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Tocopherols: Chemical Structure, Bioactivity, and Variability in Croatian Virgin Olive Oils

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Additional information is available at the end of the chapter

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Abstract

Virgin olive oil (VOO) represents a rich source of natural antioxidants, with tocopherols as the most effective group of lipophilic, phenolic antioxidants. α -Tocopherol represents more than 95% of the total tocopherols in virgin olive oil, and it possesses the highest biological activity among members of the vitamin E family. Content and composition of the tocopherols of virgin olive oil depend on several agronomic factors, as well as olive processing and oil storage conditions.

In this chapter, the tocopherol homologue activity in virgin olive oil and the biological importance are discussed. Research work is reported on the tocopherol content and composition variability in virgin olive oils of the most widespread Croatian cultivar "Oblica" and Italian cultivar "Leccino." Factors studied such as year, growing area and olive fruit ripening and their influence on the tocopherol content and composition of virgin olive oils are discussed. The effect of filtration of the oil and storage conditions on tocopherols are also examined.

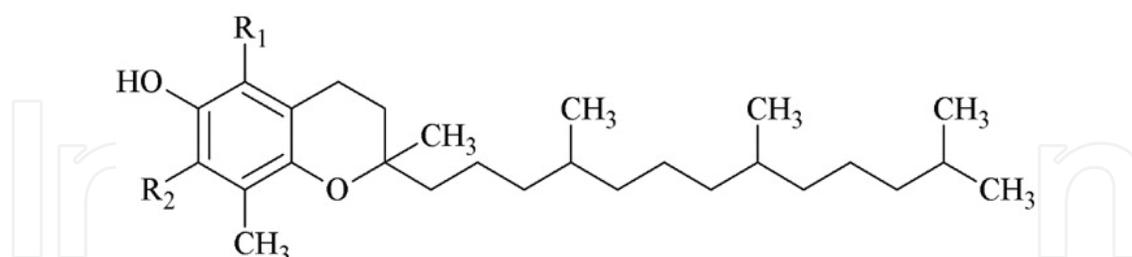
Keywords: tocopherols, structure, antioxidant activity, concentration variability, virgin olive oil

1. Introduction

1.1. Structure variation and biological activity of virgin olive oil tocopherols

Tocopherols are the natural antioxidants synthesized at various levels and in different combinations by all plant tissues. They are amphipathic molecules with the polar chromanol ring and hydrophobic saturated side chain. The general structure of tocopherols is shown in

Figure 1. The four homologues, α -, β -, γ -, and δ -tocopherol, differ in the number and position of methyl groups in the aromatic ring [1].



Tocopherol	R ₁	R ₂
α -	CH ₃	CH ₃
β -	CH ₃	H
γ -	H	CH ₃
δ -	H	H

Figure 1. Tocopherol structures. The table indicates the number and position of methyl groups on the aromatic ring.

Tocopherols act as antioxidants by scavenging peroxy radicals of polyunsaturated fatty acids or by reacting with singlet oxygen and other reactive oxygen species (ROS). ROS quenching occurs by charge transfer mechanism. Termination of polyunsaturated fatty acids oxidation chain reactions is achieved by donation of a hydrogen atom from the hydroxyl group on the chromanol ring resulting in a “tocopherol radical” formation. The tocopherol radicals are resonance stabilized within the chromanol ring and do not propagate the chain reactions or are rapidly recycled back to the corresponding tocopherol, allowing each tocopherol to participate in many peroxidation chain-breaking events. One tocopherol molecule can protect about 10^3 – 10^8 polyunsaturated fatty acids at low peroxide values [1, 2]. Tocopherol homologues possess different antioxidant activity as a result of their structural differences. The antioxidant activity of tocopherol homologues decreases in the order $\delta > \beta > \gamma > \alpha$ *in vitro*, while *in vivo*, that is vitamin E activity, decreased in order $\alpha > \beta > \gamma > \delta$ [3]. Although there is no significant difference in the absorption of tocopherols from the gastrointestinal tract, the highest vitamin E activity of α -tocopherol can be explained by its preferential retention and incorporation into lipoproteins by the hepatic α -tocopherol transfer protein, occurring in higher plasma and tissue level [1, 4].

The main role of tocopherols is the protection of lipids from peroxidation. Therefore, they are abundantly found in plant-based food, but vegetable oils are considered to be the best source of tocopherol in nutrition [3]. Their content and composition mostly depend on the type of oil [2, 5]. Tocopherol content of virgin olive oils (VOOs) varies from 97 to 785 mg/kg. Despite differences in the concentration of total tocopherols that can be attributed to agronomical,

geographical, and technological factors, α -tocopherol is the dominant homologue in virgin olive oils making more than 90% of total tocopherols. γ -Tocopherol is in virgin olive oils present in low amounts (<10%), and β -tocopherol is present only in trace amounts [6–8].

Tocopherols possess high antioxidative activity; this makes them important components in cardiovascular disease and cancer prevention [9, 10, 11]. Furthermore, tocopherols appear to act synergistically with other antioxidants [12]. This indicates that intake of tocopherol in the form of food like virgin olive oil, which is rich in other natural antioxidants such as biophenols and carotenes, might improve their efficiency.

Considering the importance of tocopherols in daily nutrition and disease prevention, it is important to know and examine the influence of certain factors on their content and composition.

2. Varieties grown in Croatia

2.1. Plant material and growing areas selected

Cultivars “Oblica” and “Leccino,” in three consecutive years (2010, 2011, and 2012), grown at two different locations in Dalmatia (Kaštela and Šestanovac), Croatia, were studied. The fruit samples were hand-harvested from the olive trees, at biweekly intervals, from the end of September till mid-November. An aliquot of 100 fruits was taken from each fruit sample to determine ripening index which is based on evaluation of the olive skin and pulp color [13]. The olive fruits were processed by centrifugal extraction using an Abencor laboratory oil mill (mc2, Ingenierias y Sistemas, Seville, Spain) within 24 h after the harvesting. Tocopherol content and composition were determined in all produced virgin olive oils using standard method (ISO 9936:2006) with normal-phase HPLC analysis. Total tocopherols were calculated as a sum of the concentration of the individual tocopherols. Results are given in milligrams of tocopherol per kg of oil.

In order to study the effect of cultivar, year, growing area location, and fruit ripening index on the tocopherol content in olive oil samples, factorial ANOVA was performed (Fisher’s F-test), followed by Tukey’s honest significant difference (HSD) test. A significance level of $p \leq 0.05$ was applied. The correlation coefficients (r) determined by Pearson correlation matrix were used to define the influence of climatic conditions during the year on the VOOs tocopherol content. The obtained data were analyzed using Statistica software version 11.0 (StatSoft, Inc., USA, 2012).

Croatia is a fringe growing area of olive trees cultivation. Selected growing area locations are from different olive growing subregions. Kaštela is located at 28 m above sea level and influenced by Mediterranean climate, while Šestanovac is located at 358 m influenced by continental climate in winter and the Mediterranean climate in summer period.

The monthly mean values of temperature and rainfalls registered for studied years at selected locations were obtained from weather stations (Meteorological and Hydrological Service of Croatia) (Figures 2 and 3).

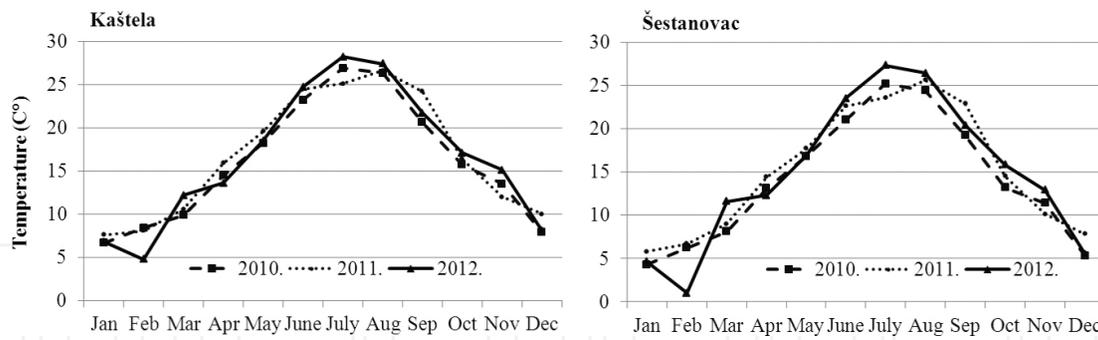


Figure 2. Microclimate temperature parameters (°C) measured for two olive growing areas in the year 2010, 2011, and 2012.

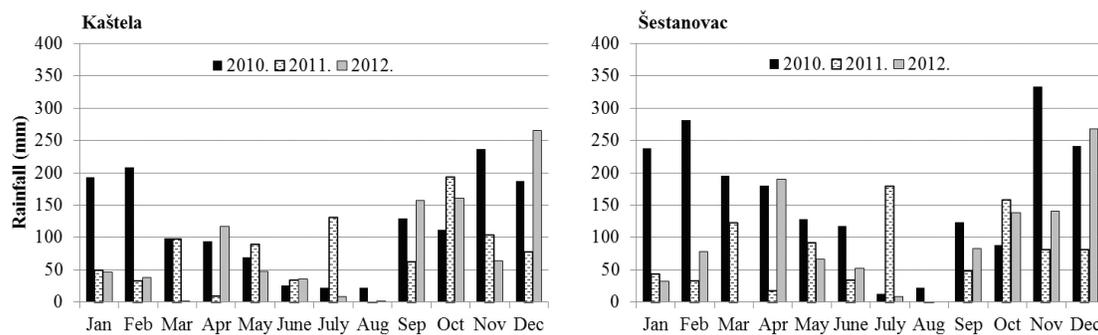


Figure 3. Monthly rainfall (mm) for two olive growing areas in the year 2010, 2011, and 2012.

Year 2010 was recorded as the highest rainfall year and with lowest average daily air temperature (Figures 1 and 2). During olives intense growth and ripening (July–November), higher mean daily temperatures were recorded in 2012, compared to overall 3 years of research, while 2011 is the year with the lowest rainfall. Kaštela could be considered as the more drought affected and warmer growing area.

2.2. The influence of the cultivar on tocopherol content and composition

The quality of virgin olive oil is influenced by several factors, but the olive cultivar stands out as the most important one [14]. The content of tocopherols in the virgin olive oils varies from 97 to 785 mg/kg [6–8], from 163 to 510 mg/kg in the Spanish oils [8], 98–370 mg/kg in the Greek oils [14], 97–403 mg/kg in oils from Turkey [6], 120–478 mg/kg in oils from Tunisia [15], and 138–298 mg/kg in the Portuguese oils [16].

Tocopherol content of studied cultivars is presented in Figures 4–6. Cultivar shows a significant impact on the content of the α -, γ -, and total tocopherols. “Oblica” VOOs have a modest total tocopherol content, ranging from 186 to 442 mg/kg. Significantly higher total tocopherol content had varietal oils from “Leccino” with average value of 510 mg/kg (337–784 mg/kg). Higher total tocopherol content in “Leccino” VOOs also was recorded for several different cultivars in the study reported by Tura et al. [17] and Koprivnjak et al. [18]. In “Oblica” and “Leccino” VOO samples δ -tocopherol was not detected.

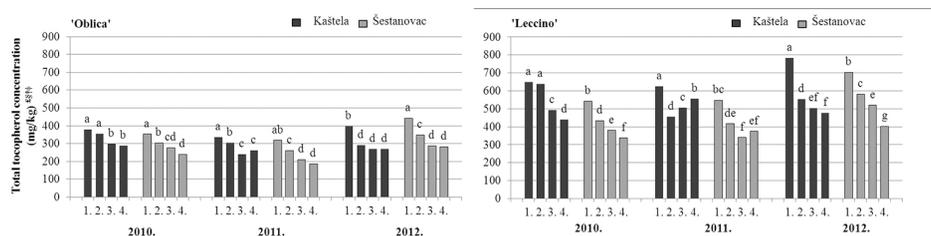


Figure 4. Total tocopherol content (mg/kg) of “Oblica” and “Leccino” virgin olive oils during ripening at two different growing locations in three successive crop years. [‡]Cultivar has a significant effect ($p < 0.05$); [§]year has a significant effect ($p < 0.05$); [†]growing location has a significant effect ($p < 0.05$); harvest period has a significant effect ($p < 0.05$). The means marked with different letters (within the same cultivation year), labeled with different letters, are significantly different (Tukey’s test, $p < 0.05$).

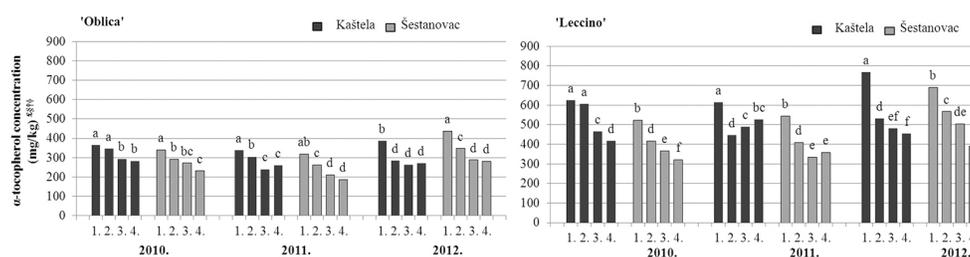


Figure 5. α -Tocopherol content (mg/kg) of “Oblica” and “Leccino” virgin olive oils during ripening at two different growing locations in three successive crop years. [‡]Cultivar has a significant effect ($p < 0.05$); [§]year has a significant effect ($p < 0.05$); [†]growing location has a significant effect ($p < 0.05$); harvest period has a significant effect ($p < 0.05$). The means marked with different letters (within the same cultivation year), labeled with different letters, are significantly different (Tukey’s test, $p < 0.05$).

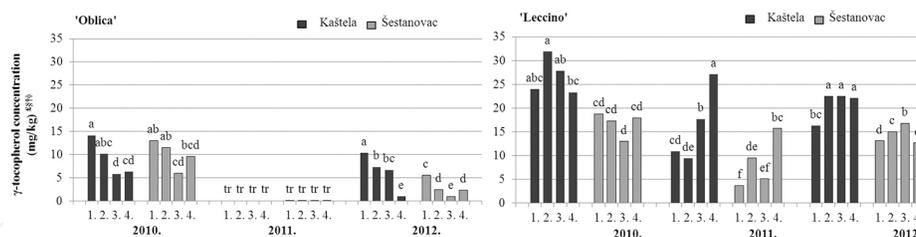


Figure 6. γ -Tocopherol content (mg/kg) of “Oblica” and “Leccino” virgin olive oils during ripening at two different growing locations in three successive crop years. [‡]Cultivar has a significant effect ($p < 0.05$); [§]year has a significant effect ($p < 0.05$); [†]growing location has a significant effect ($p < 0.05$); harvest period has a significant effect ($p < 0.05$). The means marked with different letters (within the same cultivation year), labeled with different letters, are significantly different (Tukey’s test, $p < 0.05$).

α -Tocopherol comprises more than 97% in all analyzed VOO samples (Figure 5). The γ -tocopherol content in “Leccino” oils ranged from 4 to 32 mg/kg (average 17 mg/kg), while the content in “Oblica” oils was significantly lower (Figure 6). Average concentration in “Oblica” oils was 7 mg/kg, and values ranged from traces to 14 mg/kg. The γ -tocopherol concentrations obtained in this study for both varieties are within the concentrations reported in different varietal virgin olive oils studies [16, 17, 19].

2.3. Environmental factors and tocopherols of Croatian olive oils

Production geographic area, marked by soil factors, altitude and latitude, and climatic conditions during the year, has a significant impact on the properties and chemical composition of virgin olive oils [7, 17, 20–23]. Studies of environmental factors impact on VOOs chemical composition have quite different results, due to the fact that all these factors together interact and as result varieties behave differently in different agroclimatic conditions.

Parameter	Mean temperature (°C)				
	July	August	September	October	November
α -Tocopherol conc.	0,287*	0,252*	-0,021	0,391*	0,302
γ -Tocopherol conc.	0,059	0,044	0,055	0,114	-0,068
Total tocopherol conc.	0,295*	0,244*	-0,037	0,376*	0,293
	Rainfall (mm)				
α -Tocopherol conc.	-0,190	-0,079	0,015	-0,217	0,009
γ -Tocopherol conc.	-0,067	0,252*	0,048	-0,010	-0,329
Total tocopherol conc.	-0,206*	-0,053	0,030	-0,206	-0,002

* Significant difference at $p < 0.05$.

Table 1. Correlation factors of virgin olive oil tocopherol content and a microclimate parameters (mean temperature and rainfall; **Figures 1** and **2**) in the period of olives' intensive growth and ripening.

Year and geographical production area significantly influenced the content of tocopherol in "Oblica" and "Leccino" VOOs (**Figures 4 – 6**). The correlation coefficients of rainfall and mean daily temperature during intense growth and ripening of olive fruit (July–November) with α -, γ -, and total tocopherol content were calculated and presented in **Table 1**. It is evident that the mean daily air temperatures have a significant impact on the α - and total tocopherol content. The highest average α - and total tocopherol content in "Oblica" and "Leccino" VOOs was recorded in year 2012, while in oils from year 2011, concentrations were the lowest of three studied years (α -tocopherol: "Oblica": 302 mg/kg in year 2010, 264 mg/kg in year 2011, 319 mg/kg in year 2012; "Leccino": 467 mg/kg in year 2010, 464 mg/kg in year 2011, 547 mg/kg in year 2012.) (**Figure 5**). A Spanish group of scientists [24] recorded lower tocopherol content in the year with the lowest average air temperature. For the lower α - and total tocopherol content in year 2011, a possible explanation lies in rainfall, due to the fact that tocopherols negatively correlate with rainfall in July (**Figure 2** and **Table 1**). Results are in line with a Beltrán et al. [8] publication according to which the year has a very significant impact on the α - and total tocopherol content in "Picual", "Frantoio", and "Hojiblanca" VOOs, and the highest content was recorded in the year with the lowest rainfall. In the same study, the effect of the year on the γ -tocopherol was not recorded, contrary to our results on the "Oblica" and "Leccino" VOOs (**Table 1**). The γ -tocopherol positively correlates with the rainfall in August. In the year

2010, more rainfall and, as mentioned previously, the highest γ -tocopherol content in oils of the two investigated cultivars were recorded (**Table 1** and **Figure 6**).

Higher α -, γ -, and total tocopherol content had "Oblica" and "Leccino" VOOs obtained from fruits harvested at the growing area of lower altitude (**Figures 4 – 6**). The correlation coefficient results are in accordance with the observation (**Table 1**) that oils from growing area of higher average temperatures synthesize more tocopherols. Similar results were published by Arslan et al. [6] and Aguilera et al. [22]. On the other hand, Tura et al. [17] report that the growing area had no effect on α -, β - and total tocopherol content and that only the γ -tocopherol content was influenced, with the highest content in oils of the highest altitude area.

2.4. Ripening and tocopherols of Croatian olive oils

The fruit ripening is one of the most important factors that lead to changes in the chemical composition of virgin olive oil [25]. The knowledge of varietal oil characteristics, as well as changes that take place through fruit maturation, contributes to the higher quality of VOOs. The genetic composition directly affects the ripening; thus, in each cultivar, different changes in composition of the olive fruit and its virgin olive oils are confirmed [25].

A wide range of α -, γ -, and total tocopherol content was perceived during ripening of "Oblica" and "Leccino" VOOs (**Figures 4 – 6**). Analysis of variance showed a difference in tocopherol content of VOOs derived from olives harvested at different ripening stages.

The total tocopherol content in VOOs decrease with increase in the fruit ripening index from which the oil is produced (**Figure 4**). The average total tocopherol content reduction of about 30% in VOOs from unripe to ripe olives has the same trend as the α -tocopherol; this is expected since the α -tocopherol abundance in VOOs is around 95%. The results are in line with research reported by Matos et al. [16], Bengana et al. [25] and Beltrán et al. [8]. Depending on the olive fruit pigmentation, variations in the total tocopherol content in the "Chetoui" (138–496 mg/kg) and "Chemlali" (224–350 mg/kg) were also noted [15].

In general, the α -tocopherol content decreases during ripening, although a decrease rate of the studied cultivars was not quite equal (**Figure 5**). Lower α -tocopherol content for 25% (Kaštela) and 35% (Šestanovac) in "Oblica" VOOs obtained from ripe olive fruits was recorded in comparison with initial content in oils from the unripe olive fruits. The loss of α -tocopherol is more pronounced in the "Leccino" VOOs of both locations. Decrease of the α -tocopherol through the ripening period has also been reported in other studies [8, 26–28].

The γ -tocopherol content in "Oblica" VOOs decreases as the fruit ripening index increases, although decrease rate was not the same at both studied locations (**Figure 6**). A significant decrease of 70% as average value was recorded in the oils from the Kaštela, while in oils from Šestanovac, an increase in the γ -tocopherol content in oils from ripe olive fruits was observed. This is consistent with Beltrán et al. [8] who have also reported a similar trend for "Frantoio," "Hojiblanca," and "Picual" VOOs. γ -Tocopherol in "Leccino" VOOs was not clearly associated with the increase in olive fruit ripening index (**Figure 6**), what was also reported for virgin olive oils of several different cultivars [15, 16, 19].

3. Filtration and storage: a short literature review

3.1. Filtration

Virgin olive oil after processing is a metastable mixture that can be consumed without refining [29]. From the commercial point of view, apart from major manufacturers and industrial producers who prefer filtered oils, there is an increasing interest of consumers for unfiltered oils which they consider as minimally processed [30]. Suspended substances (cellulose, hemicelluloses, pectin, proteins) can affect the quality by increasing triacylglycerols hydrolysis causing a free fatty acids increase. Therefore, filtration is the process of clarification aimed at a faster process during which the qualitative and quantitative changes in the composition of virgin olive oils may take place. In fact, there is a controversy and there are some confusing comments in relation to “cloudy” and filtered oils.

In the study of six different Spanish and Italian varietal VOOs by Fregapane et al. [31], filtration was not found to cause significant differences in the α -tocopherol content. A new filtration method based on the flow of an inert gas developed and patented by the University of Bologna and Sapio [32] had also no effect on the lipophilic phenols level [33].

3.2. Storage

The overall quality of virgin olive oil decreases over the time as a consequence of oxidative and hydrolytic degradations which also cause the partial loss of other minor constituents having health-promoting effects. Consequently, VOOs is generally consumed within one year from its production [34–36]. As it was mentioned formerly, the main role of tocopherols is the protection of lipids from peroxidation, and according to Aparicio and Luna [37], their contribution accounts for around 11%. Thus, monitoring of tocopherol levels during its shelf life, it is needed.

The degradation rate of the α -tocopherol content during storage under the store shelves conditions was reported by Psomiadou and Tsimidou [38]. Keepability test carried out on five VOOs in conventional storage at room temperature resulted in insignificant α -tocopherol losses in samples kept sealed [39]. On the other hand, in the same study, considerable α -tocopherol losses were observed in samples opened periodically within the period of two years storage as a result of renewal of oxygen supply. Rastrelli et al. [40] investigated storage conditions regarding availability of oxygen in the oil headspace and lighting. These authors reported the loss of tocopherols in VOO samples, stored 12 months in completely filled dark glass bottle, in range from 20 to 25%. Similarly rate of α -tocopherol losses was also reported in storage condition study [7], which is contrariwise almost total loss of α -tocopherol in “Arbequina” oils in same storage conditions (in darkness and at ambient temperature for 12 months) [41]. Under medium temperature at accelerated storage conditions (50°C, 36 weeks), the α -tocopherol loss was much more rapidly in oils stored in open bottles than in close bottles [42]. The research of VOOs storage conditions (room temperature, +4 and -20°C) influences on tocopherol content evidenced α -tocopherol content decreasing trend with storage time [7]. In same study, after 12 months

storage, the highest loss of α -tocopherol was recorded in oils stored at room temperature, among which the oils obtained from unripe olives showed the greatest α -tocopherol stability. Storage at lower temperatures did not always delayed α -tocopherol consumption compared to room temperature.

As it can be seen, different effect of storage conditions on tocopherol content and α -tocopherol degradation rate is shown through literature affecting the amount of tocopherol in oils within the period in which positive chemical and organoleptic properties remain preserved and VOOs are desirable to consume.

4. Conclusions

Research work on the tocopherol content and composition variability in virgin olive oils of Croatian cultivar "Oblica" and Italian cultivar "Leccino" showed that the cultivar has a major influence on the tocopherol content and composition. Location also has an impact, which can be associated with a microclimate characteristic of the growing area.

The cultivation year, climate characteristics of temperature and rainfall, has significant impact on the variation of the tocopherol content. The amount of rainfall in July correlated negatively with the total tocopherol content, and the highest content was recorded in the year with the lowest rainfall. Both cultivars have gained an average higher α -, γ -, and total tocopherol content at a warmer and of low altitude location. The fruit ripening stage has a significant impact on the composition of the tocopherols and primarily α -tocopherol. The clear decrease in α -tocopherol content as ripening progresses was observed.

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