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GxE Effects on Tocopherol Composition of Oils from Very Old and Genetically Diverse Olive Trees

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Abstract Tocopherols are compounds with high biological activity, beneficial for human health that can be found in vegetable oils like olive oil, contributing for its resistance to oxidation. In this work, the tocopherol contents of olive oils extracted from centenarian olive trees of six cultivars (cvs. Lentisca, Madural, Rebolã, Redondal, Verdeal, and Verdeal Transmontana) were evaluated during five consecutive crop seasons (2013–2017). Three tocopherol isoforms (α-, β- and γ-tocopherols) were detected in all analyzed olive oils, and their content varied significantly with the cultivar and year of production. The highest amounts were found in cv. Lentisca (456 ± 122 mg/kg olive oil), while the lowest were observed in cv. Verdeal (179 ± 45 mg/kg olive oil). Crop year was the most influential factor, with the highest contents observed in 2013 and lowest in 2014. Principal component analysis and hierarchical clustering analysis helped differentiate olive oils according to cultivar or production year. These data suggest that tocopherol composition may serve as a chemical marker to distinguish the subject cultivar olive oils from centenarian trees either by olive cultivar or by crop year, being some cultivars identified as potential candidates for guaranteeing the production of olive oils rich in these compounds.

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Introduction

Virgin olive oil, obtained by mechanical and physical methods from fruits of the olive tree (*Olea europaea* L.), incorporates a huge chemical richness in terms of bioactive compounds (Boskou, 2015). Several studies showed that some of these microcomponents play important roles in human health (Huang and Sumpio, 2008; López-Miranda et al., 2010; Nocella et al., 2018; Visioli and Bernardini, 2011). Indeed, olive oil consumption has been associated with lower incidence rates of coronary disease and cancer, improved digestive functions, and brain aging delaying, among others (Abuznait et al., 2013; Huang and Sumpio, 2008; Nocella et al., 2018; Psaltopoulou et al., 2011; Visioli and Bernardini, 2011).

Among these bioactive molecules, vitamin E, the generic name used for a group of lipid compounds, including at least four different tocopherols and tocotrienols (α-, β-, γ- and δ-) (Ahsan et al., 2015; Bramley et al., 2000) plays a key role in human health, acting as antioxidants in the lipid phase, capturing radicals in membranes, and lipoprotein particles (Esterbauer et al., 1991; Pryor, 2000). In particular, α-tocopherol shows the highest biological activity, with numerous important clinical effects, regulating heme synthesis, inhibiting platelet aggregation, and participating in the prevention of degenerative neuropathies (Bramley et al., 2000; Pryor, 2000).

Vitamin E is typically found in vegetable sources with high lipid contents, as seeds and nuts, where they act primarily as antioxidants, protecting the integrity of the lipids and increasing their oxidative stability (Blekas et al., 1995;

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Bramley et al., 2000). In olive oil, α-tocopherol is the predominant vitamin E compound, representing 90–95% in most cultivars (Kalogeropoulos and Tsimidou, 2017), whereas β- and γ-tocopherols are only found in reduced amounts. However, the total amounts of vitamin E are variable, in a clear dependence of several factors, such as the cultivar, fruit ripening, environmental conditions, agronomic factors (e.g., irrigation, fertilization, pest, and diseases incidence), conditions used during oil extraction, storage, etc. (Aguilera et al., 2005; Beltrán et al., 2010; Cayuela and García, 2017; Kalogeropoulos and Tsimidou, 2017). Among all these factors, investigations using modern cultivars indicate that genotype has greatest influence on the contents and composition of tocopherols (García-González and Aparicio, 2010; Tura et al., 2007), but unfortunately this information remains unknown for most of the minor cultivars. Fruit ripening has some importance, with α-tocopherol and total tocopherols decreasing during ripening (Beltrán et al., 2005). The edapho-climatic conditions are also recognized factors in olive oil compositional variability, in particular the year of production, which is known to significantly affect the content and composition of tocopherols due to stress conditions to which the plant is subjected throughout its reproductive cycle (Beltrán et al., 2010).

Recently, there has been an effort to find oils naturally rich in bioactive compounds. In this pursuit, some traditional olive cultivars may represent useful sources of bioactive compounds. Many genetically diverse centenarian olive trees can be found in the north of Portugal, especially in the region of Trás-os-Montes. Thus, this work aimed to characterize the tocopherol profile and contents in olive oils extracted from olives produced by centenarian trees belonging to six minor olive cultivars along five production years (2013–2017), with the purpose to explore their diversity and potential for future breeding, taking into account the olive oil with higher bioactive richness. To the Authors' best knowledge, information about the influence of genetic diversity on the tocopherol composition of olive oil is scarce in the literature, and has not been reported for the cultivars Lentisca, Rebolã, Redondal, and Verdeal.

Materials and Methods

Sampling

In Trás-os-Montes region, near Mirandela (N 41° 29.425; W 7° 15.490), Northeast of Portugal, one olive grove with centenarian trees was selected. According to the property records, the trees are more than 200 years old. Based on the tree appearance, structure, and trunk thickness, 20 trees of

six minor cultivars were randomly selected, which included cvs. Lentisca (3 trees), Madural (3 trees), Rebolã (3 trees), Redondal (3 trees), Verdeal (2 trees), and Verdeal Transmontana (6 trees). During five consecutive crop seasons (from 2013 to 2017), approximately 3 kg of fruits were manually collected from each tree. To avoid the influence of the ripening stage on the olive oil composition, the harvest occurred when the fruits were between the ripening stage two and three that corresponds to the fruit epidermis with red spots in less than half the olive (MI 2) and the fruit epidermis red or purple in more than half the olive (MI 3). So, in every year the harvest occurred during the month of November, namely on the 25th and 26th days in 2013; on the 10th and 11th days in 2014; on the second and 3rd days in 2015; on the 7th and 8th days in 2016; and, on the 13th and 14th days in 2017. The fruits were extracted shortly after harvest, i.e., less than 24 hours after harvest, in a pilot extraction plant with an Abencor analyzer (Comercial Abengoa S.A., Seville, Spain), with three main units: a mill, a thermobeater where malaxation takes place at controlled temperature, and a centrifuge. Olives were milled, and the obtained paste was homogenized and about 700 g was transferred to the thermobeater unit (25 °C, 20 min) for malaxation using a thermostatic water bath at 25 °C. In the final 5 min of each malaxation, 100 mL of water at 27 °C was added to aid the olive oil separation. The mixture was centrifuged, decanted, and the olive oil collected. After that the oils were filtered (Whatman paper n° 4) using anhydrous sulfate in order to remove the solid particles and residual water. The olive oils were put in 125 mL dark bottles and stored in the dark at room temperature. All the assays were carried out between one and 2 months after extraction and were made in triplicate.

Vitamin E Content and Profile

Vitamin E contents were analyzed by high performance liquid chromatography (HPLC) with fluorescence detection, according to ISO 9936, 2006, with some modifications as detailed by Cruz and Casal (2013). Tocopherols standards (α, β, and γ) were purchased from Sigma (Spain), while the internal standard 2-methyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol (tocol) was from Matreya Inc. (Pleasant Gap, PA, USA). Individual standards purity was monitored by spectrophotometry (UV-1800, Shimadzu, Japan) based on their molar attenuation coefficients. N-hexane was HPLC grade from Sigma-Aldrich (Germany), 1,4-dioxane was from Sigma-Aldrich (PA, USA).

Filtered olive oil (50 mg) was mixed with internal standard solution (tocol), diluted in n-hexane and homogenized. The mixture was centrifuged for 5 min at 13,000 rpm at room temperature and the supernatant obtained analyzed by HPLC, using a normal phase silica column (Supelcosil™ LC-SI;

7.5 cm × 3 mm; 3 mm) (Supelco, USA), conditioned at 25 °C and eluted with a mobile phase of 1, 4-dioxane in n-hexane (2.5%, v/v), at a flow rate of 0.75 mL/min. Analyses were carried out using an integrated system with a data transmitter (Jasco LC—NetII/ADC, Japan), pumps (Jasco PU—4180, Japan), an auto-sampler (Jasco AS—4050, Japan), oven (ECOM Eco2000, Czech Republic), a DAD (Jasco MD—4010, Japan), and fluorescence detector (FLD, Jasco FP—4025, Japan) programmed for excitation at 290 nm and emission at 330 nm. Data were analyzed with the ChromNAV Control Center—JASCO Chromatography Data Station (Japan). The different compounds of vitamin E were identified by comparing the retention times with authentic standards, confirmed by their UV spectra and spectral purity and quantified by individual calibration curves, being expressed in mg/kg of olive oil.

Statistical Analysis

One-way analysis of variance (one-way ANOVA) was applied to evaluate the existence of statistical significant effects of the tree cultivar or the crop year in the tocopherol contents of olive oils. Moreover, if a significant statistical effect was found (P-value <0.050), the post-hoc multicomparison Tukey's test was also applied aiming to identify the levels (i.e., olive tree cultivar or crop year) of each effect that were responsible for the detected significant effect. Finally, boxplots were used to show the one-way ANOVA statistical results.

The possible influences of olive tree cultivar or crop year on the olive oil tocopherol profile was also evaluated using principal component analysis (PCA). For PCA, the tocopherols' contents were centered and scaled minimizing data variability.

Finally, hierarchical clustering heatmap was used to verify if the tocopherol profiles could be used as chemical markers to differentiate the olive oil obtained from olives picked from different cultivars. This false color image with dendograms is obtained by computing the distance (dissimilarity) between both rows (olive tree cultivar) and columns (tocopherol contents), being selected the Euclidean distance for matrix computation.

The statistical analysis was performed using the Subselect (Cadima et al., 2004; Cadima et al., 2012; Kuhn and Johnson, 2013) and MASS (Venables and Ripley, 2002) packages of the open source statistical program R (version 2.15.1), at a 5% significance level.

Results

A total of 100 olive oils, from six cultivars, were extracted and analyzed during five consecutive crop years. In all the olive oil samples evaluated, three isoforms of tocopherols, namely α-, β-, and γ-tocopherol, were identified and quantified. Their relative proportion was almost constant between years and cultivars, with α-tocopherol ranging from 94.5 to 98.2%, β-tocopherol from 0.4 to 1.9%, and γ-tocopherol from 0.9 to 4.1%, with average amounts of 96.8, 1.1, and 2.2%, respectively. Fig. 1 shows a similar pattern of variation in absolute amounts (mg/kg of olive oil). For all olive cultivars evaluated, α-tocopherol was the major isoform, varying between 118.4 mg/kg of olive oil (cv. Verdeal) and 607.1 mg/kg of olive oil (cv. Lentisca). The ranking of α-tocopherol contents by cultivar was: Lentisca ≈ Redondal > Madural ≈ Rebolã > Verdeal Transmontana ≈ Verdeal (Fig. 1). Indeed, the results pointed out that α-tocopherol contents were significantly influenced by olive cultivar (P-value ≤0.001 for one-way ANOVA and/or Tukey's multicomparison test; Fig. 1).

The γ-tocopherol was the second isoform in terms of concentration, varying its contents between 1.4 (cv. Verdeal Transmontana) and 17.3 mg/kg of olive oil (cv. Lentisca). Similarly, the γ-tocopherol contents were significantly influenced by the olive cultivar (P-value ≤0.001 for one-way ANOVA and/or Tukey's multicomparison test; Fig. 1) in the following descending order: Lentisca > Redondal > Rebolã ≈ Madural ≈ Verdeal > Verdeal Transmontana (Fig. 1). Finally, β -tocopherol was the isoform with the lowest amounts, varying from 1.0 (cv. Verdeal) to 7.5 mg/kg olive oil (cv. Lentisca) (P-value ≤0.001 for one-way ANOVA and/or Tukey's multicomparison test; Fig. 1). Considering the total content of vitamin E as the sum of the three abovementioned isoforms of tocopherols, the contents varied from 124.4 (cv. Verdeal) to 629.6 mg/kg of olive oil (cv. Lentisca), being these contents also significantly dependent of the olive cultivar (P-value ≤0.001 for one-way ANOVA and/or Tukey's multicomparison test; Fig. 1). The decreasing content order was the same as for α-tocopherol.

From the overall tocopherol analysis in these six cultivars, it can be inferred that the centenarian cvs. Lentisca and Redondal are the most promising for obtaining olive oils rich in tocopherols.

These findings also helped to evaluate the possibility of using tocopherol composition to classify olive oils from centenarian trees. PCA suggested that the cultivars could be distinguished based on tocopherol composition using three principal components (PC), which explained 100% of the data variability (82.3, 11.7 and 5.9% for PC1, PC2, and PC3, respectively) (Fig. 2).

For cvs. Lentisca and Redondal, β-tocopherol was the tocopherol that most contributed to the olive cultivars clustering. For cv. Madural, α-tocopherol was the most influential isoform, whereas in cv. Rebolã, γ-tocopherol had the greatest influence. Overall, for the olive oils studied, the tocopherol content was highly dependent on the cultivar. The hierarchical clustering heatmap shown in Fig. 3 strengthen the previous
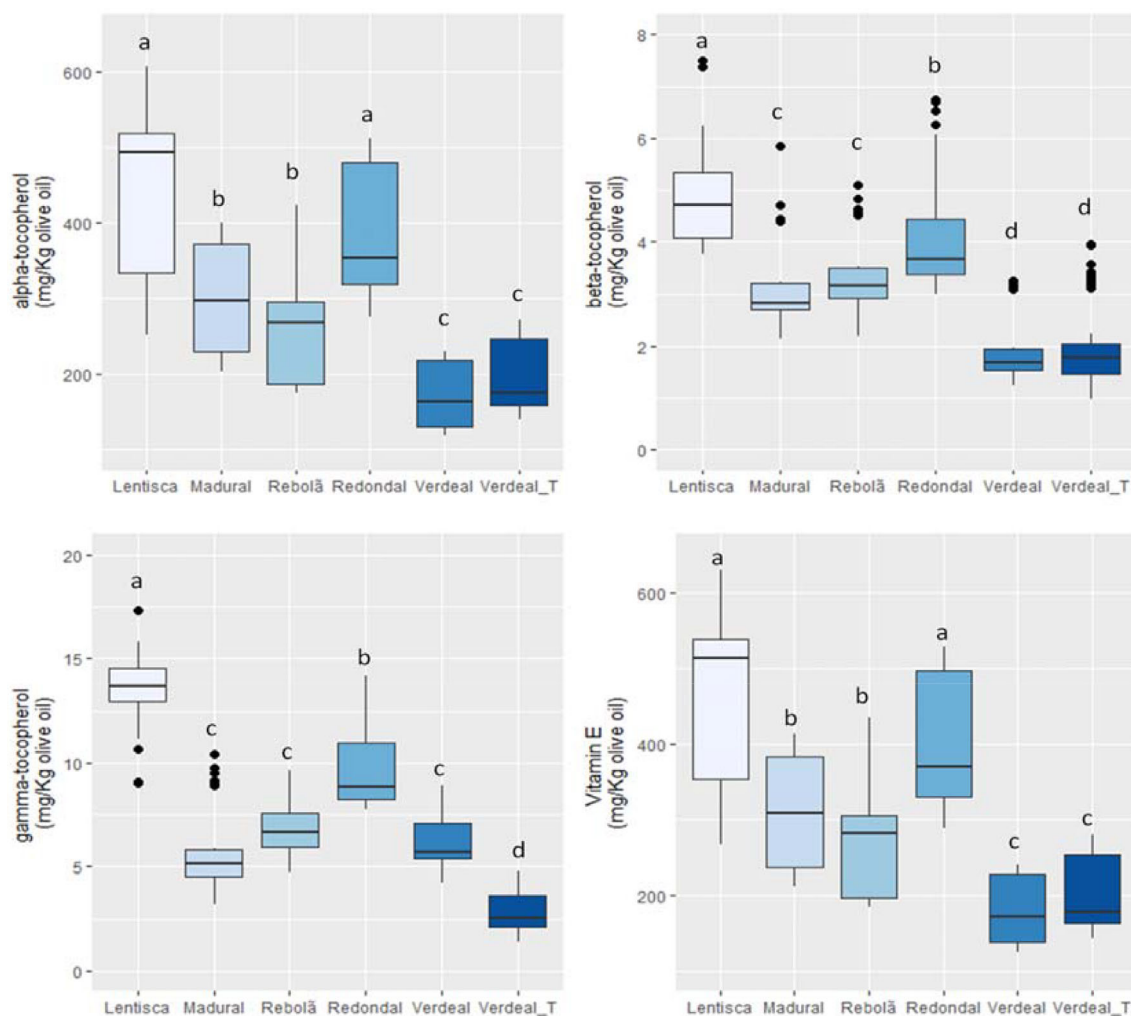
Fig. 1 Boxplots of the contents (mg/kg of olive oil) of α-, β-, and γ-tocopherols as well as of vitamin E (total tocopherol content) found in olive oils extracted from olives collected from centenarian trees from different cultivars (cvs. Lentisca, Madural, Rebolã, Redondal, Verdeal, and Verdeal Transmontana) during five consecutive crop years (2013–2017). Different lowercase letters mean significant statistical differences at a 5% significance level (one-way ANOVA followed by the Tukey's multicomparison test)

findings. Taking into account the dendograms information it is clear that the tocopherol contents may be split into two groups, one concerning the α-tocopherol and the other comprising β- and γ-tocopherols.

This information allowed grouping the olive oils into four main groups according to the olive cultivar. The first cluster mainly comprised olive oils from cvs. Lentisca, Madural, and Redondal (highest contents of α-tocopherol and medium to high contents of the other two isoforms represented by yellow and light orange colors). The second cluster included cvs. Redondal, Madural, and Rebolã (medium to high contents of the three tocopherol isoforms, corresponding mainly to light orange and orange colors). The third and fourth clusters were mainly constituted by cvs. Verdeal and Verdeal Transmontana with some olive oils of cvs. Redondal and Madural (medium to low

tocopherol contents, represented by orange and red colors). This analysis, although confirming that the olive cultivar greatly influenced the tocopherol contents of olive oils from centenarian trees, also pointed out that the crop year has also an important role, since the four identified clusters contained more than one cultivar.

Thus, considering that the climatic conditions vary from year to year, and that this work comprised five consecutive production campaigns, the effect of the crop year on the content of the different isoforms and the total of vitamin E was also evaluated, considering all the olive oils, regardless the olive cultivar. Fig. 4 shows the effect of the crop year on the content of the different tocopherol isoforms and of vitamin E. For α-tocopherol, the highest levels were recorded in 2013 and 2015, being significantly higher (P-value ≤0.0056, Tukey's multicomparison test) than those
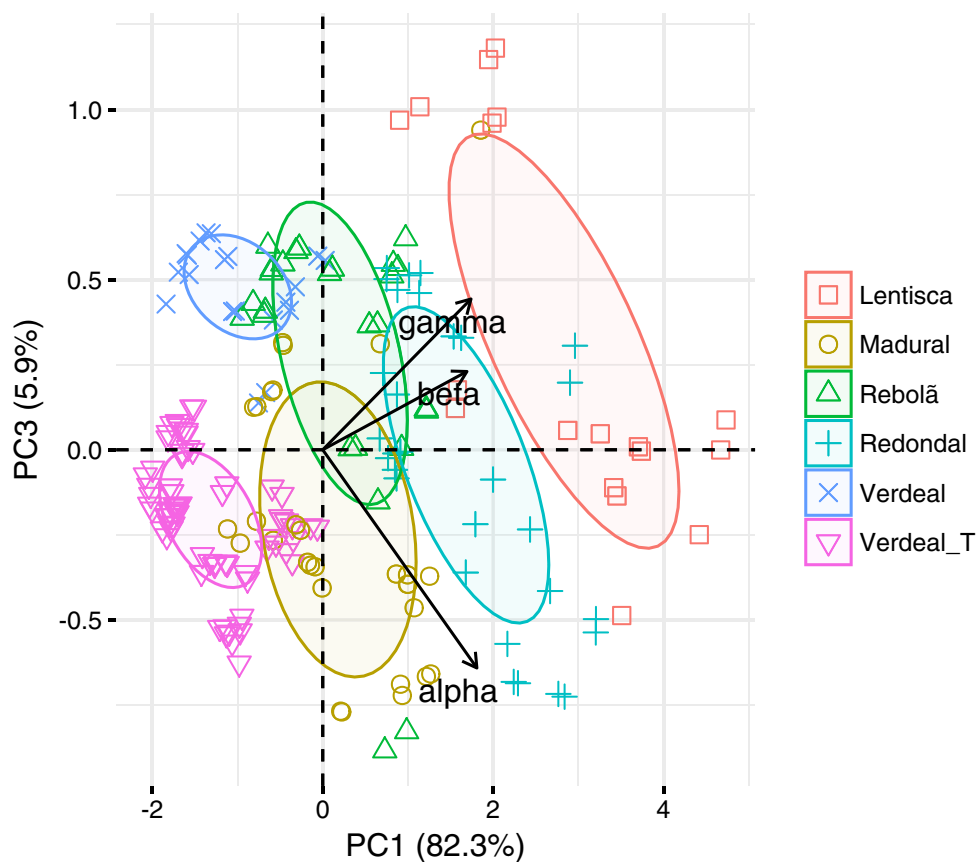
Fig. 2 Principal component analysis (PC1: 82.3%, PC2: 11.7%, and PC3: 5.9%): 3D plot showing the unsupervised pattern recognition according to olive cultivar (Lentisca, Madural, Rebolã, Redondal, Verdeal, and Verdeal Transmontana) based on the α-, β-, and γ-tocopherols contents found in olive oils obtained from olives collected from centenarian trees during five consecutive crop years (2013–2017)

determined for the other years. Thus, in decreasing order of abundance, $2013 \approx 2015 > 2017 \approx 2014 \geq 2016$ (Fig. 4). Similar to α-tocopherol, the γ-tocopherol levels were significantly higher in 2013 (8.7 mg/kg of olive oil) compared to the other crop years (P-value ≤0.0001, Tukey's multicomparison test), while in 2014 it was observed the lowest average amounts (4.1 mg/kg of olive oil). Also for β-tocopherol, statistically significant differences were observed between years (P-value ≤0.0001, Tukey's multicomparison test), with a higher significant content observed in 2015 (4.55 mg/kg of olive oil) (Fig. 4). For the total vitamin E, in decreasing order of abundance, appeared $2013 \approx 2015 > 2017 > 2016 \approx 2014$ (Fig. 4). The analysis allowed verifying that the tocopherol profiles of olive oils from centenarian trees changed according to the crop year, independently of the olive cultivar. This conclusion was further confirmed by the PCA (Fig. 5). As can be observed, olive oils may be partially grouped according to the crop year (although some overlapping of samples from different years may be observed), being the year 2013, 2015, and 2016 the most differentiated.

Discussion

In general, the total contents of tocopherol found (118.4–607.1 mg/kg), as well as those of the different isoforms, are in agreement with the literature values although, in some cases, were higher (Beltrán et al., 2010; Borges et al., 2017; Noorali et al., 2017; Tura et al., 2007). In this work, α-tocopherol represented more than 94% of the total tocopherol content in all the evaluated olive oils, which was also in accordance with the literature (Beltrán et al., 2010).

The olive oils studied were obtained from centenarian trees of six olive cultivars, namely cvs. Lentisca, Madural, Rebolã, Redondal, Verdeal, and Verdeal Transmontana, significantly differing the tocopherol amounts according to the olive cultivar (Fig. 1). The dissimilarity of the tocopherol amounts also allowed identifying patterns within the olives oils that enabled the oil differentiation and clustering by cultivar (Figs. 2 and 3).

These results further support the evidence that genetic factors (e.g., cultivar) have a great influence on the
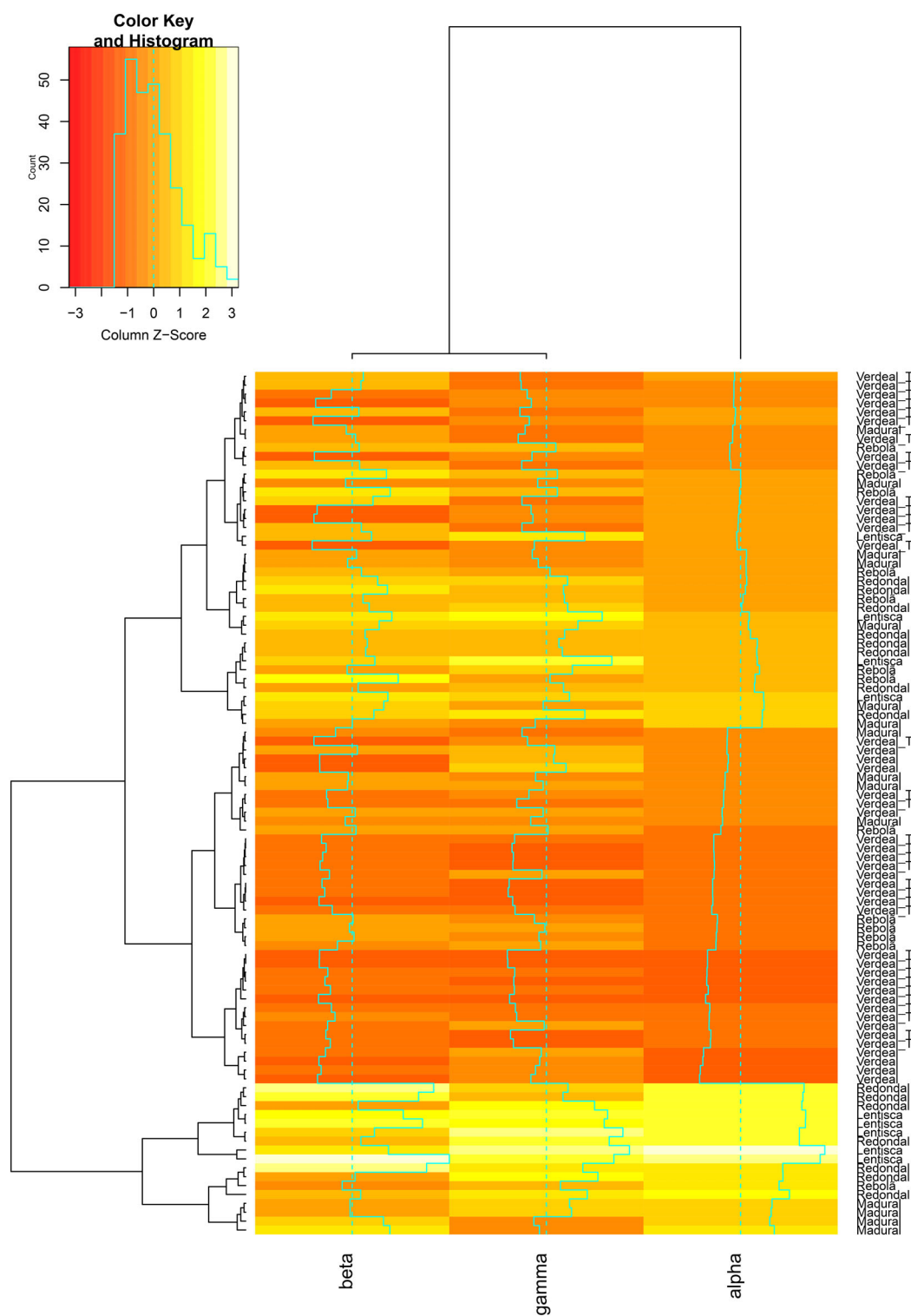
Fig. 3 Hierarchical clustering heatmap (using Euclidean distances and Ward method) and respective dendograms for variables (α-, β-, and γ-tocopherols contents) and olive oils obtained from centenarian olive trees of different cultivars (Lentisca, Madural, Rebolã, Redondal, Verdeal, and Verdeal Transmontana), during five consecutive crop seasons (2013–2017)

tocopherol concentrations found in olive oils. A study conducted with varietal olive oils from 18 Italian cultivars (Tura et al., 2007), showed that the total tocopherol

contents, varying from 39.4 to 425.9 mg/kg, were mainly dependent on the olive cultivar, having the environmental condition lower or no significance. In the same way, the
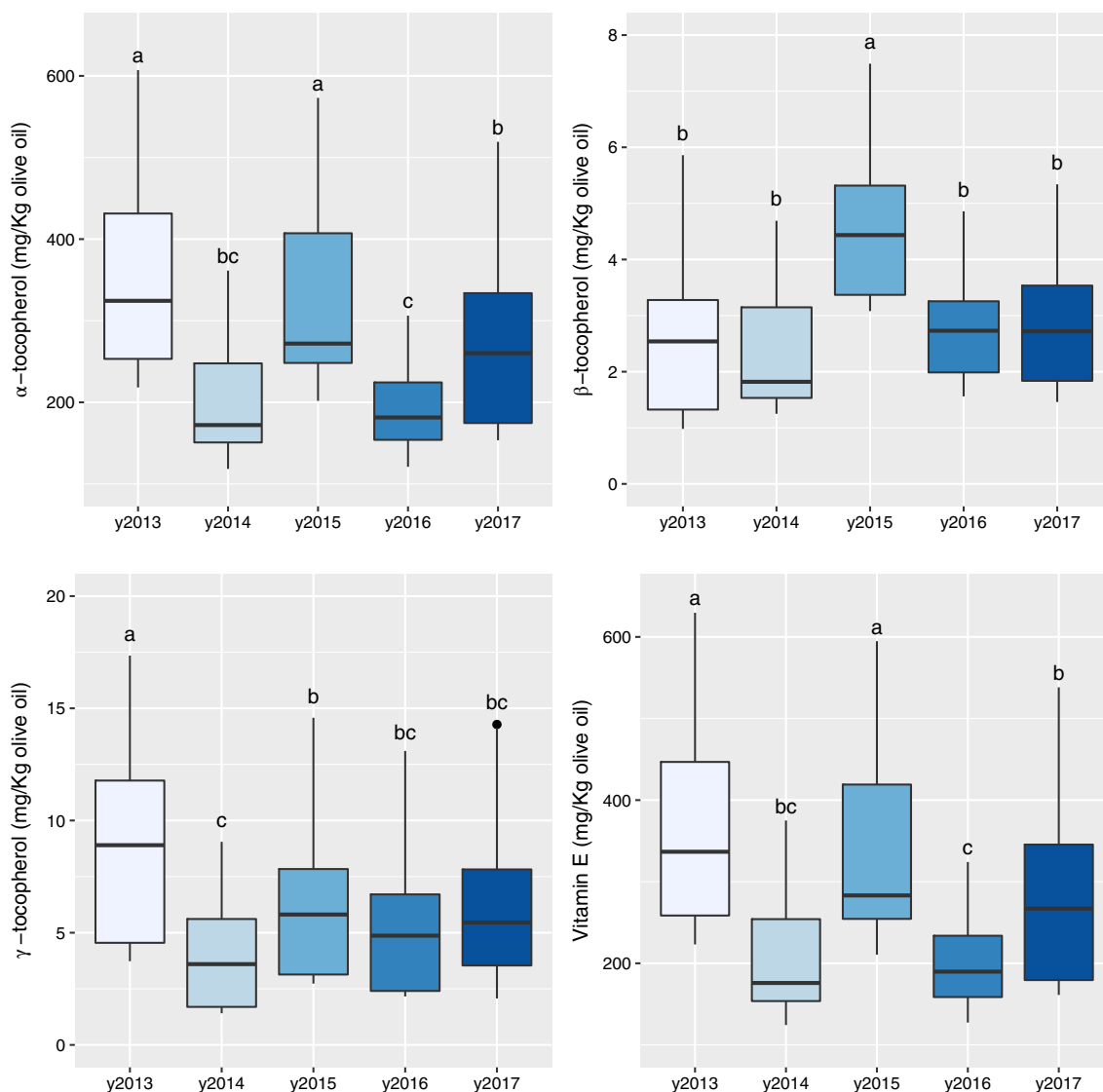
Fig. 4 Boxplots of the contents (mg/kg of olive oil) of α-, β-, and γ-tocopherols as well as of vitamin E (total tocopherol content) found in olive oils extracted from olives collected from centenarian trees from different cultivars (cvs. Lentisca, Madural, Rebolã, Redondal, Verdeal, and Verdeal Transmontana) during five consecutive crop years (2013–2017). Different lowercase letters mean significant statistical differences at a 5% significance level (one-way ANOVA followed by the Tukey's multicomparison test)

results from a study comprising 30 Spanish olive cultivars, with total tocopherol contents ranging from 84 to 463 mg/kg (Beltrán et al., 2010), also showed that the amounts of tocopherols in virgin olive oil were significantly affected by genetics or cultivar. Other works carried out in different countries such as Brazil (Borges et al., 2017), China (Xiang et al., 2017), Croatia (Špika et al., 2016), Greece (Boskou et al., 2006), Italy (Aguilera et al., 2005; Condelli et al., 2015), Spain (Beltrán et al., 2010; Franco et al., 2014), Tunisia (Laroussi-Mezghani et al., 2016), and Turkey (Arslan and Schreiner, 2012; Uluata et al., 2016), reported similar tocopherol ranges.

Concerning Portuguese olive oils, a huge amount of information is available for one of the most spread olive cultivar, cv. Cobrançosa, with reported amounts of 99–313 mg/kg of olive oils, being these values dependent from the storage conditions of the olive fruits before extraction (Pereira et al., 2002), olive fly attack (Pereira et al., 2004), decreasing along the fruit ripening (Matos et al., 2007), and increasing with the addition of olive leaves during extraction (Malheiro et al., 2013). Also, Peres et al. (2016) reported that olive oils extracted from olives of cv. Cobrançosa and other typical cultivar (cv. Galega Vulgar), picked in early stages of ripening, had different tocopherol contents, but in lower amounts compared to
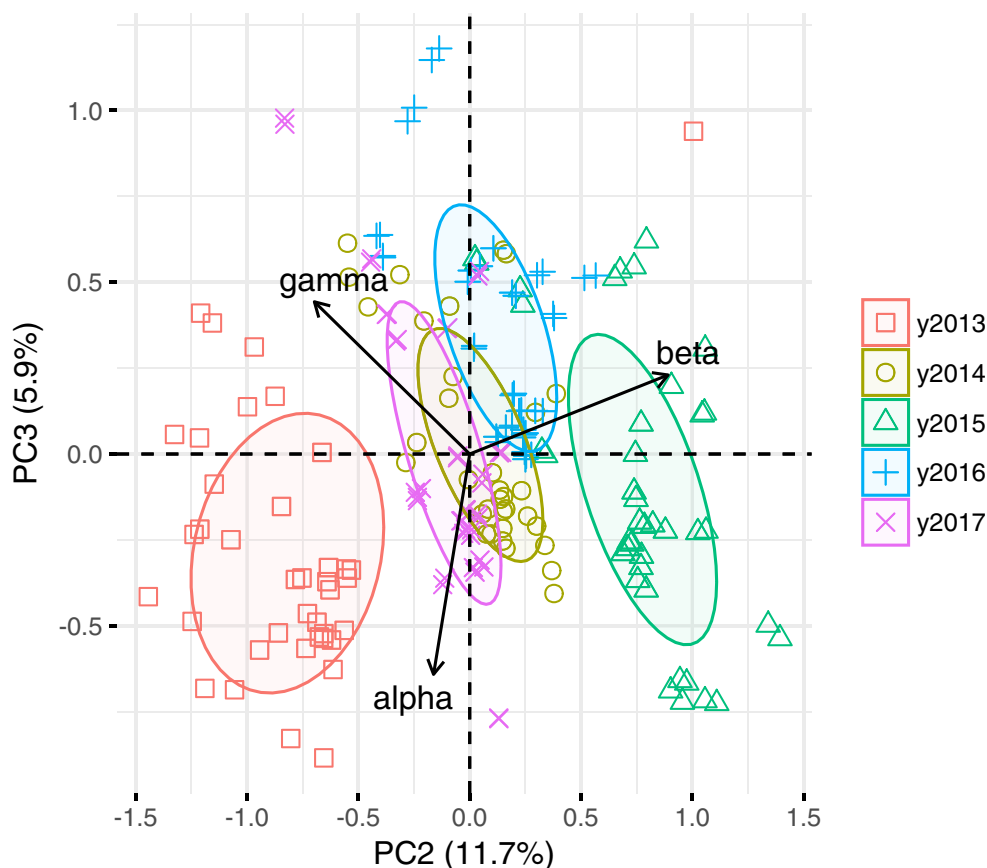
Fig. 5 Principal component analysis (PC1: 82.3%, PC2: 11.7%, and PC3: 5.9%): 3D plot showing the unsupervised pattern recognition according to crop year (2013–2017) based on the α-, β-, and γ-tocopherols contents found in olive oils obtained from olives collected from centenarian trees of different cultivars (Lentisca, Madural, Rebolã, Redondal, Verdeal, and Verdeal Transmontana)

those determined in this work for cvs. Lentisca, Madural, and Redondal. Garcia et al. (2012) studied four typical cultivars (cvs. Cordovil, Carrasquinha, Verdeal, and Negrinha de Freixo), with amounts within the expected range, from 201 mg/kg (cv. Negrinha do Freixo) to 391 mg/kg (cv. Cordovil). Additionally, these authors showed that there was a reduction trend with fruit ripening. Aware of these variations, we have eliminated this variability factor by using olives under the same ripening stage, in order to guarantee that the variations observed were strictly due to cultivar differences.

Although the effect of tree age on the oils composition was not an objective on this study, information available on this subject is scarce. There are many difficulties to develop and design a rigorous detailed study for this purpose, once it is not easy to assure the same genetic source of the plant material between different geographical origins. For a correct comparison, all trees should grow under the same edapho-climatic conditions and subjected to the same agronomic practices, being difficult to find groves under these conditions. Still, Chtourou et al. (2017) attempted to study the effect of tree age on the chemical composition of olive

oils produced from a minor Tunisian cultivar, cv. Oueslati, under different ripening stages. The authors denoted some differences between adult and young trees, with a slight increase in total tocopherols in the older ones, but the effect of fruit ripening was more pronounced than tree age. To avoid this confounder, this study only used olive oils extracted from olives at the same ripening stage, being all trees fully adult ones.

There is a general consensus that the composition of olive oils varies from year to year, as a function of the climate conditions. However, most observations are only supported by studies conducted during 2 or 3 years. In the present study, with five consecutive crops evaluated, a clear interannual variation was observed. Beltrán et al. (2010) attributed these variations to rainfall levels, with higher tocopherol amounts in oils from drier crop years, although this trend was not observed in the present study (Table 1).

The content of vitamin E in olive oils may be included as part of the health claims allowing that olive oils to be labeled as "*Source of Vitamin E*," under certain conditions (levels established in the European Regulation EU 1169/2011, 2011). According to this regulation, and considering the

Table 1 Minimum, mean, and maximum temperature (°C) in the month of August, September, and October, year 2013–2017, Mirandela, Portugal

	Month	2013	2014	2015	2016	2017
Minimum temperature (°C)	August	16.8 ± 2.2	14.3 ± 2.6	14.5 ± 2.6	15.8 ± 2.4	15.0 ± 2.8
	September	14.2 ± 2.2	14.4 ± 4.5	11.9 ± 3.0	12.9 ± 2.8	10.8 ± 3.1
	October	11.0 ± 3.8	10.9 ± 3.0	9.8 ± 3.3	9.9 ± 2.7	8.9 ± 3.7
Mean temperature (°C)	August	25.6 ± 9.3	22.7 ± 8.9	23.2 ± 9.4	25.1 ± 9.9	24.4 ± 10.0
	September	22.4 ± 8.8	21.0 ± 7.6	19.5 ± 8.3	21.4 ± 9.6	19.9 ± 9.6
	October	16.4 ± 6.4	17.1 ± 6.8	15.2 ± 6.2	16.3 ± 7.2	17.7 ± 10.0
Maximum temperature (°C)	August	34.4 ± 3.4	31.2 ± 2.6	31.9 ± 4.4	34.5 ± 3.2	33.8 ± 3.4
	September	30.5 ± 3.8	27.6 ± 4.5	27.1 ± 3.5	30.0 ± 5.5	28.9 ± 3.1
	October	21.9 ± 2.9	23.2 ± 3.0	20.6 ± 2.7	22.6 ± 3.8	26.5 ± 5.4
Rainfall (mm)	August	0.0	0.4	20.2	32.2	1.2
	September	30.0	84.8	50.4	24.2	0.2
	October	111.4	77.2	109.4	41.6	28.6
	Sum	141.4	162.4	180.0	98.0	30.0

recommendation of a daily intake of 12 mg, the ingestion of 28.5 mL of olive oil from centenarian trees of cv. Lentisca (evaluated in the present work) would be sufficient to meet vitamin E daily intake requirements. For the other cultivars, higher amounts would be required, namely 32.3 mL for cv. Redondal, 41.9 mL for cv. Madural, 48.4 mL for cv. Rebolã, 63.4 mL for cv. Verdeal Transmontana and 72.8 mL for cv. Verdeal. These values are of the same order of those reported in the literature, namely by Bayram et al. (2012), where a daily intake of 50 mL of olive oil was needed to meet the daily requirements of vitamin E. Although other vegetable oils can be regarded as richer sources of vitamin E, particularly sunflower oil, it should be retained that the tocopherols are only a part of the rich antioxidant pool of virgin olive oils. The simultaneous ingestion of other olive oil hydrophilic phenolic compounds extracted from the olive fruit, such as the hydroxytyrosol derivatives as well as mono-unsaturated fatty acids contribute also to the richness of olive oil. Still regarding tocopherols, it should also strengthen the olive oil richness in α-tocopherol, the most bioactive vitamin E isoform. Other isoforms show less clear positive associations for some health effects, namely γ-tocopherol (Cook-Mills and Mc Cary, 2010), the main isoform in several vegetables oils, as soybean oil. In this sense, the search for the highest contents in these bioactive compounds has shown that some cultivars are stronger candidates for obtaining olive oils with higher health promoting activities and higher oxidative stability.

(cvs. Lentisca, Madural, Rebolã, Redondal, Verdeal and Verdeal Transmontana) may be a good source of tocopherols, and a daily intake of 28.5–72.5 mL (depending on the cultivar) would guarantee the daily needs for vitamin E. Indeed, from a health point of view, olive oils from cv. Lentisca were the most promising ones. Overall, three tocopherol isoforms (α-, β- and γ-tocopherol) were found in all the olive oils, being α-tocopherol the main isoform. Both olive cultivar and crop year had a significant influence on the tocopherol profiles. Hopefully, the results reported in this work will attract the attention and increase the interest of olive producers to these minor and almost forgotten cultivars that are still grown in the region of Trás-os-Montes (Portugal). In fact, this study contributed to a better understanding of the genetic heritage that exists in this Portuguese region, showing the possibility of selecting and propagating some of these minor olive cultivars with the aim of obtaining differentiated olive oils rich in antioxidant compounds.

Conclusions

The work carried out showed that olive oils obtained from olives collected from centenarian trees of different cultivars

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