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The Chemical Composition of Argan Oil

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Abstract— The present study assessed the chemical composition (such fatty acids, triglycerides, tocopherols) of argan oil from plants growing in two regions of Algeria (Tindouf and Mostaganem). The extraction of oil was carried out by solvent using soxhlet apparatus. The oil yield obtained was 55.9 % for Tindouf oil (TO) and 66.5 % for Mostaganem oil (MO). The fatty acid composition was determined by GC-FID, the triglycerides and tocopherols by HPLC. The results showed that the unsaturated fatty acids were 79.88 % and 82.58 % with the predominant components were oleic acid 45.02 % and 50.3 % followed by linoleic acid 29 % and 36.8 %. The major triglycerides were dilinoleoyl-oleoyl-glycerol (LLO) 12 % and 15 %, dioleoyl-llinoleoyl-glycerol (OOL) 15.5 % and 18.8 %, palmitoyl-oleoyl-linoleoyl-glycerol (POL) 12.6 % and 14.3 %, palmitoyl-dioleoyl-glycerol (POO) 15.8 % and 16.8 % then triolein (OOO) 11.3 % and 12.1 %. The unsaponifiable fraction was 1.6 % and 1.71 %. The tocopherols showed a high amount 657. 424 and 749.38 mg/kg with the gamma tocopherol as main compound 555.827 and 689.49 mg/kg.

***Keywords*- Arania spinosa, argan oil, extraction, fatty acids, triacylglycerols, unsaponifiable fraction, tocopherols**

I. INTRODUCTION

Argania spinosa is a tropical tree which belongs to the sapotaceae family [1]. This plant is endemic of south-western Algeria and Morocco [2]. This species is originally grown in South Morocco and grows also throughout south-western of Algeria [3]; it's widespread in arid and semi-arid regions [4]. It protects soil from desertification and erosion [5], it has a very significant economic interest which lies in the over use of all parts of the plant such oil, firewood, timber [6] and forage (leaves and pulp) [7]. The plant is a source of valuable biologically active and edible oil produced from fruits of *Argania* [8]. This oil is consumed as human food and used in cosmetic preparations and in traditional medicine [9] and it is now incorporated into many cosmetic preparations [10]. This oil is mainly rich in essential polyunsaturated fatty acids. It's a source of oleic acid (47.7%) and linoleic acid (29.3 %) [11]. It is rich in minor and noble compounds like tocopherols, polyphenols, sterols, carotenoids, xanthophylls, squalene [12] and saponins [13]. Many scientific studies have reported that the oil has many pharmacological effects, such as antioxidant [14], antiproliferative [15], cardioprotective [16] and hypolipemiant [17] activities.

Therefore, the aim of this present work was to determinate the chemical composition of argan oil obtained from trees growing in two regions of Algeria (Tindouf and Mostaganem). The results will furthermore be important as an indication of the potential nutraceutical and economical utility of argan oil of Algeria as a new source of edible lipids.

II. MATERIALS AND METHODS

1. Plant materials

Argan ripe fruits were collected from trees (*Argania spinosa*) original of two regions of Algeria (Tindouf and Mostaganem) during the fruition state (June, 2011).

2. Sample preparation

Fruits (1000 g) of each location were selected. The kernel were removed, dried and ground into fine powder, then stored for the following use. The oil was extracted by using solvent extraction in 250 ml Soxhlet extractor for 8 h [18]. 30 g of seeds powder placed into a cellulose paper cone and extracted using hexane (b. p 40 - 60°C). The solvent was removed by evaporating using a rotary evaporator under reduced pressure. The residual solvent was removed by drying in an oven at 60°C for 1 h. The oil was filtered under anhydrous sodium sulfate (Na_2SO_4). The results are expressed as lipid percentage of dry matter.

3. Identification of fatty acids

The determination of fatty acids composition of the argan oil was performed by gas chromatography (GC-FID), according to the method recommended following the [19]. Fatty acid methyl esters (FAME) were prepared in the presence of a solution of potassium hydroxide (2 N) in methanol and analyzed on a THERMO FINNIGAN Instruments GC 8000 capillary gas chromatograph equipped with a FID detector and a BPX 70 fused silica capillary column (30 m × 0.32 mm , film thickness : 0.25 µm). The operational conditions were: oven temperature in isotherm was as follows: 190ºC; injector 250°C, detector 250ºC; the carrier gas was Nitrogen at a flow rate of 1 ml / min; injected amount 1µl with splitless mode.

4. Identification of triacylglycerol

The triacylglycerol (TAGs) profile obtained was carried out according to the method recommended by the norm displayed in the European Official Journal [20]. The method was realized by High performance liquid chromatography (HPLC) using a WATERS HPLC

Instrument equipped with a UV-Visible detector. The analysis was carried out according to the operatory conditions: an oil solution of 5 % in acetone. The TAGs were separated using a C_{18} column (25 cm × 4 mm and particle size of 5 µm) and was eluted with a mixture of acetonitrile–acetone (65/35: v/v) at a flow rate of 0.5 ml /min; the injected content was 20 µl and the wavelength used was 292 nm.

5. Unsaponifiable fraction

The unsaponifiable fraction was determined according to the NF method [21]. 5 g of oil were saponified with an ethanolic solution of potassium hydroxide (2N) by refluxing for 20 minutes. After cooling and addition of distilled water, the unsaponifiable fraction were extracted with ethyl ether and then washed with water until neutral reaction of washing. Evaporation of the solvent is done by a rotary evaporator and the remaining residue is taken to dryness, and the residue weighed after drying to constant weight. The content of unsaponifiables is determined by percentage calculation.

6. Identification of tocopherols

The determination of tocopherol compounds were performed according to the method described by ISO [22] using HPLC. The separation and the quantification of the isomers were determined by HPLC apparatus (Waters) equipped with a UV-Visible detector and fitted with a LiChrosorb Si 60 column (25 cm × 4mm × 5 µm). The compounds were eluted with a mixture of solvents hexane: isopropanol (99.5: 0.5) at an isocratic flow with 1 ml / min. the oil was dissolved in hexane (10%). 20 µl of the mixture was injected. The tocopherols content was analysed at 295 nm and quantified with reference to standard curves of each vitamer.

III. RESULTS

1. Percent yield

The oil yield obtained is 55.9 % for TO and 66.5 % for (MO).

2. Fatty Acids

The fatty acid compositions (Figure 1 and 2) of argan oil for the two locations are shown in table 1. Unsaturated fatty acids were the predominant components with 79.88 % (TO) and 82.58 % (MO), both oils contain as major fatty acids the oleic and linoleic acid with the highest concentration, they differ mainly only in ratio of oleic and linoleic acid. The TO contains the greatest amount of oleic acid (50.30 %) compared to the MO (45.02 %) while the MO has the greatest amount of linoleic acid (36.80 %) compared to the TO (29 %). The linolenic acid is shown in trace amount for the two oils (0.12 - 0.32 %). The major saturated fatty acids were palmitic acid with 13.84 % (TO) and 12.28 % (MO), then the stearic acid with 05.68 % (TO) and 04.72 % (MO).

Table 1: Fatty acids percentage of argan oil for two regions.

Compound	TO (%)	MO (%)
Myristic acid C14:0	00. 20	00. 15
Palmitic acid C16:0	13. 84	12. 28
Palmitoleic acid C16:1	00. 12	00. 11
Stearic acid C18:0	05. 68	04. 72
Oleic acid C18:1	50. 30	45. 02
Linoleic acid C18:2	28. 99	36. 80
Linolinic acidC18:3	00.12	00. 23
Arachidic acid C20:0	00. 39	00. 27
Gadoleic acid C20:1	00. 35	00. 42

TO: Tindouf oil; MO: Mostaganem oil

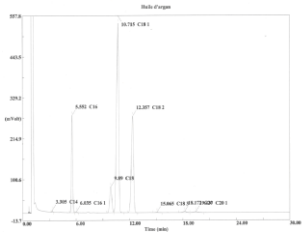

Figure1. Chromatogram of fatty acids (TO)

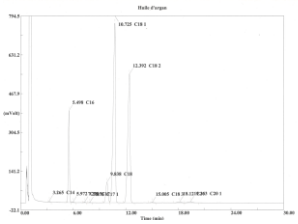

Figure2. Chromatogram of fatty acids (MO)

3. Triglycerides

The determination of triacylglycerols (TAG) was carried out by HPLC (Figure 3 and 4). The analysis had shown the presence of twelve TAG in argan oil for the two regions (Table 2). The two oils consist mostly of five predominating compounds as oleo-dilinolein (OLL), dioleo-linolein (OOL), palmito-oleio- linolein (POL), palmito-diolein (POO) and triolein (OOO) including oleic, linoleic and palmitic acid residues. The principal TAG in TO is POO (16.84 %) followed by OOL (15.51 %), POL (12.61 %), LLO (11.98 %) and OOO (12.08 %). The principal TAG in MO is OOL (18.79 %) followed by POO (15.76 %), LLO (15.01 %), POL (14.32 %) and OOO (11.28 %). The others TAGs are relatively present only in small quantities. The non-majority triglycerides are presented mainly by trilinolein (LLL) (02.83 to 02.90 %), palmito- dilinolein (PLL) (04.22 to 04.55 %), dipalmito-linolein (PPL) (02.32 to 02.42 %), dipalmito-olein (PPO) (04.04 to 05.28 %), linoleo-palmito-stearin (LPS) (2.88 to 4.31 %), and palmito-oleo-stearin (POS) (1.99 to 3.15 %). Only one compound stearo-diolein (SOO) (6.38 - 8.29 %) represented an average content for the two oils.

Table 2: Triglycerides percentage of argan oil for two regions.

TAG	TO (%)	MO (%)
LLL	02.90	02.83
LLO	11.98	15.01
PLL	04.22	04.55
OOL	15.51	18.79
POL	12.61	14.32
PPL	02.42	02.32
OOO	12.08	11.28
POO	16.84	15.76
PPO	05.28	04.04
LPS	04.31	02.68
SOO	08.29	06.38
POS	03.15	01.99

L: linoleic, O: oleic, P: palmitic, S: stearic.

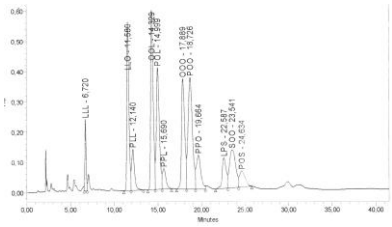

Figure 3. Chromatogram of triglycerides (TO)

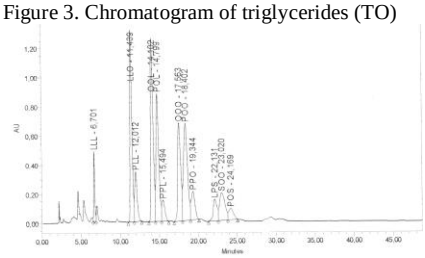

Figure 4. Chromatogram of triglycerides (MO)

4. Unsaponifiable matters

The unsaponifiable fraction determined shown that the oil of Tindouf represented a content of 1.6 % while the Mostaganem oil gived 1.71 %.

5. Tocopherols profiles

The tocopherol vitamers composition of argan oil determined by HPLC suggested that the argan oils of the two regions are presented by three isomers α, δ and γ-tocopherol (Figure 5 and 6). The total content of tocopherols in argan oil for the two regions is 657.424 mg/kg (TO) and 749.38 mg/kg (MO). The HPLC analysis shows that the predominant tocopherol is gamma-tocopherol (Table 3), representing on average 555.827 mg/kg (TO) and 689.49 mg/kg (MO). The alfa-tocopherol represented 41.22 mg/kg (MO) and 95.96 mg/kg (TO), for the delta-tocopherol showed 18.66 mg/kg (MO) and 5.64 mg/kg (TO).

Table 3 : Tocopherols percentage of argan oil for two regions.

Tocopherol	TO (mg/kg)	MO (mg/kg)
Alpha-tocopherol	95.96 (14.6 %)	41.22 (5.5 %)
Gamma-tocopherol	555.827 (84.6 %)	689.496 (92%)
Delta-tocopherol	5.64 (0.9 %)	18.66 (2.5 %)

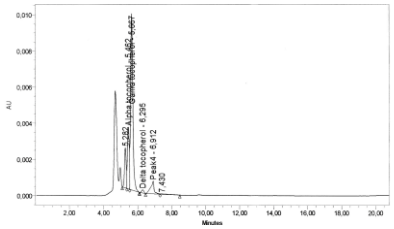

Figure 5. Chromatogram of tocopherols (TO)

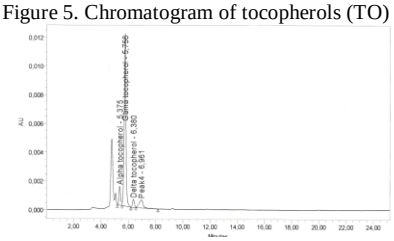

Figure 6. Chromatogram of tocopherols (MO)

IV. DISCUSSION

1. Oil content of two regions

The determination of yield oil is fundamental to predict the profitability potential source of oil from fruits harvested from plant growing in two regions. The results showed that oil from Mostaganem region give the highest content. The yield oil found is favourable well compared to those reported by many authors for Moroccan argan oil. Our results are similar to those found by many authors who worked on the Moroccan argan oil. In fact, the maximum yield reached is 54 and 55 % [23]. However, these results are found higher compared to those reported on Algerian argan oil from of Tindouf location, a result showed a yield of 33.98 % [24] and another study have reached a yield of 36 % [25].

The richness of the almonds of Mostaganem compared to Tindouf in terms of oil yield is explained by the climatic difference between the two regions, including relative humidity and precipitation [26, 27].

Thus, the difference in yield is due to genotypic effect between the trees on which the fruit has been harvested, as well as the geographical origin [28].

2. Fatty Acids

Comparing our results with those of other studies reported on Moroccan oils shows that our oils are rich in oleic acid (oils Tindouf). Thus, a study performed on Moroccan oils found a rate of 46.4% for oleic acid [29]. About linoleic acid from oil of Mostaganem location, it seems to provide a high rate of 29.97 to 34.32 % against 29.00 to 30.10 % of the oil sample from Tindouf. Compared to other studies that focus on the TO, our two oils are rich in linoleic acid against 18.1 % and 25.0 % [24, 25].

Linolenic acid was detected in low levels in our oils (0.11 %). The saturated fatty acids reveal a predominance of palmitic and stearic acid in all samples studied. These results were confirmed by found by many authors working on Moroccan oils [9, 12, 24, 25, 30, and 31].

These results are consistent with those cited by many studies 13.9 % [9] and 14.65 % [25]. As stearic acid, its best

content (6.53 %) was observed in the oil from Tindouf location followed by that from Mostaganem location (6.28 %). These results are in agreement with those published in many work 5.6 % [9] and 6.00 % [24]. the variation distinguished in fatty acid compositions of two Argan oils could be attributed to various factors, including the geographical origin of the samples [32]. In fact, some authors noted that the rate of palmitic acid increases according to the altitude and the content of oleic and linoleic acid increased with rainfall [32, 33]. Genotypic can also contribute to these variations [11, 28, and 29].

The changes observed in fatty acid composition are attributed according to various factors such as geographical origin, climate, rainfall, latitude and genetics [11].

3. Triglycerides

The triglyceride composition of the two oils studied shows the same similarity to other argan oils studied by several authors which confirms the presence of TAG mentioned previously in the composition of argan oil [25, 28, 29, 33, 32 and 34].

The small difference in composition and content between oils from several locations is affected by several factors such as cultivars, genetics, growing conditions, climatic conditions, locality, and environment [11, 28, 29 and 32]

4. Unsaponifiable matter

The unsaponifiable matter represents the small proportion of oil. Despite its low content, this fraction contains crucial and noble compounds (tocopherols, phytosterols, vitamins, triterpenes ...). These minor substances have many health benefits. Our results are similar to those reported by Maurin (0.36 - 1.1 %) [32], Charrouf (1.03 %) [29], YAGHMUR (0.3 - 1.1 %) [30] and HILALI (0.34 to 0.56 %) [28]

5. Tocopherols

From those results obtained, we can notice that oil from Mostaganem location is rich in total tocopherols (749.38 mg/kg) compared to those obtained from oil of Tindouf location (657. 424 mg/kg). These values confirm the richness of our oil in tocopherols compared to those of Morocco where concentrations range from 629 to 660 mg / kg [12] and 637 mg / kg [11]. Comparing our results with those obtained by other authors on argan oils from Tindouf, we note that However, these values are low compared to those obtained by Yousfi (1027.80 mg / kg [25]. Argan oil is richer in tocopherols relative to olive oil which contains a level of 320 mg / kg [11]. This content is considerable and is a characteristic of argan oil compared to other oils. The HPLC analysis allowed identifying and quantifying the essential constituents of total tocopherols, three isomers were found: α-tocopherol, γ-tocopherol and δ-tocopherol. The γ-tocopherol is the main vitamer with 555.827 mg/kg (84.6 %) for (TO) and 689.496 mg/kg (92%) for MO. Indeed [12] Khallouki reported a rate of γ-tocopherol with 500 mg / kg (75 %) and [25] reported a content of 700 mg / kg (68 %). The second compound is α-tocopherol. This result is confirmed by a study carry out by [25] YOUSFI on TO, he reported that the TO have a content of 325 mg / kg (31 %). Results reported by others authors range from 45 to 57 mg / kg (7 % - 6.5 %) for Khallouki [12] and Hilali [28]. The lowest level is represented by δ-tocopherol, the same value are described by Yousfi 2.86 mg / kg but at the same time [12] Khallouki showed that this vitamer is 122 mg / kg (18 %). The differences in levels of tocopherols vary depending on the geographical origin of fruits, altitude, climate, cultivars, environmental conditions and genetic factors [28].

V. CONCLUSION

In conclusion, our study shows that argan oil contains as major fatty acids, two unsaturated fatty acids, together oleic and linoleic acid (80 %) whereas linolenic acid is present as trace value (00.20 %).

Saturated fatty acids are represented mainly by palmitic acid (13 %). The predominant triacylglycerols are LLO, OOL, POL, POO and OOO. The unsaponified matter content is 1.6 -1.7 %. The tocopherols present a value of 657.43 mg/Kg and 749.38 mg/Kg with gamma tocopherol as major compound (555.83 mg/Kg - 689.49 mg/Kg). This quantity confer to our oil an important antioxidant effect whish let the oil to maintain a long stability during the conservation. The oil yield is very important (55.9 % and 6O.5 %) which allows the exploitation of this oil in many fields (food, cosmetic and pharmaceutical). Argan oil is rich in polyunsaturated fatty acids of type omega 6 and tocopherol, this gives the oil a good nutritional and biological quality, because it provides good cholesterol in the blood and fight againstcardiovasculardiseases The variations observed in values for all compounds is due to many factors, such as geographical location, climate conditions, altitude, and genetic factors

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