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# THE INFLUENCE OF THE FORMS ON THE QUALITY, CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ARGAN OIL GROWN IN MOROCCO

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**ABSTRACT**

Argan oil plays a significant socio-economic role in southwestern Morocco, with many applications in cosmetics and nutrition. In this study, the chemical composition, the quality, as well as antioxidant activity of Argan oils extracted from different fruit shapes (apiculate, oval, spherical, and fusiform) in Essaouira and Agadir were evaluated. The results collected indicated a slight resemblance in the chemical composition of fatty acids, sterols, tocopherols, and polyphenols of Argan oils. These, extracted from two different regions, also show similar yields in the oval form, with values of 53.53 % for the Essaouira region and 54.86 % for that of Agadir. However, a slight difference was noted in the shape of the fruit. Indeed, the total sterols content for the oval shape showed values of 178.64 mg/100g in the Essaouira region and 171.97 mg/100g in the Agadir region. Also, γ-tocopherol was the predominant tocopherol in all tested oils.

**Keywords:** Argan oils, Chemical composition, Yields, Antioxidant activity, DPPH

## INTRODUCTION

Argan oil is one of the worldwide known types of plant-based oils, which comes from the Argan tree (*Argania spinosa*) originating from the Moroccan southwest. As a multipurpose tree, Argan plays an important socio-economic role, and its sustainable development is essential to maintain the ecological balance and protect biodiversity **(Khallouki et al., 2017)**. Although the Argan tree is the only representative tree species of the Moroccan tropical locust family, it is the second forest species after the oak and cedar, with a lifespan of over 200 years. It is mainly cultivated in the arid and semi-arid regions of southwest Morocco, on about 870.000 hectares.

In 1998, UNESCO recognized the importance of the Argan tree, declaring it a "protected species" and the vast interior mountain plain, which protects the Argan tree, is a biosphere reserve **(Miklavčič et al., 2020)**. It has been introduced as a cultivated species in the deserts of Tunisia, Israel and South Africa, as well as in other parts of the world **(Charrouf and Guillaume, 2014)**. The importance of Argan oil in Moroccan culture stems from its traditional use in local cooking, medicine, and cosmetics. From 2019 to 2027, the global Argan oil market is expected to grow at a compound annual growth rate of 10.8% to reach USD 507.2 million by 2027 **(Size et al. 2020)**. The revenue generated creates the livelihoods that support the many rural families and communities that depend on the Arganeraie Biosphere Reserve **(Charrouf and Guillaume, 2014)**.

This luxury virgin oil is extracted from the Argan tree fruits, particularly from the seeds contained in the fruit's core **(Farres et al., 2019)**. There are several shapes of Argan tree fruits, and predominantly, depending on the width to the length ratio, it is possible to distinguish four shape types; fusiform, oval, apiculate, and spherical **(Berka et al., 2019)**. Generally, each fruit's core contains one to three seeds, of which each is composed of up to 58% of oil **(Hanana et al., 2018)**. Traditionally, this oil is prepared by Moroccan Amazigh women according to a multi-step, ancestral process **(Guillaume et al., 2019)**. However, this traditional extraction is time-consuming and carried out in unsuitable sanitary conditions. Consequently, there have been attempts to improve the Argan oil's quality by improving its extraction process **(Hilali et al., 2005)**. Due to its numerous advantages as traditional dietary medicine, Argan oil is used as a cosmetic, pharmaceutical, and nutritional product. The nutritional value and the therapeutic benefits of Argan oil consumption have been summarized by Charrouf and Guillaume **(Charrouf and Guillaume 2008)** and El Abbassi et al. **(El Abbassi et al., 2014)**, including antioxidants, antiproliferative, and antidiabetic effects, treatment of skin infections and cardiovascular disease prevention.

Argan oil contains large amounts of unsaturated (up to 80%) and saturated (up to 20%) fatty acids **(Sevindik et al., 2019)**. While several theories attribute the therapeutic effects of Argan oil to its high content of oleic and linoleic acid, many other oils with high concentrations of these fatty acids do not have the same therapeutic effects, which suggest that the 1% of non-saponifiable substances found in the oil could be mainly responsible for its therapeutic effects. These non-saponifiable materials consist of a range of biologically active compounds including carotenes (37%), tocopherols (8%), triterpenic alcohols (20%), sterols (29%) and xanthophylls (5%) **(Lall et al., 2019)**.

Carotenes, tocopherols, and xanthophylls are well-known antioxidants that stimulate intercellular respiration, neutralize free radicals, and protect the skin against the harmful effects of sun rays, while triterpenic alcohol and sterols have been associated with anticancer and antimicrobial activities **(Harlev et al., 2012; Zarrouk et al., 2019; Goik et al., 2019; Gamero-Sandemetrio et al., 2019)**. Different factors such as genotypes, environmental conditions prevalent during the growing season, location, climate, harvest time, and extraction techniques can influence the oil content and chemical composition [El Qarnifa et al. 2019]. The morphological variability of the Argan fruit was also suggested to influence the chemical composition **(Gharby et al. 2013)**.

The objectives of this paper were to identify oil content and the chemical composition (fatty acids, sterols, polyphenols, carotenoids, and chlorophylls) of different Argan fruits shapes originate from two regions in Morocco (Essaouira and Agadir) and to evaluate the quality of oils and their antioxidant activity. The relationship between the quality parameters (FFA, saponification index, K270, and iodine index), total sterols, total tocopherols, chlorophylls, carotenoids, polyphenols, linoleic (C18:2) and antioxidant activity (DPPH) of the different samples of Argan fruits shapes oils are evaluated by the Principal Component Analysis (PCA). A Hierarchical Cluster Analysis (HCA) was used to construct three clusters based on their different physicochemical parameters of the quality, total sterols, total tocopherols, chlorophylls, carotenoids, polyphenols, linoleic (C18:2), and antioxidant activity (DPPH) of the different samples of Argan fruits shapes oils.

## MATERIALS AND METHODS

### Sampling and oil extraction

A mass of 300 g of each Argan fruit shape was gathered from about thirty trees (in total 9 kg) at the end of August 2018 from two regions in Morocco ; the Essaouira region, particularly between Alhoussaynat and Almaji villages, as well as from the Azrarag, in the Agadir region. The fruits were dried in the shade for two weeks to remove the pulp and to get the Argan nuts smoothly. By breaking the Argan nuts with a rock, almonds were divided from the Argan hull. The seeds of the Argan fruit were blended in a domestic kitchen mixer grinder. Fifty grams of seeds of each form was placed in a paper thimble and put through a soxhlet extractor with a capacity of 250 mL. The oils obtained were stored in brown glass bottles at 4 °C until use. All assays were performed in triplicates to guarantee reproducibility.

### Morphological Characteristics of Kernels

The microscopic examinations of Argan kernels were managed at the Criminal Institute of Royal Gendarmerie (Rabat) accredited ISO 17025, via a FEI Quanta 650 environmental microscope. The chosen samples were adjusted on an adhesive double-sided carbon backing, and to bypass charging effects that decrease the image's quality, the samples were metalized in carbon before being advanced into the microscope enclosure. The observations were conducted under vacuum (7.4 $10^{-4}$ Pascal) at a voltage of $1^{-2}$ kV.

### Analysis of Argan oils

### Fatty acid composition

Fatty acid composition was established through an official method **(El Idrissi et al., 2022)**. Prior to the analysis, fatty acids (FAs) were converted into their corresponding methyl esters (FAMEs). The fatty acid composition was determined as their corresponding methyl esters by gas chromatography on a CPWax 52CB column (60 m × 0.25 mm i.d., 0.25 µm film thickness) via helium (flow rate 1 mL/mn) as a conveyor gas. The initial column temperature was 170 °C, the final temperature 230 °C, and the temperature was turned up by steps of 5 °C/min. The injector and detector temperature were 230 °C. The injection volume of the samples was 2 µL in a split mode (split ratio 1:50). As the relative percentage of the area of each individual fatty acid peaks, the results were presented.

### Sterol Composition

Phytosterols composition was established in accordance with the International Standard Organization method ISO 12228-1 **(ISO 12228-1 2014)** using a Varian 3800 instrument equipped with a VF-1, ms column (30 m and 0.25 mm i.d.) and using helium (flow rate 1.6 mL/min) as a conveyor gas. Column temperature was isothermal at 270 °C, injector and detector temperature culminated to 300 °C. Injected quantity was 1 µL for each analysis.

### Tocopherols Composition

The tocopherol content of the specimens were determined to use an HPLC equipped with a fluorometric detector (excitation wavelength 290 nm - emission wavelength 330 nm) on a silica column (25 cm 4 mm) in line with the ISO 9936 (2006) standard method. During the analysis, the elution is transmitted with a mixture of (isooctane: isopropanol) (99:1) at a flow rate of 1.2 mL/min (20 min). External standard curves of four tocopherols were used as well as a daily reference of quantitative and qualitative tocopherol standards **(El Moudden et al., 2020)**.

### Carotenoids and Chlorophylls analysis

In 100 mL of cyclohexane, 1 g of Argan oil is dissolved. The absorbances of chlorophylls and carotenoids are measured at 670 nm and 470 nm, respectively **(El Moudden et al., 2020)**. In conformity with the following two formulas:

$$Chlorophyll\left(mg\ Kg^{-1}\right) = \frac{A_{670} \times 10^{6}}{613 \times 100 \times d} \quad (1)$$

$$Carotenoid\left(mg\ Kg^{-1}\right) = \frac{A_{470} \times 10^{6}}{2000 \times 100 \times d} \quad (2)$$

The carotenoid content is indicated in mg Lutein per kilogram of Argan oil, and the chlorophyll content is displayed in mg Alpha Pheophytin per kilogram of Argan oil.

### Total Phenolic Compounds (TPC)

For the extraction of phenolic compounds from *Argania spinosa* seeds, a mass of 2 g of oil was extracted three times with 10 mL of methanol: water solution (8:2), after vortexing for 2 minutes and centrifugation at 3000 rpm for 10 minutes for each extraction. After incorporating the methanolic extracts, they were washed three times with 10 mL n-hexane to remove any lipid components. A rotary evaporator was used to separate the methanolic fraction and evaporate it at a lower pressure **(Ouassor et al., 2020)**.

TPC ascertainement was achieved via the Folin-Ciocalteu (FC) colorimetric method, with some modifications **(El Guezzane et al., 2021)**. In distilled water (1:10), 500 µL of the sample solution was combined with 2.5 mL of FC reagent, and 4 mL of $Na_2CO_3$ (7.5 percent, w/v) was added. The absorbance at 765 nm was measured using a UV-Vis spectrophotometer after 30 minutes of incubation in a 45 °C water bath and compared to a blank solution. Over a concentration range of 0-300 µg/mL, the TPC concentration is estimated using the regression equation of the established calibration curve with Gallic acid. The Gallic acid equivalent (mg GAE/g oil extract) was used to calculate the TPC values.

### Measurement of RSA of Argan oil by the free radical DPPH

The free radical scavenging activity of the studied oils was calculated by 1.1-diphenyl-2-picryl-hydrazil (DPPH) **(El Moudden et al., 2019)**. Shortly after, 0.2 mM solution of DPPH in ethanol was prepared and 0.5 mL of this solution was incorporated to 2.5 mL of oil samples and was left to stand at room temperature for 30 min, and then absorbency was read at 517 nm. Lower absorbency of the reaction mixture pointed higher free radical scavenging activity. The radical-scavenging activity (RSA) was determined according to the equation:

$$\%RSA = \frac{A - A_s}{A} \times 100 \quad (3)$$

Where: A is the registered absorbance of the blank sample, and $A_S$ stands for the absorbance value of the sample solution.

### Physicochemical parameters determination

Ca 3a-63, Cd 1b-87, Cd 3b-76, and Ch 5-91; FFA, iodine index, saponification index, and extinction coefficient (K270) were calculated in accordance with AOCS recommendations **(AOCS 1998)**. Using an LLG-uniSPEC 2 UV spectrometer, the amount of FFA in Argan oil was calculated as a percent of oleic acid, IV was calculated as g $I_2$/100g of oil, SV was calculated as mg KOH/1g of oil, and extinction coefficient at 270 (K270) was calculated as the specific extinction of a 1 percent (w/v) solution of Argan oil in cyclohexane.

### Statistical Analysis

#### *Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA)*

The purpose of the main component analysis in this study is to see if there is a link between the different physicochemical quality metrics utilized in different Argan fruit forms from two different areas in Morocco (Essaouira and Agadir) on the one hand, and between total sterols, total tocopherols, chlorophylls, carotenoids, polyphenols, linoleic (C18:2), and antioxidant activity (DPPH) on the other hand. The PCA was effectuated on the results of physicochemical parameters quality (FFA, saponification index, K270, and iodine index), total sterols, total tocopherols, chlorophylls, carotenoids, polyphenols, linoleic (C18:2) and antioxidant activity (DPPH) which represented the 11 variables and those of the 8 samples oils from Argan fruits shapes originate of two regions in Morocco (Essaouira and Agadir). This method facilitates the interpretation of the essential factors leading the most to explain the variation in physicochemical parameters quality following the sample oils, HCA was employed to track the interrelatedness between all samples oils and cluster characteristics, furthermore, the dendrogram was composed by the cluster method of Ward and the Squared Euclidean distance were taken as a coefficient of similarity.

#### *Correlation Matrix*

The PCA was carried out on a matrix that resumes all the sets of data from different physicochemical parameters of the quality and between total sterols, total tocopherols, chlorophylls, carotenoids, polyphenols, linoleic (C18:2), and antioxidant activity (DPPH). The individuals are represented by the 8 samples from Argan fruits shapes oil.

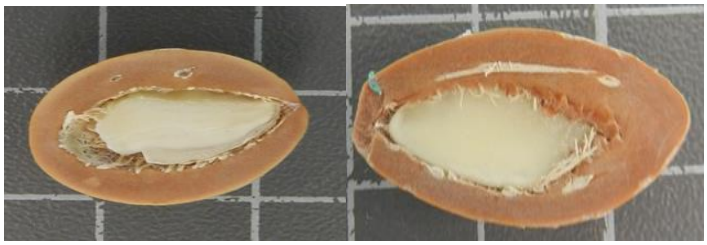
#### *Data Analysis*

The Pearson Correlation was handled to study the correlation between all the parameters used of Argan fruits shapes oils in this paper. The PCA and HCA were done by the XLSTAT 2014 software **(El Guezzane et al., 2021, El Moudden et al., 2022)**. Data were represented as mean ± standard error of the mean and were made using IBM SPSS Statistics 21 software.

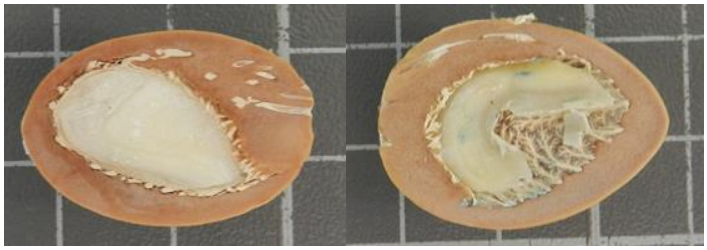
## RESULTS AND DISCUSSION

### Geometrical traits of Argan fruits

Figure 1 and Table 1 are the illustrations and the description of the size parameters of different shape types of Argan fruits (length, width). Where the spherical shape resembles a ball, the oval shape has more or less an egg shape, rounded and elongated. The fusiform is characterized by its broad shape, in the middle, and perfectly cut in both ends. The apiculate shape has a short-pointed tip containing two to three seeds in the shape of a nucleus. Regarding kernel length, apiculate and oval morphotypes from Essaouira showed the longest kernels (29 mm and 28 mm respectively) and spherical morphotypes from Essaouira and Agadir the shortest (15.50 mm and 17 mm respectively). Regarding kernel width, apiculate and fusiform fruits from Agadir have the widest kernels (27.4 mm and 24.4 mm respectively) and the oval and spherical fruits from Essaouira the narrowest (12.50 mm and 14.5 mm respectively). In general, the mean value of kernels length is greater in the case of apiculate, oval, and fusiform compared to spherical fruits. Regarding the width, apiculate and fusiform fruits remain superior.



**Spherical** **Oval**



**Fusiform** **Apiculate**

**Figure 1** Macroscopic description of the spherical, oval, fusiform and apiculate forms of Argan fruit.

**Table 1** Variation of size parameters of Argan kernels according to their form and region.

| Region | Form | **Length** (mm) | **Width** (mm) |
|---|---|---|---|
| **Essaouira** | Spherical | $15.5 \pm 0.2^{a}$ | $14.5 \pm 0.2^{ad}$ |
| | Oval | $28.5 \pm 0.5^{b}$ | $12.5 \pm 0.5^{a}$ |
| | Apiculate | $29 \pm 0.4^{b}$ | $19.5 \pm 0.4^{bc}$ |
| | Fusiform | $27 \pm 0.5^{bc}$ | $17.5 \pm 0.3^{be}$ |
| **Agadir** | Spherical | $17 \pm 0.2^{a}$ | $20.3 \pm 0.1^{c}$ |
| | Oval | $25 \pm 0.50^{cd}$ | $16.1 \pm 0.4^{de}$ |
| | Apiculate | $27 \pm 0.3^{bc}$ | $27.4 \pm 0.4^{f}$ |
| | Fusiform | $23 \pm 0.5^{d}$ | $24.4 \pm 0.4^{g}$ |

The data are presented in the form of the average of two individual repetitions (n = 2e ± SEM), the means followed by similar letters exposing in the same column are not different (P < 0.05).

### Chemical composition

As seen in Table 2, the Argan oil content was found in percentages of between 47.33 and 54.86%. Oil contents of fusiform, oval, and apiculate fruits from Essaouira and oval fruits from Agadir were higher than 50 %, oval shape showed high yield in both the region while spherical shape had the lowest yield. Gharby et al. **(Gharby et al., 2013)** showed a significant difference in oil content between apiculate (58.6%), fusiform (54.4%), spherical (53.1%), and oval (42.8%) fruits from Agadir.

**Table 2** Yield and fatty acid composition of Argan oil (%).

| Region | Shape | Oil yield | Palmitic A. (C16:0) | Stearic A. (C18:0) | Oleic A. (C18:1) | Linoleic A. (C18:2) |
|---|---|---|---|---|---|---|
| **Essaouira** | Spherical | $47.33 \pm 0.83^{a}$ | $13.06 \pm 0.06^{a}$ | $5.49 \pm 0.05^{a}$ | $45.30 \pm 0.15^{a}$ | $35.38 \pm 0.08^{a}$ |
| | Oval | $54.86 \pm 1.76^{a}$ | $12.83 \pm 0.06^{a}$ | $6.82 \pm 0.03^{b}$ | $46.42 \pm 0.7^{b}$ | $32.94 \pm 0.04^{b}$ |
| | Apiculate | $52.26 \pm 1.16^{a}$ | $12.19 \pm 0.18^{b}$ | $6.53 \pm 0.04^{be}$ | $46.50 \pm 0.06^{b}$ | $34.07 \pm 0.07^{c}$ |
| | Fusiform | $53.33 \pm 1.35^{a}$ | $13.96 \pm 0.09^{c}$ | $6.10 \pm 0.08^{ce}$ | $45.47 \pm 0.07^{a}$ | $33.80 \pm 0.08^{c}$ |
| **Agadir** | Spherical | $48.3 \pm 1.1^{a}$ | $13.22 \pm 0.12^{a}$ | $5.71 \pm 0.06^{ad}$ | $44.80 \pm 0.1^{c}$ | $35.40 \pm 0.09^{a}$ |
| | Oval | $53.53 \pm 1.73^{a}$ | $12.08 \pm 0.03^{b}$ | $6.80 \pm 0.04^{b}$ | $49.06 \pm 0.06^{d}$ | $30.24 \pm 0.04^{d}$ |
| | Apiculate | $48.56 \pm 1.36^{a}$ | $14.90 \pm 0.06^{d}$ | $5.94 \pm 0.04^{cd}$ | $46.44 \pm 0.04^{b}$ | $32.07 \pm 0.07^{e}$ |
| | Fusiform | $48.93 \pm 1.83^{a}$ | $14.72 \pm 0.05^{d}$ | $6.28 \pm 0.06^{e}$ | $47.13 \pm 0.03^{e}$ | $31.64 \pm 0.09^{f}$ |
| | Standard **(AOCS 1998)** | | 11.5 - 15.0 | 4.3 - 7.2 | 43.0 - 49.1 | 29.3 - 36.0 |

The data are presented in the form of the average of two individual repetitions (n = 2e ± SEM), the means followed by similar letters exposing in the same column are not different (P < 0.05).

Besides, oleic, linoleic, palmitic, and stearic acids were found to be the dominant fatty acids (Table 2). In the case of oleic acid, the oval fruits from Agadir showed an exceptionally high content (49.06%) followed by fusiform fruits from the same region (47.41%). Spherical fruits from Agadir showed lower content (44.80%). The linoleic acid concentration was found to be significantly higher in spherical fruits from Essaouira and Agadir (35.38% and 35.40% respectively). The lower amount was reported in oval fruits from Agadir (30.24%). Significant important palmitic acid content was found in fusiform and apiculate fruits (14.81 and 14.90%) from Agadir, and a lower amount of oval and apiculate (12.19%) fruits from Essaouira and oval fruits from Agadir (12.08%). The stearic acid content was significantly important in oval fruits from Agadir (6.80%), as well as oval and apiculate fruits from Essaouira (6.69 and 6.53% respectively). The lower amount was found in spherical fruits (5.49%) from Essaouira. Our results revealed a different tendency to those found in Gharby et al. **(Gharby et al., 2013)** a study, in which oleic acid concentration was reported to be significantly higher in Argan oil from Agadir fusiform fruits, linoleic acid was found to be lower in fusiform fruits (29.40%), palmitic acid content ranged from 11.75 % to 13.9 % in spherical and fusiform fruits respectively, and the stearic acid lower amount was found in fusiform fruits (5.7%).

The sterol fraction of Argan oil is mostly made up of spinasterol and schottenol, as is widely known (about 88 percent of the sterol fraction) **(Khallouki et al., 2003; Charrouf et al., 2008),** as well as stigmasta-8,22-diene-3-$\beta$-ol to a lower extent **(Khallouki et al., 2003; Matthäus et al., 2010)**. In our study, the total sterols level was remarkably high in oval fruit from Essaouira (178.64 mg/100g) and the lower amount (163.03 mg/100g) in spherical fruits from Agadir (Table 3). The schottenol and spinasterol were the major sterols found in Argan oils from Essaouira and Agadir. Schottenol was found to be high in apiculate shapes of Essaouira (48.97%) and the lower concentration in apiculate, oval and, fusiform shapes of Agadir (44.17, 44.53, and 44.77%, respectively) and oval shapes of Essaouira (44.82%). The spinasterols level was high considering oval fruits from Essaouira (43.85%) and lower in spherical fruits (34.40%) from Agadir. In contrast, minor components such as stigmasta-8,22-diene-3$\beta$-ol was found in higher amounts because of apiculate and oval fruits (5.43 and 5.13%, respectively) Agadir, and lower in apiculate oval and fusiform fruits from Essaouira, and fusiform from Agadir. Also, spherical fruits from Essaouira and Agadir were richer in Δ-7-Avenasterol in comparison to other shapes (6.76 and 6.72% respectively). While similar results were reported by Gharby et al., study regarding spinasterol (lower content for spherical fruits) **(El Moudden et al., 2020)**, different results were found for total sterols (content ranged from 178 mg/100g for apiculate to 164 mg/100g for oval fruits), schottenol (46.4% for the oval to 42% fusiform) and stigmasta-8,22-diene-3$\beta$-ol (content ranged from 4.7% for fusiform to 3.7% for oval fruits). However, Δ -7-Avenasterol was not reported.

**Table 3** Sterols composition of Argan oils.

| Region | Shape | Schottenol (%) | Spinasterol (%) | Stigmasta-8.22-diene-3β-ol (%) | Δ-7-Avenasterol (%) | Total Sterols (mg/100g) |
|---|---|---|---|---|---|---|
| Essaouira | Spherical | 46.04 ± 0.14[a] | 37.17 ± 0.07[a] | 4.93 ± 0.03[a] | 6.73 ± 0.03[a] | 169.53 ± 0.43[a] |
| | Oval | 44.82 ± 0.12[b] | 43.85 ± 0.05[b] | 3.85 ± 0.05[be] | 3.99 ± 0.02[b] | 178.64 ± 0.39[b] |
| | Apiculate | 48.97 ± 0.17[c] | 38.24 ± 0.04[c] | 3.59 ± 0.09[b] | 4.58 ± 0.02[c] | 166.35 ± 0.32[c] |
| | Fusiform | 47.78 ± 0.18[d] | 40.84 ± 0.04[d] | 3.74 ± 0.04[be] | 4.47 ± 0.02[c] | 171.07 ± 0.26[ae] |
| Agadir | Spherical | 48.58 ± 0.18[d] | 34.40 ± 0.04[e] | 4.14 ± 0.04[c] | 6.73± 0.01[a] | 163.03 ± 0.28[d] |
| | Oval | 44.53 ± 0.15[b] | 37.25 ± 0.05[a] | 5.13 ± 0.03[a] | 6.04 ± 0.01[d] | 171.97 ± 0.38[e] |
| | Apiculate | 44.17 ± 0.17[b] | 41.95 ± 0.05[f] | 5.43 ± 0.03[d] | 4.82 ± 0.04[e] | 174.33 ± 0.4[f] |
| | Fusiform | 44.77 ± 0.07[b] | 42.35 ± 0.05[f] | 3.94 ± 0.04[ce] | 5.63 ± 0.045[f] | 175.54 ± 0.08[f] |
| | Standard **(AOCS 1998)** | 44 - 49 | 34 - 44 | 3.2 - 5.7 | 4.0 - 7.0 | |

The data are presented in the form of the average of two individual repetitions (n = 2e ± SEM), the means followed by similar letters exposing in the same column are not different (P < 0.05).

Tocopherols have a high antioxidant capacity as well as a strong anti-free radical ability. The variability of the contents of tocopherols is presented in Table 4. Significant higher total tocopherol content was reported in apiculate fruits from Agadir (768.93 mg $kg^{-1}$) and a lower content in fusiform fruits from Essaouira (630.91 mg $kg^{-1}$). The γ-tocopherol level was much higher in apiculate fruits from Agadir (668.49 mg $kg^{-1}$) and lower in fusiform fruit from Essaouira (544.19 mg $kg^{-1}$). Conversely, the δ-tocopherol level was higher in apiculate fruits from Essaouira and Agadir (50.96 and 51.32 mg $kg^{-1}$ respectively) and lower in spherical fruits from Essaouira (39.40 mg $kg^{-1}$) and fusiform fruits from Agadir (39.31 mg $kg^{-1}$). Concerning $\alpha$-tocopherol, a higher amount was found in oval fruits from Agadir (51.54 mg $kg^{-1}$) and a lower amount in fusiform and oval fruits from Essaouira (39.80 and 39.79 mg.$kg^{-1}$, respectively). In the study of Gharby et al. **(Gharby et al., 2013)** tocopherols are mainly constituted by $\gamma$-tocopherol (between 678 mg $kg^{-1}$ for oval and 531 mg $kg^{-1}$ for spherical fruits) followed by $\alpha$-tocopherol (from 54.7 mg $kg^{-1}$ for apiculate to 39.9 mg $kg^{-1}$ for spherical fruits).

**Table 4** Tocopherols content (mg $kg^{-1}$) of Argan oils.

| Region | Shape | $\alpha$-Tocopherol | $\gamma$-Tocopherol | δ-Tocopherol | Total Tocopherol |
|---|---|---|---|---|---|
| Essaouira | Spherical | 48.83 ± 0.03[a] | 585.54 ± 0.04[a] | 39.40 ± 0.1[ae] | 711.42±0.07[a] |
| | Oval | 39.79 ± 0.09[b] | 618.53 ± 0.03[b] | 40.31 ± 0.08[a] | 698.63±0.05[b] |
| | Apiculate | 46.12 ± 0.02[c] | 589.21 ± 0.05[c] | 50.46 ± 0.44[b] | 686.29±0.09[c] |
| | Fusiform | 39.8 ± 0.05[b] | 544.19 ± 0.09[d] | 46.92 ± 0.07[c] | 630.91±0.08[d] |
| Agadir | Spherical | 41.39 ± 0.09[d] | 658.48 ± 0.08[e] | 49.15 ± 0.05[d] | 749.02±0.1[e] |
| | Oval | 51.54 ± 0.04[e] | 558.65 ± 0.05[f] | 48.97 ± 0.07[d] | 659.16±0.06[f] |
| | Apiculate | 49.12 ± 0.02[f] | 668.49 ± 0.09[g] | 51.32 ± 0.05[b] | 768.93±0.05[g] |
| | Fusiform | 49.48 ± 0.08[g] | 636.08 ± 0.08[h] | 39.31 ± 0.06[e] | 724.87±0.07[h] |
| | Standard **(Matthäus et al., 2010)** | 14.4 - 58.5 | 486 - 828 | 37.2 - 115.2 | 600 - 900 |

The data are presented in the form of the average of two individual repetitions (n = 2e ± SEM), the means followed by similar letters exposing in the same column are not different (P < 0.05).

**Pigments and polyphenols content**

A trace of chlorophyll and carotenoid pigments were found in our samples (Table 5). Chlorophyll content significantly ranged between 0.43 mg $kg^{-1}$ for fusiform fruits from Essaouira and 1.16 mg.$kg^{-1}$ for apiculate fruits from Agadir. These low levels are desired to avoid the pro-oxidative action of the chlorophyll pigments and thus to ensure better oil preservation **(Kiritsakis et al., 1988)**. It's also used to detect possible Argan oil adulteration **(Ourrach et al., 2012)**. Carotenoid content ranged from 0.25 mg $kg^{-1}$ for fusiform fruits from Essaouira to 0.63 mg $kg^{-1}$ for apiculate fruits from Agadir.

Several studies have reported the antioxidative effect and antiproliferative activity of Argan oil polyphenols **(Cherki et al., 2005; Drissi et al., 2006; Bennani et al., 2007; Charrouf et Guillaume 2007)**. The total polyphenols content in the studied samples ranged from 21.54 (mg GAE/g oil extract) for fusiform fruits from Essaouira to 23.44 (mg GAE/g oil extract) for apiculate fruits from Agadir. Data are summarized in Table 5. In the literature, the total polyphenol content ranged between 6.07 and 152.04 mg GAE/kg oil **(Marfil et al., 2011)**.

**Table 5** Pigments and polyphenols content.

| Region | Shape | Chlorophylls (mg $kg^{-1}$) | Carotenoids (mg $kg^{-1}$) | Polyphenols (mg GAE/g oil extract) |
|---|---|---|---|---|
| Essaouira | Spherical | 0.86 ± 0.06[ad] | 0.44 ± 0.04[ad] | 22.29 ± 0.09[a] |
| | Oval | 0.86 ± 0.03[bd] | 0.34 ± 0.03[ab] | 22.16 ± 0.06[a] |
| | Apiculate | 0.67 ± 0.02[c] | 0.29 ± 0.04[ab] | 21.70 ± 0.04[b] |
| | Fusiform | 0.43 ± 0.03[e] | 0.25 ± 0.02[b] | 21.54 ± 0.04[b] |
| Agadir | Spherical | 1.03 ± 0.03[df] | 0.38 ± 0.02[abd] | 22.83 ± 0.03[c] |
| | Oval | 1.02 ± 0.02[df] | 0.37 ± 0.01[abd] | 21.64 ± 0.04[b] |
| | Apiculate | 1.16 ± 0.03[f] | 0.63 ± 0.03[cd] | 23.44 ± 0.04[d] |
| | Fusiform | 0.87 ± 0.02[d] | 0.54 ± 0.04[cd] | 22.33 ± 0.03[a] |

The data are presented in the form of the average of two individual repetitions (n = 2e ± SEM), the means followed by similar letters exposing in the same column are not different (P < 0.05).

**Quality parameters and antioxidant activity**

The results in Table 6 reveals that the FFA content and UV-specific extinction coefficient are slightly different (K270) between oils obtained from all studied samples. Our results are similar to those obtained by SNIMA **(Moroccan Standard 2003)** showing that oils FFA content never exceeded the limit of 0.8 %. The higher value of the iodine index was found in spherical fruits from Essaouira and Agadir (100.76 and 100.46 g $I_2$/100g respectively) and the lower value was found in fusiform and apiculate fruits from Agadir (95.36 and 95.87 g$I_2$/100g respectively). However, the values in the saponification index ranged between 192 and 193 mg of KOH/g of oil for all Argan fruits int the Essaouira and Agadir Region.

Antioxidant activity evaluated using DPPH for all fruits of Essaouira and Agadir (Table 6). The $IC_{50}$ values for all oil samples are between 385.52 µg/mL for apiculate fruits from Agadir and 388.75 µg/mL for fusiform fruits from the Essaouira. The samples with a lower $IC_{50}$ value have a better antioxidant capacity. As a result, samples from Essaouira region seem to have a lower antioxidant capacity than Agadir's samples. The antioxidant properties observed are essentially linked to the presence of active compounds such as polyphenols and tocopherols **(Khallouki et al., 2003; Marfil et al., 2011)**. Tocopherol's antioxidant properties are easily incorporated into cell membranes (unlike phenolics that need a continuous supply) **(Khallouki et al., 2017)**.

**Table 6** Quality indexes and antioxidant activity of Argan oils.

| Region | Shape | FFA (%) | Iodine index (g of $I_2$/100g) | Saponification index (mg KOH/g) | $K_{270}$ | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|
| Essaouira | Spherical | $0.78 \pm 0.03^{a}$ | $100.76 \pm 0.06^{a}$ | $192.93 \pm 0.03^{abc}$ | $0.33 \pm 0.02^{a}$ | $387.34 \pm 0.04^{ad}$ |
| | Oval | $0.56 \pm 0.04^{b}$ | $98.14 \pm O.04^{b}$ | $192.76 \pm 0.06^{ab}$ | $0.21 \pm 0.01^{b}$ | $387.53 \pm 0.06^{ad}$ |
| | Apiculate | $0.51 \pm 0.03^{b}$ | $99.46 \pm 0.06^{c}$ | $192.74 \pm 0.04^{ab}$ | $0.20 \pm 0.01^{b}$ | $387.76 \pm 0.44^{ab}$ |
| | Fusiform | $0.67 \pm 0.02^{ab}$ | $98.06 \pm 0.06^{b}$ | $193.07 \pm 0.07^{acd}$ | $0.21 \pm 0.01^{b}$ | $388.75 \pm 0.05^{c}$ |
| Agadir | Spherical | $0.74 \pm 0.04^{a}$ | $100.46 \pm 0.06^{a}$ | $192.96 \pm 0.09^{abc}$ | $0.17 \pm 0.01^{bc}$ | $386.70 \pm 0.09^{d}$ |
| | Oval | $0.61 \pm 0.03^{ba}$ | $96.08 \pm 0.08^{c}$ | $192.69 \pm 0.09^{b}$ | $0.10 \pm 0.02^{c}$ | $388.51 \pm 0.07^{bc}$ |
| | Apiculate | $0.56 \pm 0.03^{b}$ | $95.87 \pm 0.07^{c}$ | $193.22 \pm 0.02^{cd}$ | $0.17 \pm 0.02^{bc}$ | $385.52 \pm 0.07^{e}$ |
| | Fusiform | $0.67 \pm 0.02^{ba}$ | $95.36 \pm 0.06^{d}$ | $193.37 \pm 0.07^{d}$ | $0.21 \pm 0.01^{b}$ | $386.90 \pm 0.05^{ad}$ |
| | Standard **(Matthäus et al., 2010)** | < 0.8 | 91.0 - 110.0 | 189.0 - 199.1 | < 0.35 | Ascorbic acid ($1.97 \pm 0.02^{i}$) |

The data are presented in the form of the average of two individual repetitions (n = 2e ± SEM), the means followed by similar letters exposing in the same column are not different (P < 0.05).

**Correlation Matrix**

Correlation coefficients of the various physicochemical quality characteristics (FFA, saponification index, K270, and iodine index) and total sterols, total tocopherols, chlorophylls, carotenoids, polyphenols, linoleic (C18:2), and antioxidant activity (DPPH) are shown in Table 7. Moreover, table 8 indicates the p-values of the correlation matrix coefficient. Next, DPPH (1/DPPH $IC_{50}$) represents the power to inhibit DPPH free radicals.

Table 7 presents the Pearson correlation, which made it possible to analyze the relationships between the different variables tested in this study. Table 8 also includes the p-values for the correlation matrix coefficients for all variables. As a result, we discovered a substantial (p-value < 0.05) positive connection between chlorophylls and carotenoids ($r^2 = 0.724$), as well as a significant (p-value < 0.05) positive correlation between chlorophylls and polyphenols ($r^2 = 0.741$). There was also a significant positive correlation (p-value < 0.05) between chlorophylls and total tocopherols ($r^2 = 0.757$), and antioxidant activity DPPH ($r^2 = 0.730$). In addition, the correlation positive significant (p-value < 0.05) has been detected between carotenoids and polyphenols ($r^2 = 0.784$), total tocopherol ($r^2 = 0.787$), and antioxidant activity (DPPH) ($r^2 = 0.868$) respectively. Moreover, we observed a highly significant positive correlation (p-value < 0.0001) between polyphenols and antioxidant activity (DPPH) ($r^2 = 0.975$) and total tocopherols ($r^2 = 0.936$) respectively. In addition, we observed that the highly significant positive correlation (*p*-value < 0.0001) between total tocopherols and antioxidant activity (DPPH) ($r^2 = 0.962$). Furthermore, the highly significant positive correlation (*p*-value < 0.0001) between iodine value and polyunsaturated fatty acids linoleic (C18:2) ($r^2 = 0.001$).

Samuel previously reported similar results to ours, where the significant iodine value in fluted pumpkin seed oil, indicated that it contained considerable amounts of polyunsaturated fatty acids, bringing more nutritional value in food products **(Samuel et al. 2017)**. Moreover, in a different study, Aremu reported that the low iodine value is indicative of fewer unsaturated bonds; Eze indicated considerable iodine value is an indicator of the presence of a significant percentage of unsaturated fatty acids in the seed oil ; therefore, the amount of iodine that will be absorbed by the unsaturated acids would be more important **(Aremu et al., 2006; Eze et al. 2012)**.

**Table 7** Pearson's correlation matrix coefficient between different parameters used.

| Variables | Chlorophylls | Carotenoids | Polyphenols | Total Sterols | T. Tocopherols | FFA | Iodine index | Saponification index | K270 | Linoleic (C18:2) | DPPH ($1/IC_{50}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Chlorophylls** | 1 | | | | | | | | | | |
| **Carotenoids** | 0.724 | 1 | | | | | | | | | |
| **Polyphenols** | 0.741 | 0.784 | 1 | | | | | | | | |
| **Total Sterols** | 0.103 | 0.367 | 0.046 | 1 | | | | | | | |
| **T.Tocopherols** | 0.757 | 0.787 | 0.936 | -0.013 | 1 | | | | | | |
| **FFA** | 0.002 | 0.060 | 0.101 | -0.349 | 0.095 | 1 | | | | | |
| **Iodine index** | -0.255 | -0.490 | -0.066 | -0.697 | -0.010 | 0.420 | 1 | | | | |
| **Saponification** | -0.013 | 0.600 | 0.428 | 0.292 | 0.381 | 0.261 | -0.404 | 1 | | | |
| **K270** | -0.333 | 0.021 | 0.017 | -0.016 | 0.077 | 0.481 | 0.545 | 0.224 | 1 | | |
| **Linoleic(C18:2)** | -0.301 | -0.318 | 0.118 | -0.636 | 0.145 | 0.486 | 0.928 | -0.048 | 0.652 | 1 | |
| **DPPH ($1/IC_{50}$)** | 0.730 | 0.868 | 0.975 | 0.156 | 0.962 | 0.080 | -0.169 | 0.530 | 0.064 | 0.037 | 1 |

**Table 8** *p*-values of the correlation matrix coefficient.

| Variables | Chlorophylls | Carotenoids | Polyphenols | Total Sterols | T. Tocopherols | FFA | Iodine index | Saponification index | K270 | Linoleic (C18:2) | DPPH ($1/IC_{50}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Chlorophylls** | 0 | | | | | | | | | | |
| **Carotenoids** | 0.042 | 0 | | | | | | | | | |
| **Polyphenols** | 0.035 | 0.021 | 0 | | | | | | | | |
| **Total Sterols** | 0.809 | 0.371 | 0.914 | 0 | | | | | | | |
| **T. Tocopherols** | 0.030 | 0.020 | 0.001 | 0.975 | 0 | | | | | | |
| **FFA** | 0.996 | 0.888 | 0.812 | 0.396 | 0.823 | 0 | | | | | |
| **Iodine index** | 0.542 | 0.217 | 0.877 | 0.055 | 0.981 | 0.300 | 0 | | | | |
| **Saponification** | 0.975 | 0.116 | 0.290 | 0.482 | 0.351 | 0.533 | 0.321 | 0 | | | |
| **K270** | 0.420 | 0.961 | 0.969 | 0.971 | 0.856 | 0.227 | 0.163 | 0.593 | 0 | | |
| **Linoleic(C18:2)** | 0.469 | 0.443 | 0.781 | 0.090 | 0.731 | 0.222 | 0.001 | 0.910 | 0.080 | 0 | |
| **DPPH ($1/IC_{50}$)** | 0.040 | 0.005 | < 0.0001 | 0.712 | 0.000 | 0.851 | 0.689 | 0.177 | 0.880 | 0.930 | 0 |

**Principal Component Analysis (PCA)**

The composition of total sterols, total tocopherols, chlorophylls, carotenoids, polyphenols, linoleic (C18:2), and antioxidant activity (DPPH) as well as the results of total sterols, total tocopherols, chlorophylls, carotenoids, polyphenols, and linoleic (C18:2) are taken as variables. The PCA uses the F1-F2 factorial design to project them (Figure 2). The first major component (F1) represents 42.93 percent of the overall data, whereas the second major component (F2) explains 29.03 percent. Because the cumulative percentage of the two initial principal components is 71.97 percent, its linear combination is representational of the variables because it exceeds 50 %. As a result, the first two axes are suitable to represent the information as a whole. Figure 2 shows the plane formed by axes F1

and F2 giving the correlation between the variables. The F1 axis is mainly composed of the positive correlation between, total tocopherols, chlorophylls, carotenoids, polyphenols, and antioxidant activity (DPPH). Axis F2 is formed by the positive correlation between linoleic (C18:2), iodine value, FFA, and K270 (Figure 2).

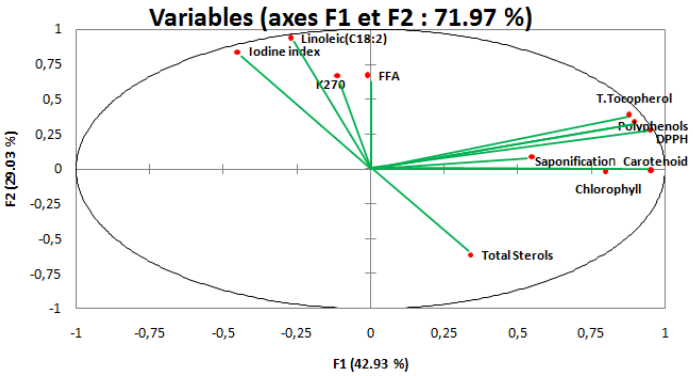



**Figure 2** PCA factorial plan carried out on the values of the different physicochemical parameters of the quality, total sterols, total tocopherols, chlorophylls, carotenoids, polyphenols, linoleic (C18:2) and antioxidant activity (DPPH) of the different samples of Argan fruits shapes oils.

Figure 3 represents that the 8 individuals are spread (of Argan oils) into 3 groups: Group I is made up of two samples fusiform and apiculate belong to the region Agadir. These samples are distinguishable by their high values of total sterols, carotenoids, chlorophylls, and total tocopherols, and a strong value of saponification index. Moreover, they had a strong antioxidant power compared to other groups. Group II is formed by the two samples spherical native from the Agadir and Essaouira. These samples are marked by high linoleic acid (C18:2) content, as well as a high FFA value, iodine index, and K270 Group III consists of 4 samples (oval Agadir, oval Essaouira, fusifrom Essaouira, and apiculate Essaouira). In these samples, the physicochemical parameters results obtained were much lower than for the other samples, also the contents of the total sterols, carotenoids, chlorophylls, and total tocopherols are lower compared to two other groups.

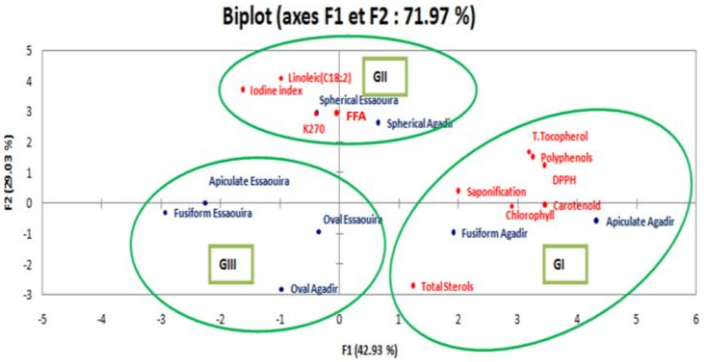



**Figure 3** Projections of individuals on the factorial plan (F1×F2). GI: Group I; GII: Group II, and GIII: Group III.

**Hierarchical clustering analysis (HCA)**

The Wards and the Squared Euclidean methods were used to classify oil samples from different Argan fruits shapes originating from two regions in Morocco (Essaouira and Agadir), in order to evaluate the similarity measure. The HCA method was used to determine the correlations for all of the 8 studied samples, using the data from the physicochemical assays, the oils composition analysis, as well as the antioxidant activity as presented in the dendrogram in Figure 4.

Based on these results of 8 samples oils were clustered into three clusters. Cluster I contains 4 samples representing for 50 % of the total samples oils characterized by a medium value means of chlorophylls 0.815 (mg $kg^{-1}$), carotenoids 0.403 (mg $kg^{-1}$), polyphenols (22.095 mg GAE/g oil extract), saponification (192.983 mg KOH/g), K270 (0.238), and linoleic acid (C18:2) (33.508%). In addition, they had a strong value means of K270 (0.238) and iodine index (98.430 g $I_2$/100g) compared to other clusters. Cluster II, formed by 2 individuals, accounted for 25 % of total samples oils, these oils had a lowest mean value of chlorophylls (0.725 mg $kg^{-1}$), carotenoids (0.310 mg $kg^{-1}$), polyphenols (21.590 mg GAE/g oil extract), total tocopherols (645.035 mg $kg^{-1}$), iodine index (97.070 $gI_2$/100g), saponification (192.915 mg KOH/g), K270 (0.155), and linoleic (C18:2) (32.02 %). Nevertheless, they had a medium mean value of total sterols (171.520 mg/100g), and FFA (0.640 %). Cluster III contained 2 samples RP, representing for 25 % of the total oils. This cluster is characterized a highest value mean of chlorophylls (1.095 mg $kg^{-1}$), carotenoid (0,505 mg $kg^{-1}$), and polyphenols (23.135 mg GAE/g oil extract). Total tocopherol (758.975 mg $kg^{-1}$), FFA (0.650%), iodine index (98.165 $gI_2$/100g), saponification (192.09 mg KOH/g), linoleic (C18:2) (33.735%) compared to group I and group II. They also characterized by a medium value means of a K270 (0.170) and iodine index (98.165 g $I_2$/100 g).

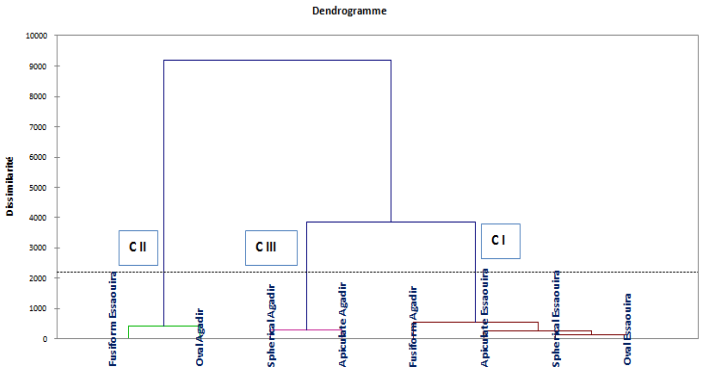



**Figure 4** Dendrogram of the samples oils studied obtained by cluster analysis (Ward and Euclidean distance). CI: Cluster I; CII: Cluster II; C III; Cluster III.

**CONCLUSION**

The present study compares the oil content; fatty acid, sterols, tocopherols and polyphenols composition, as well as the quality indexes and the antioxidant activity of different Argan oils from two Moroccan regions (Essaouira and Agadir) according to the fruit's shapes. When comparing the oils extracted from the four different Argan fruit shapes, the oval shape had the highest oil yield in Essaouira and Agadir. With regard to both regions, the differences existing between the fatty acid composition of each Argan oil extracted from the shapes of the same fruit were low in most cases. In addition, oval fruits from Essaouira showed the highest levels in total sterols. Interestingly, apiculate fruits from Agadir showed the highest amount in total tocopherols and total polyphenols, which may explain their high antioxidant power. Regarding the quality of oils, the Argan oil keeps its extra virgin label. Taken together, our results showed that each form of Argan fruit is characterized by a certain content of chemical composition in the two cities of Morocco. It is also suggested that Argan oil from Agadir can be of huge interest to the pharmaceutical, culinary and cosmetic industries.

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