Phytosterols in Cereal By-products

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ABSTRACT: Phytosterols are hypocholesterolemic. Like corn fiber oil, the lipid extracts of certain cereal by-products may be rich sources of these health-promoting compounds. The objective of this research was to examine the phytosterol content and composition of various cereal by-products. Total lipids in rice bran, wheat bran, wheat germ, durum wheat (bran and germ mixture), oat bran, oat hull, and corn fine fiber were extracted, and the sterol profiles of the extracted lipids were analyzed by GC. Rice bran contained the most lipids (22.2%), followed by wheat germ, durum wheat, oat bran, wheat bran, and oat hull; corn fine fiber contained the least amount of lipids (1.7%). Sitosterol, campesterol, and stigmasterol were the major phytosterols in these lipid extracts, whereas brassicasterol was detected only in wheat samples. Rice bran oil contained considerable amounts of cycloartenol and 24-methylenecycloartanol, which were unique to these samples. Total sterol concentrations in extracted lipids were similar for rice bran, wheat bran, wheat germ, and durum wheat (21.3–15.1 mg/g), but they were very low in oat bran lipids and oat hull lipids (3.4 and 8.2 mg/g, respectively). Corn fine fiber lipids contained the highest amount of sterols (48.3 mg/g). Rice bran appears to be the best source of phytosterols, with the highest oil content and high concentration of sterols.

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The class of sterols known as phytosterols is found mainly in plant cell walls and membranes. It has been well documented in animal and human studies that dietary phytosterols can lower serum LDL and total cholesterol concentrations by reducing intestinal cholesterol absorption (1–5).

The sterol contents of foods of plant origin have been reviewed previously (6,7). Recently, a systematic analysis of free and esterified sterols in various vegetable oils was reported (8). However, information regarding the phytosterol content and composition of some of the major cereal by-products is limited. Similar to corn fiber, many low-valued cereal by-products may contain significant amounts of phytosterols, and they could become potential sources of these health-enhancing compounds. Considerable differences were found in the phytosterol contents of different parts of the cereal kernels, and thus in various milling products, and the germ and bran fractions are known to be the best sources of sterols (7). For example, refined wheat flour and wheat bran had total sterol contents of 0.4 and 1.7 mg/g, respectively. Some studies have been conducted specifically to examine the ferulic acid and cinnamic acid esters of the phytosterols in rice bran, corn fine fiber, wheat, and rye (9–13), whereas others have focused on studying the sterol distribution within specific cereals (7). The objective of this study was to comparatively examine the total phytosterol composition and to quantify the total sterols (TS) and free sterols (FS) in some of the major cereal by-products.

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MATERIALS AND METHODS

Cereal by-products. Rice bran, wheat bran, wheat germ, oat bran, and oat hull samples were obtained from commercial milling operations of ConAgra (Omaha, NE). The durum wheat sample, which was a mixture of germ and bran, was provided by Barrila North America (Ames, IA). Corn fine fiber was obtained from the corn wet-milling research facility at the Center for Crops Utilization Research of Iowa State University. Briefly, 10 kg of corn was soaked, coarsely ground, the germ removed, and then finely ground. The coarse fiber was removed by a 50-mesh screen (300 μm size), and the fine fiber was removed by a 200-mesh screen (75 μm size).

Total lipid extraction. Chloroform/methanol (2:1, vol/vol) was used to extract total lipids from the raw materials. Samples were soaked in the solvent overnight at a 1:5–8 (wt/vol) solid to liquid ratio to ensure complete lipid extraction. Different amounts of solvent were used because of differences in the absorptive nature of the materials. A second extraction was done with a smaller amount of solvent for about 2 h with stirring. After filtration, a wash following the method of Folch *et al*. (14) was performed by adding 20% water to the combined solvent extract, and the system was thoroughly mixed to remove water-soluble substances. The chloroform fraction was collected and the solvent was evaporated to obtain the total lipids. Based on our experience, polar lipid extraction from soybean material was expected to extract the total lipids.

Sample preparation for quantification. To free the esterified phytosterols and to remove the glycerol lipids, the total lipid extract was saponified. Saponification was carried out according to the standard AOCS method, Ca 6b-53 (15). Briefly, about 1 g of the extracted total lipid was saponified with 3 mL of 50% KOH and 3 mL of ethanol in a boiling-water bath for 30 min. After the reaction, 5 mL of water was added and the unsaponifiable matter was extracted three times with 5 mL of diethyl ether, and the combined ether extract was then washed three times with 5 mL of water. The diethyl ether extract was

further washed with 5 mL of 0.5 N KOH to remove any FFA, followed by washing three times with 5 mL of water. After a second wash with 0.5 N KOH, the diethyl ether fraction was washed successively with 5-mL portions of water until the washings were no longer alkaline to phenolphthalein. The unsaponifiable matter was obtained after evaporating the ethyl ether, and the lipids were then dissolved in 1 mL of chloroform for GC analysis.

Sample preparation for FS quantification. Solid-phase extraction (SPE) and TLC were used to fractionate the FS from the total lipid extract. About 0.2 g of total lipid was dissolved in 2 mL of hexane and loaded on a 1000-mg silica SPE column (Bond Elut; Varian, Palo Alto, CA). Neutral lipids were eluted by 15 mL of 5% ethyl ether in hexane. The FS were then eluted by a solvent mixture of 15 mL ethanol/ethyl ether/hexane (50:25:25). The solvent was evaporated under N_2 to obtain the FS fraction. These solvents and elution volumes were optimized for the best separation of neutral lipids and the relatively polar FS. TLC analysis showed that there was no loss of FS in the neutral lipid fraction and that all the FS were eluted with ethanol/ether/hexane, although this fraction still contained small amounts of neutral oil. The FS fraction was redissolved in 0.2 mL of chloroform and streaked on a 20 × 20 cm, 500 μm Uniplate Silica Gel G preparative TLC plate (Alltech, Deerfield, IL), which was preactivated at 110°C for 1 h. The plate was developed with hexane/ethyl ether/acetic acid (90:10:2) to move any sterol ester contamination and other neutral lipids to the top of the plate (9), followed by benzene/ethyl ether (80:20) to separate the FS. The FS silica band was collected and the silica was extracted with 3 × 12 mL of ethanol/ethyl ether/hexane (50:25:25) to obtain the FS. The extracted FS were then subjected to a similar saponification procedure as described above to remove any possible contaminants. The unsaponifiable matter was dissolved in chloroform for GC analysis.

Phytosterol quantification by GC. Sterols in their free form were separated on a SAC-5 capillary column (0.25 mm i.d., 30 m long, and 0.25 μm film thickness; Supelco, Bellefonte, PA) with the following temperature program: hold at 250°C for 5 min, the temperature increased to 265°C at a rate of 1°C/min, and hold at 265°C for 25 min. The injector and detector temperatures were set at 280°C. Flow rate of the carrier gas (helium) was 1 mL/min and the injection split ratio was 1:50. For quantification, cholestane was added to all samples as the internal standard. The detector responses for all phytosterols were assumed to be the same as for cholestane.

GC–MS was used to provide further information for sterol identification or confirmation. The GC column and separation conditions were the same as those used for sterol quantification for unknown identification, using an Agilent 6890 Series GC system (Agilent Technologies, Palo Alto, CA) coupled with a Micromass GCT mass spectrometer (Micromass, Beverly, MA). EI ionization was used, with the scan cycle at 0.75 s and the mass range from 0 to 700.

Quantification of steryl ferulate. When the FS extract from SPE was separated on a TLC plate by developing it with hexane/ethyl ether/acetic acid (90:10:2) followed by benzene/ether (80:20), dark blue bands (compared with the yellow fluorescent sterols after spraying with 2′,7′-dichlorofluorescein and viewing under UV light) above the FS were identified as steryl ferulate (9). These bands were collected and extracted three times with 12 mL of ethanol/ethyl ether/hexane (50:25:25), and the extracts were saponified as described above. The freed sterols were then quantified by GC.

RESULTS AND DISCUSSION

Total lipids in the raw materials. The moisture and lipid contents of the raw materials are presented in Table 1. Rice bran was the most lipid-rich material, with 22.2% total lipids extracted on a dry weight basis. Wheat germ contained more lipids (11.8%) than wheat bran (6.1%). Since durum wheat material was a mixture of bran and germ, its lipid content fell in between, as 8.7%. The amount of lipids in oat bran was comparable (7.5%) to that in wheat bran, and oat hull contained a much lower level of lipids (3.3%). Corn fine fiber had the least amount of total lipids (1.7%). Moreau *et al*. (16) reported that corn fine fiber contained 1.6–2.6% lipids, which generally agrees with what we found.

Total phytosterol content. A gas chromatogram of rice bran lipid extract with separation and quantification of phytosterols is shown as Figure 1. The total quantification of phytosterols is summarized in Table 2. Responses of the FID to cholestane, cholesterol, and sitosterol were tested and found to be very similar. The slopes of the linear detector response vs. concentration relationships were within the 5% range. However, when the GC column deteriorated with time, as shown by the poorly resolved and widening peaks, the responses tended to change, with a significant reduction of signal intensity for phytosterols compared with that for cholestane. All our analyses were performed on a new GC column, and the detector responses for the three compounds were determined to be nearly identical at the time of analysis. Lampi and Piironen (17) reviewed and discussed the chromatographic responses (FID) of phytosterols; they presented evidence that an equal response has been used successfully and that it can be used for sterol quantification.

The total lipids extracted from corn fine fiber contained the most phytosterols, 48.3 mg/g of lipids (Table 2). Rice bran and wheat germ had similar levels of phytosterols in their lipid

TABLE 1
Moisture and Total Lipid Contents (%) in the Cereal By-products

Product	Moisture	Total lipids[a]
Rice bran	9.7	22.2
Wheat bran	12.3	6.1
Wheat germ	12.3	11.8
Durum wheat	11.5	8.7
Oat bran	8.6	7.5
Oat hull	9.1	3.3
Corn fine fiber	5.1	1.7
$LSD_{0.05}$		2.2

[a]Dry-weight-basis.

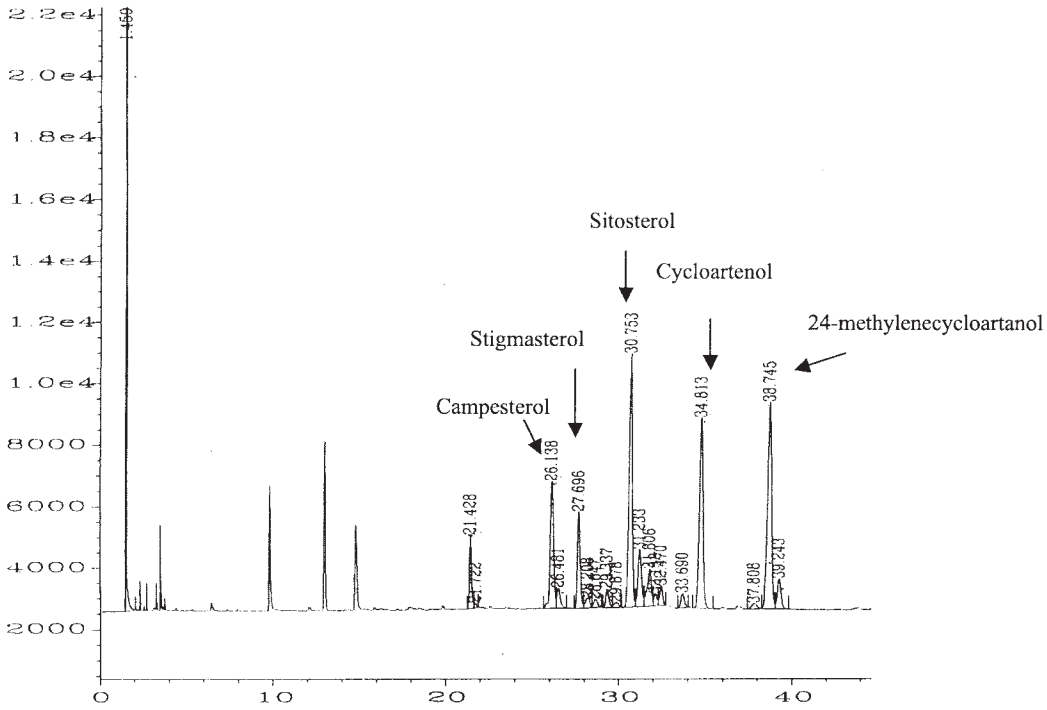

FIG. 1. A gas chromatogram of phytosterol separation and quantification of rice bran lipid extract.

extracts (20.3 and 21.3 mg/g). Wheat bran and durum wheat lipids had slightly lower levels of phytosterols (17.7 and 15.1 mg/g) compared with the wheat germ lipid sample. Oat lipids had very low phytosterol contents, 8.2 mg/g in oat hull lipids and 3.4 mg/g in oat bran lipids. The total phytosterol content in corn fine fiber (4.8% of the total lipids) was comparable to the value reported by others, which was 5.3% in total extracted lipids (16). It is worth mentioning that the same authors also reported that corn coarse fiber contained much higher total phytosterols (10.9% in total lipids) than the fine fiber.

In terms of the raw material, rice bran had the highest phytosterol content, i.e., 4.5 mg/g bran (Table 2). Wheat germ was the second-richest source of phytosterol (2.4 mg/g), followed by durum wheat (1.8 mg/g), oat bran (1.5 mg/g), and wheat bran (1.2 mg/g). Because of the low lipid content and phytosterol concentration in the lipid extract, oat hull had a relatively low sterol content of 0.7 mg/g. Corn fine fiber contained the least amount of phytosterols (0.3 mg/g), even though its total lipids had the highest concentration of phytosterols. Piironen and Lampi (7) reported that wheat bran contained 1.5–1.9 mg/g phytosterols and wheat germ contained 4.1 mg/g. Our quantification was slightly below these values. They also showed that

TABLE 2
Total Phytosterol Contents (as mg free sterols/g lipids) in Total Lipid Extracts of Cereal By-products

	Brassica-sterol	Campe-sterol	Campe-stanol	Stigma-sterol	Sito-sterol	Sito-stanol	Unknown - 1[a]	Unknown-2	Cyclo-artenol & like phytosterols	24-Methylene cycloartanol-& like phytosterols	Total (mg/g lipids)	Total (mg/g raw material)
Rice bran	—	2.61	0.36	1.80	4.94	0.84	0.86	0.34	3.33[b]	5.25[c]	20.33	4.5
Wheat bran	0.56	3.66	1.74	0.27	4.53	2.45	0.65	0.31	0.48[d]	3.01[e]	17.67	1.2
Wheat germ	0.15	4.70	0.67	0.22	11.23	0.83	1.41	0.35	0.69[d]	1.03[e]	21.28	2.4
Durum wheat	0.57	2.18	2.90	0.35	4.71	2.10	1.10	0.15	0.31[d]	0.70[e]	15.07	1.8
Oat bran	—	0.22	0.04	0.13	1.56	0.07	0.69	0.06	0.26[b]	0.38[c]	3.41	1.5
Oat hull	—	0.68	0.09	0.53	4.09	0.49	0.72	0.20	0.53[b]	0.85[c]	8.18	0.7
Corn fine fiber	—	4.85	1.14	5.01	27.63	4.15	1.74	1.47	1.44[d]	0.82[e]	48.25	0.3
$LSD_{0.05}$	0.04	0.67	0.21	0.10	0.95	0.20	0.15	0.05	0.11	0.53	2.60	

[a]This unknown peak was identified as lanosterol in rice bran lipids and as avenasterol in wheat bran and oat bran lipids. It was not identified in other samples.
[b]Identified as cycloartenol.
[c]Identified as 24-methylenecycloartanol.
[d]Unidentified compounds that had the same GC retention time as cycloartenol but had different MS spectra.
[e]Unidentified compounds that had the same GC retention time as 24-methylenecycloartanol but had different MS spectra. A dash (—) indicates not detected.

TABLE 3
Composition (%) of Total Phytosterols

Product	Brassica-sterol	Campe-sterol	Campe-stanol	Stigma-sterol	Sito-sterol	Sito-stanol	Unknown -1[a]	Unknown -2	Cycloartenol & like phytosterols	24-Methylene-cycloartano & like phytosterols
Rice bran	0.0	12.8	1.8	8.8	24.3	4.1	4.2	1.7	16.4[b]	25.8[c]
Wheat bran	3.2	20.7	9.9	1.5	25.6	13.9	3.7	1.7	2.7[d]	17.0[e]
Wheat germ	0.7	22.1	3.1	1.0	52.8	3.9	6.6	1.7	3.3[d]	4.8[e]
Durum wheat	3.8	14.4	19.3	2.3	31.3	14.0	7.3	1.0	2.1[d]	4.5[e]
Oat bran	0.0	6.5	1.2	3.9	45.7	2.0	20.1	1.8	7.5[b]	11.3[c]
Oat hull	0.0	8.3	1.1	6.4	50.0	6.0	8.8	2.4	6.5[b]	10.4[c]
Corn fine fiber	0.0	10.1	2.4	10.4	57.3	8.6	3.6	3.0	3.0[d]	1.7[e]

[a]This unknown peak was identified as lanosterol in rice bran lipids and as avenasterol in wheat bran and oat bran lipids. It was not identified in other samples.
[b]Identified as cycloartenol.
[c]Identified as 24-methylenecycloartanol.
[d]Unidentified compounds that had the same GC retention time as cycloartenol but had different MS spectra.
[e]Unidentified compounds that had the same GC retention time as 24-methylenecycloartanol but had different MS spectra.

sterols were not concentrated in the bran of oats (0.4–0.6 mg/g) (7); however, our results showed a 1.5 mg/g concentration in oat bran. Differences in variety and milling practices are likely the reason for the observed differences.

The phytosterol compositions were different among these samples, as shown in Table 3. Generally, sitosterol, campesterol, and stigmasterol were the major phytosterols in these samples. Rice bran oil contained very high concentrations of cycloartenol and 24-methylenecycloartanol, which made up over 40% of the total phytosterols. These two unique sterols were also detected in oat bran (7.5%, 11.3%) and oat hull (6.5%, 10.4%). We noted two peaks with GC retention times similar to cycloartenol and 24-methylenecycloartanol that were present in wheat samples and corn fine fiber, but the MS spectra showed they were not because of their different M.W. and MS spectra. There was not enough information to identify these peaks.

In comparing the wheat bran and wheat germ phytosterol compositions, wheat bran had much higher levels of saturated phytosterols (13.9% sitostanol and 9.9% campestanol vs. 25.6% sitosterol and 20.7% campesterol) than wheat germ (3.9% sitostanol and 3.1% campestanol vs. 52.8% sitosterol and 22.1% campesterol). It is also interesting to note that durum wheat had an even higher relative percentage of campestanol (19.3%) than campesterol (14.4%), and it also had a relatively high level of sitostanol. Phytosterols are known to be cell membrane components that modulate membrane fluidity. Usually the more unsaturated a lipid molecule is, the more fluidity it will provide to the membrane. Why cells in the bran or aleurone layer may require more saturated phytosterols than the cells in the germ is unknown. Why durum wheat, the relatively cold-weather and hardest of all wheats, contained a large amount of saturated phytosterols is also unclear. The sterol composition differences among cereal kernels were also reported by Piironen and Lampi (7). In wheat, rye, and corn, stanols were concentrated in the outer layers, whereas they were almost absent from the germ.

Other minor phytosterols were identified in these samples, as listed under the Unknown-1 column of Tables 2 and 3. Lanosterol (0.86 mg/g) was detected (by GC–MS) in rice bran oil, and avenasterol was detected (by GC–MS) in wheat and oat samples. As shown in Figure 1 for rice bran sterols, a few low-concentration sterols were identified, including lanosterol, that eluted between sitosterol and cycloartenol. Avenasterol should have eluted in this time window, but the signal was probably too weak to be confirmed by GC–MS and was not quantified. Kochhar (18) reported that rice bran oil contained about 3 mg of avenasterol/g oil, which is much higher than we could have identified in our sample. For oat bran sterols, this Unknown-1 compound was identified as avenasterol, and it was present as 20% of the total sterols. Hammond (19) reported that avenasterol constituted 34% total sterols in whole oats (*Avena sativa*), which supports our finding. Brassicasterol was detected only in wheat bran, wheat germ, and durum wheat samples.

Free phytosterol content. During fractionation of FS, we noticed that after SPE, the FS fraction still contained about 20% of the oil, which made the GC separation of sterols unsatisfactory when directly injected into the gas chromatograph because of column contamination with this neutral oil. After saponification, injections of the unsaponifiable matter gave higher sterol concentrations than that obtained by direct injection of the FS fraction (with residual oil), suggesting that some sterol esters also may have been present in this fraction, resulting in higher total FS after saponification. It is also possible that some sterol ferulate esters were eluted with FS in this fraction, because sterol ferulate esters are relative polar and can be eluted closely with FS. Therefore, TLC was used to separate FS from the neutral lipids and sterol ferulate esters contained in the FS extract from SPE. The sterol ferulate appeared as blue bands under UV light, as confirmed using a standard. These ferulate bands were especially apparent in rice bran, wheat bran, durum wheat, and corn fine fiber.

The free phytosterol contents are shown in Table 4. The majority of sterols were in free form relative to TS, e.g., 88.2% in wheat bran, 68.9% in durum wheat, 62.9% in corn fine fiber,

TABLE 4
Free Phytosterol Content (mg/g total lipids) in Total Lipid Extracts of Cereal By-products

Product	Campe-sterol	Campe-stanol	Stigma-sterol	Sito-sterol	Sito-stanol	Unknown -1[a]	Cycloartenol & like phytosterols	24-Methylene-cycloartanol & like phytosterols	Total free sterols	Total sterols (%)
Rice bran	1.22	—	1.46	3.25	—	—	0.23[b]	1.49[c]	7.65	37.6
Wheat bran	0.91	4.16	0.28	3.95	1.25	0.39	—	4.65[d]	15.59	88.2
Wheat germ	3.05	—	0.19	6.11	0.48	0.58	—	0.42[d]	10.82	50.9
Durum wheat	1.67	1.40	0.36	3.97	1.15	0.71	—	1.12[d]	10.39	68.9
Oat bran	0.23	—	0.13	1.40	—	0.30	—	0.08[c]	2.13	62.5
Oat hull	0.36	—	0.30	2.12	—	—	—	0.13[c]	2.91	35.5
Corn fine fiber	4.38	—	4.53	18.90	1.39	1.14	—	—	30.33	62.9
LSD 0.05	0.58		0.15	0.68	0.24	0.39		0.72	1.62	

[a]This unknown peak was identified as lanosterol in rice bran lipids and as avenasterol in wheat bran and oat bran lipids. It was not identified in other samples.
[b]Identified as cycloartenol.
[c]Identified as 24-methylenecycloartanol.
[d]Unidentified compounds that had the same GC retention time as 24-methylenecycloartanol but had different MS spectra. A dash (—) indicates not detected.

and 62.5% in oat bran. The wheat germ sample contained about half of its TS in free form (50.9%). In rice bran, most of the phytosterols were in the esterified form and FS made up only 37.6% of the total. The FS content was also lower in oat hull (35.5%). The high free phytosterol contents in wheat and oats were reported by Toivo *et al.* (20), but those authors analyzed the whole grain instead of different by-product fractions. For corn fine fiber, the FS were reported as 22% of the TS (16), and the esters included 49% FA esters and 31% ferulate esters.

Steryl ferulate content. Ferulate esters of the phytosterols were relatively abundant only in several samples; therefore, they were quantified only in these samples, as presented in Table 5. Rice bran is a rich source of these esters, commonly referred to as oryzanols. These steryl ferulates were reported to be 1.1–2.6% (9), 1.6% (21), and 1.6–2.7% (13) in rice bran oil; however, the concentration we determined in our sample was only 0.4%. Corn fiber was also reported as a good source of these ferulate esters, ranging from 1.7 to 2.7% in oil (16); however, our value for corn fine fiber oil was only 0.3%, which was much lower than reported. These ferulate esters in corn oil were also reported to be about 31% of the TS (16), which is much higher than our value of 6.1% of the TS. The reason for these discrepancies could be because of a difference in varieties, or any difference in the processing of these by-products.

With regard to the steryl ferulate ester composition, rice bran contained more unsaturated sterols than saturated ones (campesterol and sitosterol vs. campestanol and sitostanol), whereas wheat bran, durum wheat, and corn fine fiber contained considerably more saturated sterols than unsaturated ones. Norton (21) also reported that the predominant ferulate esters in corn fiber oil were sitostanyl and campestanyl ferulates. The principal ferulate esters in rice bran oil were cycloartenyl and 24-methylenecycloartanyl ferulates (60% of the ferulate esters), and campesteryl ferulate consisted of 18% of the total sterols. Gopala Krishna *et al.* (13) also reported 30–38% 24-methylenecycloartanyl ferulate and 24–27% campesteryl ferulate in rice bran oil. Similarly, Xu and Godber (22) reported that cycloartenyl, 24-methylenecycloartanyl, and campesteryl ferulates accounted for 80% of oryzanols in rice bran oil.

Thus, if there were an economical way to extract total lipids from corn fiber, this fiber oil would be the best source of phytosterols. Wheat germ oil and rice bran oil are the other two oils rich in phytosterols (having half the concentration as in corn

TABLE 5
Steryl Ferulate Ester Content (mg free sterols/g total lipids) in Total Lipid Extracts of Cereal By-products

Product	Campe-sterol	Camp-estanol	Stigma-sterol	Sito-sterol	Sito-stanol	Cycloartenol & like phytosterols	24-Methylene-cycloartanol & like phytosterols	Total ferulates	Total sterols (%)
Rice bran	0.75	0.26	0.11	0.36	0.16	0.45[a]	2.03[b]	4.13	20.3
Wheat bran	0.16	0.96	—	0.14	0.79	—	—	2.05	11.6
Durum wheat	0.03	0.97	0.02	0.05	0.40	—	—	1.47	9.8
Corn fine fiber	0.15	0.49	0.03	0.42	1.86	—	—	2.95	6.1
$LSD_{0.05}$	0.09	0.21	0.02	0.11	0.83	0.07	0.05	1.21	—

[a]Identified as cycloartenol.
[b]Identified as 24-methylenecycloartanol. A dash (—) indicates not detected.

fiber oil). Rice bran is the best source of total lipids and phytosterols based on raw material, followed by wheat germ. Rice bran oil is also the richest source of ferulic acid esters of phytosterols, followed by corn fiber.

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