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Which is better, eastern or western argan oil from the Kingdom of Morocco: Study of the chemical composition of argan oil according to the region of production

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Abstract

This work studied the influence of the geographical origin (the east and the west of the kingdom of Morocco) on the chemical composition of argan oil to know which is the best argan oil (oriental or western part of the Kingdom of Morocco). To carry out this work, 3 different samples were selected, two samples from the Tidzi and Tamanar regions in Essaouira province of South-West Morocco, and one sample from the Beni Snessan region of North-West Morocco. Afterwards, argan oil was prepared by mechanical pressing from roasted almonds. Physico-chemical analysis showed that the acidity and the peroxide index are higher in argan oil from the Beni Snassene region. The analysis of fatty acids and triglycerides showed that the percentage of behinic acid (C22:0) (0.38%) is higher in the argan oil collected in the plantation of Beni Snassene. The result of this work also showed that the percentage of total sterols (206 mg/100g) and the content of total tocopherols (775.5 mg/kg) in argan oil from the Beni Snassene region are higher compared to other oils. Hence, this result clearly indicates that the geographical origin can influence the chemical composition of argan oil.

Key words: Argan, Sapotaceae, Fatty Acids, Sterols, Polyphenols, Chemical composition

Introduction

The argan tree (Argania spinosa, Skeels L., Sapotaceae) is an endemic tree of southwestern Morocco, where it plays a very important socio-economic and environmental role. It is deeply rooted in the daily life of rural populations and plays a fundamental role in their livelihood.

The argan tree fights against desertification, protects the soil against water and wind erosion, and maintains its fertility. In addition to this environmental role, it has a direct economic interest by providing oil, foliage, fodder, wood and indirectly by the agricultural productions that it allows under its shade. The argan tree thus ensures the subsistence of 3 million people [1].

The main product of the argan tree is argan oil, this oil originating from Morocco, rich in vitamin E and unsaponifiables, is renowned for its nourishing, regenerating and restructuring properties. Its antioxidant content makes it an ideal active ingredient to fight against the signs of skin aging, and its fatty acids restore suppleness and softness to dry and undernourished skin [2].

Despite all these interests, we are witnessing an alarming regression of argan trees both in area and in density. In less than a century, more than 2/3 of the forest has disappeared and 600 ha are lost each year [3]. This regression is essentially due to an ecological imbalance of anthropogenic (human) origin. In the mountains, there is overgrazing and overexploitation of wood, in the plain, the argan tree is cleared because it hinders the intensification of agriculture.

Faced with this problem, the laboratory of plant chemistry and organic and bio-organic synthesis of the Faculty of Sciences of Rabat-Agdal has set itself the objective of promoting the products of the argan tree for the benefit of rural communities so that they are more motivated to protect and replant the argan tree.

This work is part of the continuity of the series of research carried out by the plant chemistry and organic and bio-organic synthesis laboratory on the argan tree. He constituted by the study of the chemical composition of argan oil according to its origin of production (eastern and western Kingdom of Morocco).

Materials and Methods

Preparation of different samples of Argan oil

In this present work, we selected 3 samples of the fruit of the argan tree from different regions (two samples from the South-West region of Morocco and one sample from the North-West region of Morocco , the distance between the two regions is equal to 1047 km), (figure 1),

Argan oil was prepared by extraction, by the method of mechanical pressing in the cooperative of Tidzi (province of Essaouira, southern Morocco) according to methods already described: extraction by mechanical pressing (roasted almonds [4-5] Table 1 provides information on the origin and method of extraction of the Argan tree from each sample.

Argan Oil Analysis:

Argan oil is extracted by mechanical pressing from roasted almonds. These oils are then analyzed in the Official Laboratory of Analysis and Chemical Research (LOARC) of Casablanca in Morocco, the Physico-chemical characteristics and the chemical composition of all the samples are determined (fatty acid, sterols, triglycerides, tocopherols). The oils are analyzed according to the analysis methods already described in the literature (European, Standard 1999)

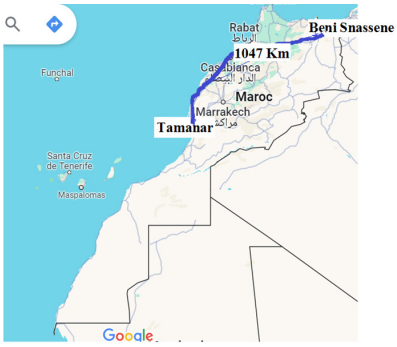

Table 1 provides information on the origin and method of extraction of each sample of argan oil.

N° Samples	Mode of extraction	The region	Province	The distance between the region and the sea (altitude)
1	Roasted almond extracted by mechanical press	Tidzi	Essaouira North-owest Morocco	25 Km (150m)
2	Roasted almond extracted by mechanical press	Tamanar	Essaouira North-owest Morocco	20 km (150m)
3	Roasted almond extracted by mechanical press	Beni Snassene	Oujda North-eastern Morocco	100 km (1533m)

Table 1: Origin and method of extraction of the 3 samples.

Physicochemical analyzes of oils:

Determination of acidity [6], the peroxide value [7], the refractive index [8] of the absorbance in the ultraviolet [9], the saponification number [10], the unsaponifiable content [11]. were measured according to the standardized methods of reference.

Determination of composition and nature in total sterols. [12]

Weigh 2.5 g of argan oil and put into a 20 ml flask. 25 ml of a solution of potassium hydroxide (1N of ethanol) is added thereto. The flask is heated under reflux for 30 minutes until the solution becomes clear.

Then, 25 ml of distilled water is added to stop the reaction.

The extraction of the unsaponifiable is carried out using 75 ml of hexane or petroleum ether. The organic phase is subjected to a series of washing with 15 ml of mixture (water / ethanol 95 °) (90/10) in a separatory funnel.

The hexane phase is transferred from the top of the ampoule into a 100ml flask. After evaporation of the solvent using a rotary evaporator, the unsaponifiable material is recovered.

The unsaponifiable agent, diluted with 300 µl of hexane or petroleum ether, is filtered.

Unsaponifiable is obtained according to the standard NFT 50-205. It is fractionated by high performance liquid chromatography (HPLC) on a silica column (25cm × 4 mm). The HP is equipped with a 205 nm-254 nm UV detector. The eluent is an isooctane / isopropanol (99/1) mixture whose flow rate is 1.2 ml / min. The duration of the analysis is 15 min, the sterol fraction recovered according to standard NF 12228 May 1999, is evaporated to dryness.

The sterols are converted to silylated derivatives (TMS) using a mixture of pyridine, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS), (9/1/1), (v / v / v). The pyridine is evaporated to dryness and the silylated derivative is diluted with 60 µl of heptane or hexane.

The TMS sterols are analyzed by gas chromatography (GC) on an apolar column (Chroma pack) (30m × 0.32mm, DI: 0.25µm, phase: CPSIL8CB).

The HP Hewlett Packard 6890 GC Series Chromatograph is equipped with a FID detector (T °: 300 ° C). The carrier gas is nitrogen and its flow rate is 1 ml / min (P.E: 8.6 bar). The analysis is performed in temperature programming (200 ° C up to 270 ° C with a speed of 10 ° C / min and an isotherm at 270 ° C for 35 min).

Analysis of cis fatty acids. [13]

The test sample of argan oil 1g is supplemented with 0.5 ml of methanolic KOH for HPLC (minimum 98%) and 10 ml of methanol in a 100 ml flask. The mixture is refluxed for 15 minutes until the solution is clear. Then 1 ml of heptane is added to the reaction mixture after cooling.

The heptanic phase containing the methyl esters is transferred to a test tube and then a solution of sodium carbonate Na_2CO_3 is added. This neutralizes all free acids by giving sodium salts with a release of carbon dioxide. The methyl esters, which are in the organic phase, are removed using a 2 ml cone pipette and placed in a test tube.

The methyl esters undergo a series of washing 20 ml are taken from the esters, withch are placed in a tube of nominal capacity of 2 ml and then filled with heptane.

The fatty acid methyl esters are analyzed by GC gas chromatography.

The HP Hewlett Packard 6890 GC Series GC chromatograph is equipped with a divider (T: 240 °C) and a FID (T: 260 °C) injector. The carrier gas is nitrogen (PE: 12.4 bar). The analysis is carried out in temperature programming (140 ° C to 200 ° C with a speed of 10 ° C / min and an isotherm at 200 ° C for 40 min) on a capillary column (polyethylene glycol) (30 m × 0 , 32 mm, DI: 0.25 µm).

Tocopherol analysis. [14]

In a 25 ml volumetric flask, 2 g of argan oil are diluted with 2,2,4-trimethyl pentane. The test sample is added to 2, 2, 4-trimethyl pentane up to the mark, then mixed thoroughly.

The tocopherols are analyzed by HPLC, on a silica column (25 cm × 4 mm), according to the AOCS method, official method CE8-89 revised 1990 updated 1992. The SHIMADZU brand device is equipped with a

fluorimetric detector (excitation wavelength 290 nm - emission wavelength 330 nm). The elution is carried out with a mixture (isooctane / isopropanol) (99/1) with a flow rate of 1.2 ml / min during the analysis time (20 min).

Triglyceride analysis [15]

To 0.15 g of the argan oil are added 0.5 ml of hexane and 15 ml of a mixture of hexane / diethyl ether (87/13). This solution is poured into a supelco brand cartridge with 0.5 g of silica gel previously activated with hexane. The triglyceride fraction is thus separated from the diglycerides and monoglycerides. It is recovered in a 100 ml flask. It is subjected to analysis after evaporation of the solvent and dilution with 1.5 ml of acetone.

The triglycerides are analyzed by HPLC on a reverse phase C18 column (250 mm × 4.6 mm, Φ silica 5 µm), according to IUPAC Method No. 2.0324. The HPLC apparatus is equipped with an HP refractometric detector 10 47A. Elution is carried out with a mixture (acetonitrile / acetone) (v / v) with a flow rate of 0.5 ml / min during the analysis time (90 min).

Results and Discussion

Analysis of physico-chemical characteristics

Table 2 shows the results of the acidity value, the peroxide value, the unsaponifiable content, the saponification value and the specific extinction values at 270 nm (k270).

Samples	1	2	3
Acidity in %	0.33	0.32	0.67
Unsaponifiable rate in %	0.55	0.72	0.63
Saponification index	197.9	196.3	189.6
Peroxide index in meq O_2/kg	1.23	0	2.40
Specific extinction at 270 nm (k270).	0.228	0.426	0.291

Table 2: The main physico-chemical constants of the 3 argan oil samples studied.

All observed acidity values are below 0.67%. This result shows that argan oil is characterized by a low acidity compared to other vegetable oils (olive ≤ 2) [16].

The acidity of sample 3 (0.67%) (Belonging to the Beni Snassene batch) is higher compared to other samples such as 1, 2 (0.33%, 0.32% respectively) (belonging to the Tidzi and Tamanar). These results suggest that the origin can influence the acidity values of argan oil.

The unsaponifiable rate of argan oil is less than 0.72% (for virgin olive oil, it is less than or equal to 1.50%) [16]. Argan oil extraction technology can influence the unsaponifiable content in argan oil. The saponification index of argan oil (table 1) was found between 189.6 and 197.9. For virgin olive oil, it is between 184 and 196) [4,16].

In general, the observed values of the specific extinction of argan oil are higher than those of olive oil, they vary between 0.228 and 0.426 for argan oil compared to 0.10 and 0.90 for olive oil. The specific extinction of sample 2 is higher (0.426).

For the results of the peroxide value, a lower peroxide value than that required for virgin olive oil was observed [17].

The peroxide number of sample 3 is higher. Indeed, this sample belonging to the batch of Beni Snassene. This result clearly indicates that some components of argan oil are extremely sensitive to oxidation.

The determination of the peroxide number seems to be a critical measure for the evaluation of the quality of argan oil.

Fatty acid analysis

The fatty acid composition of the different oils was determined after methylation of the oil and analysis of the methyl esters by gas chromatography on a capillary column. Table 3 groups together the results obtained for the 3 samples.

Samples	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{19:1}$	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$
1	**0.12**	**0.04**	**12.45**	**0.04**	**0.08**	**5.44**	**47.11**	**33.53**	**0.09**	**-**	**0.36**	**0.44**	**0.11**
2	**0.12**	**0.04**	**13.0**	**0.08**	**0.08**	**6.11**	**50.05**	**29.12**	**0.06**	**-**	**0.43**	**0.48**	**0.13**
3	**0.15**	**0.04**	**12.06**	**0.09**	**0.07**	**6.35**	**48.32**	**31.73**	**-**	**-**	**0.35**	**0.41**	**0.38**

Table 3: Fatty acid composition of the samples (in %)

The fatty acid composition corroborates with data from the literature [4,16,17].

Argan oil contains 80% unsaturated fatty acids. It is of the oleic-linoleic type and contains between 29 to 33% of essential fatty acids: linoleic acid (29 to 33%) (Vitamin F). This acid is said to be essential because it cannot be synthesized by the body and must be provided by food.

Unsaturated fatty acids play an essential role in the prevention of cardiovascular diseases and the omega 6 family (such as linoleic acid) is essential for child growth [18]. Its oleic acid content makes argan oil particularly interesting in the regulation of cholesterol.

Sample 3 contains a higher percentage of behinic acid (C22:0) (0.38%). On the other hand, this percentage does not exceed 0.13% for all the other samples, this sample is prepared from argan kernels gathered in the

Beni Snassene plantation. These variations can be considered as useful markers to ascertain the geographical origin of argan oils.

3.4 Triglyceride analysis

The triglycerides of the different samples of argan oil analyzed by high performance liquid chromatography are grouped in Table 4.

Samples	LLL	LLO	LLP	LOO	LOP	PPL	OOO	POO	OPP	LPS	PPP	SOO	SOP
1	**6.89**	**13.22**	**5.85**	**15.96**	**14.18**	**1.94**	**13.77**	**16.05**	**4.06**	**0.31**	**0.11**	**4.58**	**2.25**
2	**5.77**	**11.59**	**5.48**	**14.73**	**13.46**	**1.97**	**14.34**	**16.95**	**4.12**	**0.29**	**-**	**5.18**	**2.61**
3	**6.84**	**12.20**	**6.00**	**14.31**	**13.22**	**2.08**	**13.98**	**15.48**	**4.39**	**0.66**	**-**	**6.04**	**4.00**

Table 4: Triglyceride composition of the 3 samples (in %).

The analysis of the triglyceride fraction of argan oil by HPLC allowed the separation of individual triglycerides. We note the predominance of triglycerides LLO (11.5 to 13.2%), LOO (14.3 to 15.9%), LOP (13.2% to 14.1%), OOO (13.7 to 14 .3%) and POO (15.4 to 16.9%).

Sample 1 has a higher percentage of LLL and LOO triglycerides (6.89%, 15.96% respectively). This result agrees with the fatty acid result, which indicates that sample 1 contains a higher percentage of linoleic acid.

3.5 Sterol analysis

The composition of the sterols of the various samples of argan oil was determined by gas chromatography after silylation of the sterol fraction. The latter is obtained by fractionation of the unsaponifiable matter of argan oil by HPLC on a normal phase. This analysis was carried out in the presence of an internal control: 0.2% α-cholestanol in chloroform.

The various sterols encountered were identified by gas phase chromatography coupled with mass spectrometry and by comparison with data from the literature [19]. Their individual and total assay was possible by GC using an internal standard: 0.2% α-cholestanol in chloroform. Table 5 summarizes the results obtained for the 3 samples selected.

Samples	Campest.	Stigma 8,22	Spinast.	Schott.	Stigma 7,24	Total
1	**0.20**	**4.31**	**37.07**	**46.66**	**4.81**	**142.0**
2	**0.11**	**4.76**	**37.16**	**46.16**	**4.41**	**188.3**
3	**0.11**	**4.85**	**35.44**	**48.47**	**2.57**	**206.3**

Table 5: Sterol composition of the samples (mg/100g).

Campest.: Δ5-campesterol; Stigma 8,22: Stigmasta-8,22-diene-3β-ol; Spinast.: spinasterol; Schott: schottenol; Stigma 7,24: stigmasta-7,24-diene-3β-ol.

The total sterol content of all samples of argan oil ranges from 142 to 206 mg/100g of fat. This is not negligible compared to other seed and olive oils [17].

The sterol composition is consistent with literature data [20]. They are essentially Δ-7-stigmasterols. The majority products are schottenol (or Δ-7-stigmasterol) and spinasterol. Their proportion varies respectively between 46 and 48%, and 35 and 37%.

Schottenol and spinasterol are rarely found in vegetable oils and are characteristic of this oil. Argan oil does not contain β-sitosterol (Δ-5-stigmasterol) this has been confirmed by GC/MS. This ambiguity noted in the literature is due to the fact that β-sitosterol and spinasterol have the same retention time in GC on certain columns.

Two minority sterols have been identified in argan oil. These are stigmast-8,22-diene and stigmasta-7,24-diene (or Δ-7-avenasterol). Their proportion varies between 4.8% and 2.5% of the mixture of total sterols.

We find that the campesterol content in argan oil is very low (0.2%) compared to other seed oils and olive oil. So we can take this parameter as a marker to detect the adulteration of argan oil by other edible oils.

We found that the percentage of total sterols is higher for sample 3 (gathered in the Beni Snassene plantation) (206 mg/100g).

3.6 Tocopherol analysis

Tocopherols were analyzed by HPLC on a normal phase column, directly from the vegetable oil without saponification. They were identified by comparing their chromatogram with controls injected under the same conditions. Their dosage was possible by the use of α-tocopherol. The results obtained are grouped in Table 6.

Samples	γ-tocopherol	δ-tocopherol	α-tocopherol	β-tocopherol	total
1	**631.3**	**59.5**	**26.6**	**-**	**717.4**
2	**606.7**	**48.6**	**38.0**	**-**	**693.3**
3	**701.1**	**37.2**	**37.2**	**-**	**775.5**

Table 6: Tocopherol composition of the samples (mg/kg).

Argan oil is richer in tocopherol (693 to 775 mg/kg) than olive oil (50 to 150 mg/kg) and hazelnut oil (300 to 550 mg/kg) [21].

Tocopherols have vitamin E activity. This vitamin is a powerful antioxidant that captures free radicals and neutralizes destructive oxidation [22].

Tocopherols are natural antioxidants, gamma tocopherol has the highest antioxidant power [23]. Rich in gamma tocopherol, argan oil is a valuable nutraceutical. Tocopherols (vitamin E) and polyphenols are natural antioxidants. The latter play an essential role in the prevention of several diseases, because they are anti-free radicals. β-tocopherol has been detected in trace form in argan oil.

We found that samples 3 (the Beni Snassene region) have a higher content of total tocopherols (775.5 mg/kg). So this result shows that the geographical origin can influence the tocopherol composition of argan oil.

Conclusion

The study of the physicochemical characteristics shows that the sample of argan oil coming from the Beni Snassene batch in the east of Morocco which have an acidity and peroxide value are higher compared to the samples of Tidzi and Tamanar (west of the Kingdom of Morocco). This result suggests that geographic origin can influence acidity and peroxide index values.

The results related to tocopherols also show that the origin of argan oil can influence the composition of tocopherols. On the other hand, the sample obtained from the Beni Snassene region has a higher content of total tocopherols (775 mg/kg) compared to the other samples.

The analysis of the triglyceride fraction of argan oil shows that the Beni Snassene sample has a higher percentage of LLL (linoleic acid) and LOO (oleic acid) triglycerides (6.89%, 15.96% respectively). The results of this work clearly show that geographical origin can influence the chemical composition of argan oil.

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