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Phytosterols in Wheat Genotypes in the HEALTHGRAIN Diversity Screen

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The phytosterol contents of 130 winter wheat, 20 spring wheat, 10 durum wheat, 5 spelt, 5 einkorn, and 5 emmer wheat genotypes, grown at the same location in the same year, were analyzed with gas chromatography. Considerable variation was observed in total phytosterol contents in all wheat types. The total sterol contents ranged from 670 to 959 μg/g of dm in winter wheat and from 797 to 949 μg/g of dm in spring wheat. The highest sterol contents were found in spelt, durum wheat, and einkorn wheat. The proportions of the main phytosterols also varied substantially among the different genotypes. The most abundant phytosterol in all wheat genotypes was sitosterol (40−61% of total sterols), whereas the highest variation was seen in total stanols (7−31% of total sterols). The comprehensive data set produced in this study constitutes a valuable basis for plant breeding and selection of phytosterol-rich genotypes.

KEYWORDS: Phytosterol; bread wheat; winter wheat; spring wheat; durum wheat; spelt; diploid einkorn; tetraploid emmer; whole grain

INTRODUCTION

Phytosterols are well-known for their ability to lower serum total and low-density lipoprotein (LDL) cholesterol levels by inhibiting the absorption of cholesterol in the small intestine (*1*−*3*). It has been estimated that reducing the LDL content by 10% with a daily intake of 2 g of phytosterols will reduce the risk of coronary heart disease (CHD) by 20% over a lifetime (*4*). Recently, it was also shown that marked cholesterol lowering can be achieved with smaller doses; diets enriched with 0.74−0.83 g/day of phytosterols decreased serum total cholesterol by 4.9−10% and LDL cholesterol by 6.7−15% compared with control diets (*1, 5*). Furthermore, Hendriks et al. (*1*) found no significant differences in the reduction of cholesterol levels when they compared phytosterol doses of 0.83, 1.61, and 3.24 g/day.

Diets naturally rich in phytosterols may also substantially affect cholesterol levels. It was recently shown that people with higher intakes of natural phytosterols from the diet had lower serum cholesterol levels than those with low intakes, when the daily intake was 59−749 mg (*6*). Ostlund et al. (*3, 7*) reported that test meals containing 328 mg of natural wheat germ phytosterols or 150−300 mg of natural corn oil phytosterols considerably reduced cholesterol absorption compared with phytosterol-free test meals. In addition to lowering serum cholesterol, phytosterols may also have other beneficial effects. Both experimental and epidemiological studies among populations consuming nonenriched foods indicate that dietary phytosterols may offer protection from several cancer types, such as colon, breast, and prostate cancers (*8*). These findings thus suggest that even modest increases in the dietary intake of phytosterols may have significant health-promoting effects.

The average daily intake of phytosterols in a typical Western diet has been estimated to range from 186 to 310 mg (*6, 9*−*12*). Cereals are, along with oils and vegetables, among the most significant natural dietary sources of phytosterols. They are only moderate in their intrinsic sterol content, but provide a significant amount of sterols to the diet, due to the high amount consumed. For example, in Finland and The Netherlands cereal products comprise 38−42% of the daily phytosterol intake and are thus the most important dietary phytosterol source, whereas in Britain and Spain slightly lower values, approximately 30%, were reported (*9*−*12*). Cereal products, particularly wholegrain products, also contain a great number of other health-protective nutrients, such as dietary fiber, minerals, vitamins, and phenolic compounds (*13*). Thus, cereals play an important role in increasing the intake of natural phytosterols and other bioactive compounds.

Wheat is one of the major cereal grains and an important source of nutrients worldwide. Common bread wheat was previously reported to contain from 763 to 818 μg/g of dry matter (dm) of phytosterols (*14, 15*). Sitosterol was the dominant phytosterol, accounting for 51−54% of total sterols, and the other major phytosterols were campesterol, sitostanol, and campestanol (**Figure 1**). The content of phytosterols varies in different parts of the wheat kernel; the highest phytosterol

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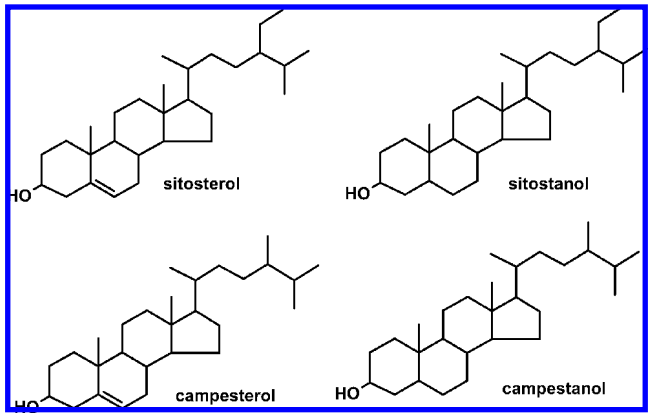

Figure 1. Chemical structures of the most abundant phytosterols in wheat.

concentrations are found in the germ and bran fractions (*15*). One of the factors affecting the phytosterol content of wheat may be genetic variation. However, few data are currently available on the influence of genetic factors on phytosterol content and composition in cereals. Varietal differences were reported in the phytosterol contents of rye and oat (*16, 17*), but for wheat such information is scarce. Furthermore, the existing data are usually limited in the number of varieties investigated, and the experimental conditions are often poorly controlled. Hence, more research is required to be able to use selection of genotypes or breeding as a measure to further enhance the phytosterol contents of wheat-based foods.

In 2005, the integrated project HEALTHGRAIN was initiated in cooperation with numerous European research centers and companies. The project is supported by the European Union as part of the sixth Framework Programme [see Ward et al. (*18*), this issue, and http://www.healthgrain.org/pub]. One part of the project was the diversity screen to study the extent of variation of phytochemicals and bioactive compounds in a gene pool of cereal grains, aiming at acquiring knowledge that can be utilized in plant breeding. The diversity screen provided a unique and wide-ranging sample material of 175 wheat genotypes, grown under strictly controlled experimental conditions in the same area. The objective of this study was to determine the extent of genetic variation in phytosterol content and composition of wheat. The focus was on winter and spring types of bread wheat, but several other wheat types were also included.

MATERIALS AND METHODS

Standards and Reagents. The compounds dihydrocholesterol (95%) and sitostanol (96%) were purchased from Sigma (St. Louis, MO), and stigmasterol (95%) was obtained from Fluka Chemie (Buchs, Switzerland). All solvents and reagents used were similar to those reported previously (*14*). Silica solid-phase extraction (SiOH-SPE) cartridges (Strata SI-1, 500 mg; Phenomenex, Torrance, CA) were used for the purification of phytosterols.

Cereals. All wheat samples were grown in experimental fields at Martonvasar in Hungary and harvested in 2005. The samples included 130 winter wheat and 20 spring wheat (*Triticum aestivum* var. *aestivum*, bread wheat), 10 durum wheat (*Triticum turgidum* var. *durum*), 5 spelt (*T. aestivum* var. *spelta*), 5 early cultivated einkorn wheat (*Triticum monococcum* var. *monococcum*), and 5 early cultivated emmer wheat (*T. turgidum* var. *dicoccum*) genotypes, which represent a selection of both current and uncommon or obsolete lines differing in their characteristics and origins (*18*). In addition, the winter wheat variety MV-Emese was provided by the HEALTHGRAIN project to all participants to be used as an in-house reference sample. After harvesting, the wheat samples were milled in a laboratory mill to give wholegrain flour of 0.5 mm particle size (*18*). The flour samples were stored in sealed plastic bags in the dark at −18 °C until analyzed. The moisture contents, total lipid contents, 1000 kernel weights, and bran yields of the wheat samples were obtained from other partners of the HEALTHGRAIN project (*18, 19*).

Sample Preparation. For phytosterol analysis, the previously published method (*14*) was modified by reducing the sample size and the amounts of reagents during the hydrolysis and extraction steps to half. Briefly, flour samples (0.5 g) in the presence of an internal standard (dihydrocholesterol, 40 μg) were subjected to acid and alkaline hydrolyses to liberate sterols from their glycoside and ester conjugates. Free sterols were extracted into a solvent mixture, purified by SiOH-SPE, derivatized to trimethylsilyl (TMS) ethers, and analyzed using gas chromatography with flame ionization detection (GC-FID). Each sample was analyzed in duplicate. The modified method gave results similar to those of the original method, and there were no statistically significant differences between the means of the sterol contents obtained with the two methods ($p < 0.05$) according to a t test (Statgraphics Plus 4.0 software; Manugistics, Inc., Rockville, MD).

Gas Chromatographic Analysis. Fifteen individual phytosterol species were analyzed, using GC-FID (Agilent Technologies 6890N GC, Santa Clara, CA) equipped with a 5% diphenyl−95% dimethyl polysiloxane column and an internal standard method, as described previously (*14*). The phytosterols analyzed belonged to the 4-desmethyl sterols [sitosterol, sitostanol, campesterol, campestanol, brassicasterol, stigmasterol, Δ^5-avenasterol, Δ^7-avenasterol, stigmasta-5,24(25)-dienol, and Δ^7-stigmastenol] and to the 4-monomethyl and 4,4′-dimethyl sterols (gramisterol, α-amyrin, cycloartenol, 24-methylenecycloartanol, and citrostadienol). The phytosterols were identified on the basis of the relative retention times of commercially available standards and literature data. In addition, gas chromatography−mass spectrometry (GC-MS) was used to confirm the identities of the phytosterols (*14*). The limit of determination was 1 μg/g of flour.

Performance of the Analytical Method. The performance of the method was verified with recovery and repeatability studies. The recoveries of the sitostanol and stigmasterol standards added at two concentration levels to wholegrain wheat flour were 98.6 ± 3.3% ($n = 8$) and 81.0 ± 2.0% ($n = 8$), respectively. The performance of the method was evaluated by analyzing the in-house reference flour once in each sample batch. To set action limits for the total phytosterol content of the in-house reference flour, 10 replicate analyses were performed, giving a coefficient of variation of 2.5%. Thus, the action limits were set at the mean ± 2 × the coefficient of variation, that is, 656 ± 32 μg/g of fresh weight (fw). If the phytosterol content of the in-house reference flour was beyond the action limits, all of the samples of the sample batch were reanalyzed. Moreover, if the difference between the total phytosterol contents of the duplicate analysis of the same sample exceeded 5%, the sample was reanalyzed, and the mean of all the replicates was calculated and reported. Possible outliers of the replicate results were identified using Dixon's Q test ($p < 0.05$), and, if found, they were rejected.

The performance of the GC-FID was assured daily by analyzing a sterol standard mixture and was shown to be good and stable. The retention time of dihydrocholesterol was 19.4 ± 0.1 min ($n = 26$), and the peak area of dihydrocholesterol in relation to cholesterol eluting next to it was 1.0457 ± 0.0038 ($n = 26$). The total phytosterol content of the in-house reference flour was also very stable, 651 ± 14 μg/g of fw ($n = 46$), implying that the repeatability of the phytosterol analysis was good. Sitosterol, campesterol, and total stanol contents of the in-house reference were 345 ± 8, 104 ± 2, and 150 ± 4 μg/g of fw ($n = 46$), respectively. All results are given as means of replicate samples on a dm basis.

Statistical Analysis. The relationships between the total phytosterol contents and 1000 kernel weights, total lipid contents, and bran yields in bread wheats were determined using Pearson correlation coefficients. The correlations with 1000 kernel weights and bran yields were calculated on a fw basis and those with total lipid contents on a dm basis. The statistical differences between the phytosterol contents of different wheat species were measured with a Kruskal−Wallis test and a notched box-and-whisker plot ($p < 0.05$). Statistical analyses were performed with Statgraphics Plus 4.0 software.

Table 1. Total Phytosterol Contents of Different Wheat Types

wheat type	no. of genotypes	total phytosterol content (μg/g of dm)		
		av	SD	range
winter wheat	130	841	55	670−959
spring wheat	20	864	41	797−949
durum wheat	10	987	79	871−1106
spelt	5	928	25	893−963
diploid einkorn	5	1054	85	976−1187
tetraploid emmer	5	857	53	796−937

RESULTS AND DISCUSSION

Total Phytosterol Contents in Wheat. The lowest average total phytosterol content among the wheat species was found in winter wheat (841 μg/g of dm), whereas the phytosterol contents of the spring wheat genotypes were somewhat higher, on average, 864 μg/g (**Table 1**). The Kruskal−Wallis test showed a statistically significant difference ($p < 0.05$) between the medians of the phytosterol contents in winter and spring wheats. The wide range in the phytosterol contents (670−959 μg/g) indicated considerable differences among the various winter wheat genotypes. The environmental factors were controlled in this study by growing all of the wheat samples under similar conditions, and thus this variability was most likely due to the genetic background. The most phytosterol-rich winter wheat genotype contained 43% more phytosterols than the genotype with the lowest sterol concentration. High variation, although not as significant as in winter wheat, was also seen in the phytosterol contents of the spring wheat genotypes (797−949 μg/g). The difference between the lowest and highest total phytosterol contents in spring wheat was 19%.

Previous knowledge on the variation in phytosterol contents of different bread wheat genotypes is scarce. The phytosterol contents of a few wheat varieties grown in the same year and area were compared by Piironen et al. (*14*) and Ruibal-Mendieta et al. (*20*). The total phytosterol contents in two Finnish varieties were 763 and 818 μg/g of dm (*14*), which are in the range observed in this study for bread wheats but slightly lower than the mean values for winter and spring wheats. In five European winter wheat varieties, the sterol contents ranged from 622 to 655 μg/g of fw (*20*) and were thus at the lower end of the range in this study for winter wheat (given on a fw basis of 594−846 μg/g). Two of the winter wheat genotypes of this study were also studied by Ruibal-Mendieta et al. (*20*), who determined considerably lower phytosterol values for Rialto (622 μg/g of fw) and Estica (655 μg/g of fw), compared with the corresponding figures in our study (786 and 741 μg/g of fw, respectively). The phytosterol contents reported by Nyström et al. (*15*) and Weihrauch and Gardner (*21*) for wholegrain wheat of unspecified variety were 783 μg/g of dm and 690 μg/g of fw, respectively. They were thus lower than the mean value of bread wheats in the present study but, nevertheless, were within range. The results obtained in this study and the previously reported data show that there is variation in the phytosterol contents of bread wheat. This variation may be partly explained by genetic factors and different environmental conditions. On the other hand, differences in the analytical methods and number of sterols analyzed may also result in substantial differences in the phytosterol contents. It was shown that the total phytosterol concentration of whole wheat flour varied considerably, depending on the analytical method used, and that acid hydrolysis was a particularly important step in the analysis of sterols (*22*). The current study was thus the first to result in a comprehensive comparison of the phytosterol contents in bread wheat genotypes without the controversial effects of environmental factors or analytical differences.

The ranges of the total phytosterol contents of durum, spelt, einkorn, and emmer wheat genotypes were 871−1106, 893−963, 976−1187, and 796−937 μg/g, respectively (**Table 1**). The wide ranges indicated considerable variation among the different genotypes of these wheat types and especially in durum wheat. Nevertheless, the variation was smaller than that observed among the winter wheat genotypes. Apparently, the greatest diversity was seen in winter wheat, due to the high number of genotypes studied, whereas for the other wheat types the number of genotypes was much smaller. The average phytosterol content of ancient emmer wheat (857 μg/g) was at the same level as those of bread wheats, whereas durum wheat, spelt, and einkorn wheat contained slightly higher levels of phytosterols (averaging 987, 928, and 1054 μg/g, respectively) than bread wheats. The highest average total phytosterol content, 25% higher than the average content in winter wheat genotypes, was found in einkorn wheat. These differences were statistically significant ($p < 0.05$) when the medians were compared using the Kruskal−Wallis test. However, when different wheat types are compared, it should be borne in mind that the number of durum wheat, spelt, einkorn, and emmer wheat genotypes analyzed was limited.

Previously, Ruibal-Mendieta et al. (*20*) observed substantially lower values and greater variation (544−807 μg/g of fw) among the phytosterol contents of 16 different spelt varieties grown in the same year and location (present range, 816−881 μg/g of fw; mean, 847 μg/g of fw). One of these spelt genotypes, Rouquin, was also determined here, and we found a significantly higher total phytosterol content (845 μg/g of fw) than that reported by Ruibal-Mendieta et al. (573 μg/g of fw). Moreover, in contrast to the present study, no differences in the sterol contents of spelt and winter wheat were observed by Ruibal-Mendieta et al. (*20*). The data obtained in these studies indicate that agricultural and environmental conditions may greatly affect phytosterol contents. No prior data of phytosterol content in einkorn, spelt, and durum wheat are available.

Total Phytosterol Contents in Individual Bread Wheat Genotypes. Table 2 shows the bread wheat varieties sorted in order of increasing phytosterol concentration into seven groups. The highest number of winter wheat genotypes fell within the interval 800−849 μg/g (44 genotypes) and the second highest (35 genotypes) within the interval 850−899 μg/g. Together these intervals covered 61% of the genotypes. In the case of spring wheat, 75% of the genotypes fell within this range. The modern commercially cultivated English variety Claire was the most phytosterol-rich winter wheat variety (959 μg/g). Almost as high phytosterol concentrations were found in the English variety Riband (955 μg/g) and in the old U.S. variety Atlas-66 (951 μg/g). High phytosterol contents (942−948 μg/g) were also observed in Rusalka (Bulgaria), Yumai-34 (China), Klein-Estrella (Argentina), and Ellvis (Germany). Ten winter wheat genotypes with the highest levels of phytosterols included six modern varieties (Claire, Riband, Yumai-34, Klein-Estrella, Ellvis, and Korweta), three old or transitional varieties (Atlas-66, Rusalka, and Maris-Huntsman), and one germplasm (CF99105). Three of these genotypes originated from the United Kingdom, whereas the others originated from other parts of the world. On the other hand, the poorest source of phytosterols was the modern French winter wheat variety Qualital (670 μg/g of dm). The German variety Herzog, the Serbian variety Sava, and the Turkish variety Gerek-79 also contained low amounts of phytosterols (739−748 μg/g). Five of the 10 genotypes with the lowest phytosterol contents were old or transitional varieties

Table 2. Total Phytosterol Contents of Winter and Spring Wheat Genotypes (Genotypes Are Listed According to Increasing Phytosterol Contents)

total phytosterol content (μg/g of dm)	no. of genotypes	winter wheat genotypes	no. of genotypes	spring wheat genotypes
<700	1	Qualital		
700−749	3	Herzog, Sava, Gerek-79		
750−799	26	Skorospelka-3B, San-Pastore, Blasco, CF99075, Martonvasari-17, Libellula, Balkan, Autonomia, MV-Suba, Agron, Sadovo-1, Geronimo, Gloria, Obriy, Aurora, Spartanka, Flamura-85, Ble-Des-Domes, Nap-Hal, Alabasskaja, Fleischmann-481, Baranjka, Bankuti-1201, Fredrick, Recital, Albatros-Odesky	1	Janz
800−849	44	Momtchil, Begra, Bezostaja-1, Granbel, Zvezda, TAM200, Avalon, Taldor, Sagittario, Iljicsovka, Tamaro, Pobeda, Vona, B16, Thesee, Ornicar, Hana, Carmen, Scout66, Fundulea-29, Arina, Ravenna, Krasnodarskaya-99, Millennium, Cubus, Fertodi-293, Karl-92, SU321, Lasta, GK-Tiszataj, Estica, Probstdorfer-Perlo, Key, Renan, Kirac66, Blue/AG, Valoris, Arthur-71, Ukrainka, Amadeus, Kirkpinar-79, Isengrain, NS-Rana-1, Plainsman-V	6	Catbird, Saratov-29, Pastor, Chinese-Spring, Pan, Milan
850−899	35	Bilancia, Magdalena-FR, Monopol, Guarni, Jubilejnaja-50, Seu-Seun-27, Campari, Spark, Nomade, Produttore, Apache, Malacca, Capo, Courtot, Alba, Buck-Catriel, Sumai-3, Akteur, Manital, Biscay, Etoile-De-Choisy, Dekan, Mv-Palotas, Kotuku, Palesio, Soissons, Atay-85, Rialto, Camp-Remy, Tommi, Disponent, Caphorn, Mieti, Kanzler, Stephens	9	Glenlea, Mexique-50, Lona, Red-Fife, Chara, Red-River, Manitoba, Azteca67, Sultan95
900−949	18	Galahad, Alliance, Gene, Augusta, Cardinal, Tremie, Moulin, CF99102, Hereward, CF99007, Lynx, Korweta, Maris-Huntsman, CF99105, Ellvis, Klein-Estrella, Yumai-34, Rusalka	4	Kukri, Sunstar, Thatcher, Cadenza
≥950	3	Atlas-66, Riband, Claire		

Table 3. Phytosterol Compositions (Micrograms per Gram of Dry Matter) of Different Wheat Types

	sitosterol			campesterol			stanols			others		
wheat type	av	SD	range	av	SD	range	av	SD	range	av	SD	range
winter wheat	438	32	342−530	130	15	95−169	199	28	97−263	74	11	49−109
spring wheat	459	34	412−527	129	10	114−153	199	31	100−232	78	13	58−104
durum wheat	438	23	410−482	159	21	126−185	271	40	223−338	119	19	91−161
spelt	457	15	437−473	133	8	120−143	247	38	181−277	91	7	82−98
diploid einkorn	500	73	454−628	195	69	142−314	229	84	80−271	130	24	106−164
tetraploid emmer	391	22	363−422	134	7	128−145	228	26	201−270	103	5	100−111

(Herzog, Gerek 79, Skorospelka 3B, San Pastore, and Libellula), 4 were modern varieties (Sava, Qualital, Blasco, and Martonvasari 17), and 1 was a germplasm (CF99075). Three of these 10 genotypes were of Italian and 2 of French origin, and the others were from other European countries. The highest concentration among the spring wheat varieties was found in Cadenza (949 μg/g) originating from the United Kingdom. Other spring wheat genotypes also rich in phytosterols were the Canadian variety Thatcher (944 μg/g) and Sunstar and Kukri of Australian origin (912 and 908 μg/g, respectively). These genotypes included both old (Thatcher and Kukri) and modern (Cadenza and Sunstar) varieties. The total phytosterol content was lowest in the Australian variety Janz (797 μg/g), whereas other genotypes with relatively low phytosterol contents (809−823 μg/g) included Catbird (United Kingdom), Saratov 29 (Russia), and Pastor (Mexico). Those spring wheat genotypes with the lowest sterol contents included two old or transitional varieties (Janz and Saratov 29), one germplasm (Catbird), and one modern variety (Pastor). The highest phytosterol contents of all varieties in this study were the Hungarian einkorn wheat variety 08-2004 (1187 μg/g) and the German durum wheat variety Durabon (1106 μg/g).

Phytosterol Composition in Wheat. The average phytosterol compositions of winter and spring wheat were similar (**Table 3**). However, significant variation in the proportions of the most abundant phytosterols was seen among different genotypes in both of these wheat types. Sitosterol was the main phytosterol in all wheat species investigated. In the winter and spring wheat genotypes, sitosterol comprised 47−59% (mean, 52%) and 49−61% (mean, 53%) of the total sterol contents, respectively. Campesterol, campestanol, and sitostanol were found in all wheat types in considerable amounts. Campesterol accounted for 11−20% (mean, 16%) of the total phytosterols in the winter wheat and 13−17% (mean, 15%) in spring wheat genotypes. The highest variation was observed in the total stanols; among the winter wheat genotypes the proportion varied from 11 to 29% (mean, 24%) and among the spring wheat genotypes, from 12 to 26% (mean, 23%). Interestingly, certain varieties with the highest relative proportion of sitosterol also had the highest campesterol and the lowest stanol proportions. On the other hand, those varieties with the lowest relative content of sitosterol had the highest proportion of stanols. The other 4-desmethyl sterols analyzed, namely, brassicasterol, stigmasterol, Δ^5-avenasterol, Δ^7-avenasterol, stigmasta-5,24(25)-dienol, and Δ^7-stigmastenol, were present as minor compounds in winter wheat and spring wheat, and each covered only 0−3% of the total phytosterol content. The total amount of these minor sterols varied from 5 to 12%. Also, 4-monomethyl and 4,4′-dimethyl sterols (gramisterol, α-amyrin, cycloartenol, 24-methylenecycloartanol, and citrostadienol) were found in low amounts, accounting for 1−2% of total sterols.

The sterol profiles determined in this study were in accordance with the profiles reported by Piironen et al. (*14*) and Nyström et al. (*15*). In two Finnish wheat varieties (*14*), sitosterol, campesterol, and stanols comprised 51−54, 16−17, and 23−24% of the total phytosterol content, respectively. Similarly, Nyström et al. (*15*) found 53% sitosterol, 16% campesterol, 22% stanols, and 10% other sterols in wholegrain wheat.

In durum, spelt, einkorn, and emmer wheat genotypes, the proportion of sitosterol was somewhat lower than in bread

wheats: 40−48% (mean, 45%), 47−53% (mean, 49%), 45−53% (mean, 47%), and 45−46% (mean, 46%), respectively. The contribution of campesterol (mean, 14−18%) was similar to that of winter and spring wheats. In durum wheat, spelt, and emmer wheat, total stanols comprised an average of 27% of total phytosterols, which is slightly more than in einkorn wheat (22%) or in bread wheat. On the other hand, variation in the proportion of total stanols was small in durum wheat, spelt, and emmer wheat (20−31%), whereas in einkorn wheat it was as high as in bread wheats (7−28%). In comparison to winter and spring wheats, the level of the minor 4-desmethyl sterols was similar (total, 7−12%) and that of 4-monomethyl and 4,4′-dimethyl sterols slightly higher (2−3%) in the other four wheat types.

The sterol profile of spelt was previously studied by Ruibal-Mendieta et al. (*20*). Sitosterol and campesterol contributed approximately 70 and 20% of the total sterols, respectively. These values were considerably higher than those determined in the present study. In contrast to our findings, Ruibal-Mendieta et al. (*20*) also reported that stanols represented <5% of the total sterols. These differences in the sterol profiles apparently resulted from analytical differences.

Relating the Total Phytosterol Contents of Bread Wheats to Other Kernel Characteristics. The correlations between the phytosterol contents of bread wheat genotypes and the total lipid contents (dm), 1000 kernel weights (fw), and bran yields (fw) were evaluated. There was a relatively weak positive relationship ($r = 0.215$, df = 128, $p < 0.05$) between the phytosterol and total lipid contents of winter wheats. In spring wheat genotypes, a moderately strong positive relationship ($r = 0.600$, df = 18, $p < 0.01$) was seen between these variables. When all bread wheats were taken into account, a moderately strong positive correlation ($r = 0.286$, df = 148, $p < 0.01$) between the total phytosterol and lipid contents was observed. Phytosterols as lipid-soluble compounds may be found in greater amounts in wheat kernels with high lipid content. Comparison of the phytosterol concentrations and the 1000 kernel weights of the winter wheat genotypes revealed a moderately strong negative relationship ($r = -0.336$, df = 128, $p < 0.01$) between these variables. On the other hand, a moderately strong positive relationship ($r = 0.298$, df = 128, $p < 0.01$) was observed between their phytosterol contents and bran yields. No significant association was found between the sterol content and 1000 kernel weight or bran yield in spring wheat, possibly due to the smaller number of genotypes studied. However, when calculated for all bread wheats together, a relatively strong negative relationship ($r = -0.382$, df = 148, $p < 0.01$) was found between the 1000 kernel weights and sterol contents and a positive association ($r = 0.273$, df = 148, $p < 0.01$) between the sterol contents and bran yields. These associations indicate that large kernels contain less phytosterols than smaller kernels and may be explained by the higher proportion of bran fraction in smaller kernels. Bran is known to be rich in sterols (*15*).

In conclusion, this study provided new and valuable data on the diversity in phytosterol content and composition of wheat genotypes. The highly controlled experiment enabled reliable comparison of the genotypes without the controversial effects of environmental or analytical factors. Thus, the data are useful for plant breeding and selection of phytosterol-rich genotypes. Among winter and spring wheat genotypes, considerable differences were shown in both phytosterol contents and compositions. Smaller kernel sizes and higher bran yields resulted in higher phytosterol contents in bread wheats. The other wheat types, namely, durum wheat, spelt, and the early cultivated forms of wheat, contained in some cases even greater amounts of phytosterols than bread wheats. For plant breeders it is also important to know whether the concentrations of bioactive compounds in certain genotypes remain stable on a yearly basis, regardless of growth location and weather conditions. Thus, more research is needed to define the effects of environmental conditions and yearly variation on phytosterol contents of the most interesting wheat genotypes.

ABBREVIATIONS AND NOMENCLATURE USED

α-Amyrin, urs-12-en-3β-ol; av, average; Δ^5-avenasterol, stigmasta-5,24(28)-dien-3β-ol; Δ^7-avenasterol, (24*Z*)-5α-stigmasta-7,24(28)-dien-3β-ol; brassicasterol, (22*E*)-ergosta-5,22-dien-3β-ol; campestanol, (24*R*)-5α-ergostan-3β-ol; campesterol, (24*R*)-ergost-5-en-3β-ol; CHD, coronary heart disease; cholesterol, cholest-5-en-3β-ol; citrostadienol, (24*Z*)-4α-methyl-5α-stigmasta-7,24(28)-dien-3β-ol; cycloartenol, 9,19-cyclolanost-24-en-3β-ol; dihydrocholesterol, 5α-cholestan-3β-ol; dm, dry matter; FID, flame ionization detector; fw, fresh weight; GC, gas chromatography; gramisterol, 4α-methyl-5α-ergosta-7,24(28)-dien-3β-ol; LDL, low-density lipoprotein; 24-methylenecycloartanol, 24-methylene-9,19-cyclolanostan-3β-ol; MS, mass spectrometry; SD, standard deviation; SiOH, silica; sitostanol, 5α-stigmastan-3β-ol; sitosterol, stigmast-5-en-3β-ol; SPE, solid-phase extraction; stigmasta-5,24(25)-dienol, stigmasta-5,24(25)-dien-3β-ol; Δ^7-stigmastenol, stigmast-7-en-3β-ol; stigmasterol, (22*E*)-stigmasta-5,22-dien-3β-ol; TMS, trimethylsilyl.

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