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Phytosterol Variability in Almond Germplasm

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Abstract. **Phytosterols are important dietary components that contribute to reducing serum cholesterol levels. The objective of this research was to assess genetic diversity for total content and profile of free and esterified phytosterols in a world germplasm collection of almond [*Prunus amygdalus* Batsch; syn. *P. dulcis* (Mill.) D.A. Webb]. Steryl glycosides and acylated steryl glycosides were not measured. Fruit from 160 almond accessions were collected in 2009 and 2010. Kernel phytosterol content ranged from 1126 to 2769 mg·kg^{-1} in 2009 and from 1191 to 2777 mg·kg^{-1} in 2010. The phytosterol fraction was mainly made up of β-sitosterol (from 59.1% to 84.1% in 2009 and from 55.9% to 84.6% in 2010) and Δ^5-avenasterol (from 8.9% to 25.4% in 2009 and from 8.5% to 28.2% in 2010). Significant genotypic effects were observed for kernel phytosterol content and concentration of major phytosterols. Kernel oil content was positively correlated with kernel phytosterol content in both years. The results suggested that almond germplasm contains genetic variability for both phytosterol content and profile that can be used for developing cultivars with increased levels of phytosterols and contrasting phytosterol profiles. Positive correlation between kernel phytosterol content and kernel oil content suggests the feasibility of simultaneous selection for both traits.**

Phytosterols or plant sterols are natural constituents of plants. They resemble mammalian cholesterol, both in their chemical structure and their biological function (Piironen et al., 2000). Phytosterols regulate the fluidity and permeability of membranes and also play many other functions in plants, for example as precursors of brassinosteroids, an important group of plant hormones involved in many aspects of plant growth (Hartmann, 1998).

Phytosterols are also important dietary components. Vegetable oils and oil-based products are the richest dietary sources of phytosterols followed by cereal grains, cereal-based products, and nuts (Piironen et al., 2000). Sterols in foods exist as free sterols, fatty acid esters, steryl glycosides, and acylated steryl glycosides (Phillips et al., 2005a). Because of the similarity in the chemical structures of phytosterols and cholesterol, dietary phytosterols reduce intestinal absorption of both dietary and endogenously produced cholesterol, thus reducing serum cholesterol levels (Plat and Mensink, 2005). Because of their cholesterol-lowering properties, phytosterol-derived products such as phytostanol esters have become important ingredients in a wide range of functional foods (Bacchetti et al., 2011).

Almond is the most important tree nut crop in terms of commercial production, which is limited to areas characterized by a Mediterranean climate (Kester and Asay, 1975). Kernel quality has become an important criterion for modern almond cultivars (Socias i Company et al., 2008). Almond germplasm collections have been evaluated for variation in kernel quality traits such as oil content, fatty acid composition (Kodad et al., 2011), and tocopherol content (Kodad et al., 2006). However, there is little information on the variability in phytosterol content in almond germplasm, because the literature only provides results from analyses of commercial almond samples. For example, the analysis of a single sample of almond kernels from a local market (Normén et al., 2007) revealed a phytosterol content of 2080 mg·kg^{-1}. Another study on four different samples of almond kernels from the U.S. market (Phillips et al., 2005b) reported an average phytosterol content of 1990 mg·kg^{-1} (from 1930 to 2080 mg·kg^{-1}). Similarly, the analysis of three almond samples from the U.S. market (Robbins et al., 2011) showed an average kernel phytosterol content of 2107 mg·kg^{-1}. The U.S. Department of Agriculture (USDA, 2011) National Nutrient Database reports a kernel phytosterol content of 1720 mg·kg^{-1}. Oil phytosterol levels between 2178 and 2750 mg·kg^{-1} have been reported for raw almond oils (Miraliakbari and Shahidi, 2008), whereas a phytosterol content of 1999 mg·kg^{-1} has been reported for commercial almond oil (Dulf et al., 2010). The USDA (2011) National Nutrient Database reports an almond oil phytosterol content of 2660 mg·kg^{-1}. Phillips et al. (2005a) found that 78% of the phytosterols in almonds are in the form of free and esterified sterols, whereas the remaining 22% are in the form of steryl glycosides and acylated steryl glycosides. The latter compounds have similar

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cholesterol-lowering properties to free and esterified phytosterols (Lin et al., 2009). However, their relevance for oil quality is limited, because very low levels of steryl glycosides and acylated steryl glycosides are extracted by conventional hexane extraction (Moreau et al., 2003).

There are some discrepancies in the literature about the composition of almond phytosterols. Although all the studies have identified β-sitosterol as the predominant phytosterol in almond, they differ in the proportions of the other phytosterols. Several studies (Dulf et al., 2010; Normén et al., 2007; Phillips et al., 2005a, 2005b; Robbins et al., 2011) have reported a phytosterol fraction mainly made up of β-sitosterol (from 72.1% to 83.9%) and Δ^5-avenasterol (from 9.9% to 12.9%) and to a lesser extent campesterol (2.5% to 3.4%) and stigmasterol (1.6% to 3.4%). However, other studies have reported different phytosterol profiles characterized by absence or lower levels of Δ^5-avenasterol (Cherif et al., 2009; Maguire et al., 2004; Miraliakbari and Shahidi, 2008).

One of the most important world collections of almond cultivars is located at Centro de Investigación y Tecnología Agroalimentaria (CITA) of Aragón, Spain, with ≈250 accessions introduced from all over the world (Espiau et al., 2002). This collection shows a very large variability reflecting the wide genetic diversity of almond (Socias i Company and Felipe, 1992). The objective of this research was to assess genetic diversity for total content and profile of free and esterified phytosterols in the CITA world germplasm collection.

Materials and Methods

Plant material. The study was conducted on 160 almond accessions of the CITA world germplasm collection. The accessions are maintained as living plants grafted on the almond × peach [*Prunus persica* (L.) Batsch] hybrid clonal rootstock INRA GF-677 using standard management practices (Espiau et al., 2002).

Sample collection and preparation. Twenty nuts per accession from open pollination were harvested in 2009 and 2010 at the mature stage when fruit mesocarp was fully dried and split along the fruit suture and peduncle abscission was complete. After blanching, the kernels were ground in a laboratory mill and stored until analysis at 4 °C for a maximum of 60 d.

Determination of oil content. Oil was extracted from 4 to 5 g of ground almond kernel in a commercial fat extractor (Soxtec Avanti 2055; Tecator, Barcelona, Spain) for 2 h using petroleum ether as a solvent and keeping the heating source at 135 °C (Kodad and Socias i Company, 2008). The oil content was expressed as the difference in weight of the dried kernel flour sample before and after extraction.

Analysis of phytosterol content. Phytosterol content was analyzed in two replicates per sample following a previously described procedure for the analysis of free and esterified phytosterols (Fernández-Cuesta et al., 2012). In short, 200 mg of almond flour was placed in 10-mL propylene tubes and 200 μL of an internal standard solution prepared by dissolving cholesterol (99% purity, reference C8667; Sigma-Aldrich, St. Louis, MO) in hexane–ethanol (3:2) solution at a concentration of 0.1%. Alkaline hydrolysis was performed by adding 2 mL of a solution of potassium hydroxide dissolved in ethanol at a concentration of 2%. Phytosterols were extracted by vortexing with 1 mL hexane and 1.5 mL water. The upper hexane layer was transferred to 2-mL glass vials that were maintained in an oven at 37.5 °C overnight. Fifty microliters of hexane and 50 μL of silylating mixture composed of pyridine:hexamethyldisilazane:trimethylchlorosilane (9:3:1 by volume, Silan-Sterol-1, reference 355650.0922; Panreac Química, Barcelona, Spain) were added to the dried pellets and the vials were left at room temperature for 15 min. The solution was transferred to 2-mL vials containing 200-μL inserts and centrifuged at 2594 g_n for 10 min (Unicen 21; Ortoalresa, Madrid, Spain). The vials were capped and conserved at –20 °C. Gas chromatographic analysis was performed on a gas chromatograph (Clarus 600; Perkin Elmer, Waltham, MA) equipped with a ZB-5 capillary column (i.d. = 0.25 mm, length = 30 m, film thickness = 0.10 μm; Phenomenex, Torrance, CA). Hydrogen was used as carrier gas at a pressure of 125 kPa. The split injector and flame ionization detector were maintained at 320 °C. The oven thermal regime was the following: the initial temperature of 240 °C was increased at 5 °C·min^{-1} to a final temperature of 265 °C and held for 10 min. Total analytical time was 15 min. Peak identification was conducted at the reference laboratory of the Instituto de la Grasa, Seville, Spain. Kernel phytosterol content was expressed as milligrams per kilogram of kernel. Oil phytosterol content, expressed as milligrams per kilogram of kernel oil, was estimated from kernel phytosterol content and kernel oil content using the following formula:

$$\text{oil phytosterol content} = (\text{kernel phytosterol content} \times 100)/\text{oil content}$$

Such estimation represents the maximum expected phytosterol content in the raw oil, although the actual content will depend on the efficiency of the extraction system. The concentration of individual phytosterols was expressed as percentage of total free and esterified phytosterols.

Statistical analyses. Data were analyzed by the General Linear Model procedure of SPSS Statistics (Version 17.0; IBM Corp., Armonk, NY). Analysis of variance was performed with genotypes as fixed factors and years as random factors. Mean values were analyzed by Duncan's multiple range test.

Results

Considering all accessions, phytosterol content (free and esterified phytosterols) in the kernel averaged 1904 mg·kg^{-1} (from 1126 to 2769 mg·kg^{-1}) in 2009 and 1861 mg·kg^{-1} (from 1191 to 2777 mg·kg^{-1}) in 2010. Kernel oil content averaged 60.7% (from 53.9% to 69.2%) in 2009 and 59.9% (from 47.2% to 66.7%) in 2010. Oil phytosterol content, calculated from phytosterol and oil contents in the kernel, averaged 3131 mg·kg^{-1} (from 2013 to 4497 mg·kg^{-1}) in 2009 and 3108 mg·kg^{-1} (from 1898 to 4554 mg·kg^{-1}) in 2010 (Table 1). The phytosterol fraction was mainly made up of β-sitosterol (72.4% in 2009 and 73.9% in 2010) and Δ^5-avenasterol (14.8% in 2009 and 16.2% in 2010), which together accounted for 87.2% and 90.1% phytosterols in 2009 and 2010, respectively. Other phytosterols detected in minor amounts were campesterol (2.7% in 2009 and 2.8% in 2010), stigmasterol (0.8% in 2009 and 0.7% in 2010), Δ^7-campesterol (3.2% in 2009 and 2.5% in 2010), clerosterol (1.3% in 2009 and 1.2% in 2010), sitostanol (0.7% in 2009 and 0.2% in 2010), $\Delta^{5,24}$-stigmastadienol (0.9% in 2009 and 0.3% in 2010), Δ^7-stigmastenol (1.5% in 2009 and 1.0% in 2010), and Δ^7-avenasterol (1.8% in 2009 and 1.1% in 2010) (Table 1). The

Table 1. Average values and ranges of variation for phytosterol content in the kernel, kernel oil content, phytosterol content in the kernel oil, and concentrations of individual phytosterols in almond kernels from 160 accessions collected in 2009 and 2010.

	2009			2010		
Trait	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Kernel oil (mg·kg^{-1} kernel)	60.7	53.9	69.2	59.9	47.2	66.7
Kernel phytosterol (% kernel)	1904	1126	2769	1861	1191	2777
Oil phytosterol (mg·kg^{-1} oil)[z]	3132	2013	4497	3108	1898	4554
Campesterol (% phytosterols)	2.7	1.4	5.8	2.8	1.5	6.1
Stigmasterol (% phytosterols)	0.8	0.3	2.9	0.7	0.2	2.6
Δ^7-Campesterol (% phytosterols)	3.2	0.9	7.5	2.5	0.1	9.8
Clerosterol (% phytosterols)	1.3	0.5	2.6	1.2	0.4	2.8
β-Sitosterol (% phytosterols)	72.4	59.1	84.1	73.9	55.9	84.6
Sitostanol (% phytosterols)	0.7	0.0	1.6	0.2	0.0	1.4
Δ^5-Avenasterol (% phytosterols)	14.8	8.9	25.4	16.2	8.5	28.2
$\Delta^{5,24}$-Stigmastadienol (% phytosterols)	0.9	0.0	2.7	0.3	0.0	1.8
Δ^7-Stigmastenol (% phytosterols)	1.5	0.1	4.8	1.0	0.1	3.5
Δ^7-Avenasterol (% phytosterols)	1.8	0.2	4.6	1.1	0.4	3.8

[z]Calculated from kernel phytosterol content and kernel oil content.

collection contained large variability in the phytosterol profiles with the concentration of β-sitosterol ranging from 59.1% to 84.1% in 2009 and from 55.9% to 84.6% in 2010 and the concentration of Δ^5-avenasterol ranging from 8.9% to 25.4% in 2009 and from 8.5% to 28.2% in 2010 (Table 1). Supplemental information with the results for all accessions is available online (CITA, 2012).

The analysis of variance showed differences among genotypes for kernel oil content, kernel phytosterol content, oil phytosterol content, and the concentration of individual phytosterols, except for Δ^7-campesterol and clerosterol (Table 2). Year effect was significant for the concentration of individual phytosterols, except for clerosterol, but not for kernel oil content, kernel phytosterol content, or oil phytosterol content. Genotype × year interaction was significant for kernel oil content, kernel and oil phytosterol content, and concentration of some phytosterols such as campesterol, Δ^7-campesterol, clerosterol, β-sitosterol, and Δ^5-avenasterol.

Table 2. Analysis of variance (mean squares) for kernel oil content, kernel phytosterol content, oil phytosterol content, and concentrations of individual phytosterols in 160 almond accessions grown in Zaragoza, Spain, in 2009 and 2010.

Trait	Genotype[z]	Yr	Genotype × yr
Kernel oil content (% kernel)	29.4**	1.4	7.1**
Kernel phytosterol content (mg·kg^{-1} kernel)	237,018.6**	297,754.4	129,715.9**
Oil phytosterol content (mg·kg^{-1} oil)	517,962.1**	846,111.1	336,221.5**
Campesterol (% phytosterols)	1.2**	3.3*	0.6**
Stigmasterol (% phytosterols)	0.4**	1.4**	0.2
Δ^7-Campesterol (% phytosterols)	4.9	84.0**	3.9*
Clerosterol (% phytosterols)	0.3	0.1	0.3*
β-Sitosterol (% phytosterols)	51.6**	368.8**	18.2**
Sitostanol (% phytosterols)	0.3*	43.4**	0.2
Δ^5-Avenasterol (% phytosterols)	31.1**	323.2**	5.8**
$\Delta^{5,24}$-Stigmastadienol (% phytosterols)	0.7**	45.1**	0.3
Δ^7-Stigmastenol (% phytosterols)	1.8**	48.7**	1.2
Δ^7-Avenasterol (% phytosterols)	1.4**	66.0**	0.7

[z]Significant at *$P < 0.05$ or **$P < 0.01$, F-test.

Taking into account both years, 'Elvira' had significantly ($P < 0.05$) lower kernel phytosterol content (1268 mg·kg^{-1}) and oil phytosterol content (2257 mg·kg^{-1}) than the other accessions. Conversely, 'Cristomorto' had significantly higher kernel phytosterol content (2472 mg·kg^{-1}), whereas both 'Cristomorto' and 'Fourcoronne' had significantly higher oil phytosterol content (4005 and 3999 mg·kg^{-1}, respectively). The accessions also showed great variability in their phytosterol profiles. Breeding clone 472 and 'Bartre' were characterized by the lowest (61.7%) and highest (81.4%) concentrations of β-sitosterol, respectively. 'G-3-3' possessed the lowest concentration of Δ^5-avenasterol (9.3%), whereas the highest concentrations of this phytosterol (greater than 24%) were identified in 'Tree', Breeding clone 478, 'Ponç', and Breeding clone 472 (Table 3).

Correlation coefficients between both years were significant for kernel oil content ($r = 0.64$; $P < 0.01$), kernel phytosterol content ($r = 0.29$; $P < 0.01$), oil phytosterol content ($r = 0.21$; $P < 0.01$), and concentrations of campesterol ($r = 0.36$; $P < 0.01$), stigmasterol ($r = 0.41$; $P < 0.01$), β-sitosterol ($r = 0.48$; $P < 0.01$), sitostanol ($r = 0.18$; $P < 0.05$), Δ^5-avenasterol ($r = 0.69$; $P < 0.01$), $\Delta^{5,24}$-stigmastadienol ($r = 0.38$; $P < 0.01$), Δ^7-stigmastenol ($r = 0.22$; $P < 0.01$), and Δ^7-avenasterol ($r = 0.37$; $P < 0.01$). They were not significant for Δ^7-campesterol and clerosterol.

Taking into account only correlations observed in both years (Table 4), kernel oil content was positively correlated with kernel phytosterol content. Kernel phytosterol content was positively correlated with the concentrations of sitostanol and $\Delta^{5,24}$-stigmastadienol and negatively correlated with the concentrations of campesterol and β-sitosterol. Kernel oil content was positively correlated with the concentrations of Δ^7-campesterol and Δ^5-avenasterol and negatively with the concentration of β-sitosterol. The latter was negatively correlated with the concentrations of stigmasterol, Δ^7-campesterol, Δ^5-avenasterol, $\Delta^{5,24}$-stigmastadienol, Δ^7-stigmastenol, and Δ^7-avenasterol.

Table 3. Phytosterol content in the kernel, kernel oil content, phytosterol content in the kernel oil, and concentration of β-sitosterol and Δ^5-avenasterol in nine almond accessions with contrasting levels of phytosterol traits.[z]

Accession no.	Accession name	Kernel phytosterol (mg·kg^{-1} kernel)	Oil content (% kernel)	Oil phytosterol (mg·kg^{-1} oil)	β-sitosterol (% phytosterols)	Δ^5-avenasterol (% phytosterols)
165	Fourcouronne	2335	58.4	**3999**	74.6	14.2
189	Bartre	1739	56.0	3109	**81.4**	10.6
193	Elvira	**1268**	56.2	**2257**	76.9	12.6
231	Cristomorto	**2472**	61.8	**4005**	74.1	12.2
246	Ponç	2353	**64.2**	3670	65.3	**25.2**
472	Breeding clone	1723	60.3	2855	**61.7**	**25.4**
478	Breeding clone	2191	63.2	3466	64.8	**24.6**
516	Tree	2424	61.9	3921	62.4	**24.5**
543	G-3-3	1495	**51.1**	2940	76.1	**9.3**

[z]Groups with minimum and maximum values in Duncan's multiple range test ($P < 0.05$) using average values from fruit collected in 2009 and 2010. The trait for which the accession shows extreme values, both low and high, is given in bold.

The strongest positive correlations between individual phytosterols involved campesterol with both stigmasterol and clerosterol, stigmasterol with clerosterol, $\Delta^{5,24}$-stigmastadienol with stigmasterol, Δ^7-campesterol, clerosterol, Δ^7-stigmastenol and Δ^7-avenasterol, and Δ^7-stigmastenol with Δ^7-avenasterol (Table 4).

Discussion

Previous studies have identified large genetic variation in oil content, fatty acid composition (Kodad et al., 2011; Kodad and Socias i Company, 2008), and tocopherol content and composition (Kodad et al., 2006) in almond germplasm. The present study, based on a large and geographically diverse germplasm collection, has shown that almond germplasm also contains large variation in free and esterified phytosterol content and profile. The accession with maximum kernel phytosterol content had 2472 mg·kg^{-1} as a 2-year average compared with a maximum content of 2107 mg·kg^{-1} previously reported. It is important to note that the method used in the present research, based on the analysis of almond kernels, does not measure steryl glycosides and acylated steryl glycosides, which account for ≈22% of total phytosterols in almond kernels (Phillips et al., 2005a).

The phytosterol fraction of all accessions was dominated by β-sitosterol, whereas Δ^5-avenasterol was in all cases the second most abundant phytosterol, which is in agreement with previous studies (Dulf et al., 2010; Normén et al., 2007; Phillips et al., 2005a, 2005b; Robbins et al., 2011) but in disagreement with other studies in which Δ^5-avenasterol was not identified or it was found in lower proportions (Cherif et al., 2009; Maguire et al., 2004; Miraliakbari and Shahidi, 2008). Compared with a maximum concentration of 12.9% Δ^5-avenasterol in the literature (Normén et al., 2007), several accessions contained a much greater concentration of this phytosterol, up to 25.4% as a 2-year average. To our knowledge no similarly high levels of Δ^5-avenasterol have been reported for any other tree nut. Robbins et al. (2011) reported a maximum average value of 16.8% for pine nut (*Pinus* L.). Δ^5-avenasterol is a major phytosterol in oats (*Avena sativa* L.), accounting for ≈30% (Määttä et al., 1999). High levels of Δ^5-avenasterol up to 36% of the sterol fraction have also been reported in olive (*Olea europaea* L.) oil (Boskou, 2011). Whereas most phytosterols have no antioxidant effect, those containing an ethylidene group in the side chain such as Δ^5-avenasterol or Δ^7-avenasterol are effective antioxidants at high temperatures, because they protect the oil against polymerization and also retard the loss of tocopherols (Rossell, 2001). To our knowledge nutritional implications of elevated Δ^5-avenasterol levels have not been investigated; hence, the availability of almond accessions with low and high levels of this phytosterol provides an excellent opportunity for such research.

Both the analysis of variance and the correlation study between the data of 2 years revealed a significant genotypic variation for phytosterol content and profile in almond germplasm. Studies of annual oilseed crops such as soybean [*Glycine max* (L.) Merr.] and rapeseed (*Brassica napus* L.) concluded a significant effect of the genotype on seed phytosterol content (Amar et al., 2008; Vlahakis and Hazebroek, 2000). In rapeseed, heritability estimates for phytosterol content ranged from 0.84 to 0.91 in three populations, suggesting that the trait is amenable to selection (Amar et al., 2008).

Kernel phytosterol content was positively correlated with kernel oil content in both years, suggesting that a simultaneous selection for both traits is feasible. A study of rapeseed found a positive correlation between oil and phytosterol content in one population, a negative correlation in another population, and absence of correlation in a third population (Amar et al., 2008). The high positive correlation between oil phytosterol content and kernel phytosterol content probably reflects the existence of much larger genetic variation in phytosterol content than in oil content within the germplasm collection. A strong positive correlation between oil and kernel phytosterol content has been reported in sunflower (*Helianthus annuus* L.) (Fernández-Cuesta et al., 2012). The strong negative correlation between the concentrations of the two major phytosterols β-sitosterol and Δ^5-avenasterol, also observed in sunflower (Fernández-Cuesta et al., 2012), suggests the possibility of selecting for contrasting levels of both compounds.

Although there is some controversy on the health benefits of diet supplementation with high levels of phytosterols (Weingärtner et al., 2011), experimental evidence points to the effect of dietary phytosterols on reducing serum cholesterol levels (Plat and Mensink, 2005). The results of this research suggest that almond germplasm contains genetic variation that can be used for developing cultivars with increased levels of these compounds. Also, genetic variation for phytosterol profile, particularly for β-sitosterol and Δ^5-avenasterol levels, indicates that selection for contrasting levels of these phytosterols is feasible, although additional information on the

Table 4. Correlation coefficients among kernel oil content, kernel phytosterol content, oil phytosterol content, and concentrations of individual phytosterols in 160 almond accessions grown in 2009 (upper row) and 2010 (lower row).

Trait[z]	Oil[y] (% kernel)	KPhy ($mg \cdot kg^{-1}$)	OPhy ($mg \cdot kg^{-1}$)	Camp (% phy)	Stig (% phy)	D7Camp (% phy)	Clero (% phy)	BSito (% phy)	Sito (% phy)	D5Av (% phy)	D5,24 (% phy)	D7Stig (% phy)
KPhy	0.46**											
($mg \cdot kg^{-1}$)	0.36**											
OPhy	0.21**	0.97**										
($mg \cdot kg^{-1}$)	0.04 NS	0.95**										
Camp	–0.27**	–0.32**	–0.27**									
(% phy)	–0.07 NS	–0.24**	–0.24**									
Stig	–0.02 NS	–0.11 NS	–0.12 NS	0.58**								
(% phy)	0.06 NS	–0.22**	–0.25**	0.71**								
D7Camp	0.25**	0.34**	0.30**	–0.13 NS	0.14 NS							
(% phy)	0.29**	0.14 NS	0.05 NS	0.10 NS	0.35**							
Clero	–0.11 NS	–0.13 NS	–0.11 NS	0.57**	0.44**	0.08 NS						
(% phy)	–0.11 NS	–0.20*	–0.18*	0.45**	0.29**	–0.22**						
BSito	–0.21**	–0.23**	–0.19*	–0.08 NS	–0.29**	–0.58**	–0.30**					
(% phy)	–0.38**	–0.17*	–0.06 NS	–0.29**	–0.39**	–0.58**	–0.14 NS					
Sito	–0.02 NS	0.21**	0.23**	0.02 NS	0.10 NS	0.02 NS	–0.03 NS	–0.02 NS				
(% phy)	–0.11NS	0.21**	0.26**	–0.08 NS	–0.16*	–0.29**	0.05 NS	0.18*				
D5Av	0.20*	0.14 NS	0.10 NS	–0.24**	–0.15 NS	0.24**	0.03 NS	–0.67**	–0.42**			
(% phy)	0.33**	0.14 NS	0.05 NS	–0.13 NS	–0.09 NS	0.14 NS	–0.13 NS	–0.71**	–0.25**			
D5,24	0.06 NS	0.22**	0.22**	0.14 NS	0.32**	0.36**	0.33**	–0.60**	0.42**	0.08 NS		
(% phy)	0.28**	0.28**	0.21**	0.18*	0.23**	0.35**	0.21**	–0.42**	0.15 NS	–0.06 NS		
D7Stig	–0.04 NS	0.08 NS	0.09 NS	0.05 NS	0.18*	0.07 NS	0.03 NS	–0.40**	0.41**	–0.17*	0.48**	
(% phy)	0.11 NS	0.06 NS	0.04 NS	0.28**	0.28**	0.13 NS	0.43**	–0.41**	0.13 NS	–0.09 NS	0.47**	
D7Av	0.15 NS	0.11 NS	0.08 NS	0.05 NS	0.24**	0.13 NS	0.01 NS	–0.47**	0.23**	–0.02 NS	0.30**	0.55**
(% phy)	0.10 NS	0.26**	0.24**	0.04 NS	0.12 NS	0.14 NS	0.23**	–0.39**	0.20*	–0.02 NS	0.45**	0.79**

[z]Oil = kernel oil content; KPhy = kernel phytosterol content; OPhy = oil phytosterol content; Camp = campesterol; Stig = stigmasterol; D7Camp = Δ^7-campesterol; Clero = clerosterol; BSito = β-sitosterol; Sito = sitostanol; D5Av = Δ^5-avenasterol; D5;24 = $\Delta^{5;24}$-stigmastadienol; D7Stig = Δ^7-stigmastenol; D7Av = Δ^7-avenasterol; % Phy = percentage of total phytosterols.
[y]Nonsignificant (NS) or significant at $*P < 0.05$ or $**P < 0.01$, two-tailed *t* test.

nutritional advantages of both profiles is required to define selection criteria properly.

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