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Vitamin E Composition of Walnuts (*Juglans regia* L.): A 3-Year Comparative Study of Different Cultivars

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The tocopherol and tocotrienol composition of walnuts (*Juglans regia* L.) was determined for nine cultivars (cvs. Arco, Franquette, Hartley, Lara, Marbot, Mayette, Mellanaise, Parisienne, and Rego). Walnuts were harvested over three consecutive years from two different geographical origins (Bragança and Coimbra, Portugal), for a total of 26 samples. The methodology employed was a normal-phase high-performance liquid chromatography coupled to a series arrangement of a diode array detector followed by a fluorescence detector, allowing the simultaneous analysis of all tocopherols and tocotrienols. The analyses showed that all samples presented a similar qualitative profile composed of five compounds: α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, and γ-tocotrienol. γ-Tocopherol was the major compound in all samples, ranging from 172.6 to 262.0 mg/kg, followed by α- and δ-tocopherols, ranging from 8.7 to 16.6 mg/kg and from 8.2 to 16.9 mg/kg, respectively. Multivariate analysis of the data obtained showed the existence of significant differences in composition among cultivars. These differences were also significant when cultivars were grouped by year of production, showing that besides genetic factors, the vitamin E composition was influenced by environmental factors.

KEYWORDS: Vitamin E; tocopherols; tocotrienols; *Juglans regia* L.; walnuts, chemometrics; multivariate analysis; predictive biplots

INTRODUCTION

Vitamin E is a term used to refer a group of minor but ubiquitous lipid-soluble compounds, encompassing four tocopherols (α-, β-, γ-, and δ-) and four tocotrienols (α-, β-, γ-, and δ-). These compounds are believed to be involved in a diversity of physiological and biochemical functions, mainly due to their action as antioxidants but also by acting as membrane stabilizers (*1*). Research has demonstrated that, in vivo, the relative antioxidant activity of tocopherols is in the order α > β > γ > δ (*2*). Consequently, much attention has been paid to α-tocopherol, contrary to what has happened with the other homologues, which have received little attention beyond the evaluation of their relative antioxidant activity. Nevertheless, in recent years, many studies focusing on the biological activities and health effects of the other vitamin E isoforms have been conducted (*3*−*7*). Some studies did not find strict correlation between antioxidant capacity and biological activity potency, indicating that all of the vitamers may contribute to the total bioactivity in foods (*2*, *7*).

Major dietary sources of vitamin E compounds are vegetable oils, nuts, cereals, green vegetables, and fruits. In the Mediterranean countries the predominant isomer consumed is probably α-tocopherol, because olive oil is one of the principal components of the typical Mediterranean diet (*8*−*10*). On the other hand, γ-tocopherol is reported to be the major vitamin E component in the North American diet (*6*) and the second most common tocopherol in human serum (10−20%) (*6*). Even though in the past γ-tocopherol has received relatively low attention, recently much research concerning this compound has been conducted. There are already studies suggesting that γ-tocopherol may be associated with the reduction of blood cholesterol levels (*11*) and of death from cardiovascular disease (*3*, *12*, *13*), as well as several epidemiological and experimental results suggesting that this compound is probably a potent cancer chemopreventive agent (*6*, *12*, *14*, *15*).

γ-Tocopherol has been reported to be the major vitamer in walnuts, but, as far as we know, few studies have been conducted on the determination of the vitamin E composition

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of these nuts. In the published works, the determination of the tocopherol composition of walnuts is done by reversed-phase high-performance liquid chromatography (RP-HPLC), the γ and β vitamers being quantified together because RP-HPLC does not allow the complete resolution of these two homologues (*16*−*18*). As far as we know, until the work reported herein, there have been no available data concerning the tocotrienol composition of walnuts. Because walnuts are widely consumed, especially when vegetarian diets are considered, and because vitamin E compounds are reported to have different biological activities and potencies, it is important to quantify each of the vitamers separately because this information can be useful in the assessment of dietary intake of beneficial components. In a previous study, a method for the separation, identification, and quantification of both tocopherols and tocotrienols in walnuts has been developed and validated (*19*), and the results of its application to several samples of different cultivars, year crops, and geographical locations are now reported. Because it is known that factors such as the cultivar, geographical origin, and environmental factors can affect compositional values, statistical analysis was carried out using the obtained data to check differences in vitamin E composition.

MATERIALS AND METHODS

Samples. A total of nine walnut (*Juglans regia* L.) cultivars [Franquette (Cv1), Lara (Cv2), Marbot (Cv3), Mayette (Cv4), Mellanaise (Cv5), Parisienne (Cv6), Arco (Cv7), Hartley (Cv8), and Rego (Cv9)] were studied. Samples of the cvs. Franquette, Lara, Marbot, Mayette, Mellanaise, and Parisienne were harvested in Bragança, in northeastern Portugal (41° 49′ N, 6° 46′ W, 670 m elevation) in three consecutive years (2001, 2002, and 2003). In 2003, samples from another geographical origin were also included in the study: cvs. Franquette, Lara, Mayette, Mellanaise, and Parisienne (in common with the Bragança location) and three others (*cvs.* Arco, Hartley, and Rego) were harvested in Coimbra (40° 03′ N, 8° 37′ W, 10 m elevation), in central Portugal. All of the samples were collected during September. After harvesting, walnuts were dried in an oven at 30 °C during at least 3 days, and a final sample of ~2 kg was randomly taken. The fruits were stored in the shell, closed in plastic bags flushed with nitrogen, and frozen to −20 °C until the analyses.

Reagents and Standards. Tocopherols (α, β, γ, and δ) and tocotrienols (α, β, γ, and δ) were purchased from Calbiochem (La Jolla, CA). 2-Methyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol (tocol) (Matreya Inc., Pleasant Gap, PA) was used as internal standard (IS). A stock solution of the IS (10 mg/mL) was prepared in *n*-hexane, kept at −4 °C, protected from light, and diluted to working solutions (500 μg/mL) as necessary. Butylated hydroxytoluene (BHT) was used as antioxidant and was obtained from Aldrich (Madrid, Spain). A working solution of BHT was prepared in *n*-hexane at a concentration of 10 mg/mL. *n*-Hexane was of HPLC grade from Merck (Darmstadt, Germany) and 1,4-dioxane was from Fluka (Madrid, Spain). All other reagents were of analytical grade.

Sample Preparation. The samples were prepared using the validated methodology reported by Amaral et al. (*19*). Before the analysis, walnuts were manually cracked, shelled, and chopped in a coffee mill. For each sample, ~600 mg of finely chopped walnuts was accurately weighed in glass screw-cap test tubes (Supelco, Bellefonte, PA), and subsequently the internal standard (150 μL of tocol solution) and the antioxidant (100 μL of BHT solution) were added. The sample was homogenized for 1 min by vortex mixing, after the addition of each of the following reagents: ethanol (2 mL), extracting solvent (*n*-hexane, 4 mL), and saturated NaCl solution (2 mL). Subsequently, the sample was centrifuged (2 min, 5000*g*) and the clear upper layer removed to another screw-cap test tube. The sample was re-extracted twice using 2 mL of *n*-hexane. The combined extracts were taken to dryness under a nitrogen stream on a Reacti-Therm module (Pierce, Rockford, IL) operating at ambient temperature, and the residue was reconstituted to a volume of 1.5 mL with *n*-hexane. The extract was dried with anhydrous sodium sulfate, centrifuged (20 s, 10000*g*), transferred into a dark 2.0 mL vial, and loaded into the HPLC programmable autosampler.

HPLC Analysis. The HPLC system consisted of a Jasco (Tokyo, Japan) high-performance liquid chromatograph, equipped with a PU-980 pump, an AS-950 autosampler with a 10 μL loop, an MD-910 multiwavelength diode array detector (DAD), and an FP-920 programmable fluorescence detector. The chromatographic separation of the compounds was achieved with a 250 × 3 mm i.d. Inertsil 5 SI normal phase column from Varian (Middelburg, The Netherlands) operating at ambient temperature (~20 °C). The mobile phase was a mixture of *n*-hexane and 1,4-dioxane (96.5:3.5, v/v). Elution was performed at a solvent flow rate of 1 mL/min with an isocratic program. The effluent was monitored with a diode array detector connected in series with a fluorescence detector programmed at the excitation and emission wavelengths of 290 and 330 nm, respectively. The gains set in the fluorescence detector were as follows: 0 min, gain 10; 12.0 min, gain 100; 14.9 min, gain 10. The compounds were identified by comparing their retention times and UV spectra with authentic standards. Quantification was made by fluorescence detection based on the internal standard method. Chromatograms were recorded and processed using the Borwin-PDA controller software (JMBS, Fontaine, France).

Statistical Analysis. To evaluate discrimination between years of production, a principal component analysis (PCA) was carried out with cross-validation of the number of components (*20*). Data organized in principal components was divided in three groups enclosing observations according to year. Differences between these groups were evaluated by canonical discriminant analyses, and predictive biplots were constructed (*21*) to relate the observed differences directly to the compounds' initial values.

To analyze differences between cultivars, data were directly divided in groups according to cultivars, and a forward stepwise selection discriminant analysis was used to select the most discriminant vitamin E compounds, followed by a canonical analysis with a predictive biplot to observe the most important details (*22*). MANOVA and Hotelling T^2 tests were used to further evaluate which are the different cultivars, and Student *t* tests were used to check univariate differences between cultivars (*23*).

RESULTS AND DISCUSSION

The methodologies for the simultaneous determination of tocopherols and tocotrienols are scarce and recent (*24*−*27*) and, as far as we know, they have not been applied to the analysis of vitamin E of walnuts, as yet. The studies so far applied to walnuts (*16*−*18*) used a RP-HPLC methodology, which does not allow the separation of β and γ vitamers, which means that these two components have been quantified together. In this work, NP-HPLC has been chosen mainly because it has the advantage of allowing the separation of all vitamin E compounds (*28*−*31*) in a short period of time.

It is known that these kinds of compounds are sensitive to light, heat, and oxygen (*28*, *29*), so all standard and sample preparations were performed in a dark room with subdued red light and, whenever possible, during all of the procedures the samples were kept on ice. BHT was used as an antioxidant to prevent oxidation of the vitamers. The accuracy, precision, and robustness of the method were improved by the use of an internal standard (tocol), and the quantification of the compounds was made by the internal standard method.

All samples presented a similar qualitative profile with five vitamers: α-, β-, γ-, and δ-tocopherols and γ-tocotrienol. **Figure 1** shows a typical chromatogram obtained for walnut samples under the experimental conditions described. The average contents of each vitamer for every cultivar, year of production, and geographical origin are shown in **Table 1**. In all samples γ-tocopherol was the major compound, ranging from 172.6 to 262.0 mg/kg, followed by α- and δ-tocopherols, which presented

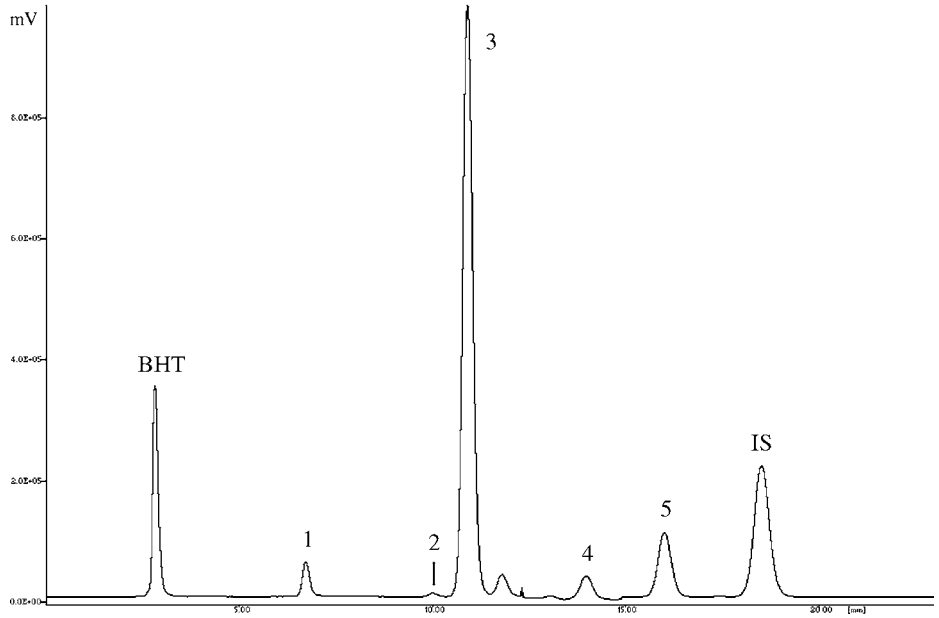

Figure 1. Chromatogram of a walnut sample. Peaks: I.S., internal standard (tocol); 1, α-tocopherol; 2, β-tocopherol; 3, γ-tocopherol; 4, γ-tocotrienol; 5, δ-tocopherol.

Table 1. Tocopherol and Tocotrienol Contents[a] (Milligrams per Kilogram) of the Studied Cultivars by Year of Production and Geographical Location

	α-tocopherol	β-tocopherol	γ-tocopherol	γ-tocotrienol	δ-tocopherol	total
			Bragança 2001			
Franquette	14.36 ± 0.42	1.46 ± 0.05	261.60 ± 4.62	2.57 ± 0.06	16.91 ± 0.14	296.90
Lara	15.76 ± 0.14	1.82 ± 0.03	208.13 ± 0.55	3.16 ± 0.04	12.98 ± 0.04	241.85
Marbot	12.65 ± 0.04	0.90 ± 0.03	262.01 ± 0.91	2.69 ± 0.02	13.05 ± 5.13	291.31
Mayette	11.98 ± 0.06	0.85 ± 0.03	258.62 ± 0.96	2.39 ± 0.04	12.82 ± 0.29	286.65
Mellanaise	10.42 ± 0.00	1.07 ± 0.02	229.87 ± 0.22	2.60 ± 0.09	15.17 ± 0.05	259.14
Parisienne	16.55 ± 0.26	1.13 ± 0.03	252.68 ± 2.17	2.91 ± 0.02	16.78 ± 0.08	290.04
			Bragança 2002			
Franquette	12.50 ± 0.05	1.08 ± 0.05	240.89 ± 1.81	3.36 ± 0.09	13.20 ± 0.14	271.03
Lara	11.44 ± 0.66	0.84 ± 0.05	229.93 ± 6.90	3.28 ± 0.08	11.01 ± 0.43	256.50
Marbot	10.77 ± 0.14	0.89 ± 0.04	255.01 ± 4.99	3.18 ± 0.09	13.62 ± 0.35	283.46
Mayette	11.33 ± 0.35	0.86 ± 0.06	213.24 ± 5.23	3.33 ± 0.07	12.19 ± 0.31	240.95
Mellanaise	10.53 ± 0.03	0.78 ± 0.02	213.87 ± 1.09	3.07 ± 0.09	10.38 ± 0.06	238.63
Parisienne	13.26 ± 0.19	0.89 ± 0.05	214.22 ± 2.80	2.46 ± 0.08	12.20 ± 0.14	243.04
			Bragança 2003			
Franquette	8.96 ± 0.03	0.88 ± 0.01	189.85 ± 3.05	2.38 ± 0.06	9.43 ± 0.16	211.51
Lara	13.25 ± 0.48	1.56 ± 0.09	229.79 ± 6.88	3.79 ± 0.14	13.09 ± 0.39	261.47
Marbot	10.36 ± 0.08	0.83 ± 0.03	197.24 ± 1.52	2.83 ± 0.01	8.24 ± 0.12	219.51
Mayette	13.02 ± 0.16	1.16 ± 0.03	200.77 ± 1.95	2.30 ± 0.06	11.38 ± 0.05	228.63
Mellanaise	8.69 ± 0.36	0.60 ± 0.04	204.17 ± 9.22	3.19 ± 0.15	8.61 ± 0.34	225.26
Parisienne	11.44 ± 0.15	0.76 ± 0.03	213.94 ± 3.01	2.25 ± 0.02	11.21 ± 0.16	239.59
			Coimbra 2003			
Arco	13.75 ± 0.42	1.26 ± 0.04	209.69 ± 5.97	4.16 ± 0.14	9.43 ± 0.32	238.29
Franquette	10.16 ± 0.07	0.73 ± 0.02	191.01 ± 0.51	2.48 ± 0.00	8.68 ± 0.02	213.05
Hartley	16.77 ± 0.63	1.21 ± 0.07	204.96 ± 7.95	3.63 ± 0.06	9.79 ± 0.39	236.37
Lara	13.54 ± 0.12	1.57 ± 0.04	193.74 ± 3.02	3.79 ± 0.11	10.37 ± 0.17	223.00
Mayette	15.55 ± 0.52	1.37 ± 0.06	247.99 ± 8.45	5.14 ± 0.14	15.57 ± 0.29	285.62
Mellanaise	9.43 ± 0.39	0.99 ± 0.04	172.58 ± 5.71	2.10 ± 0.06	8.90 ± 0.32	194.00
Parisienne	11.84 ± 0.18	0.91 ± 0.04	210.27 ± 4.36	3.71 ± 0.09	12.19 ± 0.34	238.92
Rego	14.02 ± 0.32	1.45 ± 0.06	239.98 ± 5.31	3.13 ± 0.11	14.80 ± 0.38	273.38

[a] Values are expressed as mean ± standard deviation of three determinations for each sample.

similar values, ranging from 8.7 to 16.6 mg/kg and from 8.2 to 16.9 mg/kg, respectively.

Lavendrine and co-workers (*16*), when analyzing the vitamin E composition of walnuts, used a RP-HPLC/UV methodology that allowed them to quantify three compounds: α-, γ-, and δ-tocopherols. Probably the peak quantified as γ-tocopherol was, in reality, the sum of β and γ homologues. The authors stated that the inability of the method to separate β and γ homologues was not inconvenient because the absence of β-tocopherol in walnuts has been reported. In the work herein, the presence of β-tocopherol in the matrix was confirmed, although in minimal amounts (ranging from 0.60 to 1.82 mg/kg).

The two cultivars studied by Lavendrine and co-workers (*16*) (cvs. Franquette and Hartley) are in common with the ones studied herein. The values reported by the above authors for cv. Franquette are, in general, in good agreement with the ones reported here, whereas those for cv. Hartley are, in general, higher than the ones here reported for the same cultivar.

Compared to the mean values herein reported, Delgado-Zamarreño and co-workers (*17*, *18*) obtained higher values for δ-tocopherol and lower values for γ-tocopherol. The mean value reported herein for α-tocopherol (13.0 mg/kg, mean value) is higher than the one reported in 2001 (*17*) by the above authors (7.9 mg/kg), but lower than the one reported in 2004 (*18*) (26 mg/kg) for the same compound. In both works, Delgado-Zamareño and co-workers (*17*, *18*) used RP-HPLC and did not achieve a separation between β- and γ-tocopherols, and so they have quantified these two vitamers together. Even so, the reported values were lower than the ones obtained for individual γ-tocopherol in this work. Because no data about cultivars or geographical origin were mentioned by the authors, no conclusions can be drawn that could explain the above-mentioned differences. Concerning tocotrienol composition, only γ-tocotrienol was found in walnut samples, ranging from 2.10 mg/kg in cv. Mellanaise to 5.14 mg/kg in cv. Mayette. As far as we know, this is the first report on the presence of tocotrienol in walnuts.

In the cultivars under study, total tocopherol and tocotrienols content ranged from 194 to 297 mg/kg. Some food compositional tables present values that are, to a large extent, lower (38 mg/kg) (*32*) and higher (309−455 mg/kg) (*33*) than values obtained in this work. This may probably be due to relatively old and scarce data. The actualized U.S. Department of Agriculture's (USDA) Nutrient Database (*34*) shows data for four compounds: α-, β-, γ-, and δ-tocopherols (7.0, 1.5, 208.3, and 18.9 mg/kg, respectively), but no reference is made to tocotrienols. The results reported herein are in good agreement with those reported by the USDA.

To check differences with respect to vitamin E composition, statistical analysis was carried out. It is known that discriminant analysis may use available information to discriminate between groups, even if this information is random (*35*). Therefore, a PCA was carried out to organize all of the information. Three important data structures were obtained, as suggested by cross-validation of the number of components, and consequently two components were deleted, thus removing unimportant information from the data. The selected three components contained >90% of the initial information, and the information from each vitamer organized in these three components always exceeded 85%. Because the components can be viewed as data structures or sets of organized information, a canonical variates analysis based on the first three principal components was carried out. Canonical variates are explained in terms of principal components, so it is very difficult to attribute a meaning to the observed differences between years of production. This problem can be overcome by the application of predictive biplots (*21*), for which construction and problem solving have already been dealt with (*22*, *23*, *36*, *37*), and can be extended to situations when several multivariate statistics are coupled together. For that reason, a biplot was made, relating directly the sample units to the tocopherol axes, equipped with a set of convenient scale markers (milligrams per kilogram), as shown in **Figure 2**. This graph contains 100% of the information conveyed by principal components, and it is therefore displaying slightly >90% of the available information. Instead of attributing a meaning to the canonical variables in terms of the underlying principal components, this predictive biplot relates the position of any sample point directly to the original compounds and respective initial measurement units. It is seen that α-, γ-, and δ-tocopherols are the most important in the discrimination of nuts from different production years. The majority of nuts from 2001 and 2002 have between 210 and 260 mg/kg of γ-tocopherol, but

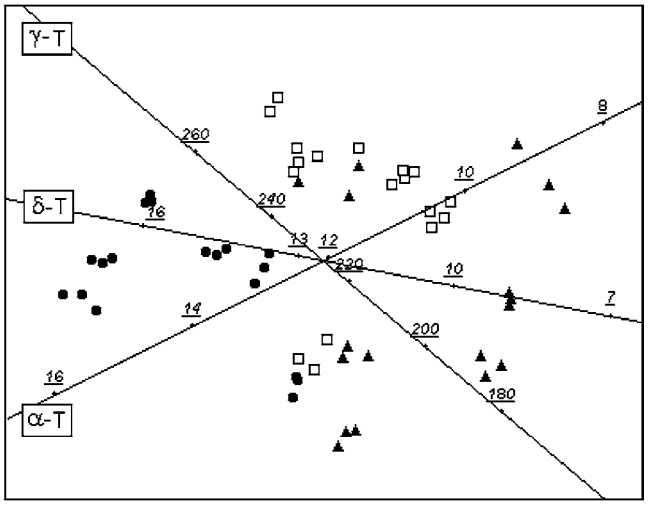

Figure 2. Predictive biplot applied to combined PCA/CVA analyses: (●) 2001; (□) 2002; (▲) 2003. Small points over tocopherol axes are scale markers labeled with respective units for measurement.

they differentiate from each other because the former have 12−16 mg/kg of α-tocopherol and 13−17 mg/kg of δ-tocopherol and the latter have 10−13 mg/kg of α-tocopherol and 10−14 mg/kg of δ-tocopherol. These two years differ from 2003 mainly in the levels of γ-tocopherol, which in 2003 varied, in the majority of samples, between 190 and 220 mg/kg. These results suggest that vitamin E composition can be influenced by climatic conditions, because significant differences can be observed among years of production when the same six cultivars, growing in the same experimental field under the same agricultural practices, are considered. The same approach has been taken in the study of the triacylglycerol composition of the same samples, and similar results were obtained (*38*).

To evaluate if vitamin E composition can be useful for discrimination among cultivars, a different approach was followed, because the number of cultivars exceeds the number of tocopherols, and the total number of samples per cultivar was relatively small. A MANOVA was applied directly to the initial data using cultivars as the grouping factor, showing that differences exist, and Hotelling T^2 tests were carried out to compare cultivars in pairs. Differences between cultivars were significant, as was seen by the significant Rao's R and Pillai−Bartlett test. However, the η^2, which reflects the degree of group membership, shows that only ~32% of the available information reflects the differences between cultivars. To display these differences, a canonical variate analysis was carried out, this time with the data divided in six groups, according to cultivar membership. The plot of canonical variates 1 versus 2 is shown in **Figure 3**. Due to the proportions of parameters, groups, and significant dimensions, it is difficult to determine exactly which are the main differences among cultivars. In **Figure 3** it becomes obvious that some cultivars are really very alike (e.g., Mayette and Marbot), whereas others are very distinct (e.g., Parisienne and Lara). Only α-tocopherol and γ-tocotrienol seem to have some predictive power, which reflects the fact that differences between cultivars cannot be described by a simple data structure, and possibly the differences between cultivars must be explained on the basis of all possible combinations. Consequently, Hotelling T^2 tests were carried out to determine which cultivars are different from the others in terms of their tocopherol and tocotrienol compositions (**Table 2**). It is seen that there are overall significant differences among cultivars, with the exceptions of the pairs Mellanaise and Marbot and Mayette and

Table 2. Hotelling T^2 Tests for the Overall Difference between Two Cultivars and Results of Student's *t* Tests for the Significance of Differences between Two Cultivars in Each Tocopherol

	Franquette	Lara	Marbot	Mayette	Mellanaise	Parisienne
Franquette	–	γ-TTR	β-T	none	α-T; β-T	none
Lara	71.77	–	α-T; γ-TTR	β-T; γ-TTR	α-T; γ-TTR	β-T; γ-TTR
Marbot	79.35	164.19	–	none	α-T	α-T; γ-TTR
Mayette	124.60	305.02	7.54	–	α-T	none
Mellanaise	596.05	366.28	6.92	26.03	–	α-T; γ-TTR
Parisienne	412.13	261.99	75.30	21.08	77.81	–

[a] Lower triangle: F_{obs} values (Hotelling T^2); any value greater than the critical $F_{(5,12)} = 8.89$ means that two cultivars are significantly different at the level of $\alpha = 0.001$. Upper triangle: tocopherols for which t_{obs} values were found to be significant on the univariate test of the hypothesis that two group means are equal. "None" means that differences were not found to be significant for any tocopherol. T, tocopherol; TTR, tocotrienol.

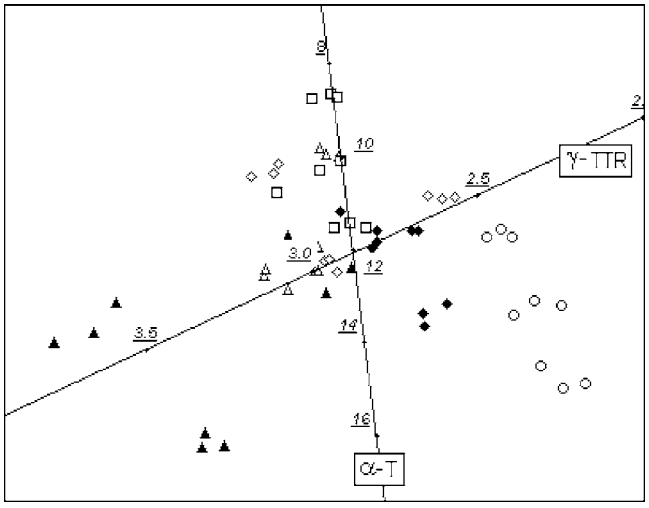

Figure 3. Biplot based on the first two canonical dimensions: (△) Franquette; (▲) Lara; (◇) Marbot; (◆) Mayette; (□) Mellanaise; (○) Parisienne. Underlined numbers are scale values.

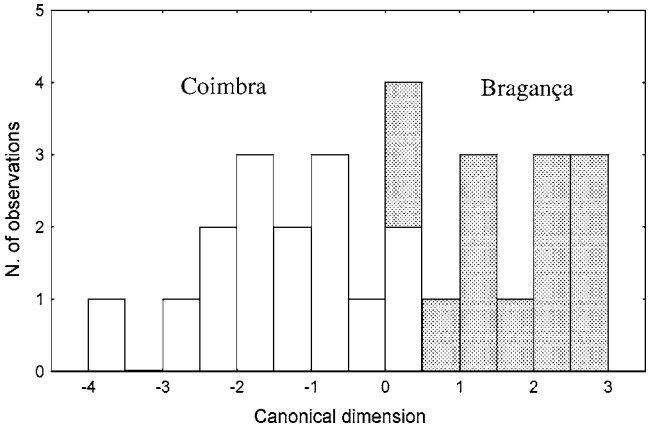

Figure 4. Display of the overall differences of walnuts according to region.

Marbot. The results presented in **Table 2** show that significant differences exist between many cultivars, and that these differences are mainly related to the levels of α-tocopherol and γ-tocotrienol, confirming the general display of **Figure 3**.

In 2003, samples from another geographical origin were included in the study. When walnuts from the two regions were compared, because only two groups are being considered (locations Coimbra and Bragança), the overall differences can be displayed in only one canonical dimension. Although more samples should be studied to reach conclusions with regard to differences with respect to geographical origins, these data seem to indicate that significant differences exist, as can be seen in **Figure 4**.

This work represents a contribution for the chemical characterization of walnuts, particularly the definition of their vitamin E (tocopherols and tocotrienols) profile. Probably due to the diversity of methodology employed (RP-HPLC or NP-HPLC) and the lack of a standardized method for the analysis of vitamin E vitamers, there are discrepancies among published data. Taking into account the fact that different biological activities and potencies have been found for the several vitamin E components, quantitative data on each of them are probably more useful when the nutritional and health benefits of foods are assessed. For this purpose, the methodology used in the present work can be very useful because it proved to be suitable for the simultaneous and individual determination of all tocopherol and tocotrienol vitamers in walnuts. Considering the data obtained for all of the studied samples, the results herein reported suggest that, besides genetic factors, the vitamin E composition can be influenced by environmental factors. Diet seems to play an important role in health maintenance and disease prevention and potential benefits are ascribed to vitamin E vitamers, so the qualitative and quantitative vitamin E composition herein reported for this matrix should encourage the consumption of walnuts.

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NOTE ADDED AFTER ASAP PUBLICATION

In the original ASAP posting of May 26, 2005, the column headings in Table 2 were labeled incorrectly. This was corrected on June 1, 2005.

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