

Seasonal variation of fatty and essential oil in terebinth (*Pistacia terebinthus* L.) fruit

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Abstract

A member of Anacardiaceae family, terebinth (*Pistacia terebinthus* L.) is a perennial tree that is evergreen. Terebinth is spread on a very wide area in Turkey, from the Mediterranean coasts to South-Eastern Anatolia. In the present study, to determine seasonal variation of fatty oil and essential oil in terebinth fruits, harvests were made when the fruit colour was Green (GF), Red (RF), Red-Black (RBF) and Black (BF). Upon ripening, fatty oil rate in the fruit raised from 13.40% to 37.08%, while essential oil rate reduced from 0.116% to 0.082%. In all growth periods, oleic, linoleic and palmitic acids made-up the main composition of the oil, and through growth there was increase in the amounts of oleic and palmitic oil acids while a decrease was reported in linoleic acid rate. In terms of essential oil compounds, α - pinene, limonene, p-cymen-8-ol and p-anisaldehyde composed the main compounds, the highest α - pinene rate was observed during BF period as 37.23%, the highest limonene rate was observed during RBF period as 22.01%, while the highest p-cymen-8-ol and p-anisaldehyde rates have been observed during the GF harvest period by 11.29% and 7.25%, respectively. In conclusion is that the fruit should be harvested during the black fruit colour, when the fruit is fully ripened, in order to get a good quality fruit and aroma and people picking the fruit from nature should be trained about this.

Keywords: essential oil composition; fruit oil; fatty acids; ontogenetic variability; terebinth

Introduction

Pistacia terebinthus L. is a perennial tree spreading naturally from the Mediterranean to West Asia (Kavak *et al.*, 2010). And in Turkey, it can grow naturally in a wide area covering Mediterranean, Aegean, Black Sea and Southeastern Anatolia Regions (Tufan *et al.*, 2017). Turpentine chi Otica (gum) is obtained by opening the wound in the trunk of the tree. This terebinth has a pleasant odour, has the consistency of honey, is transparent with a light yellow colour melts easily in alcohol. Gallnut, formed by some insects on plant leaves, carry high amounts of tannin. Such gallnut is used to dye valuable silk fabrics, to colour wine and also used as incense material (GDF, 2019). Young shoots and fruits are used for nutrition. The fruits have been used as an appetiser in human nutrition in southern Turkey for several thousand years. As a folk medicine, the decoction of its leaves is used in gastric treatments and its fruits are used for the treatment of gastralgia (internally), rheumatism, coughs (externally), and as a stimulant, diuretic, anti-tussive and anti-rheumatic (Ozcan, 2004).

Received: 27 Nov 2020. Received in revised form: 08 Mar 2021. Accepted: 09 Mar 2021. Published online: 10 Mar 2021.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal will use article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

In Turkey, fruits of this plant are roasted and marketed as dry fruit or grinded and sold as “terebinth (meningic) coffee”. Furthermore, oil extracted from its fruits is used in making soap (Baytop, 1999). Terebinth fruits are rich in oil containing high concentrations of unsaturated fatty acids and carotenoids, phenolic compounds and tocopherols, tannin and resinous substances, and dietary fiber (Matthäus and Ozcan, 2006; Kızıl and Turk, 2010).

Studies related to fatty oil in the fruit led to the conclusion that oil ratio ranges between 38.74-47.0% and oleic, palmitic and linoleic oil acids are the main oil acids (Ozcan, 2004; Matthäus and Ozcan, 2006; Kızıl and Turk, 2010; Durmaz and Gokmen, 2011; Kaya and Ozer, 2015).

Essential oil is present in the leaves, young suckers, flower and fruits of terebinth plant. Essential oil rates and the main components of this oil, such as limonene, β - pinene and α - pinene have been observed at different rates during different growth periods of the plant (Couladis *et al.*, 2003; Pulaj *et al.*, 2016). This change in the essential oil rates and its components was present during harvest seasons too. A study conducted by Zrira *et al.* (2003) on the leaves and flowers of *P. lentiscus* cultivar from the same family reported significant differences between essential oil and its components. It has been similarly observed that the essential oil rates of fruits from plants grown in different locations in their natural environments ranged between 0.08- 0.16% (Ozcan *et al.*, 2009).

Growing naturally in South Eastern Region of Turkey, terebinth trees are used as rootstock and are grafted with *Pistacia vera* vaccine. This, however, causes a reduction in terebinth population. Furthermore, terebinth fruits are picked without any regulations and sold in domestic and international markets (Inan and Harbi, 2018). Uncontrolled and untimely picking leads to a change in the quality and aroma (especially in terebinth coffee production) if fruits are picked before ripening, and to a reduced yield in late picking. The purpose of this study was to determine the oil contained in terebinth fruits during different growth periods (ontogenetic variability) as well as the composition of fatty, essential oil and its components.

Materials and Methods

Plant material

Fruits of the *Pistacia terebinthus* L. cultivar spreading naturally Nemrut Mountain National Park in Adiyaman province in the south eastern Anatolian region of Turkey (37°56'33" N, 38°39'27" E and altitude 819 m) composed the material of this study. Fruits have been picked from the same tree in 4 different periods; at the beginning of ripening, when green (GF: green fruit) (23.08.2018), red (RF: red fruit) (07.09.2018), red-black (RBF: red-black fruit) (20.09.2018) and when fully ripened in black (BF: black fruit) colour (01.10.2018). Harvested fruits have been dried at room temperature (Figure 1).

Fatty oil rate

After being dried at room temperature, the collected fruits have been grinded, 5 g of sample has been taken with 3 repetitions for each harvest period and subjected to 6 hours of extraction in Soxhlet system. The solvent used in the system was petroleum ether. At the end of the duration, balloons have been kept at a 105 °C stove for 1 hours, before being cooled in a desiccator. Then the samples have been weighed and the results converted into %.

Fatty acid composition

100 μ l samples have been taken from oils extracted in each harvest season, KOH solution with 3 ml N-Heptan and 400 μ l 2N methanol has been added and following esterification, fatty components have been defined at the Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops Medical and Aromatic Crops Analysis Laboratory through gas-chromatographic method. Defining the fatty oil components was performed by using a Thermo Scientific ISQ Single Quadrupole gas-chromatography device under the

following conditions. A TG-Wax MS, 5% Phenyl Polysilphenylene-silohexane column with a 0.25 mm internal diameter x 60 m length, 0.25 μm film thickness has been used. Carrier gas used in the study was helium (99,9%) with a flow rate of 1 mL m^{-1} . Ionization energy has been set as 70 eV and mass interval has been set as m/z^{-1} 1,2-1200 amu. Scan mode has been used for data collection. MS transfer line temperature was 250 $^{\circ}\text{C}$, MS ionization temperature was 220 $^{\circ}\text{C}$, injection port temperature was 220 $^{\circ}\text{C}$, and column temperature was 50 $^{\circ}\text{C}$ at the beginning and then raised up to 220 $^{\circ}\text{C}$ with a temperature increase rate of 3 $^{\circ}\text{C} \text{m}^{-1}$. The structure of each compound has been identified with Xcalibur program, by using mass spectrums (Wiley 9).



Figure 1. Green (GF), Red (RF), Red-Black (RBF) and Black turpentine fruits

Essential oil rate

Fruits were air-dried in the shade at room temperature and then extracted separately using hydrodistillation method for 3 h in Clavenger type apparatus. Essential oils were dried over anhydrous sodium sulfate and stored in the dark vials at -4°C , until analyzed.

Gas chromatography-mass spectrometry analysis (GC-MS) of the essential oils

The GC-MS analysis were carried out with Thermo Scientific ISQ Single Quadrupole model device. A TG-Wax MS model column (5% Phenyl Polysilphenylene-siloxane, 0,25 mm inside diameter 30 m in length, having 0,25 μm film thickness) was used with the carrier gas helium. The MS transfer line temperature was set to 250 $^{\circ}\text{C}$, MS ionization temperature was set to 220 $^{\circ}\text{C}$. The column temperature starts at 50 $^{\circ}\text{C}$ and has risen up to 220 $^{\circ}\text{C}$ with 3 $^{\circ}\text{C} \text{min}^{-1}$ temperature rise rate. The split ratio was set to 10:1 Mass spectra were recorded at 70 eV; the mass range was from 1,2-1200 m/z^{-1} . Scan Mode has been used in data collection. Structure of each compound has been defined by the Xcalibur program by using mass spectrums.

Statistical analyses

Thousand seed weight and oil rate data obtained in harvest periods have been subjected to variance analysis with a randomized blocks experimental design by using Mstac analysis program.

Results and Discussion

P. terebinthus fruits were picked starting from the second half of July. Thousand seed weights, oil and essential oil rates of the picked fruits are provided in Table 1. Statistically meaningful differences have been observed between the harvest periods in terms of thousand seed weights and oil rates. The highest thousand seed weight and oil rate have been respectively observed as 58.25 g and 37.08% at full ripening period (in black fruits). The lowest values, on the other hand, have been observed as 33.3 g and 13.40% during the first harvest when fruits were green in color. Something quite the contrary has been observed in essential oil rates. Essential oil rate in fruits started to reduce with ripening. The highest essential oil rate (0.116%) has been observed during GF harvest period, and no statistically meaningful difference has been observed between the essential oil rates (between 0.093% and 0.088% respectively) in RF and RBF harvest seasons. The lowest essential oil rate (0.082%) has been observed during the BF harvest season.

Table 1. The effects of different harvest periods on thousand seed weight, fatty oil and essential oil rates of *P. terebinthus* fruits

Harvest period	Harvest date	Thousand seed weight (g)	Fatty oil rate (%)	Essential oil rate (%)
GF: Green fruit	23.08.2018	33.30 d	13.40 d	0.116 a
RF: Red fruit	07.09.2018	36.23 c	19.27 c	0.093 b
RBF: Red-black fruit	20.09.2018	42.48 b	33.04 b	0.088 bc
BF: Black fruit	01.10.2018	58.25 a	37.08 a	0.082 c
LSD (1%) *		0.96	2.04	0.0095

*LSD (1%): Least Significant Differences Test

Our findings related to thousand seed weight and oil rates are lower than those reported by Ozcan (2004), Kızıl and Turk (2010), and Kaya and Ozer (2015). Thousand seed weights defined by us in RBF and BF harvest periods are higher than those reported by Gultekin *et al.* (2007). Ozcan *et al.* (2009) collected ripened fruits from different locations with essential oil rates ranging between 0.08- 0.16%, and in a similar study, Pulaj *et al.* (2016) reported a range between 0.02-0.12%. Essential oil rates observed by us are within these values. Couladis *et al.* (2003) reported an essential oil rate of 0.54% in non-ripened fruits, and a rate of 0.73% in ripened fruits. These values are much higher than our findings. However, the essential oil rate observed by us at the GF harvest period is higher than those reported by Ozcan (2004) and Kızıl and Turk (2010). The differences between our study findings and the above examples are probably due to collecting the materials from different locations, age of the crops, harvest periods, climate and soil conditions.

Fatty acid composition

Oil acid variations, as observed in oils extracted from *P. terebinthus* fruits in different harvest periods, are given in Table 2. In all four harvest periods, oils extracted from fruits have been observed to contain 7 oil acids with a ratio of over 1%. Oleic, linoleic and palmitic acids compose the main compositions of oil. With ripening, there was an increase in oleic, palmitic, palmitoleic and stearic acid contents, and a decrease in linoleic, cis-13-octadecenoic and linolenic acids. The highest oleic acid content has been observed in fruits picked during the BF period by 46.13%, while the lowest ratio has been observed in the RF period fruits by 41.67%. Something similar has also been observed in palmitic acid. Observed at a rate of 18.78% during the GF period, palmitic acid content became as high as 20.31% at the BF period. However, the 25.71% linoleic acid content at the GF period receded to 18.15% at the BF period (Figure 2). This is a significant indicator as it confirms that the oil composition of fruit varies in different growth periods.

Studies related to fruit oil report that in terms of unsaturated fatty acids, oleic acid content varies between 34.80-55.16% and linoleic acid content ranges between 16.22-29.64%, and in terms of saturated fatty

acids, palmitic acid content varies between 13.67-25.67% (Ozcan, 2004; Kızıl and Turk, 2010; Durmaz and Gokmen, 2011; Durak and Ucak, 2015; Tufan *et al.*, 2017).

Table 2. The effects of different harvest periods on the fatty acid compositions (%) of *P. terebinthus* fruits

Fatty Acid	GF	RF	RBF	BF
Palmitic acid (C16:0)	18.78	18.19	19.78	20.31
Palmitoleic acid (C16:1)	2.34	3.35	3.17	4.04
Stearic acid (C18:0)	2.28	2.72	3.26	3.65
Oleic Acid (C18:1)	42.87	41.67	43.32	46.13
cis-13-octadecenoic (13c-18:1)	2.69	2.13	0.24	0.69
Linoleic acid (C18:2)	25.71	24.80	23.12	18.15
Linolenic acid (C18:3)	1.05	1.43	1.00	0.42
Total	95.72	94.29	93.89	93.39

GF: Green Fruit, RF: Red Fruit, RBF: Red-Black Fruit, BF: Black Fruit

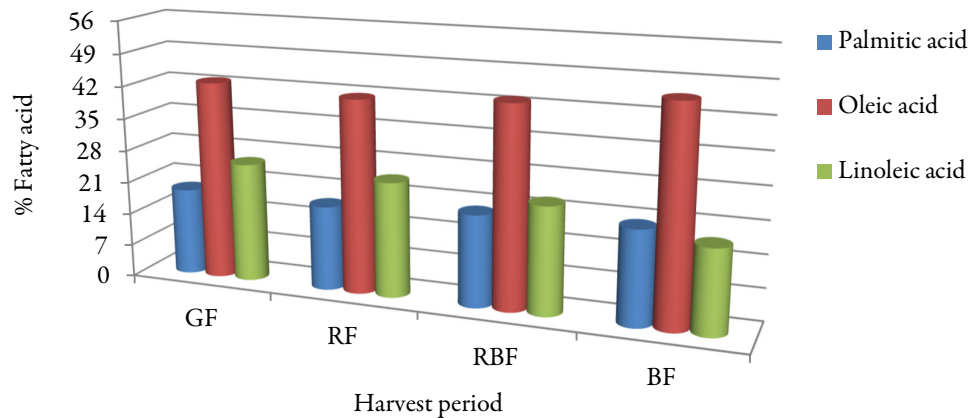


Figure 2. Some important fatty acid changes in the oil obtained from the *P. terebinthus* fruits according to the harvest times; GF: Green Fruit, RF: Red Fruit, RBF: Red-Black Fruit, BF: Black Fruit

Essential oil components

The essential oil components from the *P. terebinthus* fruits harvested in different growth periods are given in Table 3. As the fruit ripened, components such as α -pinene, limonene, o-cymene, verbenol and α -terpineol had an increased content while others such as β -pinene, piperitone, d-verbonen, caryophyllene oxid, spathulenol, p-anisaldehyde and 3-allylguaiacol were significantly reduced. Camphane, α -campholene aldehyde and p-acetonylanisole went through an initial increase followed with a decrease at the final harvest period. Observed as a main component in all four harvest periods, α -pinene increased to 30.17% at RF period and then reached a top value at BF period by 37.23%. Limonene, one of the other important components, kept raising from GF period (9.65%) to RBF period (22.01%) but was then reduced to 19.89% at the final harvest period. p-cymen-8-ol rate has been observed to be 11.29% at the first harvest period, and was reduced to 5.62% and 5.23% respectively at the RF and RBF periods, followed by a slight increase at the BF period, reaching 6.55%. o-cymene rate increased as the fruit ripened and increased from 2.13% to 7.54% (Figure 3). Pinocarvone and piperitone rates decreased to zero in BF period (Table 3). A study by Couladis *et al.* (2003) reported that the main components of the essential oils extracted from non-ripened and ripened fruits were limonene, β -pinene and α -pinene respectively, and it also reported a decrease in limonene and α -pinene components and increase in β -pinene component with ripening. Ozcan *et al.* (2009) reported different main components in the essential

oils of terebinth fruits collected from different parts of Turkey, fruits picked from some areas had α -pinene as the main component, while others had limonene, and there were also some with caryophyllene oxide and p-cymene-8-ol as the main components, and they concluded that this could possibly be due to environmental and climactic conditions. The use of essential oil components as a flavorant in terebinth coffee is reportedly effective, and even though α -pinene is the main component, other components are also contributing for the flavour and by roasting (cooking) the fruit, the rates of components may change (Gogus *et al.*, 2011; Amanpour *et al.*, 2019).

Table 3. The effects of different harvest periods on the essential oil components (%) of *P. terebinthus* fruits

	Component name	GF	RF	RBF	BF
1	α -Pinene	31.28	30.17	34.12	37.23
2	Camphane	1.25	1.23	2.01	0.38
3	β -Pinene	1.86	1.92	1.00	0.51
4	β -Phellandrene	0.56	0.32	0.30	1.22
5	Limonene	9.65	18.23	22.01	19.89
6	o-Cymene	2.13	2.21	3.21	7.54
7	α -Campholene aldehyde	0.23	0.21	1.24	0.19
8	Bornyl Acetate	0.00	0.24	0.31	1.19
9	Pinocarvone	1.12	1.25	0.48	-
10	Verbenol	1.05	1.10	2.63	2.76
11	α -Terpineol	1.10	0.86	1.12	6.94
12	α -Terpinyl acetate	1.23	0.47	0.52	0.80
13	trans- Carveol	1.32	0.23	0.32	0.89
14	p-Cymen-8-ol	11.29	5.62	5.23	6.55
15	Piperitone	2.43	1.10	0.98	-
16	D-verbonen	3.98	3.03	3.00	2.58
17	Caryophyllene oxid	2.93	2.85	0.23	0.16
18	Spathulenol	3.86	3.45	2.98	2.02
19	p-Anisaldehyde	7.25	7.00	3.25	2.13
20	3-Allylguaiacol	6.28	5.89	6.22	1.18
21	p-Acetonylanisole	2.31	5.21	3.09	0.08
	Total	93.11	92.59	94.25	94.24

GF: Green Fruit, RF: Red Fruit, RBF: Red-Black Fruit, BF: Black Fruit

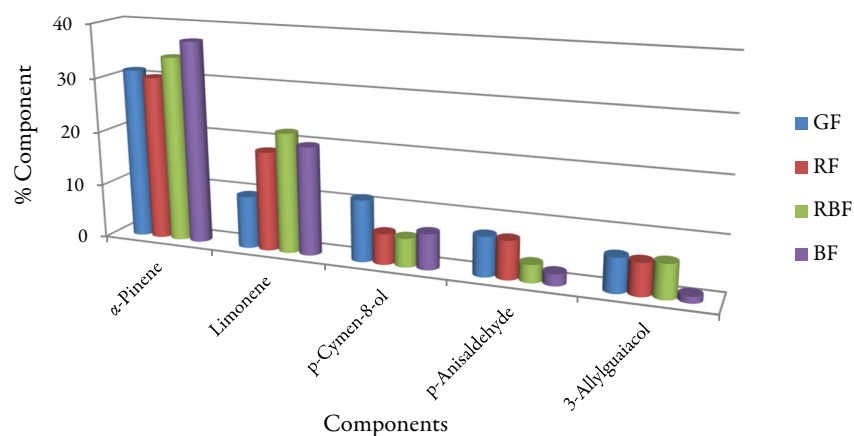


Figure 3. Some important components change in the essential oil obtained from the *P. terebinthus* fruits according to the harvest times

Conclusions

It is known that the fatty and essential oil rates, fatty oil and essential oil components in terebinth fruit are affected by the region they are grown, climate conditions and soil structure. This study has examined the variations in these characteristics in different growing periods. It has been concluded that when terebinth is going to be used as coffee or dry fruit, crops must be harvested at the full-ripening (BF) period to ensure high quality fruits. This needs to be taken into consideration when picking from nature and the people picking terebinth fruits need to be trained about this issue.

Authors' Contributions

The author read and approved the final manuscript.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The author declares that there are no conflicts of interest related to this article.

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