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Identification and quantitation of tocopherols, carotenoids and triglycerides in edible *Pistacia lentiscus* oil from Tunisia

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Abstract

The fruits of *Pistacia lentiscus* are a source of a fixed oil used in some forested areas for culinary purposes and in traditional medicines. The aim of this study was to evaluate the levels of tocopherols, carotenoids and triglycerides in this oil using high-performance liquid chromatography (HPLC). The results demonstrated the presence of two vitamin E isomers; α- and γ-tocopherols, at respective concentrations of 119 mg and 23 mg per kg of oil. β-carotene, lutein and zeaxanthin were the principal carotenoid molecules identified. β-carotene was the main molecule present, with a level of 6.13 mg/kg oil. Di-unsaturated triglycerides comprised more than half of the total triglycerides (TAG) present. Dioleopalmitin (OOP), palmitooleolinolein (POL) and triolein (OOO) were the principal TAG molecules, at respective percentages of 26%, 17% and 13%. This study highlighted the nutritional value of this non-wood forest product and therefore increases the possibility of its use by the food industry.

1. Introduction

The Mediterranean diet, which is considered as a healthy model, is characterized by an abundance of plant-based foods. Olive oil is the best example and is the principal source of fat in this diet. This oil, which is rich in unsaturated fatty acids, plays an important role in the prevention of cardiovascular diseases and cancers [1]. As well as olives and olive oil, the Mediterranean region is home to a wealth of plants that are a source of vegetable oils of considerable nutritional value. *Pistacia lentiscus* L. is one of these plants. It is a wild species that is widespread in the Mediterranean region. The fruits of this species contain a fixed oil that is widely used and appreciated by the inhabitants of some forested areas in Sardinia, Algeria and Tunisia. In addition to its culinary uses, this oil is used locally in traditional medicine for wound healing and the treatment of gastric diseases and asthma. This natural product has been the subject of just a few studies which have described determinations of its antioxidant and antibacterial activities [2], its protective effect against poisoning by some heavy metals [3] and its healing effects [4,5]. The biochemical characterization of this oil has been limited to a few studies that determined its composition in fatty acids and sterols [2,6,-8].

To our knowledge, no studies have yet been published regarding the triglyceride, tocopherol and carotenoid contents in this oil. The aim of the present study was therefore to determine the levels of these substances in *P. lentiscus* fixed oil, which would enable a clearer understanding of its biochemical characteristics and thus highlight its nutritional and pharmacological properties.

2. Material and Methods

2.1. Plant material

Pistacia lentiscus L. fruits were harvested during November from wild plants growing in the Bellif region of north-western Tunisia. Fruits were harvested from at least five trees.

The plant was identified by Dr A. Khaldi from I.N.R.G.R.E.F-Tunisia and certified specimens (VS1-PL2009) were deposited at the Herbarium run by I.N.R.G.R.E.F.

2.2. Oil extraction

The oil was extracted using a pressing method. Briefly, fruits were first of all ground using an ordinary chopper. The resulting paste was mixed for 30 minutes in a water bath and was then placed in a hydraulic press to allow separation of the liquid phase from the meal. All floating oil was removed and stored in under cold and dark conditions for subsequent chemical analyses.

2.3. Quantitative analysis of tocopherols and carotenoids

A 400 mg of oil was accurately weighed into a screw-capped centrifugation tube and then 0.2 g δ-tocopherol (internal standard), 15 ml absolute ethanol and 4 ml of 76% potassium hydroxide solution were added under a stream of nitrogen. The tubes were incubated for 30 min at 70°C. under slow and constant stirring. Five ml sodium chloride (25g/l) were added after cooling and the suspension was extracted three times with 15 ml portions of n-hexane-ethyl acetate (85:15, v/v). The organic phase was evaporated to dryness at 40°C and the residue was dissolved in 0.5 ml methanol. After passing through a 0.45 µm filter, the samples were injected for chromatographic analysis [9].

The HPLC apparatus was a Jasco PU-1580 Plus intelligent pump (JASCO International Co., Ltd., Japan) equipped with an automatic injector system AS300 (Thermo, San José, CA, USA) and a Jasco MD-1510 plus multi-wavelength detector (JASCO International Co., Ltd., Japan). HPLC analyses were carried out using reverse-phase high-performance liquid chromatography (RP-HPLC) with a Nucleosil C18 column (25 x 4.6 mm id, 5 µm particle size) and a VIDAK C18 column (25 x 4.6 mm id, 5 µm particle size). The isocratic solvent system was acetonitrile/methanol at 50 mM ammonium acetate/water/dichloromethane (700:150:50:100, v/v/v/v); the flow rate was 2 mL min^{-1}, and detection was performed at 450 nm for lutein and zeaxanthin and 298 nm for tocopherols. Six quantities of β-carotene (range: 25-1000 ng), lutein (range: 20-500 ng), zeaxanthin (range: 4-500 ng), α-tocopherol (range: 1-20 µg) and γ-tocopherol (range: 40-1600 ng) were injected into the RP-HPLC system (each standard being dissolved in 1 mL of the HPLC mobile phase: acetonitrile/methanol containing 50 mM ammonium acetate/water/dichloromethane (700:150:50:100, v/v/v/v). The linear regression equation for each standard curve was then obtained by plotting the amount of the standard compound injected against the peak surface area. The regression equation and correlation coefficient (r^2) were calculated using ChromNav software (JASCO).

2.4. HPLC analysis of triglycerides (TAG)

50 mg of oil were accurately weighed and then added to 5 ml acetone. The TAGs were then separated by RP-HPLC according to their molecular weight and unsaturation degree, depending on the number of partition NP (or equivalent carbon number: ECN).

The HPLC apparatus used was a Spectra System P1000XR pump (Thermo, San José, CA, USA) and an RID-10A refractive index detector (Shimadzu, Japan). TAG separation was carried out by RP-HPLC with a Hypersil-Kestone C18 column (4.6 x 150, 5µm particle size) and Kinetex C18 column (4.6 x 150, 2.6µm particle size). The isocratic solvent system was a mixture of acetone/acetonitrile (70/30; v / v) at a flow rate = 1 mL min^{-1}.

2.5. Statistical analyses

All analytical determinations were performed in triplicate. The values of different parameters were expressed as the mean ± standard deviation.

3. Results and discussion

3.1. Tocopherols and carotenoids

Figure 1 shows the chromatographic profiles of the carotenoids and tocopherols identified in the oil under study. The content of the identified tocopherols and carotenoids of the oil is summarized in Table 1. Two forms of vitamin E were detected and quantified; α-tocopherol and γ-tocopherol (α-T and γ-T). The content of α-T was about 119 mg/kg of oil, which was similar to that determined for *Pistacia terebinthus* oil (110 mg/kg) [10] and

for olive oil (141.94 mg/kg) [11]. The concentration of γ-T was about 23 mg/kg of oil. Tocopherols are a class of lipid-soluble antioxidants, which are essential ingredients in human nutrition.

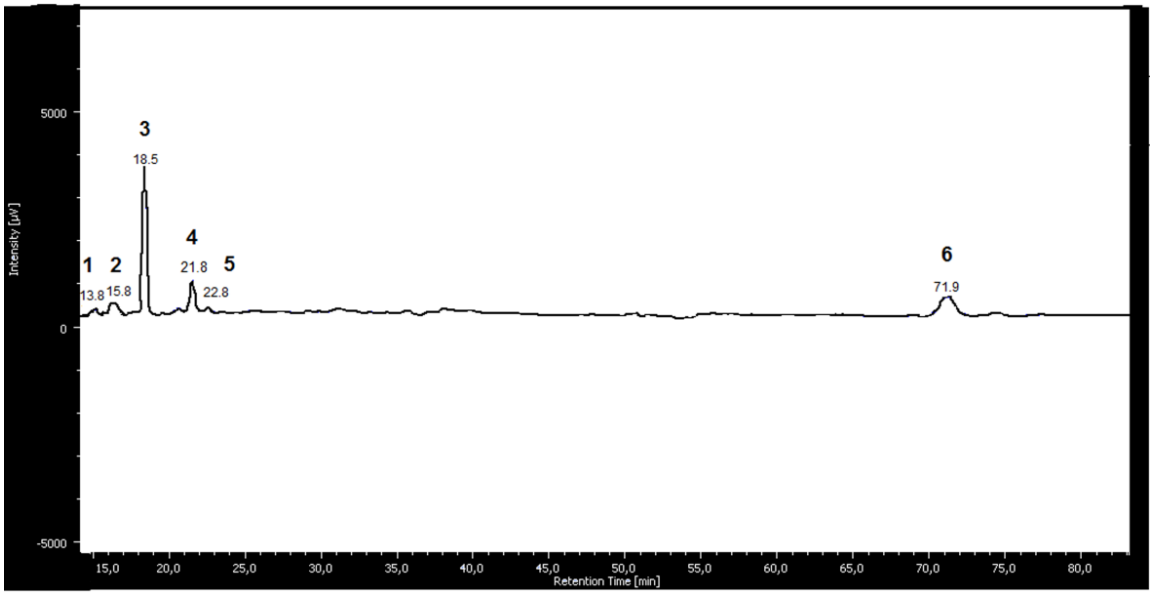

Figure 1: UV-RP-HPLC chromatogram of Carotenoids and tocopherols (1: γ-tocopherol, 2: δ-tocopherol, 3: α-tocopherol, 4: lutein, 5: zeaxanthin, 6: β-carotene)

Epidemiological evidence has indicated that vitamin E supplementation results in a lower risk of cardiovascular disease and cancer; it also helps to support the immune function and can prevent or slow down a number of degenerative diseases associated with ageing (such as arthritis), and disorders of the nervous system [12,13]. Alpha-tocopherol, which is mostly derived from seed oils, is characterized by significant vitamin activity [14]. This compound inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation into smooth muscle cells, platelets and monocytes [15]. It is known for its antioxidant and anti-inflammatory effects [16].

Table 1. Tocopherol and carotenoid contents (mg/kg of oil) in *Pistacia lentiscus* L. seed oil

Compounds	Quantity (mg/kg of oil)
α-tocopherol	119.99 ± 1.05
γ-tocopherol	23.52 ± 0.47
Lutein	2.07 ± 0.58
zeaxanthin	1.35 ± 0.12
β-carotene	6.13 ± 0.49
Total tocopherols	143.51
Total carotenoids	9.55

As for carotenoids, the molecules identified in *Pistacia lentiscus* oil were lutein, zeaxanthin and β-carotene, with the respective contents of 2, 1.35 and 6 mg/kg of oil. These compounds are considered to be powerful natural antioxidants which protect the oil against oxidative degradation and maintain cell membrane integrity in the body [17]. All carotenoid species that contain a β-ring can be converted into retinol and are thus precursors for vitamin A, hence the major importance of carotenoids to human nutrition [18]. β-carotene participates with other micronutrients in the prevention of cancers, atherosclerosis, cataracts and premature ageing [19]. The high β-carotene content in *P. lentiscus* seed oil thus means that it is a good natural source of carotenoids when compared with olive oil (1.58-2.84 mg/kg of oil) [9]. Gester [20] studied the anti-carcinogenic effects of carotenoids and

demonstrated the considerable potential of β-carotene as an active agent in the stabilization of cells initiated during carcinogenesis. Lutein and zeaxanthin are present in large quantities in the eye; they have an antioxidant effect and can prevent macular degeneration that affects older individuals [21]. These two molecules are considered to protect the eyes because they affect the ability of macular pigment to absorb blue light before it is perceived by photoreceptor cells and also display important antioxidant potential [22]. The oral and topical administration of antioxidants procures important antioxidant activity in the skin and therefore a high degree of protection against ultraviolet rays [23].

3.2. Triglycerides

The chromatographic profile of mastic oil revealed the presence of 14 triglycerides, as shown in Figure 2. The seven principal TAG molecular species identified were: dioleopalmitin (OOP), palmitooleolinolein (POL), triolein (OOO), dipalmitoolein (PPO), dioleolinolein (OOL), dilinoleopalmitin (PLL) and dipalmitolinolein (PLP).

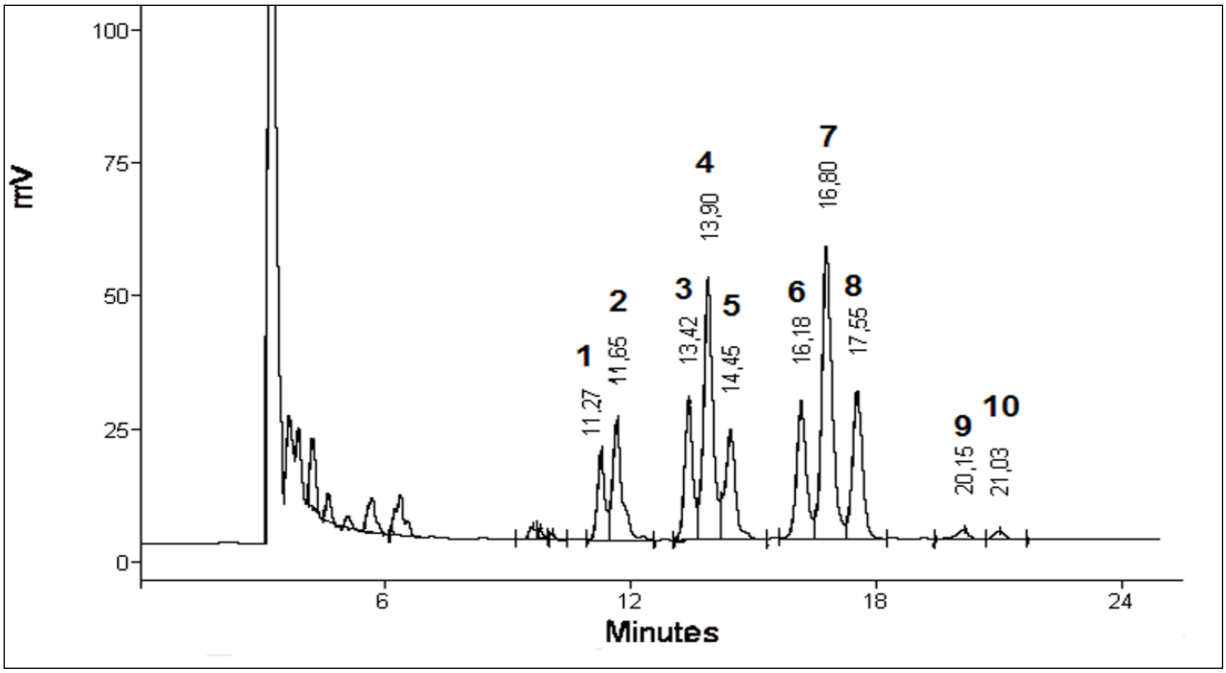

Figure 2: RP-HPLC chromatogram of TAG contained in *Pistacia lentiscus* fixed oil (1: LOL, 2: PLL, 3: OOL, 4: POL, 5: PLP, 6: OOO, 7: POO, 8: PPO, 9: ALO, 10: SSO)

The percentage contents of these molecular species are summarized in Table 2. The oil under study was characterized by an abundance of palmitodiolein (OOP) at a level of 26%, followed by palmitooleolinolein (POL) which accounted for 17% of all TAGs. Triolein (OOO) was present at a level of about 13%. This composition shows that *P. lentiscus* oil is rich in oleic, palmitic and linoleic acids. This is consistent with the findings of Mezni et al. [2] and Trabelsi et al. [8] regarding the fatty acid composition of this fixed oil. In addition, the profile of TAGs contained in this oil was different from that of olive oil and similar to that determined for the oil of *Pistacia atlantica*. Both oils contain the same principal molecular species but at different proportions; palmitooleolinolein (POL) is the major element in *Pistacia atlantica* oil [24] whereas palmitodiolein (OOP) predominates in mastic tree oil. This can be explained by the fact that Atlantic Pistachio oil is richer in linoleic acid [25]. Triglycerides are the most important group of lipids in human nutrition, storing the most important fatty acids in the human body, which are mainly located in adipose tissue [26]. TAGs with ECN $_{48}$ (49.81%) predominated, followed by TAGs with ECN$_{46}$ (34.08%). Mastic tree oil is rich in long-chain triglycerides (LCT). Diunsaturated TAGs account for more than 53% of the total composition of TAGs. Dipalmitoolein (PPO) is the principal component in diunsaturated TAGs (10.43%). Several studies have shown that a diet rich in LCT enables a reduction in plasma cholesterol [27] and promotes the intestinal absorption of β-carotene in humans [28].

Table 2. TAG Composition of *Pistacia lentiscus* fixed oil

Triglyceride	Percentage (%)
OOP	26±0.83
POL	17.39±0.49
OOO	13.38±0.44
PPO	10.43±0.36
LOO	9.66±0.54
PLL	7.7±1.88
PLP	7.03±0.26
OLL	3.99±0.12
ALO	1.56±0.13
SOO	1.01±0.01
O LLn	0.85±0.11
LLnP	0.32±0.02
OOLn	0.21±0.02
LLL	0.17±0.09
ECN_{42}	1.02±0.2
ECN_{44}	11.9±2.02
ECN_{46}	34.08±1.29
ECN_{48}	49.81±1.63
ECN_{50}	2.57±0.14
ECN_{52}	0.32±0.02
Monounsaturated	17.46
Diunsaturated	53.98
Triunsaturated	28.26

P, palmitic; O, oleic; L, linoleic; S, stearic; Ln, linolenic and A, arachidic acids. ECN_{42} = LLL + OLLn; ECN_{44} = OLL+ OOLn + PLL. ECN_{46}: LOO + POL + PPL; ECN_{48} = OOO + POO + PPO; ECN_{50} = ALO + SOO; ECN_{52}: LLnP. Monounsaturated: POO, POL, PLL, ALO, SOO, LLnP ; Diunsaturated: PPO, PLP; Triunsaturated: OOO, LOO, OLL, OLLn, OOLn, LLL.

Conclusion

This study enabled identification for the first time of the composition of tocopherols, carotenoids and triglycerides in *P. lentiscus* fixed oil.

This oil contained very high levels of α-tocopherol and β-carotene. This reflects its important antioxidant activity and therefore the high nutritional value of this natural product.

These properties would enable the use of this oil by the food, pharmaceutical and cosmetic industries.

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