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Chemical composition of Lentisk (*Pistacia lentiscus* L.) seed oil

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We were interested in Pistacia lentiscus fixed oil which was extracted from ripe seeds. The characterization of this oil was performed on fatty acids, triacylglycerols, tocopherols and sterols composition. We also evaluated its mineral composition. The results showed that the prominent class of fatty acids was represented by monounsaturated fatty acids accounting for 52.4% of the whole fatty acids. It was followed by saturated fatty acids and polyunsaturated fatty acids accounting respectively for 26.42, 21.18 and 11%. The major fatty acid (FA) was oleic acid with an amount of 51.06%. Linoleic acid (C18: 2) which is an essential FA accounted for 20.71% of total fatty acids. The majority of triacylglycerols are in mono and polyunsaturated forms. The major constituents were stearoyl-oleyl-linoleylglycerol and palmitoyl-dioleylglycerol acounting together for 27.58% of total TAGs. Concerning sterols, their quantity in Lentisk oil was about4.17 mg/ kg of oil. This quantity is comparable to that of oil seed rapeseed. We noted the prevalence of β-sitosterol with an amount of 55.5%. Furthermore, P. lentiscus oil contained 8111.137 mg of tocopherols/kg of oil. α-tocopherol which has the highest antioxidant activity accounted for 97% of whole tocopherols in Lentisk oil. Lentisk oil was rich in minerals. The most abundant mineral was Na, followed by K, Ca, Mg, Fe and Cu. These minerals are essential and indispensable for the human body, for their nutritional value.

Key words: *Lentisk, Pistacia lentiscus*, ripe seed oil, fatty acids, triacylglycerols, sterols, tocopherols.

INTRODUCTION

Lentisk (Pistacia lentiscus L). is an evergreen shrub or tree and an aromatic member of the Anacardiaceae family producing bright red globose berries. It grows in several Mediterranean region (Bonnier and Douin, 1990). The fruits, galls, resin and leaves of the *Lentisk* have a long tradition in folk medicine dating from the times

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of the ancient Greeks (Charef et al., 2008). The same authors reported that in Algeria the oil of the fruit is used by the population in traditional medicine in many ways, as an anti-diarrhoeal and also as constituent of cattle feed. Several studies focused on the phytochemical composition of the resin, the leaves and the galls of *Lentisk* and also on its essential oils (Castola et al., 2000; Duru et al., 2003; Romani et al., 2002) but in contrast, fewer studies are related to the composition of the fruit oil (Ucciani, 1995).

It is used as an antibacterial (Iauk et al., 1996) and antiulcer (Al-Said et al., 1986) agent. The essential oil of *Lentisk* are extensively used in the perfumery and in food and pharmaceutical industries as reported by Calabro and Curro (1974). *Lentisk* oil may partially help in the protection against mercury intoxication, and it could also be considered a safe nutritional source, at least by maintaining total cholesterol and LDL-cholesterol in their normal ranges (Maarouf et al., 2008). Many works were focused on *Lentisk* essential oil composition and activities whereas few studies have focused on its fixed oil. The fixed oil extracted from mature fruits is commonly used in Tunisian traditional medicine as an anti-ulcer, wound healing and antiseptic (Rejeb et al., 2006; Mezni et al., 2012). The aim of this study was to ascertain fatty acids, triacylglycerols, tocopherols and sterols composition of *Lentisk* fixed oil. We also evaluated its mineral composition.

MATERIALS AND METHODS

Oil extraction

Oil was extracted from Lentisk mature seeds using hexane; the ground dried Lentisk seeds (40 g) were placed into a cellulose paper cone and extracted with 400 ml hexane using a soxhlet extraction apparatus for 8 h. The solvent was removed via a rotary vacuum distillation at 40 to 50°C flushing with nitrogen to blanket the oil during storage. The residue was weighed and stored at –20°C until it was analysed. Oil weight was determined from 40 g of the seed powder to calculate the lipid content. The result was expressed as the lipid percentage in the seed powder dry matter.

Analysis of fatty acids composition

The fatty acid methyl esters (FAME) composition was determined by the conversion of oil to fatty acid methyl esters prepared by adding 1 ml of n-hexane to 40 mg of oil followed by 200 µl of sodium methoxide (2 M). The mixture is heated in the bath at 50°C for few seconds followed by adding 200 µl HCl (2 N). The top layer (1 µl) was injected onto a GC (Agilent 6890N, California, USA) equipped with a flame ionisation detector (FID) and a polar capillary column (HP-Innowax polyethylene glycol, 0.25 mm internal diameter, 30 m in length and 0.25 µm film in thickness) to obtain individual peaks of FAME. The FAME peaks were identified comparing their retention times with individual standards, FAME being injected in the same analytical conditions and analysed with the Agilent Technologies Chemstation A09.01 Software. The relative percentage of each FA was calculated on the basis of the peak area of a FA species to the total peak area of whole FA in the oil sample.

Analysis of triacylglycerols composition

The triacylglycerols (TAGs) profile was obtained by a reverse phase high performance liquid chromatography (HPLC) (Agilent 1100, California, USA) equipped with a G1354 quaternary pump, a G1313A standard auto sampler, and a G1362A refractive index detector. The chromatogram was carried out using Agilent Technology Chemstation software. The TAGs were separated using a commercially packed Hypersil ODS column (125 × 4 mm) with a particle size of 3 µm and were eluted from the column with a mixture of acetonitrile/acetone (65/35) at a flow rate of 0.5 ml/min; the TAGs were detected with a refractive index detector. Ten microliters of 0.05 g oil diluted in 1ml (chloroform/acetone 50/50, v/v) was injected into the HPLC. The total run time was 45 min. TAG peaks were identified by comparison with chromatograms of sunflower and corn oil obtained in the same analytical conditions.

Analysis of tocopherols composition

Prior to the HPLC analysis, the seed oil 0.5 g was diluted with 5 ml hexane and 5 µl samples were injected. The tocopherol composition of *M. pomifera* seed oils was determined using HPLC according to norm ISO 9936. The sample was analysed by an HPLC (Agilent 1100, CA, USA) consisting of a G1354 quaternary pump, a G1313A standard auto sampler, a G1321A fluorescence detector set at λ excitation = 295 nm, and λ emission = 330 nm and a chemstation software. A normal phase column (Pinnacle II silica) (150 × 3.2 mm × 3 µm) was used with hexane/isopropanol (99.5/0.5, v/v) as a mobile phase. The system was operated isocratically at a flow rate of 0.5 ml/min. The separations were carried out at 30°C. The quantification was based on an external standard method. The mixed tocopherol standards in a hexane solution (2 mg/ml) were prepared from the standard compounds: α-, β-, γ- and the δ-tocopherols (Sigma Chemical Co., St. Louis, MO, USA).

Analysis of phytosterols composition

Separation of sterols (ST) was performed according to the method ISO 12228. Lipids (250 mg) were refluxed for 15 min with 5 ml ethanolic KOH solution (3%, w/v) after addition of cholesterol (1 mg; Fluka) as an internal standard and a few antibumping granules. The mixture was immediately diluted with 5 ml of ethanol. The unsaponifiable part was eluted over a glass column packed with slurry of aluminium oxide (Scharlau) in ethanol (1:2, w/v) with 5 ml of ethanol and 30 ml of diethyl ether at a flow rate of 2 ml/min. The extract was evaporated in a rotary evaporator at 40°C under reduced pressure, and then ether was completely evaporated under a steam of nitrogen. For the characterization of sterols, a silica gel F254 plate (Fluka) was developed in the solvent system n-hexane/diethyl ether (1:1, v/v). For the detection of sterols, the thin-layer plate was sprayed with methanol; the sterol bands were scraped from the plate and recovered by extraction with diethyl ether. The sterols trimethylsilyl ether (TMS) derivatives were prepared by adding 100 µl of a silylant reagent N-methyl-N-(trimethylsilyl) trifluoroacetamide/ pyridine (1/10, v/v) in a capped glass vial and heated at 105°C for 15 min.

Preparation of standard solutions

A mixture of standard solutions of sterols was prepared by derivatization (cholesterol, sitosterol, stigmasterol, ergosterol and campesterol). The sterols trimethylsilyl ether derivatives were analysed using the GC system (Agilent 6890N, California, USA) equipped with a FID and the GC chemstation software. A HP-5 (5%

Table 1. *Lentisk* seed oil fatty acids composition.

FA	Amount (% of TFA)
C16 : 0	23.52 ± 3.01[b]
C16 : 1	1.19 ± 0.12[d]
C17 : 0	0.10 ± 0.01[g]
C18 : 0	1.41 ± 0.02[c]
C18 : 1	51.06 ± 4.37[a]
C18 : 2	20.71 ± 2.25[b]
C18 : 3	0.47 ± 0.10[e]
C20 : 0	0.14 ± 0.02[f]
C20 : 1	0.15 ± 0.01[f]
C22 : 0	1.25 ± 0.02[d]
SFA	26.42 ± 5.09[b]
MUFA	52.4 ± 7.18[a]
PUFA	21.18 ± 2.23[b]

pheynyl methyl polysiloxane column) was used (0.32 mm i.d. × 30 m in length; 0.25 µm film in thickness; an Agilent 19091J-413, CA, USA). A carrier gas (helium) flow was 1.99 ml/min (split–splitless injection with a split ratio of 1:200). The detector and the injector were set at 320°C, and the injected volume was 1_l. The total analyses were set at 71 min to ensure the elution of all ST. The operational conditions were: injector temperature 320°C, column temperature: a gradient of 4°/min from 240 to 255°C. Sterols peak identification was carried out according to the ISO 12228 reference method and confirmed by GC–MS (NIST 2002 database) operating in the same conditions as used for the GC–FID.

Mineral composition

The mineral constituents (Ca, Na, K, Fe, Mg and Cu) present in *Lentisk* seed were analysed, using an atomic absorption spectrophotometer (NOUVA400, ANALYTIKJENA, Germany) and a flame ionisation spectrophotometer (Flame Photometer 410, SCHERWOOD, Germany).

Statistical analyses

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

RESULTS AND DISCUSSION

Oil characterization

Oil yield of *Lentisk* was of 35.37%. This yield is appreciable and is similar to that of some oleaginous seeds. *Lentisk* oil is visquous with a green colour. Charef et al. (2008) reported that the crude fat content of *P. lentiscus* varied from 32.8% for black fruits to 11.70% for red ones. According to Karlenskind (1992), the black fruit of *Letisk* can be considered as an oleaginous seed as peanut, olive, sunflower and cotton seeds whose oil yield range from 30 to 45%.

Fatty acid and triacylglycerol composition

The prominent class of FA was represented by monounsaturated FA (MUFA) accounting for 52.4%. It was followed by saturated FA (SFA) and polyunsaturated FA (PUFA) and accounting respectively for 26.42, 21.18 and 11% of the whole FA (Table 1). The major FA was oleic acid (C18: 1) with an amount of 51.06%. This FA is reputed for its role in preservation of cardiovascular diseases and its nutritional value (Corbett, 2003). Linoleic acid (C18: 2) which is an essential FA (EFA) accounted for 20.71% of whole FA. Furthermore, palmitic acid (C16: 0) was detected at a significant percentage of 23.52%.

C18: 2 had favorable nutritional implications and beneficial physiological effects in the prevention of coronary heart disease and cancer (Oomah et al., 2000). Generally, FA and TG are able to reduce trans epidermal water loss and so increase skin hydration (Dweck, 2002). C18: 1 and C18: 2 are known for their anti-inflammatory properties. Linoleic and alpha linoleic acids provide lipids necessary for cell membrane repair and cellular respiration (Loden and Andersson, 1996). Djerrou et al. (2010) reported that in *Lentisk* oil, the three dominant FA were 18: 1 (55.3%), C18: 2 (17.6%) and 16:0 (16.3%). The FA composition of our sample is similar to that of Algerian Lentisk oil (2) whose major FA was C18: 1 with an amount varying from 55.3 to 64%. In Algerian *Lentisk* oil, C18: 2 was characterized by a significant percentage (17.6 to 28.4%). In *Pistacia terebinthus* oil, the dominating FA of the oil is C18: 1, which accounted for 43.0 to 51.3% of the total FA (Matthäus and Özcan, 2006). In addition, our results for *Lentisk* oil agree well with the data recorded by Ucciani (1995) in his dictionary. It is to note that the UFA/SFA ratio was equal to 2.78. Furthermore, the profile of FA confirms the similarity between *Pistacia lentiscus* oils and other edible vegetable oils such as sunflower, peanut, cotton, olive and avocado.

Furthermore, the low saturated/unsaturated FA ratio (0.35) reveales a high content in UFA which may give it nutritional and dietetic virtues. The FA profile of *Lentisk* seed oil is similar to that of *Pistacia vera* (Chahed et al., 2006) and *Pistacia atlantica* (Ghalem and BenHassaini, 2007). *Lentisk* seed oil could be used for nutritional purposes as an interesting source of omega 6 and omega 9 FA. The TAGs composition of *Lentisk* showed that the majority of TAGs are in mono and polyunsaturated forms (Table 2). Considering the fatty acid composition; the major constituents were stearoyl-oleyl-linoleylglycerol (SOL) and palmitoyl-dioleylglycerol + (POO) accounting together for 27.58% of total TAGs. Stearoyl-dilinoleoylglycerol (SLL) and palmitoyl-oleyl-linoleoylglycerol (POL) represented 21.5% of total TAGs whereas Trioleylglycerol (OOO), dioleyl-linoleylglcerol (OOL) and dipalmitoyl-oleylglycerol (PPO) were signify-cantly represented with respective amounts of 12.04, 9.83 and 8.51%. Furthermore, the trilinoleyl-glycerol (LLL)

Table 2. *Lentisk* seed oil triacylglycerols composition

Tag	Amount (% of total tags)
LLLn	-
LLL	1.32 ± 0.28^{f}
OLLn	-
OLL	5.67 ± 1.62^{e}
PLL	7.97 ± 1.86^{de}
OOL	9.83 ± 2.03^{cd}
SLL + POL	21.50 ± 2.06^{b}
PPL	5.58 ± 1.12^{e}
OOO	12.04 ± 1.43^{c}
SOL + POO	27.58 ± 2.36^{a}
PPO	8.51 ± 1.09^{d}

LLL: Trilinoleoyl-glycerol, OLLn: Oleyl-linoleoyl-linolenoylglycerol, OLL: Oleyl-dilinoleoyl-glycerol, PLL: Palmitoyl-dilinoleoyl-glycerol, OOL: Dioleyl-linoleoylglcerol, SLL: Stearoyl-dilinoleoylglycerol, POL: Palmitoyl-oleyl-linoleoylglycerol, PPL: Dipalmitoyl-linoleoylglycerol, OOO: Trioleylglycerol, SOL: Stearoyl-oleyl-linoleoylglycerol, POO: Palmitoyl-dioleylglycerol, PPO: Dipalmitoyl-oleylglycerol

Table 3. *Lentisk* seed oil tocopherols composition.

Tocopherol	Quantity (mg/ g of oil)	Amount (% of total tocopherols)
α-tocophérol	7.59 ± 0.61^{a}	93.62^{a}
β-tocophérol	0.47 ± 0.02^{b}	5.79^{b}
γ-tocophérol	0.48 ± 0.04^{b}	0.59^{c}
δ-tocophérol	-	-

was characterized by a low percentage (1.32%)

Unsaponifiable compostion of *P. lentiscus* seed oil

The unsaponifiable fraction of oils contains tocopherols, sterols and phenolic components. However, no study has been published which concern this fraction. It is important to mention that Mattähaus and Özcan (2006) quantified FA, tocopherols and sterols in *Pistacia terebinthus Chia.* The stability of vegetable oils under the conditions of oxidation is due to the presence of high levels of natural antioxidants, the most important are the tocopherols, which come in four isomeric forms: α, β, γ and δ. *Lentisk* oil contained 8111.137 mg of tocopherols/ kg of oil. It should be noted that this oil is rich in tocopherols some vegetable oils such as corn oil (1111.4 mg/ kg of oil) and rapeseed oil (820 mg/ kg of oil) and sunflower seed oil (734 mg/ kg of oil) according to Ayerdi (2008). α-tocopherol which had the highest antioxidant activity accounted for 93.62% of whole tocopherols in *Lentisk* oil. The isomers β and γ were detected with respective amounts of 5.79 and 0.59% (Table 3). δ-tocopherol was not detected. Matthäus and Özcan (2006) reported that seed oil of *P. terebinthus* was characterized by the predominance of α- and γ isomers of tocopherols. It also contained different tocotrienols, with γ-tocotrienol as the dominate compound of this group. The effect of the environment on the temperature seems to be a key factor in the accumulation of tocopherols (Ayerdi, 2008). According to this author, the amount of α–tocopherol increases in sunflower oil during the maturation parallel with a decrease in that γ-tocopherol which is the precursor of α-tocopherol. The results of quantitative determinations of α-tocopherol in *P. lentiscus* var. chia, in and *P. terebinthus* leaves by TLC-densitometry and colorimetry. The maximum content of α-tocopherol was found in the leaves of *P. lentiscus* var. chia (Kivçak and Akay, 2010). Consumption of food rich in natural antioxidants is protective against some types of cancer and may also reduce the risk of cardiovascular and cerebrovascular events Aruoma (1998). These actions of antioxidants have been attributed to their ability to scavenge free radicals, thereby reducing oxidative damage of cellular biomolecules such as lipids, proteins, and nucleic acids (Ferguson, 1995).

This richness in tocopherols, including the predominance of α-tocopherol, which is a very good antioxidant fatty phases, contributes to the natural protection and conservation of the oil against oxidation. It is important to mention that *Lentisk* oil has a good vitaminic activity due to its high content of vitamin E. According to Table 4, the oil of *Lentisk* contained β-sitosterol as the major phytosterol (55.55%), followed by cholesterol (44.45%). However, stigmasterol and other sterols were not detected. They may disappear during maturation. It should be noted that the respective quantities of β-sitosterol and cholesterol in our sample were of 231.67 mg/ 100 g of oil and 185.35 mg/ 100 g of oil.

The whole quantity of sterols in *Lentisk* oil was of 4.17 mg/kg of oil. This quantity is comparable to that of oilseed rape which is one of the most important oil seed crops in the world. Phytosterols content in new oilseed rape varieties ranges between 5.13 and 9.79 g/kg oil. It is important to note that the phytosterols content ranges between 1.41 and 15.57 g/ kg oil and depends of plant species (Gul and Amar, 2006).

In *P. terebinthus* L. seed oil, the total content of sterols ranged between 1341.3 and 1802.5 mg/kg, with β-sitosterol as the predominant sterol accounting for more than 80% of the total amount of sterols (Matthäus and Özcan, 2006). The levels of tocopherols and phytosterols in oil seeds (rapeseed and soybean) are highly dependent on both genetic factors and plant environmental conditions of cultivation (Abidi, 2003). In recent years increased interest in phytosterols lies in their potential to reduce plasma low-density lipoprotein cholesterol level, decreasing coronary mortality and therefore acting as naturally preventive dietary product (Gul and Amar, 2006). It has been found that plants that

Table 4. *Lentisk* seed oil sterols composition.

Sterol	Quantity (mg/ 100 g of oil)	Amount (% of total ST)
β-sitostérol	231.67 ± 10[a]	55.55
Cholesterol	185.35 ± 22[b]	44.45

Table 5. *Lentisk* seed oil mineral composition.

Mineral	Quantity (mg/ 100 g of oil)
Na	25.36 ± 3.25[a]
K	2.17 ± 0.05[b]
Ca	0.25 ± 0.04[c]
Mg	0.19 ± 2.23[d]
Fe	0.004 ± 0.00[tr]
Cu	0.0001 ± 0.00[tr]

have cicatrizing and vulnerary properties often have a high level of plant sterols (Dweck, 2002). Nuts contain bioactive constituents that elicit cardio-protective effects including phytosterols, tocopherols and squalene.

Mineral composition of the seed of *P. lentiscus*

The mature seeds of *Lentisk* are rich in minerals. The most abundant mineral is Na, followed by K, Ca, Mg, Fe and Cu (Table 5). These minerals are essential and indispensable to the human body, for their nutritional value. The mineral composition of *P. lentiscus* seed revealed its nutritional value for human and/or animal consumption. According to Ferguson (1995), pistachio is a rich source of phosphorus, potassium, magnesium, calcium and iron. The importance of mineral composition is due to their nutritional properties and beneficial health effects, as well as their meeting of dietary guidelines required for a healthy diet (Welna et al., 2008).

According to this study, the seeds of *Lentisk* seemed to be a good source of oil. This oil had an interesting FA composition. It is rich in C18: 1 which is reputed for its role in prevention of cardiovascular diseases and also in C18: 2 which is an essential FA with beneficial physiological effects in the prevention of coronary heart disease and cancer. This oil is characterized by an interesting composition of nutritional point of view. In a further work, we will try to complete this research by exploring phenols composition and some physico-chemical properties of *Lentisk* oil. Such study could lead to industrial applications particularly in cosmetics industry.

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