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Tocopherol Concentration in Almond Oil: Genetic Variation and Environmental Effects under Warm Conditions

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S *Supporting Information*

ABSTRACT: The concentration of the different tocopherol homologues in almond kernel oil was determined in 17 almond cultivars grown in two different experimental orchards, in Spain and Morocco. The three main homologues showed a large variability, ranging from 210.9 to 553.4 mg/kg of oil for α-tocopherol, from 4.64 to 14.92 mg/kg for γ-tocopherol, and from 0.2 to 1.02 mg/kg for δ-tocopherol. The year effect was significant, independent of the experimental site, for all homologues and total tocopherol, the values of α-tocopherol, γ-tocopherol, and total tocopherol being higher in 2009 than in 2008, whereas the value of δ-tocopherol was higher in 2008. The location effect was also significant, the values of γ- and δ-tocopherol being higher in Spain than in Morocco, whereas for α-tocopherol the location effect was dependent on the genotype. These effects could not be explained by the temperature differences between sites, but probably other undetermined environmental factors might explain the effect of the location, such as rainfall and irrigation supplementation during fruit growing and ripening.

KEYWORDS: almond, climate, *Prunus amygdalus* Batsch, tocopherol content, tocopherol homologues

INTRODUCTION

Most vegetable oils, especially oils with high levels of unsaturated fatty acids, contain tocopherols in differing amounts. Tocopherols are natural monophenols occurring in plants as a family of four different homologues depending on the position and number of methyl groups and are identified as α-, β-, γ-, and δ-tocopherol. These components are believed to be involved in a diversity of physiological, biological, and biochemical functions, mainly due to their action as antioxidants[1] but also by acting as membrane stabilizers.[2] Their main biochemical function is believed to be the protection of polyunsaturated fatty acids against peroxidation.[3] Vitamin E (α-tocopherol), the antioxidant polyphenols, and dietary fiber from almonds help to prevent heart disease and cancer.[3,4] The comparison of an almond diet with others such as cellulose and wheat bran control diets on the evolution of colon cancer showed that while the number of polyps remained unchanged, almond diets activated five antiproliferative genes as compared to just two in the control diets.[4] Thus, tocopherol content in seed oils is considered as a value-added compound.[5] Major dietary sources of vitamin E are vegetable oils, nuts, cereals, green vegetables, and fruits. In Mediterranean countries the predominant homologue consumed is probably α-tocopherol, because olive oil is one of the principal components of the typical Mediterranean diet.[6] Recent studies also point out that almond (*Prunus amygdalus* Batsch) is consumed on a large scale in Mediterranean countries[7] and could be considered as an important complementary source of α-tocopherol in this region.

Information on tocopherol concentration is important to determine the end use of the kernels and for predicting their storage life.[8,9] Almond kernels, with a higher tocopherol concentration than other nuts, had longer storage ability.[10] Consequently, kernel quality may be increased by higher levels of α-tocopherol, due to its lipid-stabilizing function and nutritive value as vitamin E, taking into account present consumer trends for foods without synthetic additives.[11] Almond oil contains a high concentration of the α-tocopherol homologue,[12,13] 10 times higher than the levels of γ- and δ-tocopherol, which are present in similar amounts.[12] The concentrations of these three homologues are highly variable between genotypes, showing that they depend on each genotype and may be considered a cultivar trait, suggesting that selection of almonds kernels for high tocopherol content is feasible.[12] In addition, these components have been shown to be under genotypic control.[14] Despite this genetic control, a clear year effect on the amount of tocopherol in almond oil has been proven, mainly a temperature effect, because hot summers induce higher concentrations of tocopherol not only in almond but also in other species.[12]

Although a location effect on the tocopherol concentration in almond oil has been reported, no statistical analysis was applied, nor was the reason for the effect elucidated.[13] Consequently, the main aim of the present study was to determine the location and the interaction genotype × location effect on the tocopherol content in almond oil for a set of commercial cultivars.

MATERIALS AND METHODS

Plant Material and Experimental Area. Seventeen almond cultivars grown in the collections at two different experimental stations

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Table 1. Tocopherol Concentration for Each Cultivar and Site over Two Consecutive Years

		α-tocopherol (mg/kg oil)		δ-tocopherol (mg/kg oil)		γ-tocopherol (mg/kg oil)		total tocopherol (mg/kg oil)	
cultivar	site	2008	2009	2008	2009	2008	2009	2008	2009
Atocha	Spain	415.4[a] ± 7.6	399.3 ± 14.6	0.55 ± 0.06	0.61 ± 0.13	9.49 ± 0.69	17.38 ± 0.62	425.4[a] ± 8.4	417.2[a] ± 7.6
	Morocco	355.5 ± 7.1	368.2 ± 2.2	0.41 ± 0.07	0.27[a] ± 0.05	6.4[a] ± 0.47	8.6[a] ± 0.02	362.3 ± 13.9	377.1 ± 5.2
Castilla	Spain	191.5 ± 7.8	294.6 ± 39.8	0.72 ± 0.09	0.11 ± 0.03	16.9 ± 0.59	4.68 ± 0.26	196.9 ± 8.4	311.6[a] ± 8.4
	Morocco	222.2[a] ± 7.3	135.7[a] ± 18.6	0.10[a] ± 0.06	0.11 ± 0.07	2.4[a] ± 0.7	2.77 ± 0.73	224.7[a] ± 40.1	138.5 ± 26.8
Colorada	Spain	324.1 ± 7.2	390.4 ± 35.4	0.34 ± 0.06	0.21 ± 0.15	4.17 ± 0.23	7.42 ± 0.55	328.6 ± 9.7	398.1 ± 7.8
	Morocco	328.4 ± 7.7	431[a] ± 9.6	0.46 ± 0.01	0.14 ± 0.01	5.26 ± 0.07	5.45 ± 0.01	334.2 ± 36.1	436.6[a] ± 9.2
Desmayo Largueta	Spain	535.9 ± 8.4	514.6 ± 34.6	2.18 ± 0.25	0.59 ± 0.08	18.71 ± 0.99	13.94 ± 0.28	556.8[a] ± 9.6	529.1 ± 7.8
	Morocco	449.4[a] ± 7.3	536.1 ± 4.7	0.51[a] ± 0.02	0.83 ± 0.33	7.34[a] ± 0.02	12.49 ± 0.52	457.2 ± 34.9	549.4 ± 10.1
Desmayo Rojo	Spain	450.4 ± 14.6	452.4 ± 9.6	0.43 ± 0.04	0.55 ± 0.09	6.35 ± 0.49	13.64 ± 0.29	457.1[a] ± 15.2	466.5[a] ± 8.6
	Morocco	385.9[a] ± 7.6	354.3[a] ± 12.9	0.23[a] ± 0.01	0.11[a] ± 0.01	5.16 ± 0.19	4.97[a] ± 1.18	391.3 ± 9.3	359.4 ± 10.2
Del Cid	Spain	398.7 ± 15.1	368.7 ± 37.8	0.28 ± 0.02	0.22 ± 0.17	5.41 ± 0.57	13.46 ± 1.2	404.3[a] ± 15.6	382.4 ± 7.3
	Morocco	346.2[a] ± 8.4	340.7 ± 5.3	0.19 ± 0.07	0.55[a] ± 0.12	6.39 ± 0.21	8.81[b] ± 0.33	352.7 ± 39.1	350.1 ± 15.1
Ferraduel	Spain	375.2[a] ± 14.5	361.3 ± 7.1	0.26 ± 0.04	0.12 ± 0.05	5.42 ± 0.58	5.5 ± 0.08	380.9 ± 15.4	366.9 ± 8.3
	Morocco	395.4 ± 8.2	381.8 ± 3.2	0.42[a] ± 0.02	0.17 ± 0.07	4.26 ± 0.08	3.51 ± 0.06	400.1[a] ± 7.1	385.4 ± 6.4
Ferragnès	Spain	371.7 ± 8.0	377 ± 7.2	1.55 ± 0.21	0.21 ± 0.05	3.45 ± 0.62	7.97 ± 1.99	376.6 ± 8.8	385.2 ± 8.6
	Morocco	296[a] ± 8.5	454.4[a] ± 13.7	0.34[a] ± 0.05	0.12 ± 0.03	4.49 ± 0.04	7.45 ± 0.63	300.9 ± 5.3	461.9[a] ± 13.5
F. Brézenaud	Spain	470.5 ± 14.9	380.7 ± 6.7	1.12 ± 0.17	0.84 ± 0.26	22.24 ± 0.33	20.88 ± 0.96	493.9[a] ± 15.4	402.4 ± 7.2
	Morocco	493 ± 7.1	426.4 ± 3.3	0.54[a] ± 0.05	0.24[a] ± 0.07	8.1[a] ± 0.14	7.07[a] ± 0.14	501.6 ± 5.9	433.7 ± 4.5
Khoukhi	Spain	285.2 ± 7.3	405.9 ± 2.1	0.62 ± 0.02	0.68 ± 0.02	4.2 ± 0.21	13.02 ± 0.61	289.9[a] ± 7.5	419.5 ± 6.9
	Morocco	317.3 ± 7.4	451.1 ± 46.2	0.69 ± 0.13	0.28[a] ± 0.01	5.67 ± 0.30	8.99[a] ± 0.33	323.6 ± 11.5	460.4 ± 44.6
Lauranne	Spain	389.2 ± 8.8	446.5 ± 5.9	0.23 ± 0.02	0.60 ± 0.10	5.32 ± 0.45	13.9 ± 0.77	394.8 ± 9.3	461.1 ± 8.3
	Morocco	339.4 ± 7.7	405.9 ± 4.7	0.31 ± 0.01	0.18[a] ± 0.00	8.47[a] ± 0.66	6.44[a] ± 0.12	348.2 ± 6.6	412.5 ± 11.2
LeGrand	Spain	395.7 ± 8.0	346.9 ± 5	0.44 ± 0.01	0.42 ± 0.13	8.85 ± 0.25	10.17 ± 0.39	404.9 ± 8.3	357.5 ± 7.4
	Morocco	455.1[a] ± 7.2	546.6[a] ± 17.3	0.48 ± 0.02	0.13[a] ± 0.04	13.06[a] ± 0.21	8.82 ± 0.39	468.6[a] ± 4.4	555.5[a] ± 12.3
Marcona	Spain	329.4 ± 9.0	364.5 ± 33.2	0.36 ± 0.03	0.22 ± 0.01	4.34 ± 0.05	9.4 ± 0.58	334.1 ± 9.2	374.1 ± 8.6
	Morocco	393.9[a] ± 8.3	340.9 ± 7.3	0.54 ± 0.06	0.11 ± 0.02	7.20 ± 0.27	4.39[a] ± 0.06	401.6 ± 13.4	345.4 ± 7.8
Picantilli	Spain	406.1 ± 8.7	367.5 ± 2.8	1.02 ± 0.02	0.63 ± 0.09	14.15[a] ± 0.25	28.3 ± 1.09	421.3 ± 8.9	396.4 ± 7.2
	Morocco	434.1 ± 7.2	445[a] ± 2.7	0.44[a] ± 0.06	0.11[a] ± 0.01	8.87[a] ± 0.13	6.46[a] ± 0.27	443.4 ± 3.9	451.6[a] ± 2.9
Retsou	Spain	456.5 ± 7.8	550.8 ± 18.7	0.54 ± 0.05	0.71 ± 0.18	9.03 ± 0.26	25.7 ± 0.12	466.1 ± 8.4	577.2 ± 18.7
	Morocco	617.2[a] ± 24.3	589.5 ± 6.2	0.40 ± 0.02	0.25[a] ± 0.09	13.65[a] ± 0.91	11.3[a] ± 0.62	631.3[a] ± 18.7	601.1[a] ± 10.9
Vivot	Spain	227.1 ± 7.2	450.5 ± 37.3	0.32 ± 0.03	0.17 ± 0.10	4.07 ± 0.09	6.39 ± 0.47	231.4 ± 8.6	457.1 ± 7.2
	Morocco	305.1[a] ± 7.3	430.7 ± 42.7	0.11[a] ± 0.01	0.23 ± 0.18	5.39 ± 0.03	6.15 ± 0.26	310.6[a] ± 21.5	437.1 ± 37.9
Yaltinskij	Spain	328.5 ± 7.7	456.7 ± 20.2	0.26 ± 0.01	0.21 ± 0.15	11.35 ± 0.48	11.62 ± 0.89	340.1 ± 8.2	468.5 ± 7.9
	Morocco	453.5[a] ± 7.8	460.5 ± 32.9	0.31 ± 0.01	0.13 ± 0.01	6.24[a] ± 0.05	4.92[a] ± 0.13	460.1[a] ± 21.3	465.5 ± 33.1

[a] Significant difference at $P < 0.01$ between the localities during each year, of each component for every genotype.

Table 2. Mean Values of the Tocopherol Homologues for Each Year and Location

tocopherol homologue	site	year 2008	year 2009	mean
α-tocopherol (mg/kg oil)	Spain	373.5 ± 84.57	407.5 ± 61.99	390.5 ± 77.11
	Morocco	387.5[a] ± 89.06	417.5[a] ± 98.47	402.5[a] ± 98.32
δ-tocopherol (mg/kg oil)	Spain	0.66[a] ± 0.52	0.41[a] ± 0.24	0.54[a] ± 0.42
	Morocco	0.38 ± 0.16	0.23 ± 0.19	0.3 ± 0.19
γ-tocopherol (mg/kg oil)	Spain	9.02[a] ± 5.61	13.14[a] ± 6.54	11.08[a] ± 6.44
	Morocco	6.9 ± 2.82	6.97 ± 2.56	6.9 ± 2.70
total tocopherol (mg/kg oil)	Spain	382.5 ± 91.1	421.8 ± 65.5	402.2 ± 80.1
	Morocco	394.8 ± 94.1	424.7 ± 103.4	409.8 ± 100.3

[a] Significant difference at $P \leq 0.01$ at each location and year.

were studied. The cultivars were 'Atocha', 'Castilla', 'Colorada', 'Desmayo Largueta', 'Desmayo Rojo', 'Del Cid', 'Vivot', and 'Marcona', originally from Spain; 'Ferragnès', 'Ferraduel', 'Lauranne', and 'Fournat de Brézenaud' from France; 'Retsou' from Greece; 'Picantilli' from Italy; 'Yaltinskij' from Ukraine; 'Khoukhi' from Tunisia; and 'Le Grand' from the United States. One experimental station was the CITA de Aragón in Zaragoza, in northeastern Spain (41° 38′ N, 0° 53′ W; 220 m above sea level), and the other was the INRA field of Meknès, in central Morocco (33° 55′ N, 5° 13′ W; 499 m above sea level). In both collections, the common rootstock was the peach × almond hybrid GF 677, and each cultivar was represented by three adjacent trees trained in the open vase manner to have a well-lighted canopy. Flood irrigation every 2 weeks was applied in Spain, but not in Morocco.

Nuts were harvested at maturity, when fruit mesocarp was fully dried and split along the fruit suture and peduncle abscission was complete. In each location, during the two years two replicates of 20 fruits were randomly collected around the canopy from a tree after open pollination to study the stability and plasticity of the studied traits for each cultivar.

Oil and Tocopherol Determination. After blanching, the kernels were ground in an electrical grinder. Oil was extracted from 4−5 g of ground almond as already described.[15] The oil content was expressed as the difference in weight of the dried kernel sample before and after extraction.

Tocopherol concentrations were determined in samples of 0.3 g of almond oil as already described.[12] The chromatographic conditions allowed the simultaneous determination of all tocopherol homologues by identifying the different peaks with comparison of retention times with standards and confirmed by their characteristic spectra using a Kontron 440 photodiode array detector (PDA) (Kontron, Eching, Germany), which also confirmed their purity. To quantify all of the isomers, calibration curves were drawn. Standard linearity was verified in each case by analysis of six standards in triplicate, each containing 20−200 mg/kg for α-tocopherol, 0.1−8 mg/kg for γ-tocopherol, and 0.05−5 mg/kg for δ-tocopherol. Tocopherol compositions were the mean values of three replicates from each sample and were expressed as mg/kg oil.

Statistical Analysis. All statistical analyses were performed using SAS programs (SAS Institute, Cary, NC). The analysis of variance was done using the PROC GLM procedure (see the Supporting Information). The additive linear model for the statistical analysis was

$$P_{ijk} = \mu + g_i + y_j + (g \times y)_{ij} + e_k + (g \times e)_{jk} + (y \times e)_{jk} + \varepsilon_{ijk}$$

where P_{ijk} is the phenotypic value of the ith genotype in the jth year and k site, μ is overall mean, g_i is the genotype effect, y^j is the year effect, e^k is the site effect, $(g \times y)_{ij}$ is the genotype and year interaction effect, $(g \times e)^{ik}$ is the genotype and site interaction effect, $(y \times e)_{jk}$ is the year and site interaction effect, and ε_{ijk} is the residual effect. The mean separation was done with the LSD test at $P \leq 0.05$ or $P \leq 0.01$.

RESULTS AND DISCUSSION

Variation between Genotypes and Years. The 17 cultivars studied showed a high genotypic variability for the three tocopherol homologues and total amount of tocopherol. The values varied from 210.9 mg/kg ('Castilla') to 553.4 ('Retsou') for α-tocopherol, from 4.64 mg/kg ('Ferraduel') to 14.92 ('Retsou') for γ-tocopherol, from 0.2 mg/kg ('Vivot') to 1.02 ('Desmayo Largueta') for δ-tocopherol, and from 217.9 mg/kg ('Castilla') to 568.8 ('Retsou') for total tocopherol (Table 1). This genotype effect was expected because it had been previously reported in other almond cultivars and selections.[12,13] Despite the genetic relationship of 'Ferragnès' and 'Ferraduel', as both come from the cross 'Cristomorto' × 'Aï', their values for tocopherol concentrations were dissimilar and followed a different pattern of variation, as did those for 'Lauranne', from the cross 'Ferragnès' × 'Tuono'. In addition, the study on the transmission of these components in almond has confirmed not only that the concentration in the different tocopherol homologues depends primarily on the genotype but also that they are under polygenic control.[14]

The year effect, however, has not been so far reported in almond. When the statistical analysis was applied to the data of both years independently at each site (data not shown), the results showed that the year effect was significant, independent of the experimental site, for all homologues and for total tocopherol (Table 2). The effect of the climatic conditions of the year, mainly temperature, had already been reported to be significant in determining the concentrations of the different tocopherol homologues in almond.[12] Similarly, the year effect on the tocopherol concentration in different oils has been widely reported[16−18] and clearly indicates that these components depend on the temperature and the plant water status during fruit ripening.

In both experimental sites, the values of α-tocopherol, γ-tocopherol, and total tocopherol obtained in 2009 were higher than in 2008, as opposed to the values of δ-tocopherol, which were higher in 2008 (Table 2). The temperatures were higher in 2009 in both locations (Table 3) and could explain the highest values obtained of α-tocopherol, γ-tocopherol, and total tocopherol in the second year. The amount of rain was higher in both

Table 3. Monthly Mean Temperatures and Total Rainfall during the 2008 and 2009 Fruit-Growing Seasons at the Two Sites

		temperature (°C)		rainfall (mm)	
month	site	2008	2009	2008	2009
May	Morocco	19.3	21.5	17	2
	Spain	16.5	18.6	162.4	13.9
June	Morocco	25.9	26.3	3.5	8
	Spain	20.2	23.2	19.8	3.9
July	Morocco	27.9	28.8	0	0.3
	Spain	23.4	24.7	16.6	26.5
August	Morocco	26.5	28.3	0	0
	Spain	23.2	24.8	5,6	16.6

locations in 2008 (Table 3), thus possibly affecting the concentrations of α-tocopherol, γ-tocopherol, and total tocopherol in the same direction as the temperature. The significance of the genotype × year and genotype × site interactions indicates that the magnitude of the variation of these traits depends on the specific characteristics of each genotype, which behave differently depending on the definite climatic and environmental conditions. Moreover, some genotypes show stable and similar year to year values of some tocopherol homologues in both experimental sites, such as 'Atocha', 'Desmayo Rojo', 'Desmayo Largueta', and 'Ferraduel' for α-tocopherol and 'Atocha', 'Ferraduel', 'Fournat de Brézenaud', and 'Yaltinskij' for γ-tocopherol (Table 1), confirming that the year to year stability of each tocopherol homologue depends on the specific characteristics of the genotype.[12]

Variation between Sites. The location effect on the tocopherol concentration in almond kernel oil has been rarely considered,[13] with only the tocopherol concentration being reported as showing differences between locations. However, because no statistical analysis was applied, this effect could not be elucidated. In our study, the analysis of variance showed a significant location effect for the content of all tocopherol homologues, but not in the amount of total tocopherol. In both years, the mean values of α-tocopherol were consistently higher in Morocco, and those of γ- and δ-tocopherol were consistently higher in Spain (Table 2). In fact, some cultivars, such as 'Atocha', 'Desmayo Rojo', 'Del Cid', and 'Lauranne' showed significantly higher values of α-tocopherol concentration at the CITA experimental site in both years, but others, such as 'Desmayo Largueta' and 'Ferragnès', only in 2008 and still others, 'Castilla', 'Marcona', and 'Vivot', only in 2009 (Table 1). For γ- and δ-tocopherols, the cultivars 'Picantilli', 'Atocha', 'Desmayo Largueta', and 'Desmayo Rojo' showed consistentlt higher values in Spain (Table 1), whereas 'Ferraduel' showed higher values in Morocco.

For total tocopherol, 'Atocha', 'Del Cid', 'Lauranne', and 'Desmayo Rojo' showed consistently higher values in Spain, whereas 'Khoukhi', 'Retsou', and 'Legrand' showed higher values in Morocco (Table 1). 'Ferraduel' and 'Fournat de Brézenaud' showed similar values in both locations. The lack of significant differences between locations for total tocopherol could be due to the metabolism of the different homologues, as the environmental conditions could favor the synthesis of one homologue or another from an original pool.

These results confirm the differential response of each genotype to the variability of the growing conditions between sites, independent from the origin of the genotype. In another nut tree, such as walnut, the contents of the different tocopherol homologues of six cultivars grown in two different areas in Portugal were found to be influenced by the geographical origin of the samples,[17] although no explanation was given. The effect of drought stress on almond kernel oil and its composition is not clear, and the results of several studies undertaken in this field are ambiguous. Although irrigation did not significantly affect almond oil content and fatty acid composition in one case,[19] other studies have shown that kernel oil composition was affected by an irrigation supplement.[20] The tocopherol concentration has been reported to be affected by drought stress in olive oil,[21] but not in *Brassica napus*,[22] by temperature in almond[12] and soybean,[23] and by a combination of both in shea butter (*Vitellaria paradoxa* C.F. Gaertn.).[16] In almond, however, no studies have been undertaken to assess the effect of irrigation and drought stress on its tocopherol concentration.

Several environmental factors differed between the two experimental sites. The main differences were temperature and irrigation, as trees were irrigated in Spain but not in Morocco. The mean temperatures during fruit growing and ripening were consistently higher in Morocco than in Spain (Table 3), thus expecting, according to previous results,[12] higher concentrations of α-tocopherol in the samples from Morocco. The low rainfall of the INRA station could also explain the high concentration of α-tocopherol in these samples (Table 3). However, several cultivars showed higher values of α-tocopherol at the CITA experimental site, explaining the significant effect of the genotype × site interaction. The values of γ- and δ-tocopherol were higher at the CITA experimental site (Table 2), showing that the effect of the growing region on each tocopherol homologue depends on the specific characteristics of the genotype. This genotype effect was also observed in 10 olive cultivars from different growing regions of Australia, where the effect of the growing region was significant only for the amount of α-tocopherol in 2 of these 10 cultivars.[24]

The year and site effects reported here show how the nutritional and antioxidant properties of the almond kernels, due to their tocopherol content,[3] can be affected by the geographic location, including the climate of each site. Thus, more attention might be directed to elucidate the influence of the environmental conditions, mainly temperature and drought, on the concentration of tocopherol in almond, because the environmental conditions may increase or decrease this concentration. The present results may be very informative for the industry and the growers, because the growing region and the cultivars chosen have a clear impact on the final quality and nutritional value of the product.

■ ASSOCIATED CONTENT

Supporting Information. Analysis of variance for tocopherol concentration of 17 almond cultivars. This material is available free of charge via the Internet at http://pubs.acs.org.

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