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ORIGINAL ARTICLE

Fatty acids and triacylglycerols composition from Tunisian *Acacia* species seed oil

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Abstract Recently, plant seeds that have not been enough explored and exploited are cheap sources of a lot of natural molecules for industrial applications. The aim of the present study was to evaluate for the first time the composition of fatty acids and triacylglycerols (TAG) of mature unexploited seeds of some *Acacia* species (*Acacia cyclops*, *Acacia ligulata* and *Acacia salicina*) harvested in Tunisia in order to reveal their potential for human consumption.

Results showed that, *Acacia* seed oils were mainly unsaturated (more than 71%). The polyunsaturated fatty acids were the major fractions (52–68%) with the linoleic acid as the major fatty acid (more than 52%), followed by oleic acid (15–27%) as monounsaturated fatty acid. The TAG composition was significantly different among the three *Acacia* species. PLL, PLO, LnLO, OLL, OOL, and OOO were the major forms. *Acacia* seed oil could be used as potential source of oil with high industrial value; nevertheless *in vivo* tests are essential to confirm its safety before use.

1. Introduction

Owing to the increasing demand for edible oils many studies on the characterization of plant seed and fruit oils have been undertaken (Stupp et al., 2008; Silva et al., 2009). Plants such as olive, corn, sunflower or soybean, being overexploited; studies were focused on newer sources of edible oils such as unexploited plant seeds that represent important sources of suitable oils with high nutritional, pharmaceutical and industrial importance. Vegetable oils are complex mixtures containing a wide range of compounds. They are mainly composed of triacylglycerols, diacylglycerols, free fatty acids, phospholipids, glycolipids and other minor components (Wu, 2007). Triacylglycerols (TAG) are the major storage lipids in plant seeds, and of great nutritional and nutraceutical value as well as, a common source of edible oils for human consumption and industrial purposes (Lung and Weselake, 2006). TAG composition has been considered as a measurement of the

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quality and purity of vegetable oils (Aparicio and Aparicio-Ruiz, 2000). In fact, TAG are one of the prime determinants in the study of oxidation of oils (Christopoulou et al., 2004; Hamilton et al., 1997).

The properties of the oils are dependent on the fatty acids composition which has gained much attention owing to its beneficial implications for human health (Sakouhi et al., 2011). Indeed, the presence of polyunsaturated fatty acids increases the potential beneficial properties of the oils. Previous studies have reported the essential role of polyunsaturated fatty acids in the fluidity and selective permeability of membranes (Ward and Singh, 2005). Moreover, oleic acid and palmitoleic acid, as monounsaturated fatty acids, have an important physiological role (Colquhoun et al., 1996).

Acacia is a cosmopolitan genus of the family of leguminosae containing more than 1350 species widespread in the warm sub-arid and arid portions of the word (Maslin et al., 2003).

Acacia species are found in pure stands or together with other species in naturalized environment and/or in dry deciduous forests. They show strong resistance to drought and salinity (Abari et al., 2011).

Several studies have shown that *Acacia* species are rich in secondary metabolites such as alkaloids, cyanogenic glycosides, cyclitols, gums, terpenes, flavonoids and condensed tannins (Maslin et al., 2003). *Acacia* is used in traditional medicine for their antibacterian, antioxidant, anti-inflammatory, antispasmodic, anti-arythmique and astringent proprieties (El Abbouyi et al., 2004).

In the literature, and to the best of our knowledge, (1) we did not find information about fatty acid and TAG composition of *Acacia cyclops*, *Acacia ligulata* and *Acacia salicina*, and (2) very few studies have been carried out on chemical composition of Tunisian *Acacia* seeds. The goal of the present study was to shed light on the chemical composition of fatty acids and triacylglycerols of *A. cyclops*, *A. ligulata* and *A. salicina* seeds from Tunisia in an attempt to highlight the possibility of using *Acacia* seeds as a raw material source of oil in industrial products.

2. Experimental

2.1. Seed materials

Seeds were collected in June 2010 at random from at least three wild trees of three *Acacia* species (*A. cyclops*, *A. ligulata* and *A. salicina*) located in Tunisia. Mature collected seeds were directly mixed and placed in an oven for 48 h at 60 °C until the difference between successive weight (before and after putting the samples in the oven) is less than or equal to 5 mg. Tables 1 and 2 provide more information about their geographical origin and climatic conditions.

2.2. Reagent and standards

Triacylglycerols standards: glyceryltripalmitate (⩾99%) was purchased from Sigma Aldrich (St. Louis), and chloroform was purchased from Fisher Scientific Company (Ottawa, Canada).

Methanol, and n-hexane, solvents of LC grade were purchased from Panreac Quimica SA. (Barcelona, Spain). Isopropanol and acetic acid were from Fisher Scientific SA (Loughborough, Spain). Petroleum ether was purchased from Sigma Aldrich. Sodium chloride was purchased from Panreac Quimica SA (Barcelona, Spain).

2.3. Oil extraction

The oil from seeds was extracted by a Soxhlet extractor as reported previously (Nasri et al., 2013) following ISO method 659:1998. The solvent was evaporated under reduced pressure, using a rotary evaporator at 50 °C, flushing with nitrogen to blanket the oil during storage. Oil was weighed and stored at −20 °C. All the analyses were conducted in triplicate.

2.4. Fatty acids analysis

Fatty acids analysis was performed using the method published by Youzbachi et al. (2012). Fats were transmethylated

Table 1 Geographic and climatic data of the three sampling station species (annual).

Acacia species	Location	Latitude	Longitude	Altitude	Climate	Soil type	Temperature (°C)	Average rainfall (mm)	Rainfall days (mm)	Humidity (%)
A. cyclops	Nabeul	36°27′N	10°44′E	10.64	Sub-humid	Alluvial	8.4–30.6	5–59	1–12	70–83
A. ligulata	Gabes	33°53′N	10°06′E	4.67	Arid-sahara	Gypsum	8–31	1–44	1–44	61.9–73.8
A. salicina	Tunis	36°49′N	10°09′E	21.84	Sub-humide	Sandy clay	7.2–32.7	2–67	1–13	64.3–78.2

Table 2 Climatic data of the three sampling station species (June).

Acacia species	Location	Temperature (°C)	Average Rainfall (mm)	Rainfall days (mm)	Humidity (%)
A. cyclops	Nabeul	17.8–26.8	8	4	65
A. ligulata	Gabes	19.1–28	3	1	73.8
A. salicina	Tunis	17.3–29	10	3	67.2

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using boron trifluoride in methanol. The fatty acid methyl esters formed by transmethylation were analyzed in an HP 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) fitted with a CPSIL-88 column 100 m × 0.25 mm i.d., film thickness 0.20 μm (Varian, Les Ulis, France). Hydrogen was used as a carrier gas (inlet pressure 210 kPa). The oven temperature was held at 60 °C for 15 min, increased to 85 °C at a rate of 3 °C/min and then to 190 °C at a rate of 20 °C/min, finally being held at 190 °C. The injector and flame ionization detector were maintained at 250 °C and 280 °C, respectively. Fatty acid methyl esters were identified by comparison with standards. The data were computed using the Galaxie software (Varian, Les Ulis, France) and reported as a percentage of total fatty acids. All analyses were performed in triplicate.

2.5. Triacylglycerols determination

Triacylglycerols containing sodium adducts (+23 *m*/*z*) were detected using Waters QTOF-1 mass spectrometer electrospray ionization (operating in positive ion mode).

50 μl of unmodified plant oil weighed and diluted in 950 μl of chloroform. Five μl of this solution (depending on observed intensities) was mixed with 1 ml of a 90:10 methanol/chloroform solution containing 5.75×10^{-5} of both PPP standards. The final solution was then spiked with 50 μl of a 3 mg/ml NaCl solution. The TAG-containing solution was injected into a 15 μl sample loop and carried by a methanol phase with a flow of 60 μl/min to the electrospray capillary, which was held at 3 kV. The source temperature was 80 °C and the desolvation temperature was set to 150 °C. The capillary voltage was 3 kV and the cone voltage was 45 V. ESI-MS was focused on mass ranges of 700–1100 *m*/*z*. Isotopic overlap between species differing by 2 Da (1 double bond) was accounted for using the theoretical natural abundances of Carbon, Oxygen and Hydrogen isotopes and in-house software.

To quantify the different TAG species a total mass spectral intensity were used. As an example, the sodium adducted PPL and POP will have an *m*/*z* of 853.7 and 855.7, respectively. Theoretical percentage of PPL containing two C_{13} isotopes (855.7 *m*/*z*), relative to PPL that containing no C_{13} isotopes, is 18.4%. This directly corresponds to the *m*/*z* of PPO. The experimental intensities occurring from PPL (853.7) and PPL-1C_{13} (854.7 *m*/*z*) were summed with the theoretical intensities of PPL-2C_{13} (855.7 *m*/*z*) and PPL-3C_{13} (856.7 *m*/*z*) to give the total molecular species intensity.

After that the corrected intensity of POP (855.7 *m*/*z*) was calculated by subtracting the theoretical intensity of PPL-2C_{13} (855.7 *m*/*z*) from the experimental intensity at that *m*/*z*. The same was done for POP-1C_{13}; experimental intensity 856.7 less the theoretical intensity of PPL-3C_{13} to give corrected intensity of POP-1C_{13}. The obtained corrected intensities were used to calculate the theoretical intensities of their 2C_{13} and 3C_{13} counterparts, as reported by Tlili et al. (2011) and Trabelsi et al. (2014).

Quantification was achieved by comparison with the total ion count of the internal standard (Tripalmitin) with intensity corrections made for isotopic peak overlap according to the following equation:

$$[\mathrm{TAG}] = \mathrm{TAG}_{\mathrm{intensity}} \frac{[\mathrm{STD}]}{\mathrm{STD\ intensity}}$$

where TAG: Triacylglycerols; STD: Internal Standards.

2.6. Statistical analysis

The experimental data were analyzed using the analysis of variance (ANOVA) and the Statistical Analysis System (XLSTAT 2008). Differences at $p \leqslant 0.05$ were considered statistically significant by Duncan's new multiple range test.

3. Results and discussion

3.1. Oil content

Table 3 shows the seed-oil content of the three *Acacia* species. The mean content of total lipids was 9.35 ± 2.68% on a dry weight basis (dw%), and varied between *Acacia* species. *A. cyclops* contained about 6.83%, *A. ligulata* contained 9.04% and *A. salicina* contained the highest value (12.18%). Indeed, Youzbachi et al. (2012) have reported that *Acacia cyanophylla* in Tunisia contains about 10% of oil. Other previous studies have reported that the oil content in *Acacia* species in India is between 4% and 10% (Sundar Rao et al., 1983; Maity and Mandal, 1990; Khan et al., 2012), and in 20 species of *Acacia* in Australia is between 3% and 22% (Brown et al., 1987). These differences were probably due to genetic factor and also to location affect since the crops chemical composition can vary with the crop varieties, soil and climatic conditions of the area (Breene et al., 2007).

3.2. Fatty acids composition

The results of the analysis of the fatty acid fraction of *Acacia* seed oil are summarized in Table 3. Thirteen fatty acids were identified (C12 to C22).

The most abundant saturated fatty acid was palmitic acid followed by stearic acid. A difference in palmitic acid contents was noted within species; *Acacia cyclpos* contained the highest level (10.25%) followed by *A. ligulata* (7.71%) than *A. salicina* (5.36%). The highest value of staric acid was detected once again in *A. cyclpos* (2.10%) while the amounts in *A. ligulata* and *A. salicina* were 1.61% and 1.39%, respectively. Lauric acid was detected only in *A. cyclops* with a low level (0.09%).

Acacia seed oil can be regarded as linoleic–oleic oil because of the abundance of linoleic acid, precursor of ω6, followed by oleic acid. *A. ligulata* contained the highest level of linoleic acid (67.75%) while the levels in *A. salicina* and *A. cyclops* were 54.31% and 52.39%, respectively. *A. cyclops* presented the high value of oleic acid (27.13%) while the values in *A. ligulata* and *A. salicina* were 17.95% and 15.06%, respectively.

The observed differences could be due to genetic and/or environmental factors. In fact, our samples were from different locations in Tunisia. These regions are characterized by different environmental conditions, such as temperature and rainfall (Tables 1 and 2). Indeed, previous studies on numerous plant-seeds have suggested the effect of the environmental conditions (precipitation and temperature) on the composition of the fatty acids. Aslam et al. (2009), Schulte et al. (2013) and BenLajnef et al. (2015) have reported the effect of temperature and precipitation on palmitic acid, oleic acid and linoleic acid composition, which support our findings.

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Table 3 Comparison of oil content (%) and fatty acids composition (% of total fatty acids) of *Acacia* seed oil of this study and literature.

	Acacia cyclops	*Acacia ligulata*	*Acacia salicina*	*Acacia cyanophylla*[a]	*Acacia tortilis*[b]	*Acacia mollissima*[c]
Oil content %	6.83 ± 1.26	9.04 ± 2.34	12.18 ± 3.56	10.17	4	7.16
Fatty acid (% of total fatty acids)						
12:0	0.09 ± 0.01	tr	tr	0.07	nd	nd
14:0	0.14 ± 0.01	0.08 ± 0.00	0.03 ± 0.01	0.13	nd	0.1
16:0	10.25 ± 0.12	7.71 ± 0.06	5.36 ± 0.09	9.63	15.10	10.5
16:1n-9	0.06 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	nd	nd	nd
16:1n-7	0.18 ± 0.00	0.52 ± 0.01	0.15 ± 0.01	0.41	nd	nd
18:0	2.10 ± 0.04	1.61 ± 0.00	1.39 ± 0.03	1.7	1.85	0.8
18:1n-9	27.13 ± 0.04	17.95 ± 0.01	15.06 ± 0.19	21.52	19	16.6
18:1n-7	0.94 ± 0.07	1.77 ± 0.01	0.91 ± 0.29	1.37	nd	nd
18:2n-6	52.39 ± 0.01	67.75 ± 0.04	54.31 ± 0.18	63.08	64.79	68.1
18:3n-3	0.32 ± 0.04	0.74 ± 0.01	0.32 ± 0.26	0.24	nd	0.8
20:0	1.08 ± 0.00	0.50 ± 0.01	0.79 ± 0.02	0.47	nd	0.6
20:1n-9	0.56 ± 0.01	0.11 ± 0.00	0.39 ± 0.21	0.13	nd	nd
22:0	0.63 ± 0.06	0.26 ± 0.01	0.49 ± 0.02	0.48	nd	nd
SFA	14.29	10.16	8.06	12.68	16.95	12
MUFA	28.87	20.37	16.54	23.68	19	16.7
PUFA	52.71	68.49	54.63	63.08	64.79	68.9
UFA	81.58	88.86	71.17	86.76	83.79	85.6
PUFA/SFA	3.69	6.74	6.77	4.97	3.82	5.74
ω6	52.39	67.75	54.31	63.08	64.79	68.1
ω3	0.32	0.74	0.32	0.24	nd	0.8

SFA. saturated fatty acid; MUFA. Monounsaturated fatty acids; PUFA. Polyunsaturated fatty acids. UFA. Unsaturated fatty acids; tr: trace; nd: note determined.

[a] youzbachi et al. (2012).
[b] Khan et al. (2012).
[c] Banerji et al. (1988).

Youzbachi et al. (2012) have suggested that in 12 Tunisian *A. cyanophylla*, linoleic acid (61.11–65.45%) and oleic acid (19.67–22.85%) are the major fatty acids. Moreover, previous study on 20 species of edible Australian *Acacia* seeds has reported that the major detected fatty acids are linoleic (12–71%), oleic (12–56%) and plamitic (7–35%) (Brown et al., 1987). Furthermore, other authors have reported that in Indian *Acacia* species linoleic acid and oleic acid are the main fatty acids (Khan et al., 2012; Maity and Mandal, 1990; Banerji et al., 1988).

Acacia seed oil of this study contained more than 70% of unsaturated fatty acids and *A. ligulata* showed the highest level with 88.86% (Table 3). These high values were essentially due to the high values of polyunsaturated fatty acids (ca. 68%, ca. 54% and ca. 52% for *A. ligulata*, *A. salicina* and *A. cyclops*, respectively). The monounsaturated fatty acids were between 16.54 (*A. salicina*) and 28.87 (*A. cyclops*). Youzbachi et al. (2012) have reported that the unsaturated fatty acids content in *A. cyanophylla* is about 86% (23% and 63% for monounsaturated and polyunsaturated, respectively). Khan et al. (2012) have suggested that the polyunsaturated content of *Acacia tortilis* is about 83%. In *Acacia mollissima* the amount is 85.6% (Banerji et al., 1988).

The level of saturated fatty acids was between 8.06% (*A. salicina*) and 14.29% (*A. cyclops*). The values reported for *A. mollissima*, *A. cyanophylla* and *A. tortilis* are 12%, 12.68%, and 16.95%, respectively (Khan et al., 2012; Youzbachi et al., 2012; Banerji et al., 1988).

The detected unsaturated fatty acids confer a dietary and an industrial importance to *Acacia* seeds. Indeed, it is known that polyunsaturated fatty acids can influence some physical properties of the cellular membranes such as fluidity and permeability (Ward and Singh, 2005). Moreover, the benefits of polyunsaturated fatty acids in some diseases, such as cardiovascular diseases and autoimmune disorders, have been reported (Reiffel and Mcdonald, 2006).

Results showed that the polyunsaturated/saturated ratio of *Acacia* seed oil was between 3.69 (*A. cyclops*) and 6.77 (*A. salicina*). The obtained values were similar to those reported by Khan et al. (2012) for 15 *Acacia* species from India (1.98–7.48). This ratio was slightly high than the value reported by Nehdi (2011) for soybean (2.68) and close to some fenugreek cultivars as reported by Skakovskii et al. (2013). Indeed, a high polyunsaturated/saturated ratio is regarded favorable for reduction of serum cholesterol and prevention of heart diseases (Oomah et al., 2002).

The ω3 and ω6 polyunsaturated fatty acids are essential components of the human diet because humans are unable to synthesize these fats, both of which are considered to be the precursor of eicosanoids. Moreover, linoleic fatty acid is indispensable for the healthy growth of human skin (Bruckert, 2001). Results clearly showed that *Acacia* seeds oil contained more than 52% of linoleic acid (ω6), while α-linolenic acid (ω3) did not exceed 0.74%. These findings were similar to other reports for other *Acacia* species (Khan et al., 2012; Youzbachi et al., 2012; Banerji et al., 1988).

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Therefore, *Acacia* seed oil could be used as promising source of oil with high value for pharmaceutical and cosmetic sectors; nevertheless its safety must be tested before use. Previous work has shown that *Acacia* species contain cyanogenic glycosides (Maslin et al., 2003). In fact, cyanogenic glycoside, especially in the presence of β-glycosidases, is potentially poisonous. Indeed, Seigler (2003) has reported that among the known cyanogenic plants, some species have been described that have cyanogenic glycosides and not the enzymes, other plants have the enzymes and not the glycosides, and other species have both; and this last category is potentially toxic to livestock.

3.3. Triacylglycerols composition

Fig. 1 shows a typical TAG chromatogram. Total mass spectral intensities (Fig. 2) were used to quantify the different TAG species. Fourteen molecular species of TAG were detected (Table 4). The TAG composition was different between the three *Acacia* species. The predominant molecular species of triacylglycerols were those containing fatty acids 18:2 and 18:1, which was in agreement with the above results showing that the major fatty acids were 18:2n-6 and 18:1n-9. Results showed that the species PLL, PLO, LnLO, OLL, OOL, and OOO were the major forms in the all *Acacia* seed oils.

It can be concluded that the main forms of TAG in *A. cyclops* were LnLO (19.25%), followed by SSL (16.36%), OOO (12.88%) and OLL (12.14%). In *A. ligulata*, the major fraction was LnLO (43.66%) followed by OLL (21.55%) and PLL (11.23%). The dominant fraction in *A. salicina* was LnLO (37.7%), followed by OLL (24.59%) and PLL (12.26%). PPS, PSO, SSL and SSO were identified only in *A. cyclops* (1.17%, 0.85%, 16.36 and 2.03%, respectively). These quantitative variations observed in the major TAG composition of the three *Acacia* species may be due to differences in the levels of major fatty acids: oleic, linoleic and palmitic acids. Indeed, *A. cyclops* presented the high values of C16:0, C18:0 and C18:1, which could explain the high level of OOO, PPS, PSO, SSL and SSO in this species compared to *A. ligulata* and *A. salicina*. This ascertainment was in agreement with those of Jellum (1970) and Harrabi et al. (2010) for corn kernel. Moreover, the separation and the identification of TAG from vegetable oil seem to be a hard task due to the presence of numerous TAG species with similar physicochemical properties (Trabelsi et al., 2014).

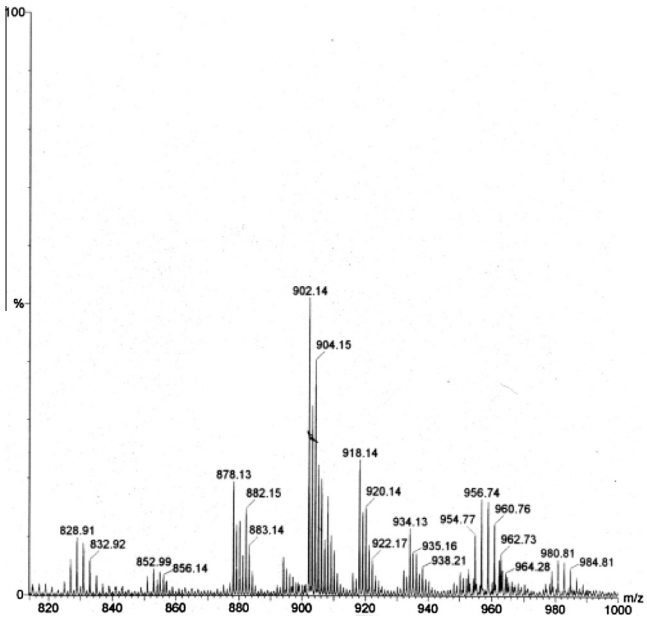

Figure 2 Spectrum of the different molecular species of TAG from *Acacia* species.

The obtained results were slightly different to that of some common seed oils in which the linoleic acid is the major fatty acid such as soybean oil (16% OLL, 7% LLLn, 2% OOO, and 8% OOL) and *Pinus pinea* seed oil which has 24.38% LLO, 17.82% LOO, 11.31% PLO, and 7.5% POO (Padley, 1994; Nasri et al., 2009). Our results agreed with other authors

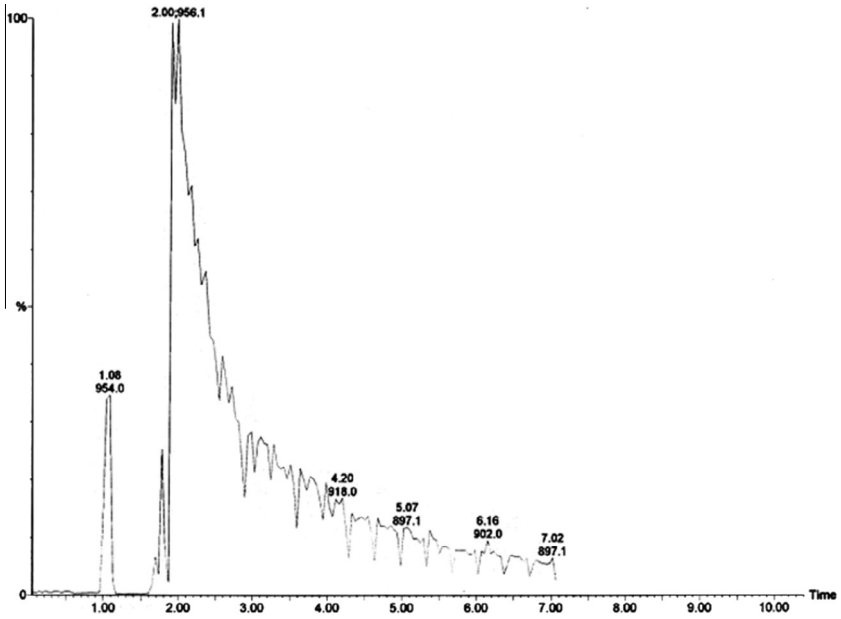

Figure 1 Chromatogram of TAG from *Acacia* species.

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Table 4 Triacylglycerol content (mg TAG/100 mg oil) and composition (%) of *Acacia* seed oil.

Molecular species of TAG	*Acacia cyclops*	%	*Acacia ligulata*	%	*Acacia salicina*	%
PPLn (851 *m/z*)	0.34 ± 0.07	1.32	ND	ND	0.18 ± 0.04	0.37
PPL (853 *m/z*)	0.23 ± 0.06	0.89	ND	ND	0.37 ± 0.058	0.77
PPO (855 *m/z*)	0.32 ± 0.04	1.24	ND	ND	0.22 ± 0.045	0.46
PPS (857 *m/z*)	0.30 ± 0.06	1.17	ND	ND	ND	ND
PLL (877 *m/z*)	1.87 ± 0.21	7.30	3.67 ± 0.54	11.23	5.82 ± 1.57	12.26
PLO (879 *m/z*)	2.01 ± 0.25	7.84	3.00 ± 0.64	9.18	3.75 ± 1.01	7.90
POO (881 *m/z*)	1.20 ± 0.02	4.68	0.57 ± 0.27	1.74	0.74 ± 0.17	1.55
PSO (883 *m/z*)	0.22 ± 0.02	0.85	ND	ND	ND	ND
LnLO (901 *m/z*)	4.93 ± 0.62	19.25	14.26 ± 0.63	43.66	17.89 ± 2.42	37.70
OLL (903 *m/z*)	3.11 ± 0.40	12.14	7.04 ± 0.12	21.55	11.67 ± 0.39	24.59
OOL (905 *m/z*)	1.56 ± 0.25	6.09	2.92 ± 0.89	8.94	4.94 ± 0.59	10.41
OOO (907 *m/z*)	3.30 ± 0.31	12.88	1.20 ± 0.20	3.67	1.87 ± 0.96	3.94
SSL (909 *m/z*)	4.19 ± 0.38	16.36	ND	ND	ND	ND
SSO (911 *m/z*)	2.03 ± 0.15	7.92	ND	ND	ND	ND

Each value is mean ± standard deviation (SD) of a triplicate analysis performed on different samples P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid; ND, not detected.

who reported that the TAG content can vary among populations and also can vary among species (Crane et al., 2005; Nasri et al., 2009).

Spady and Dietschy (1985) have suggested that saturated dietary TAG inhibit the activity of hepatic LDL receptors in the Hamster. Other studies have reported the benefic effect of triacylglycerol on glucose homeostasis and on the prevention of obesity and atherosclerosis (Kojima et al., 2010; Pegorier et al., 1985). *Acacia* seed oil could be used as potential source of TAG with high industrial value; however its safety must be proved.

4. Conclusion

In conclusion, the present study revealed that the seed oils of *Acacia* species, widely distributed in Tunisia, seems to be a promising source of polyunsaturated fatty acids. Oleic acid and linoleic acid were the major fatty acids. Different molecular species of TAG were detected in *Acacia* seed oil. From the molecular species of triacylglycerols PLL, PLO, LnLO, OLL, OOL, and OOO were the major forms. The composition of TAG though the species studied was in accordance with their fatty acid composition. Oil from *Acacia* seeds can be proposed as potential source of oil with economic benefit to populations in developing countries. It was interesting to bring attention that *in vivo* tests are needed to confirm the beneficial quality of *Acacia* seed oil.

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