

Chemical Composition and Lipid Fraction Characteristics of Aleppo Pine (*Pinus halepensis* Mill.) Seeds Cultivated in Tunisia

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The proximate composition of Aleppo pine (*Pinus halepensis* Mill.) seeds cultivated in Bizerta (Tunisia) were investigated for their fatty acid composition, and their thermal and physical properties of lipid fraction. The proximate analysis of pine seeds showed the following composition (on a dry-weight basis): protein 22.7%, oil 43.3%, ash 8.3% and total carbohydrate 25.7%. Potassium, magnesium and calcium were the predominant mineral elements present in the seeds and reached, together, about 1%. Oleic and linoleic acids were the major unsaturated fatty acids (27.3 and 48.8%, respectively), while the main saturated one was palmitic acid (8.75%). Myristic, myristoleic, palmitoleic, margaric, margaroleic, stearic, linolenic, arachidic, eicosenoic, eicosadienoic, eicosatrienoic, behenic and lignoceric acids were also detected. Thermal profile of *Pinus halepensis* Mill. seed oil, determined by its DSC melting curve, showed that the entire liquefaction of the Aleppo pine seed oil occurs at -8°C . CieLab colour parameters (L^* , a^* , b^*), oxidative stability by Rancimat test and viscosity were also determined. Physico-chemical properties of the oil include: saponification number 190, peroxide value 3.18, iodine index 117, and a low acidity of 0.61%. Results suggested that the production of oil from *Pinus halepensis* seeds could provide a potential use in food, pharmaceutical, cosmetics and other non-food industries.

Key Words: *Pinus halepensis* Mill. seeds, oil, fatty acids, thermal profile

INTRODUCTION

Aleppo pine (*Pinus halepensis*), described by Miller in 1768, is a circummediterranean species (Nahal, 1986). This woody and perennial pine tree, cultivated in the Mediterranean area is useful for reforestation (Le Thiec and Manninen, 2003). *Pinus halepensis* species belonging to the *Pinaceae* family and is the most widely distributed pine in this region, where it covers more than 25,000 km² (Maestre et al., 2003). The main Aleppo pine forests are found in Spain, France

and North Africa (Raeda and Arianoutsou, 2000) especially in Algeria and Tunisia where they constitute a clump (Nahal, 1986). In Tunisia, Aleppo pine covers near 300,000 ha from the mountains to the south of Mejerda and to the west of Zaghouan, to the central region of Tunisia (Bizerta, Ben Arous, Nabeul, Zaghouan, Beja, Jendouba, El Kef, Kairouan and Kasserine). *Pinus* species are essentially grouped as soft pines (haploxyton) and hard pines (diploxyton). *Pinus halepensis* Mill. belongs to diploxyton pine groups, which produce the most commercialised pine seeds in the world. In fact, these seeds, lodged in large cones, are highly nutritious and very appreciated by consumers.

Aleppo pine seeds are traditionally used throughout Tunisia, and other Islamic countries, for preparing a sweet pudding of ground pine seeds, called 'Assida-Zgougou'. Recently, it has also been employed as an ingredient in ice-creams and candies. A few works have been published on the Tunisian Aleppo pine

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seeds (Nasri and Triki, 2004) which focus on the fatty acid composition, the unsaponifiable fraction and the phenolic compounds of the corresponding oil, however not on their detailed composition and physicochemical properties.

The aim of this work was to study the chemical composition of *Pinus halepensis* seeds cultivated in Tunisia and to determine the fatty acid composition and physico-chemical properties of their lipid fraction.

MATERIALS AND METHODS

Materials

Seeds

Aleppo pine seeds (*Pinus halepensis* Mill.) were purchased (20 kg) from a herbal market in Bizerta (Tunisia), where they were harvested. Seeds were directly stored at 15°C for a maximum of 3 days and then cleaned manually to remove foreign matter. Seed samples were separately milled in a heavy-duty grinder for 4 min to pass 1–2 mm screens and then fractioned into 40 powdered samples (500 g) which are preserved in hermetic bags at –20°C until analysis.

Methods

Oil extraction and preservation

Aleppo pine seeds (50 g) were placed in a dark flask (capacity = 1 L) and homogenised with 250 mL of hexane. The mixture was moved for 4 h in a shaker (Selecta, Spain) at 180 U/min, and then centrifuged for 15 min at 1,000 × g. The extraction was conducted at 24 ± 1°C. The supernatant was filtered through a Whatman No. 2 filtering paper. The extraction procedure was repeated twice and the collected solvent was removed using a rotary evaporator at 40°C. The seed oil obtained was finally drained under a stream of nitrogen and then stored in a freezer (–20°C) for subsequent physico-chemical analyses.

Analytical Methods

All analytical determinations were performed at least in triplicate. Values were expressed as the mean ± standard deviation.

Chemical Analysis of Powdered Seeds

The dry matter was determined according to the Association of Official Analytical Chemists (AOAC, 1990).

Oil was extracted from 15 g of Aleppo pine seed powder in a Soxhlet extractor for 8 h using hexane as a

solvent at 45°C. The result was expressed as the percentage of lipids in the dry matter of seed powder.

Total protein was determined by the Kjeldahl method. Protein was calculated using a nitrogen conversion factor of 5.30 for pine nut seeds (Greenfield and Southgate, 1992; Nergiz and Dönmez, 2004).

To remove organic matter, about 0.5 g of powdered seed samples were incinerated in the muffle furnace at 550°C for about 12 h. The ashes were dissolved in HNO₃ (Larrauri et al., 1996) and the mineral constituents (Ca, Na, K, Mg, Fe, Zn, Cu and Mn) were determined using an atomic absorption spectrophotometer (Hitachi Z 6100, Japan). The phosphorus content was analysed by the phosphomolybdovanadate method (AOAC, 1990). The total ash was expressed as a percentage of dry matter.

The carbohydrate content was estimated by difference of mean values and dry matter content.

Analysis of Oil Extract

AOCS official methods (American Oil Chemists' Society, AOCS, 1997) were used to evaluate the peroxide, iodine, saponification and acidity values (methods number Cd 8-53, Cd 3d-63, Cd 3-25 and Cd 3d-63, respectively). Determination of refractive index (at 20°C) was determined with a refractometer (Abbe-Zeiss).

K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 and 270 nm, respectively, with a UV spectrophotometer (Secoman, Anthelie 70 MI 0291, No. 344, France) using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Chlorophyll content was determined by spectrophotometry following the methodology described by Mínguez et al. (1991).

Total phenols, expressed as gallic acid (mg/kg of oil) were determined colorimetrically at 725 nm using Folin-Ciocalteu reagent as previously done by Gutfinger (1981) on virgin olive oil.

Viscosity was measured with a Stress Tech Rheologica Rheometer (Rheologica Instruments AB, Lund, Sweden). Measurements were performed at 25°C with a steel cone-plate (C40/4) under a constant shear rate of 100/s.

Absorbance of oil solutions in hexane was measured with a UV-240 spectrophotometer (Shimadzu-Corporation, Kyoto, Japan).

The CieLab coordinates (L^* , a^* , b^*) were measured with a spectrophotometer MS/Y-2500 (Hunterlab, Inc., Reston, VA, USA), calibrated with a white tile (Besbes et al., 2004a). Absorbance of oil solutions in hexane was measured with a spectrophotometer UV-240 (Shimadzu Corporation, Kyoto, Japan) with light of wavelengths between 205 and 800 nm (Besbes et al., 2004a).

Oxidation stability was evaluated by measuring the oxidation induction time, with the use of the Rancimat 679 apparatus (Metrohm AG, Herison, Switzerland). A flow of air (15 L/h) was bubbled through the oil

Table 1. Chemical composition of *Pinus halepensis* and *Pinus pinea* seeds.

Composition	<i>Pinus halepensis</i> seeds (mean \pm SD, n = 3)	<i>Pinus pinea</i> seeds ¹
Dry matter (%)	94.8 \pm 0.1	94.9
Oil (g/100 g)	43.3 \pm 0.5	44.9
Crude protein (g/100 g)	22.7 \pm 0.5	31.6
Ash (g/100 g)	8.3 \pm 0.1	4.5
Total carbohydrate (g/100 g)	25.7 \pm 0.2	13.9

¹Nergiz and Dönmez (2004).**Table 2.** Minerals of *Pinus halepensis* seeds compared to other seeds.

Minerals	Mineral content of seeds (mg/kg)			
	<i>Pinus halepensis</i> (mean \pm SD, n = 3)	<i>Xylopia aethiopica</i> ¹	<i>Pinus pinea</i> ²	Deglet Nour ³
Potassium	6171 \pm 12.0	8676.3	7130	2290
Magnesium	3303 \pm 9.8	1266.5	3250	517
Calcium	1167 \pm 4.9	13020.9	138	388
Phosphorus	568 \pm 0.8	1840.0	5120	683
Sodium	69.6 \pm 0.1	68.5	117	104
Iron	271 \pm 1.8	203.5	102	23
Copper	22.5 \pm 0.1	43.5	15	Nd
Zinc	134.9 \pm 0.4	38.5	64	Nd
Manganese	51.3 \pm 0.1	Nd	69	Nd

¹Barminas et al. (1999); ²Nergiz and Dönmez (2004); ³Besbes et al. (2004a). Nd: not determined.

(2.5 g) heated at 100°C and the volatile degradation products were trapped in distilled water, increasing the water conductivity. The induction time was defined as the time necessary to reach the inflection point of the conductivity curve (Halbault et al., 1997).

Fatty acid composition was analysed by gas-liquid chromatography after derivatisation to fatty acid methyl esters (FAMES) with 2 M KOH in methanol at room temperature according to the IUPAC standard method (IUPAC, 1992). Analyses of FAMES were carried out with a Hewlett-Packard 5890 Series II gas chromatograph (H. P. Co., Amsterdam, The Netherlands) equipped with a hydrogen flame ionisation detector and a capillary column: HP Inovax cross-linked PEG (30 m \times 0.32 mm \times 0.25 μ m film). The column temperature was programmed from 180 to 240°C at 5°C/min and the injector and detector temperatures were set at 250°C. Identification and quantification of FAMES was accomplished by comparing the retention times of peaks with those of pure standards purchased from Sigma and analysed under the same conditions. The results were expressed as a percentage of individual fatty acids in the lipid fraction.

Thermal characteristics of *Pinus halepensis* seed oil was performed using a modulated differential scanning calorimeter (DSC 2920 Modulated DSC-TA Instruments, Newcastle, DE, USA). Oil samples (2 \pm 0.10 mg) were weighed directly in a DSC-pan (SFI-Aluminium, TA Instrument T11024). The samples were quickly cooled to -50°C with a speed of 15°C/min, maintained at this temperature for 15 min and heated to 90°C with

a heating speed of 15°C/min. The same operation (cooling and heating) was repeated and the DSC thermographs were recorded during the second melting. An empty DSC-pan was used as reference. The instrument was calibrated for temperature and heat flow using eicosane ($T_p = 36.80^\circ\text{C}$, $H = 247.70\text{ J/g}$) and dodecane ($T_p = -9.65^\circ\text{C}$, $H = 216.73\text{ J/g}$). Solid fat content was determined from the DSC melting curve according to Deroanne (1977).

RESULTS AND DISCUSSION

Chemical Composition of Aleppo Pine Seeds

The average composition of *Pinus halepensis* seeds is shown in Table 1. The moisture content and the amount of oil were 5.15% and 3.34%, respectively. Quite similar results were reported by Nergiz and Dönmez (2004) for *Pinus pinea* seed oil. Wolff and Bayard (1995) reported that the oil content of some varieties of pine seeds varied from 31 to 68%, however Nasri and Triki (2004) found that Tunisian *Pinus halepensis* Mill. contained 34.63%, an amount lower than our results for the same species. In general, pine seeds are rich in oils, their content varies due to differences in species and environmental factors (Nergiz and Dönmez, 2004). The average protein content of the seeds (22.66%) was lower than that reported for *Pinus pinea* by those authors.

Aleppo pine seeds mineral profile was compared to those of other seeds such as *Pinus pinea*, *Xylopia*

aethiopica and *Phoenix dactylifera* L. (Table 2). *Pinus halepensis* seeds contained significant amounts of important mineral elements (Table 2, macro and micro constituents). Potassium was the most abundant element in the *Pinus halepensis* seeds, followed by magnesium and calcium. The other elements, in descending order by quantity, were P, Fe, Zn, Na, Mn and Cu. *Pinus halepensis* seeds seemed to have lower amounts of phosphorus and sodium than those reported for Deglet Nour and *Pinus pinea* seeds, also calcium and copper were lower than those for *Xylopiya aethiopica*, while magnesium, iron and zinc were detected to be the highest, compared with those reported for all the oil seeds studied. *Pinus halepensis* seeds provide appreciable amounts of microelements (Mn, Zn, Cu and Fe) from a nutritional point of view. They could significantly contribute to the daily requirements of all the elements studied.

Profile of Aleppo Pine Seed Oil

As previously studied, the lipid fraction was found to be the most abundant in *Pinus halepensis* seeds. Consequently, we were interested in determining fatty acid composition, and thermal and physical profiles of Aleppo pine seed oil.

Fatty Acid Composition

Essential fatty acids and their long-chain derivatives are important structural elements of cell membranes and are essential for the formation of new tissues (McKevith, 2005).

Fatty acid composition of *Pinus halepensis* seed oil (Table 3) showed that linoleic and oleic acids account for more than 76% of the total fatty acids. *Pinus halepensis* seed oil is rich in unsaturated fatty acids. This result was in accordance with those reported for *Pinus pinea* seed oil (Nergiz and Dönmez, 2004; Nasri et al., 2005). Previous studies showed that the oils of *Pinus* varieties contained oleic and linoleic acids at relatively high levels (Sagrero-Nieves, 1992; Wolff and Marpeau, 1997) in agreement with our results. In this study, saturated fatty acids (SAFA) accounted for 16.8% of total fatty acids. Among them, the main saturated acids were palmitic, stearic and behenic, with minute amounts of arachidic, lignoceric, myristic and margaric. The high polyunsaturated fatty acids (PUFA) to SAFA ratio of 3:1 is attributed to a high level of PUFA. This is a health benefit, as consumption of diets high in PUFA have been shown to have an inverse correlation to coronary heart disease (Thompson et al., 1993). Therefore, the consumption of these seeds in sufficient amounts could contribute positively to human health.

The $\omega 6/\omega 3$ ratio is very important in terms of human nutrition. An estimate of the ratio in our diet 100 years ago was between 3:1 and 5:1 (Guilliams, 2000) and for the ideal daily consumption varies from 2:1 to 4:1 (Eramus, 1993). *Pinus halepensis* seed oil showed a lower $\omega 6/\omega 3$ ratio compared to *Pinus pinea* seed oil (~10:1 as against ~70:1). This ratio is very variable in usual vegetable oils used in human food, such as olive oil, sunflower oil, soybean oil, palm oil and corn oil (13:1; 199:1; 7:1; 45:1 and 82:1, respectively; Guilliams, 2000).

Table 3. Fatty acid composition of *Pinus halepensis* seed oil.*

Fatty Acid (FA)	<i>Pinus halepensis</i> seed oil (g/100g of total FA, mean \pm SD, n = 3)	<i>Pinus pinea</i> seed oil ¹
Myristic C _{14:0}	0.09 \pm 0.02	0.05 \pm 0.004
Myristoleic C _{14:1}	0.08 \pm 0.01	Nd
Palmitic C _{16:0}	8.75 \pm 0.51	6.49 \pm 0.078
Palmitoleic C _{16:1}	0.22 \pm 0.11	0.224 \pm 0.03
Margaric C _{17:0}	0.08 \pm 0.01	Tr
Margaroleic C _{17:1}	Tr	Nd
Stearic C _{18:0}	4.32 \pm 0.18	3.47 \pm 0.1
Oleic C _{18:1}	27.3 \pm 1.7	38.6 \pm 0.6
Linoleic C _{18:2}	48.8 \pm 2.4	47.6 \pm 0.3
Linolenic C _{18:3}	4.86 \pm 0.36	0.68 \pm 0.001
Arachidic C _{20:0}	0.62 \pm 0.01	0.54 \pm 0.03
Eicosenoic C _{20:1}	0.83 \pm 0.06	0.79 \pm 0.06
Eicosadienoic C _{20:2}	0.42 \pm 0.05	Nd
Eicosatrienoic C _{20:3}	0.49 \pm 0.03	Nd
Behenic C _{22:0}	2.89 \pm 0.23	0.13 \pm 0.01
Lignoceric C _{24:0}	0.18 \pm 0.11	3.02 \pm 0.02
SAFA	16.8 \pm 1.3	13.7 \pm 0.2
MUFA	28.4 \pm 2.3	39.6 \pm 0.7
PUFA	54.2 \pm 3.4	48.3 \pm 0.3

*Nergiz and Dönmez (2004). SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; Tr: trace ($\leq 0.05\%$); Nd: Not detected.

Thermal Properties

Differential scanning calorimetry (DSC) is a fast and direct way to assess the quality of oil (Gloria and Aguilera, 1998). Any endothermic or exothermic event is registered as a peak in the chart, and its area is proportional to the enthalpy gained or lost, respectively. *Pinus halepensis* seed oil exhibited a thermogram with three thermal structural transitions between -39.83 and -12.05°C , indicative of crystalline melting (Figure 1). The obtained peaks were asymmetric and may indicate the presence of three components, having different weights. The first small endothermic peak at -39.83°C may represent the melting of the unstable α crystal form, followed by the crystallisation of the unstable β' form and the last one may correspond to the more stable β form. This hypothesis should be supported later by the X-ray diffraction method to identify the crystalline forms. The fact that thermograms seemed to correspond to a number of compounds higher than those clearly shown, suggests the presence of mixed glyceride groups with different melting points in the used conditions (Herrera and Anon, 1991). After total solidification, mixed glyceride crystals should be formed by intersolubility. They are associated in different crystalline groups with different

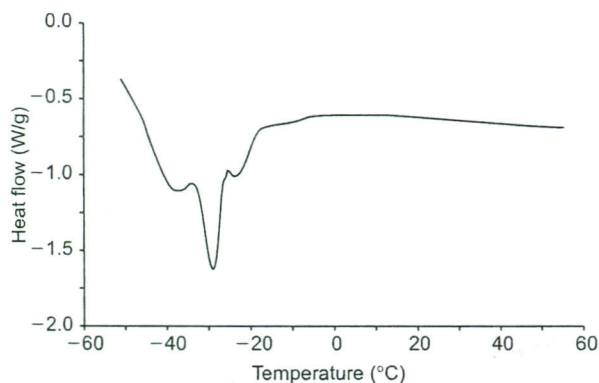


Figure 1. Melting thermogram of Aleppo pine seed oil.

Table 4. Thermal parameters, from DSC melting curves, of *Pinus halepensis* seed oil.

Parameter	<i>Pinus halepensis</i> (mean \pm SD, n = 3)
Peak transition temperatures ($^{\circ}\text{C}$)	
T1	-39.83 ± 0.18
T2	-29.37 ± 0.18
T3	-23.36 ± 0.31
Onset temperature ($^{\circ}\text{C}$)	-42.3 ± 1.3
Peak temperature ($^{\circ}\text{C}$)	-29.37 ± 0.18
Total melting enthalpy (J/g)	52.5 ± 3.3

melting points. *Pinus halepensis* seed oil exhibited a melting peak (-29.37°C), a melting enthalpy (52.5J/g) and an onset temperature (-42.3°C , Table 4), values lower than those reported by Besbes et al. (2004a) for date seed oils. This was due to the higher PUFA in Aleppo pine seed oil (54.2 as against 14.0 for Aleppo pine and Deglet Nour seed oils, respectively).

Solid fat content decreased when temperature increased (Figure 2) and remained constant for temperatures above -8°C , that corresponded to the entire liquefaction of Aleppo pine seed oil. Liquefaction of Deglet Nour seed oil (Besbes et al., 2004a) began at 15°C . This can be explained by the fact that Deglet Nour seed oil had a higher SAFA content than the Aleppo pine seed oil (44.3 as against 16.8, respectively).

Colour

Aleppo pine seed oil showed lower L^* , a^* and b^* values than those reported for Deglet Nour seed oil (57.31, -1.82 and 30 as against 66, -0.4 and 55, for Aleppo pine and Deglet Nour seed oils, respectively; Besbes et al., 2004a). This means that *Pinus halepensis* seed oil was slightly darker than Deglet Nour seed oil. Such a colour seems to attract consumers (Hsu and Chung, 1998). The CieLab (L^* , a^* , b^*) values of other vegetable oils, such as palm, soybean, sunflower, olive and corn ranged from, 63.4 to 69.5, 3.8 to 4.4 and 9.2 to 10.4, respectively (Hsu and Yu, 2002). *Pinus halepensis* seed oil b^* value was higher than those of other vegetable oils. *Pinus halepensis* seed oil was more yellow-coloured than vegetable oils studied by Hsu and Yu (2002). This may suggest the presence of more yellow pigments (carotenoids) in *Pinus halepensis* seed oil. Aleppo pine seed oil showed another colour particularity: Hunter a^* negative value (-1.82) was markedly lower than the Hunter a^* value of common vegetable oils.

Pinus halepensis seed oil showed some absorbance in the UV-C (100–290nm) with a maximum of 2.39 units at 210nm for oil diluted to 1:800, in the UV-B

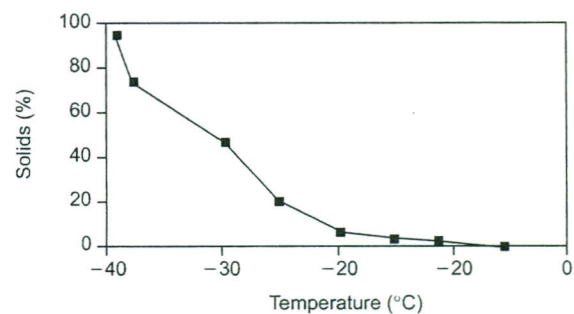


Figure 2. Solid-temperature curve for Aleppo pine seed oil determined by DSC.

(290–320 nm) ranging from 0.3 to 0.1 for oil diluted to 1:100, and UV-A (320–400 nm) ranging from 0.1 to 0.02 for oil diluted to 1:100. Therefore, Aleppo pine seed oil may provide protection against both UV-A (an origin of oxidative stress to skin) and UV-B. The optical transmission of *Pinus halepensis* seed oil, especially in the UV range (290–400 nm) was comparable to that of date seed and raspberry seed oils. This is comparable to titanium dioxide preparations as a sun protection factor for UV-B (SPF) and a protection factor for UV-A (PFA) (Besbes et al., 2004a; Oomah et al., 2000). *Pinus halepensis* seed oil also contained more yellow colouring than raspberry seed oil studied by Oomah et al. (2000). The latter is characterised by a lower absorbance than Aleppo pine seed oil (absorbance ranging from 0.08 to 0.11 against 0.17 to 0.19 at 440–460 nm, respectively, for oils diluted to 1:10). This confirmed the results of colour measurements. Yellow colours, due partly to carotenoids, are beneficial, since they simulate the appearance of butter without the use of primary colorants, such as carotenes and annatos, commonly used in the oil and fat industry (Oomah et al., 2000).

Oxidative Stability

Oxidation is the main problem affecting oils, leading to aldehyde production which imparts strong disagreeable flavours and odours, referred to as rancidity. Generally, the rate of oxidation will depend on the degree of unsaturation of the oil and its temperature as well as the presence of antioxidants (McKevith, 2005). Oils deteriorate after an induction period in which relatively small changes occur (Gordon, 1991). Stability, although is not a standard parameter of quality, is useful to provide information about the hypothetical shelf-life of an oil (Aparicio et al., 1999).

Stability, expressed as the oxidation induction time, was about 24.5 h for *Pinus halepensis* seed oil (Table 5). This oxidation time is lower than that reported by Besbes et al. (2004a) for date seed oil (Deglet Nour cultivar). Such difference may be explained by the fact that *Pinus halepensis* seed oil contained more PUFA than date seed oil (Deglet Nour cultivar) (54.2 as against 14, respectively). The latter also has a higher antioxidant content (phenolic compounds) (526 as against 186 µg/g for date seed and *Pinus halepensis* seed oils, respectively, Table 5) which mainly determines a greater resistance to auto-oxidation (Perrin, 1992; Tsimidou et al., 1992; Baldioli et al., 1996). Phenolics in vegetable oils may generally exert a beneficial effect on their oxidative stability (Farag et al., 2003). Aparicio et al. (1999) mentioned a significant correlation of phenols, oleic/linoleic ratio and tocopherols with oil stability, and it was also observed between total phenol content and oxidative stability by Rancimat (Gutfinger, 1981; Salvador et al., 2001). Phenolic

Table 5. Oxidation induction time and viscosity of seed oil from *Pinus halepensis* and Deglet Nour seed oils.

	<i>Pinus halepensis</i> (mean ± SD, n = 3)	Deglet Nour ¹
Induction time (h)	24.4 ± 2.5	44.8
Viscosity (mPa.s)	7.17 ± 0.05	18.3

¹Besbes et al. (2004a).

compounds would contribute around 50% to the stability of olive oil, while the oleic/linoleic ratio would reach only 27%. The contribution of total tocopherols and colouring pigments (chlorophylls and carotenoids) were around 9 and 13%, respectively (Aparicio et al., 1999). The oxidative stability of other vegetable oils, such as canola, coconut, corn, grapeseed, olive, peanut, safflower, soybean and sunflower ranged from 6.1, 11.25, 4.75, 2.07, 4.85, 5.07, 2.27, 2.22 and 3.25 h, respectively at 110 °C (Tan et al., 2002). This shows that *Pinus halepensis* seed oil oxidative stability was higher than that of most oleic–linoleic vegetable oils.

Viscosity

Viscosity of *Pinus halepensis* seed oil (Table 5) was lower than that reported by Besbes et al. (2004a) for date seed oil (Deglet Nour variety) (7.17 mPa.s as against 18.3 mPa.s), which may be explained by the fact that the latter contained less UFA (65.45 as against 82.6, for Deglet Nour and *Pinus halepensis* seed oils, respectively). It is worth noting that the viscosity of *Pinus halepensis* seed oil is lower than that of most vegetable oils (26 mPa.s, 47.3 mPa.s and 49.4 mPa.s, for raspberry, safflower and grape seed oils, respectively (Oomah et al., 2000)). The viscosity of olive oil reported by Lalas and Tsakins (2002) varies from 45 to 80 mPa.s.

Some Quality Parameters

Pinus halepensis seed oil has a relatively higher phenol content (Table 6) compared to most edible oils except for olive oil, which is considered as a rich source of phenolic compounds in the Mediterranean diet. Salvador et al. (2001) reported that total phenolic content measured by the Folin–Ciocalteu method ranged from 19 to 380 mg/kg, and from 124 to 516 mg/kg according to Nissiotis and Tasioula-Margari (2002). The amount of phenols in crude seed oils is an important factor when evaluating the quality of the oil because these compounds have been correlated with colour, the shelf-life of oil and, in particular, its resistance to oxidation (Cinquanta et al., 1997). *Pinus halepensis* seed oil could be considered as a potential source of natural phenolic compounds. Despite their

Table 6. Physicochemical characterisation of *Pinus halepensis* seed oil.

Parameter	<i>Pinus halepensis</i> (mean \pm SD, n = 3)
Polyphenols (as mg gallic acid/kg of oil)	186 \pm 19
Chlorophyll (mg/kg)	0.07 \pm 0.06
Refractive index (at 20°C)	1.4750 \pm 0.0100
Acidity (% oleic acid)	0.61 \pm 0.08
Saponification index (mg of KOH/g of oil)	190 \pm 13
Iodine index (g of I ₂ /100g of oil)	117 \pm 10
Peroxide index (meq O ₂ /kg of oil)	3.18 \pm 0.05
ϵ_{232}	0.51 \pm 0.01
ϵ_{270}	1.5 \pm 0.31

participation in conferring specific flavour to oil (Caponio et al., 1999), dietary phenolic compounds might have a positive effect in the prevention of coronary heart disease and cancer (Owen et al., 2000; Tuck and Hayball, 2002) a protective and a therapeutic potential in oxidative damage-related pathologies (Auger et al., 2004).

The chlorophyll pigments (Table 6) are important quality parameters because they correlate with colour, which is a basic attribute for evaluating oil quality (Salvador et al., 2001). These pigments are involved in auto-oxidation and photo-oxidation mechanisms (Gutierrez et al., 1992; Mínguez-Mosquera et al., 1990). Interesse et al. (1971) reported that the oxidative stability of olive oil is greatly affected by the presence of chlorophylls and their derivatives, especially in the presence of light as they have the ability to transfer energy from light into chemical molecules. The chlorophyll content of *Pinus halepensis* seed oil is lower than that reported by Aparicio et al. (1999) for virgin olive oil (0.07 as against 9.32–12.67 μ g/g, respectively).

Refractive index of Aleppo pine seed oil (1.4750) was similar to that of olive oils studied by Lalas and Tsakins (2002) and date seed oils studied by Besbes et al. (2004b). The saponification value of 190 is comparable to *Xylopiya aethiopica* oil (Barminas et al., 1999). The lower acidity of *Pinus halepensis* seed oil showed that it might be edible and could have a long shelf-life. It is known that the increase of free acidity is mainly due to enzyme activity (Boskow, 1996). The iodine index of 117 indicates that *Pinus halepensis* seed oil is a highly unsaturated oil and suggests that it contains high levels of oleic and linoleic acids, as previously shown in Table 3. The iodine value of *Pinus halepensis* seed oil is situated inside the interval range of the values mentioned by Tan et al. (2002) in some edible oils (9.37 to 145 g of I₂/100g oil). It provides a measure of degree of oil unsaturation and a means of predicting shelf-life. Lower iodine value means a more stable oil with a longer shelf-life. Peroxide value (3.18 meq O₂/kg of oil) of *Pinus halepensis* seed oil is lower than that of canola (6.68 meq O₂/kg of oil), grapeseed (10.6 meq/kg), olive

(8.5 meq/kg), peanut (6.82 meq/kg), safflower (5.07 meq/kg) and sunflower (9.1 meq/kg) oils (Tan et al., 2002). Salvador et al. (2001) reported that less than 5% of the oils had a peroxide value higher than the upper limit of 20 established for the 'extra-virgin' olive oil

Aleppo pine seed oil showed low absorptivity values at 232 and 270 nm, thus containing little amounts of primary (hydroperoxides) and secondary oxidation products. Compared to *Nigella* seed oil studied by Ramadan and Mörsel (2004), absorptivities at 232 and 270 nm of *Pinus halepensis* seed oil were relatively lower (0.51 and 1.5 as against 3.6 and 1.2, respectively).

The results revealed that Aleppo pine (*Pinus halepensis*) seeds are a rich source of many important nutrients that appear to have a very positive effect on human health. The seed lipids of the investigated samples were rich in linoleic acid, which has a beneficial effect on blood lipids, lowering blood pressure and serum cholesterol. They constitute a good alternative source of essential fatty acids compared with common vegetable oils and could contribute to the overall dietary intake of the mineral elements studied. This unique fatty acid composition, relatively high polyphenol content and quality, and hence high protection against oxidative stress, relatively good shelf-life and other desirable physicochemical characteristics lead to more diverse and novel applications of Aleppo pine seed oil in the food, pharmaceutical, cosmetic and other non-food industries.

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