Plant Sterols in Cereals and Cereal Products

V. Piironen,[1,2] J. Toivo,[1,3] and A.-M. Lampi[1]

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ABSTRACT

The total plant sterol contents (free sterols and covalently bound structures) of the main cereals cultivated in Finland were determined. Furthermore, sterol contents were determined for different flour and bran fractions in the milling process of wheat and rye, as well as plant sterol contents in various milling and retail bakery products. The sample preparation procedure included acid and alkaline hydrolysis to liberate sterols from their glycosides and esters, respectively. Free sterols were extracted and, after recovery using solid-phase extraction, derivatized to trimethylsilyl ethers for gas chromatography (GC) analysis. We used GC with a mass spectrometer (MS) for identification. When two cultivars of rye, wheat, barley, and oats grown in the same year were compared, the highest plant sterol content was observed in rye (mean content 95.5 mg/100 g, wb), whereas the total sterol contents (mg/100 g, wb) of wheat, barley, and oats were 69.0, 76.1, and 44.7, respectively. In addition, the 10 rye cultivars and breeding lines compared had total sterol contents of 70.7–85.6 mg/100 g. In the milling process of rye and wheat, the plant sterols fractionated according to the ash content of the corresponding milling product. In all cereal grain and milling product samples, sitosterol was the main sterol. The level of stanols differed in the different milling process samples; it was lower in the most refined rye and wheat flours (≈15%) than in the bran fractions (≈30% in the bran with 4% ash content). Rye bread with whole meal rye flour as the main or only ingredient was a good source of sterols. Sterol content was higher than that of wheat bread, whereas plant sterol content of other bakery products was affected by the type and amount of fat used in baking.

Plant sterols are one of the food components currently being especially actively studied. They have decreased serum cholesterol levels in several studies (Miettinen et al 1995; Hendriks et al 1999; Hallikainen et al 2000; Jones et al 2000) and they may also be beneficial in preventing colon cancer (Rao and Janezic 1992; Awad et al 1998). Their potency in decreasing serum cholesterol levels and thus protecting against cardiovascular diseases has led to the development of functional foods enriched in plant sterol. In the products currently on the market, plant sterols are derived either from tall oil or soy. The beneficial health effects of plant sterols led to a rise in interest in plant sterols of various natural materials and on the means to optimize these levels (Piironen et al 2000a).

The most important natural sources of plant sterols in human diets are oils and margarines. Cereal products are also recognized as significant plant sterol sources, whereas plant sterol content of vegetables, given on fresh weight basis, is considerably lower (Weihrauch and Gardner 1978; Dutta and Appelqvist 1996; Normen et al 1999; Piironen et al 2000b). The most abundant sterols in these natural sources are 4-desmethylsterols, such as sitosterol, campesterol, stigmasterol, Δ5-avenasterol, and Δ7-avenasterol; sitosterol was the predominant sterol. Other sterols like saturated stanols and sterols synthesized earlier in the biosynthesis pathway, such as the 4-monomethyl and 4,4-dimethyl sterols, usually occur in lower amounts. In cereals, plant sterols occur as free sterols, steryl esters with fatty acids, or phenolic acids, steryl glycosides, and acylated steryl glycosides. The level of these components vary in different cereals and in different parts of the kernel (Seitz 1989; Norton 1994; Moreau et al 1998; Toivo et al 1999).

Although cereals and cereal products are generally regarded as significant plant sterol sources, recent data on sterol contents is scarce (Chung and Ohm 2000; Piironen et al 2000a). In older articles, mainly percentage compositions (levels of main sterols or sterol classes) are given. On the other hand, when quantitative results (sterol contents in mass units) are given, they usually include only free sterols and their esters, but steryl glycosides, which comprise a significant part of the total sterol content in cereals (Toivo et al 1999; Chung and Ohm 2000), are excluded from the results. This is caused by shortcomings in the analytical methods used. The sample preparation for plant sterol analysis usually starts with saponification, which liberates sterols from their esters, and continues by extracting the unsaponifiables. After partial purification of the sterols, they are quantified as trimethyl silyl ethers by gas chromatography (GC). Alternatively, lipids are extracted first and saponified after this step (Piironen et al 2000a). Sterols are not liberated from their glycosides in these methods. They can be hydrolyzed by acid or enzymatic hydrolysis methods. Previously, plant sterol contents obtained without the acid hydrolysis step were reported as 64–80% of that of samples prepared with this treatment (Jonker et al 1985; Piironen et al 1999; Toivo et al, *in press*). To our knowledge, only Jonker et al (1985), Dutta and Appelqvist (1996), Piironen et al (1999), Määttä et al (1999), and Toivo et al (*in press*) determined the plant sterol composition in a few cereals or cereal products after a sample preparation method also capable of hydrolyzing steryl glycosides. The specificity of the analytical methods has also improved as chromatographic columns and mass spectrometric (MS) identification techniques have developed. Furthermore, variation caused by genetic factors or growing conditions are not well known. Fractionation of sterols in milling processes and the significance of different milling and bakery products as natural dietary plant sterol sources should also be studied by modern specific methods.

The aim of the present study was to determine the total plant sterol contents (the sum of free and covalently bound sterols) of the main cereals cultivated in Finland. Furthermore, distribution of plant sterols in the milling process of rye and wheat as well as the contents of plant sterols in milling and bakery products available for retail sale were determined. In this study, the plant sterol contents of cereals and cereal products were determined using a sample preparation method capable of hydrolyzing all bound sterols classes to the corresponding free sterols, which were determined by GC.

MATERIALS AND METHODS

Sampling

Whole grain samples of two common Finnish wheat (*Triticum aestivum L.*), rye (*Secale cereale L.*), oats (*Avena sativa L.*), and barley (*Hordeum vulgare L.*) cultivars were obtained from Boreal Plant Breeding in 1997 (Table I). In addition, samples of 10 rye cultivars and breeding lines were taken from the same source in 1999 (Table II). Four of them were Finnish population cultivars, three were Finnish population breeding lines, two were German

[1] Department of Applied Chemistry and Microbiology, P.O. Box 27, (Latokartanonkaari 11), FIN-00014 University of Helsinki, Finland.

[2] Corresponding author: Department of Applied Chemistry and Microbiology, P.O. Box 27 (Latokartanonkaari 11), FIN- 00014 University of Helsinki, Finland. Fax: + 358 9 191 58222. E-mail: vieno.piironen@helsinki.fi

[3] Present address National Technology Agency, P.O. Box 69, FIN-00101 Helsinki, Finland.

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hybrid cultivars, and one was an Estonian population cultivar. All the cultivars were grown in experimental fields in the same area of Finland. Samples from milling processes of rye and wheat were taken directly from an industrial plant (Oululainen Mill, Fazer Co., Finland) in 1997 and 1998; samples of oat bran and wheat germ were collected once (Melia Ltd., Finland). Samples (300 g to 2 kg) were delivered to the laboratory. Grain samples were homogenized with a mill (Cyclotec 1093 sample mill, Tekator, Sweden or Mill KT-30, Koneteollisuus Oy, Finland). If needed, other samples were prepared with a household homogenizer (Bamix, Type M 133, Esge AG, Mettlen, Switzerland) just before analysis.

Samples of milling and bakery products available for consumers were purchased from 10 retail stores in the Helsinki area representing the major food chains in Finland (mainly in 1997). One package (250 g to 2 kg) of each milling product and bread was bought from each shop. Sampling of some flour samples was repeated. Subsamples representing the same item and bought at the same sampling time were pooled by combining equal amounts of each subsample in the pool. The bakery products were cut into cubes before pooling. Flours did not require any pretreatment, while samples of the other milling products and bakery products were prepared with the household homogenizer before the analysis.

Sample Preparation for Sterol Analysis

The solvents were the same as used by Toivo et al (2000). As an internal standard either cholesterol (99+%, Sigma-Aldrich) or dihydrocholesterol (≈95%, Sigma-Aldrich) was used. The internal standard was added to the sample (1 g) weighed in a 39-mL test tube at the beginning of the determination. For acid hydrolysis, 5 mL of 6*M* HCl was added and the tube contents were mixed for 10 sec with a test tube mixer (Vibrofix VF1, Janke and Kunkel, Ika Labortechnik, Germany) before the tube was placed into a shaking water bath (80–85°C) for 60 min. The tube was mixed manually every 10 min. After the tube was cooled, lipids were extracted with 20 mL of a hexane and diethyl ether (1:1) solvent mixture by shaking for 10 min using a sample tube rocker (AEG, C. Desaga GmbH, Heidelberg, Germany). The organic layer was separated by letting the tubes stand for 15 min (and centrifuging if needed). The organic layer was transferred to a round-bottom flask and evaporated to dryness in a rotary evaporator at 50°C. For saponification, 8 mL of absolute ethanol was added to the residue and the solution was transferred to a 50-mL test tube. After adding 0.5 mL of saturated aqueous KOH solution, the tube contents were mixed (10 sec) using the test tube mixer and the tube was placed into a shaking water bath (80–85°C) for 30 min (shaking 100 times per minute) and then cooled. For extraction of the unsaponifiables, 12 mL of water and 20 mL of cyclohexane were added, followed by shaking for 10 min using the sample tube rocker. An aliquot of 15 mL of the organic layer was transferred to a round-bottom flask and evaporated to dryness in a rotary evaporator at 50°C. The residue was dissolved in either 1 mL of chloroform (when using a C18 cartridge to purify the unsaponifiable fraction) or in 1 mL of hexane (when using a SiOH cartridge). Initially, C18 cartridges were used. Use of SiOH cartridges later in the study improved the repeatability of the method and shortened the time needed for the solid phase extraction.

A C18 cartridge (MegaBond Elut, 1.0 g, Varian, Palo Alto, CA) was activated by 5 mL of methanol and 5 mL of water. The sample solution was first filtered through a nylon acrodisc 13 syringe filter (0.45 µm, Pall Gelman Laboratory, Ann Arbor, MI). The solution was eluted by gravity flow for a couple of minutes and then by vacuum (5–10 Hg; vacuum manifold, Visaprep DL, Supelco, Bellefonte, PA) for 5 min. The sterol fraction was eluted with 15 mL of methanol and chloroform (5:95). The solution was eluted to a round-bottom flask and evaporated to dryness as above.

A SiOH cartridge (Varian Bond Elut, 0.5 g) was activated by 5 mL of hexane. After filtering and applying the sample as a hexane extract, the cartridge was washed with 5 mL of hexane and 5 mL of a hexane and diethyl ether (90:10) solvent. The sterol fraction was eluted with 5 mL of a hexane and diethyl ether (50:50) solvent and evaporated to dryness at 50°C under a gentle stream of nitrogen (N-EVAP analytical evaporator, model 111, Organomation Assoc., Berlin, MA). The sterol fraction residue was dissolved in 500 µL of dichloromethane and an aliquot of 100 µL was evaporated to dryness and silylated. The samples were silylated with BSTFA

TABLE I
Plant Sterol Contents in Grains of Barley, Oats, Rye, and Wheat Cultivars (mg/100 g, wb)[a]

Sample	Dry Matter %	Brassicasterol	Campesterol	Campestanol	Stigmasterol	Sitosterol	Sitostanol
Barley							
Kustaa	87.4	1.3 ± 0.7	15.0 ± 0.2	0.7 ± 0.4	2.4 ± 0.1	43.7 ± 0.6	1.0 ± 0.4
Pokko	87.6	tr	19.2 ± 0.1	0.9 ± 0.3	3.6 ± 0.1	48.4 ± 0.0	1.0 ± 0.2
Oats							
Lisbeth	88.5	3.5 ± 1.4	4.3 ± 0.1	tr	1.7 ± 0.1	27.4 ± 1.2	0.9 ± 0.3
Veli	88.4	2.8 ± 0.6	3.7 ± 0.0	· · ·	1.5 ± 0.3	27.4 ± 1.1	0.8 ± 0.5
Rye							
Amando	87.4	0.5 ± 0.3	16.2 ± 0.3	8.4 ± 0.1	3.3 ± 0.1	45.6 ± 0.2	13.0 ± 0.2
Anna	86.7	tr	18.3 ± 0.2	8.2 ± 0.1	3.4 ± 0.1	49.7 ± 0.1	11.7 ± 0.4
Wheat							
Aura	87.2	tr	10.8 ± 0.4	7.1 ± 0.2	1.6 ± 0.0	36.0 ± 0.5	8.3 ± 0.5
Mahti	87.4	2.5 ± 2.2	12.1 ± 0.2	5.9 ± 0.1	2.2 ± 0.1	36.8 ± 0.7	11.2 ± 1.5

TABLE I (continued)

Sample	Δ5-Avenasterol	Δ7-Avenasterol	Other Sterols	Total Plant Sterols
Barley				
Kustaa	6.0 ± 0.2	0.9 ± 0.1	0.5 ± 0.1	72.0 ± 2.1
Pokko	4.4 ± 0.1	1.2 ± 0.0	1.3 ± 1.1	80.1 ± 1.2
Oats				
Lisbeth	4.4 ± 0.3	1.2 ± 0.2	2.6 ± 2.9	46.6 ± 4.4
Veli	1.7 ± 0.4	0.8 ± 0.3	3.2 ± 0.1	42.7 ± 3.1
Rye				
Amando	1.8 ± 0.1	1.1 ± 0.2	2.5 ± 0.3	92.4 ± 1.8
Anna	2.2 ± 0.3	1.5 ± 0.1	3.3 ± 0.0	98.5 ± 1.0
Wheat				
Aura	0.9 ± 0.4	0.6 ± 0.0	0.8 ± 0.1	66.5 ± 0.1
Mahti	· · ·	tr	0.6 ± 0.1	71.5 ± 4.0

[a] Mean ± SD (n = 3); tr = traces of sterol content (<0.5 mg/100 g, wb).

and TMCS (99:1) in pyridine as described by Toivo et al (2000). The TMS ether derivatives were dissolved in 200 µL of hexane before analyzing by GC.

GC Analysis of Sterols

A Hewlett Packard (HP) 5890 series II gas chromatograph equipped with an autosampler, on-column HP 7673 injector and a flame ionization detector (300°C) was used (Toivo et al 1998, 2000). The column was an RTX-5w/INTEGRA fused silica capillary column (diphenyl and dimethyl polysiloxane [5:95]; 60-m × 0.32-mm i.d., 0.1-µm film with 10-m Integra-Guard column, Restek Corp., Bellefonte, PA). Helium was used as the carrier gas with a constant flow of 0.8 mL/min. The initial column temperature was 70°C and it was programmed to increase to 245°C at 60°C/min, stabilize for 1 min, and thereafter increase to 275°C at 3°C/min. The final isothermal period was 35 min.

Gas chromatography with a mass spectrometer (GC-MS) (FinniganMat Incos 50, San Jose, CA, connected to a Varian 3400 gas chromatograph) (Toivo et al 2000) was used to further identify the sterols. Operating conditions included an RTX-5w/INTEGRA fused silica capillary column as described above, splitless injection of 1 µL at 270°C, constant oven temperature 270°C, and helium carrier gas at 16 psi. For mass spectroscopy, an ionization energy of 70 eV in the electron impact mode was used, and spectra were scanned within *m/z* 100–600. The ion-source and transfer line temperatures were 160 and 270°C, respectively.

Identification of sterols was based on relative retention times of commercially available sterols (sitosterol 95%, campesterol 98%, stigmasterol 95%, and sitostanol 96.7% [Sigma]), comparison with literature data (Akihisa et al 1991; Kamal-Eldin et al 1992) and mass spectra analyses. Quantification was based on an internal standard method. Calibration curves were calculated for sitosterol, campesterol, sitostanol, and stigmasterol at six levels (1–200 µg) with a constant dihydrocholesterol or cholesterol level (20 µg). The contents of campestanol and brassicasterol were calculated with the campesterol curve, and the contents of other sterols were calculated with the sitosterol curve if the corresponding standard compound was not available. A blank test without any internal standard was run for each material to ascertain that there were no interfering peaks at the same retention time. When cholesterol was used as an internal standard**,** trace amounts of cholesterol found in the samples were taken into account when calculating the results. Later, dihydrocholesterol which does not occur naturally in cereal samples, was used as an internal standard.

The overall validation of the method is reported by Toivo et al (*in press*). In the routine work, an in-house reference sample (whole meal wheat) was used to monitor the analytical level each day. The coefficient of variation was 6.3% for sitosterol and 6.0% for total sterols when cholesterol was used as the internal standard, and 2.4% when dihydrocholesterol was the internal standard. The recovery of stigmasterol added to grain and milling fraction samples was 102.5 ± 9.4% ($n = 16$). In our previous study, we showed that the recovery of cholesterol, cholesteryl palmitate, and glycosidic sterols added to whole wheat flour ($n = 6$) was 93.8, 100.1, and 91.2%, respectively (Toivo et al, *in press*). Furthermore, the GC separation and quantification were monitored daily by injecting a sterol standard mixture. The detection limit determined using standard solutions and calculated was 0.1 mg/100 g of sample. The lowest sterol concentration given when the normal sample size and sample preparation procedures were applied was 0.5 mg/100 g.

Moisture Determination

The moisture contents of the samples were determined by heating the samples (1–3 g) at 103 ± 2°C overnight.

RESULTS AND DISCUSSION

In this study, the total plant sterol contents of cereal and cereal product samples were analyzed using a sample preparation procedure enabling the determination of both free sterols and their bound derivatives (esters and glycosides). Good separation of the internal standard and sterols was achieved by GC (Fig. 1).

Cereals and Different Cultivars

The four cereals, rye, wheat, barley and oats, differed in total sterol content. When the samples of two cultivars of each cereal grown in the same year in the same area of Finland were analyzed, the highest plant sterol contents (mean 95.5 mg/100 g, wb) were determined in rye (Table I). In barley and wheat, the average contents were 76.1 and 69.0 mg/100 g, respectively, whereas in oats the total sterol content was only 44.7 mg/100 g. When 10 rye cultivars grown in the same year in the same area in Finland were

TABLE II
Plant Sterol Contents in Grains of 10 Rye Cultivars (mg/100 g, wb)[a]

Sample	Dry Matter %	Brassicasterol	Campesterol	Campestanol	Stigmasterol	Sitosterol	Sitostanol
Anna	90.5	tr	13.6 ± 0.4	6.9 ± 0.1	2.4 ± 0.1	40.5 ± 0.5	9.2 ± 0.1
Akusti	91.5	tr	13.6 ± 0.3	6.8 ± 0.1	2.5 ± 0.1	41.0 ± 0.8	8.9 ± 0.1
Bor 7068	91.4	tr	13.4 ± 0.6	6.4 ± 0.3	2.4 ± 0.1	38.6 ± 1.8	7.7 ± 0.3
Bor 9214	91.5	tr	13.1 ± 0.1	6.6 ± 0.1	2.2 ± 0.0	38.9 ± 0.4	9.0 ± 0.2
Bor 9414	91.4	· · ·	14.9 ± 0.4	6.8 ± 0.3	2.6 ± 0.0	42.8 ± 1.2	8.4 ± 0.4
Elvi	91.4	· · ·	13.4 ± 0.3	6.2 ± 0.0	2.4 ± 0.0	37.8 ± 0.7	7.7 ± 0.1
Esprit	91.4	· · ·	13.7 ± 0.4	5.8 ± 0.1	2.6 ± 0.0	36.0 ± 0.4	6.4 ± 0.1
Picasso	91.4	· · ·	13.8 ± 1.0	6.1 ± 0.4	2.6 ± 0.1	35.8 ± 1.8	6.7 ± 0.3
Riihi	91.4	tr	12.8 ± 0.5	7.3 ± 0.3	2.2 ± 0.1	39.4 ± 1.4	10.3 ± 0.4
Voima	91.4	· · ·	14.0 ± 0.4	6.8 ± 0.4	2.3 ± 0.2	42.2 ± 1.0	9.0 ± 0.4

TABLE II (continued)

Sample	Δ5-Avenasterol	Δ7-Avenasterol	24-Methylcycloartanol	Other Sterols	Total Plant Sterols
Anna	1.5 ± 0.2	1.4 ± 0.0	0.5 ± 0.0	5.8 ± 0.1	81.9 ± 0.9
Akusti	1.1 ± 0.0	1.0 ± 0.0	0.5 ± 0.0	5.0 ± 0.1	80.4 ± 1.3
Bor 7068	1.3 ± 0.1	1.4 ± 0.1	0.7 ± 0.2	5.4 ± 0.2	77.3 ± 2.7
Bor 9214	1.3 ± 0.0	1.3 ± 0.0	0.5 ± 0.0	5.5 ± 0.1	78.6 ± 0.8
Bor 9414	1.3 ± 0.1	1.3 ± 0.1	0.7 ± 0.3	5.8 ± 0.2	85.6 ± 2.7
Elvi	1.1 ± 0.0	1.1 ± 0.0	tr	5.3 ± 0.1	75.3 ± 1.0
Esprit	1.0 ± 0.1	0.9 ± 0.0	· · ·	4.3 ± 0.2	70.7 ± 1.1
Picasso	0.9 ± 0.0	0.9 ± 0.0	tr	4.2 ± 0.2	71.3 ± 3.4
Riihi	1.5 ± 0.2	1.2 ± 0.0	tr	5.1 ± 0.3	80.3 ± 2.7
Voima	1.6 ± 0.1	1.2 ± 0.0	tr	5.3 ± 0.0	82.7 ± 2.4

[a] Mean ± SD ($n = 3$); tr = traces of sterol content (<0.5 mg/100 g, wb).

compared, total plant sterol contents varied at 70.7–85.6 mg/100 g (Table II). The total plant sterol contents of the four Finnish population cultivars (Akusti, Voima, Riihi, Anna) were very close to each other, whereas somewhat higher variation was seen between the breeding lines (Bor 7068, 9214, 9414). The lowest contents were measured in the German hybrid cultivars (Picasso, Esprit).

Based on the results of the four cereals, the difference between the cultivars (7–11%) was not significant in terms of cereals as dietary plant sterol sources. The total variation caused by differences in growing conditions, affected by growing location and year and by wider genetic variation may, however, be more significant. This was indicated by the difference in the total plant sterol contents of the rye cultivars for the two years included in this study, and especially by the marked difference when the cultivar samples were compared with the grain samples from the milling process (Table III). These samples were mixed samples containing both domestic and imported grains.

Previously, Jonker et al (1985) determined plant sterol contents in whole wheat, Dutta and Appelqvist (1996) in mixed Finnish samples of wheat, oats, barley and rye, and in one wheat cultivar, and Määttä et al (1999) in oats using sample preparation procedures including acid hydrolysis. Unfortunately, the sterol contents are given only as mg/g of lipids by Dutta and Appelqvist (1996). The total sterol contents were 44.6, 12.1, 35.6, and 71.2 mg/g of lipids in wheat, oats, barley, and rye, respectively, showing considerable variation between cereals. The plant sterol content was highest in rye and lowest in oats, which is similar to this study. The value given by Jonker et al (1985) for unspecified whole wheat (44.7 mg/100 g) is lower than determined in this study. The value found in this study for oats is in the range (350–491 µg/g, dw) reported by Määttä et al (1999) and lower than the value found in this study. Weihrauch and Gardner (1978) reported plant sterol contents of 58 and 69 mg/100 g in oats and wheat, respectively.

The variation between different studies may be caused by differences in analytical methods (either the extractability of sterols by the methods used or chromatographic separation) or by real differences in the samples. Especially in the older studies, the samples are not described well enough to enable exact comparison. The effects of genetic factors or growing conditions are rarely studied for cereals but are studied somewhat more extensively for oil crops. When Määttä et al (1999) compared seven oats cultivars, each grown in three locations in Sweden, they found statistically significant differences between cultivars but not between growing locations. Both genetic background and planting location significantly affected total sterol contents of oil crops (Vlakis and Hazebroek 2000). When soybean was grown in pots kept in a greenhouse at three different temperatures, the total plant sterol contents increased with higher temperatures. In all our cereal samples, sitosterol was the main plant sterol, varying at 49–64% of the total sterol contents. Its level was constant in all rye samples (49–51%). The other most significant sterols were campesterol, campestanol, sitostanol, stigmasterol, and two avenasterols. In rye, campestanol accounted for 8–9% and sitostanol for 9–14% of total sterols. The corresponding figures for wheat were 8–11 and 12–16%, whereas stanols were not detected or detected only in very low amounts in oats and barley. Previously, sitosterol accounted for 39–44% of sterols in wheat, 32% in oats, 37–46% in barley, and 40% in rye (Dutta and Appelqvist 1996). They did not find stanols in barley or oats. The relative proportions of the various sterols is in accordance with the data reviewed by Chung and Ohm (2000), although the exact percentages may differ depending on the separation efficiency of the analytical method used. The amount of avenasterols may be underestimated in this study because they are partly isomerized in the sample preparation method applied (Kamel-Eldin et al 1998).

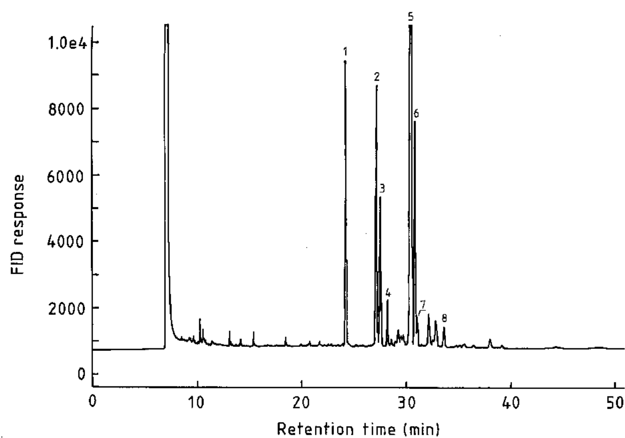

Fig. 1. Gas chromatography of a whole meal wheat flour sample (in-house reference sample). Peak identification: 1, dihydrocholesterol; 2, campesterol; 3, campestanol; 4, stigmasterol; 5, sitosterol; 6, sitostanol; 7, Δ5-avenasterol; 8, Δ7-avenasterol.

Milling Process

In the milling of rye and wheat, plant sterols were distributed according to the ash content of the corresponding milling product (Table III). The ash content indicates the level of the outer layers of the kernel in the product. When the total sterol contents of two lots of wheat grains were 72.6 and 83.0 mg/100 g, the total sterol contents of the corresponding flours with ash contents of 0.6% were 43.0 and 39.8 mg/100 g of the fraction, and those of the bran fraction with the 4% ash content were 168 and 177 mg/100 g, respectively. Similarly, total sterol contents of 109 and 113 mg/100 g in rye grains led to total sterol contents of 47.4 and 62.1 mg/100 g at the lowest ash level 0.7% and to 176 and 188 mg/100 g in the bran fractions with an ash content of 4%. Furthermore, the results showed that the sterol composition differs in different parts of the kernel. The level of stanols in rye products was ≈15% (ash level 0.7%), 19% in whole meal flour (ash content 1.8%), and 29% in bran (Fig. 2). The corresponding figures for wheat flours

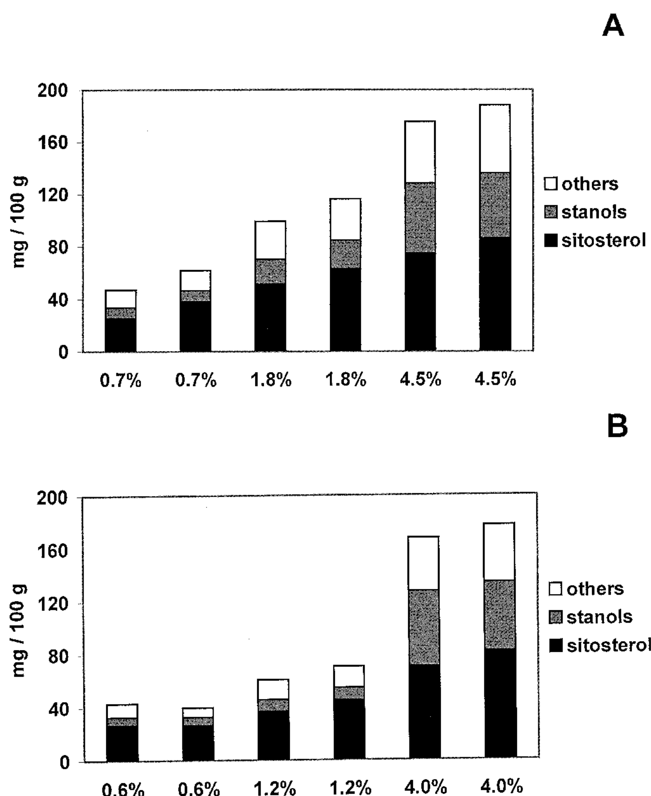

Fig. 2. Fractionation of plant sterols in milling process of rye (**A**) and wheat (**B**). Samples with different ash contents were taken twice.

TABLE III
Plant Sterol Contents of Milling Fractions of Rye and Wheat (mg/100 g, wb)[a]

Sample	Dry Matter %	Brassicasterol	Campesterol	Campestanol	Stigmasterol
Rye 1997					
Whole grain	88.0	· · ·	20.4 ± 1.1	9.4 ± 0.6	3.7 ± 0.2
Flour, 0.7% ash	86.7	tr	8.4 ± 0.1	3.6 ± 0.0	1.6 ± 0.4
Flour, 1.0% ash	87.3	0.5 ± 0.2	12.6 ± 0.4	4.3 ± 0.3	2.5 ± 0.1
Flour, 1.8% ash	87.5	· · ·	19.0 ± 0.1	8.4 ± 0.1	3.6 ± 0.0
Bran, 4.5% ash	88.0	1.6 ± 0.5	26.6 ± 0.7	23.8 ± 0.2	7.0 ± 0.0
Rye 1998					
Whole grain	88.3	· · ·	21.0 ± 0.3	8.1 ± 0.0	3.4 ± 0.1
Flour, 0.7% ash	89.5	· · ·	11.8 ± 0.3	2.7 ± 0.0	1.1 ± 0.1
Flour, 1.0% ash	87.5	· · ·	15.3 ± 0.2	4.2 ± 0.2	1.8 ± 0.0
Flour, 1.8% ash	89.4	· · ·	22.9 ± 0.2	8.5 ± 0.1	3.5 ± 0.0
Bran, 4.5% ash	91.0	· · ·	32.8 ± 0.4	22.5 ± 0.3	7.4 ± 0.0
Wheat 1997					
Whole grain	87.4	· · ·	12.1 ± 0.1	6.0 ± 0.4	1.9 ± 0.4
Flour, 0.6% ash	87.2	0.6 ± 0.4	7.6 ± 0.2	2.3 ± 0.1	0.5 ± 0.2
Flour, 1.2% ash	86.5	· · ·	11.1 ± 0.3	4.0 ± 0.0	0.9 ± 0.2
Bran, 4.0% ash	90.8	0.6 ± 0.1	24.3 ± 0.6	25.6 ± 0.2	7.4 ± 0.1
Bran, 4.5% ash	88.8	· · ·	35.4 ± 0.8	17.6 ± 0.1	5.9 ± 0.1
Wheat 1998					
Whole grain	87.2	· · ·	15.0 ± 0.7	7.3 ± 0.1	1.5 ± 0.2
Flour, 0.6% ash	86.5	· · ·	7.1 ± 0.1	2.0 ± 0.1	· · ·
Flour, 1.2% ash	88.5	· · ·	15.3 ± 0.2	2.8 ± 0.0	tr
Bran, 4.0% ash	91.6	· · ·	28.9 ± 1.9	24.0 ± 1.7	6.1 ± 0.6
Bran, 4.5% ash	90.8	· · ·	34.7 ± 3.0	16.5 ± 0.7	6.3 ± 0.5

TABLE III (continued)

Sample	Sitosterol	Sitostanol	Δ5-Avenasterol	Δ7-Avenasterol	Other Sterols	Total Plant Sterols
Rye 1997						
Whole grain	54.4 ± 1.0	12.6 ± 0.7	2.1 ± 0.2	2.1 ± 0.4	4.6 ± 0.4	109.2 ± 4.1
Flour, 0.7% ash	25.4 ± 0.4	4.6 ± 0.2	1.4 ± 0.1	0.7 ± 0.2	1.6 ± 0.1	47.4 ± 0.5
Flour, 1.0% ash	36.0 ± 1.2	6.0 ± 0.7	1.4 ± 0.1	1.0 ± 0.0	2.2 ± 0.1	66.4 ± 2.6
Flour, 1.8% ash	51.4 ± 0.2	10.7 ± 0.2	1.8 ± 0.2	1.6 ± 0.0	2.8 ± 0.1	99.3 ± 0.6
Bran, 4.5% ash	74.7 ± 0.7	29.9 ± 0.1	2.8 ± 0.2	3.1 ± 0.1	6.4 ± 1.3	175.5 ± 2.4
Rye 1998						
Whole grain	60.7 ± 0.4	13.8 ± 1.3	· · ·	0.5 ± 0.1	5.8 ± 0.8	113.4 ± 2.7
Flour, 0.7% ash	38.3 ± 0.6	5.6 ± 0.2	tr	· · ·	2.4 ± 0.4	62.1 ± 1.1
Flour, 1.0% ash	46.4 ± 1.7	7.9 ± 0.5	0.7 ± 0.1	tr	3.3 ± 1.0	79.5 ± 2.2
Flour, 1.8% ash	63.3 ± 0.5	13.0 ± 1.0	tr	0.9 ± 0.2	4.2 ± 2.0	116.3 ± 2.6
Bran, 4.5% ash	86.1 ± 0.9	27.3 ± 0.8	tr	1.6 ± 0.1	10.3 ± 0.4	188.1 ± 2.2
Wheat 1997						
Whole grain	40.6 ± 0.4	9.1 ± 1.3	1.3 ± 0.2	0.9 ± 0.1	0.8 ± 0.4	72.6 ± 1.3
Flour, 0.6% ash	26.8 ± 0.5	3.6 ± 0.2	tr	0.6 ± 0.1	0.6 ± 0.2	43.0 ± 1.0
Flour, 1.2% ash	36.8 ± 0.2	4.8 ± 0.1	1.7 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	60.7 ± 0.4
Bran, 4.0% ash	70.4 ± 0.5	31.5 ± 0.1	1.7 ± 0.0	2.7 ± 0.6	3.8 ± 0.1	167.8 ± 1.5
Bran, 4.5% ash	93.8 ± 1.2	20.9 ± 1.2	5.0 ± 0.7	3.1 ± 0.1	3.0 ± 0.9	184.7 ± 1.5
Wheat 1998						
Whole grain	48.6 ± 1.9	9.8 ± 0.2	· · ·	· · ·	0.8 ± 0.5	83.0 ± 3.2
Flour, 0.6% ash	26.6 ± 0.3	4.1 ± 0.2	· · ·	· · ·	· · ·	39.8 ± 0.2
Flour, 1.2% ash	45.2 ± 0.3	6.5 ± 0.2	· · ·	· · ·	tr	70.4 ± 0.8
Bran, 4.0% ash	81.8 ± 4.1	28.3 ± 1.2	· · ·	1.2 ± 0.1	7.0 ± 0.4	177.3 ± 9.4
Bran, 4.5% ash	92.5 ± 6.0	20.7 ± 1.5	· · ·	1.0 ± 0.1	5.9 ± 0.6	177.6 ± 12.2

[a] Mean ± SD (*n* = 3); tr = traces of sterol content (<0.5 mg/100 g, wb).

(ash contents of 0.6 and 1.2%) and bran (ash content 4%) were 15, 14, and 32%, respectively.

Previously, distribution of sterols in rye or wheat milling fractions had not been studied in detail, but the bran and germ fractions are, in general, regarded as the richest parts of the kernel (Dutta and Appelqvist 1996). Unfortunately, no germ fractions of rye or wheat could be obtained from the process. The structure of rye kernel hinders the separation of the germ in the normal milling process. A separate wheat germ sample taken from another mill contained 289 mg of sitosterol/100 g, 122 mg of campesterol/100 g and 17.8 mg of stanols/100 g; total sterol content was 476 mg/100 g. This showed that among the wheat milling fractions studied, the level of stanols was the lowest in the germ fraction. On the other hand, an oat bran sample taken from the same mill showed that sterols in oats are not as concentrated in the bran fraction as they are in wheat and rye; the total sterol content was 44.6 mg/100 g. Similarly, the total sterol content determined by Dutta and Appelqvist (1996) for oat bran was only 9.4 mg/g of lipids while that of wheat bran was 44.9 mg/g.

Retail Samples of Milling and Bakery Products

Tables IV and V give plant sterol contents of several milling and retail bakery products. The sterol contents of the milling products of rye and wheat were in line with those of the samples obtained directly from industry. This met our expectations because plant sterols are not so unstable that significant losses could be expected under common storage and transportation conditions of milling products (Neurooz-Zahed and Appleqvist 1992). Among the wheat samples, the highest sterol content was determined in wheat germ (411 mg/100 g) and the lowest in the most refined flours (37–48 mg/100 g). Similarly, the plant sterol content of barley meal and rolled oats were at expected levels when compared with the corresponding grains. As in rye and wheat, refining rice decreased the plant sterol content significantly; the plant sterol content of brown

TABLE IV
Plant Sterol Contents of Flours and Other Milling Products (mg/100 g, wb)[a]

Sample	Dry Matter %	Brassicasterol	Campesterol	Campestanol	Stigmasterol	Sitosterol
Wheat flour, 0.7% ash	87.2	· · ·	8.5 ± 0.3	3.1 ± 0.2	tr	32.1 ± 1.6
Wheat flour, ≈0.6% ash[b]	87.3	· · ·	8.3 ± 0.0	2.9 ± 0.1	0.7 ± 0.2	29.6 ± 0.1
Wheat flour, ≈0.6% ash[b]	88.6	· · ·	6.3 ± 0.3	2.5 ± 0.1	0.5 ± 0.0	22.8 ± 1.1
Wheat flour, 0.6% ash[b]	87.6	· · ·	7.6 ± 0.2	3.0 ± 0.1	tr	29.9 ± 0.8
Wheat flour, 1.2–1.4% ash	88.6	· · ·	13.3 ± 0.5	3.5 ± 0.1	0.8 ± 0.0	43.6 ± 0.7
Wheat flour, whole grain	88.8	· · ·	12.3 ± 0.1	6.9 ± 0.1	2.1 ± 0.3	40.5 ± 0.2
Wheat bran	91.0	· · ·	25.9 ± 0.5	33.8 ± 1.1	1.2 ± 0.0	81.4 ± 1.7
Wheat germ	91.6	2.4 ± 0.0	103.4 ± 3.9	5.4 ± 1.0	1.9 ± 0.1	253.7 ± 8.7
Rye meal[b]	90.0	· · ·	17.8 ± 0.2	7.7 ± 0.2	3.3 ± 0.0	49.2 ± 0.6
Rye meal[b]	89.9	· · ·	14.6 ± 0.2	6.0 ± 0.1	2.9 ± 0.1	41.6 ± 0.6
Barley meal[b]	88.3	· · ·	12.6 ± 0.3	· · ·	1.7 ± 0.1	42.2 ± 1.1
Barley meal[b]	90.2	· · ·	8.4 ± 0.2	0.6 ± 0.0	1.0 ± 0.1	35.2 ± 0.6
Oats, rolled[b]	89.6	3.1 ± 0.3	4.2 ± 0.1	tr	1.1 ± 0.1	27.2 ± 0.8
Oats, rolled[b]	90.2	1.2 ± 0.1	4.0 ± 0.0	0.7 ± 0.0	1.4 ± 0.0	34.8 ± 0.5
Buckwheat, whole grain	87.7	· · ·	9.3 ± 0.6	· · ·	tr	77.5 ± 3.0
Millet, whole grain	88.4	1.1 ± 0.1	11.2 ± 0.2	· · ·	1.8 ± 0.0	37.1 ± 0.6
Rice, polished	86.4	· · ·	4.5 ± 0.5	1.3 ± 0.0	3.2 ± 0.0	19.5 ± 1.0
Rice, brown	87.0	· · ·	14.6 ± 0.7	1.6 ± 0.2	10.4 ± 0.0	37.5 ± 0.6
Corn flakes	94.9	· · ·	5.8 ± 0.1	1.8 ± 0.1	1.3 ± 0.0	22.4 ± 1.0

TABLE IV (continued)

Sample	Sitostanol	Δ5-Avena-sterol	Cycloartenol +Δ7-Stigmastenol	Δ7-Avena-sterol	24-Methyl-cycloartanol	Other Sterols	Total Plant Sterols
Wheat flour, 0.7% ash	4.9 ± 0.4	0.7 ± 0.1	0.5 ± 0.0	tr	· · ·	· · ·	50.7 ± 2.6
Wheat flour, ≈0.6% ash[b]	4.6 ± 0.2	0.8 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	· · ·	· · ·	48.1 ± 0.4
Wheat flour, ≈0.6% ash[b]	3.3 ± 0.2	0.6 ± 0.0	· · ·	tr	· · ·	1.0 ± 0.1	37.3 ± 1.9
Wheat flour, 0.6% ash[b]	4.6 ± 0.2	0.7 ± 0.0	tr	tr	· · ·	· · ·	46.6 ± 1.2
Wheat flour, 1.2–1.4% ash	5.3 ± 0.1	1.3 ± 0.0	· · ·	0.9 ± 0.0	· · ·	· · ·	68.7 ± 1.3
Wheat flour, whole grain	9.2 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	· · ·	· · ·	74.4 ± 0.9
Wheat bran	39.0 ± 1.0	2.0 ± 0.3	10.9 ± 0.2	3.1 ± 0.1	· · ·	· · ·	195.1 ± 3.2
Wheat germ	6.8 ± 0.9	13.4 ± 0.9	16.2 ± 1.1	9.0 ± 0.6	· · ·	· · ·	411.4 ± 16.7
Rye meal[b]	10.7 ± 0.2	1.9 ± 0.1	3.4 ± 0.0	1.5 ± 0.1	· · ·	· · ·	95.4 ± 1.1
Rye meal[b]	7.7 ± 0.2	1.4 ± 0.0	· · ·	1.3 ± 0.0	0.5 ± 0.0	5.1 ± 0.3	81.0 ± 1.3
Barley meal[b]	tr	4.0 ± 0.1	tr	1.1 ± 0.0	· · ·	· · ·	62.1 ± 1.7
Barley meal[b]	1.9 ± 0.1	3.5 ± 0.1	tr	0.7 ± 0.0	0.9 ± 0.0	2.6 ± 0.1	55.1 ± 0.6
Oats, rolled[b]	0.6 ± 0.1	2.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	· · ·	· · ·	40.8 ± 1.3
Oats, rolled[b]	tr	3.1 ± 0.0	· · ·	1.0 ± 0.0	1.1 ± 0.0	7.4 ± 0.2	54.8 ± 0.2
Buckwheat, whole grain	2.3 ± 0.1	4.0 ± 0.2	2.8 ± 0.5	· · ·	· · ·	· · ·	96.3 ± 4.2
Millet, whole grain	· · ·	8.7 ± 0.4	7.8 ± 0.2	· · ·	· · ·	· · ·	77.0 ± 1.2
Rice, polished	1.7 ± 0.1	· · ·	tr	· · ·	· · ·	· · ·	29.2 ± 3.0
Rice, brown	1.7 ± 0.1	1.3 ± 0.1	4.5 ± 0.0	0.7 ± 0.0	· · ·	· · ·	72.3 ± 1.3
Corn flakes	6.0 ± 0.5	· · ·	0.9 ± 0.0	· · ·	· · ·	· · ·	38.1 ± 1.7

[a] Mean ± SD (*n* = 3); tr = traces of sterol content (<0.5 mg/100 g, wb).
[b] Sampling was repeated.

rice was 72 mg/100 g and that of polished rice was 29 mg/100 g. Millet and buckwheat products, rarely consumed in Finland, were analyzed for comparison. Their total sterol contents of 77.0 and 96.3 mg/100 g were at levels found in rye, wheat, and barley products.

In Finland, rye is mainly used as whole meal flour for breadbaking. Therefore, rye bread with whole meal rye flour as the main or only ingredient was a good source of sterols (80–90 mg/100 g). Its sterol content was higher than that of wheat bread (≈40 mg/100 g). In other bakery products made from wheat flours with low ash contents, the plant sterol contents were affected by the type and amount of added fat. Their plant sterol contents were 39–85 mg/100 g; their highest levels were the same as those of the rye breads. Previously, 5.2–64.0 mg of sterols/100 g were determined in different types of breads (Jonker et al 1985).

CONCLUSIONS

Cereal products in general can be regarded as significant natural plant sterol sources. The plant sterol contents determined for different cultivars grown in the same area within the same year did not differ significantly. However, the results indicated that the total variation in cereal raw materials is higher. Furthermore, there are considerable differences between various milling products of the same cereal, leading to different levels in bakery products. Among milling products, whole meal products, bran fractions, and germ fractions are the richest sterol sources. In the Finnish diet, rye is an important cereal accounting for ≈20% of the total use of cereals as food. It is mainly used as whole meal flour for breadbaking and thus its contribution to plant sterol intake is significant. In bakery products made from refined flours, the plant sterol content is affected by the type and amount of added fat. Based on the results, increasing the utilization of whole meal flours and bran fractions in cereal products would further strengthen the position of cereal products as natural plant sterol sources.

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TABLE V
Sterol Contents of Other Cereal Products (mg/100 g, wb)[a]

Sample	Dry matter %	Cholesterol	Brassicasterol	Campesterol	Campestanol	Stigmasterol
White bread	66.3	· · ·	0.7 ± 0.1	8.3 ± 0.3	2.1 ± 0.1	0.6 ± 0.0
Yeast bread	65.7	1.8	1.0 ± 0.1	8.1 ± 0.1	2.0 ± 0.2	0.6 ± 0.1
Rye bread[b]	67.5	· · ·	· · ·	14.1 ± 0.5	7.5 ± 0.3	2.8 ± 0.4
Rye bread[c]	66.1	· · ·	2.5	16.0 ± 0.4	7.5 ± 0.1	2.9 ± 0.1
Crispbread, rye	93.1	0.6	1.1 ± 0.1	17.6 ± 0.2	7.4 ± 0.1	3.1 ± 0.0
Doughnut, raised	72.6	26.8 ± 2.9	1.3 ± 0.3	29.4 ± 3.2	1.7 ± 0.5	0.5 ± 0.2
Cinnamon roll	79.3	14.2 ± 0.5	2.2 ± 0.1	22.9 ± 0.9	2.0 ± 0.5	1.9 ± 0.1
Bunloaf	76.0	14.5 ± 0.3	1.2 ± 0.1	12.8 ± 0.3	1.8 ± 0.1	1.5 ± 0.0

TABLE V (continued)

Sample	Sitosterol	Sitostanol	Δ5-Avenasterol	Cycloartenol + Δ7-Stigmastenol	Δ7-Avenasterol	Other Sterols	Total Plant Sterols
White bread	23.9 ± 0.9	3.2 ± 0.3	0.8 ± 0.1	0.5 ± 0.1	tr	· · ·	40.5 ± 1.4
Yeast bread	22.8 ± 0.1	3.2 ± 0.2	0.8 ± 0.1	tr	tr	· · ·	39.2 ± 0.3
Rye bread[b]	43.4 ± 0.7	7.0 ± 0.7	2.2 ± 0.4	2.1 ± 0.1	1.2 ± 0.0	· · ·	80.3 ± 1.7
Rye bread[c]	45.8 ± 0.7	9.9 ± 0.1	2.8 ± 0.7	3.2 ± 0.4	1.5 ± 0.1	· · ·	90.2 ± 0.6
Crispbread, rye	48.3 ± 0.4	11.0 ± 0.2	1.8 ± 0.1	3.6 ± 0.2	1.5 ± 0.1	· · ·	95.3 ± 1.1
Doughnut, raised	46.3 ± 5.1	1.6 ± 0.1	2.4 ± 0.2	· · ·	tr	1.3 ± 0.3	84.8 ± 10.0
Cinnamon roll	44.9 ± 1.5	3.1 ± 0.0	2.4 ± 0.0	· · ·	· · ·	1.1 ± 0.5	80.4 ± 2.8
Bunloaf	27.8 ± 0.6	2.7 ± 0.0	1.2 ± 0.0	· · ·	· · ·	1.0 ± 0.0	50.1 ± 0.8

[a] Mean ± SD (n = 3); tr = traces of sterol content (<0.5 mg/100 g, wb).
[b] Contained rye and wheat flour.
[c] Rye flour was only cereal ingredient.

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