Valorization of argan oil: all you need to know about argan oil from Morocco

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ABSTRACT
This work makes it possible to study the argan oil which has extracted by several extraction methods (artisanal, mechanical pressing of roasted almonds, mechanical pressing of unroasted almonds, solvent extraction, pulped by a goat), after extraction, a detailed Physico-chemical study was done on this oil to certify its quality, the study of this work makes it possible to achieve an official standard for argan oil and studies the influence of the extraction method and the origin of production on the chemical composition. The study shows that roasting appears as a parameter influencing the acidity value of argan oil. Analysis of the peroxide index shows that the samples of argan oil extracted by the artisanal method and that those pulped by goats have a higher peroxide content. The sterol composition as per the data in the literature. They are essentially Δ-7-stigmasterols. The main products are schottenol and spinasterol. The composition of fatty acids and sterols shows no significant variation. These results are consistent with those reported in the literature. This shows that the origin and the method of extraction do not influence the nutritional qualities of argan oil. The results for the tocopherols show that the extraction method (plump by a goat) and roasting can influence the composition of the tocopherols. On the other hand, the sample obtained by the artisanal method has a lower content of total tocopherols. Roasting decreases the total content of α-tocopherol. After this study, it is urgent to ensure the quality of this oil, so Physico-chemical analyzes were carried out to detect fraud in pure argan oil. Finally, we determined the influence of the fruit form of the argan tree (spindle-shaped, apiculate, oval, spherical) on the Physico-chemical parameters of argan oil.

INTRODUCTION
The argan tree (*Argania Spinosa* (L.) Skeels) is an endemic tree of Morocco, where it constitutes the second forest species of the country, after the green oak and just before the thuja. It is a tree that can live up to 200 years. Some 250-year-old subjects could be observed. The argan forest covers about 870 000 ha (Díaz-Barradas et al., 2010) and has more than 20 million trees (Charrouf and Guillaume, 2018). This tree of the Sapotaceae family is particularly resistant to dry and arid conditions in southwestern Morocco. It can indeed withstand temperatures ranging from 3 to 50 °C and be content with deficient rainfall.

The argan tree grows wild and abundant in the arid and semi-arid areas of southwestern Morocco, where it plays an irreplaceable role in the ecological balance and the preservation of biodiversity. Thanks to its powerful root system, it contributes
to the maintenance of the soil. It makes it possible to fight against the erosion hydric and windy which threat of desertification a good part of the region (Justamante et al., 2017).

The argan tree is also of great economic interest because it is a multipurpose tree. Each part of the tree is usable and is a source of income or food for the user: the wood is used as fuel, the leaves and pulp of the fruit constitute food for goats and camels and the oil extracted from the almond kernel is used in human nutrition and traditional medicine.

The fruits of the Argan tree are green then turn towards the brown at maturity. They look like an olive but are bigger and rounder.

Inside the argan fruit, there is a nut with a tough shell. The shell is represented about a quarter of the weight of the fruit of the argan tree. The nut can contain up to three almonds, and argan oil is extracted from the almonds of the argan fruit. Argan oil plays a significant role in the nutrition of indigenous peoples. It is an edible oil extracted from almonds. For the Souss regions, it provides 25% of the fat intake. Its annual production is of the order of 4000 tons, that is 1.6% of the Moroccan consumption in edible oils, and 9% of the national production (Bani-Aameur, 2001). The oleaginous almond represents only 3% of the weight of the fresh fruit (Bani-Aameur, 2001). The extraction of the oil is done in an artisanal way, it results, on the one hand, a loss of 45% of oil in the cake, corresponding to 1,530 tons (Charrouf and Guillaume, 2018), and on the other hand a loss of hygienic and sanitary nature. The rate of oil extraction could be increased, and its quality could be improved by the introduction of appropriate technology that would ensure the extraction of 95% of the existing oil in the kernel (Sinsin et al., 2020).

### Different Type of Aragn Oil Extraction

#### Artisanal Extraction

The artisanal extraction of argan oil is done in several stages:

1. Purging: the women remove the skin of the fruit with two stones, the separation of the pulp and the nut is done as and when the operation of pulping.
2. Crushing or deshelling: It is done with the same stones as Depulpage, the nut is crushed by crushing it strongly. Sorting is done at the end of the operation.
3. Roasting almonds: It is made in earthenware containers on a softwood fire.
4. Grinding almonds: It is made in a cut stone wheel. The result is a brown dough.
5. Mixing and pressing: The paste obtained is kneaded by hand in a bowl. It is supplemented with lukewarm water in a small quantity until a smooth paste is obtained, which is then vigorously kneaded and then manually pressed until the oil is obtained.
6. Bottling: The artisanal oil is usually put in recycled bottles and very often poorly cleaned.

#### Extraction By Mechanical Pressing

The extraction of argan oil is carried out from the almonds of the argan fruit by several (Charrouf and Guillaume, 1999; Waroux and Lambin, 2013). In most cases, the extraction yield has been improved compared to the artisanal method.

All operations were mechanized except the crushing which has remained traditional. A detailed technical study of this process is described in the literature (Sinsin et al., 2020). A machine of local manufacture carries out the pulping operation. It allows you to tear the fruit pulp and separate it from the nuts via a blower. The work done on the tearing of the pulp gives a yield of 70-95%.

On the other hand, the work done by the wind tunnel is negligible and needs to be improved. For a Kg of raw fruit, it takes on average 4 minutes. The roaster operates with butane gas. It allows for roasting 6 Kg to 10 Kg of almonds at a time. It takes 20 to 30 minutes to roast 6 to 10 kg of almonds. A screw press type KOMET D85 carries out the pressing. It can produce 6 to 8 litres of oil per hour. The oil obtained by mechanical pressing is heavily loaded with solid material (remaining cake). It requires decantation of 4 to 10 days before filtration on a filter press.

The flow of the latter is 17 litres/hour.

Thanks to the mechanical extraction one can obtain two types of oil:

1. Food oil, nutty flavour, obtained by mechanical pressing of roasted almonds.
2. Virgin oil, or cosmetic oil intended for cosmetic purposes, obtained from unroasted almonds.

So the mechanical pressing reduces the time, and the roughness of the work also allows to obtain a better quality oil and with good performance (Guillaume and Charrouf, 2016).
The argan oil thus produced does not require the addition of water for extraction (the oil can be stored longer).

Previous Studies on Aragn Oil

The work summarizes all the studies that have been done on argan oil:

1. Study of the chemical composition of argan oil according to its extraction method and its origin of production
2. Multi analyzes for adulteration research of pure argan oil by other edible oils.
3. The influence of the diversity of the morphological characters of the argan fruit on the chemical composition of argan oil, fruit pulp and oilcake could give new life to the argan tree.
4. Studying the effect of storage on the chemical composition of argan oil
5. The study of the roasting effect of almonds on the chemical composition of argan oil would help produce a better quality organoleptic oil.

MATERIALS AND METHODS

Preparation of oils

Preparation of different samples of argan oil and olive oil

Argan oil was prepared by extraction, by the mechanical pressing method and by artisanal extraction in the cooperative of Tidzi (province of Essaouira, southern Morocco) according to the methods already described: extraction by mechanical pressing (almonds roasted (Taarji et al., 2018).

Physicochemical Analyses Of Oils

Determination of acidity (Fernández et al., 2005), the peroxide value (Pizarro et al., 2013), the refractive-index (Hasenhuettl, 2016) of the absorbance in the ultraviolet (Rowland et al., 2002), the saponification number (Jobling, 2007), the unsaponifiable content (Sylvester et al., 1945) were measured according to the standardized methods of reference.

Determination Of Composition And Nature In Total Sterols

Operating Mode

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. The flask is heated under reflux for 30 minutes until the solution becomes clear; afterwards, 25 ml of distilled water are 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 a 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 on a silica column (25cm × 4mm). The HPLC device 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 Hewlett Packard brand and series 6890 GC chromatograph is equipped with an FID detector (T°: 300 °C). The carrier gas is nitrogen, and its flow rate is 1 ml/min (PE: 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) (Honfo et al., 2014).

The silylation reaction of sterols is shown in Figure 1

Analysis of CIS Fatty Acids

Operating Mode

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. Then a solution of sodium carbonate Na₂CO₃ 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, which are placed in a tube of the 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 an 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 (polyethene glycol) (30 m × 0.32 mm, Dl: 0.25 μm) (Baer et al., 2010).

The reaction of the methyl esters of the triglyceride fatty acids is as follows (Figure 2).

### Tocopherol Analysis

#### Operating Mode

In a 25 ml volumetric flask, 2 g of argan oil is introduced, and then we filled the flask 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 (Taarji et al., 2018). 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) (Lara-Ortega et al., 2017).

### Triglyceride Analysis

#### Operating Mode

0.15 g of argan oil is placed in a beaker after adding 0.5 ml of hexane and 15 ml of a hexane/diethyl ether mixture (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 1047A, during the analysis time (90 min). The elution is made by the mixture (acetonitrile / acetone) (v / v) with a flow rate of 0.5 ml / min (Álamo et al., 2004).

### RESULTS AND DISCUSSION

#### Influence of Origin and Extraction Method on Argan Oil Physico-Chemical Characteristics and Composition

As part of the development of argan oil, several studies were made on argan oil, starting by a comparative study of the different physicochemical parameters of argan oil according to its mode of extraction and its origin of production. To carry out this work, 21 samples of argan fruits located in different geographic locations of Morocco were selected. They were extracted in different ways (by mechanical pressing, artisanal, and from fruit removed by goats, extraction by the solvent) to realize a standard of argan oil from Morocco for the first time.
The study of physicochemical characteristics shows that all acid values of argan oils are lower than 1.40% (Charrouf and Guillaume, 2008). This result shows that argan oil is characterized by low acidity compared to other vegetable oils (olive ≤ 2%). The study shows that roasting appears as a parameter influencing the acidity value of argan oil. Indeed, the acidity value is higher in argan oil samples prepared from unroasted almonds. More than that the argan oil sample from Tamanar, (a village south of Morocco) lot has a higher acid value than the samples. This result suggests that geographical origin can influence acidity values (Hilali et al., 2005).

The results concerning the unsaponifiable rate show that argan oil is characterized by a low unsaponifiable rate (≤ 0.81%), but unsaponifiable of olive oil is (Olive ≤ 1.50%).

The extraction technology of argan oil can influence the unsaponifiable rate of argan oil. Indeed, the unsaponifiable rate of the sample prepared by hexane is lower (0.34 to 0.56%) than those prepared by mechanical or artisanal press (0.6% to 0.81%).

The results of the saponification index of argan oil were found to be between 180.0 and 199.0 (virgin olive oil varies between 184 and 196).

Analysis of the saponification index shows that the sample of argan oil extracted by hexane or extracted by the artisanal method have a saponification index lower than that of the samples extracted by mechanical pressing. Still, the study the argan oil shows that torrefaction and depulping by the goat influence the increase of this parameter (Hilali et al., 2005).

The results of the peroxide index on the 21 samples of argan oil are lower (≤ 6 meq O₂ / kg) than those required for virgin olive oil (≤ 20 meq O₂ / kg).

The analysis of the peroxide value shows that the samples of the argan oil extracted by the artisanal method and which those deflated by the goats have a higher peroxide content compared to the other samples. The determination of the peroxide value appears to be a critical measure for assessing the quality of argan oil.

The refractive index was determined at 20 °C. This index varies between 1.4644 and 1.4705.

The specific extinction has been determined at 270 nm in general, the values found are higher than that of olive oil; they vary between 0.228 and 0.426.

Specific extinction and refractive index give no precise information on the origin and method of extraction of argan oil.

The fatty acid analysis shows that argan oil contains 80% unsaturated fatty acids. It is oleic-linoleic and contains between 29% and 35% of essential fatty acids: linoleic acid (29 to 34%) (Vitamin F). Its oleic acid content makes this oil particularly interesting in the regulation of cholesterol.

Sterol analysis shows that the total sterol levels of argan oil range from 130 to 233 mg / 100g of fat (Charrouf and Guillaume, 2008). (Olive 98-184, hazelnut 75-195).

The sterol composition is under the data of the literature. These are essentially ∆-7-stigmastersol. The major products in argan oil are schottenol (47.75%) (or ∆-7-stigmatasterol) and spinasterol (36.21%). Schottenol and spinasterol are rarely found in vegetable oils and are characteristic of argan oil. Argan oil does not contain β-sitosterol (∆-5-stigmasterol), Schottenol and spinasterol, which are very rare in vegetable oils, maybe a parameter in the detection of adulteration of this oil. Two minority sterols were identified based on their GC / MS mass spectrum and compared with literature data. It is stigmatiste-8,22-diene and stigmasta-7,24-28-diene (or ∆-7-avenasterol); their proportion varies between 2.6%
The results of the composition of fatty acids and sterols show that the origin and the method of extraction do not influence the nutritional qualities of argan oil.

Argan oil is richer in tocopherols (597 to 775 mg/kg) than olive oil (50 to 150 mg/kg) and hazelnut oil (300 to 550 mg/kg) (Charrouf et al., 2006).

The results for tocopherols show that the extraction method and roasting can influence the composition of tocopherols (Harhar et al., 2011). On the other hand, the sample obtained by the artisanal method has a lower content of total tocopherols. Roasting decreases the total α-tocopherol content.

Analysis of the triglyceride fraction of argan oil allowed the separation of the individual triglycerides. The predominance of triglycerides LLO (12% -14%), LOO (13% -15%), LOP (14%), OOO (12% -14%), and OOP (14% -17%) in the Argan Oil. These triglycerides represent approximately 73% of each triglyceride fraction of argan oil.

The result of determining the percentage of palmitic acid at position-2 in triglycerides is low and varies between 0.16 and 0.43%.

The results of triglycerides and palmitic acid at the 2-position of triglyceride give no specific information on the geographical origin and the extraction process of the fruit of the argan tree.

The determination of the wax content of certain fats can be used in particular to differentiate press oils from extraction oils.

The analysis of the wax content can give ideas on the extraction process, which allows the introduction of an exogenous wax, or by the use of almonds stored under poor conditions.

In general, argan oil contains a lower percentage of waxes (≤70mg/kg) (Charrouf and Guillaume, 2008) than other virgin oils such as olive oil (≤350mg/kg) (Karleskind, 1992).

Determination of the wax content shows that the sample extracted from roasted almonds has a higher percentage of waxes than the sample extracted from unroasted kernels.

These results show that roasting can increase the percentage of waxes in argan oil.

The study of the concentration of benzo-α-pyrene suggests that roasting does not produce significant amounts of benzo-α-pyrene, this work has shown that the argan oil extracted by mechanical pressing is the best and the results from this work contributed to the development of a national standard for argan oil.

**Study of the Chemical Composition of Arag Oil According to its Shape of the Arag Fruit**

In the perspective of domestication of the argan tree with economically interesting trees, a comparison between the oils produced from the different forms of the fruit of the argan tree has been launched. Regarding the quality indices, no significant influence of all the parameters has been shown apart from the acidity, the peroxide index and the extinction E232 and E270 of the oil of the fusiform form which has shown maximum values (Gharby et al., 2012).

Regarding the quality criteria, the shape of the fruit does not influence the percentages for triglycerides and tocopherols. For the latter, their content in oils of the apiculate and oval forms is of the order of 770 mg/kg, whereas the other two do not exceed 626 mg/kg (Mcgown, 2006). Given the nutritional value of tocopherols and the difference of 150 mg/kg between forms. The shape of the fruits slightly influences the fatty acid and sterol composition. Indeed the results clearly show that tree selection based on fruit shape could allow the natural production of argan oil rich in linoleic acid and schotenol from spherical fruits and an oil rich in oleic acid from fusiform fruits. These results are to be taken into account for the domestication of the argan tree (Gharby et al., 2012).

The mode of extraction influenced the phospholipid content regardless of geographical origin. This criterion recorded the highest values in the food press oil, followed by the oils produced by traditional methods. For cosmetic press oil, the phospholipid content is lower. This demonstrates the link with roasting. The same result was observed for beta carotene.

Regarding the oxidative stability, evaluated by Rancimat, it seems more influenced by the extraction methods than by the three geographical origins studied. Indeed, the fastest oxidation was observed for oils produced from unroasted kernels, followed by those produced by traditional methods and that prepared by mechanical pressing of roasted kernels (Gharby et al., 2013). The stability of the latter is twice that of the first. Regarding the shape of the fruit, it does not influence the stability of the oil because the induction time of all the oils extracted from the different forms is between 18 and 21 hours.

The stability results obtained by Rancimat were confirmed by the stability study at 60 °C. Indeed, the edible oil obtained by mechanical pressing of roasted almonds is more stable to oxidation for 35 days (Gharby et al., 2013).
Multi Analyzes for Adulteration Research of Pure Aragan Oil by Other Edible Oils

After establishing Moroccan argan oil standards, there is an urgent need to ensure the quality of argan oil. So, to detect argan oil fraud with other edible oils such as soybean, rapeseed, sunflower, apricot, hazelnut, olive, sesame, and peanut oils is a real analytical challenge. These oils have, in fact, physico-chemical characteristics and a very close fatty acid composition which greatly hinder their detection when they are mixed. The analysis of fatty acids makes it possible to demonstrate a falsification of argan oil by soybean oil and rapeseed oil from a level of 1% based on the percentage of the acid linolenic (C18: 3) that does not exceed 0.1% in pure argan oil (Hilali et al., 2005). On the other hand, olive oil, hazelnut oil and sesame oil can only be detected at a rate of 5%. The result of the fatty acid composition does not make it possible to demonstrate a falsification of argan oil by sunflower oil, apricot oil and peanut oil.

The determination of the sterol composition prompted us to take the percentage of campestral as a marker of adulteration. The latter does not exceed 0.4% in pure argan oil (Hilali et al., 2007). The result of the sterol composition shows that the detection threshold of edible oils having a percentage greater than 10% of campesterol such as soybean, rapeseed, sunflower, sesame and peanut oils in argan oil is 1% against oils with a percentage of campesterol less than 10% the threshold of detection of adulteration is 2% for apricot oil and 5% in the case of olive oil and hazelnut (Hilali et al., 2007).

Study of the Storage Effect on the Physico-Chemical Composition of Argan Oil

The quality of argan oil lead to making the following recommendations;

1. The duration of the drying should be limited to 2 - 3 weeks.
2. Fruits and nuts can be kept for 24 months without affecting the quality of the oil; it is recommended to store nuts rather than whole fruits to save space in the warehouse.
3. The storage conditions of the kernels, after sufficient drying, do not affect the quality of the oil.
4. Roasting increases the stability of argan oil in addition to its significant influence on organoleptic properties (Guillaume et al., 2019).

CONCLUSION

Great efforts have been made to develop argan oil by improving its extraction technology and the creation of argan oil extraction good quality cooperatives and achieve the preservation of the argan tree because argan oil is very important in nutrition. Argan oil contains 80% unsaturated fatty acids and contains 38% of essential fatty acids: linoleic acid (38%) (Vitamin F), while the percentage of linoleic acid in the oil of the olive is 10.83%. The total sterol content of the argan oil is 154 mg / 100g fat. The major products in argan oil are schottenol (47.75%) (or Δ-7-stigmasterol) and spinasterol (36.21%). Schottenol and spinasterol are rarely found in vegetable oils and are characteristic of argan oil. Argan oil is richer in tocopherol (717 mg / kg) than olive oil (320 mg / kg). Argan oil is rich in γ-tocopherol (80 to 90%). The study of this work shows that extraction technology can influence the Physico-chemical parameters of argan oil. Our study demonstrated the high quality of argan and olive oil extracted by mechanical pressing.

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