not be eluted using the solvents described in our method. The free oil fraction had a significantly greater neutral lipid fraction than that in the trapped oil and the trapped oil had a significantly greater polar lipid fraction than that in the free oil. The fatty acid composition of the phopholipid classes of the trapped oil is shown in Table 9. Phosphatidylinositol (PI) had the most saturated fatty acids, palmitic and stearic compared to phosphatidylcholine (PC) and phosphatidylethanolamine (PE). PE and PC had more unsaturated fatty acids than PI.

Qualitative TLC showed that the neutral lipid fraction of the free oil contained mainly TAGs, free fatty acids, diacylglycerols, monoacylglycerols, phytosterols, and tocopherols. The polar lipid fraction of the trapped oil contained mostly polar lipid classes such as PC, PI, PE, and some TAGs. The presence of a high concentration of polar lipids in the trapped oil fraction may explain why this fraction is difficult to extract by centrifugation alone.

Conclusions

The CCDS oil precipitate had a high free fatty acid content and very high palmitic acid content compared to CCDS oil. The solid appearance at room temperature was mainly attributed to the presence of saturated fatty acids in the free fatty acid fraction. In addition, the presence of wax at high concentrations may also contribute to the physical characteristics of the CCDS oil precipitate. This product is not a rich source of phytosterols. The CCDS oil precipitate can be used for making biodiesel and waxes can be removed by winterization. Since the CCDS oil precipitate is high in free fatty acid content, acid catalyzed transesterification followed by base catalysis can be used as done in this study. For oil recovery from CCDS, the presence of a high concentration of polar lipids in the trapped oil fraction may explain why this fraction is difficult to extract by centrifugation alone. Therefore, polar solvent may be used for complete oil extraction, or other physical and chemical means for breaking polar interactions need to be used to improve oil extraction.

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