JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Sterol and Fatty Acid Compositions, Oxidative Stability, and Nutritional Value of Six Walnut (*Juglans regia* L.) Cultivars Grown in Portugal

JOANA S. AMARAL,[†,#] SUSANA CASAL,[‡] JOSÉ A. PEREIRA,[§] ROSA M. SEABRA,*[,†] AND BEATRIZ P. P. OLIVEIRA[‡]

REQUIMTE, Serviços de Farmacognosia and Bromatologia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha 164, 4050-047 Porto, Portugal, and Escola Superior de Tecnologia e de Gestão and Escola Superior Agrária, Instituto Politécnico de Bragança, Quinta de Sta. Apolónia, Apartado 134, 5301-857 Bragança, Portugal

Six cultivars (Franquette, Marbot, Mayette, Mellanaise, Lara, and Parisienne) of walnuts (*Juglans regia* L.) were collected during the 2001 crop, from Bragança, Portugal. Chemical composition, including moisture, total oil content, crude protein, ash, carbohydrates, and nutritional value, was evaluated. Fat was the predominant component, ranging from 62.3 to 66.5%. Total oil was extracted and analyzed for fatty acids, sterols, oxidative stability, and peroxide value. Fatty acids and sterols were determined by gas−liquid chromatography coupled to a flame ionization detector. Eighteen fatty acids were quantified. Polyunsaturated fatty acids and, in particular, linoleic acid were predominant. β-Sitosterol, Δ^5-avenasterol, and campesterol were the major sterols found. Differences were observed among the studied cultivars, especially in peroxide values and in the sterol profile.

KEYWORDS: ***Juglans regia* L.; walnut; chemical composition; fatty acid composition; sterol composition; nutritional value**

INTRODUCTION

Nuts are important components of the Mediterranean diet, which is thought to be a healthy one and has been considered as one of the best in coronary heart disease (CHD) prevention (*1*). From preagriculture times to the present day, nuts have been eaten as part of the human diet, providing macronutrients and micronutrients, as well as other bioactive constituents. A recent decline in the consumption of nuts was probably due to concerns about ingesting fatty foods. Walnuts, for example, are ∼60% fat (fresh weight). Although total fat intake is certainly related to health risks, there is now general agreement that the more important issue, where health is concerned, is the type of fat or fatty acids that are consumed. In fact, fatty acid composition can influence various physiological and biochemical processes, including blood pressure regulation, glucose metabolism, lipidic metabolism, platelet aggregation, and erythrocyte deformability (*2*).

Walnut fat is mostly unsaturated, and unsaturated fatty acids have been associated with beneficial effects on serum lipids. Compared with most other nuts, which contain mostly monounsaturated fatty acids (MUFA), walnuts are highly enriched in omega-6 and omega-3 polyunsaturated fatty acids (PUFA), which are essential dietary fatty acids. Epidemiological and clinical trials suggest that omega-3 PUFA might have a significant role in the prevention of CHD. Several mechanisms were suggested for that action, including antiarrhythmic, hypolipidemic, and antithrombotic roles (*3*, *4*). Several epidemiological studies to evaluate the effect of nuts on CHD have been conducted during the past decade (*5*−*8*). They showed that the inclusion of whole food nut consumption in the diet had a significant protective benefit with respect to fatal and nonfatal CHD events. Studies were also conducted specifically on walnuts, finding that frequent consumption of moderate quantities of walnuts favorably modified the lipoprotein profile and decreased serum levels of total cholesterol (*9*, *10*).

Nuts are also rich in phytosterols, which, due to their structural similarity with cholesterol, inhibit its intestinal absorption, thereby lowering total plasma cholesterol and low-density lipoprotein (LDL) levels (*11*, *12*). Epidemiologic and experimental studies have been made suggesting that dietary phytosterols may offer protection from cancers such as colon, breast, and prostate cancers (*13*, *14*).

Assessment of the identity and quality of vegetable oils obtained from different sources is a challenge to the food industry in their efforts to meet consumer expectations. Generally this involves many tests, including analysis of fatty acid and sterol compositions because these are probably the most important major and minor components, respectively (*15*).

* Corresponding author (telephone 351.222078934; fax 351.222003977; e-mail rseabra@ff.up.pt).
[†] Serviço de Farmacognosia.
[#] Present adress: Escola Superior de Tecnologia e de Gestão.
[‡] Serviço de Bromatologia.
[§] Escola Superior Agrária.

10.1021/jf030451d CCC: $25.00
Published on Web 11/19/2003

Tabulated data on food composition generally include only mean values on fat composition, but there are several factors such as the cultivar, geographical origin, and agricultural practices that can affect compositional values (*16*, *17*).

More detailed research focusing on the nutritional quality and health-promoting components of walnuts will enhance our knowledge and encourage walnut consumption. Although there have been some data published on the subject (*15*, *18*−*24*), they include mean values, and there is no information concerning the cultivars grown in Portugal. In this investigation, six cultivars of walnuts growing in the same experimental orchard and subjected to the same agricultural practices were analyzed for total fat, moisture, crude protein, ash, carbohydrates, oxidative stability, and peroxide value. The chemical study was extended to 18 fatty acids and 8 phytosterols to determine any differences in the compositional profiles of the analyzed cultivars.

MATERIALS AND METHODS

Samples. Six walnut (*J. regia* L.) cultivars, Franquette, Marbot, Mayette, Mellanaise, Lara, and Parisienne, were studied. The trees were identified and carefully marked. The walnut fruits were gathered by hand in "Quinta de Santa Apolónia", an orchard in Bragança, in the northeastern region of Portugal (6° 46′ W, 41° 49′ N, 670 m elevation). The orchard has 24 walnut trees (2 of cvs. Mellanaise, Marbot, Mayette, and Parisienne; 4 of cv. Lara; and 12 of cv. Franquette) with a planting density of 7 × 7 m. All trees are >18 years old. They are pruned when necessary, and they receive organic fertilization but not phytosanitary treatments. The surrounding soil is tilled to control infestation and irrigated in summer (July and August).

The walnut fruits were harvested in the crop year 2001 (September), and a final sample of ∼2 kg was taken. After harvest, walnut fruits were immediately transported to the laboratory and held in an oven (UL60, Memmert, Germany) for 3 days at 30 °C. The fruits were stored in the shell, closed in plastic bags, and frozen to −20 °C, until the analyses.

Sample Preparation. Before chemical analysis the walnuts were manually cracked and shelled and then chopped in a 643 MX coffee mill (Moulinex, Spain). To obtain oil for further analysis, finely chopped nuts (∼15 g) were extracted with light petroleum ether (bp 40−60 °C) in a Soxhlet apparatus, and the remaining solvent was removed by vacuum distillation. The extracted oil was kept in tubes over anhydrous sodium sulfate and flushed with nitrogen. The tubes were carefully wrapped in aluminum foil and stored at 4 °C until the analyses.

Chemical Analysis. Analyses of moisture, total fat, ash, and protein contents were carried out in duplicate. Moisture was determined at 100 ± 2 °C (∼5 g test sample) with an SMO 01 infrared moisture balance (Scaltec, Goettingen, Germany) (*25*). Ash, crude protein (N × 5.3), and total fat contents were determined according to AOAC Official Methods (*26*). Carbohydrate content was estimated by difference of the other components using the following formula: carbohydrate content = 100% − (% moisture + % protein + % fat + %ash) (*27*). Energy was expressed as kilocalories, using the factors proposed by Greenfield and Southgate (*28*).

Oxidative Stability (OS). The oxidation induction time was measured on a Rancimat apparatus (Metrohm CH series 679, Herisau, Switzerland). Air with a 20 L/h flow was bubbled through the oil (∼3.0 mL) heated at 110 ± 0.2 °C. The volatile compounds released during oxidation were collected to a cell containing water, and the increasing water conductivity was continually measured. The time taken to reach the conductivity inflection point was recorded as the induction time.

Peroxide Value (PV). PV was evaluated following the NP-904 (1987) method (*29*). It consists of the reaction in darkness of a mixture of oil and chloroform/acetic acid 2:3 (v/v) with a saturated potassium iodide solution. The free iodine released was titrated with a sodium thiosulfate solution. The peroxide value was expressed as milliequivalents of active oxygen per kilogram of oil (mequiv/kg). A second evaluation was carried out 1 year later. Freshly extracted oils were used for these determinations, and at least two determinations were performed for each sample.

Table 1. Systematic and Abbreviated Names of Fatty Acids Used as Standards

series	IUPAC	abbrev
saturated	butanoate	C4:0
	hexanoate	C6:0
	octanoate	C8:0
	decanoate	C10:0
	undecanoate	C11:0
	dodecanoate	C12:0
	tridecanoate	C13:0
	tetradecanoate	C14:0
	pentadecanoate	C15:0
	hexadecanoate	C16:0
	heptadecanoate	C17:0
	octadecanoate	C18:0
	eicosanoate	C20:0
	heneicosanoate	C21:0
	docosanoate	C22:0
	tricosanoate	C23:0
	tetracosanoate	C24:0
monounsaturated	*cis*-9-tetradecenoate	C14:1
	cis-10-pentadecenoate	C15:1
	cis-9-hexadecenoate	C16:1
	cis-10-heptadecenoate	C17:1
	trans-9-octadecenoate	C18:1ω9*t*
	cis-9-octadecenoate	C18:1ω9
	cis-11-octadecenoate	C18:1ω7
	cis-11-eicosenoate	C20:1
	cis-13-docosenoate	C22:1ω9
	cis-15-tetracosenoate	C24:1ω9
polyunsaturated	*trans*-9,12-octadecadienoate	C18:2ω6*tt*
	cis-9,12-octadecadienoate	C18:2ω6
	cis-9-*trans*-12-octadecadienoate	C18:2ω6*ct*
	trans-9-*cis*-12-octadecadienoate	C18:2ω6*tc*
	cis 6,9,12-octadecatrienoate	C18:3ω6
	cis-9,12,15-octadecatrienoate	C18:3ω3
	cis-11,14,eicosadienoate	C20:2ω6
	cis-8,11,14-eicosatrienoate	C20:3ω6
	cis-11,14,17-eicosatrienoate	C20:3ω3
	cis-5,8,11,14-eicosatetranoate	C20:4ω6
	cis-13,16-docosadienoate	C22:2ω6
	cis-5,8,11,14,17-eicosapentaenoate	C20:5ω3
	cis-4,7,10,13,16,19-docosahexenoate	C22:6ω3

Fatty Acid Composition. Fatty acids were determined by gas−liquid chromatography with flame ionization detection (GLC-FID)/capillary column based on the method used by Oliveira et al. (*30*) with minor modifications. Fatty acid methyl esters (FAMEs) were prepared by hydrolysis with a 11 g/L methanolic potassium hydroxide solution, methyl esterification with BF_3/MeOH, and extraction with *n*-heptane. The fatty acid profile was analyzed with a Chrompack CP 9001 chromatograph (Chrompack, Middelburg, The Netherlands) equipped with a split−splitless injector, a FID, and a Chrompack CP-9050 autosampler. The temperatures of the injector and detector were 230 and 270 °C, respectively. Separation was achieved on a 50 m × 0.25 mm i.d. fused silica capillary column coated with a 0.19 μm film of CP-Sil 88 (Chrompack). Helium was used as carrier gas at an internal pressure of 120 kPa. The column temperature was 160 °C, for a 1 min hold, and then programmed to increase to 239 °C at a rate of 4 °C/min and then held for 10 min. The split ratio was 1:50, and the injected volume was 1.2 μL. The results are expressed in relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. A Supelco (Bellefonte, PA) mixture of 37 FAMEs (standard 47885-U) was used (**Table 1**). In addition, the fatty acid isomers *cis*-9-*trans*-12-octadecadienoate (C18:2ω6*ct*), *trans*-9-*cis*-12-octadecadienoate (C18:2ω6*tc*), and *cis*-11-octadecenoate (C18:1ω7) were identified with individual standards also purchased from Supelco.

Sterol Composition. Sterol composition was evaluated by GLC-FID/capillary column following the NP EN ISO 12228 (1999) method (*31*). Briefly, after the addition of 1.0 mL of internal standard solution

Table 2. Systematic and Common Names of Phytosterols Used as Standards

common name	systematic name
cholesterol	cholest-5-en-3β-ol
cholestanol	5α-cholestan-3β-ol
campesterol	24*R*-methylcholest-5-en-3β-ol
campestanol	24*R*-methylcholestan-3β-ol
stigmasterol	24*S*-ethylcholesta-5,22-dien-3β-ol
clerosterol	24*S*-ethylcholesta-5,25-dien-3β-ol
β-sitosterol	24*R*-ethylcholest-5-en-3β-ol
β-sitostanol	24-ethylcholestan-3β-ol
Δ^5-avenasterol	24*Z*-ethylidenecholest-5-en-3β-ol
Δ^7-avenasterol	24*Z*-ethylidenecholest-7-en-3β-ol

(betulin, 1.0 mg/mL), ~250 mg of oil was saponified with an ethanolic potassium hydroxide solution; the unsaponifiable fraction was isolated by solid-phase extraction on an aluminum oxide column, and the steroid fraction was obtained after TLC with *n*-hexane/diethyl ether 1:1 (v/v) as developing solvent and a methanol spray to visualize the band. The trimethylsilyl ethers were obtained by the addition of 1-methylimidazole and *N*-methyl-*N*-(trimethylsilyl)-heptafluorobutyramide (MSHFBA). The sterol profile was analyzed on the same equipment used for fatty acid analysis, with a 30 m × 0.25 mm i.d., 0.25 μm DB-5MS column (J&W Scientific, Folsom, CA) with a maximum operating temperature of 325 °C. The temperatures of the injector and the detector were both 320 °C. The column temperature was 250 °C and programmed to increase at a rate of 2 °C/min to 300 °C and then held for 12 min. The carrier gas used was helium at an internal pressure of 100 kPa. The split ratio was 1:50, and the injected volume was 1.5 μL. The total sterol content was determined considering all peaks of sterols eluted between cholesterol and Δ^7-avenasterol. Identification was achieved by comparing the relative retention times from samples with those obtained with standards. Standards used for identification were purchased from Sigma (St. Louis, MO) and included cholestanol, cholesterol, campesterol, stigmasterol, β-sitosterol, β-sitostanol, and betulin (**Table 2**). Campestanol, clerosterol, Δ^5-avenasterol, and Δ^7-avenasterol were tentatively identified by comparison with references (*15*, *18*, *31*). β-Sitostanol and Δ^5-avenasterol eluted very close, and therefore they were quantified as Δ^5-avenasterol.

RESULTS AND DISCUSSION

Table 3 shows the results of the chemical composition obtained for the six cultivars of walnuts analyzed. Fat was the predominant component, followed by protein and carbohydrates, confirming the high energy value of nuts. In general, the values determined were in agreement with those published previously (*19*, *20*). The values obtained by Savage et al. (*19*) when studying 12 cultivars of walnuts grown in New Zealand were, on average, higher for moisture (6.1%) and crude protein (14.6%) but slightly lower for total fat (63.0%) than those obtained with walnuts grown in Portugal (4.2, 13.7, and 64.6%, respectively). Rugeri et al. (*20*) studied four cultivars of walnuts grown in Italy and obtained, on average, higher values for crude protein (15.7%) and total fat (67.4%). The cv. Franquette, in comparison with the analyses by Rugeri et al. (*20*), had different values for almost all parameters. This can possibly be explained by the different geographical origin or other factors such as agricultural practices. The energy values determined ranged from

Table 3. Proximate Chemical Composition (Grams per 100 g of Fresh Weight) of Six Walnut Cultivars Grown in Portugal (Mean ± SD)

	cultivar					
	Franquette	Marbot	Mayette	Mellanaise	Lara	Parisienne
moisture	4.4 ± 0.03	3.6 ± 0.11	4.1 ± 0.17	4.3 ± 0.03	4.1 ± 0.11	4.4 ± 0.54
crude protein	15.2 ± 0.25	12.2 ± 0.63	13.0 ± 0.06	15.0 ± 0.18	13.8 ± 0.13	13.2 ± 0.10
total fat	62.3 ± 0.13	66.3 ± 0.06	66.3 ± 0.38	63.0 ± 0.44	66.5 ± 0.01	63.1 ± 0.22
ash	2.0 ± 0.02	1.8 ± 0.01	1.8 ± 0.10	2.0 ± 0.01	2.1 ± 0.05	2.1 ± 0.03
carbohydrates	16.2 ± 0.08	16.1 ± 0.68	14.8 ± 0.38	15.7 ± 0.23	13.4 ± 0.08	17.2 ± 0.83
energy (kcal)	686	710	708	690	708	689

Table 4. Fatty Acid Composition (Percent) of Oil Extracted from Analyzed Walnut Samples (Mean ± SD)

	cultivar					
	Franquette	Marbot	Mayette	Mellanaise	Lara	Parisienne
C14:0	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
C16:0	7.48 ± 0.02	7.14 ± 0.01	7.00 ± 0.01	7.02 ± 0.01	6.94 ± 0.02	6.32 ± 0.00
C16:1ω7	0.05 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
C17:0	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
C18:0	2.43 ± 0.00	2.77 ± 0.01	2.55 ± 0.01	2.65 ± 0.03	2.22 ± 0.01	2.41 ± 0.01
C18:1ω9t	0.02 ± 0.00	0.02 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
C18:1ω9	16.99 ± 0.04	16.51 ± 0.08	18.09 ± 0.09	14.49 ± 0.12	14.26 ± 0.02	17.45 ± 0.06
C18:1ω7	1.53 ± 0.04	1.26 ± 0.06	1.21 ± 0.08	1.24 ± 0.08	1.29 ± 0.04	1.11 ± 0.06
C18:2ω6ct	0.08 ± 0.00	0.09 ± 0.01	0.08 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.07 ± 0.00
C18:2ω6	59.22 ± 0.07	58.90 ± 0.12	57.46 ± 0.02	61.31 ± 0.08	62.50 ± 0.02	62.45 ± 0.02
C20:0	0.07 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
C18:3ω6	0.06 ± 0.00	0.07 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.03 ± 0.00
C20:1ω9	0.19 ± 0.00	0.19 ± 0.00	0.19 ± 0.01	0.17 ± 0.00	0.18 ± 0.01	0.22 ± 0.00
C18:3ω3	11.69 ± 0.02	12.74 ± 0.01	12.98 ± 0.01	12.51 ± 0.00	12.16 ± 0.02	9.64 ± 0.01
C20:2ω6	0.07 ± 0.00	0.06 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
C22:0	0.05 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.03 ± 0.00
C22:2ω6	nd[a]	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	nd
C22:5ω3	nd	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	nd
total SFA	10.11 ± 0.02	10.12 ± 0.05	9.72 ± 0.02	9.85 ± 0.00	9.34 ± 0.01	8.90 ± 0.01
total MUFA	18.78 ± 0.08	18.06 ± 0.01	19.59 ± 0.01	15.99 ± 0.18	15.82 ± 0.03	18.87 ± 0.01
total PUFA	71.12 ± 0.09	71.90 ± 0.04	70.66 ± 0.02	74.02 ± 0.08	74.83 ± 0.03	72.24 ± 0.02

[a] Not detected.

Table 5. Oxidative Stability (OS) and Peroxide Value (PV) of Oil Extracted from Walnut Samples

	cultivar					
	Franquette	Marbot	Mayette	Mellanaise	Lara	Parisienne
OS (h)	3.3	3.0	3.4	3.2	2.8	2.7
PV[a]	3 ± 0	4 ± 0	2 ± 0	2 ± 0	18 ± 0	5 ± 0
PV[b]	3 ± 0	19 ± 1	3 ± 0	16 ± 0	12 ± 1	5 ± 1

[a] First evaluation. [b] Second evaluation 1 year later.

686 kcal in cv. Franquette to 710 kcal in cv. Marbot and are similar to the values reported by Ruggeri et al. (*20*).

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of the studied cultivars are shown in **Table 4**. The major fatty acid found in Portuguese samples was linoleic acid (C18:2ω6), followed by oleic acid (C18:1ω9), α-linolenic acid (C18:3ω3), palmitic acid (C16:0), and stearic acid (C18:0). Besides these five main fatty acids, 13 more were identified and quantified. Some of them were already described, but, as far as we know, C18:1ω9t, C18:2ω6ct, C18:3ω6, C20:2ω6, C22:0, C22:2ω6, and C22:5ω3 are now reported, for the first time, in this matrix. PUFA were the main group of fatty acids in walnut oil extracted from the studied cultivars, ranging from 70.7 to 74.8%; MUFA group ranged from 15.8 to 19.6%, and SFA were the minor group, ranging from 8.9 to 10.1%. Considering total MUFA content, cv. Lara had the lowest value but contained the highest PUFA content, also being the cultivar with the highest value of linoleic acid. The cv. Mayette had the highest MUFA value and the lowest PUFA value but, interestingly, was the cultivar with the highest value of α-linolenic acid. The main fatty acids were found, in general terms, in amounts similar to those already described for walnuts grown in New Zealand (*21*, *22*) and in Italy (*20*) with some differences, namely, stearic (C18:0), oleic (C18:1ω9), linolenic (C18:3ω3), and eicosenoic (C20:1ω9) acids. *cis*-Vaccenic acid (C18:1ω7), reported to occur for the first time in walnuts by Savage et al. (*22*), was also found in Portuguese samples. Trans isomers of unsaturated fatty acids were detected in trace amounts in all of the studied cultivars as well as γ-linolenic (C18:3ω6) and C20:2ω6. C22:2ω6 and C22:5ω3 were also present in minute quantities but were not detected in cvs. Franquette and Parisienne.

In what concerns oxidative stability (**Table 5**) the six cultivars showed some homogeneity. All of them presented low values for OS, which confirms that walnut oil is relatively unstable when compared to other common plant oils (*22*). This can be ascribed to its high content of PUFA.

The results found for PV (**Table 5**) are within the range of values found for other walnuts (*22*) except for cv. Lara, which exhibited a very high value. One year after the first analyses, the pattern of PV values has changed: the PV of cv. Lara decreased, the PV of cvs. Marbot and Mellanaise rose considerably, and the values for the other three cultivars remained constant. This behavior is different from that described in the work published by Savage et al. (*23*). In this mentioned work, all nine analyzed cultivars showed a similar curve for PV: the values rose during the first and second years and decreased in the third year. This decrease is explained by the decomposition of peroxides during the progression of the autoxidation process. The behavior observed with the cultivars now under study points to differences among them. These results cannot be explained by the parameters that were studied in this work, because the values registered for total PUFA are not distinct enough to be responsible for such differences. Most probably this can be explained by the presence of different amounts of antioxidants and/or pro-oxidants in the samples.

Total phytosterols are present in amounts from 0.1 to 0.2% of total oil. These values are in agreement with those already described for walnuts (*15*, *18*) and are in the same range of those found in olive, peanut, and hazelnut oils but lower than those found in the majority of other oils (*15*, *18*). The identified compounds are listed in **Table 6**. The major ones (β-sitosterol, Δ^5-avenasterol, and campesterol) were already reported to occur in walnuts and in similar amounts (*15*, *18*, *31*). Besides these compounds, cholesterol and clerosterol were also found in all samples, and three others (stigmasterol, Δ^7-sigmasterol, and Δ^7-avenasterol) were detected at least in some cultivars. Samples were also checked for cholestanol and campestanol, but neither of them was detected.

According to some authors (*18*) sterol distribution is rather typical for each plant family, and the evaluation of the total sterol content and the sterol profile is a good tool to assess authenticity. However, no data were found in the literature concerning cultivars. In this work some differences among the samples were observed, pointing to different phytosterol profiles. The cvs. Parisienne and Mellanaise showed the lowest content of β-sitosterol and, consequently, total phytosterols. Cv. Parisienne is also the cultivar with the lowest amounts of cholesterol, campesterol, and Δ^5-avenasterol. Cvs. Franquette and Mayette were the only cultivars in which Δ^7-stigmasterol was present. Stigmasterol was not detected in cvs. Lara and Parisienne. In cvs. Mayette and Parisienne Δ^7-avenasterol was undetectable. Cv. Franquette was the only cultivar that contained all phytosterols identified.

In conclusion, walnuts are a rich source of PUFA (~70%). Among these fatty acids, linolenic acid, which has been associated with health benefits, is present in a considerable amount (~12%). Walnuts also contain several phytosterols, which have been used as "nutraceuticals" as it appears that they

Table 6. Sterol Content (Milligrams per 100 g of Oil) of Oil Extracted from Walnut Samples (Mean ± SD)

	cultivar					
	Franquette	Marbot	Mayette	Mellanaise	Lara	Parisienne
cholesterol	1.4 ± 0.03	2.0 ± 0.04	3.8 ± 0.12	1.4 ± 0.03	0.9 ± 0.09	0.8 ± 0.04
campesterol	8.6 ± 0.20	9.6 ± 0.30	8.1 ± 0.17	6.5 ± 0.03	10.8 ± 0.18	6.1 ± 0.02
stigmasterol	0.5 ± 0.07	0.8 ± 0.03	0.6 ± 0.07	0.5 ± 0.03	nd[a]	nd
clerosterol	1.5 ± 0.11	2.0 ± 0.06	1.5 ± 0.08	1.1 ± 0.06	1.2 ± 0.09	2.0 ± 0.04
β-sitosterol	138.3 ± 1.80	175.7 ± 0.24	151.4 ± 0.49	109.8 ± 0.06	170.6 ± 0.21	109.3 ± 0.04
Δ^5-avenasterol[b]	7.3 ± 0.11	11.4 ± 0.41	13.3 ± 0.01	7.3 ± 0.03	9.8 ± 0.09	2.5 ± 0.02
Δ^7-stigmasterol	0.7 ± 0.05	nd	0.9 ± 0.01	nd	nd	nd
Δ^7-avenasterol	1.4 ± 0.29	1.1 ± 0.06	nd	0.5 ± 0.02	2.8 ± 0.06	nd
total	159.0 ± 5.00	202.6 ± 1.67	179.6 ± 0.73	127.1 ± 0.15	196.1 ± 0.36	120.7 ± 0.06

[a] Not detected. [b] β-sitostanol + Δ^5-avenasterol.

can inhibit intestinal absorption of cholesterol. Differences were observed among the studied cultivars, especially in PV and in sterol profile. Cv. Mayette is the cultivar with the lowest values of PUFA and PV and with the highest values of MUFA, α-linolenic acid, and OS. More studies need to be made in the coming years to confirm the conclusions here reported, but the data registered so far seem to indicate that the six analyzed cultivars are chemically different. Further studies, focusing on antioxidant and pro-oxidant components present in walnuts, should also be made to arrive at some explanation for the different behaviors in what concerns PV.

LITERATURE CITED

(1) Medeiros, D. M. Olive oil and health benefits. In *Handbook of Nutraceuticals and Functional Foods*; Wildman, R., Ed.; CRC Press: London, U.K., 2000; pp 261−267.

(2) Iso, H.; Sato, S.; Umemura, U.; Kudo, M.; Koike, K.; Kitamura, A.; Imano, H.; Okamura, T.; Naito, Y.; Shimamoto, T. Linoleic acid, other fatty acids, and the risk of stroke. *Stroke* **2002**, *33*, 2086−2093.

(3) Harper, C. R.; Jacobson, T. A. The fats of life: The role of Omega-3 Fatty Acids in the Prevention of Coronary Heart Disease. *Arch. Intern. Med.* **2001**, *161*, 2185−2192.

(4) Bucher, H. C.; Hengstler, P.; Schindler, C.; Meier, G. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *Am. J. Med.* **2002**, *112*, 298−304.

(5) Sabaté, J.; Radak, T.; Brown Jr., J. The role of nuts in cardiovascular disease prevention. In *Handbook of Nutraceuticals and Functional Foods*; Wildman, R., Ed.; CRC Press: London, U.K., 2000; pp 478−495.

(6) Fraser, G. E.; Sabaté, J.; Beeson, W. L.; Straham, M. A possible protective effect of nut consumption on risk of coronary heart disease. *Arch. Intern. Med.* **1992**, *152*, 1416−1424.

(7) Hu, F. B.; Stampfer, M. J.; Manson, J. E.; Rimm, E. B.; Colditz, G. A.; Rosner, B. A.; Speizer, F. E.; Hennekens, C. H.; Wilett, W. C. Frequent nut consumption and the risk of coronary heart disease in women: prospective cohort study. *Br. Med. J.* **1998**, *317*, 1341−1345.

(8) Albert, C. M.; Willett, W. C.; Manson, J. E.; Hennekens, C. H. Nut consumption and the risk of sudden and total cardiac death in the Physician's Heath Study. *Circulation* **1998**, *98* (Suppl. 1), I-582.

(9) Sabate, J.; Fraser, G. E.; Burke, K.; Knutsen, S. F. M.; Bennett, H.; Lindsted, K. D. Effects of walnuts on serum lipid levels and blood pressure in normal men. *N. Engl. J. Med.* **1993**, *328*, 603−607.

(10) Zambon, D.; Sabate, J.; Munoz, S.; Campero, B.; Casals, E.; Merlos, M.; Laguna, J.; Ros, E. Substituting walnuts for monounsaturated fat improves the serum lipid profile of hypercholesterolemic men and women: a randomised crossover trial. *Ann. Intern. Med.* **2000**, *137*, 538−546.

(11) Wong, N. C. The beneficial effects of plant sterols on serum cholesterol. *Can. J. Cardiol.* **2001**, *17*, 715−721.

(12) Plat, J.; Mensink, R. P. Effects of plant sterols and stanols on lipid metabolism and cardiovascular risk. *Nutr. Metab. Cardiovasc. Dis.* **2001**, *11*, 31−40.

(13) Awad, A. B.; Fink, C. S. Phytosterols as anticancer dietary components: Evidence and mechanism of action. *J. Nutr.* **2000**, *130*, 2127−2130.

(14) Awad, A. B.; Williams, H.; Fink, C. S. Phytosterols reduce in vitro metastatic ability of MDA-MB-231 human breast cancer cells. *Nutr. Cancer* **2001**, *40*, 157−164.

(15) Firestone, D.; Reina, R. J. Authenticity of vegetable oils. In *Food Authentication*; Ashurst, P. R., Dennis, M. J., Eds.; Chapman and Hall: London, U.K., 1996; pp 198−285.

(16) Parcerisa, J.; Codony, R.; Boatella, J.; Rafecas, M. Triacylglycerol and phospholipid composition of hazelnut (*Corylus avellana* L.) lipid fraction during fruit development. *J. Agric. Food Chem.* **1999**, *47*, 1410−1415.

(17) Areias, F.; Valentão, P.; Andrade, P. B.; Ferreres, F.; Seabra, R. M. Flavonoids and phenolic acids of sage: Influence of some agricultural factors. *J. Agric. Food Chem.* **2000**, *48*, 6081−6084.

(18) Kamm, W.; Dionisi, F.; Hischenhuber, C.; Engel, K. Authenticity assessment of fats and oils. *Food Rev. Int.* **2001**, *17*, 249−290.

(19) Savage, G. P. Chemical composition of walnuts (*Juglans regia* L.) grown in New Zealand. *Plant Foods Hum. Nutr.* **2001**, *56*, 75−82.

(20) Ruggeri, S.; Cappelloni, L.; Gambelli, S.; Carnovale, E. Chemical composition and nutritive value of nuts grown in Italy. *Ital. J. Food Sci.* **1998**, *3*, 243−252.

(21) Zwarts, L.; Savage, G. P.; McNeil, D. L. Fatty acid content of New Zealand-grown walnuts (*Juglans regia* L.). *Int. J. Food Sci. Nutr.* **1999**, *50*, 189−194.

(22) Savage, G. P.; Dutta, P. C.; McNeil, D. L. Fatty acid and tocopherol contents and oxidative stability of walnut oils. *J. Am. Oil Chem. Soc.* **1999**, *76*, 1059−1063.

(23) Savage, G. P.; McNeil, D. L. Oxidative stability of walnuts during long term in shell storage. *Acta Hortic.* **2001**, *544*, 591−597.

(24) Tsamouris, G.; Hatziantoniou, S.; Demetzos, C. Lipid analysis of Greek walnut oil (*Juglans regia* L.). *Z. Naturforsch.* **2002**, *57C*, 51−56.

(25) James, C. S. Theory of analytical methods for specific food constituents. In *Analytical Chemistry of Foods*; Blackie Academic and Professional, Chapman and Hall: London, U.K., 1996; pp 37−67.

(26) AOAC. *Official Methods of Analysis of AOAC International*, 17th ed.; Horwitz, W., Ed.; AOAC: Arlington, VA, 2000; Vol. II, 40, pp 1−3.

(27) Grosso, N. R.; Nepote, V.; Guzmán, C. A. Chemical composition of some wild peanut species (*Arachis* L.). *J. Agric. Food Chem.* **2000**, *48*, 806−809.

(28) Greenfield, H.; Southgate, D. A. T. In *Food Composition Data. Production, Management and Use*; Elsevier Applied Science: London, U.K., 1992.

(29) NP-904. Gorduras e óleos comestíveis. Determinação do índice de peróxido, 1987.

(30) Oliveira, M. B.; Alves, M. R.; Ferreira, M. A. Multivariate analysis of fatty acid *cis* and *trans* in margarines determined by HRGC/FID/capillary column. *J. Chemom.* **2001**, *15*, 71−84.

(31) NP EN ISO 12228. Animal and vegetable fats and oils—Determination of individual and total sterols contents—Gas chromatographic method, 1999.

Received for review June 18, 2003. Revised manuscript received September 25, 2003. Accepted September 30, 2003. J.S.A. is grateful to Programa para o Desenvolvimento Educativo para Portugal (PRODEP III) for financial support.

JF030451D