JOURNAL
OF NEW SCIENCES
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Chemical characteristics of fixed oil of lentisk tree (*Pistacia lentiscus* L.)

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Abstract – This study characterised the fixed oil of the lentisk tree from four regions of northern Tunisia (Dmain, Ouled Salah, Oued Maaden and Tabouba), by determining its fatty acid composition, fat content, and structural and refractive indexes. There were no significant differences in the fat content across all four regions, while oleic and palmitic acids were higher in Dmain, Ouled Salah, Oued Maaden. The refractive index was lowest in the Tabouba region, iodine not significantly different across all regions, while the lowest saponification index was in Ouled Salah. Results suggest that the geographical origin does not influence the characteristics of oil but rather, the production method has more influence on the quality of the oil.

Keywords: Fixed oil, chemical analysis, fatty acids, quality indexes.

1. Introduction

Several million households worldwide depend heavily on non-wood forest products (NWFPs) for their livelihood and as a source of income (Brown & Lassoie 2010). About 80% of the population in developing countries, such as Tunisia, use NWFPs to meet nutritional and health needs, while also providing raw materials for industrial processing (Croitoru 2007). Some NWFPs are important export commodities, with at least 150 NWFPs currently on international trade, including honey, gum arabic, mushrooms, essential and vegetable oils (Shackleton & Gumbo 2010). These forest species deserve more attention and scientific work in order to value them and, eventually, contribute more towards their conservation and the income of households in forest areas. Consequently, this study aims to determine the chemical characteristics of the fixed oil of *P. lentiscus*, an important medicinal plant with economic value which is on a continuous increase (Mezni 2015).

2. Materials and methods

2.1. Biological material

The four fixed oil samples for chemical analyses represent the Agricultural Development Groups (ADGs) of the Dmain, Ouled Salah, Oued Maaden and Tabouba regions of the Nefza Delegation, located in the governorate of Beja in northern Tunisia. The different analyses were repeated three times for each region.

2.2. Experimental apparatus

The artisanal method of extracting fixed oil from *P. lentiscus* L. in northern Tunisia began by harvesting the fruits of this shrub. The branches were then cleaned by placing the leaves in cold water. The tab obtained was then ground, kneaded and pressed. Finally, the juice obtained was filtrated in order to obtain oil (Mezni 2015).

2.3. Chemical analysis
2.3.1. The quality indexes

The quality indexes used for classifying the oil were the acid index, the peroxide value, the water content and the content of insoluble impurities.
* The analysis of the acid index was determined according to NFT 60-204 (1985)

The acid index was then calculated by applying the following formula:

$$AI= (56.1 *V*C) /m$$

With:

AI: acid index

V: volume (ml) of the titrated solution of potassium hydroxide used

C: concentration (mol / l) of the titrated solution of potassium hydroxide

M: mass (g) of the test portion.

* The analysis of the peroxide value was determined according to ISO 3960 (2001). The calculation of this index was made based on the following formula:

$$PV = ((V-V0) * C (thio) * F * 1000) / m)$$

With:

PV: Peroxide value

V: volume of the sodium thiosulfate solution used for the determination (ml)

V0: volume of the sodium thiosulfate standard solution used for the blank test (ml)

F: factor of sodium thiosulfate solution 0.01 N

C (thio): concentration of the sodium thiosulfate standard solution (0.01 mol / l)

m: mass of the test portion.

* The water content analysis was determined according to NT ISO 662 (1980).

The content of water and volatiles (W) expressed as a percentage of mass were given by the following relation:

$$W= (M1-M2 / M1-M0) * 100$$

With

M1: mass of the capsule and the test portion before heating (g)

M2: mass of the capsule and the test portion after heating (g)

M0: mass of the empty capsule (g).

* The analysis of the content of insoluble impurities was determined according to NFT standard ISO 663 (1993). The content of insoluble impurities (IMP) was calculated by applying the following formula:

$$IMP = ((MI-M0) * 100) / E$$

With:

IMP: content of insoluble impurities

MI: mass (g) of the container with insoluble impurities after drying to constant mass. M0: mass (in g) of the container

E: mass of the test portion (g).

2.3.2. The structural indexes

The structural indexes represent the saponification index and the iodine value.

* The analysis of the saponification index was determined according to the standard ISO 3657 (2002). The saponification index (SI) was then calculated as follows;

$$SI = ((V0-V1) * C * 56.1) / m$$

SI: Saponification index

V0: volume of the standard HCl solution for the blank test (ml)

V1: volume of the HCl standard solution for the determination

C: exact concentration of the titrated HCl solution used (mol / l)

M: test portion (g).

* The analysis of the iodine value was determined according to ISO 3961 (1987). The value of this index (Wi) was calculated as follows:

$$Wi = 12.69 * C * (V1-V2) / m$$

With

Wi: Iodine value

C: the concentration of the sodium thiosulfate solution (mol / l)

V1: volume of the sodium thiosulfate solution used for the blank test (ml)

V2: volume of the sodium thiosulfate solution for determination (ml)

M: test portion (g).

2.3.3. Fat content

The fat content was determined according to ISO 659 (2009). The fat content was then calculated using the equation:

$$FC = ((P2-P1) / m) * 100$$

With:

FC: Fat content

P2: Mass of the tare containing the test portion (g)

P1: mass of the empty tare (g)

M: test portion (g).

2.3.4. Fatty acids

The determination of the fatty acids was carried out using a gas chromatograph (GC). The fatty acid analysis was determined according to ISO 5509 (2000).

2.3.5. Refractive index

The refractive index analysis was determined using a refractometer according to ISO 6320 (2000).

3. Results and discussion

3.1. The quality indexes

The acid index obtained in this study varied from 7.55 to 58.86, with the highest ($p<0.05$) in the Tabouba region. The peroxide index ranged from 6.43 to 27.04, with the lowest recorded in the Ouled Salem region (Fig. S1 A & B). Analysis of the water content shows no significant differences ($p>0.05$) observed in all regions (Fig. S1C), while the Tabouba region recorded the highest insoluble impurities (3.652%) (Fig. S1D). It can be suggested that there is no correlation between the extraction method, the impurity level and the geographical origins of three of the production areas in the current study (Dmain, Ouled Salem and Oued Maaden). However, the high content of impurities in oil from the Tabouba region supports our hypothesis of some problems in the production method in this region. This can be explained by some similarities, in one way or another, between extraction methods that are all artisanal, applying a high spin and causing a lot of impurities.
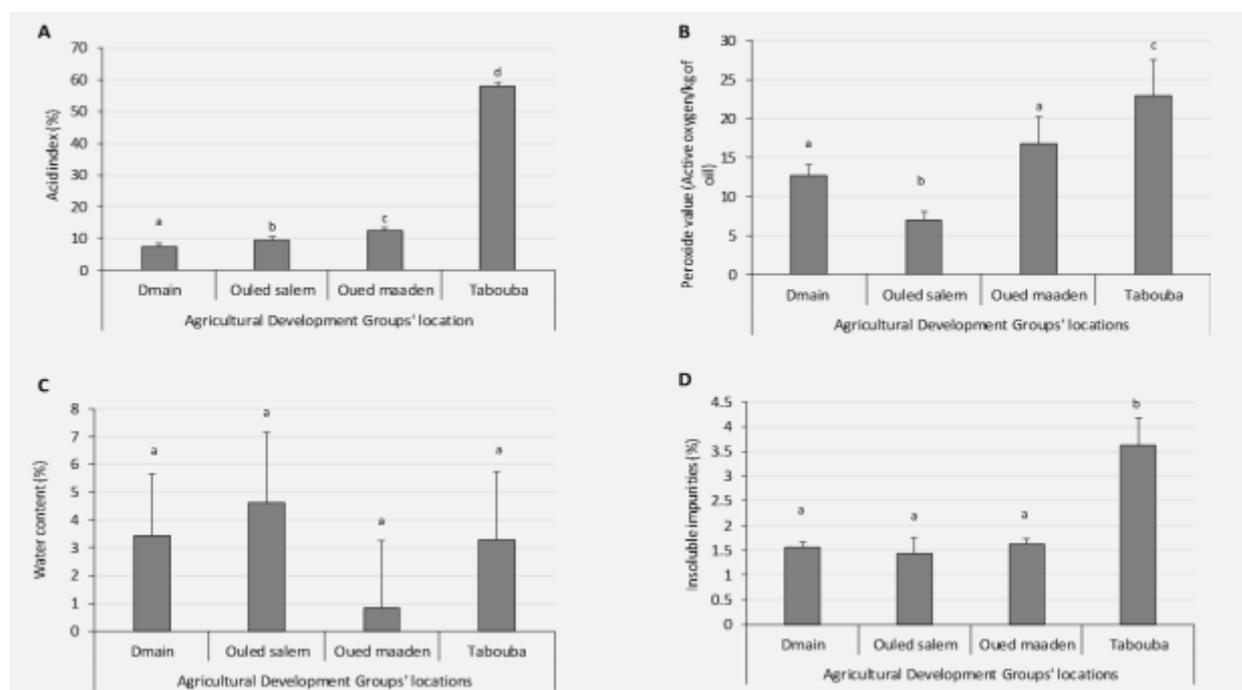
Figure S1. Evolution of quality indexes according in the four regions (Dmain, Ouled Salah, Oued Maaden and Tabouba), **A**: acid index, **B**: peroxide value, **C**: water content, **D**: insoluble impurities. Error bars with different letters indicate significant differences between means.

3.2. The structural indexes

The iodine values ranged from 5.81 to 15.33 g/100g of oil, with no significant differences obtained across the regions, while the saponification index was between 126.76 and 191.87 g/100g of oil (Fig S2 A & B). The Ouled Salem region recorded the lowest structural indexes (Fig. S2B). Neither the geographical location nor the extraction process had any influence on the iodine value of the oils. The saponification index of an oil varied according to its geographical origin (region of provenance), and also according to the process used during its extraction.
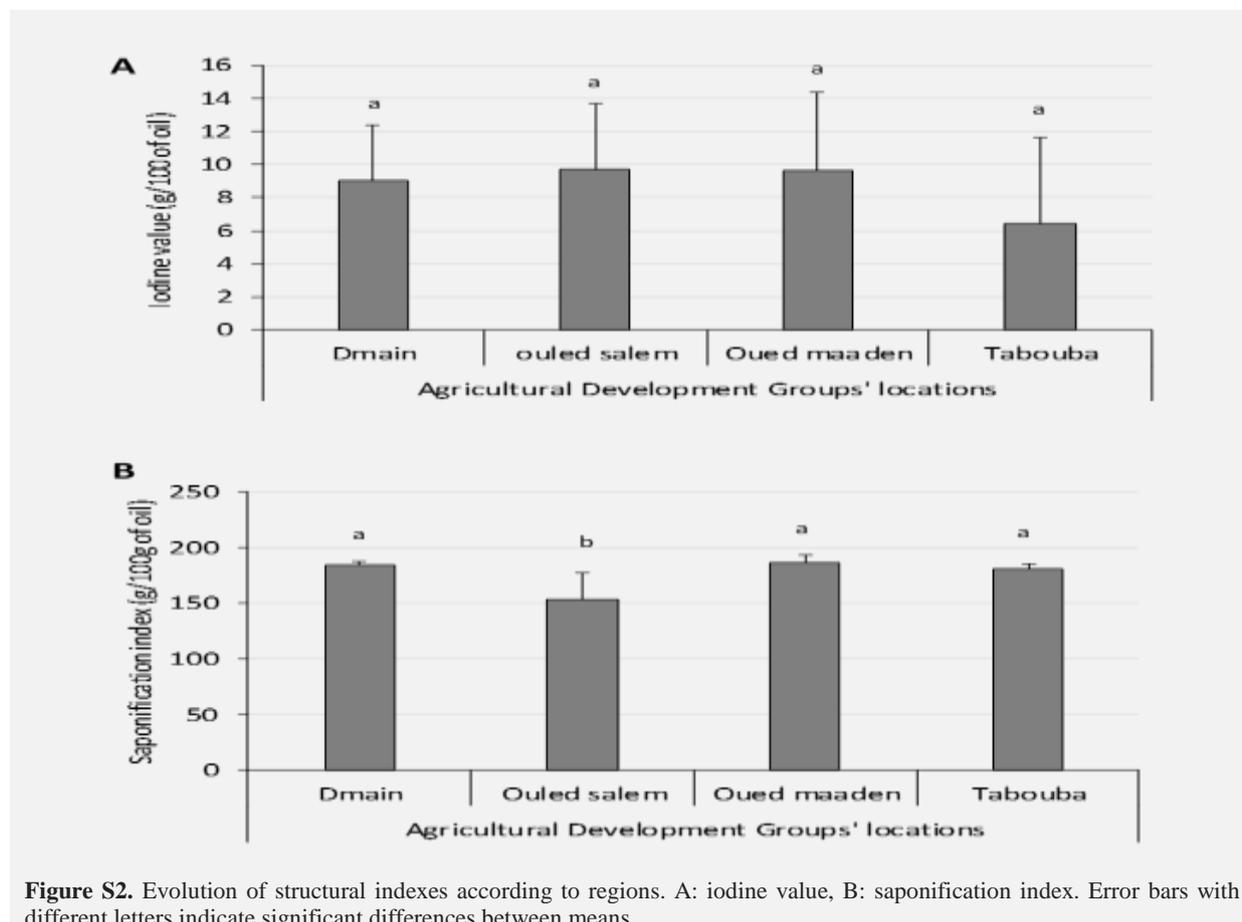

Figure S2. Evolution of structural indexes according to regions. A: iodine value, B: saponification index. Error bars with different letters indicate significant differences between means.

3.3. Fat content

The fat content in the oil was not significantly different across all four regions (range 92.10- 100%; Fig. S3), implying that it is not dependent on the geographical origin of the oils, but possibly dependent on the species. The richness of the fixed oil of *P. lentiscus*, in unsaturated fatty acids, indicates a significant nutritional value of the oil. According to the content of each fatty acid in the most consumed vegetable oils, the fixed oil of lentisk meets the criteria of high quality oil, as it has a composition similar to that of cotton seed oil and rice bran oil.

3.4. Fatty acids

Oleic, palmitic and palmitoleic acids were higher in the Dmain, Ouled Salah, Oued Maaden compared to the Tobouba region, whereas stearic acid was highest in the Tabouba region (Table S1). Most fatty acids present in fixed oils of *P. lentiscus* are affected by the origin (region) and by the individual plant effect within the same site. Depending on the harvest site and the individual from which the fruit was harvested, changes in the oleic and linoleic acid contents observed in all oil samples are probably related to genetic and landscape factors (e.g. pedoclimatic and topography).
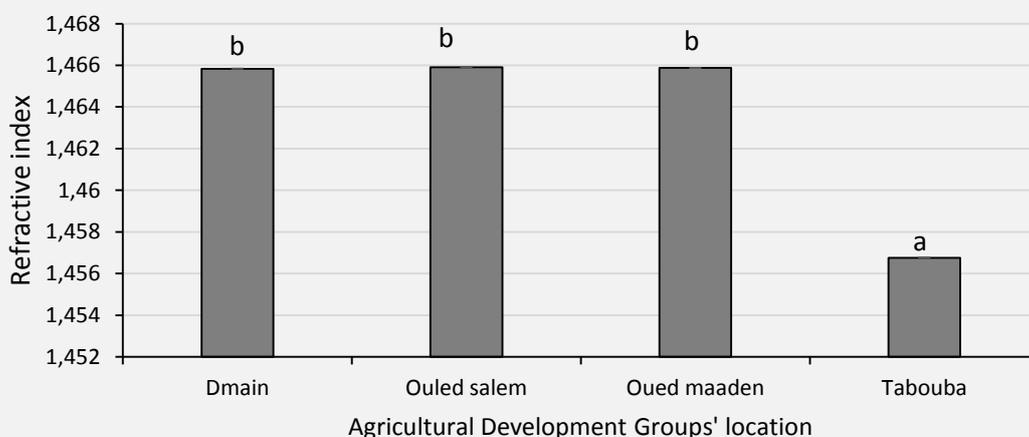
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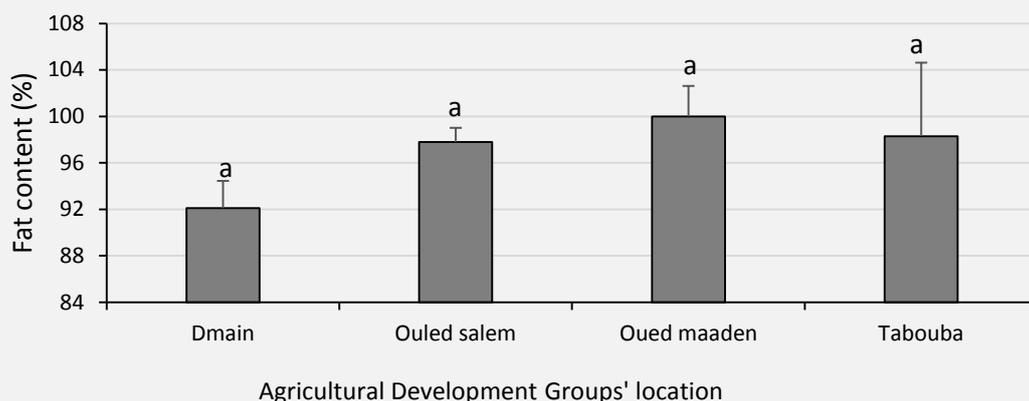
Figure S3. Evolution of fat content in the four regions (Dmain, Ouled Salah, Oued Maaden and Tabouba). Error bars with different letters indicate significant differences between means.

Table S1. The fatty acid composition (saturated fatty acids, subdivided into two subgroups and unsaturated fatty acids, subdivided into three subgroups) of samples from the four Agricultural Development Groups.

	Fatty acid	Dmain (%)	Ouled Salem (%)	Oued Maaden (%)	Tabouba (%)	The average (%)
Unsaturated fatty acid	**Oleic (C18 :1)**	48,92	48,04	46,27	41,99	**46,305**
	Linoleic (C18:2)	20,88	23,13	21,98	24,63	**22,655**
	Palmitoleic (C16:1)	1,75	1,66	1,88	1,33	**1,655**
Saturated fatty acid	**Palmitic (C16 :0)**	27,16	25,64	28,35	23,41	**26,14**
	Stearic (C18 :0)	0,51	0,54	0,75	1,27	**0,7675**

3.5. Refractive index

The refractive index was lowest in the Tabouba region, compared to the other three regions (Fig. S4). The refractive index for the current study was lower than the values reported by Karleskind (1992) who reported 1.47 for olive, 1.45 for palm and 1.47 for avocado oils. Because the refractive index in the Tabouba region was significantly lower than the other regions, it can be assumed that this oil was not pure and it may have been mixed with other liquids, such as water, the refractive index depends on the chemical composition of the oil.

Figure S4. Evolution of the refractive index by regions (Dmain, Ouled Salah, Oued Maaden and Tabouba). Error bars with different letters indicate significant differences between means.

4. Conclusion

The acid and peroxide indexes were highest in the Tabouba region, with no significant differences in all regions for the iodine value, fat content and the water content. The main unsaturated fatty acids contained in the fixed lentisk oils were oleic, linoleic and palmitoleic acids, representing more than 70% of the total acid composition. The richness of the fixed oil of *P. lentiscus* in unsaturated fatty acids demonstrates a high nutritional value. The different characterisation parameters of *P. lentiscus* oils have asserted that the geographical origin does not influence the characteristics of oil but rather, the production method with respect to the quality standards during the production process are the guarantors of the quality of the oil. This is confirmed in some indices for the oil from the Tabouba region, which have detected some failures in the production process.

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