

Determination of the Fatty Acid Composition of the Fruits and Different Organs of Lentisk (*Pistacia lentiscus* L.)

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Abstract: This paper reports the fatty acid composition of the oil extracts from seeds and *in vivo* and *in vitro* grown organs of *Pistacia lentiscus* L. were determined by using gas chromatography. The main fatty acids were linoleic (LA), palmitic (PALM), oleic (OLA) and linolenic (ALA) acids in the fruits, resins and in both *in vivo* and *in vitro* grown root, leaf and stem sections of male or female tree. The major fatty acids were represented by polyunsaturated fatty acids (PUFA) accounting for 56.94 %, 64.44 % and 55.57 % in root, leaf and stem part of male tree grown *in vivo*, respectively. The prominent class of fatty acid composition of different male organs of *P. lentiscus* L. regenerated *in vitro* was represented by PUFA accounting for 63.24 %. The monounsaturated fatty acid (MUFA), OLA and PUFA, LA were determined in the oils of the two genotypes studied.

Key words: Lentisk, fatty acids, *Pistacia lentiscus* L., *in vivo*, *in vitro*.

Introduction

Lentisk, *Pistacia lentiscus* L., is an evergreen and dioecious shrub of the Anacardiaceae family consisting of nine species and five subspecies ¹. *P. lentiscus* L. is native in extreme ecosystems of thermo-Mediterranean region, from Morocco and Iberia in the west through southern France and Turkey to Iraq and Iran in the east ². The tree is mainly grown in seaside stony areas and spread in forests under pine trees in different parts of Turkey. Today, the Mastic tree (*Pistacia lentiscus* var. chia) is cultivated for its aromatic resin on the Greek island of Chios, in the Aegean Sea. *P. lentiscus* var. chia (cultivated only in the Greek

island of Chios) is by far the second economically important species in the *Pistacia* genus because the fruits, its resins and leaves of lentisk have a long tradition in folk medicine dating from the time of the ancient Greeks ³. Despite its limited distribution in the world, this plant is now being used internationally for several therapeutic properties such as its antifungal ⁴, antibacterial ⁵, antimicrobial ⁶, antioxidant ⁷, antiulcer ⁸ agent and antiproliferative effects ⁹. Independent ethnobotanical and ethnopharmacological surveys conducted recently in Israel, Jordan and the Palestinian area are limited to treating stomach aches, heartburn, jaundice and respiratory

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problems^{10,11,12,13}. Fruits of lentisk give edible oil which is rich in mono/poly unsaturated fatty acids as OLA and LA¹⁴. Fatty acids have many important biochemical functions such as energetic, metabolic and structural activities¹⁵. Lentisk oil is characterized by its good nutritive quality that it contains 73 %¹⁶ and 69 %¹⁷ of essential PUFA (OLA and LA). Oils with a high proportion of OLA are more stable than others and contribute to reduction in cholesterol and cardiovascular diseases in humans¹⁸. Thus, LA, the most common form of PUFA, is an unsaturated fatty acid ($\omega 6$), vital, involved in lipid metabolism and maintaining the integrity of the integuments¹⁹. It is also effective in reducing blood cholesterol concentrations, HDL-cholesterol and VLDL-cholesterol and lesser reduction of LDL-cholesterol²⁰. The brain is particularly sensitive to oxidative attack because of its high level of unsaturated lipids and high rate of oxidative metabolism²¹, and mercury is one of the agents which inhibits lipid synthesis in the nervous system²², causing a reduction in tissue cholesterol levels²³. *P. lentiscus* oil could be used as a reserve to replace the damaged unsaturated fatty acids in cell membranes. LA is an essential fatty acid; the high content of LA makes oil very important to the industry such as protective coatings, plastics, urethane derivatives, surfactant, dispersants, cosmetics, lubricants and varieties of synthetic intermediate, stabilizers in plastic formulations and in the preparations of other long chain compounds²⁴. The fatty acids profile is a main determinant of the oil quality. Fats are used in the food industry for production of a wide variety of products, ranging from margarines to chocolate or used directly as salad and cooking oils. The use of the oil in industry is determined by the composition of fatty acids and this is highly dependent on its natural origin^{25, 26}. Several studies have been performed to determine the phytochemical composition of the leaves, the fruits and the resins of lentisk grown *in vivo*^{27, 28,29, 30}. However, a comparative investigation on fatty acid composition of the different organs of lentisk trees such as leaf, stem and root and fruits of female and male trees has not been carried out although there are only three studies on the oil and fatty acid composition^{14, 27, 30}. Therefore, the

aim of this study is to learn more about fatty acid composition of the different organs of *in vivo* and *in vitro* grown lentisk. This research is a part of our investigations on exploiting fatty acids and bioactive natural products with prospects for using them in industrial applications.

Materials and methods

Plant materials

The plant material of *P. lentiscus* L. was collected from Çiftlikköy district around the vicinity of the Çesme peninsula in the province of Izmir. The plants were identified by Dr. A. Selçuk Ertekin from Department of Biology, Faculty of Science, the University of Dicle. Samples [fruits, leaves, roots, and stems] are dried at room temperature, and kept separately.

Production of *in vitro* regenerants

Shoot tips of mature-male tree of *P. lentiscus* L. were collected from natural growing areas in Çesme. Shoot tips were surface sterilized by immersion in a 5 % [v/v] sodium hypochlorite solution (Commercial Axion) for 5 min. Then, the shoots were washed three times with sterile distilled water before being placed in contact with 50 ml of MS³¹ medium containing 1.0 mg/l BA (benzyl amino purine) and 30 g/l sucrose for proliferation in Magenta vessels (GA-7, Sigma Ltd.). Four weeks after culture, the actively proliferating shoots were obtained. By repeating the process of cutting and subculturing the shoot tips from *in vitro* generated shoots, a large number of shoots were proliferated to identify the fatty acids in the total lipids.

Lipid extraction and esterification of fatty acids from the explants

The air-dried *in vivo* and *in vitro* samples was grounded. From each of the milled samples, 5 g was taken and stored in chloroform-methanol (2:1, v/v) at room temperature in order to extract lipids from plant³². Autoxidation of unsaturated components was minimized by adding 50 μ l of two percent butylated hydroxytoluene (BHT) in chloroform to each sample during the extraction process. Total lipid extracts were dried under a stream of N₂. Then, associated fatty acids were transmethylated by refluxing in acidified (sulfuric

acid) methanol for 90 min at 85°C. Fatty acid methyl esters (FAME) were extracted from the reaction vials three times with hexane and concentrated.

Gas chromatography conditions

FAME were analyzed by capillary gas chromatography using a Shimadzu GC-2010 Plus equipped with a flame ionization detector (FID) and a fused silica capillary column (DB-23) (Bonded 50 percent cyanopropyl, 30 m x 0.25 mm, 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA). The flow rates of compressed air and hydrogen were 300 ml/min and 30 ml/min, respectively. The carrier gas was helium, at a flow through the column of 0.50 ml/min. The split ratio was 1:20. The temperature profiles were as follows: initial temperature, 170°C (initial time, 2 min); final temperature, 210°C; the injector temperature was maintained at 250°C; and the flame ionization detector (FID) at 250°C.

The fatty acids were identified by comparisons

of their retention times with those of standard purified fatty acids (Sigma Chemical Co., St., Louis, MO, USA).

Statistical analyses

All the analysis was conducted in triplicate. The values of different parameters were expressed as the mean values. Statistical analyses were performed using statistical package SPSS 13.0 for windows. Significance was determined by analysis of variance (ANOVA) and comparisons for two groups were made with Student's *t*-test (two-tailed). The level of significance was defined as $P \leq 0.05$.

Result and discussion

Fatty acids (FA) composition of the different organs of male and female trees grown in vivo

The major fatty acid was represented by PUFA accounting for 56.94 %, 64.44 % and 55.57 % in root, leaf and stem part of male tree, respectively (Table 1). It was followed by SFA accounting

Table 1. Fatty acid compositions of different organs of male and female lentisk grown *in vivo**

Fatty Acids**	Female: Type of organ***			Male: Type of organ***		
	Root [%]	Leaf [%]	Stem [%]	Root [%]	Leaf [%]	Stem [%]
C14:0	0.38 a	4.79 b	0.62 c	0.46 a	6.10 b	2.95 c
C15:0	0.17	nd	nd	0.17 a	nd	0.48 b
C16:0	21.60 a	21.12 a	21.28 a	19.27 a	19.29 a	26.86 b
C17:0	0.10 a	nd	0.06 b	0.22	nd	nd
C18:0	1.63 a	0.87 b	1.03 c	1.54 a	1.11 b	1.10 b
ΣSFA	23.90	26.79	23.01	21.68	26.51	31.40
C16:1n-7	0.53 a	3.61 b	0.26 c	0.31 a	2.20 b	nd
C18:1n-9	19.76 a	8.03 b	19.43 a	20.65 a	6.83 b	13.02 c
C20:1n-9	0.45	nd	nd	0.39	nd	nd
ΣMUFA	20.74	11.64	19.70	21.37	9.04	13.02
C18:2n-6	45.10 a	24.77 b	45.25 a	47.00 a	28.87 b	40.78 c
C18:3n-3	10.25 a	36.78 b	12.02 a	9.94 a	35.57 b	14.78 c
ΣPUFA	55.34	61.55	57.27	56.94	64.44	55.57

*Each value is mean of a triplicate analysis

**SFA: saturated fatty acids

MUFA: monounsaturated fatty acids

PUFA: polyunsaturated fatty acids

C number of carbon atoms in the fatty acids

***Indicates that means in a row followed by the same lowercase letters are not statistically different at $P \leq 0.05$ level of significance according to the Student's *t* test

for 21.68 %, 26.51 %, 31.40 %, and MUFA accounting for 21.37 %, 9.04 %, 13.02 % in root, leaf and stem part of male tree, respectively (Table 1). Similar to male genotype, the order of the major fatty acids were PUFA, SFA and MUFA for female genotype. Leaves of both genotypes had the highest total PUFA and SFA contents and the MUFA contents were the lowest in leaves of both genotypes. Significant differences were determined between the groups (root, leaf and stem) for the individual fatty acids in the both genotypes. Ten different fatty acids were identified, with their carbon numbers ranging from C14:0 to C20:1n-9. Generally, LA, PALM, OLA and ALA were the major components in the oils identified from the different organs of both the female and male *P. lentiscus* L. trees. In the different parts of male tree, regarding LA, higher percentages were obtained from root (47.00 %) and stem (40.78 %) than leaf (28.87 %) section. The major fatty acid of leaves of both genotypes was with an amount of 35.57 % for male and 36.78 % for female. In plants, leaf lipids usually contain large proportions of C18:3n-3, which is an important component of chloroplast membrane polar lipids. Mammals who feed on these plants convert C18:3n-3 to EPA and DHA, the long chain omega-3 fatty acids found in fish³³. In this study, LA and ALA are determined as major fatty acids, which have to be in human diets because they cannot be synthesized by human. Similar results were reported for several plant species^{15,34,35}. PALM, an intermediate in biosynthesis of sexual pheromones of some insects, was found at almost similar percentages in the root, leaf and stem section of male or female tree, but the amount of PALM was significantly higher in the stem of male tree. OLA occurred at the lowest proportions in leaf of male and female trees, contributing to approximately 6.83 % and 8.03 % of the total fatty acid content, respectively. It was the highest in root of male tree, with a proportion of 20.65 %. However, the amounts of OLA in the root and stem of a female tree were similar. Lower levels of fatty acids were also detected in both genotypes studied as traces such as C17:0, C16:1n-7, C14:0, C15:0, C18:0, and eicosenoic acid (C20:1n-9) was detected only in the root oils of both genotypes,

0.45 % in female and 0.39 % in male genotype. These results were similar to those reported by Charef *et al.*³⁰. Leaves of both genotypes contain higher amounts of myristic acid (14:0) and palmitoleic acid (16:1n-7) than their root and stem parts. LA amounts determined in root and stem was higher than leaves of both genotypes. These results show that quantitative fatty acid composition of leaves of both genotypes differs from root and stem parts. The reason for this may be the function of these organs in the plant; leaves are very rich in chloroplasts containing chlorophyll for photosynthesis. Roots and stems anchor the plant to the ground and support the body and stem carry up nutrients where it is necessary.

Fatty acids composition of the different organs of male trees grown *in vitro*

The fatty acid compositions of oil from different male organs of *P. lentiscus* L. produced *in vitro* are summarized in Table 2. The prominent class of fatty acid composition of different male organs of lentisk regenerated *in vitro* was represented by PUFA accounting for 63.24 % for leaf and 48.51 % for stem. It was respectively followed by SFA with 27.17 % and 38.49 % and by MUFA with 9.58 % and 12.98 % for leaf and stem (Table 2). The amount of the C16:0 and C18:0 was high in stem parts of lentisk because the total amount of SFAs in stem was higher than leaf part of *in vitro* grown tissues. Eight different fatty acids were identified, with their carbon numbers ranging from C14:0 to C18:0. Similar to *in vivo* grown materials of male tree, ALA, LA, PALM, OLA were the major components in the oils identified from leaf and stem sections of male trees grown *in vitro* (Table 2). The major fatty acid in leaf was ALA with an amount of 32.06 %. This fatty acid is reported for its role in its nutritional value³⁶. LA which is an essential FA (EFA) accounted for 31.17 % of whole fatty acid. Furthermore, PALM was determined at a significant percentage of 23.10 %. LA had favorable nutritional implications and beneficial physiological effects in the prevention of coronary heart disease and cancer³⁷. Minor amounts of C14:0, C15:0, C16:1n-7, C17:0, C18:0 are also detected in the leaf and stem sections of

Table 2. Fatty acids composition of different male organs of lentisk regenerants produced *in vitro**

Fatty Acids**	Type of organ***	
	Leaf [%]	Stem [%]
C14:0	1.66 a	1.43 b
C15:0	0.28 a	0.36 a
C16:0	23.10 a	31.94 b
C18:0	2.12 a	4.74 b
ΣSFA	27.17	38.49
C16:1n-7	1.00 a	0.60 b
C18:1n-9	8.58 a	12.38 b
ΣMUFA	9.58	12.98
C18:2n-6	31.17 a	32.55 a
C18:3n-3	32.06 a	15.95 b
ΣPUFA	63.24	48.51

*Each value is mean of a triplicate analysis

**SFA: saturated fatty acids

MUFA: mono unsaturated fatty acids

PUFA: poly unsaturated fatty acids

C number of carbon atoms in the fatty acid

***Indicates that means in a row followed by the same lowercase letters are not statistically different at $P \leq 0.05$ level of significance according to the Student's t-test

in vitro grown male trees. The percentages of ALA and LA determined from *in vivo* grown leaf and stem samples were similar to *in vitro* grown leaf and stem organs (Table 2). These results show that quantitative fatty acid composition of leaf and stem grown *in vitro* shows a similar trend of *in vivo* grown leaf and stem of a female tree (Table 2).

Fatty acids composition of the mature and immature seeds collected from two female genotypes

The major class of fatty acid was represented by MUFA accounting for 52.78 % in black fruit (mature) and 49.33 % in the red fruits of the genotype 1. It was followed by PUFA accounting for 27.21 % and 29.37 %, and by SFA accounting for 20.0 % and 21.28 % in the black and the red fruits of the genotype 1, respectively (Table 3). Thirteen different fatty acids were identified in the seed oils (Table 3). Contrary to the different organs of male and female, fatty acid composition is identical for the genotypes collected from

different regions. *P. lentiscus* L. given in Table 1, only three major fatty acids (OLA, LA and PALM) were the major components in the oils identified from mature and immature seeds of two genotypes. However, the reported fatty acid composition in the mature and immature seeds showed identical values for major fatty acids in the black and red fruits of *P. lentiscus* L. reported by Charef *et al.*³⁰. The unsaturated fatty acids like C18:1n-9 and C18:2n-6 were found in the oils of the two genotypes studied. C18:1n-9 was determined to be the dominant fatty acid constituent, ranging from 48.37 to 51.58 % in the oils from the genotype 1 and 40.45 to 46.45 % in the oils from the genotype 2. For the C18:2 fatty acid, the content was from 26.31-27.34 % in the oils of the genotype 1 and 31.09-37.64 % in the oils of the genotype 2. Concerning the SFA in the oils of the two genotypes are PALM and stearic (STA); however, PALM was the major SFA constituent, ranging 18.63-19.39 % in the oils of the genotype 1 and 17.12-19.11 % in the oils of the genotype 2. C14:0, C15:0, C16:1n-7, C17:0, C18:0,

Table 3. Fatty acids composition of seeds of two female genotypes of *P. lentiscus* L. collected from the Çesme Peninsula, Turkey*

Fatty Acids**	Genotype 1***		Genotype 2***	
	Mature Seeds [%]	Immature Seeds [%]	Mature Seeds [%]	Immature Seeds [%]
C14:0	0.04 a	0.14 b	0.05 a	0.20 b
C15:0	0.01 a	0.05 b	0.01 a	0.03 b
C16:0	18.63 a	19.39 a	19.11 a	17.12 a
C17:0	0.08 a	0.10 b	0.14 a	0.11 a
C18:0	1.22 a	1.58 b	0.76 a	1.47 b
ΣSFA	20.00	21.28	20.09	18.95
C16:1n-7	0.99 a	0.80 a	1.14 a	1.19 a
C18:1n-9	51.58 a	48.37 b	46.45 a	40.45 a
C20:1n-9	0.20 a	0.16 a	0.17 a	0.36 b
ΣMUFA	52.78	49.33	47.78	42.01
C18:2n-6	26.31 a	27.34 a	31.09 a	37.64 b
C18:3n-3	0.75 a	1.51 b	0.94 a	1.39 b
C20:2n-6	nd	0.05	0.01	nd
C20:3n-6	nd	0.21	nd	nd
C20:4n-6	nd	nd	0.01	nd
C20:5n-3	0.14 a	0.24 b	0.07	nd
ΣPUFA	27.21	29.37	32.12	39.03

*Each value is mean of a triplicate analysis

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C number of carbon atoms in the fatty acids

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C18:3n-3, C20:1n-9 and C20:5n-3 were found in the seeds of both genotypes in very tiny amounts that did not exceed 1.22 % (C18:0) in the mature seeds and 1.58 % immature seeds (C18:0). OLA, LA and PALM are the major fatty acids reported in mature (black) and immature (red) fruit oils of *P. lentiscus* L. Our results for mature and immature fruits of *P. lentiscus* fruit oil agree with the data recorded by Charef *et al.*³⁰. ALA was present at very low values in the mature and immature fruits of both genotypes. This is in agreement with the results reported by Trabelsi *et al.*²⁷ for mature seeds, but not immature ones. It was noted that this low level of ALA concentration has been produced by genetic modifications in the desaturation step from LA to ALA controlled by

omega-3 fatty acid desaturases³⁸. It should be noted that the MUFA such as OLA have great importance because of their nutritional implication and effect on oxidative stability of oils³⁹ and the intake of ALA in the diet protected against fatal ischemic heart disease⁴⁰.

Fatty acids composition of the resin of male and female lentisk

Resin is a natural product of *P. lentiscus* L., used as active additives in pharmaceuticals and cosmetics. Seven fatty acids were identified in the resin oils of the both genotypes (Table 4). Similar to other materials of male or female lentisk tree, ALA, LA, PALM, OLA were the major components in the oils identified from resins of male

Table 4. Fatty acid composition of the resin of male and female lentisk *

Fatty acids	Mastic gum	
	Female [%]	Male [%]
C14:0	4.77 a	2.33 b
C16:0	38.11 a	33.85 b
C18:0	4.14 a	0.46 b
ΣSFA	47.02	36.64
C16:1n-7	0.52 a	2.84 b
C18:1n-9	6.18 a	9.12 b
ΣMUFA	6.70	11.96
C18:2n-6	40.84 a	46.32 b
C18:3n-3	5.43 a	5.04 a
ΣPUFA	46.27	51.36

*Each value is mean of a triplicate analysis

**SFA: saturated fatty acids

MUFA: mono unsaturated fatty acids

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C number of carbon atoms in the fatty acids

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and female trees. The unsaturated fatty acids, C18:1n-9 and C18:2n-6 were determined in the oils of both the type of resins. C18:2n-6 was determined to be the dominant fatty acid constituent, accounting for 40.84 % and 46.32 % in the oils from the resins of male female genotype, respectively. For the C18:1n-9, the content was from 6.18 % and 9.12 % in the oils from the resins of female and male lentisk. Concerning the SFA in the oils of the two genotypes, PALM was determined to be dominant SFA constituent in the oils from both types of resins. It is known that a high level of PAM is required for the production of margarine, shortening and other fat products ⁴¹.

The percentages of the major and minor oils have similar but not identical fatty acid composition in the different genotypes, resins and organs (root, leaf, stem) of lentisk grown *in vivo* and *in vitro*. The PUFA /SFA ratio was above 1.5 in all data (oil percentages from different organs of male and female lentisk trees) presented in Table 1. According to current dietary guidance for healthy nutrition, PUFA/SFA ratio above 1.5 is associated with good health ⁴².

Our study shows that the oil can have beneficial

health effects in the different organs and seeds of some genotypes grown *in vivo* and leaves of *in vitro* grown male tree compared to other types (resin and seeds of some genotypes). The unsaturated/saturated ratios for mature and immature seeds presented in this study was also reported another genotype of lentisk ³⁰ and acorn ⁴³. Our study of the fatty acid composition in the leaf and stem sections of both *in vivo* (Table 1) and *in vitro* (Table 3) grown male trees showed identical values for OLA. OLA has great importance because of their nutritional implication and effect on oxidative stability of oils ⁴⁴. Apart from resin, ALA, which is an important component of chloroplast membrane polar lipids, is one the major components in the oils identified from the different organs of male and female trees in both *in vivo* and *in vitro* grown. Mammals who feed on ALA containing plants convert 18:3 to EPA and DHA, the long-chain omega-3 fatty acids found in fish ³³. The results of this study also shows us the nutritional potential of the oil of *P. lentiscus* materials (leaf, stem, root, seeds and resin), which can offer opportunities for rational exploitation for medical purposes and in the food industries. This study also give us basic informa-

tion for the establishment of tissue cultures in order to produce large scale cell suspensions. Further information on the composition properties of lentisk regenerants would assist in efforts to achieve more industrial applications for health, cosmetics and functional food composition of this plant.

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