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Tocopherol and Tocotrienol Content of Hazelnut Cultivars Grown in Portugal

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Hazelnuts from 19 cultivars collected during 3 consecutive-year crops, in 2 different geographical localities, for a total of 79 samples, were evaluated for their contents in tocopherols and tocotrienols by normal-phase high-performance liquid chromatography coupled to a series arrangement of a diode array and a fluorescence detector. Seven compounds were identified and quantified. All samples presented α-, *β*-, *γ*-, and *δ*- tocopherols and *β*-tocotrienol; α- and *γ*-tocotrienols were detected in some of the studied samples. α-Tocopherol was the major compound in all samples, ranging from 105.9 to 226.8 mg/kg of hazelnut. Considering the generality of the obtained results, an identical qualitative and quantitative pattern was found, which can define a chemical fingerprint that may be helpful in the assessment of identity and quality of hazelnut oils. Statistical analyses were carried out in order to check for differences among cultivars, year crops, and geographical localities. Although some differences were observed when different-year crops and geographical localities were considered, neither of these factors seemed to produce considerable differences in terms of tocopherol and tocotrienol contents. Some minor differences were observed among cultivars.

KEYWORDS: Vitamin E; tocopherols; tocotrienols; *Corylus avellana* L.; hazelnut; multivariate analysis

INTRODUCTION

Hazelnuts are consumed all over the world, not only as a fruit but also in a diversity of manufactured food products, such as snacks, chocolates, cereals, bakery, ice creams, and other dessert formulations. In 2004, over 8.5 million tons of nuts were produced throughout the world, almost 700 thousand tons of which were of hazelnuts (*Corylus avellana* L.). Turkey dominates the world market with over 70% of the production, followed by Italy (12%), the USA (6%), and Spain (2%) (*1*). Portuguese production is still comparatively low and mostly restricted to small scattered orchards near the Porto Wine region and in the Beira Alta region (*2*).

The Mediterranean diet is seen, nowadays, as a healthy practice, being considered one of the best in coronary heart disease (CHD) prevention (*3*). Hazelnuts, many of which are produced in the Mediterranean area, are considered important ingredients of this popular diet. In comparison to other nuts, hazelnuts are among the ones with highest contents of monounsaturated fatty acids (MUFAs) and lowest contents of saturated fat (*4*, *5*). There is evidence showing that a MUFA-rich diet can lower the risk of CHD and also has preventive effects on atherosclerosis (*6*−*8*). Hazelnuts are also rich in phytosterols (*5*), which are known to decrease blood cholesterol and have also been associated with anticancer and immune system modulating properties (*9*−*12*).

Hazelnuts have a high content of fat, thus being easily susceptible to rancidity that reduces both shelf life and sensory characteristics. This feature is thought to depend on several factors, such as the concentration of unsaturated fatty acids, the presence of natural antioxidants, such as α-tocopherol, and the presence of prooxidant minerals, namely iron and copper (*13*, *14*). The vitamin E family of compounds (α-, *β*-, *γ*-, and *δ*-tocopherols and α-, *β*-, *γ*-, and *δ*-tocotrienols), mainly due to their antioxidant activity, is particularly important in the prevention of lipid oxidation processes. These compounds are also believed to be involved in a diversity of physiological and biochemical functions. In the past, α-tocopherol was believed to be the most important vitamer. However, recent evidence shows that the other vitamers may also contribute to the total bioactivity in foods, playing different roles in the human body, so that different health benefits can arise from these vitamers (*15*-*19*). Although α-tocopherol has been reported to be the

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major vitamer in hazelnuts, few studies have been conducted on both the tocopherol and the tocotrienol composition of this type of nut. Keeping in mind the importance of these compounds in the shelf life and sensory characteristics of nuts, but also considering that the different vitamers are reported to possess a large range of biological activities and potencies, the tocopherol and tocotrienol compositions of several different hazelnut cultivars were qualitatively and quantitatively evaluated. In a previous study an analytical method for the separation, identification, and quantification of both tocopherols and tocotrienols in hazelnuts has been developed and validated (*20*) and the results of its application to several samples of different cultivars grown in Portugal, in several-year crops and in different geographical localities, are now reported. Statistical analysis was carried out to check for significant differences with regard to hazelnut tocopherol and tocotrienol composition. In addition to contributing to the knowledge of hazelnut chemical composition, this work aims to study possible influences of variables such as cultivars, climate, and geographical origin on the vitamin E composition of hazelnuts.

MATERIALS AND METHODS

Samples. A total of 19 hazelnut (*Corylus avellana* L.) cultivars (Butler, Campanica, Cosford, Couplat, Daviana, Ennis, Fertille de Coutard, Grossal, Gunslebert, Lansing, Longa d'Espanha, Merveille de Bollwiller, Morell, Negreta, Pauetet, Round du Piemont, Santa Maria de Jesus, Segorbe, and Tonda de Giffoni) were studied. Samples of all cultivars (with the exception of *cv.* Daviana, which was not obtained in 2003, due to its low productivity in that year) were collected in an experimental orchard at Vila Real, in the north inland region of Portugal (district of Vila Real, 41° 19′ N, 7° 44′ W, 470 m asl) during three consecutive years (2001−2003). In 2002 and 2003, samples from another geographical location (Felgueiras, district of Oporto, 41° 22′ N, 8° 11′ W, 50 km from the Atlantic Ocean, 320 m asl) were also included in this study: in 2002, 10 cultivars were studied (Butler, Campanica, Cosford, Couplat, Ennis, Fertille de Coutard, Merveille de Bollwiller, Morell, Pauetet, and Tonda de Giffoni), and in 2003, 3 more were added (*cvs.* Longa d'Espanha, Negreta, and Segorbe). The hazelnuts were harvested during September, as they fell to the ground, and a final sample of about 2 kg was randomly taken. After harvest, hazelnuts were sun-dried and stored in 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 10 mg/mL working solution of BHT was prepared in *n*-hexane. *n*-Hexane was 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 methodology reported by Amaral et al. (*20*). Immediately before the analysis, hazelnuts were manually cracked, shelled, and chopped in a 643 MX home coffee mill (Moulinex, Spain). The internal standard (150 μL of tocol solution) and the antioxidant (100 μL of BHT solution) were added to approximately 300 mg of sample accurately weighed in glass screw-cap tubes (Supelco, Bellefonte, PA) and homogenized for 1 min with 2 mL of ethanol by vortex mixing. Subsequently, 4 mL of *n*-hexane was added and again vortex mixed for 1 min. After the addition of 2 mL of saturated NaCl aqueous solution, the mixture was vortexed for 1 min and then centrifuged (2 min, 5000 g) and the clear upper layer was carefully removed to another glass screw-cap tube. The sample was re-extracted twice with *n*-hexane. The combined extracts were taken to dryness under a nitrogen stream, at room temperature, on a ReactiTherm module (Pierce, Rockford, IL) and transferred to microcentrifuge tubes with 1.5 mL of *n*-hexane. The extract was dehydrated with anhydrous sodium sulfate, centrifuged (20 s, 10 000 g), transferred into a dark injection vial, and analyzed by HPLC.

Owing to the instability of these compounds in the presence of air, light, and heat (*21*, *22*), in addition to using BHT as an antioxidant to prevent oxidation losses of the compounds, all standard and sample preparations were performed in a dark room under red light and, as possible, samples were kept on ice.

HPLC Analysis. HPLC analysis was achieved as described by Amaral et al. (*20*) with an analytical HPLC unit (Jasco, Japan), consisting of a PU-980 pump, an AS-950 autosampler with a 10 μL loop, a MD-910 multiwavelength diode array detector (DAD), and a 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 (95.5/4.5 v/v) eluted at a solvent flow rate of 0.7 mL/min for approximately 25 min. 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: gain 10 from 0 to 9.4 min; gain 100 until 12.7 min, and afterward gain 10. The compounds were identified by comparing their retention time and UV spectra with authentic standards. Quantification was performed on the basis of the internal standard method using fluorescence detection, since it provided a higher sensitivity than the DAD detector. Data were analyzed using a Borwin-PDA Controller Software (JMBS, France).

Statistical Analysis. Multivariate analyses of data involved the following: (i) MANOVA to evaluate general differences, all groups and variables being taken into consideration; (ii) Hotelling T^2 tests applied to pairs of groups, to evaluate the hypothesis "the two groups are significantly different in at least one compound", calculate T^2 values, and calculate and list the respective F values and corresponding probabilities; (iii) Student's t tests to evaluate the power of each tocopherol or tocotrienol in the discrimination between any two groups under consideration; (iv) a forward stepwise discriminant analysis (DA) to select the most discriminant variables (tocopherols and tocotrienols); (v) canonical variate analysis (CVA) based on a subset of the most discriminant variables, to further analyze the differences between groups and display those differences in convenient canonical variate plots. All analyses were carried out in the Statistica for Windows statistical package (Statistica for Windows, StatSoft Inc., Tulsa, OK), and comments to statistical results were based on literature (*23*, *24*).

RESULTS AND DISCUSSION

In the majority of hazelnut samples analyzed, seven vitamers were identified: α-, *β*-, *γ*-, and *δ*-tocopherols and α-, *β*-, and *γ*-tocotrienols. All the compounds were separated in a short period of time (less than 17 min). A typical chromatogram of a hazelnut sample containing all the identified compounds is shown in **Figure 1**. Vitamin E compositions (mg/kg of hazelnut) expressed in terms of mean values and standard deviations for each vitamer, with regard to every cultivar, year of production, and geographical locality, are presented in **Table 1**. As expected, α-tocopherol was the major compound in all samples, ranging from 105.9 mg/kg in *cv.* Merveille de Bollwiller to 226.8 mg/kg in *cv.* Fertille de Coutard, both collected in 2002 in Felgueiras. α- and *γ*-tocotrienols were present in low levels and were not detected in some of the studied cultivars.

Several studies have been performed on hazelnut vitamin E content (*25*−*36*). In most of them, determinations were performed on the extracted oil and not on the whole nut, which can explain some differences when values are compared. Converting the results presented in **Table 1** to mg/kg of oil (data not shown), the values obtained were, in general, in good agreement with those studies already published. Some variations can also be imputed to the analytical methodology used. Gas−

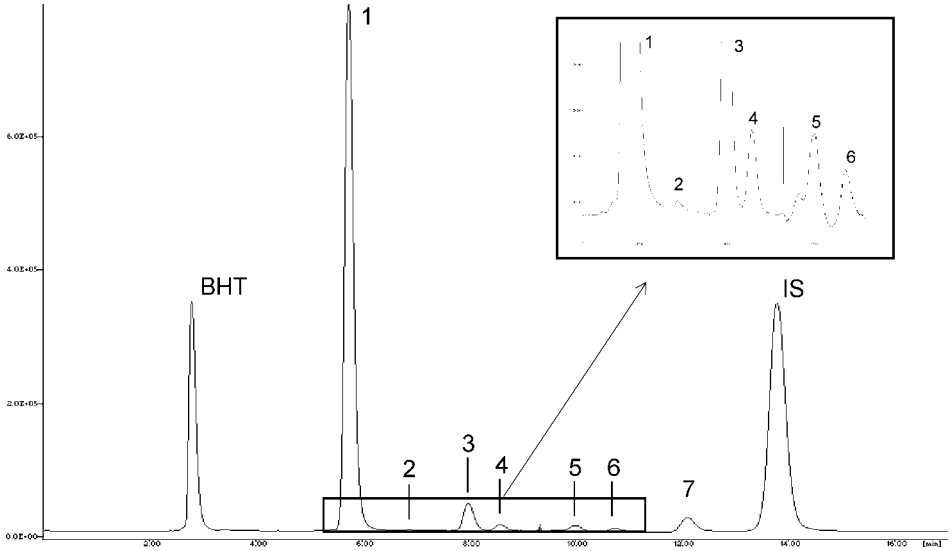

Figure 1. HPLC with fluorescence detection chromatogram of a hazelnut sample. Peaks: IS, internal standard (tocol); 1, α-tocopherol; 2, α-tocotrienol; 3, β-tocopherol; 4, γ-tocopherol; 5, β-tocotrienol; 6, γ-tocotrienol; 7, δ-tocopherol; BHT, butylated hydroxytoluene.

liquid chromatography (*25*−*27*), spectrophotometric color reaction (*28*, *29*), and RP-HPLC using electrochemical detection in the coulometric mode (*30*) have been used to determine vitamin E contents in hazelnuts. Most of these methodologies only allowed the determination of α-tocopherol. In other studies, the vitamer separation was performed by NP-HPLC and UV detection was used (*31*, *32*); Alasalvar et al. (*31*), when studying the oil of one Turkish cultivar by the referenced methodology, reported contents for α-, β-, and δ-tocopherols identical with those reported herein but much higher values of γ-tocopherol (on average, almost 5 times higher). Although we cannot explain the reason, in this work two samples (*cv.* Morell from Vila Real and *cv.* Couplat from Felgueiras, both in the 2003 crop) presented a very high γ-tocopherol value (15.7 and 20.8 mg/kg of sample, respectively) compared to all other samples (5.0 mg/kg, on average). Oils from hazelnuts collected in five countries were studied by Crews et al. (*32*), presenting a wide range of α-tocopherol values. In comparison to the values herein reported for several cultivars grown in Portugal, one sample from Croatia presented a much higher γ-tocopherol value (65 mg/kg of oil), and one sample from Italy presented an extremely high α-tocotrienol value (209 mg/kg of oil); in addition, in almost all samples analyzed in the cited work, β- and γ-tocotrienols were not detected, with two exceptions from Spain, whose values were also extremely high in comparison to those reported herein. These differences are probably due to the detection method used, since the authors used a UV detector, which does not allow the confirmation of the identity of the compounds as the DAD does by comparing the compounds' spectra with those obtained with standards. Consequently, it is possible that the extremely high values reported do not correspond to the real compound contents but were the result of some coeluted impurity. In addition, using the fluorescence detector to perform the quantification of the compounds allowed us to obtain a much higher sensitivity, with limits of quantification less than 1 mg/kg for all vitamers (*20*). In the Crews et al. (*32*) study, values lower than 10 mg/kg were not detectable, which indicates the possibility that β- and γ-tocotrienols could exist in those samples but were not detected. This hypothesis is supported by the results obtained by Benitez-Sánchez et al. (*33*), who reported the occurrence of 7 vitamers (α-, β-, γ-, and δ-tocopherols and α-, β-, and γ-tocotrienols) when studying 17 hazelnut oils from 5 countries using a NP-HPLC/MS/DAD/fluorescence detector apparatus. In general, the individual contents of the vitamers herein reported are in good agreement with those reported by these last authors (*33*), with the exception of β-tocopherol contents, which presented lower values in the cited study (*33*); this can be possibly explained by the fact that refined oils and oils obtained from roasted hazelnuts (where losses are expected to occur) were also included in that study. In some of the cultivars grown in Portugal, α- and γ-tocotrienols were not detected, which is also in agreement with the data reported by Benitez-Sánchez et al. (*33*). The results herein reported are also in good agreement with those reported by Bada et al. (*34*), Bernardo-Gil et al. (*35*), and Savage et al. (*36*) when analyzing hazelnuts grown in different countries. All these last three studies used a NP-HPLC/fluorescence detector methodology, which allowed the determination of four tocopherols. Nevertheless, in those studies tocotrienols were not determined.

To check differences in tocopherol and tocotrienol composition among cultivars, years of crops, and localities, statistical analysis was carried out. As already stated, *cv.* Morell (Vila Real, 2003 crop) presented an abnormally high γ-tocopherol content, being considered an outlier and consequently being removed from all multivariate analysis; otherwise, these analyses would be describing the differences between *cv.* Morell and the other cultivars, instead of providing a general picture of differences and similarities existing in the whole data set. To check if there were significant differences essentially due to climatic factors, a multivariate analysis of variance (MANOVA) was carried out with the results obtained for the cultivars from the same locality (Vila Real) and the year of the crop as the grouping factor. The result showed that at least one group is different from the others, as expressed by a significant Wilks λ value. Hotelling T^2 tests were subsequently carried out with the year of the crop as the grouping factor, therefore comparing years in pairs. The results showed that, at least for some of the considered compounds, all groups are statistically different. Subsequent Student's *t* tests for the differences between groups helped to check which were the main tocopherols responsible for the observed differences (for details, see part I in the Supporting Information). Bearing in mind that these statistical approaches can lead to overoptimistic results because they do not take into consideration the undesirable effects of collinearity (*23*, *24*), a multivariate analysis of data was performed, aiming to obtain a global picture of major differences. A forward

Table 1. Tocopherol and Tocotrienol Contents (in mg/kg, Mean ± SD of Three Determinations for Each Sample) of the Studied Cultivars by Year of Production and Geographical Locality

	compd							
cultivar	α-tocopherol	β- tocopherol	γ-tocopherol	δ-tocopherol	α-tocotrienol	β-tocotrienol	γ-tocotrienol	total
				2001				
				Vila Real				
Butler	188.89 ± 2.16	9.21 ± 0.17	4.88 ± 0.11	1.07 ± 0.01	nd[a]	0.26 ± 0.01	0.31 ± 0.02	204.61
Campanica	183.58 ± 0.67	6.89 ± 0.04	7.89 ± 0.03	1.11 ± 0.02	nd	0.22 ± 0.01	0.28 ± 0.01	199.98
Cosford	177.15 ± 2.83	8.50 ± 0.18	7.66 ± 0.14	1.60 ± 0.02	nd	0.11 ± 0.00	0.40 ± 0.02	195.42
Couplat	193.50 ± 1.64	7.38 ± 0.05	4.93 ± 0.02	1.18 ± 0.02	nd	0.12 ± 0.00	0.42 ± 0.01	207.53
Daviana	165.96 ± 1.27	7.67 ± 0.05	4.47 ± 0.01	0.61 ± 0.00	nd	0.19 ± 0.01	0.37 ± 0.02	179.27
Ennis	151.85 ± 0.18	7.51 ± 0.04	3.97 ± 0.03	0.61 ± 0.01	nd	0.15 ± 0.00	nd	164.09
F. Coutard	182.84 ± 1.57	5.34 ± 0.02	5.25 ± 0.05	0.31 ± 0.01	nd	0.11 ± 0.01	nd	193.85
Grossal	160.22 ± 1.20	4.98 ± 0.01	2.29 ± 0.03	0.29 ± 0.01	nd	0.21 ± 0.01	0.21 ± 0.14	168.19
Gunslbert	170.80 ± 0.73	7.04 ± 0.03	6.44 ± 0.02	1.43 ± 0.00	1.30 ± 0.04	0.21 ± 0.01	0.27 ± 0.01	187.48
Lansing	136.98 ± 0.04	3.40 ± 0.04	3.54 ± 0.09	0.42 ± 0.01	1.24 ± 0.04	0.22 ± 0.01	0.25 ± 0.01	146.05
L. Espanha	200.73 ± 1.72	11.47 ± 0.13	8.95 ± 0.11	2.10 ± 0.01	nd	0.24 ± 0.01	0.58 ± 0.02	224.07
M. Bollwiller	133.96 ± 0.57	6.17 ± 0.03	2.42 ± 0.03	0.96 ± 0.02	nd	0.11 ± 0.00	nd	143.61
Morell	166.85 ± 0.94	8.24 ± 0.01	5.89 ± 0.04	1.33 ± 0.02	nd	0.11 ± 0.00	0.44 ± 0.01	182.86
Negreta	164.25 ± 1.40	7.75 ± 0.06	5.66 ± 0.04	0.96 ± 0.00	nd	0.09 ± 0.00	0.41 ± 0.01	179.12
Pauetet	154.41 ± 0.26	5.28 ± 0.05	4.41 ± 0.03	0.60 ± 0.01	nd	0.10 ± 0.00	0.23 ± 0.02	165.03
R. Piemont	180.17 ± 0.29	4.92 ± 0.02	4.04 ± 0.02	0.32 ± 0.01	nd	0.20 ± 0.01	0.58 ± 0.02	190.23
Segorbe	219.96 ± 0.59	11.55 ± 0.02	6.13 ± 0.05	0.68 ± 0.01	nd	0.12 ± 0.00	0.78 ± 0.04	239.23
S. M. Jesus	140.99 ± 0.16	4.78 ± 0.02	5.69 ± 0.02	0.52 ± 0.03	nd	0.17 ± 0.00	0.31 ± 0.02	152.47
T. Giffonni	214.75 ± 1.56	4.87 ± 0.03	2.64 ± 0.02	0.24 ± 0.01	nd	0.33 ± 0.02	nd	222.82
mean	173.04	7.00	5.11	0.86	1.24[b]	0.17	0.38[b]	186.63
range	134.0–220.0	3.4–11.5	2.3–9.0	0.2–2.1	1.2–1.3	0.1–0.3	0.2–0.8	143.6–239.2
				2002				
				Vila Real				
Butler	140.65 ± 0.28	5.34 ± 0.05	3.08 ± 0.05	0.64 ± 0.00	nd	0.21 ± 0.00	0.35 ± 0.01	150.26
Campanica	186.83 ± 2.19	6.25 ± 0.09	9.83 ± 0.03	1.21 ± 0.02	nd	0.19 ± 0.01	0.35 ± 0.01	204.65
Cosford	171.12 ± 0.76	8.49 ± 0.03	9.53 ± 0.04	2.77 ± 0.01	nd	0.13 ± 0.00	0.30 ± 0.01	192.34
Couplat	188.63 ± 1.53	8.38 ± 0.02	4.18 ± 0.03	1.20 ± 0.01	1.21 ± 0.05	0.15 ± 0.00	0.39 ± 0.03	204.13
Daviana	176.54 ± 2.09	6.10 ± 0.11	4.39 ± 0.10	0.70 ± 0.02	nd	0.16 ± 0.01	0.29 ± 0.01	188.19
Ennis	165.71 ± 0.52	5.93 ± 0.05	6.18 ± 0.09	0.88 ± 0.03	nd	0.14 ± 0.00	nd	178.85
F. Coutard	146.86 ± 0.09	4.01 ± 0.03	3.69 ± 0.04	0.27 ± 0.00	nd	0.29 ± 0.00	nd	155.13
Grossal	196.00 ± 3.41	5.78 ± 0.09	4.15 ± 0.04	0.46 ± 0.03	1.03 ± 0.11	0.33 ± 0.03	0.33 ± 0.02	208.08
Gunslbert	194.49 ± 2.21	9.27 ± 0.09	8.22 ± 0.12	2.42 ± 0.03	1.22 ± 0.09	0.37 ± 0.01	0.46 ± 0.03	216.44
Lansing	145.56 ± 1.26	4.30 ± 0.10	7.12 ± 0.04	0.47 ± 0.03	nd	0.10 ± 0.01	0.25 ± 0.01	157.80
L. Espanha	173.88 ± 2.46	6.73 ± 0.13	8.03 ± 0.16	1.14 ± 0.01	nd	0.33 ± 0.02	0.40 ± 0.02	190.51
M. Bollwiller	158.38 ± 0.70	5.91 ± 0.04	3.31 ± 0.01	0.76 ± 0.02	nd	0.12 ± 0.01	nd	168.48
Morell	188.20 ± 4.08	6.78 ± 0.12	5.03 ± 0.07	0.69 ± 0.01	1.18 ± 0.05	0.33 ± 0.01	0.43 ± 0.01	202.65
Negreta	186.19 ± 0.40	5.61 ± 0.01	6.51 ± 0.04	0.69 ± 0.01	nd	0.18 ± 0.01	0.34 ± 0.02	199.53
Pauetet	168.80 ± 1.68	5.97 ± 0.08	6.86 ± 0.07	0.76 ± 0.01	nd	0.09 ± 0.00	0.28 ± 0.01	182.76
R. Piemont	165.88 ± 0.43	4.18 ± 0.04	6.10 ± 0.05	0.40 ± 0.00	nd	0.10 ± 0.00	0.41 ± 0.02	172.89
Segorbe	221.19 ± 0.64	9.41 ± 0.10	5.45 ± 0.04	0.57 ± 0.01	nd	0.25 ± 0.02	0.47 ± 0.01	237.33
S. M. Jesus	167.18 ± 0.43	3.61 ± 0.12	5.79 ± 0.04	0.26 ± 0.00	nd	0.30 ± 0.01	0.49 ± 0.03	177.62
T. Giffonni	214.33 ± 1.52	4.57 ± 0.06	4.23 ± 0.01	0.25 ± 0.01	nd	0.28 ± 0.01	0.30 ± 0.04	223.95
mean	176.65	6.14	5.88	0.87	1.18[b]	0.22[b]	0.36[b]	190.08
range	145.6–221.2	3.6–9.4	3.1–9.8	0.3–2.8	1.0–1.2	0.1–0.4	0.3–0.5	155.1–237.3
				Felgueiras				
Butler	184.30 ± 0.43	9.81 ± 0.00	5.21 ± 0.06	1.45 ± 0.00	nd	0.33 ± 0.01	0.27 ± 0.01	201.37
Campanica	190.30 ± 0.42	5.43 ± 0.15	5.95 ± 0.08	0.90 ± 0.02	nd	0.23 ± 0.01	0.24 ± 0.01	203.05
Cosford	110.15 ± 1.99	4.32 ± 0.09	2.67 ± 0.07	0.64 ± 0.03	nd	0.05 ± 0.00	0.00 ± 0.05	117.83
Couplat	223.57 ± 0.28	12.01 ± 0.03	8.61 ± 0.06	1.58 ± 0.01	nd	0.26 ± 0.00	0.61 ± 0.00	246.63
Ennis	123.89 ± 2.66	5.46 ± 0.14	5.85 ± 0.15	1.08 ± 0.02	nd	0.12 ± 0.00	3.20 ± 0.12	139.59
F. Coutard	226.83 ± 1.85	8.01 ± 0.15	9.75 ± 0.18	1.03 ± 0.03	1.22 ± 0.03	0.28 ± 0.01	0.33 ± 0.01	237.45
M. Bollwiller	105.87 ± 0.21	4.07 ± 0.07	2.60 ± 0.02	0.61 ± 0.01	nd	0.05 ± 0.00	nd	113.21
Morell	221.22 ± 3.19	9.12 ± 0.17	13.39 ± 0.76	2.37 ± 0.15	1.10 ± 0.04	0.40 ± 0.00	0.34 ± 0.00	247.93
Pauetet	201.04 ± 3.26	7.40 ± 0.15	7.60 ± 0.17	1.14 ± 0.03	nd	0.09 ± 0.00	0.25 ± 0.01	217.52
T. Giffonni	219.94 ± 0.59	5.44 ± 0.01	4.74 ± 0.04	0.43 ± 0.01	nd	0.12 ± 0.00	0.24 ± 0.01	230.92
mean	180.71	7.11	6.64	1.12	1.16[b]	0.19	0.61[b]	196.55
range	105.9–226.8	4.1–12.0	2.6–13.4	0.4–2.4	1.1–1.2	0.0–0.4	0.0–3.2	113.2–247.9
				2003				
				Vila Real				
Butler	156.29 ± 7.26	5.53 ± 0.20	2.11 ± 0.09	0.40 ± 0.02	0.95 ± 0.05	0.23 ± 0.02	0.33 ± 0.02	165.84
Campanica	165.50 ± 0.37	4.82 ± 0.04	3.75 ± 0.03	0.40 ± 0.01	nd	0.35 ± 0.01	0.62 ± 0.04	175.45
Cosford	177.47 ± 0.76	8.97 ± 0.01	5.49 ± 0.01	1.32 ± 0.02	nd	0.17 ± 0.00	0.22 ± 0.01	193.65
Couplat	158.79 ± 0.68	7.60 ± 0.04	4.31 ± 0.06	1.09 ± 0.02	1.12 ± 0.03	0.09 ± 0.00	0.48 ± 0.01	173.46
Ennis	126.78 ± 0.42	7.29 ± 0.05	1.04 ± 0.02	0.25 ± 0.01	1.11 ± 0.04	0.29 ± 0.01	nd	135.65
F. Coutard	174.54 ± 5.24	4.30 ± 0.09	1.69 ± 0.05	0.23 ± 0.00	nd	0.32 ± 0.02	nd	181.07
Grossal	188.63 ± 0.97	6.56 ± 0.01	2.25 ± 0.04	0.32 ± 0.01	nd	0.16 ± 0.00	nd	197.91
Gunslbert	181.55 ± 0.98	10.77 ± 0.12	3.74 ± 0.07	0.86 ± 0.02	nd	0.21 ± 0.00	0.25 ± 0.01	197.36
Lansing	110.15 ± 1.01	3.29 ± 0.01	1.72 ± 0.02	0.23 ± 0.01	1.34 ± 0.03	0.18 ± 0.01	nd	115.57

Table 1. (Continued)

	compd							
cultivar	α-tocopherol	β- tocopherol	γ-tocopherol	δ-tocopherol	α-tocotrienol	β-tocotrienol	γ-tocotrienol	total
				2003 (Continued)				
				Vila Real (Continued)				
L. Espanha	193.98 ± 0.86	7.51 ± 0.04	5.97 ± 0.03	1.15 ± 0.02	nd	0.30 ± 0.00	0.49 ± 0.02	209.39
M. Bollwiller	159.76 ± 1.03	5.30 ± 0.06	1.66 ± 0.06	0.35 ± 0.01	nd	0.14 ± 0.00	nd	167.21
Morell	188.44 ± 1.01	6.28 ± 0.08	15.69 ± 0.08	2.38 ± 0.01	nd	0.20 ± 0.01	0.57 ± 0.03	213.55
Negreta	170.08 ± 2.31	4.15 ± 0.00	4.02 ± 0.01	0.36 ± 0.00	0.30 ± 0.01	nd	178.91	
Pauetet	177.83 ±2.89	6.64 ± 0.06	4.67 ± 0.04	0.53 ± 0.01	nd	0.21 ± 0.01	0.38 ± 0.02	190.25
R. Piemont	193.64 ± 2.36	5.88 ± 0.10	5.80 ± 0.10	0.58 ± 0.01	nd	0.17 ± 0.01	0.54 ± 0.03	206.61
Segorbe	203.43 ± 1.36	8.74 ± 0.06	2.25 ± 0.04	0.25 ± 0.01	nd	0.21 ± 0.02	0.37 ± 0.01	215.26
S. M. Jesus	201.54 ± 1.90	9.39 ± 0.09	4.51 ± 0.04	0.81 ± 0.02	nd	0.10 ± 0.00	0.32 ± 0.03	216.67
T. Giffonni	177.78 ± 2.83	3.42 ± 0.09	1.89 ± 0.06	0.14 ± 0.00	nd	0.21 ± 0.01	nd	183.45
mean	172.56	6.47	4.03	0.65	0.92[b]	0.21	0.39[b]	184.29
range	110.1–203.4	3.3–10.8	1.0–15.7	0.1–2.4	1.0–1.3	0.1–0.4	0.2–0.6	115.6–216.7
				Felgueiras				
Butler	148.48 ± 0.30	9.04 ± 0.03	1.94 ± 0.04	0.54 ± 0.02	1.07 ± 0.02	0.32 ± 0.02	0.41 ± 0.00	161.79
Campanica	212.95 ± 1.35	5.87 ± 0.03	4.42 ± 0.05	0.72 ± 0.02	nd	0.15 ± 0.00	0.28 ± 0.02	224.39
Cosford	169.08 ± 1.13	8.34 ± 0.06	4.94 ± 0.01	1.09 ± 0.02	nd	0.07 ± 0.00	0.30 ± 0.02	183.82
Couplat	220.75 ± 1.63	13.54 ± 0.23	20.79 ± 0.65	4.39 ± 0.15	nd	0.15 ± 0.01	0.51 ± 0.02	260.14
Ennis	132.92 ± 1.11	6.49 ± 0.05	2.34 ± 0.06	0.50 ± 0.01	1.10 ± 0.04	0.11 ± 0.01	nd	142.35
F. Coutard	116.29 ± 1.11	3.41 ± 0.02	6.27 ± 0.06	1.11 ± 0.03	nd	0.13 ± 0.01	0.28 ± 0.01	127.51
Gunslbert	202.99 ± 1.22	11.97 ± 0.06	4.40 ± 0.02	2.40 ± 0.00	nd	0.07 ± 0.00	0.35 ± 0.02	222.19
L. Espanha	221.33 ± 0.56	13.10 ± 0.03	8.44 ± 0.02	1.75 ± 0.01	nd	0.10 ± 0.00	0.61 ± 0.05	245.33
M. Bollwiller	154.84 ± 1.36	8.80 ± 0.02	3.73 ± 0.05	4.13 ± 0.03	2.48 ± 0.11	3.80 ± 0.06	1.88 ± 0.04	179.66
Morell	214.44 ± 1.82	10.20 ± 0.05	3.55 ± 0.08	1.37 ± 0.03	nd	0.10 ± 0.00	0.84 ± 0.03	230.49
Negreta	218.67 ± 1.48	6.77 ± 0.04	4.02 ± 0.03	0.75 ± 0.01	nd	0.09 ± 0.01	0.25 ± 0.02	230.56
Pauetet	217.04 ± 1.43	8.23 ± 0.16	5.62 ± 0.11	1.20 ± 0.01	nd	0.19 ± 0.00	0.38 ± 0.01	232.66
Segorbe	222.86 ± 0.30	8.93 ± 0.06	2.54 ± 0.04	0.58 ± 0.00	nd	0.11 ± 0.00	0.49 ± 0.03	235.51
T. Giffonni	214.29 ± 1.52	5.50 ± 0.05	3.18 ± 0.05	0.36 ± 0.02	nd	0.12 ± 0.00	0.24 ± 0.02	223.68
mean	190.49	8.59	5.44	1.49	1.55[b]	0.39	0.53[b]	207.15
range	116.2–222.9	3.4–13.5	1.9–20.8	0.4–4.4	1.1–2.5	0.1–3.8	0.2–1.9	127.5–260.1

[a] nd = not determined. [b] Mean of the determined values.

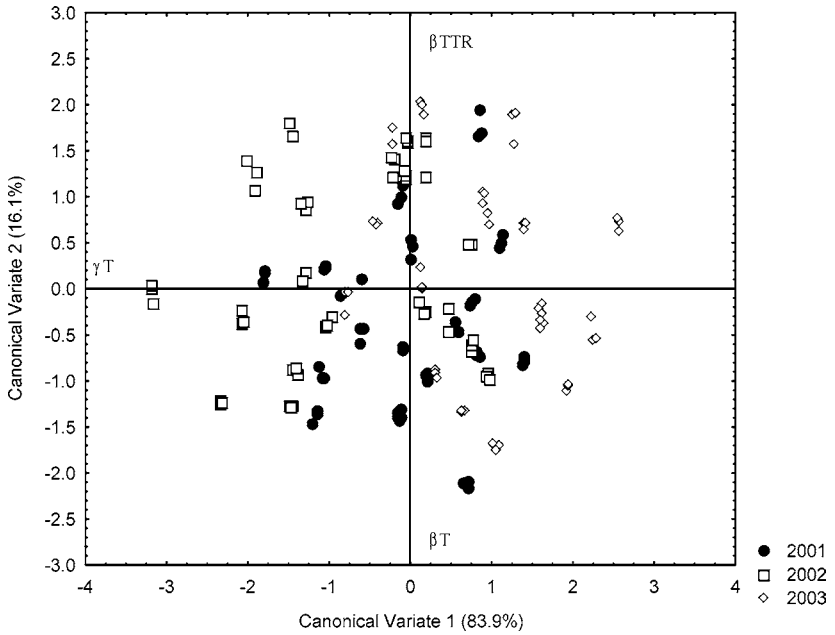

Figure 2. Results from CVA with the year of production as the grouping factor, of samples from Vila Real. Plot of canonical variate 1 versus variate 2. Vitamer labeling on the canonical axes is important for their interpretation. Percentage values refer to the amount of information explained by each canonical dimension. Abbreviations: γT, γ-tocopherol; βT, β-tocopherol; βTTR, β-tocotrienol).

stepwise discriminant analysis was subsequently applied to data from samples grouped by year of production, allowing the selection of three vitamers as the most discriminant ones (β- and γ-tocopherols and β-tocotrienol), and a canonical variate analysis was developed to enable the visualization of all results. **Figure 2** shows the results of an exploratory canonical analysis carried out with the available data, expressed as a plot of variate 1 versus variate 2. The first dimension represents 84.0% of the information in the data. Although some differences were revealed in the Hotteling T^2 tests (for details, see part I in the Supporting Information), the plot demonstrates that, even though some minor differences exist, it is not possible to distinguish

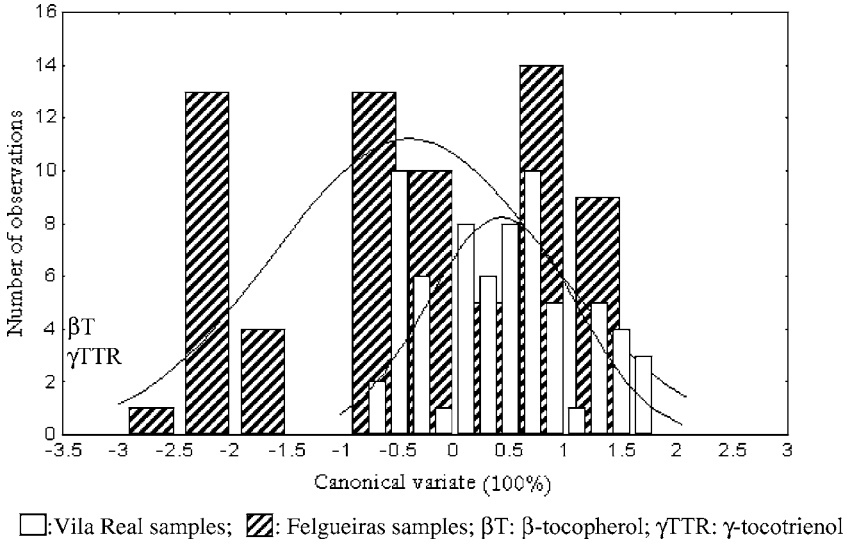

Figure 3. Display of the overall differences according to geographical locality.

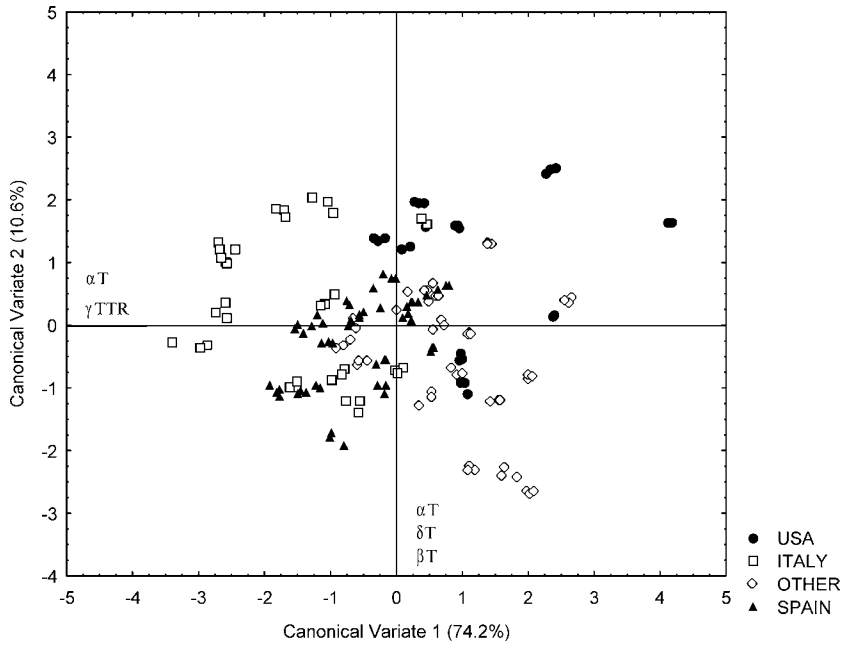

Figure 4. Results from CVA with the origin of the cultivars as the grouping factor, of samples from Vila Real considering crops from all years. Plot of canonical variates 1 versus variate 2. Vitamer labeling on the canonical axes is important for their interpretation. Percentage values refer to the amount of information explained by each canonical dimension. Abbreviations: αT, α-tocopherol; γTTR, γ-tocotrienol; γT, γ-tocopherol; βT, β-tocopherol).

three distinct groups, and in general the samples from the three different years of crops are fairly alike. As can be seen in the plot, the small differences between years of crops are mainly explained by γ- and β-tocopherol and β-tocotrienol contents. With regard to the major compound (α-tocopherol), no significant differences were found among the three different years of crops (part I in the Supporting Information and **Figure 2**).

To evaluate possible differences related to geographical locations, samples were obtained from two different localities: Vila Real and Felgueiras. Statistical analyses were carried out, considering only the results obtained for the common cultivars in 2002 and 2003 crops. A forward stepwise discriminant analysis allowed the selection of two vitamers as the most discriminant ones when samples were grouped by geographical locality, and a canonical variate analysis was developed to enable the visualization of all results. Because only two groups are being considered (Vila Real and Felgueiras), the overall differences can only be displayed in one canonical dimension. **Figure 3** shows the results of an exploratory canonical analysis carried out with the available data. Although for other matrices differences related to geographical locations have been described (*37*, *38*), these data seem to indicate that this factor does not generate substantial differences in terms of the hazelnut's vitamin E composition. Even though the Felgueiras location displays a higher dispersion of results, with some cultivars presenting slight differences in the generality of the obtained results (mainly cultivars Couplat from both years, and Longa d'Espanha, Merveille de Bollwiller, and Morell from 2003, presenting higher levels of β-tocopherol and γ-tocotrienol), **Figure 3** shows that the samples collected from Vila Real are identical with most of the samples from Felgueiras. Nevertheless, since only two years were taken in account, more samples should be studied in order to confirm this conclusion regarding differences with respect to geographical locations.

In a previous work, when a discriminant analysis was performed using fatty acid as variables, Parcerisa et al. (*26*)

reported that hazelnut cultivars, all grown in the same orchard, were grouped according to the cultivar's origin. Although α-tocopherol contents were also determined in the cited work, the same approach was not performed with the results obtained for this parameter, probably due to the scarcity of data, since only one vitamer was determined. In the work herein, a similar approach was performed using tocopherols and tocotrienols as variables. Although the samples were collected from trees cultivated in Portugal, the several cultivars under analysis are native from various countries. Therefore, four groups were made according to the origin of the hazelnut's cultivars (*39*): the USA (Butler, Ennis, and Lansing), Italy (Campanica, Round de Piemont, Santa Maria de Jesus, and Tonda de Giffoni), Spain (Couplat, Grossal, Morell, Negreta, Pauetet, and Segorbe), and others (comprising the French cultivar Fertille de Coutard, the British cultivars Cosford, Daviana, and Longue d'Espanha and the German cultivars Gunslebert and Merveille de Bollwiller). A statistical analysis similar to that described for the evaluation of significant differences related to the production years was carried out using the origin of the cultivars as the grouping factor. MANOVA, Hotteling T^2 tests, and Student's *t* tests were carried out with the origin of cultivars as the grouping factor, thereby comparing groups in pairs. The results showed that all groups are different, although the differences for each considered pair were due to different sets of variables. The list of variables found to be responsible for the observed differences between groups ($p < 0.05$) are given in part II of the Supporting Information (upper triangle). Interestingly, with the exception of the pair Italy/Spain, all groups were significantly different in terms of the major compound (α-tocopherol), in opposition to what happened when years of crops and localities were considered. Subsequently, a CVA was performed using only the results obtained from Vila Real locality, and the differences between groups were displayed in a plot condensing 84.8% of all information (**Figure 4**). This plot shows that although the four groups were not clearly separated, some differences among them are visible. This plot also points to the existence of considerable differences within groups, since the variable α-tocopherol is important in both dimensions, making the differences between groups less visible. Additionally, the Spain group seems to display intermediate characteristics, since it is lying mainly in the plot's central part. The USA group appears to be distinct from the Italy group, mainly due to the lower contents of α-tocopherol and γ-tocotrienol; differences between the Spain and USA groups were also evident. Although some natural variability in the compound's contents exists among cultivars and year crops within the same origin group, the Spanish cultivar Segorbe and the Italian cultivar Tonda de Giffoni were among those with less variability, presenting in all cases high contents of α-tocopherol. The "others" group was the one presenting a higher dispersion of results. Nevertheless, it is possible to observe that some cultivars were distinct from the remaining ones, presenting higher contents of β- and δ-tocopherols and a lower content of γ-tocotrienol. Some of the Italian and Spanish cultivars seemed to be very alike, which is in good agreement with the results of the Hotteling T^2 tests (for details, see part II in the Supporting Information), since as already stated, the Italy/Spain pair was the only one that did not present significant differences in terms of the major compound (α-tocopherol).

In conclusion, these results confirm that hazelnuts are a good source of α-tocopherol and, although in minor amounts, they also contain other tocopherols and tocotrienols. Since there is strong evidence that tocopherols and tocotrienols can play an important role in human health, the knowledge of all tocopherol and tocotrienol contents in foods is becoming of utmost importance, especially where clinical and epidemiological studies are concerned. Statistical analysis of the obtained data points out that, even though some minor differences exist, in general the samples from the three years of crops are fairly alike. Identically, the geographical location does not seem to produce a significant influence in hazelnut vitamin E composition.

This work also represents a contribution to hazelnut characterization. In the literature it has been reported that tocopherols can be a useful parameter in the detection of the adulteration of olive oil with hazelnut oil (*33*, *40*, *41*). In this way, the data obtained can also be valuable in updating databases and available composition tables concerning hazelnut chemical composition, which could be helpful in the evaluation of possible adulterations.

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Supporting Information Available: Tables giving results of statistical tests for the data given in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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