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EFFECT OF ROOTSTOCK ON FAT CONTENT AND FATTY ACID COMPOSITION OF IMMATURE PISTACHIO KERNELS

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Abstract

This study was conducted on pistachio trees (*Pistacia vera* L., cv Siirt) grafted on four different rootstocks (*Pistacia atlantica* Desf., *P. khinjuk* Stocks, *P. terebinthus* L. and *P. vera* L.) to elucidate effects of the rootstocks in the fat content and fatty acid composition of early harvested green pistachio kernels. Fat content and fatty acid compositions were determined after harvesting. The fat content of pistachio kernels ranged from 24% on *P. terebinthus* to 35.4% on *P. atlantica*. The fatty acid compositions ranged from 10.83% on *P. atlantica* to 12.17% on *P. khinjuk* in saturated fatty acids (SFAs); 68.29% on *P. khinjuk* to 70.09% on *P. atlantica* in monounsaturated fatty acids (MUFAs) and 18.42% on *P. vera* to 19.54% on *P. khinjuk* in polyunsaturated fatty acids (PUFAs). The unsaturated/saturated fatty acid ratio varied between 7.22 and 8.23. In the present study, we found that the fatty acids were affected by the rootstock in pistachio trees.

Key words: fat content, fatty acid, pistachio, rootstock

Introduction. The genus *Pistacia* is a member of the Anacardiaceae family and consists of at least eleven species. Except for *P. mexicana* and *P. texana*, which originated in the USA and Mexico, all other species are distributed mainly within the Mediterranean region, Western and Central Asia and the Middle East [1]. Turkey has a large *Pistacia* populations belonging to six species: *P. vera* L., *P. terebinthus* L., *P. khinjuk* Stocks, *P. atlantica*, Desf., *P. eurycarpa* Yalt. and *P. lentiscus*. The main rootstock in pistachio cultivation is *P. vera*, and it is followed by *P. terebinthus, P. khinjuk* and *P. atlantica* [2].

Pistachio has a high nutritional value due to its high protein, vitamin, mineral and unsaturated fat content and the fat content of pistachio kernels varies from 40% to 63% [3]. Pistachio kernels contain both monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) and saturated fatty acid composition of pistachio kernels ranged from 7.45% to 10.14%, monounsaturated fatty acids

changed between 55.23% and 77.61%, and polyunsaturated fatty acids were between 13.82% and 33.11% depending on cultivars [4]. The unsaturated fatty acids are a nutritional requirement due to their health benefits. KRIS-ETHERTON et al. [5] reported that MUFAs, such as oleic acid, have been shown to decrease plasma triacylglycerol and cholesterol concentrations. Similarly, PUFAs, such as linoleic and linolenic acids, assist to the prevention of cancer, atherosclerosis, heart disease, and diabetes [6]. The major unsaturated fatty acids found in pistachio oil are oleic, linoleic and linolenic acids [4]. The ratios of these fatty acids to each other are important to the economic and nutritional value of the nut. The oils with lower linoleic and linolenic acid contents may have a longer shelf life, and monounsaturated fatty acids may be more desirable due to their potential health benefits [7]. The positive nutritional advantages of pistachios in lowering blood cholesterol should not be overlooked. Consumption of pistachio as 20% of daily caloric intake leads to significant improvement in high-density lipoprotein (HDL) and inhibits low-density lipoprotein (LDL) cholesterol oxidation. Pistachio may be beneficial for both prevention and treatment of coronary artery disease. These advantages come from the high levels of mono- and polyunsaturated fatty acids and possibly the tocopherol content.

Green kernel colour, which depends on genotype and environmental conditions is an important quality characteristic for pistachio due to its appearance and taste. According to KUNTER et al. [8], there are two methods to get pistachio with green kernel: a) growing pistachio trees in high altitudes, and b) early harvesting. Recently, early harvesting is the most common practice in Turkey to get pistachio with green kernels to use them especially in the industry.

Rootstocks are used primarily to aid scion propagation. Their resistance to disease and pests are also very important in plant production. Many scion cultivars are extremely difficult to propagate clonally on their own roots, or sensitive to disease and pests and therefore, rootstocks are good choices especially for the production of horticulturally important plant species.

Rootstocks may affect cultivar in respect of vigour, yield, fruit quality, climatic adaptability, and susceptibility to pests and diseases. Organic compounds characteristic of the rootstock sometimes can be translocated into the top of the tree. Rootstocks and scion interact in many ways including at least 14 fruit quality factors influenced by the rootstock [9].

There are a few studies dealing with fat content and fatty acid composition of pistachios. However, no detailed study has been reported so far about the effect of rootstocks on fat and fatty acid compositions of immature (green kernel) pistachio fruits. Therefore, the objective of this study is to determine the effect of rootstocks on fat content and fatty acid composition of early harvested pistachios.

Material and methods. ***Plant material.*** Immature (green kernel) fruits (∼25–30 days before maturation) of pistachio trees (cv. Siirt) grafted on *P. atlantica*, *P. khinjuk*, *P. terebinthus* and *P. vera* rootstocks were hand-harvested

in experimental orchard of Harran University, Faculty of Agriculture, located in the Şanlıurfa province of Turkey in August 2015. A sample of 25 kernels was randomly selected to evaluate each rootstock in three replicates. The nuts were analyzed after drying them for 5–6 days at room temperature.

Oil extraction. For oil extraction, 20 g of nut sample was extracted by using diethyl ether solvent for 1 h using automatic Soxhlet equipment (Gerhardt Soxtherm), and the residue was placed in drier and weighed up to a fixed value. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (FAMEs) [10].

GC-FID analysis. Fatty acids were analyzed with Clarus 500 gas chromatography (GC) using an autosampler (PerkinElmer, Shelton, CT, USA) equipped with a flame ionization detector (FID) and a fused-silica capillary SGE column (30 m × 0.32 mm, ID 0.25 µm, BP20 0.25 UM; PerkinElmer, Austin, TX, USA). The oven temperature was held at 140 °C for 5 min and then raised to 200 °C at a rate of 4 °C min^{-1} and to 220 °C at a rate of 1 °C min^{-1}, while the injector and the detector temperatures were set at 220 and 280 °C, respectively. The sample volume was 1 µl; the carrier gas controlled at 16 psi and split ratio was 1:100. Fatty acids were determined by comparing FAMEs retention indices with a standard 37-component FAME mixture (Supelco, Bellefonte, PA, USA).

Statistical analysis. GC analysis was done in triplicate and results were expressed as a mean GC area (%) value ± standard deviation. Results were analyzed by ANOVA using a completely randomized design. Means were separated by Duncan's multiple range test at $P \leq 0.05$.

Results and discussion. The total fat content of 'Siirt' pistachio cultivar grafted on different rootstocks ranged from 24.0% on *P. terebinthus* to 35.4% on *P. atlantica*. It was 33.5% on *P. vera* and 34.9% on *P. khinjuk*. The total fat content of pistachio kernels was highly affected by rootstock, especially on *P. terebinthus*, which significantly decreased fat content of pistachio kernels (Table 1).

The fat content of early harvested pistachio in this study was lower than that of mature nuts analysed in the previous studies. For example; fat content of 'Siirt' kernels grown in Şanlıurfa province was 42.11% [4] and 51.78% [11]. On the other hand, fat content of 'Siirt' cultivar growing in different ecologies was determined as 53.9% by Çinar and Okay [12], 54.2% by Garcia et al. [13] and 51.77% by Kafkas et al. [14]. Differences in the fat content of pistachio cultivar may be due to differences in factors such as growing conditions and ecology [4]. It is known that embryo development is related to the total fat accumulation and indicated increases in total dry weight accompanied by increases in fat accumulation in pistachio [15]. According to Panahi and Khezri [16], the appropriate time of harvest is one of the most important factors affecting the quality of pistachio, and total crude fat and sugar contents showed a peak in the middle of September. Agar et al. [11] reported that fat content increased from 5.5% (on 16th July) to 32.9% (on 3rd September) during the embryo development stage and reached

Table 1

Fat and saturated fatty acid content (%) of Siirt pistachio cultivar grafted on different rootstocks

Rootstocks	Fat (%)	Saturated fatty acids (%)					Unsaturated fatty acids (%)			
							Monounsaturated fatty acids		Polyunsaturated fatty acids	
		Myristic C14:0	Palmitic C16:0	Stearic C18:0	Arachidic C20:0	Behenic C22:0	Palmitoleic C16:1	Oleic C18:1	Linoleic C18:2	α-Linolenic C18:3
P. atlantica	35.40±1.40 a*	nd	8.63±0.04 a	1.71±0.13 c	0.27±0.05 a	0.22±0.03 a	0.55±0.02	69.54±0.39 a*	18.11±0.11 ab	0.97±0.02 ab
P. khinjuk	34.90±0.80 a	0.79±0.06 b	8.66±0.15 a	2.34±0.03 a	0.23±0.01 ab	0.15±0.02 c	0.52±0.01	67.77±0.01 b	18.29±0.02 a	1.25±0.13 a
P. terebinthus	24.00±3.80 b	1.47±0.03 a	8.30±0.02 b	2.10±0.08 b	0.20±0.02 b	nd	0.90±0.45	67.75±0.02 b	18.35±0.05 a	0.93±0.42 ab
P. vera	33.50±2.80 a	nd	8.70±0.18 a	2.53±0.09 a	0.23±0.03 ab	0.21±0.02 b	0.61±0.02	69.30±0.48 a	17.70±0.53 b	0.72±0.06 b
$LSD_{\%5}$	3.816	0.063	0.268	0.199	0.063	0.002	ns	0.703	0.558	0.484

*The letters following the numbers indicate different groups determined by Duncan's test ($p \leq 0.05$) nd: not detected

its maximum level at the date of harvest maturity in 'Siirt' pistachio cultivar. Tavallali and Rahemi [17] reported that type of rootstocks increased fat content of pistachio kernel up to 64%, and 'Sarakhs' rootstock had better performance on increasing crude fat. The results obtained from the present study confirmed that rootstocks had an important effect on kernel fat content and fatty acid composition of pistachio. Fat is the main component of the edible pistachio kernel.

The fatty acid compositions and their ratios are reported in Table 1 and Fig. 1 as well as the percentage of the mean value of 9 fatty acids for each rootstock. The main fatty acids were oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0) and stearic acid (C18:0), and their contents were significantly affected by rootstocks.

Green kernel pistachio analyzed and presented in the present study had very low saturated fatty acids. Palmitic acid was the main saturated fatty acid, followed by stearic, myristic, arachidic and behenic acids, respectively. Palmitic acid content varied between 8.30 and 8.70%, whereas stearic acid ranged from 1.71 to 2.53%. Myristic acid was not detected in the kernel of 'Siirt' pistachio

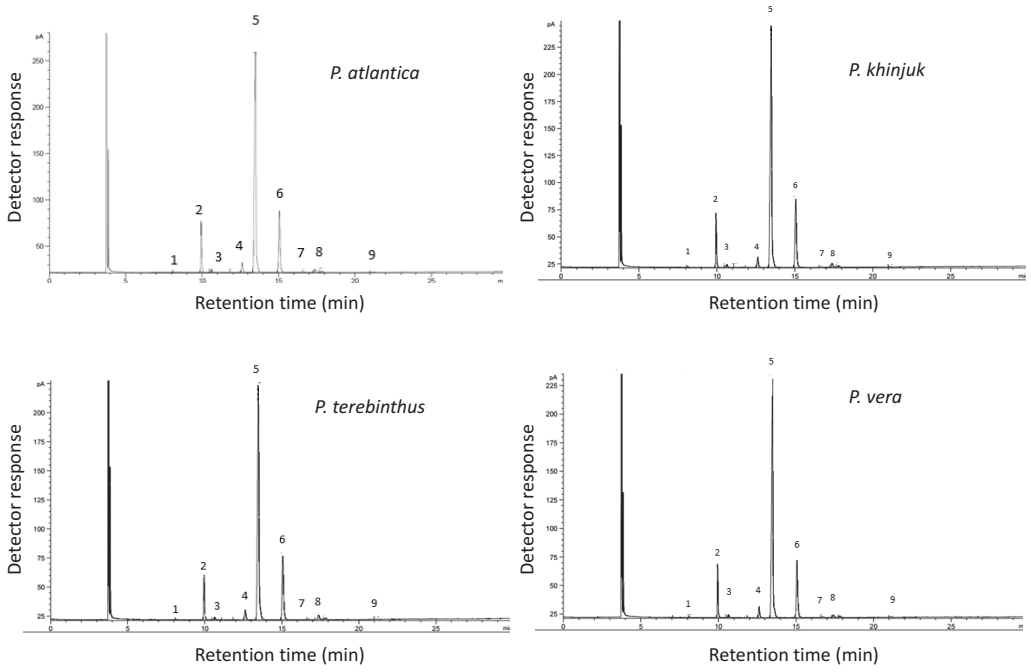

Fig. 1. Representative gas chromatograms showing separation and relative quantification of fatty acids generated from extracted fat samples of immature seed of Siirt pistachio cultivar grafted on *Pistacia atlantica*, *P. khinjuk*, *P. terebinthus and P. vera* rootstocks. Fatty acid methyl ester peaks are as follows: (1) Myristic acid, (2) Palmitic acid, (2) Palmitoleic acid, (3) Margaric acid, (4) Stearic acid, (5) Oleic acid, (6) Linoleic acid, (7) Arachidic acid, (8) α-Linolenic acid, and (9) Behenic acid

Table 2

Total saturated and unsaturated fatty acid contents of Siirt cultivar grafted on different rootstocks

Rootstocks	Saturated fatty acids (SFA) (%)	Unsaturated fatty acids (USFA) (%)			Unsaturated/ Saturated
		Mono-unsaturated fatty acids (MUFA) (%)	Poly-unsaturated fatty acids (PUFA) (%)	Total Unsaturated fatty acids (USFA) (%)	
P. atlantica	10.83±0.21 c*	70.09±0.38 a	19.08±0.09 ab	89.17	8.23
P. khinjuk	12.17±0.16 a	68.29±0.02 b	19.54±0.14 a	87.83	7.22
P. terebinthus	12.07±0.08 ab	68.65±0.47 b	19.28±0.37 a	87.93	7.28
P. vera	11.67±0.27 b	69.91±0.46 a	18.42±0.58 b	88.33	7.57
$LSD_{\%5}$	0.419	0.814	0.797	–	–

*The letters following the numbers indicate different groups determined by Duncan's test ($p \leq 0.05$)

grafted on *P. atlantica* and *P. vera*, and behenic acid was not detected at that on *P. terebinthus* (Table 1, Fig. 1). Acar et al. [4] determined palmitic acid as 7.23%, stearic acid as 0.76%, arachidic acid as 0.10%, myristic acid as 0.08% and behenic acid as 0.00% in 'Siirt'cultivar.

Unsaturated fatty acids were designated as monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Oleic acid was the main monounsaturated fatty acid in this study. Oleic acid content ranged from 67.75 to 69.54%, with a significant difference over different rootstocks. The low and high oleic acid contents were observed on *P. terebinthus* and *P. atlantica* rootstocks, respectively. Linoleic acid was the predominant polyunsaturated fatty acid varied between 17.70 and 18.35% (Table 1, Fig. 1). Acar et al. [4] determined oleic acid as 76.85%, linoleic acid as 13.60%, palmitoleic acid as 0.41% and linolenic acid as 0.22% in 'Siirt' pistachio cultivar. In this study, oleic acid was lower and linoleic acid higher than the contents reported by Acar et al. [4]. Agar et al. [11] reported that, oleic acid had increased from 52.0% (on 16th July) to 71.1% (on 3rd September) while linoleic acid decreased from 26.2% (on 16th July) to 16.8% (on 3rd September) during the embryo development stage in 'Siirt' pistachio cultivar. According to Cinar and Okay [12], oleic acid was 69.77%, linoleic acid was 18.51%, palmitoleic acid was 0.53% and linolenic acid was 0.35% in 'Siirt' cultivar, while Agar et al. [18] found oleic acid content as 66.03%, linoleic acid as 21.35%, palmitoleic acid as 0.99% and linolenic acid as 0.30%. The results obtained in this study are in agreement with those reported by Agar et al. [18] and by Cinar and Okay [12] for 'Siirt' cultivar.

The total amount of saturated and unsaturated fatty acids and their ratios (unsaturated/saturated) are listed in Table 2. The fatty acid composition of pistachio genotypes led to the following results: saturated fatty acids (SFAs) ranged in the interval of 10.83–12.17%; monounsaturated fatty acids (MUFAs) ranged

in the interval of 68.29–70.09%; polyunsaturated fatty acids (PUFAs) ranged in the interval of 18.42–19.54%. The unsaturated/saturated fatty acids ratios varied between 7.22 and 8.23, and these results are in agreement with previous studies [4,14,18]. It was also observed that the proportion of these fatty acids changed significantly among the rootstocks. According to some researchers, the fatty acid composition of nuts can be affected by ecological conditions, variety, location, geographical origin, and technical and cultural practices [18]. Carbonell-Barrachina et al. [19] found that yield, nut weight, and mineral content were higher on *P. atlantica* than *P. integerrima* and *P. terebinthus* rootstocks. In the present study, we found that the fatty acids in pistachio nuts also affected by the rootstock (Tables 1, 2, Fig. 1).

Oleic and linoleic acid, the two major fatty acids in pistachio, are of interest due to their beneficial effects on human health. Consumers are especially interested in unsaturated FAs, because PUFAs are a natural preventive of cardiovascular disease [20]. It has been reported that MUFAs were as effective as PUFAs in reducting low-density-lipoprotein cholesterol in humans [21].

In conclusion *P. atlantica* was found to have potential health-promoting pistachio rootstock with the highest total fat and total unsaturated fatty acid contents, and had the lowest saturated fatty acid content.

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