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Murat eker [a]; Muhammet Kemal Gül [b]; Meryem pek [a]; Cell Toplu [c]; Nilüfer Kalec [a]
[a] Department of Horticulture, [b] Department of Field Crops, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, Turkey [c] Department of Horticulture, Faculty of Agriculture, Mustafa Kemal University, Antakya-Hatay, Turkey

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Screening and comparing tocopherols in the rapeseed (*Brassica napus* L.) and olive (*Olea europaea* L.) varieties using high-performance liquid chromatography

MURAT ŞEKER[1], MUHAMMET KEMAL GÜL[2], MERYEM İPEK[1], CELİL TOPLU[3], & NILÜFER KALECİ[1]

[1]*Department of Horticulture and,* [2]*Department of Field Crops, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, Turkey, and* [3]*Department of Horticulture, Faculty of Agriculture, Mustafa Kemal University, Antakya-Hatay, Turkey*

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Abstract
Rapeseed and virgin olive oils are a good source of tocopherols. Tocopherols are the most important compounds having antioxidant activity in both crops. Little is known about the tocopherol contents of rapeseed and olive oil grown in Turkey. The aims of this research were to investigate some new rapeseed varieties and olive genotypes grown in northwest Turkey and to compare the tocopherol fractions and contents of both crops. For rapeseed, the data were collected in two growing seasons (2004–2005, 2005–2006) from a field experiment with 19 new rapeseed varieties. For olives, virgin olive oils produced from 21 different varieties were examined in the 2004–2005 and 2005–2006 growing seasons.

The separation and identification of tocopherols and the analysis of their contents were successfully achieved using the high-performance liquid chromatographic method. According to the obtained results, γ-tocopherol (44.200–118.900 mg/kg) was the major fraction of total tocopherol, followed by α-tocopherol (19.300–68.500 mg/kg) and δ-tocopherol (0.00–2.600 mg/kg[1]) for rapeseeds. Regarding olive varieties, the α-tocopherol content changed between 52.000 and 194.750 mg/kg, followed by γ-tocopherol ranging from 0.00 to 39.750 mg/kg. The total tocopherol content ranged between 83.900 and 173.800 mg/kg for rapeseed and between 52.100 and 213.075 mg/kg for olives. This study revealed that an important variability exists for tocopherol content and composition in rapeseed and olive varieties.

Keywords: *Quality, tocopherol, α-tocopherol, γ-tocopherol, rapeseed, virgin olive oil*

Introduction

Tocopherols are natural antioxidants synthesized only by higher plants. They exist in four forms—α-tocopherol, β-tocopherol , γ-tocopherol and δ-tocopherol—differing in both molecular structure and biological effectiveness. Tocopherols are important dietary nutrients and have beneficial effects in the prevention of cardiovascular diseases. The most abundant tocopherols in oil crops are α-tocopherol and γ-tocopherol. While α-tocopherol is mainly important as vitamin E, γ-tocopherol is the superior antioxidant in oil products. Due to the chemical characteristics of the

Correspondence: Murat Şeker, Department of Horticulture, Faculty of Agriculture, Çanakkale Onsekiz Mart University, 17020 Çanakkale, Turkey. Fax: 90 286 218 05 45. E-mail: mseker@comu.edu.tr

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tocopherols, the ratio of α-tocopherol and γ-tocopherol is important as well as the total amount of tocopherols in oilseed crops. As a consequence, high levels of tocopherols in oilseeds are favourable for both health-promoting purposes and vegetable oil product quality (Kamal-Eldin and Appelqvist 1996; Marwede et al. 2005).

Rapeseed is one of the most important oil suppliers and an important tocopherol source. The main tocopherol fractions in rapeseed oil are γ-tocopherol and α-tocopherol. The proportion of δ-tocopherol is not important in the seed oil according to the study reported by Goffman and Becker (2001). In different research it is reported that the total tocopherol content varied between 80 and 1,000 mg/kg in seed oil (Marquard 1976; Abidi et al. 1999; Dolde et al. 1999; Marwede et al. 2004).

Tocopherols are very sensitive organic compounds and their content is influenced by environmental factors. Genotype × environment interactions and variation results have been presented in different studies (Marquard 1976; Abidi et al. 1999; Dolde et al. 1999). Marwede et al. (2005) mapped some Quantitative Trait Locus for tocopherol content and reported from environmental effects.

Rapeseed is the major vegetable oil supplier in Europe. Thus, the scientific interest and economic importance resulted in a high oil yield (40% and 47% of oil) and the improvement of detailed quality research in the past few decades. Besides tocopherols, rapeseed oil is predominantly composed of fatty acids, such as oleic acid, linoleic and linolenic acid, glycolipids, phytosterols and carotenoids (Gül and Şeker 2006).

Virgin olive oil (VOO) can be extracted directly from olive fruits and VOO can be consumed without further treatments. Its chemical composition consists of major and minor components. The main components, which include glycerols, represent more than 98% of the total oil weight. Minor components, which amount to about 2% of the total oil weight, include more than 230 chemical compounds (e.g. aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants). The main antioxidants of VOO are carotenes and phenolic compounds including lipophilic and hydrophilic phenols. While the lipophilic phenols, among which are tocopherols, can be found in other vegetable oils, some hydrophilic phenols of VOO are not generally present in other oils and fats (Servili and Montedoro 2002). There are many olive varieties that yield VOO in the northwest region of Turkey.

There is no specific study on rapeseed and olive oil regarding the tocopherol content of crops grown in the northwest region of Turkey. The olive is an economically important crop for industry and human nutrition, and rapeseed has greater importance year by year in Turkey. A determined tocopherol content could have important potential for the oil industry as well as for breeding studies.

The objective of the present study was to investigate the screening of and compare tocopherol fractions and contents in rapeseed and olive varieties grown in northwest Turkey.

Materials and methods

Plant materials and field experiments for rapeseed

Nineteen new rapeseed varieties with a standard genotype (Licrown) were used in the field experiments. The genotypes used in this experiment were kindly provided by KWS Saat AG and NPZ Saatzucht, Germany.

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The field trials were conducted during the growing season 2004–2005 at the Experimental Station of Onsekiz Mart University in Çanakkale. The experiment was designed in a completely randomized block design with three replications. Plots consisted of four rows and were 1.20 m $\times$ 5 m in size. The field was fertilized with 260 kg/ha nitrogen. One-third of the nitrogen was applied with the planting and the remaining was in March, between the rows. The crop was harvested manually and all plants for each plot were threshed together. Samples were taken randomly from each plot for quality analysis.

Olive varieties employed and preparation of oil samples

A total of 21 varieties have been studied during the study. The trees were at the Experimental Plot of the Olive Production Station of The Ministry of Agriculture and Rural Affairs located at Edremit–Balikesir and the Experimental Station of Onsekiz Mart University in Çanakkale. Fruit samples have been manually harvested from three trees of each variety. All the trees were more than 20 years old and under optimum management techniques. A total of 100 fruit samples from each trees of the varieties were collected from the beginning of December to the end of January in 2004–2005 and 2005–2006. Each variety was harvested when 75% of the fruit were coloured pink/purple. Trees of the same variety were harvested on the same day. A maturity index was used for the determination of the optimum ripeness stage of olive varieties. The olives were sorted into skin and flesh colour, and then the maturity index equation was applied as reported by Boskou (2002). The maturity index was between 2.4 and 3.8 (data not presented) for all surveyed varieties. The harvested olives were crushed with an inox hammer crusher, malaxed for 30 min at 28°C and the oil was separated out by a vertical centrifuge at 2,000 rounds/min.

Sample preparation for high-performance liquid chromatography

Sample preparation for rapeseed was conducted according to Thies (1997). About 1 g seeds were ground in a coffee mill for 10 sec. Then 50 $\pm$ 5 mg milled (in a coffee mill) rapeseed meal was weighed and transferred to a 5 ml volumetric PE-test tube. For olive oil samples, 30 mg oil was used. Then, 0.5 ml of a 0.001% solution (w/v) of β-tocopherol in isooctane was added as internal standard (Calbiochem Inc., San Diego, CA, USA). After adding 1.5 ml isooctane the tubes were closed with a PE-cap and agitated on a vortex-mixer for 2–3 sec. After storage for 15 h in the dark at room temperature, the solution was agitated again and centrifuged at 5,000 $\times g$ for 7 min. An aliquot of 0.8 ml of the clear supernatant was filtered through a disposable filter holder (0.45 μm), and stored in 0.8 ml high-performance liquid chromatography vials. Then 10 μl of the solution were injected into the high-performance liquid chromatography system for tocopherol analysis.

The variance analysis was performed using the general linear model, and phenotypic correlations were investigated using proc corr of the SAS statistical software. Mean separations were done by multiple comparison tests (SAS, 1998).

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Results

Rapeseed

The tocopherol compositions of the rapeseed varieties are presented in Table I. α-tocopherol, γ-tocopherol and δ-tocopherol were detected in all rapeseed samples. The analysis of variance indicated significant differences between varieties. Across these 19 varieties, α-tocopherol averaged 37.890 mg/kg. The average α-tocopherol contents of the varieties grown in the northwest of Turkey ranged from 25.00 mg/kg for 'Talent' to 48.283 mg/kg for 'Viking'. γ-Tocopherol represented the vitamin E form present in the highest quantity. 'Baros' showed the highest γ-tocopherol content (110.950 mg/kg), followed by 'Elan' (108.733 mg/kg). δ-Tocopherol was measurable in rapeseed varieties but at levels usually less than 0.2 mg/kg. In our data 'Adder', which presented the highest δ-tocopherol (0.212 mg/kg), had a significantly higher value than the other varieties. 'H602014' had the lowest δ-tocopherol value (0.038 mg/kg). As far as the total tocopherol content is concerned, a wide variability was noticed. Some varieties had high contents, such as those obtained from varieties such as 'Viking' (155.817 mg/kg) and 'Elan' (150.883 mg/kg), but, on the other hand, 'Baros' had the lowest total tocopherol content (93.000 mg/kg).

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Olives

In Table II the tocopherol contents of some olive varieties are presented. The tocopherol content of olive varieties examined in this study varied significantly.

Table I. Comparison of rapeseed genotypes for tocopherol contents (mg/kg).

Genotype	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherol
Talent	25.000^{h}	85.400^{g}	1.3333^{cdef}	111.717^{i}
Aragon	38.617^{ef}	87.683^{fg}	1.2833^{def}	127.517^{fg}
Elan	41.050^{cd}	108.733^{a}	1.1000^{efg}	150.883^{ab}
Rasmus	43.067^{c}	94.683^{de}	1.8500^{abc}	139.600^{cde}
Viking	48.283^{a}	106.367^{ab}	1.1500^{efg}	155.817^{a}
Express	41.417^{cd}	100.250^{bcd}	1.4833^{bcde}	143.117^{bcd}
Alesi	26.700^{h}	85.967^{g}	0.9000^{fgh}	113.600^{hi}
Triangle	45.333^{b}	98.933^{cde}	1.9167^{ab}	146.117^{b}c
Adder	46.850^{ab}	85.900^{g}	2.1167^{a}	136.333^{de}
Licrown	25.467^{h}	93.083^{ef}	1.2500^{def}	112.633^{i}
H602016	40.033^{de}	83.250^{g}	0.9333^{fg}	134.083^{ef}
H602014	37.433^{f}	85.483^{g}	0.3833^{h}	121.067^{gh}
Prince	37.567^{f}	84.217^{g}	1.3000^{def}	124.383^{g}
Action	41.817^{cd}	95.050^{de}	0.7000^{gh}	126.717^{fg}
Artus	41.567^{cd}	86.633^{fe}	1.6000^{abcde}	138.267^{cde}
Titan	37.750^{f}	104.633^{abc}	0.9333^{fg}	125.300^{g}
Mendel	42.450^{c}	58.800^{h}	1.7333^{abcd}	148.800^{ab}
Baros	33.400^{g}	110.950^{a}	0.8000^{fgh}	93.000^{j}
Tenno	26.117^{h}	87.367^{fg}	1.3000^{def}	135.533^{de}
Least Significant Differences 5%	2.1	6.9	0.5	7.9
Minimum	19.300	44.200	0.00	83.900
Maximum	68.500	118.900	2.6	173.800
Mean	37.890	91.757	1.267	130.762

Note: Means within a column followed by the same superscript letter are not significantly different at the $P = 0.05$ level.

Table II. Comparison of olive genotypes for tocopherol contents.

Genotype	α-Tocopherol	γ-Tocopherol	Total tocopherol
Arbequina	160.25^{cd}	20.75^{bc}	180.950^{d}
Ascolana	180.00^{abc}	18.25^{bc}	198.400^{b}
Ayvalik	99.50^{kl}	0.00^{g}	99.475^{m}
Çakir Yağlik	138.00^{efg}	6.00^{cd}	144.300^{h}
Domat	118.50^{hijk}	0.00^{g}	118.575^{k}
Edincik Su	132.25^{efgh}	9.00^{c}	141.175^{i}
Erkence	149.50^{de}	0.00^{g}	149.525^{fg}
Gemlik	108.25^{jk}	0.50^{fg}	108.700^{l}
Gordales	139.75^{efg}	4.50^{de}	145.975^{gh}
Gökçeada	52.00^{m}	0.00^{g}	52.100^{o}
Hojiblanca	144.25^{def}	7.50^{cd}	151.925^{ef}
Karamürsel Su	109.75^{ijk}	21.75^{b}	131.300^{j}
Kiraz	128.00^{fghi}	0.75^{efg}	128.950^{j}
Leccino	173.50^{bc}	39.75^{a}	213.075^{a}
Manzanilla	188.25^{ab}	4.75^{de}	192.950^{c}
Memecik	145.75^{def}	0.00^{g}	145.725^{gh}
Memeli	100.00^{kl}	0.50^{fg}	100.400^{m}
Negral	194.75^{a}	4.75^{de}	199.575^{b}
Samanli	140.50^{efg}	7.00^{cd}	147.600^{fgh}
Uslu	83.75^{l}	1.75^{efg}	85.525^{n}
Verdial	124.00^{ghij}	21.75^{b}	145.675^{gh}
Least Significant Difference 5%	1.95	0.40	4.70
Minimum	52.00	0.00	52.100
Maximum	194.75	39.75	213.075
Mean	133.83	8.06	141.994

Note: Means within a column followed by the same superscript letter are not significantly different at the $P = 0.05$ level.

Although α-tocopherol was the most abundant tocopherol fraction in olive varieties, there was also γ-tocopherol in some varieties in a small quantity. The average α-tocopherol contents of the olive varieties ranged from 52.00 mg/kg for 'Gökçeada' to 194.75 mg/kg for 'Negral'. The tocopherol fraction of olive varieties is made up of α-tocopherol, which accounts for between 80% and 100% of the total tocopherols in different varieties. Although there were some statistically significant variations among the γ-tocopherol contents of olive varieties, it was not detected in 'Ayvalik', 'Domat', 'Erkence', 'Gökçeada' and 'Memecik'. When the total tocopherol content was taken into account, a wide variability was noticed. The values ranged between 52.10 mg/kg for 'Gökçeada' and 213.075 mg/kg for 'Leccino'.

Discussion

The used high-performance liquid chromatography method was the same method as in the previous studies (Goffman et al. 1999; Marwede et al. 2004). Our results of tocopherol fractions (α-tocopherol = 19.300–68.500 mg/kg, γ-tocopherol = 40.420–118.900 mg/kg and δ-tocopherol = 0.000–2.600 mg/kg) and the total tocopherol contents (83.900–173.800 mg/kg) are medium to low. In these two previous research studies, however, the values ranged from 233.8 to 306.5 mg/kg and from 314 to 350 mg/kg, respectively (Goffman et al. 1999; Marwede et al. 2004). However, the

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tocopherol components according to the size are the same and γ-tocopherol was the major tocopherol component for all varieties.

The reasons for low tocopherol values could be different. Firstly, in this study, the small number of varieties, a single location for field experiments, the growing conditions and the temperatures of the seed-drying time can be described as reasons for the low tocopherol values. The climatic conditions, especially high temperatures and intensity of light during harvest and the seed-drying period, were not optimum for protection of the tocopherol content. The environmental conditions of temperature as sources of variability for the tocopherol content have been described in some previous experiments (Marquard 1976; Dolde et al. 1999).

In another study, high temperatures and light intensity are given as strong factors for decreasing the tocopherol content (Marquard 1976).

The variance analyses have shown significant genotypic differences for tocopherol contents in olives. The total tocopherol content in extra virgin olive oil varied between 52.10 mg/kg and 213.075 mg/kg. The average α-tocopherol contents of the olive varieties ranged from 52.00 mg/kg to 194.75 mg/kg. This significant variation shows that genotype strongly affects the content of tocopherols in olive oils. The variation for total tocopherol content in the varieties evaluated in the present study is very similar to that previously reported (Boskou 2002). High concentrations of α-tocopherol were observed in most of the samples selected from various regions of Greece (Psomiadou et al. 2000). The researchers found the values ranging between 98 and 370 mg/kg (> 200 mg/kg in 60% of samples). Manzi et al. (1998) had similar results as measured by normal-phase high-performance liquid chromatography for α-tocopherol (160.95–206.76 mg/kg) (Manzi et al. 1998; Boskou 2000; Psomiadou et al. 2000; Stark and Madar 2002).

Generally the concentrations of tocopherols in olive oils vary greatly as they tend to range from 5 to 300 mg/kg oil (Boskou 2000), with α-tocopherol representing about 95% of the tocopherol fraction (Stark and Madar 2002). High-quality olive oils tend to have tocopherol values over 100 mg/kg oil (Baskou 2000). In our data, the highest tocopherol content was found in the 'Leccino' variety, which therefore appears to be a good candidate for improving oil quality and breeding olives for higher tocopherol content.

Compared with the major oil crops, olives have a middle to low total tocopherol content. However, tocopherol concentrations in olive oils are generally lower than in many other vegetable oils (Visioli et al. 2000). Wheat germ oil has the highest tocopherol content of all vegetable oils, up to about 2,500 mg/kg, and also the highest content of α-tocopherol, which represents around 60% of the total content (Zacchi et al. 2006). Soybean oil contains on average about 1,000 mg/kg tocopherol content (Kamal-Eldin and Andersson 1997), and sunflower oil about 800 mg/kg total tocopherol content (Demurin et al. 1996). Oils with high levels of antioxidants would in fact be more stable in autoxidation and thermoxidation processes than others that contain less antioxidants.

Olive oil characteristics are dependent on genetic, agronomic and environmental factors as well as on the quality and physiological condition of the olives from which it is extracted, as pointed out by Manzi et al. (1998). The olive oil quantity and quality vary greatly among different cultivars. Therefore, a selection scheme for elite olive cultivars relative to oil quantity and quality should include the selection of genotypes suitable for superior oil yield and quality. Furthermore, these quantitative traits could

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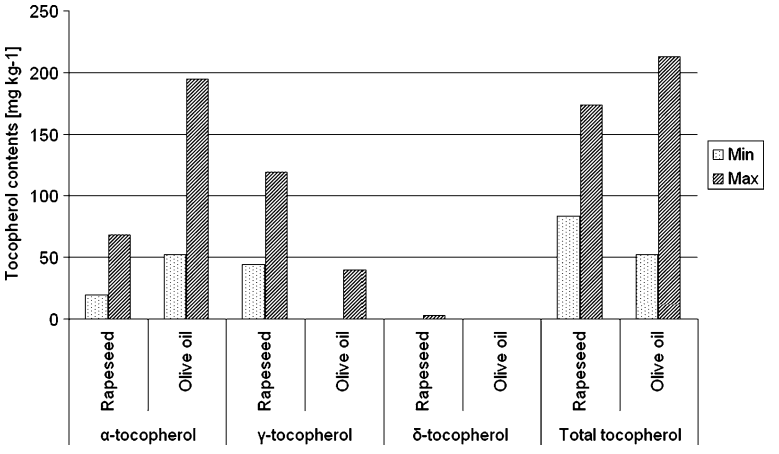


Figure 1. Comparison of rapeseed and olive oil for tocopherol contents.

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vary greatly due to soil constituents, environmental conditions and agricultural practices. On the basis of our previous observations, it is possible to affirm that, under the environmental conditions found in the northwest of Turkey, it is possible to produce extra virgin olive oils. However, precise applications, such as fruit harvesting at the optimum ripening stage, transport and prompt extraction of the oil, utilization of advanced technologies for the extraction and suitable storage of the products, must be taken into account.

In summary, the present study revealed considerable variability for the tocopherol content and composition in rapeseed and olive varieties. The mean value of the total tocopherol content in rapeseed seeds is 130.762 mg/kg (Figure 1). This value is doubly high in the seed oil. However, our results are lower than in previous research. To save the quality parameters such as tocopherols in the seeds and seed oil, storage conditions are of great importance. Although the tocopherol contents are in accordance with previous research, some varieties had been found to have higher levels in this study, probably because of the environmental conditions. In the consideration of both crops, the total tocopherol content in rapeseed oil is similar to olive oil. However, the active proportion of vitamin E in olive oil is significantly higher than that in rapeseed oil. To improve the quality of vegetable oils, olive oils can be added to rapeseed oil with a high active vitamin E proportion.

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