ECRONICON OPEN ACCESS

EC ORTHOPAEDICS
Research Article

Study of the Chemical Composition of Argan Oil According to the form of the Fruit

Miloudi Hilali[1]*, Hanae El Monfalouti[1], Larbi El Hammari[2], Nadia Maata[3] and Badr Eddine Kartah[1]

[1]*Laboratory of Plant Chemistry and Organic and Bioorganic Synthesis, Faculty of Science, University Mohammed-V, Av. Ibn Battouta, Agdal, Rabat, Morocco*

[2]*Laboratory of Applied Chemistry of Materials, Faculty of Science, University Mohammed-V, Av. Ibn Battouta, Agdal, Rabat, Morocco*

[3]*Official Laboratory of Chemical Analysis and Research 25, Street Nichakra Rahal, Casablanca, Morocco*

***Corresponding Author:** Miloudi Hilali, Laboratory of Plant Chemistry and Organic and Bioorganic Synthesis, Faculty of Science, University Mohammed-V, Av. Ibn Battouta, Agdal, Rabat, Morocco.

Received: May 14, 2021; **Published:** July 29, 2021

Abstract

This work is to study the influence of phenotypic variability of the fruit of the argan tree with different morphological characters (fusiform, oval, apiculate and spherical) on the fat content, in proteins, the rate of unsaponifiable and on the chemical composition of fatty acids and sterols.

The result of this work shows that the oval shape is the best shape of the argan fruit because their kernel contains more than 50% fat and a higher percentage of unsaponifiable.

The fatty acid and sterol results show that argan oil has 80% unsaturated fatty acids. It is of the oleic–linoleic type, also the result of this work shows that the main products of the sterolic composition of argan oil are schottenol (or Δ-7- stigmasterol) (42.8 and 46.4%) and spinasterol (39.8 and 45.6%). the study of the chemical composition shows that there is no correlation with the shape of the fruit of the argan tree and the composition of fatty acids, The results obtained do not allow the conclusion that the form can influence the chemical compositions of fatty acids and sterols. As a result, the variations in the chemical composition of fatty acids and sterols found in the analysis of the chemical composition of argan fruit types are not only related to the shape, but also depend on the nature of the soil, as well as the altitude, longitude, and distance from the sea.

Keywords: *Argan; Chemical Composition; Form; Influence; Fatty Acid; Sterols; Correlation*

Introduction

The argan tree (*Argania spinosa* (L.) Skeels) (Figure 1) is a Moroccan endemic tree that ranks second in the country's forest species after the holm oak and only ahead of the thuya. It's a tree with a lifespan of up to 200 years. Subjects as old as 250 years have been observed. The argan forest covers approximately 800,000 ha and has more than 20 million trees [1]. This Sapotaceae tree is particularly hardy in southwestern Morocco's dry and arid climate. It can in fact withstand temperatures ranging from 3 to 50°C and be satisfied with very low rainfall [2].

In the arid zone of south-west Morocco, the argan tree grows wild and in abundance, where it plays an essential role in ecological harmony and biodiversity protection. Thanks to its powerful root system, it helps maintain the soil and helps fight against water and wind erosion, which threatens much of the region with desertification.

Because of it's multipurpose uses, the argan tree is of high economic interest. Each part of the tree is useful and provides income or food to the user: the wood is used as fuel, the leaves and fruits are used as fodder for goats and camels, and the almond oil is used in human food and traditional medicine.

The argan tree plays a vital socio-economic and environmental role [3] in these geographic areas. It is a state forest with broad rights of use dedicated to local communities due to its unique statutory status (Dahir of March 4, 1925 and requirements relating to agrarian activities under the argan tree of July 20, 1983). Free course fee, right to harvest fruit and collect wood for domestic use.

Unfortunately, the argan grove has been diminished as a result of wealth, as well as changes in the rural way of life and environment. Its agricultural overexploitation, soil erosion, the advance of the desert are all attacks on this unique heritage. More than half of the forest has vanished in less than a century and the average tree density has risen from 100 to 300 trees per ha [4]. Yet all research shows that the argan tree is not an endangered fossil.

Despite all these interests, we are witnessing an alarming decline in argan groves both in area and density. In less than a century, more than 2/3 of the forest has disappeared and there are 600 ha of lost each year [5].

Faced with this problem, the Laboratory of Plant Chemistry and Organic and Bio-organic Synthesis of the Faculty of Sciences of Rabat 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 therefore part of the continuation of the series of research carried out by the Laboratory of Plant Chemistry and Organic and Bio-organic Synthesis within the Faculty of Sciences of Rabat on the argan tree to improve and enhance the products of the argan tree to preserve and preserve the argan.

Materials and Methods

Preparation of samples

To study the influence of the shape of the argan fruit on the chemical composition of argan oil, with the help of indigenous populations, we have selected 4 different fruit shapes (fusiform, apiculate, spherical and oval) [6] that we harvested from the same place (Tamanar) in the province of Essaouira (plain area southwest of Morocco).

After the harvest of the fruit of the argan tree, of each form, 100 samples are taken. These are pulped and crushed to remove the kernel. After the hexane extraction with soxhlet, we determined: the fat content, the protein level, the unsaponifiable content, the compositions of male acids and sterols in the fat.

Physicochemical analyzes of oils

All tests were carried out at the Moroccan Government's Official Laboratory of Chemical Analysis and Research (LOARC) in Casablanca. Percentage of fat, the unsaponifiable content, percentage of protein, sterols cis fatty acids, were measured according to the standardized methods of reference.

Determination of ISO 659 fat content [7]

The lipid content is determined according to the AOAC method (AOAC International, 1990). 25g of sample were added to the filter paper cartridges, then placed on the Soxhlet. 250 mL of hexane was poured into a flask. For 4 hours the flask was heated. After removal of the solvent by distillation, the flask is dried at a temperature of 70 - 80°C, then weighed after cooling in a desiccator.

The fat content is determined according to the following formula:

$$L\ (\%) = ((P2 - P1)/P3) \times 100$$

P1: Weight of empty balloon (g)

P2: Weight of the flask with the extracted oil (g)

P3: Weight of the test portion (g).

Determination of the protein content [8]

The Kjeldahl method is used to calculate the protein content (ISO 5983). This method is based on the quantification of the nitrogen content, then the protein content is calculated by multiplying the total nitrogen content N (%) by the coefficient 6,25. This method takes place in two stages: The first is the digestion phase which consists of a chemical attack with sulfuric acid to remove organic matter. 1g of sample was mixed with 1g of Kjeldahl catalyst (copper and potassium sulfate) and 15 mL of sulfuric acid, the mixture was prepared in a mineralization flask by applying progressive heating. When the solution becomes clear, it is cooled with 100 ml distilled water. The second stage is the distillation which consists in solubilizing the mineral nitrogen in the form of ammonia, this stage is carried out in a Rota vapor by adding 20 ml of soda to 35 Materials and Methods 102% in the flask and 25% of boric acid in a 250 mL flask. The ammonia is recovered in a solution of boric acid. The last step is titration, it was carried out by adding a few drops of the Tachiro indicator (mixture of methylene blue and methyl red) to the flask containing ammonia and boric acid. The excess ammonia is then dosed with 0.05 N sulfuric acid by simple titration.

The total nitrogen content is determined by the following formula:

$N (\%) = (Co \times 2 \times V \times 14)/P$

$P (\%) = N (\%) \times 6.25$

N: Percentage of nitrogen (%)

P: Percentage of protein (%)

Co: Normality of sulfuric acid (0.05)

V: Volume of sulfuric acid poured (mL)

P: Weight of test portion (g).

Determination of unsaponifiable content [9]

The unsaponifiable content refers to the percentage of substances in a product that are not volatile after saponification with potassium hydroxide and extraction with a specific solvent.

Procedure

5g of argan oil and 50 ml of KOH (1N) (ethanolic) solution are placed in a 250 ml flask. We bring the mixture to a gentle boil for an hour. then add 100 ml of water from the top of the cooler distilled and allowed to cool last wash should not give a pink color by adding a drop of phenolphthalein solution. The ethereal phase is transferred to a 500 ml flask previously dried and tared. The solvent is evaporated at rotavapor and the residue is dried in an oven at 103 ± 2°C for 15 minutes until the difference between two successive weighings is less than 0.00015g, let P1 be the mass of the residue After weighing the residue, it is dissolved in 4 ml of diethyl ether. Then add 20 ml of 95°

ethanol previously neutralized and a few phenolphthalein drops. The titration is carried out with an ethanolic KOH solution titrated at 0.1N for dosing free fatty acids.

Determination of composition and nature in total sterols [10]

Operating mode

In a 250 ml flask, weigh 2.5g of argan oil, then add 25 ml of potassium hydroxide (1N ethanol) solution. Heat the flask at reflux for 30 minutes, or until the solution clears. Finally, to stop the reaction, 25 ml of distilled water are added.

The un-saponifiable is extracted with 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 100 ml flask. After evaporation of the solvent using a rotary evaporator, the unsaponifiable material is recovered.

The unsaponifiable agent is filtered on a silica column (25 cm 4 mm) after being diluted with 300l of hexane or petroleum ether. A UV detector with a wavelength range of 205 nm to 254 nm is included in the HPLC unit. The eluent is a 99/1 mixture of isooctane and isopropanol with a flow rate of 1.2 ml/min. The duration of the analysis is 15 minutes, 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 × 0.32 mm, DI: 0.25 µm, phase: CPSIL8CB).

The FID detector (T°: 300°C) is included in the HP Hewlett Packard 6890 GC Series Chromatograph. Nitrogen is the carrier gas, and the flow rate is 1 ml/min (P.E: 8.6 bar). The study is carried out using temperature programming (200°C to 270°C at a rate of 10°C/min and a 35-minute isotherm at 270°C).

Analysis of cis fatty acids [11]

Operating mode

In a 100 ml flask, 1g of argan oil is combined with 0.5 ml of methanolic KOH for HPLC (minimum 98%) and 10 ml of methanol. Reflux the mixture for 15 minutes, or until the solution is clear. After cooling, 1 mL of heptane is added to the reaction mixture.

The methyl esters-containing hepatic phase is moved to a test tube, and then a sodium carbonate Na_2CO_3 solution is added. This neutralizes all free acids by releasing carbon dioxide and producing sodium salts.

Using a 2 ml cone pipette, remove the methyl esters from the organic process and position them in a test tube.

The methyl esters are washed several times, yielding 20 mL, which are then put in a tube with a nominal capacity of 2 mL and filled with heptane. The fatty acid are analyzed by GC gas chromatography. A divider (T: 240°C) and an FID (T: 260°C) injector are included in the HP Hewlett Packard 6890 GC Series GC chromatograph. The carrier gas is nitrogen (PE: 12.4 bar). On a capillary column (polyethylene

glycol) (30 m 0,32 mm, DI: 0.25 m), the study is carried out in temperature programming (140°C to 200°C at a speed of 10°C/min and an isotherm at 200°C for 40 minutes).

Results and Discussion

Our field investigation revealed four different argan fruit shapes: fusiform, oval, apiculate and spherical [12]. With the help of the indigenous populations, we selected an area located in the plain. So, we have 4 samples containing different shapes and in places to see the influence of the shape on the chemical composition.

According to people, the crushing factor increases from spindle to spherical shape.

It emerges from these results that the argan kernel is very rich in fat (54%) (Table 1) and in lipid extract, the values found for the fat of the almond vary from 49% for an apiculate form to 54%. for an oval shape. Moreover, the unsaponifiable rate varies from 0.22% to 0.60% for an oval shape.

			Almond		
Lot	**N° samples**	**Form of the fruit**	**% Fat**	**% unsaponifiable in oil**	**% Protein**
Lot Plain	1	Apiculate	49	0,22	25
	2	Fusiform	51	0,36	21,5
	3	Spherical	50	0,36	23
	4	Oval	54	0,60	22

Table 1: *The percentage of fatty matter, % unsaponifiable and proteins of the almond of the fruit.*

When it comes to protein levels, almonds are rich in protein. For the almond, variations are from 21.5% for a fusiform shape to 25% for an apiculate shape (Figure 1) [13].

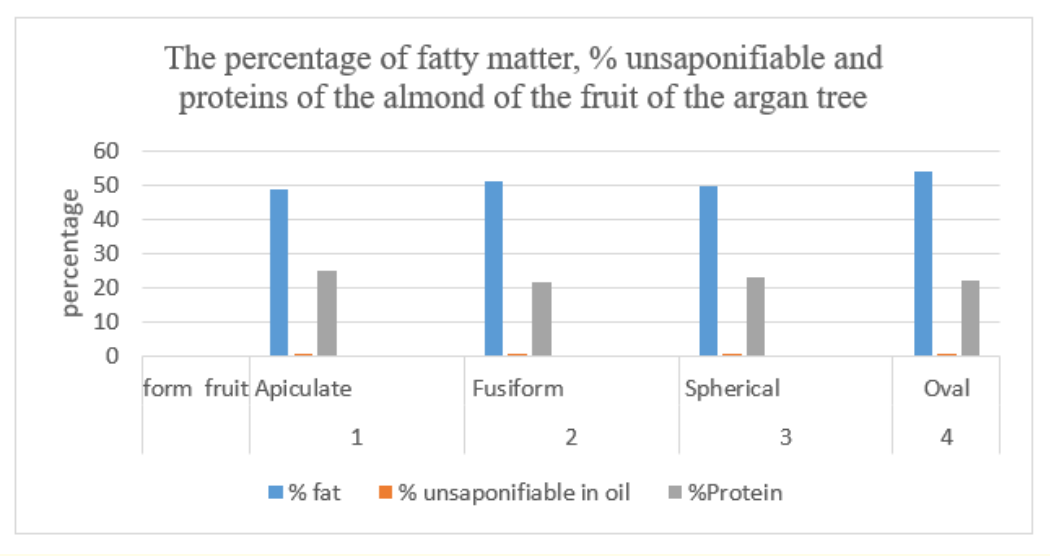

Figure 1: *The percentage of fatty matter, % unsaponifiable and proteins of the almond of the argan tree.*

This result shows clearly and clearly that the shapes of the argan tree can modify the percentage of fat, the protein and the unsaponifiable content of the argan fruit kernel, and that the best shape is oval because contains the highest percentage of fat and protein.

Analysis of fatty acids

The fatty acid composition of the different oils was determined after methylation of argan oil and analysis of methyl esters by gas chromatography on a capillary column. Table 2 groups together the results obtained for the 4 samples.

Fatty acid/N° sample-form	1 apiculate	2 fusiform	3 spherical	4 oval
Myristic C14 :0	0,15	0,12	0,16	0,18
Canoic pentade C15 :0	0,07	0,05	0,05	0,05
Palmitic C16 :0	14,52	13,69	15,11	14,15
Palmitoleic C16 :1	0,12	0,14	0,16	0,10
Heptadecanoic C17 :0	0,05	0,08	0,07	0,09
Stearic C18 :0	6,39	5,41	4,78	5,35
Oleie C18 :1	46,97	48,46	44,13	46,78
Linoleic C18 :2	30,75	31,02	34,56	32,32
Linolenc C18 :3	0,42	0,41	0,40	0,44
Arachidic C20 :0	0,34	0,35	0,36	0,34
Behenic C22 :0	-	0,10	0,08	0,08

Table 2: *Fatty acid composition of argan oil in samples 1 to 4.*

The fatty acid composition corroborates with the data in the literature [13-15].

Virgin argan oil contains 80% unsaturated fatty acids. It is of the oleic - linoleic type and contains between 30 to 34% of essential fatty acids: linoleic acid (30 to 34%) (Vitamin F) (Table 2). This acid is said to be essential because it cannot be synthesized by the body and must be supplied through food.

Unsaturated fatty acids play an essential role in the prevention of cardiovascular disease and the omega 6 family (like linoleic acid) is essential for the growth of the child [16].

Its oleic acid content makes argan oil particularly beneficial in regulating cholesterol.

The other fatty acids present are: myristic acid C14: 0 (0.12 to 0.18%), palmitic C16: 0 (13 to 15%) and stearic C 18: 0 (4.7 to 6.4%) (Figure 2). The percentage of linolenic acid (C18: 3) in argan oil does not exceed 0.1%. We note the presence in virgin argan oil of long chain fatty acids such as C20: 0 (0.34%), and C22: 0 (0.1%) [17].

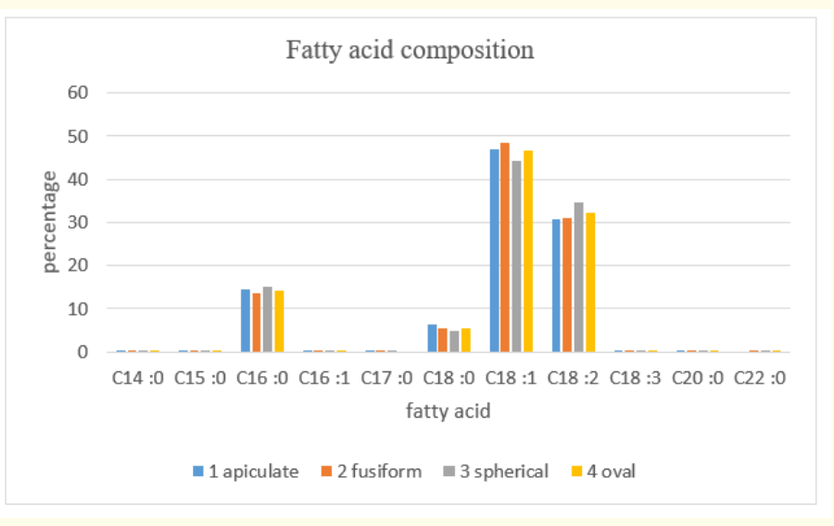

Figure 2: *Fatty acid composition of samples.*

From the study of the fatty acid composition of the fruits of batch A, it emerges that there is no correlation with the shape of the fruit and the composition of the fatty acids.

Analysis of sterols

The sterols of the different samples of virgin argan oil were determined by gas chromatography after silylation of the sterol fraction. The latter is obtained by fractionating the unsaponifiable in virgin 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 chromatography coupled with mass spectrometry and by comparison with data from the literature [18]. Their individual and total assay was possible by GPC using an internal standard: 0.2% α-cholestanol in chloroform.

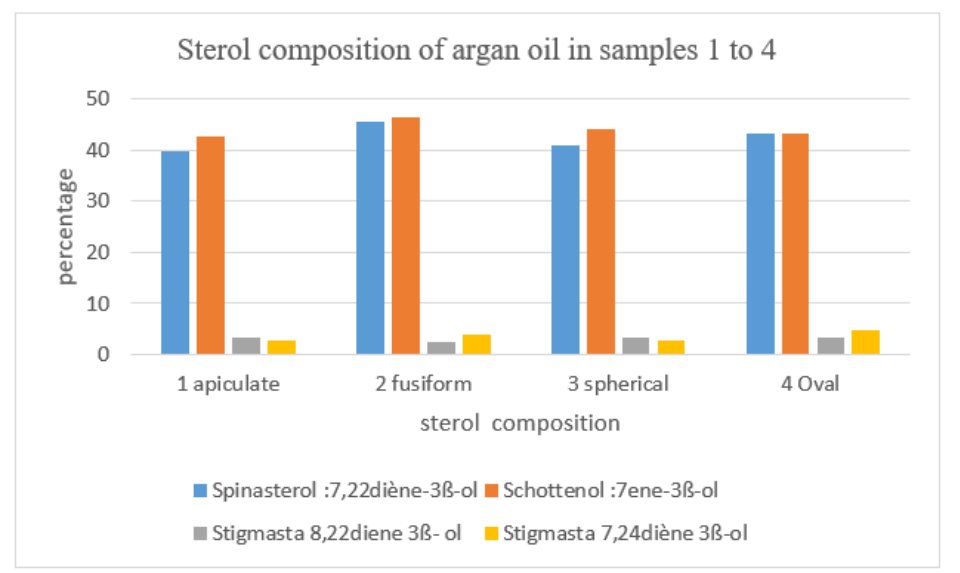

Figure 3: *Sterol composition of argan oil in samples 1 to 4.*

Sterol/N° samples	1 apiculate	2 fusiform	3 spherical	4 Ovale
Spinasterol :7,22diène-3βol	39,88	45,63	41,03	43,18
Schottenol :7ene-3βol	42,79	46,37	44,10	43,27
Stigmasta 8,22diene 3ß ol	3,26	2,53	3,35	3,46
Stigmasta 7,24diène 3ß ol	2,83	3,90	2,63	4,72

Table 3: *Sterol composition of argan oil in samples 1 to 4.*

The sterolic composition is consistent with the data in the literature [17]. They are essentially Δ-7-stigmasterols. The predominant products are schottenol (or Δ-7-stigmasterol) and spinasterol (Figure 3). Their proportion varies respectively between 42.8 and 46.4%, and from 39.8 and 45.6% (Figure 3) [19].

Schottenol and spinasterol are rarely found in vegetable oils and are characteristic of this oil. Two minority sterols have been identified in argan oil. These are stigmast-8,22-diene and stigmasta-7,24-28-diene (or Δ-7-avenasterol). Their proportion varies between 2.5% and 4.7% of the mixture of total sterols [20].

We find that the campesterol content in argan oil is very low. We can take this parameter as a marker to detect adulteration of argan oil.

From a study of the sterol composition of argan fruit from 4 samples, it appears that there is no correlation with the shape of the argan fruit and the composition of sterols [21].

Conclusion

This study the influence of the shape of the argan fruit on the chemical composition of argan oil we selected with the help of indigenous populations 4 different forms of fruit that we collected from the same place in the province. from Essaouira (Tamanar south of Morocco).

From these results of this work, we can say that the oval shape represents the best shape of the argan tree.

Therefore, there is no correlation with the shape of the fruit and the composition of fatty acids and sterols, The results obtained do not allow to conclude that the shape can influence the compositions of fatty acids and sterols.

The differences observed in the study of the chemical composition of fatty acids and sterols are not linked only to the form but also depend on the nature of the soil and the altitude, the longitude and the distance from the sea.

Acknowledgments

The authors would like to thank my colleagues in the Plant Chemistry Laboratory for their invaluable assistance. To all the people who have contributed directly or indirectly to the realization of this work, we send them my warmest thanks. The authors have stated that they are not taking any funding to do this work or to publish this article.

Competing Interests

Authors have declared that no competing interests exist.

Bibliography

1. Chamich M. “La forêt d’Arganier: quelle gestion pour quelle resource?” *Revue Forestière Française* (2014).
2. Mateille T., *et al*. “Aridity or low temperatures: What affects the diversity of plant-parasitic nematode communities in the Moroccan argan relic forest?” *Applied Soil Ecology* 101 (2016): 64-71.
3. Justamante MS., *et al*. “Vegetative propagation of argan tree (Argania spinosa (L.) Skeels) using in vitro germinated seeds and stem cuttings”. *Scientia Horticulturae* 225 (2017): 81-87.
4. De Waroux YLP and Lambin EF. “Monitoring degradation in arid and semi-arid forests and woodlands: the case of the argan woodlands (Morocco)”. *Applied Geography* 32.2 (2012): 777-786.
5. Khayi S., *et al*. “First draft genome assembly of the Argane tree (Argania spinosa)”. *F1000 Research* (2018): 7.
6. Hilali M., *et al*. “Plant cell biotechnology and molecular biology 21 (2020): 25-26.

7. ISO 659. Graines oléagineuses. Détermination de la teneur en huile (Méthode de référence) (2014).

8. ISO, I.. 5983–1: Animal Feeding Stuffs—Determination of Nitrogen Content and Calculation of Crude Protein Content—Part 1: Kjeldahl Method. International Organization for Standardization: Geneva, Switzerland (2005).

9. ISO 3596-1/2000 (méthode de référence): détermination de la teneur de l'insaponifiable de matière grasse (2000).

10. ISO 6799: Animal and vegetable fats and oils Determination of composition ofthe sterol. Determination of composition of the sterol (1996).

11. Analysis of cis fatty acids: Reference: NF ISO 5509. COFRAC code: CC30 Normalization (2015).

12. Gharby S., *et al*. "Can fruit-form be a marker for argan oil production?" *Natural Product Communications* 8 (2013): 1.

13. Hilali M., *et al*. "In Search of economically interesting trees in the argan grove". *Plant cell Biotechnology and Molecular Biology* 21 (2020): 25-26.

14. Hilali M., *et al*. "Evaluation of the chemical comoposition of argan oil according to its mode of extraction, its origin of production and altitude". *Online Journal of Animal and Feed Research* 10.3 (2020): 111-118.

15. Hilali M., *et al*. "Physicochemical Characterizes of Argan Oil (Argania spinosa L.) Affected by Roasting and Pulping by Goats". *International Journal of Veterinary Science* (2020).

16. Russo GL. "Dietary n− 6 and n− 3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention". *Biochemical Pharmacology* 77.6 (2009): 937-946.

17. Hilali M., *et al*. "Influence of origin and extraction method on argan oil physico-chemical characteristics and composition". *Journal of Agricultural and Food Chemistry* 53.6 (2005): 2081-208.

18. Hilali M., *et al*. "Detection of argan oil adulteration using quantitative campesterol GC-analysis". *Journal of the American Oil Chemists' Society* 84.8 (2007): 761-764.

19. Guillaume D., *et al*. "Argan [Argania spinosa (L.) Skeels] Oil. In Fruit oils: chemistry and functionality". *Springer, Cham* (2019): 317-352.

20. El Kharrassi Y., *et al*. "Biological activities of Schottenol and Spinasterol, two natural phytosterols present in argan oil and in cactus pear seed oil, on murine miroglial BV2 cells". *Biochemical and Biophysical Research Communications* 446.3 (2014): 798-804.

21. El Abbassi A., *et al*. "Physicochemical characteristics, nutritional properties, and health benefits of argan oil: A review". *Critical Reviews in Food Science and Nutrition* 54.11 (2014): 1401-1414.

Volume 12 Issue 8 August 2021