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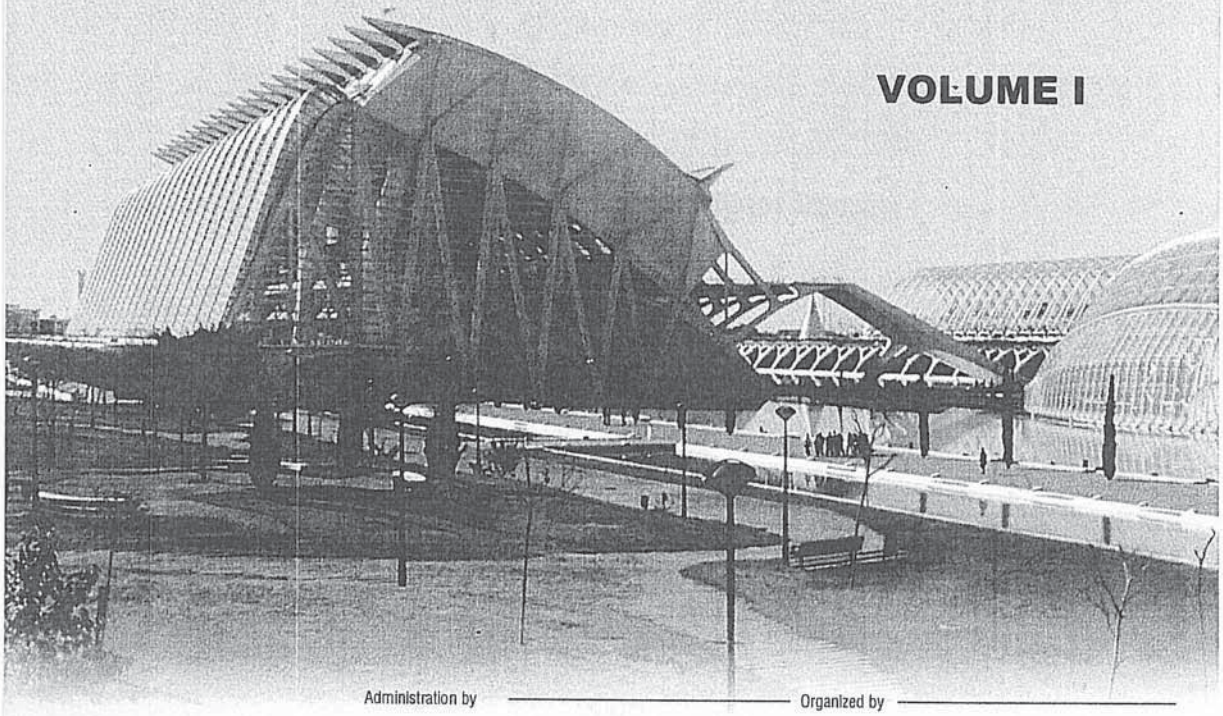

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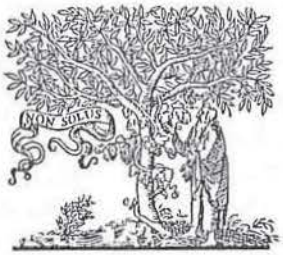

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Tocopherols and tocotrienols composition of hazelnuts

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Introduction

Vitamin E is a term used to generally refer a group of compounds: α-, β-, γ-, and δ- tocopherols and tocotrienols. These compounds are believed to be involved in a diversity of physiological and biochemical functions, mainly due to their antioxidant activity by inhibiting lipid peroxidation in biological membranes (Mishima, 2003; Theriault, 1999). Besides the antioxidant activity of these compounds, several studies on the health effects of the non-a-tocopherol vitamers have been recently published (Mishima, 2003; Theriault, 1999; Li 1999, Campbell, 2003). Due to their antioxidant activity, this family of compounds is also particularly important in the prevention of lipid oxidation processes that reduce foods shelf life and sensorial characteristics.

Vegetable oils are probably the main dietary source of vitamin E, being nuts, cereals, and fruits other valuable sources (Woollard, 2003). Owing to the richness in vitamin E present in nuts, and since hazelnuts are widely used as raw material in a large range of products, this study aims to analyse several hazelnuts cultivars in terms of their Vitamin E qualitative and quantitative compositions.

Materials and methods

Samples. Samples were from Vila Real, Portugal (19 cultivars) and from Felgueiras, Portugal (10 cultivars), all collected in the 2002 year crop.

Standards and reagents. Individual stock solutions (~ 5mg/mL) of each isomer (α-, β-, γ- and δ- tocopherols and tocotrienols, Calbiochem, CA, USA) were prepared in *n*-hexane. A stock standard mixture, with the different isomers in relative proportions similar to those presented by the samples, was prepared and diluted to working standard mixtures. Tocol (Matreya Inc., PA, USA) was used as internal standard (IS); a stock solution

at 10 mg/mL in *n*-hexane was kept at -4 ºC, protected from light, and diluted to working solutions (500 μg/mL) as necessary. BHT (10 mg/mL, in *n*-hexane) was from Aldrich (Madrid, Spain). *n*- Hexane was HPLC grade from Merck (Darmstad, Germany) and 1,4-dioxane was from Fluka (Madrid, Spain).

Extraction procedure. Samples were extracted using a previously validated methodology (Amaral, 2005). Chopped hazelnuts (~300 mg) were accurately weighted in glass screw cap tubes and subsequently the IS (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). After that, the sample was centrifuged (2 min, 5000 rpm) and the clear upper layer transferred to another tube. The sample was re-extracted twice. The combined extracts were taken to dryness under a nitrogen stream, at room temperature, transferred to microcentrifuge tubes with 1.5 mL of *n*-hexane and, finally, water was removed with anhydrous sodium sulphate. The extract was centrifuged (10000 rpm, 20 s), transferred into a dark injection vial and analysed by HPLC.

HPLC analysis. The HPLC equipment consisted of an integrated system with a PU-980 pump, an AS-950 auto-sampler, a MD-910 multiwavelength diode array detector (DAD), and a FP-920 fluorescence detector (Jasco, Japan). Data were analyzed using a Borwin-PDA Controller Software (JMBS, France). The chromatographic separation was achieved with an Inertsil 5 SI (250 x 3 mm) normal-phase column from Varian (Middelburg, Netherlands) operating at room temperature. The mobile phase was a mixture of *n*-hexane and dioxane (95.5:4.5, v/v) at a flow rate of 0.7 mL/min, and the injection volume was 10 μL. The effluent was monitored with a DAD connected in series with a fluorescence detector, programmed for excitation at 290nm and emission at 330nm. The compounds were identified by chromatographic comparisons with authentic standards and by their UV spectra. Quantification was made by fluorescence detection based on the internal standard method.

Results and discussion

Three extractions procedures were previously assayed (Amaral, 2005) and the best results were obtained using an extraction procedure with *n*-hexane which was used to analyze the studied samples. α-, β-, γ- and δ- tocopherols, and b- tocotrienol were detected in all the studied cultivars. Some samples also presented a- and g- tocotrienols in minor amounts. **Figure 1** shows a typical chromatogram of a hazelnut sample containing the seven identified compounds. The average contents of each vitamer for every cultivar are shown in **Table 1**. In all samples a-tocopherol was the major compound, ranging from 105.9 mg/kg to 226.8 mg/kg. In general, Vitamin E total contents are higher in the Felgueiras location when compared to Vila Real location. Since the cultivars where identical in these two locations, these can indicate that climate or different agricultural practices can influence

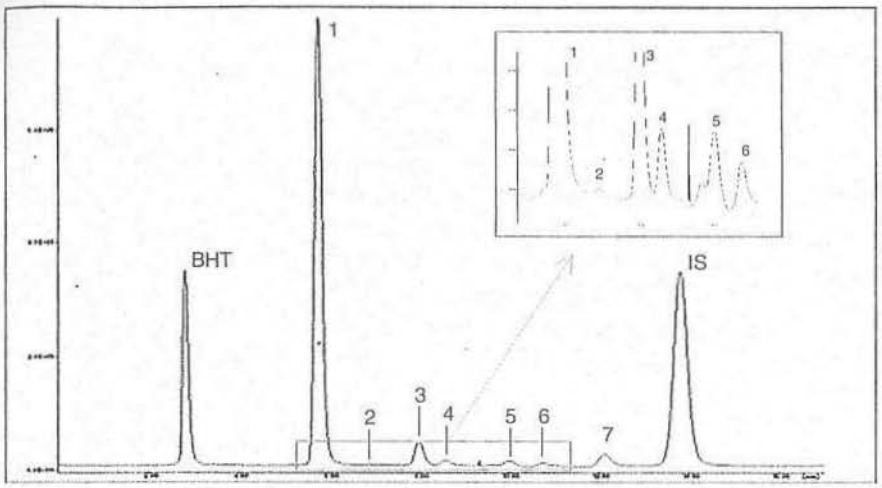

Figure 1. HPLC with fluorescence detection chromatogram of a hazelnut sample. Peaks: I.S., internal standard (tocol); 1, α-tocopherol; 2, α-tocotrienol; 3, β-tocopherol; 4, γ-tocopherol; 5, β-tocotrienol; 6, γ-tocotrienol; 7, δ-tocopherol; BHT, butylated hydroxytoluene.

Table 1. Tocopherol and Tocotrienol contents (mg/kg hazelnut) of the studied cultivars by geographical location. nd: not determined; T: tocopherol; TTR: tocotrienol

Geographical location	Cultivar	α-T	α-TTR	β-T	γ-T	β-TTR	γ-TTR	δ-T	Total
Vila Real	Butler	140.65 ± 0.28	nd	5.34 ± 0.05	3.08 ± 0.05	0.21 ± 0.00	0.35 ± 0.01	0.64 ± 0.00	150.26
	Campanica	186.83 ± 2.19	nd	6.25 ± 0.09	9.83 ± 0.03	0.19 ± 0.01	0.35 ± 0.01	1.21 ± 0.02	204.65
	Cosford	171.12 ± 0.76	nd	8.49 ± 0.03	9.53 ± 0.04	0.13 ± 0.00	0.30 ± 0.01	2.77 ± 0.01	192.34
	Couplat	188.63 ± 1.53	1.21 ± 0.05	8.38 ± 0.02	4.18 ± 0.03	0.15 ± 0.00	0.39 ± 0.03	1.20 ± 0.01	204.13
	Daviana	176.54 ± 2.09	nd	6.10 ± 0.11	4.39 ± 0.10	0.16 ± 0.01	0.29 ± 0.01	0.70 ± 0.02	188.19
	Ennis	165.71 ± 0.52	nd	5.93 ± 0.05	6.18 ± 0.09	0.14 ± 0.00	nd	0.88 ± 0.03	178.85
	Fertille Coutard	146.86 ± 0.09	nd	4.01 ± 0.03	3.69 ± 0.04	0.29 ± 0.00	nd	0.27 ± 0.00	155.13
	Grossal	196.00 ± 3.41	1.03 ± 0.11	5.78 ± 0.09	4.15 ± 0.04	0.33 ± 0.03	0.33 ± 0.02	0.46 ± 0.03	208.08
	Gunslbert	194.49 ± 2.21	1.22 ± 0.09	9.27 ± 0.09	8.22 ± 0.12	0.37 ± 0.01	0.46 ± 0.03	2.42 ± 0.03	216.44
	Lansing	145.56 ± 1.26	nd	4.30 ± 0.10	7.12 ± 0.04	0.10 ± 0.01	0.25 ± 0.01	0.47 ± 0.03	157.80
	Longue Espanha	173.88 ± 2.46	nd	6.73 ± 0.13	8.03 ± 0.16	0.33 ± 0.02	0.40 ± 0.02	1.14 ± 0.01	190.51
	Merveille Bollwiller	158.38 ± 0.70	nd	5.91 ± 0.04	3.31 ± 0.01	0.12 ± 0.01	nd	0.76 ± 0.02	168.48
	Morell	188.20 ± 4.08	1.18 ± 0.05	6.78 ± 0.12	5.03 ± 0.07	0.33 ± 0.01	0.43 ± 0.01	0.69 ± 0.01	202.65
	Negreta	186.19 ± 0.40	nd	5.61 ± 0.01	6.51 ± 0.04	0.18 ± 0.01	0.34 ± 0.02	0.69 ± 0.01	199.53
	Pauetet	168.80 ± 1.68	nd	5.97 ± 0.08	6.86 ± 0.07	0.09 ± 0.00	0.28 ± 0.01	0.76 ± 0.01	182.76
	Roud Piemont	165.88 ± 0.43	nd	4.18 ± 0.04	6.10 ± 0.05	0.00 ± 0.00	0.41 ± 0.02	0.40 ± 0.00	176.97
	Segorbe	221.19 ± 0.64	nd	9.41 ± 0.10	5.45 ± 0.04	0.25 ± 0.02	0.47 ± 0.01	0.57 ± 0.01	237.33
	St. Mª de Jesus	167.18 ± 0.43	nd	3.61 ± 0.12	5.79 ± 0.04	0.30 ± 0.01	0.49 ± 0.03	0.26 ± 0.00	177.62
	Tonda Giffonni	214.33 ± 1.52	nd	4.57 ± 0.06	4.23 ± 0.01	0.28 ± 0.01	0.30 ± 0.04	0.25 ± 0.01	223.95
Felgueiras	Butler	184.30 ± 0.43	nd	9.81 ± 0.00	5.21 ± 0.06	0.33 ± 0.01	0.27 ± 0.01	1.45 0.00	201.37
	Campanica	190.30 ± 0.42	nd	5.43 ± 0.15	5.95 ± 0.08	0.23 ± 0.01	0.24 ± 0.01	0.90 ± 0.02	203.05
	Cosford	223.57 ± 0.28	nd	12.01 ± 0.03	8.61 ± 0.06	0.26 ± 0.00	0.61 ± 0.00	1.58 ± 0.01	246.63
	Couplat	110.15 ± 1.99	nd	4.32 ± 0.09	2.67 ± 0.07	0.05 ± 0.00	0.00 ± 0.05	0.64 ± 0.03	117.83
	Ennis	123.89 ± 2.66	nd	5.46 ± 0.14	5.85 ± 0.15	0.12 ± 0.00	3.20 ± 0.12	1.08 ± 0.02	139.59
	Fertille Coutard	226.83 ± 1.85	1.22 ± 0.03	8.01 ± 0.15	9.75 ± 0.18	0.28 ± 0.01	0.33 ± 0.01	1.03 ± 0.03	247.45
	Merveille Bollwiller	105.87 ± 0.21	nd	4.07 ± 0.07	2.60 ± 0.02	0.05 ± 0.00	nd	0.61 ± 0.01	113.21
	Morell	221.22 ± 3.19	1.10 ± 0.04	9.12 ± 0.17	13.39 ± 0.76	0.40 ± 0.00	0.34 ± 0.00	2.37 ± 0.15	247.93
	Pauetet	201.04 ± 3.26	nd	7.40 ± 0.15	7.60 ± 0.17	0.09 ± 0.00	0.25 ± 0.01	1.14 ± 0.03	217.52
	Tonda Giffonni	219.94 ± 0.59	nd	5.44 ± 0.01	4.74 ± 0.04	0.12 ± 0.00	0.24 ± 0.01	0.43 ± 0.01	230.92

hazelnuts Vitamin E contents. Differences in the vitamin E profiles among cultivars also seem to exist.

In conclusion, the values here reported represent a contribution for the chemical caracterization of this kind of nut. The same cultivars are being studied along different year crops, in order to confirm if differences exist among years and geographical locations.

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