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Fatty Acid, Phytosterol, and Polyamine Conjugate Profiles of Edible Oils Extracted from Corn Germ, Corn Fiber, and Corn Kernels

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Abstract This study compared the profiles of fatty acids, phytosterols, and polyamine conjugates in conventional commercial corn oil extracted from corn germ and in two "new-generation" corn oils: hexane-extracted corn fiber oil and ethanol-extracted corn kernel oil. The fatty acid compositions of all three corn oils were very similar and were unaffected by degumming, refining, bleaching, and deodorization. The levels of total phytosterols in crude corn fiber oil were about tenfold higher than those in commercial corn oil, and their levels in crude corn kernel oil were more than twofold higher than in conventional corn oil. When corn kernel oil was subjected to conventional degumming, refining, bleaching, and deodorization, about half of the phytosterols was removed, whereas when corn fiber oil was subjected to a gentle form of degumming, refining, bleaching, and deodorization, only about 10% of the phytosterols was removed. Finally, when the levels of polyamine conjugates (diferuloylputrescine and *p*-coumaroyl feruloylputrescine) were examined in these corn oils, they were only detected in the ethanol-extracted crude corn kernel oil, confirming earlier reports that they were not extracted by hexane, and providing new information that they could be removed from ethanol-extracted corn kernel oil by conventional degumming, refining, bleaching, and deodorizing.

Keywords Corn · *Zea mays* · Fatty acids · Phytosterols · Germ · Oil

Introduction

Recently we published a report comparing the levels of lutein and zeaxanthin in corn germ oil, corn fiber oil, and corn kernel oil [1] and concluded that corn kernel oil contained the highest levels of both. We also reported the levels of tocopherols and tocotrienols in corn germ oil, corn fiber oil, and corn bran oil [2]. In another study we reported the occurrence of two unusual polyamine conjugates [diferuloylputrescine (DFP) and *p*-coumaroyl feruloylputrescine (CFP)] in corn kernel oil (obtained by extracting ground corn with ethanol), but they were absent in corn germ oil (obtained by extracting corn germ with hexane) [3]. Until now, all commercial corn oil has been obtained by pressing and/or extracting corn germ (obtained mostly from corn wet mills) with hexane and refining it via conventional refining, bleaching, and deodorization (RBD) [4, 5]. In recent years processes have been reported and patented to produce corn fiber oil by extracting corn fiber with hexane or other solvents [6, 7] and to produce corn kernel oil by extracting ground corn with ethanol [8, 9]. Many publications have described the fatty acid profile and phytosterol profiles of corn germ oil (commercial corn oil) [4, 5], but only one has reported the fatty acid profile and phytosterol profile of corn fiber oil (unrefined) [10] and only one has reported the fatty acid profile and phytosterol profile of corn kernel oil (hexane extraction, unrefined)

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[11]. Because of the interest in all three types of these corn oils, this study was designed to compare the fatty acid, phytosterol, and polyamine conjugate profiles of corn germ oil (hexane extracted followed by conventional degumming, refining, bleaching, and deodorization, RBD), corn fiber oil (hexane extracted, unrefined and with gentle RBD), and corn kernel oil (ethanol extracted, unrefined and with conventional RBD).

Materials and Methods

Materials

Corn kernels (yellow dent No. 2) were purchased from a local feed store. Corn fiber (a mixture of pericarp and endosperm fiber) was obtained from a commercial corn wet mill. Commercial corn oil (Mazola®, RBD) was purchased at a local supermarket.

Sample Preparation

Corn kernel oil was prepared by extracting ground corn (common yellow dent No. 2) with ethanol (100%). The ethanol was removed from the miscella with a rotary evaporator. One kilogram of crude oil was degummed (with aqueous citric acid), refined (with NaOH), bleached (with Tonsil 167 FF bleaching clay, Sud Chemie, Louisville, KY, USA), and deodorized (at 260–265 °C for 120 min, with vacuum). The latter are conditions commonly used for commodity edible oils. The levels of free fatty acids in the corn kernel oils were 4.57% before and 0.18% after degumming, refining, bleaching, and deodorization (AOCS official method Ca 5a-40). Corn fiber oil was obtained by extracting ground corn fiber (the mixture of pericarp fiber and endosperm fiber was obtained from a commercial corn wet mill and ground to 20 mesh with a Wiley mill). The ground fiber was extracted with hexane (1 kg fiber/10 L hexane). The hexane was removed from the miscella by rotary evaporation. One kilogram of the crude corn fiber oil was degummed (with aqueous citric acid), refined (with Britesorb™ CNS Purifier, PQ Corporation, Southgate, CA, USA), bleached (with Tonsil 126 FF bleaching clay, Sud Chemie, Louisville, KY, USA), and deodorized (220 °C for 20 min, with vacuum). The latter is a modified and "gentle" process developed to retain more of the phytosterols and other phytonutrients. The levels of free fatty acids in the corn fiber oils were 6.65% before and 0.93% after degumming, refining, bleaching, and deodorization (AOCS official method Ca 5a-40). A comparison of the conventional RBD method used for corn kernel oil and the "gentle" RBD method used for corn fiber oil reveals that the major differences were in the two steps of refining (caustic refining with NaOH was used for corn kernel oil and a resin, Britesorb™ CNS Purifier, was used to remove free fatty acids in corn fiber oil) and deodorization (for corn kernel oil deodorization was at a higher temperature and longer duration, 260–265 °C for 120 min, compared with 220 °C for 20 min used for corn fiber oil). The degumming step was the same for both and the bleaching step was similar (except that two slightly different types of bleaching clay were used).

GC Analysis of FAMEs

Between 0.1 and 0.2 g of each lipid sample (in duplicate) was converted to fatty acid methyl esters (FAMEs) using the AOAC official method [12]. Briefly, 1 mL 20 mg/mL C13:0 (internal standard) dissolved in chloroform was added to each sample in flat-bottomed flasks. Methanolic NaOH (10 mL) was added, and the mixture was refluxed for 10 min, after which time 10 mL BF_3 reagent was added. Reflux continued for an additional 5 min. *n*-Heptane (10 mL) was then added, followed by an additional 1 min of reflux, after which time the reaction mixture was allowed to cool and then transferred to a measuring cylinder/centrifuge tube. The flat-bottomed flask was rinsed with 10 mL saturated NaCl solution, and the wash was transferred to the centrifuge tube. The contents of the centrifuge tube was mixed thoroughly and then kept still for 10 min to allow for phase separation. The organic phase containing the FAMEs was transferred to a gas chromatography (GC) vial and used for analysis. The FAMEs were analyzed in parallel with a FAME standard (Supelco 37-component FAME mix; Supelco, Bellafonte, PA) using an Agilent Technology 6890 N gas chromatograph equipped with a flame ionization detector (FID). An SP-2560, 100 m × 0.25 mm i.d., 0.20 μm film column was used for separation. Duplicate injections (1 μL) for each sample were performed in the split mode at a split ratio of 50:1. Helium was the carrier gas, the linear velocity was 18 cm/s, and the flow rate was 1 mL/min. The oven temperature was initially held at 120 °C for 5 min, then programmed to 240 °C at 3 °C/min, and held isothermally for 20 min. The injection port temperature was 200 °C while that of the detector was 250 °C. The different amounts of FAMEs were analyzed and integrated by an online computer, and values for duplicate samples were averaged to give the fatty acid profile of each sample.

GC Analysis of Sterols

Total sterols were analyzed by the method of Piironen et al. [13] that uses both acid and alkaline hydrolysis, derivatization to trimethylsilyl (TMS) ethers, and analysis using gas chromatography with flame ionization detection

(GC–FID). Dihydrocholesterol was used as an internal standard. In addition to the sterols previously identified [13] a few minor sterols, namely stigmastadienol, gramisterol, β-amyrin, cycloartenol, Δ7-stigmastenol, and citrostadienol, were identified using gas chromatography–mass spectrometry (GC–MS) and were also quantified using GC–FID. The conditions of the GC–MS system were as previously reported [14], and the identification of the sterols was based on relative retention times and mass spectral properties of plant sterols found in the literature [15, 16]. Total plant sterol content of rapeseed oil sample (in-house reference sample) was analyzed daily as a control. The sterol content of the control sample was 890 mg/100 g f.w., which is in line with the reference range (average ± standard deviation, SD) of 902 ± 13 mg/100 g f.w. ($n = 37$).

HPLC Analysis of Polyamine Conjugates

Polyamine conjugates (diferuloylputrescine and *p*-coumaroyl feruloylputrescine) were analyzed using a method that we reported previously [3]. The analyses were performed on a Hewlett-Packard Model 1100 high-performance liquid chromatograph (HPLC, Agilent Technologies, Palo Alto, CA) with an autosampler, with detection via two detectors in series; the effluent first entered a Hewlett-Packard Model 1100 diode array ultraviolet (UV)–visible detector, and then a Sedex Model 55 evaporative light scattering detector (ELSD) (Richard Scientific, Novato, CA) operated at 40 °C, with nitrogen as a nebulizing gas, at a pressure of 2.0 bar. The HPLC column was a LiChrosorb Diol 5 µm (3 × 100 mm, packed by Varian/Chrompack, Walnut Creek, CA), and the isocratic mobile phase was hexane/isopropanol/acetic acid (66.6:33.3:0.1 by volume) at a flow rate of 0.5 mL/min. A sample of purified DFP/CFP mixture (determined to be at least 99% pure by HPLC–ELSD, and estimated to be 85% DFP and 15% CFP by HPLC with UV absorbance measurement at 320 nm) was used to construct a calibration curve which exhibited a linear relationship between area of UV 320 nm and mass in the range of 1–30 µg DFP and 1–10 µg CFP.

The fatty acid and polyamine conjugate experiments were performed in triplicate; mean and standard deviation values are reported. The phytosterol analyses were performed in duplicate; mean values are reported.

Results and Discussion

Corn oil (commercial corn oil, extracted from corn germ) has long been known to be one of the "high-linoleate" vegetable oils [4, 5]. This property is shared with oils from soy, peanut, safflower, cottonseed, and several others. We previously reported that the fatty acid composition of corn fiber oil (unrefined) was similar to that of commercial corn oil, with the former having slightly less linoleate: 56.4% versus 60.1% linoleate, respectively [10]. In the current study we prepared fresh corn fiber oil by extracting wet-milled corn fiber with hexane as previously described [6]. We also prepared fresh corn kernel oil by extracting ground corn with 100% ethanol as previously described [8, 9]. We then sent samples of both corn fiber oil and corn kernel oil to a commercial facility that had them refined, bleached, and deodorized (RBD). After alkaline hydrolysis and conversion of the fatty acids to fatty acid methyl esters (FAMEs), the fatty acid composition of both oils (crude and RBD) and that of commercial corn oil (corn germ oil, RBD) were then analyzed (Table 1).

Linoleic acid was the most abundant fatty acid in all five oils; its concentration ranged from 54.55% in crude corn fiber oil to a high of 57.26% in commercial corn oil, and the levels in both corn kernel oil samples were within these limits. Oleic acid was the next most abundant fatty acid; its concentration ranged from 22.33% to 27.65%. Palmitic was next highest in concentration (10.56–12.29%), followed by linolenic (0.92–4.35%), stearic, arachidic, and palmitoleic acids. The fatty acid composition of the two RBD oils was very similar to those of each of the crude counterparts. Among the data points for corn fiber oil and corn kernel oils, RBD caused some increases and some decreases in individual fatty but there were no consistent trends. In summary, the fatty acid compositions of all five corn oils were very similar, as we reported previously for crude corn fiber oil compared with commercial corn oil [10]. Because corn kernel oil is actually comprised of a combination of oil from the germ and the fiber, it is not surprising that its fatty acid composition is similar to that of germ oil and fiber oil.

Corn oil has also long been known to contain higher than average levels of phytosterols, both free phytosterols (~0.4%) and esterified (fatty acyl) phytosterols (~0.4%) [4, 5]. We previously reported that corn fiber oil contained very high levels of phytosterols [6]. In addition to free (~1.5%) and esterified phytosterols (~4%), corn fiber oil also was found to contain ferulate phytosteryl esters (6–9%) [6].

In the next experiment, the five samples of oils were alkaline-hydrolyzed to convert all of the esterified phytosterols to free forms, and then the total levels of phytosterols (free + esterified) were analyzed by GC (Table 2). The levels of total phytosterols in the crude corn fiber oil were, as expected, extremely high (8.71%) and were only slightly lower in the RBD (gentle conditions) corn fiber oil (7.94%). The levels of total phytosterols in the crude corn kernel oil were 2.40% and were much lower in the RBD corn kernel oil (1.11%). The levels of total

Table 1 Fatty acids in crude and RBD oils measured as fatty acid methyl esters

	Corn kernel oil		Corn fiber oil		Corn germ oil[a]
	Crude (wt%[a])	RBD[b] (wt%)	Crude (wt%)	RBD[c] (wt%)	(wt%)
Myristic 14:0	0.11 ± 0.04[d]	0.04 ± 0	0.06 ± 0	0.05 ± 0	0.04 ± 0
Palmitic 16:0	11.46 ± 0.02	10.56 ± 0.05	12.29 ± 0.03	11.15 ± 0.04	10.72 ± 0.02
Palmitoleic 16:1	0.10 ± 0.03	0.05 ± 0	0.11 ± 0.01	0.11 ± 0	0.05 ± 0.01
Stearic 18:0	1.83 ± 0	1.76 ± 0.01	2.34 ± 0	2.37 ± 0.01	1.85 ± 0.01
Oleic 18:1	24.66 ± 0.03	25.76 ± 0.03	22.33 ± 0.02	22.77 ± 0.01	27.65 ± 0.02
Linoleic 18:2	57.23 ± 0.22	55.52 ± 0.21	54.55 ± 0.03	55.46 ± 0.24	57.26 ± 0.04
Linolenic 18:3 (*n*-6)	1.68 ± 0.03	0.92 ± 0	4.20 ± 0.01	4.35 ± 0.01	1.22 ± 0.01
Arachidic 20:0	0.45 ± 0.01	0.41 ± 0	0.61 ± 0	0.62 ± 0	0.45 ± 0
Other	2.48	4.98	3.51	3.12	0.76

[a] Commercial edible corn oil

[b] With corn kernel oil, a conventional alkali refining, bleaching, and deodorization process was used to simulate the RBD steps employed in the processing of commercial corn oil and most other edible oils

[c] With corn fiber oil, a modified "gentle" refining, bleaching, and deodorization process was used to ensure that the ferulate esters were not degraded

[d] Mean ± SD ($n = 3$)

phytosterols in the commercial corn oil were 0.84%, consistent with previously published values [4, 5]. The most abundant phytosterol in both crude and RBD corn fiber oil was sitostanol (3.2% and 3.9%, respectively), followed by sitosterol, campestanol, and campesterol, with lesser amounts of eight other phytosterols. These results are consistent with those in our previously published phytosterol profile for corn fiber oil except that our units in the previous report were relative percentage of total phytosterols, with 43.1% sitostanol, 34.3% sitosterol, 14.7% campestanol, and 4.9% campesterol [10]. The most abundant phytosterols in the crude corn kernel oil was sitosterol (1.15%), followed by campesterol, sitostanol, and campestanol. The phytosterol composition of corn kernel oil is consistent with the fact that it is comprised mainly of a mixture of oils from both the germ and the fiber regions of

Table 2 Total sterols (mg/100 g oil) in oil samples after alkaline hydrolysis

	Corn kernel oil		Corn fiber oil		Corn germ oil
	Crude	RBD	Crude	RBD	RBD
Campesterol	363	135	680	594	151
Campestanol	147	74	1,279	1,182	13
Stigmasterol	127	46	209	142	54
Sitosterol	1,145	510	2,115	1,897	503
Sitostanol	352	184	3,246	2,964	30
Δ5-Avenasterol	126	38	393	375	29
Stigmasta-5,24(25)-dienol	11	27	57	65	5
Gramisterol + α-amyrin	19	12	106	92	9
Cycloartenol Δ7-stigmastenol	45	44	236	249	18
Δ7-Avenasterol	25	10	145	144	5
24-Methylene cycloartanol	25	20	128	121	15
Citrostadienol	16	11	117	116	8
Total	2,399	1,109	8,709	7,939	840

Corn kernel oil was refined, bleached, and deodorized (RBD) by conventional processes. Corn fiber oil was refined, bleached, and deodorized (RBD) using gentle processes. Commercial corn germ oil was purchased locally

Total sterols: mean ($n = 2$)

the kernel [5]. We previously reported [10] that most of the stanols are in the aleurone cells in the corn fiber and most of the stanols are esterified to ferulate.

We previously reported that, when ground corn was extracted with ethanol, the corn kernel oil contained significant levels (5%) of two polyamine conjugates: diferulylputrescine and *p*-coumaroyl feruloylputrescine [3]. However, when ground corn, corn germ or corn bran was extracted with hexane, no polyamine conjugates were detected in the oils [3]. In the current study we confirmed that we were unable to detect any polyamine conjugates in commercial corn oil and corn fiber oil (both extracted with hexane) (Table 3). Also, as previously reported, high levels of both polyamine conjugates were detected in corn kernel oil extracted with ethanol (Table 3). However, no polyamine conjugates were detected in the RBD corn kernel oil, indicating that refining, bleaching, and deodorizing effectively removed all traces of polyamine conjugates. These results are important because the safety of polyamine conjugates is unknown. If RBD corn kernel oil contained polyamine conjugates, their safety would need to be established before the oil could be used for edible applications. However, since polyamine conjugates appear to be effectively removed by conventional degumming, refining, bleaching, and deodorizing, the issue of their safety is not a major concern. As noted in our previous publication [3], we believe that the increased polarity of ethanol is the main reason that polyamine conjugates were only extracted with ethanol but not with hexane (Table 3). Because the polyamine conjugates have similar HPLC retention times (and similar polarities) to common phospholipids, phosphatidylethanol amine and phosphatidylcholine [3], we speculate that they are probably removed during the degumming process.

Table 3 The effect of conventional refining, bleaching, and deodorizing (RBD) on the polyamine conjugates in various corn oils

	Wt% oil[a]	
	Diferuloylputrescine	*p*-Coumaroyl putrescine
Corn fiber oil (hexane extracted, unrefined)	0	0
Corn fiber oil (hexane extracted, RBD)	0	0
Ethanol-extracted corn kernel oil (unrefined)	0.13 ± 0.02	0.46 ± 0.03
Ethanol-extracted corn kernel oil (RBD)	0	0
Commercial corn oil (RBD)	0	0

[a] Mean ± SD ($n = 3$)

The major differences between the conventional RBD methods used with corn kernel oil and the "gentle" RBD method used with corn fiber oil were described in the Section "Materials and Methods". From a mechanistic standpoint the conventional caustic refining method for corn kernel oil resulted in lower levels of free fatty acids than the gentler method (0.18% FFA in caustic refined corn kernel oil versus 0.93% FFA in corn fiber oil refined with Britesorb CNS Purifier). Resins such as Britesorb CNS are commonly used as filter aids to extend the usable life of frying oils, but they have also been reported to reduce the levels of free fatty acids by about 85% [17]. The major difference in the conventional deodorization method used for corn kernel oil (260–265 °C for 120 min, with vacuum) versus the gentle deodorization method used for corn fiber oil (220 °C for 20 min, with vacuum) is that levels of total phytosterols were reduced by 54% with the conventional deodorization method and by only 9% with the gentle deodorization method (Table 2).

In conclusion, this study compared the profiles of fatty acids, phytosterols, and polyamine conjugates in conventional commercial corn oil and in two "new-generation" corn oils. The fatty acid compositions of all three corn oils were very similar and were unaffected by RBD. The levels of total phytosterols in crude corn fiber oil were about tenfold higher than those in commercial corn oil, and their levels in crude corn kernel oil were more than twofold higher than in conventional corn oil. When crude corn kernel oil was subjected to conventional RBD, about half of the phytosterols was removed, whereas when crude corn fiber oil was subjected to a gentle RBD, only about 10% of the phytosterols was removed. Finally, when the levels of polyamine conjugates were examined in these corn oils, they were only detected in the crude ethanol-extracted corn kernel oil, confirming earlier reports that they were not extracted by hexane, and providing new information that they could be removed from ethanol extracted corn kernel oil by conventional refining, bleaching, and deodorizing.

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