Research Paper

Phytosterols in 17 Turkish hazelnut (*Corylus avellana* L.) cultivars

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The phytosterol contents of the oils from 17 Turkish hazelnut cultivars were determined by gas chromatography with a flame ionization detector. The total phytosterol content varied from 1180.4 (Uzunmusa-Ordu) to 2239.4 mg/kg (Cavcava), and the average was 1581.6 ± 265.1 mg/kg. One of the most significant commercial cultivars, Tombul, contained quite low total phytosterols (1297.7 mg/kg). Total and individual phytosterol contents of hazelnut cultivars were significantly different at $p < 0.01$, except for phytostanol and campestanol. The main component was β-sitosterol which ranged from 82.8 to 86.7% in all cultivars. This was followed by campesterol, Δ^5-avenasterol, sitostanol and stigmasterol. Interestingly, the same cultivars from different regions showed similar total phytosterol contents, and fall almost within the same range according to Duncan's test, which may indicate that the phytosterol content is highly related to the cultivar.

Keywords: *Corylus avellana* / Hazelnut / Phytosterol

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1 Introduction

Turkey is the main hazelnut (*Corylus avellana* L.) producing country in the world, providing 65–70% of the world production and accounting for more than 80% of the world hazelnut trade. Hazelnut production in Turkey utilizes over 540,000 ha of land, with an annual production of about 358,000 t in 2004. Turkey is followed by Italy (120,000 t), Spain (30,000 t) and the USA (25,000 t) in the rate of annual production. Germany is the most important hazelnut-importing country in the world, responsible for approximately 35% of the total world import [1].

On the Anatolia peninsula, 17 cultivars of hazelnut are found and each has a different commercial significance [2, 3]. The production areas can be divided into four regions: Giresun, Ordu, Trabzon, and Akçakoca. The major cultivar produced in Turkey is Tombul, which occupies 29.75% of all production areas. This is followed by the cultivars Çakıldak (15.19%), Mincane (14.08%), İncekara (12.35%), Palaz (11.90%), Foşa (7.02%), Kalınkara (2.66%), Cavcava (1.90%), Sivri (1.55%), and Uzunmusa (0.89%). The Acı, Kan, Kargalak and Kuş cultivars are not grown commercially. The other three cultivars (Yassıbadem, Yuvarlakbadem and Karafındık) have very limited commercial value.

Hazelnuts are particularly valuable for their rich lipid composition, which accounts for around 60% of the hazelnut kernel. Proteins, carbohydrates, sterols, vitamin E and minerals such as boron are also important constituents of hazelnuts [4–8]. Phytosterols comprise a major proportion of the unsaponifiable fraction of most vegetable oils [9]. Clinical studies have repeatedly shown that phytosterols, taken as dietary supplements or as supplemental ingredients in foods, reduced serum cholesterol and low-density lipoprotein cholesterol levels in normal and mildly hypercholesteremic subjects [10]. The mechanism involved may be related to inhibition of dietary and biliary cholesterol absorption from the intestinal lumen [11]. In addition to the cholesterol-lowering effect,

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phytosterols have been suggested to possess anti-inflammatory, antibacterial, antifungal, anti-ulcerative and antitumor activities [12–17].

The aim of this study was to examine the phytosterol compositional differences of all 17 Turkish hazelnut cultivars, including those of greater and lesser commercial significance.

2 Materials and methods

2.1 Materials

Twenty-one samples of 17 hazelnut cultivars (*Corylus avellana* L.) harvested in August 2006 were used in this study. Acı, Cavcava, İncekara, Kalınkara, Kan, Kargalak, Kuş, Mincane and Yuvarlakbadem cultivars were obtained from the province of Giresun. Çakıldak, Kara, Palaz and Tombul cultivars were from the province of Ordu. Sivri, Yassıbadem and Uzunmusa cultivars were taken from both Giresun and Ordu. The Foşa cultivar was from both Giresun and Trabzon. Samples from Giresun were obtained from the collection yards of the Hazelnut Research Institute in Giresun, Turkey. Ordu and Trabzon samples were collected from farms in Ordu and the town of Arsin in Trabzon.

Hazelnut samples (100 g) from each cultivar and location were randomly sampled from a 3-kg portion. The cultivars were air-dried, deshelled and milled (using a Moulinex-750 model grinder) to pass a 0.2-mm sieve. Ground hazelnuts were vacuum-packed in PE bags and stored at –28 °C until the time of analysis. Approximately 15 g of ground hazelnuts were weighed into thimbles and the oils were extracted over 6 h with refluxing *n*-hexane in a glass Soxhlet apparatus [18]. Solvent was removed under vacuum at 40 °C and the last traces were removed with a nitrogen purge. The oil content of the cultivars was 60–65%. Until analyzed, the oils were kept in dark bottles under nitrogen in a freezer at –28 °C.

Campesterol, stigmasterol and cholestanol (internal standard) were purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA). Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethyl-chlorosilane (TMCS) was commercially available from Supelco (Bellefonte, PA, USA). β-Sitosterol and all other chemicals, which were of analytical grade, were obtained from Merck (Darmstadt, Germany).

2.2 Methods

2.2.1 Sterol analysis

Preperation of unsaponifiable matter and determination of the composition of the sterol fractions were done according to AOCS Official Method Ch 6-91 [19]. Derivatives of the sterols (silyl ethers) were analyzed using a gas chromatograph (GC 2010; Shimadzu, Kyoto, Japan) equipped with a fused-silica capillary column HP-5 (30 m × 0.25 mm ID and 0.25 μm film thickness) (Chrom Tech., Apple Walley, MN, USA) and quantified using cholestanol as internal standard. The impurity (cholesterol) of the internal standard was taken into account in the calculations. The split ratio was 50 : 1 and the carrier gas was helium at 0.8 mL/min. Injector, column and detector (FID) temperatures were 280, 260 and 290 °C, respectively, for the whole run. Three injections of 1 μL were made for each derivatized oil.

Identification of indivual sterols was performed by GC-MS under some of the conditions as for GC-FID. The system was connected to an Agilent 5973 mass selective detector (MSD) and an Agilent 7683 autosampler. The transfer line from the gas chromatograph to the mass spectrometer was set at 300 °C. MSD parameters were as follows: scan mode, 50–600 amu; threshold, 400; sample rate, 2.9 scans/s; ionizing voltage, 70 eV; and EM voltage, 2000 V. The available phytosterol standards of β-sitosterol, campesterol and stigmasterol were also used for verification of the results.

2.2.2 Statistical analysis

Data for each of the 17 cultivars ($n = 3$) were recorded as mean ± standard error and analyzed by SPSS (SPSS Inc., Chicago, IL, USA) for Windows (ver. 10.1.). One-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out to test any significant differences between cultivars.

3 Results and discussion

A GC-FID chromatogram of the Çakıldak cultivar is given in Fig. 1. The total phytosterol content in the Turkish hazelnut oil samples ranged from 1180 to 2239 mg/kg (Table 1). The Uzunmusa and Cavcava cultivars contained the lowest and highest phytosterol levels, respectively. ANOVA indicated that there were significant differences among the cultivars at the $p < 0.01$ level in terms of total sterol content.

The Duncan's test results (Table 2) indicated that the Uzunmusa, Tombul, Çakıldak and Kalınkara cultivars were in group A to BC, which is the group containing the lowest levels of total phytosterols. On the contrary, the Cavcava, Yuvarlakbadem, Yassıbadem and Mincane cultivars contained the highest amounts of total phytosterols (they were in group I to K).

Tombul, which is the most important commercial cultivar, was in the low-phytosterol-containing group (AB). The other important commercial cultivars such as Çakıldak and İncekara were also classified in comparably low-phytosterol-containing groups (BC and CDE); however, other important commercial cultivars such as Palaz and Mincane were in the GH or I group. In comparison, the commercially less significant cultivars such as Acı, Kuş, Yuvarlakbadem, and Yassıbadem tended to be in the groups with higher levels of phytosterols (DEF to *XX*J groups).

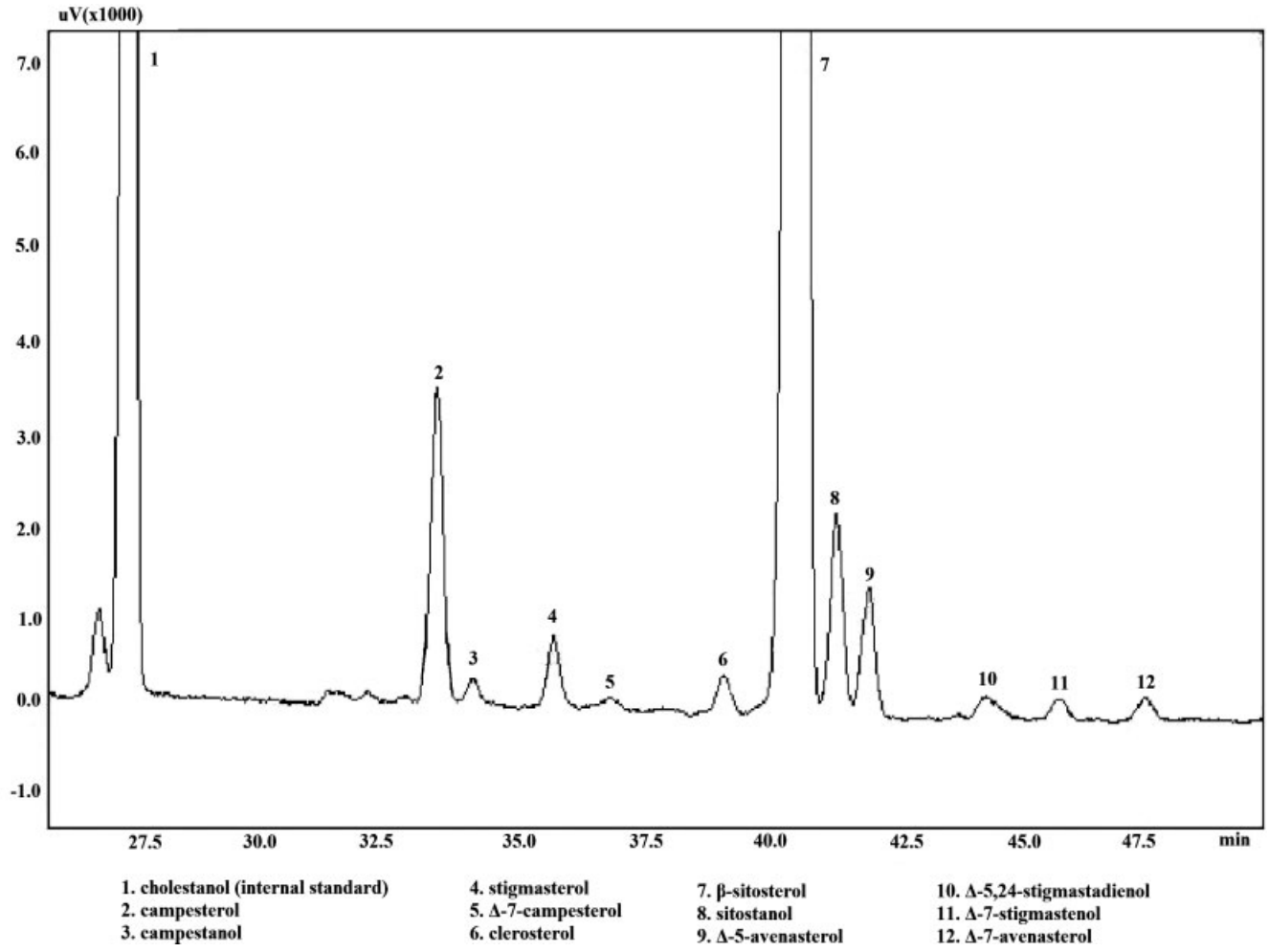

Figure 1. GC-FID chromatogram of the Çakıldak cultivar.

Benitez-Sanchez *et al.* [20] determined total phytosterol contents of 1242–1657 mg/kg in four cultivars (Tombul, Palaz, Foşa and Mincane). In the current study, the phytosterol levels of these cultivars were found within the range of 1298–1807 mg/kg.

Weihrauch and Gardner [9] and Maguire *et al.* [21] reported 1200 and 1096 mg/kg total phytosterol contents in hazelnut oils. Alasalvar *et al.* [22] found 1135 mg/kg of total phytosterols in Tombul hazelnut oil harvested from the Giresun region of Turkey in 2001. This finding is lower than our result of 1298 mg/kg for Tombul in 2006. Parcerisa *et al.* [23] found 1394 and 1621 mg/kg of total phytosterol contents in two Turkish hazelnut cultivars (Tombul, and Imperial, respectively). Bada *et al.* [24] studied the phytosterol contents of hazelnut oils from various countries and found that Spanish samples contained between 1458 and 3469 mg/kg phytosterols, one French sample had 1534 mg/kg, whereas one commercial Turkish oil had a level of 1411 mg/kg. These differences among the sterol contents of hazelnut oils are probably due to varietial differences or growing, climatic and environmental conditions.

In relation to the phytosterol distribution of hazelnut oil, β-sitosterol was the single most predominant one and varied between 1003 (Uzunmusa, Ordu) and 1932 mg/kg (Cavcava). This was followed by campesterol (59.4–109.0 mg/kg) and Δ^5-avenasterol (27.8–98.9 mg/kg).

Parcerisa *et al.* [23] found 1128.5 (Tombul) and 1394.5 mg/kg (Imperial) of β-sitosterol in two Turkish hazelnut cultivars. The β-sitosterol content of the Tombul cultivar in Alsalavar *et al.* [22] was quite similar to our findings. They reported 1050.5 mg/kg, while we found 1092.1 mg/kg. Maguire *et al.* [21] and Benitez-Sanchez *et al.* [20] found 991.7 and 1105–1376 mg/kg of β-sitosterol in Turkish hazelnut oils, respectively.

The percent distribution of β-sitosterol varied between 82.8 and 86.7%, with the lowest proportion in the Foşa (Trabzon) and the highest in the Yassıbadem (Giresun) cultivar.

Table 1. Phytosterol content in hazelnut cultivars (mg/kg).[†]

Cultivar	Campesterol	Campestanol	Stigmasterol	Δ^7-Campesterol	Clerosterol	β-Sitosterol	Sitostanol	Δ^5-Avenasterol	$\Delta^{5,24}$-Stigmastadienol	Δ^7-Stigmastenol	Δ^7-Avenasterol	Total
Acı	74.9 ± 1.41	3.7 ± 0.13	14.9 ± 0.10	4.7 ± 0.26	8.9 ± 0.19	1344.2 ± 23.44	40.6 ± 0.31	61.4 ± 1.45	8.0 ± 0.78	14.7 ± 1.13	13.6 ± 0.65	1589.59 ± 38.65
Cavcava	91.6 ± 1.90	4.3 ± 0.58	20.3 ± 0.84	3.9 ± 0.22	16.3 ± 0.71	1932.1 ± 41.16	46.2 ± 2.67	98.9 ± 6.97	12.9 ± 1.14	6.8 ± 0.74	5.8 ± 0.19	2239.34 ± 78.87
Çakıldak	71.8 ± 2.86	4.4 ± 0.82	13.7 ± 0.73	1.8 ± 0.26	7.7 ± 0.37	1158.0 ± 49.40	45.0 ± 2.85	27.8 ± 1.61	6.3 ± 0.87	6.0 ± 0.32	5.2 ± 0.64	1347.65 ± 85.40
Foşa (Giresun)	79.6 ± 1.17	4.0 ± 0.72	17.3 ± 0.22	2.8 ± 0.31	7.0 ± 0.57	1333.0 ± 8.20	47.3 ± 0.08	65.9 ± 0.64	8.3 ± 0.45	5.0 ± 0.11	8.2 ± 0.83	1578.39 ± 14.90
Foşa (Trabzon)	80.9 ± 1.68	5.8 ± 0.62	22.7 ± 0.87	15.1 ± 1.01	7.5 ± 0.52	1275.7 ± 5.86	48.3 ± 1.10	62.5 ± 3.13	6.5 ± 0.85	6.1 ± 0.61	8.6 ± 0.44	1539.83 ± 12.92
İncekara	78.1 ± 1.36	5.1 ± 0.22	13.8 ± 0.33	2.5 ± 0.43	11.5 ± 1.13	1207.9 ± 35.52	46.2 ± 2.01	52.5 ± 2.77	8.6 ± 0.45	14.2 ± 0.52	10.3 ± 0.53	1450.77 ± 62.13
Kalınkara	70.4 ± 2.12	4.9 ± 0.52	13.4 ± 0.51	2.8 ± 0.57	8.2 ± 0.31	1139.4 ± 29.15	37.0 ± 1.63	49.7 ± 1.24	6.8 ± 0.06	12.4 ± 0.14	8.4 ± 0.46	1353.41 ± 50.90
Kan	74.9 ± 3.67	3.5 ± 0.49	15.3 ± 0.67	3.8 ± 0.74	9.0 ± 1.38	1244.3 ± 53.14	38.6 ± 1.55	56.5 ± 1.95	5.6 ± 0.53	12.2 ± 0.81	11.4 ± 1.28	1475.10 ± 86.65
Karafındık	92.0 ± 1.91	3.9 ± 0.39	13.1 ± 0.56	3.6 ± 0.98	12.0 ± 0.22	1533.8 ± 54.44	38.9 ± 3.48	58.6 ± 2.95	8.4 ± 0.90	10.7 ± 0.49	11.9 ± 1.08	1786.80 ± 89.36
Kargalak	68.3 ± 0.27	4.4 ± 0.86	11.8 ± 0.14	2.5 ± 0.51	8.1 ± 0.48	1167.3 ± 32.68	50.9 ± 1.14	40.2 ± 1.87	6.6 ± 0.27	18.1 ± 0.68	11.3 ± 0.32	1389.48 ± 47.55
Kuş	75.0 ± 0.84	4.0 ± 0.71	13.3 ± 0.37	2.4 ± 0.83	10.5 ± 1.21	1285.9 ± 55.51	42.5 ± 2.48	61.2 ± 3.99	8.0 ± 0.82	9.4 ± 0.27	9.8 ± 0.21	1522.03 ± 91.02
Mincane	85.5 ± 2.52	5.8 ± 1.20	17.2 ± 0.97	5.0 ± 1.03	9.2 ± 0.79	1529.8 ± 30.46	57.8 ± 2.73	51.8 ± 2.84	9.2 ± 1.28	23.9 ± 0.61	11.7 ± 0.61	1807.11 ± 62.94
Palaz	94.1 ± 1.92	5.1 ± 0.66	13.7 ± 0.26	5.5 ± 1.11	8.5 ± 0.83	1389.0 ± 42.73	41.0 ± 4.05	47.7 ± 5.50	6.4 ± 0.53	37.0 ± 1.01	16.7 ± 0.23	1664.68 ± 81.92
Sivri (Giresun)	83.1 ± 1.22	6.2 ± 0.97	16.1 ± 0.34	5.9 ± 0.99	9.8 ± 0.34	1329.7 ± 18.18	39.7 ± 0.17	50.8 ± 0.09	4.8 ± 0.69	6.7 ± 0.01	8.9 ± 0.34	1561.95 ± 22.73
Sivri (Ordu)	84.9 ± 1.86	4.9 ± 0.37	12.8 ± 0.29	1.7 ± 0.49	10.3 ± 0.43	1359.4 ± 30.33	35.5 ± 1.02	37.3 ± 1.19	8.2 ± 0.53	6.5 ± 0.12	7.7 ± 0.31	1569.29 ± 46.47
Tombul	73.9 ± 1.46	3.5 ± 0.33	11.3 ± 0.41	2.7 ± 0.69	9.4 ± 1.15	1092.1 ± 53.18	36.4 ± 3.01	40.8 ± 5.48	6.7 ± 0.64	11.5 ± 0.97	9.7 ± 0.63	1297.75 ± 93.15
Uzunmusa (Giresun)	61.6 ± 0.52	4.2 ± 0.12	12.7 ± 0.86	3.3 ± 0.69	7.5 ± 0.17	1010.0 ± 25.32	37.4 ± 1.36	45.0 ± 1.34	7.4 ± 0.21	5.5 ± 0.44	5.8 ± 0.21	1200.43 ± 41.05
Uzunmusa (Ordu)	59.4 ± 1.23	3.0 ± 0.93	11.7 ± 0.87	4.0 ± 1.04	5.6 ± 0.70	1003.1 ± 15.11	30.2 ± 0.83	40.1 ± 0.81	5.3 ± 0.52	8.6 ± 1.17	9.1 ± 1.31	1179.97 ± 32.25
Yassıbadem (Giresun)	88.5 ± 1.59	4.1 ± 0.29	13.9 ± 0.67	2.4 ± 0.46	11.6 ± 1.47	1622.1 ± 49.78	44.6 ± 2.64	48.4 ± 2.04	8.8 ± 0.82	15.9 ± 1.04	10.0 ± 0.89	1870.54 ± 86.28
Yassıbadem (Ordu)	91.8 ± 0.81	5.0 ± 0.50	17.1 ± 1.22	6.5 ± 0.99	8.4 ± 0.40	1555.7 ± 13.74	45.6 ± 1.49	55.3 ± 0.95	6.3 ± 0.46	8.7 ± 0.98	10.2 ± 0.63	1810.83 ± 23.07
Yuvarlakbadem	109.0 ± 1.89	5.4 ± 0.36	22.4 ± 1.09	3.3 ± 1.08	12.5 ± 1.06	1669.6 ± 27.88	61.4 ± 3.59	65.3 ± 1.72	10.9 ± 2.04	9.7 ± 1.27	10.0 ± 2.92	1979.66 ± 53.29

[†] Data expressed as means ± standard error of the mean (SE) of triplicate experiments.

Table 2. Duncan's test results in hazelnut varieties.[†]

Cultivar	Campesterol	Campestanol	Stigmasterol	Δ^7-Campesterol	Clerosterol	β-Sitosterol	Sitostanol	Δ^5-Avenasterol	$\Delta^{5,24}$-Stigmastadienol	Δ^7-Stigmastenol	Δ^7-Avenasterol	Total
Acı	CD	ABC	DEF	BCDEF	BCD	FG	BCDEF	GHI	BCDE	G	G	FG
Cascava	I	ABCD	H	ABCDE	I	J	EFGH	J	G	AB	AB	K
Çakıldak	BC	ABCD	BCDE	A	ABC	BC	DEFGH	A	ABCD	A	A	BC
Foşa (Giresun)	DEF	ABC	G	ABC	AB	FG	FGH	I	CDEF	A	BC	EFG
Foşa (Trabzon)	EFG	CD	I	G	ABC	DEFG	GH	HI	ABCDE	A	BCDE	EFG
İncekara	DE	ABCD	BCDE	AB	EFGH	CDE	EFGH	DEFG	DEF	FG	CDEF	CDE
Kalınkara	BC	ABCD	ABCDE	ABC	BCD	BC	AB	CDEF	ABCDE	EF	BCD	BC
Kan	CD	AB	EFG	ABCDE	BCDE	CDEF	BCD	EFGHI	ABC	EF	DEFG	CDEF
Karafındık	I	ABC	ABCDE	ABCDE	GH	H	BCDE	FGHI	DEF	CDE	FG	HI
Kargalak	B	ABCD	ABC	ABC	ABCD	BCD	H	BC	ABCDE	H	DEFG	BCD
Kuş	CD	ABC	ABCDE	AB	DEFGH	EFG	BCDEFG	GHI	BCDE	CD	CDEF	DEF
Mincane	GH	CD	G	CDEF	BCDEF	H	I	DEFG	EF	I	EFG	I
Palaz	I	ABCD	BCDE	DEF	BCD	G	BCDEFG	CDE	ABCD	J	H	GH
Sivri(Giresun)	EFGH	D	FG	EF	CDEFG	FG	BCDE	DEF	A	AB	CDEF	EFG
Sivri (Ordu)	FGH	ABCD	ABCD	A	DEFGH	FG	AB	B	CDE	AB	ABC	EFG
Tombul	CD	AB	A	ABC	BCDEF	AB	AB	BC	ABCDE	DE	CDEF	AB
Uzunmusa (Giresun)	A	ABCD	ABCD	ABCD	ABC	A	BC	BCD	ABCDE	A	AB	A
Uzunmusa (Ordu)	A	A	AB	ABCDE	A	A	A	BC	AB	BC	CDEF	A
Yassıbadem (Giresun)	HI	ABCD	CDE	AB	FGH	HI	CDEFGH	CDE	DEF	G	CDEF	IJ
Yassıbadem (Ordu)	I	ABCD	G	F	BCD	H	DEFGH	EFGH	ABCD	BC	CDEF	I
Yuvarlakbadem	J	BCD	I	ABCD	H	I	I	I	FG	CD	CDEF	J

[†] Mean values in a column with different letters are significantly different at the $p < 0.01$ level, according to Duncan's test. A is the lowest value and K is the highest value. Other notations indicate the values between these two extremes.

The phytostanols campestanol and sitostanol have recently been found in Turkish hazelnut oils. Our values of 3.0–6.2 mg/kg for campestanol and 30.2–61.4 mg/kg for sitostanol are similar to the findings of Bada *et al.* [24] of 3.4–6.2 and 34.1–61.4 mg/kg, respectively.

Δ^5-Stigmastadienols have recently been identified in Spanish, French and Turkish hazelnut oils and their contents were reported between 0.63 and 1.04% [24]. We have identified a specific compound of this family, $\Delta^{5,24}$-stigmastadienol, which varied between 0.31 and 0.62%.

We analyzed the same cultivars (Sivri, Uzunmusa and Yassıbadem) from two different regions (Ordu and Giresun). When considering β-sitositerol and total phytosterol, the differences between the average values for the cultivars were significant ($p < 0.001$). However, they were found to be insignificant with respect to region and cultivar × region interaction. Duncan's test also verified these results (Table 3). On the other hand, for the Foşa cultivar from Giresun and Trabzon, the results of the *t*-test revealed that the region had a significant effect on the β-sitosterol ($p < 0.01$) but not on the total phytosterol content.

The major phytosterols of hazelnut oil are similar to those of olive oil, except for a lower content of Δ^5-avenasterol and a higher content of Δ^7-stigmastenol in hazelnut oil [20]. The Δ^7-stigmastenol content of olive oil is an important indicator of adulteration with other vegetable oils and particularly with fruit oils, which must be less than 0.5% [25]. As it can be concluded from Table 1, the Δ^7-stigmastenol content of Turkish hazelnut cultivars ranged between 0.30 and 2.22% and some cultivars contain less than 0.5% of Δ^7–stigmastenol, which suggests that it is very difficult to detect adulteration between olive and some hazelnut oils using the sterol composition.

4 Conclusions

The results revealed that the total phytosterol content and the distribution of individual phytosterols varied significantly between the 17 Turkish hazelnut cultivars. However, the effect of growing region was not significant for differences in total phytosterol and β-sitosterol contents while it was significant for the β-sitosterol content of the Foşa cultivar. Hazelnuts are oil-rich fruit and cholesterol-lowering phytosterols are significant components of them. Because of the high oil contents

Table 3. Differences in β-sitositerol and total phytosterol contents in the same cultivars obtained from the Giresun and Ordu regions (mg/kg).[†]

Cultivar	β-Sitosterol content		Total phytosterol content	
	Giresun region	Ordu region	Giresun region	Ordu region
Sivri	1010.0 ± 25.32^{aA}	1003.1 ± 15.11^{aA}	1200.4 ± 41.05^{aA}	1180.0 ± 32.25^{aA}
Uzunmusa	1329.7 ± 18.18^{bA}	1359.4 ± 30.33^{bA}	1561.9 ± 22.73^{bA}	1569.3 ± 46.47^{bA}
Yassıbadem	1622.1 ± 49.78^{cA}	1555.7 ± 13.74^{cA}	1870.5 ± 86.28^{cA}	1810.8 ± 23.07^{bA}

[†] Data expressed as means ± standard error of the mean (SE) of triplicate experiments. Means in a column (a–c across cultivar) not having a common letter are different ($p < 0.05$). Means in a row (A–C across region) not having a common letter are different ($p < 0.05$) for each analyte.

(61 ± 3.4%) of the 17 evaluated cultivars [26], hazelnuts can be considered as a valuable source of phytosterols.

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Conflict of interest statement

The authors have declared no conflict of interest.

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