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Influence of harvest date on fatty acid composition and antioxidant activity of *Pistacia lentiscus* L. edible oils

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Abstract

Pistacia lentiscus seed oil is a natural product widely used for culinary and medicinal purposes in Algerian and Tunisian forest areas. Until now, the quality of this oil is still not well known. This study aims to optimize its quality by determining the best period to harvest fruits. Quality criteria include fatty acid composition and antioxidant activity of this oil. Fruits were harvested during three maturity stages: unripe, ripe and over ripe. Oleic acid was the major fatty acid (more than 40%), followed by palmitic (22.28-28.6%) and linoleic (13.63-20.5%) acids. Antioxidant activity was estimated using DPPH scavenging ability and Trolox Equivalent Antioxidant Capacity (TEAC) assays. Oil extracted from the pulp at over-ripeness showed the highest percentage of inhibition of DPPH with about 37% corresponding to 80 ng of Trolox/g of oil.

Keywords: antioxidant activity, fatty acids, oil, *Pistacia lentiscus* L., ripeness.

1. Introduction

In the nutrition and health fields, fat is considered the most energy-dense nutrient; it serves as an important source of energy in the absence of glucose [1]. For this reason, there is a continuous increase in the intake of fats, and particularly vegetable oils such as olive, sunflower and palm oils. In the food industry, there is a tendency to search for new sources of oil that may have nutritional value.

Pistacia lentiscus fixed oil is edible oil used in the Tunisian forest area for culinary and medicinal purposes. In traditional medicine, it is especially used to heal wounds and cure gastric diseases. It is also used for liver diseases, dermatological troubles and as a treatment for cough and asthma [2]. Scientifically, there is little information about the biochemical and biological properties of this oil. The fatty acid composition was determined by Tej-Yaakoubi and Dhaou [3] and the effect of ripeness on these compounds was studied by Trabelsi et al. [4]. Analysis of its glycerophosholipid content was recently studied by Trabelsi et al. [5]. Concerning the biological properties of this natural product, the wound healing effect of the unsaponifiable fraction of this oil was tested by Boulebda et al. [6] and its protective effect against poisoning by some heavy metals was determined by Maarouf et al [7].

This study aims to optimize the quality of this oil by determining its fatty acid composition and its antioxidant activity relative to the different parts of the fruit and the harvest date (maturation stage). This study is original due to the fact that antioxidant activity and fatty acid composition of the different parts of the fruit of this species have not been studied before.

2. Material and methods

2.1. Plant material

Pistacia lentiscus L. fruits were harvested from wild plants in the Ain Draham region located in the northwest of Tunisia (N 36°42'; E 8°41'). The plant was identified by Dr A. Khaldi from I.N.R.G.R.E.F-Tunisia and voucher specimen (VS1-PL2009) was deposited at the Herbarium of I.N.R.G.R.E.F. Fruit was harvested in three phenological stages: unripe (October 2009: green-red), ripe (December 2009: majority black) and over-ripe (January 2010: fully black). The oil was extracted separately from the whole fruit, pulp and seeds.

2.2. Oil Extraction

Oil was extracted by a Soxhlet apparatus for 6 hours using hexane as a solvent. The final extract was obtained after concentration and solvent removal by evaporation at 60°C. The oil was then stored in cold darkness for later chemical analysis. The oil content was determined according to ISO method 659:1998 [8].

2.3. Fatty acid methyl ester preparation and gas chromatographic analysis

Methyl esters of the fatty acids were prepared by the saponification and esterification procedure described by Metcalfe et al. [9] and injected into a gas chromatograph coupled with mass spectrometry (GC/MS) (Agilent, France) equipped with a capillary column of type HP5 MS, 30 m long and with a 250 µm internal diameter; the thickness of the film was 0.250 µm. The temperature of the injector was 250 °C. The vector gas was helium and its flow fixed at 0.8 ml/min. Analyses were conducted in Split mode (50:1) with a temperature of 150°C (0min) - 5°C / 240°C.

2.4. DPPH radical scavenging activity

The DPPH radical scavenging activity was measured using the method described by Brand-Williams et al. [10]. 50 µl of oil in ethanol (50 µl/950 µl by volume) was added to 1 ml of ethanolic DPPH solution (60 µM). The decrease in absorbance at 517 nm was measured after one hour of incubation in darkness at room temperature.

The radical scavenging activity was expressed as the inhibition percentage (IR) and monitored using the following equation:

$$\mathbf{IR = [(AC-AS) / AC] \times 100}$$

AC: absorbance of control (ethanol) and AS: absorbance of sample solution.

2.5. Trolox Equivalent Antioxidant Capacity

The TEAC (Trolox Equivalent Antioxidant Capacity) assays were carried out with different concentrations of Trolox (1; 0.25; 0.125; 0.06; 0.03 and 0.01 mg/ml) as a standard reference compound. A linear relationship was established for the decrease in absorbance versus Trolox concentration. The percentage of DPPH inhibition was determined and then reported to Trolox Equivalent values using this linear relationship [11].

2.6. Statistical analysis

Data processing was done using the GLM procedure (General Linear Models) of the SAS (9.0) program. An analysis of variance of the studied parameters was performed. Significant correlations were retained.

3. Results and discussion

3.1. Oil content

The accumulation of oil in *P. lentiscus* fruits during maturation is reported in figure 1. A gradual increase of the oil content was found. This result is similar to that found by Trabelsi et al. [5]. Differences in oil content were marked at different stages of ripening and for the different constitutive parts of the fruit. During the unripe harvest, a low lipid accumulation was noted for all the studied oils. The pulp showed the highest amount of oil during the three stages of ripeness. This amount varied from 11.95% (unripe) to 45.97% (over-ripe). The lowest oil content was reached by the oil extracted from seeds. Its contents were respectively 4.37%, 9.66% and 14.84% for the unripe, ripe and over-ripe stages.

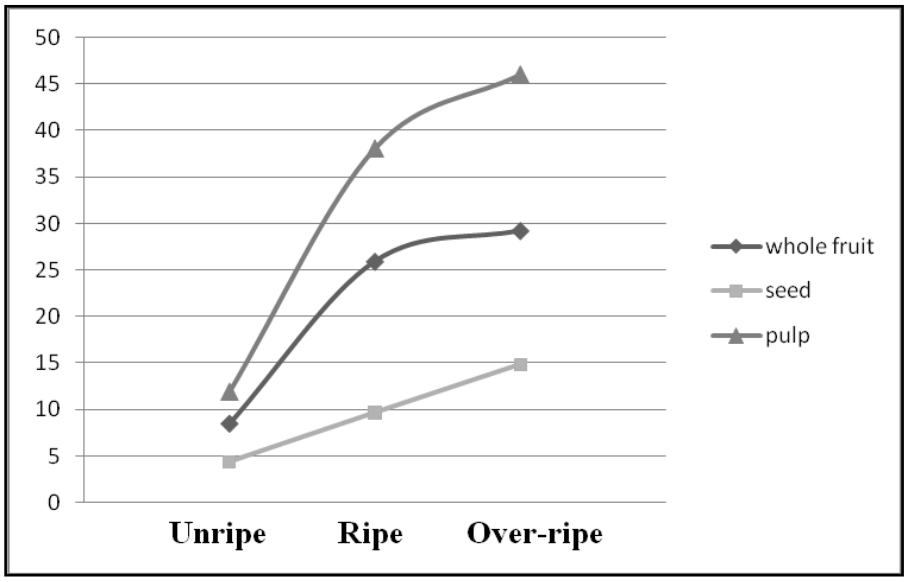

Figure 1. Changes in the oil content of the *Pistacia lentiscus* constutitive parts of fruit during maturation

3.2. Fatty acids composition

The results of fatty acid composition of oils extracted from pulp, seeds and whole fruit are summarized in the Table below. The nine oils studied contain the same five main fatty acids with significant differences of concentration. These fatty acids are oleic (C18:1), palmitic (C16:0), linoleic (C18:2), stearic (C18:0) and palmitoleic (C16:1) acids. These results are concordant with our previous study and with Tej- Yaakoubi and Dhaou's [3] findings which showed that oleic is the main fatty acid in *Pistacia lentiscus* oil followed by palmitic and linoleic acids. On the other hand, the results of this study showed that oleic, palmitic and stearic acid rates have significant variations according to the three stages of ripeness. The highest rate of oleic acid was observed in the oil extracted from the ripe pulp (55%). These data agree with those produced by other authors on oleaginous species such as olive trees [12].

Table: Fatty acid composition of *Pistacia lentiscus* L. fatty oils during ripeness (%)

	Unripe			**Ripe**			**Over ripe**		
	Fruit	Pulp	Seed	Fruit	Pulp	Seed	Fruit	Pulp	Seed
Oleic	49.5±3	47.7±0.9	43.20±2.7	53.3±1.7	55.08±2	44.87±4	51.36±2.4	52.65±1.7	50.4±4
Palmitic	22.45±1	23.8±0.5	22.28±1.5	28.6±1.3	27.71±1	24.73±0.4	27.26±1.2	26.97±1	23.4±3
Linoleic	19.3±5.6	19.4±3.2	20.25±5.3	13.7±3.4	13.63±3	16.72±6.5	16.6±3.8	16.46±2.4	20.5±2
Palmitoleic	1.34±0.5	1.44±0.4	0.83±1.1	1.3±0.43	0.78±0	0.76±0.07	1.29±0.4	1.37±0.4	1.3±0
Stearic	2.54±0.4	2.4±0.09	4.18±0.6	1.39±0.0	0.73±0	2.95±0.61	1.25±0.1	1.09±0.0	2.6±0
∑saturated	24.99	26.23	26.46	30.04	28.44	27.68	28.51	28.06	26.12
∑unsaturated	70.14	68.65	64.28	68.47	69.49	62.35	69.25	70.48	72.41
Unsaturated/ saturated	2.80	2.61	2.42	2.27	2.44	2.25	2.42	2.51	2.77

Values represent percentage mean ± standard deviation.

Unripe: oil extracted from unripe fruits (harvested in October 2009)

Ripe: oil extracted from ripe fruits (harvested in December 2009)

Over ripe: oil extracted from over ripe fruits (harvested in January 2010)

Unripe seed oil was the richest in stearic acid (4%). Decrease of the stearic acid content the riper the fruit (from 4 to 2% for seed oil, for example) is accompanied with an increase in the oleic acid rate (from 43 to 50% for the same oil). This trend can be explained by the fact that stearic acid is the precursor of the biosynthesis of oleic one. The reaction is carried out by the stearyl desaturase, the only desaturase able to synthesize oleic acid in plant cells [13].

Palmitic acid is the most important saturated acid. Its rate increases with maturation and the highest values are reached by oils extracted from ripe and over-ripe samples. This increasing rate observed during the maturation process was already demonstrated by Trabelsi et al. [4]. *Pistacia lentiscus* oil is relatively rich in palmitic acid content reaching 28% of total fatty acids, when compared with other edible oils such as olive oil (7.5-20%) and sunflower oil (5-6%) [14,15]. This saturated acid has been thought for many years to raise cholesterol levels if consumed, although a Canadian study examined the effects of high consumption of palmitic acid in healthy volunteers and concluded that it does not raise cholesterol if it is combined with linoleic acid, as is the case of the studied oil [16].

The mean content of total unsaturated fatty acids is 62% to 70%, on average. The high unsaturated to saturated fatty acid ratio indicates that this oil can have beneficial health effects, especially for protecting against cardio-vascular diseases [17]. Specific epidemiological studies [18] that have controlled for a number of potentially confounding variables have also reported the protective effects of monounsaturated fatty acids against coronary heart diseases.

3.3. Determination of the antioxidant activity

Antioxidant activity, determined by both DPPH radical scavenging activity and Trolox Equivalent Antioxidant Capacity (TEAC), was significantly different between the studied oils (Figure 2 (a,b)).

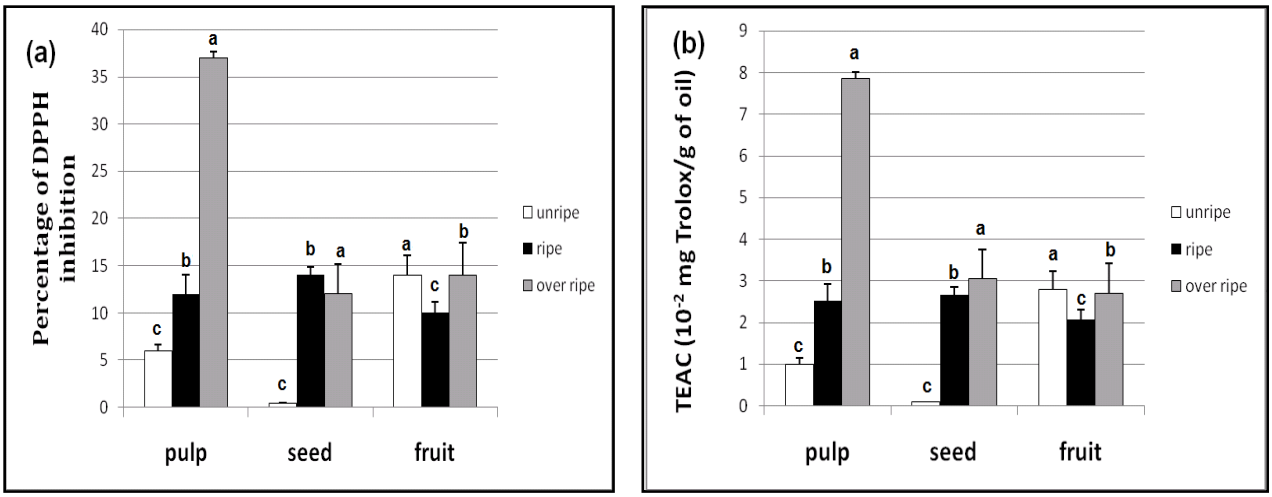

Figure 2: Antioxidant activity of *Pistacia lentiscus* fatty oils.

(a) Percentage of DPPH inhibition values (b) TEAC values

Unripe: oil extracted from unripe fruits (harvested on October 2009)

Ripe: oil extracted from ripe fruits (harvested on December 2009)

Over ripe: oil extracted from over ripe fruits (harvested on January 2010)

The highest antioxidant activity was obtained with the oil extracted from the over-ripe pulp (37% corresponding to 80 µg of Trolox/g of oil), followed by that extracted from the unripe whole fruit (14% corresponding to 28 µg of Trolox/g of oil). In contrast, the lowest antioxidant power was found coming from the unripe seed oil (0.4% corresponding to 0.9 µg of Trolox/g of oil).

This antioxidant power could be explained by the existence of natural antioxidants, including phenolics. These compounds have a hydroxyl group (OH) enabling them to scavenge free radicals and giving them an important antioxidant capacity. The "deactivation" of oxidant species by polyphenolic antioxidants (POH) is based, with regard to food systems that are deteriorated by peroxyl radicals (R•), on the donation of hydrogen, which interrupts chain reactions. Phenoxyl radicals (PO•) generated according to this reaction may be stabilized through resonance and/or intramolecular hydrogen bonding or combine to yield dimerisation products [19]. Compared to other edible oils, such as olive oil, *P. lentiscus* showed the highest radical scavenging activity compared to Gorinsteina et al. [20] whose findings showed that the percentage of DPPH inhibition of virgin olive oil was about 29%. Notwithstanding the different constitutive parts of the fruit, the most important antioxidant activity values were recorded at over-ripeness and the lowest values were noted for the unripe samples.

This increase in antioxidant activity during the process of maturation is probably due to a proportional increase in the rate of phenols. Several studies on different plant species have demonstrated the existence of a significant correlation between antioxidant activity and the rate of phenols [19,21,22]. Similarly, the difference recorded between antioxidant activities of the studied oils suggests a difference in their phenolic composition. According to the study by Grati-Kammoun et al. [23] done on olive oil, this variation depends of the nature of phenols; some phenols increase while others decrease during fruit maturation.

For the over-ripe samples, the antioxidant activity of whole fruit oil (seed + pulp) was significantly lower than pulp oil (14% and 37%, respectively). This decrease could be attributed to the presence of seeds during the extraction process. This finding supports previous research into this area which links the presence of stones and the decrease in the rate of phenols in olive oil. Servilli and Montedoro [24] have demonstrated that stone-related changes could be associated with its endogenous enzymes. This is related to oxidative reactions catalyzed by endogenous oxidoreductases such as polyphenoloxidase which is highly concentrated in the seeds. This enzyme can induce phenolic oxidation during processing.

Conclusion

Results of this study showed that *Pistacia lentiscus* seed oil is rich in unsaturated fatty acids, accounting for more than 70% of the total composition of fatty acids. This edible oil has high antioxidant activity. These biochemical properties reflect great nutritional value. This can make it possible to better use and add value to this non-wood forest product, especially in the pharmaceutical and nutritional fields. This investigation can help determine the best harvesting periods and, at the same time, optimize oil quality in order to promote its economic value.

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References

1. German J.B., Dillard C.J. *Crit. Rev. Food Sci. Nutr*. 46 (2006) 57-92.
2. Mezghani S. L'exploitation traditionnelle du maquis au nord de la Tunisie : possibilité d'une meilleure utilisation, Office de l'élevage et des pâturages, (1992).
3. Tej Yaakoubi M., Dhaou S. *Revue des régions arides*. 1 (2007) 30.
4. Trabelsi H., Aicha Cherif O., Sakouhi F., Villeneuve P., Renaud J., Barouh N., Boukhchina S. Mayer P. *Food Chem*. 131 (2012) 434.
5. Trabelsi H., Renaud J., Herchi W., Khouja M.L., Boukhchina S., Mayer P. *JAOCS.* 90 (2013) 611-618.
6. Boulebda N., Belkhiri A., Belfadel F., Ben Seguini A., Bahri L. *Pharm. Net. World*. 1 (2009) 66.
7. Maarouf T., Cherif A., Housaine N. *Eur. J. Sci. Res*. 24 (2008) 591-600.

8. ISO International Standard ISO 659:1998. Oil seeds determination of hexane extract (or light petroleum extract), called ‘‘oil content’’. ISO, Geneva, (1999).
9. Metcalfe L.D., Schimitz A.A., Pelka J.R. *Anal. Chem.* 38 (1996) 514.
10. Brand-williams W., Cuvelier M.E., Berset C. *Food Sci. Technol*. 28 (1995) 25.
11. Barton H., Folta M., Zachwieja Z. *Now. Lekar*. 74 (2005) 510.
12. Ranalli A., Pollastri L., Contento S., Di loreto G., Iannucci E., Lucera L., Russi, F. *J. Agric. Food Chem*. 50 (2002) 3775.
13. Trémolières A. Les lipides végétaux: Voies de biosynthèse des glycérolipides, Boeck Supérieur, (1998).
14. French M.A., Sundram K., Clandinin M.T. *Asia Pac. J. Clin. Nutr*. 11 (2002) 401.
15. León L., Uceda M., Jiménez A., Martín L.M., Rallo L. *SJAR*. 2 (2004) 353-359.
16. Turhan H., Citak N., Pehlivanoglu H., Mengul Z. *Bulg. J. Agric. Sci*. 16 (2010) 553-558.
17. Bourre J.M. *J. Nutr. Heal. Aging*. 8 (2004) 163.
18. Artaud-Wild S.M., Connor S.L., Sexton G., Connor W.E. *Circulation.* 88(1993) 2771–2779.
19. Stellman J.M., Dufresne C. Encyclopédie de sécurité et de santé au travail. International labour organisation, (2000).
20. Gorinsteina S., Martin-Bellosob O., Katricha E., Lojekc A., Cizc M., Gligelmo-Miguelb N., Haruenkitd R., Parke Y., Jungf S., Trakhtenbergg S. *J. Nutr. Biochem.* 14 (2003) 154.
21. Alali F.K., Tawahax K., El-elimatz T., Syouf M., El-fayad M., Abulaila K., Joy Nielsen S., Wheaton W.D., Falkinham J.O., Oberlies N.H. *Nat. Prod. Res*. 21 (2007) 1121.
22. Zhang Y., Wang Z. *C.R. Biol*. 332(2009) 816–826.
23. Grati-Kammoun N., Khlif M., Ayadi M., Rekik H., Rekik B., Hamdi M.T. *Revue Ezzaitouna* 5 (1999) 1-2.
24. Servilli M., Montedoro G. *Eur. J. Lipid Sci. Technol*. 104 (2002) 602–613.

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