

Article

Nutritional Assessment and Comparison of the Composition of Oil Extracted from Argan Nuts Collected from a Plantation and Two Natural Forest Stands of ARGAN Trees

Chaimaa Sabiri ¹, Bouchra Tazi ², Nadia Maata ³, Souad Rahim ¹, Hassan Taki ¹, Ahmed Bennamara ⁴, Lhoussaine Saad ³ and Abdelfettah Derouiche ^{1,*}

¹ Research Unit, Human Nutrition, Laboratory of Biology and Health, Faculty of Sciences Ben Msik, Hassan II University of Casablanca, Avenue Driss El Harti B.P 7955, Sidi Othmane, Casablanca 20660, Morocco

² Department of Basic Sciences, National School of Agriculture (ENAM), km 10, Route Haj Kaddour, B.P. S/40, Meknes 50000, Morocco

³ Laboratory of Analysis and Chemical Research (LOARC), 25, Rue Rahal Nichakra, Casablanca 20100, Morocco

⁴ Laboratory of Biomolecules and Organic Synthesis, Department of Chemistry, Faculty of Sciences Ben M'Sik, University Hassan II of Casablanca, Avenue Driss El Harti B.P 7955, Sidi Othmane, Casablanca 20660, Morocco

* Correspondence: derouicheaf@yahoo.com; Tel.: +212-638-083834

Abstract: Argan oil (AO), extracted from the argan tree's fruits, is principally composed of mono-unsaturated fatty acids, polyphenols, tocopherols, and sterols. This unique chemical composition is likely to be responsible for its beneficial effects. The argan tree (*Argania spinosa*) grows endemically in the southwest of Morocco. This study aimed to evaluate the chemical composition of three types of argan oil from three geographical locations: argan oil extracted from argan nuts collected from a plantation (Casablanca, AOC) and two forest stands of argan trees growing naturally in their native environment of the south-west of Morocco ((regions of Essaouira (AOE) and Taroudant (AOT)). The composition of the three oils corresponds to the known composition of argan oil in terms of fatty acids and unsaponifiable fraction. The chemical analyses revealed that the argan oil extracted from the plantations (AOC) is significantly richer in linoleic acid, linolenic acid, and tocopherols compared to the oil from the two natural stands (AOE and AOT) of argan trees. These results suggest that it is possible to facilitate an assisted migration of the argan tree outside its natural area into sites exposed to sea spray without affecting the quality of its argan oil.

Keywords: argan stand; argan plantation; argan oil; chemical composition; physicochemical parameters



Citation: Sabiri, C.; Tazi, B.; Maata, N.; Rahim, S.; Taki, H.; Bennamara, A.; Saad, L.; Derouiche, A. Nutritional Assessment and Comparison of the Composition of Oil Extracted from Argan Nuts Collected from a Plantation and Two Natural Forest Stands of ARGAN Trees. *Forests* **2023**, *14*, 180. <https://doi.org/10.3390/f14020180>

Academic Editors: Mohammed S. Lamhamedi, Damase P. Khasa and Steeve Pépin

Received: 27 November 2022

Revised: 9 January 2023

Accepted: 11 January 2023

Published: 18 January 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The forests of the argan tree, or *Argania spinosa* (L.) Skeels, a species endemic to Morocco, currently extend only to the arid and semi-arid zones of southwestern Morocco. Argan trees cover an area of 828,000 hectares, and they are the second-most important forest species in Morocco after the holm oak [1]. During the Tertiary period, when it first appeared, the argan tree is thought to have spread over a large part of Morocco [2]. In the Quaternary period, it would have been pushed back to the southwest by the glacial invasion. This probably explains the presence of two small areas of argan trees in the upper Grou valley to the southeast of Rabat and the northwestern foothills of the Beni-Snassen, near Oujda [3]. *Argania spinosa* trees adjust their physiological status and leaf attributes to environmental conditions, allowing plants to thrive under a dry climate [4]. All parts of the argan tree are usable and present great economic, medicinal, and therapeutic interests [5] thanks to extracts from its organs (fruit and leaves) [6]. Despite the economic, social, and environmental importance of this tree, little interest has been given to its installation outside its biotope in Morocco and abroad [7]. The failure of the extension, planting, and reforestation operations of the argan tree is due to major obstacles such as rainfall, humidity, cold, and different traits related to root architecture [8]. Pre-inoculation with an effective

strain of an endomycorrhizal fungus (*Glomus intraradices* Schenck & Smith) provides a clear advantage for the growth of argan tree seedlings. This technique deserves to be considered in the current practice of nurseries, as it is already practiced in some countries for other woody species [9,10].

Argan oil is known for its richness in unsaturated fatty acids and its minor compounds, polyphenols, tocopherols, sterols, etc., which give it its antioxidant properties. The quantification of these minor compounds is important and their determination is necessary, as their variation can be accompanied by nutritional health modifications. Indeed, some compounds, such as polyunsaturated fatty acids (essential fatty acids) or vitamin E (tocopherols), are responsible for the nutritional interest in argan oil. This particular biochemical profile of argan oil has often led to its being considered as a new functional food or health food [11]. Argan oil is composed of triacylglycerols, of which 80% comprises unsaturated fatty acids, which are known for their role in primary and secondary prevention of cardiovascular disease [12]. These are dominated by oleic acid at 48%, followed by linoleic acid at approximately 32%. The saturated fatty acids in argan oil are palmitic acid (approximately 13%) and stearic acids (approximately 5%). The unsaponifiable fraction of argan oil contains equal amounts (20%) of sterols and triterpenes. Schottenol and spinasterol are the two main sterols, and these molecules seem to have protective properties for the epidermis. Finally, α -tocopherol (vitamin E) plays the role of a regulator in the immune system and in inflammation [12]. In addition, the polyphenols contained in argan oil play a promoting role in the prevention of and therapy for diseases with underlying inflammatory conditions, including cancer, neurodegenerative diseases, obesity, diabetes, type II diabetes, and cardiovascular disease [13]. Research conducted on argan oil attributes nutritional, medicinal (pharmacological), and cosmetic benefits to it. Its consumption regulates the lipid profile [14], protects against cardiovascular diseases, atherosclerosis, and certain cancers [15–17], and also increases androgenic activity [18]. Argan oil has been used traditionally for many centuries. The development of processes to extract oil by mechanical cold presses from non-torrified almonds has motivated some laboratories to integrate it into cosmetic products, such as soaps, shampoos, and creams [17,19]. It is used to nourish hair, prevent hair loss, and fortify dull and brittle hair [20]. It is also used for the treatment of chapped, dry, or dehydrated skin and acne. Long-term use reduces the speed of wrinkle appearance and helps with the healing of scars [21,22]. The quality of the oil is linked to the concentration of many trace metals. Some metals are known to increase the rate of oil oxidation. The assessment of elemental concentration in vegetable oil is vital because of their elicit toxic effect on the human health if consumed in excessive quantities. The main objective of this study is to evaluate and compare the composition of argan oil from an argan tree plantation outside its natural area with those from two natural forest stands grown in southwestern Morocco.

2. Materials and Methods

2.1. Plant Material

The first argan oil originated from “Tafingoult”, a small rural commune in Taroudant Province of the Souss-Massa region (Morocco), at the following location (latitude: 30°46′0″ N; longitude: −8°22′60″ W). It is characterized by a hot and cold semi-arid climate (Köppen-Geiger climate classification) [23]. The soil of “Tafingoult” belongs to the category of silty soils of the textural class known as “sandy silt”, and the most abundant species is *Argania spinosa*, followed by *Tetraclinis articulata* and *Quercus rotundifolia*.

The second argan oil originated from “Smimou”, a town in Essaouira Province of the Marrakech-Safi region (Morocco) at the following location (latitude: 31°12′49″ N; longitude: −9°42′21″ W). It is characterized by a hot and cold semi-arid climate (Köppen-Geiger climate classification) [24]. The soil of “Smimou” belongs to the category of silty soils of textural class known as “silt”, and the most abundant species is *Argania spinosa*, followed by *Tetraclinis articulata*.

The planted argan trees in Casablanca were planted in 2008. The argan plants used were grown in the “Ounagha forest nursery” in Essaouira in polyethylene bags until they were suitable for transplantation (1 year). The argan tree plantation is located in an urban area of Casablanca, in “Hay Laymoune”, and in the vicinity of the city in “Dar Bouazza” (see Figures S1–S5).

2.2. Sampling and Processing of Argan Nuts

Three types of argan oil were extracted by cold mechanical pressure from unroasted argan kernels. These are the argan oils from the fruits of the plantation in “Casablanca” (AOC) and the two other oils from forest trees in the prefectures of “Taroudant” (AOT) and “Essaouira” (AOE). The three samples were obtained from the dried fruits of the 2018 collection, when the planted trees were 8 years old, and the wholes were treated in parallel under the same conditions of pulping, crushing, and extracting [25]. The argan fruits were collected in October 2018 and dried in the traditional way by exposure to the sun, then depulped. The nuts were crushed and the almonds were directly extracted in May 2019. The extraction was performed using 446 g of kernels from each provenance (Figure 1). The obtained oils were stored after decantation in sterile, hermetically sealed, coated bottles to avoid the impact of external factors. The first analyses were carried out during the week preceding the extraction. All of the results were obtained 3 weeks after the extraction of the samples. All of the analyses were carried out at the Official Laboratory of Chemical Analysis and Research of Casablanca, “LOARC”, and the National School of Agriculture Meknes (ENAM).

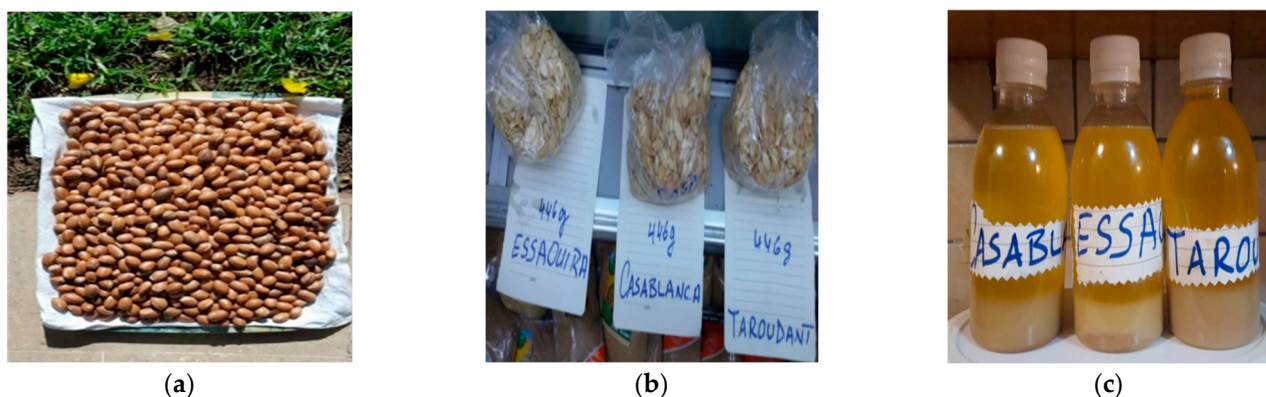


Figure 1. Sampling and extraction of argan oil from the plantation and two natural forest stands of argan trees. (a) Argan nuts from a plantation in Casablanca. (b) Composite samples of argan kernels collected from the three sampling sites (Essaouira, Casablanca, and Taroudant). (c) Argan oil extracted by cold mechanical pressure.

2.3. Physico-Chemical Parameters

The physicochemical parameters used for classifying the three argan oils (Casablanca, Taroudant, and Essaouira) were the acidity, the peroxide value, and the spectrophotometric characteristics.

2.3.1. Acidity

Acidity is an indicator used to determine the content of free fatty acids resulting from the hydrolysis of triglycerides. It is expressed as a percentage of oleic acid and detected by ISO 660 [26]. In this experiment, 10 g of argan oil was added to a mixture of solvents of ethanol (50 mL) and diethyl ether (*v:v*) previously neutralized by potassium hydroxide (KOH 0.1 N) in the presence of phenolphthalein. The fatty acids were titrated with a potassium solution (0.1 N) until the appearance of pink coloration. The volume of the added potassium solution was noted, and then the acidity was expressed as a percentage of free oleic acid according to the following formula:

$$\text{Acidity \%} = (N \times V \times M) / (\text{Wt of sample (g)} \times 10) \quad (1)$$

where:

N: normality of standard KOH solution used for titration (0.1 mole/liter);

V: volume (mL) of standard KOH solution used for titration;

M: molecular weight of oleic acid (282 g/mole);

Wt: weight (g) of the sample.

2.3.2. Peroxide Value

The peroxide value of each type of oil was determined according to the protocol ISO 3960 [27]. A 5 g sample of argan oil was solubilized in a mixture of acetic acid (30 mL) and isooctane (20 mL), and 1 mL of saturated potassium iodide (KI) solution was then added to the mixture. After a one-minute reaction, 100 mL of distilled water was added with 1 mL of starch solution. The obtained mixture was then titrated with a 0.01% sodium thiosulfate solution until a black color was obtained. The added volume was noted, and the peroxide value was calculated using the formula:

$$\text{Peroxide Value in meq O}_2/\text{kg} = (V \times 1000 \times N) / (\text{Wt of sample (g)}) \quad (2)$$

where:

V: volume (mL) of sodium thiosulfate used for the determination;

N: normality of sodium thiosulfate solution ($\text{Na}_2\text{S}_2\text{O}_3$);

Wt: weight (g) of the sample.

2.3.3. Spectrophotometric Characteristics

An analytical method was used to determine abnormal oxidized compounds in virgin argan oil, according to the International Oleic Council standard [28]. A 0.25 g sample was adjusted to 25 mL with cyclohexane, and after homogenization, the absorbance was measured at 232 nm, 266 nm, 270 nm, and 274 nm using double beam UV-Vis Spectrophotometer (6850 UV/Vis. Spectrophotometer-JENWAY). The variation of the specific extinction (Δk) was calculated using the formula:

$$\Delta K = \Delta K = K_{270 \text{ nm}} - [(K_{274 \text{ nm}} + K_{266 \text{ nm}}) / 2] \quad (3)$$

where:

K_{270 nm}: absorbance at 270 nm.

K_{274 + K₂₆₆ nm}: absorbance at 270 nm plus or minus 4 nm (K₂₇₄ + K₂₆₆).

2.4. Chemical Composition

2.4.1. Fatty Acids

A test sample containing between 0.3 and 0.4 g of argan oil was introduced into a glass screw tube with 4 mL of methanolic potassium hydroxide solution. After stirring to esterify the mixture, extraction of the methyl esters was carried out by adding 4 mL of the isooctane solution. In order to increase the density of water and to separate the two phases (water and oil), 1 g of sodium bisulfate monohydrate was added to the mixture, which was agitated at the vortex and left to rest for 4 min. A 0.5 mL intake of the mixture was then diluted by hexane (up to 10 times) in a vial. The vial was finally placed in the in the autosampler rack of gas chromatography (GC) for the injection of the solution [29]. The analysis of fatty acid methyl esters was processed by gas chromatography (GC; HP 6890, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector and a capillary column (Carbowax 20M, 30 m × 0.32 mm, 0.25 μm thickness, Agilent Technologies, Santa Clara, CA, USA). The nitrogen flow rate was 2.5 mL/min. The temperature was programmed

from 140 to 240 °C at 10 °C/min. The injected quantity was 1 µL. Fatty acids were identified by comparing their retention times to those of the standards.

2.4.2. Sterol Composition

The fat, with the addition of α -cholestanol (250 µL) as internal standard, was saponified with potassium hydroxide in ethanolic solution (25 mL). Then, the unsaponifiable matter was extracted with diethyl ether (200 mL). The sterol fraction was separated from the unsaponifiable extract by silica gel plate chromatography (TLC) at about 2 cm; then, the sterols recovered from the silica gel were analyzed by capillary column gas chromatography (Varian 3800 instrument) equipped with a VF-1 ms column and using helium (20 to 35 cm/s); hydrogen (30 to 50 cm/s) as carrier gas. The column temperature was isothermal at 260 ± 5 °C, and the temperature of the injector and detector was 280 °C–300 °C. The injected quantity was 1 µL for each analysis [30].

2.4.3. Tocopherol Composition

For this step, 0.25 g of the sample was dissolved in 25 mL of isooctane. The separation of the different tocopherols was achieved by high performance liquid chromatography (HPLC). The tocopherol content was determined by HPLC using Shimadzu instruments equipped with a C18-Varian column (250 mm \times 4.6 mm \times 5 µm). Detection was performed using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330 nm). The tocopherols content was determined using calibration factors determined from standard solutions [31].

2.4.4. Heavy Metals

To measure the totality of metals, a total mineralization of the sample to be analyzed was recommended. For the mineralization assay, 0.3 g of oil was mixed with 7 mL of nitric acid (HNO₃) and 1 mL of hydrogen peroxide (H₂O₂). The mixture was placed in the microwave for 1 h. After cooling, the content was poured into a 50 mL flask and adjusted with distilled water. Finally, the determination of metals was achieved by Atomic Absorption Spectrometry (AAS) using double beam UV-Vis Spectrophotometer (6850 UV/Vis. Spectrophotometer-JENWAY) [32]. The concentration of lead, mercury, and cadmium in the samples was calculated using the following formula:

$$\text{Pb, Cd (mg/Kg)} = \frac{(\text{Sample concentration (mg/L)} - \text{blanc concentration (mg/L)})}{(20 \times \text{weight of the sample (g)})} \quad (4)$$

$$\text{Hg (mg/Kg)} = \frac{(\text{Sample concentration (mg/L)} - \text{blanc concentration (mg/L)})}{(50 \times \text{weight of the sample (g)})} \quad (5)$$

2.5. Statistical Analyses

Statistical analyses of the different variables were performed using the analysis of variance. The hypothesis of the normality of the error terms of all the variables was checked. Mean comparisons were made using the Bonferroni test at a 5% significance level. All statistical analyses were carried out using SPSS Statistics Software (IBM SPSS Statistics 25.0) [33]. Each reported value is the mean of duplicate or triplicate samples prepared for each type of argan oil. The results of the ANOVA and post hoc Bonferroni tests were considered statistically significant if $p < 0.05$.

3. Results

3.1. Physico-Chemical Parameters

3.1.1. Acidity

The obtained results revealed that Casablanca argan oil had a high acidity level of 0.37% compared to that of the Essaouira and Taroudant oils, which had levels of 0.25% and 0.2%, respectively, without reaching the significance level (Table 1).

Table 1. Comparison of physico-chemical parameters of argan oil from a plantation (Casablanca) and two natural forest stands (Taroudant and Essaouira) of argan trees.

Samples	% Acidity ¹	Peroxide Value meqO ₂ /Kg	OD at 232 nm ¹ (K232 nm)	OD at 270 nm ¹ (K270 nm)	Variation of Specific Extinction at 270 nm (ΔK)
Casablanca	0.37 ± 0.02 ^a	0	1.37 ± 0.06 ^b	0.19 ± 0.00 ^b	0.00 ± 0.00 ^a
Taroudant	0.25 ± 0.04 ^a	0	1.70 ± 0.00 ^a	0.20 ± 0.00 ^b	0.00 ± 0.00 ^a
Essaouira	0.2 ± 0.03 ^a	0	1.50 ± 0.01 ^{a,b}	0.34 ± 0.00 ^a	0.00 ± 0.00 ^a

¹ Mean values ± standard deviation (*n* = 2). Values in the same column with different letters are significantly different at *p* < 0.05, according to the Bonferroni test.

3.1.2. Peroxide Value

The results of the analyses of the three oils (Casablanca, Essaouira, and Taroudant) (Table 1) show a definitive absence of hydroperoxides (0 meqO₂/kg).

3.1.3. Spectrophotometric Characteristics

Casablanca oil recorded ultraviolet absorbance values that were close to those of the other two oils studied (Table 1). The absorbance values obtained at 232 nm and 270 nm in the argan oils of Casablanca, Taroudant, and Essaouira were in the order of 1.37 and 0.19 (ΔK = 0.00), 1.702 and 0.198 (ΔK = 0.00), and 1.501 and 0.348 (ΔK = 0.00), respectively.

3.2. Chemical Composition

3.2.1. Fatty Acids

Analysis of the fatty acid profile results indicated that the differences in the percentages of certain fatty acids, including C-14:0, C-16:1, C-17:0, C-17:1, and C-18:0, were not statistically significant (Table 2). The comparison of the results of AOC analysis with those of AOT and AOE revealed that AOC was significantly less rich in oleic acid compared to AOT and AOE (42.66%, 47.55%, and 47.14%, respectively). In addition, AOC was significantly richer in linoleic acid than AOT and AOE (36.95%, 31.65%, and 34.28%, respectively). For linolenic acid, AOC was significantly richer, with a percentage of 0.11% compared to 0.08% for AOT and AOE. Trans fatty acids were absent in all of the studied samples (Table 2).

Table 2. Comparison of cis and trans fatty acid concentration (%) of argan oil from argan trees grown on a plantation (Casablanca: AOC) and in two natural forest stands (Taroudant: AOT and Essaouira: AOE).

Fatty Acids ¹	Casablanca %	Taroudant %	Essaouira %
Myristic acid (C-14:0)	0.13 ± 0.00 ^a	0.14% ± 0.00 ^a	0.14 ± 0.00 ^a
Palmitic acid (C-16:0)	12.97 ± 0.00 ^b	13.42 ± 0.06 ^a	12.35 ± 0.04 ^c
Palmitoleic acid (C-16:1)	0.10 ± 0.00 ^a	0.11 ± 0.00 ^a	0.12 ± 0.00 ^a
Margaric acid (C-17:0)	0.09 ± 0.00 ^a	0.08 ± 0.00 ^a	0.07 ± 0.00 ^a
Heptadecenoic acid (C-17:1)	0.02 ± 0.00 ^a	0.02% ± 0.00 ^a	0.01% ± 0.00 ^a
Stearic acid (C-18:0)	6.16 ± 0.06 ^a	6.11 ± 0.04 ^a	5.04 ± 0.01 ^b
Oleic acid (C-18:1 (w-9))	42.66 ± 0.11 ^b	47.55 ± 0.12 ^a	47.14 ± 0.00 ^a
Linoleic acid (C-18:2 (w-6))	36.95 ± 0.18 ^a	31.65 ± 0.18 ^b	34.28 ± 0.01 ^c
Linolenic acid (C-18:3 (w-3))	0.11 ± 0.00 ^a	0.08 ± 0.00 ^b	0.08 ± 0.00 ^b
Arachidic acid (C-20:0)	0.42 ± 0.00 ^a	0.40 ± 0.00 ^a	0.34 ± 0.00 ^c
Gadoleic (C-20:1)	0.40 ± 0.00 ^b	0.43 ± 0.00 ^a	0.43 ± 0.00 ^a
Trans fatty acids	0.00 ^a	0.00 ^a	0.00 ^a
Elaidic acid (C ₁₈ :1T)	0.00 ^a	0.00 ^a	0.00 ^a
Linolelaidic acid (C ₁₈ :2T)	0.00 ^a	0.00 ^a	0.00 ^a

¹ Mean values ± standard deviation (*n* = 2). Values in the same column with different letters are significantly different at *p* < 0.05, according to the Bonferroni test.

3.2.2. Sterol Composition

The sterol assay results presented in Table 3 show that the difference between the percentages of sterol derivatives, especially campesterol, spinasterol, schottenol, and others, in the three studied oils was statistically non-significant, and that AOC and AOE were significantly richer in cholesterol than AOT ($0.08 \pm 0.001\%$, 0.09 ± 0.01 and $0.2 \pm 0.05\%$, respectively). For delta-7-avenissterol, AOC was significantly richer than AOE ($4 \pm 0.08\%$, $3.55 \pm 0.04\%$). The total sterol content in AOC compared to AOT constituted almost identical percentages, with a non-significant difference (143.17 ± 6.56 and 143.33 ± 4.09 , respectively) (Table 3).

Table 3. Comparison of sterol concentration (%) of argan oil from argan trees grown on a plantation (Casablanca: AOC) and in two natural forest stands (Taroudant: AOT and Essaouira: AOE).

Sterol Composition ¹	Casablanca %	Taroudant %	Essaouira %
Cholesterol	0.08 ± 0.00^a	0.2 ± 0.05^b	0.09 ± 0.01^a
Campesterol	0.19 ± 0.02^a	0.20 ± 0.02^a	0.18 ± 0.02^a
Spinasterol	33.67 ± 0.44^a	33.79 ± 0.17^a	33.14 ± 0.49^a
Schottenol	39.97 ± 0.85^a	39.84 ± 0.65^a	40.65 ± 0.39^a
Delta-7-Avenissterol	4 ± 0.08^a	$3.86 \pm 0.09^{a,b}$	3.55 ± 0.04^b
Others	9.98^a	10.01^a	10.91^a
Total sterols (mg/100 g)	143.33 ± 6.56^b	143.17 ± 4.09^b	151.74 ± 4.09^a

¹ Mean values \pm standard deviation ($n = 2$). Values in the same column with different letters are significantly different at $p < 0.05$, according to the Bonferroni test.

3.2.3. Tocopherol Composition

The results of the tocopherol assay presented in Table 4 show that AOC was significantly richer in gamma-tocopherols, with $90.53\% \pm 0.22$ compared to AOT and AOE ($87.15 \pm 0.06\%$ and $87.88 \pm 0.06\%$, respectively). On the other hand, AOC and AOE were significantly richer in total tocopherols than AOT (758.47 ± 5.65 , 749.43 ± 0.04 and 662.80 ± 3.06) (Table 4).

Table 4. Comparison of tocopherol concentration (%) of argan oil from argan trees grown on a plantation (Casablanca: AOC) and in two natural forest stands (Taroudant: AOT and Essaouira: AOE).

Tocopherols Composition ¹	Casablanca	Taroudant	Essaouira
Alpha-tocopherols %	4.24 ± 0.05^a	7.04 ± 0.11^a	4.49 ± 0.21^a
Gamma-tocopherols %	90.53 ± 0.22^a	87.15 ± 0.06^b	87.88 ± 0.06^c
Delta-tocopherols %	5.23 ± 0.17^a	5.81 ± 0.17^a	7.64 ± 0.14^a
Total tocopherols (mg/kg)	758.47 ± 5.65^a	662.80 ± 3.06^b	749.43 ± 0.04^a

¹ Mean values \pm standard deviation ($n = 2$). Values in the same column with different letters are significantly different at $p < 0.05$, according to the Bonferroni test.

3.2.4. Heavy Metals

The results showed similar concentrations for the three oils studied. Indeed, mercury and cadmium were represented by a rate lower than 0.015 mg/kg, and lead by a concentration lower than 0.07 mg/kg.

4. Discussion

This study demonstrated that the free acidity of the analyzed samples remained below 0.8% , revealing that the three samples should be classified as extra virgin argan oil according to the Moroccan standard N.M. 08.5.090 [34]. These results were in accordance with those reported in previous studies [35,36], which estimated an acidity ranging from 0.12% to 0.64% [35].

In terms of peroxide value, the three argan oils (Casablanca, Taroudant, and Essaouira) were free of hydroperoxides (0 meqO₂/kg), and, therefore, did not contain primary oxidation products due to the presence of antioxidants contained in the fruit of the argan

tree, which enriches the oil with these substances during its extraction [21]. Thus, the analyzed argan oils (Casablanca, Taroudant, and Essaouira) complied with the Moroccan standard NM. 08.5.090, which sets a value of 15 meq O₂/kg of oil for extra virgin argan oil [37]. These results are superior to those obtained by Gharby et al. (2011), whose values ranged from 0.03 to 0.93 meq O₂/kg oil, and Azizi et al. (2022), who found values that varied between 1.07 and 3.30 meq O₂/kg. This may be due to our control of the different stages of processing of our products, from the harvesting of the fruits to the obtaining and conservation of the oil, as well as during the analysis of the different parameters.

The determination of the specific extinction of argan oil allowed us to highlight its quality and to confirm the results given by the peroxide value determination. The absorbance results of the three oils at 270 nm conformed with the Moroccan standard (NM.08.5.090), since these values were all lower than (or equal to) 0.35 in the results reported by Rahmani [37]. The specific extinction of argan oil was also determined by Gharby et al. in 2011 [35], for a cosmetic oil from Taroudant. The results revealed K270 values that generally varied between 0.02 and 0.16. The specific extinction of argan oil was also determined by Hilali [38] on samples taken from 21 trees. This study showed values of K270 that generally varied between 0.228 and 0.426.

Moroccan standard NM. 08.5.090 for argan oil has not yet set a value for extinction at 232. Regarding extra virgin olive oil, it specifies this for K232 nm values below 2.50 [39]. By extrapolation, we deduced that the absorbance of our samples at 232 nm was low. Indeed, the lowest value determined for AOC was 1.37 ± 0.06 . The maximum value was recorded for AOT (1.70 ± 0.00) and the average value for AOE (1.50 ± 0.00). The values of the specific extinction variation (ΔK) for the three studied samples remain within the limits set by the Moroccan standard (the standard recommends a ΔK that is always ≤ 0.01) [37]. According to the obtained values of absorbance in the ultraviolet, and comparing our results with the Moroccan standard, we can classify our oils in the category of “Extra virgin oil” according to the Moroccan standard N.M. 08.5.090.

As far as contaminants are concerned, the results obtained in this study show that cadmium and mercury contamination was very limited, since the levels recorded were lower than 0.015 mg/kg, which does not exceed 0.002 mg/kg [40]. All samples contained less than 0.07 mg/kg of lead, and were, therefore, in compliance with European regulations, which tolerate a value less than or equal to 0.1 mg/kg.

It was noted that the three samples analyzed showed approximately identical percentages of saturated fatty acids and some monounsaturated fatty acids, such as C16:1, C17:1, and C20:1, with the predominance of palmitic and stearic acid. These results are similar to the results reported in the study carried out by Khallouki in 2003 [41]. AOC was significantly richer in linoleic acid ($36.95\% \pm 0.18\%$) and linolenic acid ($0.11\% \pm 0.00\%$) compared to AOT and AOE, which had, respectively, $31.65\% \pm 0.18\%$ and $34.28\% \pm 0.01\%$ for linoleic acid and 0.08% for linolenic acid. A comparison of our results to those published by Khallouki reveals that the composition of AOC in linolenic acid ($36.95\% \pm 0.18\%$) is similar to that found by Khallouki (36%) [41]. The percentage of linolenic acid in AOC ($0.11\% \pm 0.002\%$) found in this study was higher than the results reported by Khallouki, who did not detect the presence of linolenic acid, and higher than the result reported by Gharby et al. in 2021, who had worked on cosmetic argan oil prepared from regurgitated nuts before and after deodorization (0.10 ± 0.01 and 0.07 ± 0.01 , respectively) [42]. The comparison of our results with those of Gharby showed that AOC was richer with linoleic acid ($36.95\% \pm 0.180\%$) compared to the levels (32.1 ± 0.15 and 31.36 ± 0.20) reported by Gharby [42]. AOC was also richer in linoleic acid than the three argan oils that were analyzed by Zarrouk (33.71 ± 0.73 , 33.38 ± 0.40 and 31.99 ± 0.31) [43]. Argan oil from a plantation in Casablanca was less rich in oleic acid than the other oils analyzed. It would have compensated for the lack of oleic acid by a higher percentage of linoleic acid. The variations observed in the fatty acid composition of argan oil could be attributed to various factors, including the geographical origin and the effect of climate, especially rainfall [44,45]. The rest of the fatty acids analyzed in AOC presented rates close to those found in argan

oils from the other two regions, Taroudant and Essaouira (AOE and AOT). After extraction, the three samples did not undergo any treatment, which explains the absence of trans fatty acids; thus, they were classified as pure oils. Indeed, the presence of trans fatty acids in argan oil suitable for consumption is an indicator of the presence of refined oil. The Moroccan standard tolerates a percentage of trans fats less than or equal to 0.05% [34]. The most dominant sterols in the three analyzed samples were schottenol (AOC: 39.97%, AOT: 39.84%, and AOE: 40.65%) and spinasterol (AOC: 33.67%, AOT: 33.79%, and AOE: 33.14%). This is in line with the studies established by Khallouki in 2003 [41] and Zarrouk in 2019 [43], which also detected a dominance of these components. AOE contains a total sterol content that exceeds that present in the other two analyzed oils, AOT and AOE.

The γ -tocopherol is the most represented vitamin in argan oil tocopherols. Its concentration was significantly higher in AOC compared to AOT and AOE ($90.53\% \pm 0.22\%$, $87.15\% \pm 0.06$ and $87.88\% \pm 0.06$, respectively). These results are in accordance with the results reported by Azizi et al. (2022), who indicated that the γ -tocopherols were the most abundant, followed by α -tocopherols and, finally, the δ -tocopherols. On the other hand, the total tocopherol content in AOC was statistically higher (758.47 ± 5.65 mg/kg of oil), compared to that of AOT (662.80 ± 3.06 mg/kg of oil). The total tocopherol content of AOC exceeded that reported by Khallouki et al., 2003 (629 mg/kg oil) [41] and Gharby et al., 2021 (75 ± 5.5 and 60 ± 3.5 mg/100 g of oil) [42].

5. Conclusions and Research Needs

The chemical and phytochemical proprieties of argan oil (*Argania spinosa* L. skeels) from three regions of Morocco (an argan plantation in the region of Casablanca and two spontaneous cultures) were analyzed and compared. It was found that the argan trees, although they were grown outside their biotope in the region of Casablanca, produced high-quality oil which was richer in linoleic and linolenic acid, as well as in certain unsaponifiable compounds such as sterols and tocopherols. These properties constitute an added value for its use in the nutritional and pharmaco-cosmetic field. The findings of this study will be useful for selecting the domestication of the argan tree as a real opportunity to mitigate of the pressure on this resource, to develop it, and to conserve it, which will have a positive impact on development both in the socio-economic and ecological fields. However, in order to better understand the effect of genotype environment-interaction on the yield and quality of argan oil, it is necessary to produce experimental designs of the same argan genotypes in different reforestation sites. The production and multiplication of the selected genotypes requires the development of clonal or varietal forestry specific to the argan tree.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f14020180/s1>, Figure S1: Photo of the three argan trees taken from the terrace in the background tramway of Casablanca; Figure S2: Argan tree N°1; Figure S3: Argan tree N°2 with tramway of Casablanca in the background in left; Figure S4: Argan tree N°3 with tramway of Casablanca in the background in right; Figure S5: Branch of argan tree planted in Casablanca with argan fruits, and the tramway of Casablanca in the background.

Author Contributions: Conceptualization, A.D.; methodology, A.D., L.S., N.M. and C.S.; software, C.S.; validation, A.D., B.T., S.R., H.T. and A.B.; formal analysis, C.S. and A.D.; resources, A.D., L.S. and N.M.; data curation, C.S.; writing—original draft preparation, C.S.; writing—review and editing, C.S. and A.D.; visualization, C.S., A.D. and B.T.; supervision, A.D. and B.T.; project administration, A.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: We would like to thank Ben Abdenbi Fatouma, Naih Aicha and Mbarak Mohamadi for their active participation in the supply of the argan oil and Derouiche Yazid, UNC Charlotte USA, for his proofreading of this document.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Kabouss, A.E.; Charrouf, Z.; Oumzil, H.; Faïd, M.; Lamnaouer, D.; Miyata, Y.Y.; Miyahara, K. Characterization of flavonoids of Argan tree leaves (*Argania spinosa* (L.) Skeels, Sapotaceae) and study of their antimicrobial activity. *Actes Inst. Agron. Vet.* **2001**, *21*, 157–162.
- Boukhobza, M.; Pichon-Prum, N. Argan tree, an economic and medicinal resource for Morocco. *Phytotherapy* **1988**, *27*, 21–26.
- Mousadik, A.E.; Petit, R.J. Chloroplast DNA phylogeography of the argan tree of Morocco. *Mol. Ecol.* **1966**, *5*, 547–555. [[CrossRef](#)] [[PubMed](#)]
- Díaz-Barradas, M.C.; Zunzunegui, M.; Ain-Lhout, F.; Jáuregui, J.; Boutaleb, S.; Álvarez-Cansino, L.; Esquivias, M.P. The specific form of extinction in ultraviolet light. *Plant Soil* **2010**, *337*, 217–231. [[CrossRef](#)]
- Charrouf, Z.; Guillaume, D. Secondary metabolites from *Argania spinosa* (L.). *Skeels. Phytochem. Rev.* **2002**, *1*, 345–354. [[CrossRef](#)]
- Pumareda, L.; Henry, F.; Charrouf, Z.; Pauly, G.; Flaconne, G. Valorization of argan leaves: Environmental impact. *BFT* **2006**, *287*, 35–44.
- Nouaïm, R. *L'arganier au Maroc: Entre Mythes et Réalités*; L'HARMATTAN: Paris, France, 2005; p. 230.
- Msanda, F.; Aboudi, A.E.; Peltier, J.P. Biodiversity and biogeography of the Moroccan argan forest. *Cah. Agric.* **2005**, *14*, 357–364.
- Nouaïm, R.; Chaussod, R. Mycorrhizal dependency of two clones of micro propagated Argan tree (*Argania spinosa*): I. Growth and biomass production. *Agrofor. Syst.* **1994**, *27*, 53–65. [[CrossRef](#)]
- Nouaïm, R.; Chaussod, R. Role of mycorrhizae in the hydrous and mineral nutrition of plants, especially woody plants in arid zones. Mycorrhization of forest plants in arid and semi-arid environments and the fight against desertification in the Mediterranean Rim. *Cah. Options Méditerran.* **1997**, *20*, 9–26.
- Kamal, R.; Kharbach, M.; Eljemli, M.; Bouklouze, A.; Cherrah, Y.; Aloui, K. In vivo anti-inflammatory activity of argan oil. *Phytothérapie* **2019**, *18*, 255–261. [[CrossRef](#)]
- Lewis, E.D.; Meydani, S.N.; Wu, D. Regulatory role of vitamin E in the immune system and inflammation. *IUBMB Life* **2019**, *71*, 487–494. [[CrossRef](#)] [[PubMed](#)]
- Yahfoufi, N.; Alsadi, N.; Jambi, M.; Matar, C. The Immunomodulatory and Anti-Inflammatory Role of Polyphenols. *Nutrients* **2018**, *10*, 1618. [[CrossRef](#)] [[PubMed](#)]
- Derouiche, A.; Cherki, M.; Drissi, A.; Bamou, Y.; Messal, M.E.; Oudghiri, A.I.; Lecerf, J.M.; Adlouni, A. Nutritional intervention study with argan oil in man: Effects on lipids and apolipoproteins. *Ann. Nutr. Metab.* **2005**, *49*, 196–201. [[CrossRef](#)] [[PubMed](#)]
- Cherki, M.; Derouiche, A.; Drissi, A.; Messal, M.E.; Bamou, Y.; Oudghiri, A.I.; Lecerf, J.M.; Adlouni, A. Consumption of argan oil may have an antiatherogenic effect by improving paraoxonase activities and antioxidant status: Intervention study in healthy man. *Nutr. Metab. Cardiovasc.* **2005**, *15*, 352–360. [[CrossRef](#)]
- Derouiche, A.; Saïle, R.; Drissi, A.; Cherki, M.; Bamou, Y.; Oudghiri, A.I.; Lecerf, J.M.; Adlouni, A. We-P14:437 Effect of tow naturals products oils, Argan and Olive, in the nutritional prevention on atherosclerosis in man. *Atheroscler. Suppl.* **2006**, *7*, 443. [[CrossRef](#)]
- Adlouni, A. L'huile d'argan, de la nutrition à la santé. *Phytotherapy* **2010**, *8*, 89–97. [[CrossRef](#)]
- Derouiche, A.; Jafri, A.; Driouch, I.; Khasmi, M.E.; Adlouni, A.; Benajiba, N.; Bamou, Y.; Sail, R.; Benouhoud, M. Effect of argan and olive oils consumption on the hormonal profile of androgens among Moroccan healthy adult men. *NPC* **2013**, *8*, 51–53. [[PubMed](#)]
- Guillaume, D.; Charrouf, Z. Argan oil. *Altern. Med. Rev.* **2001**, *16*, 275–276.
- Charrouf, Z.; Guillaume, D. Phenols and polyphenols from *Argania spinosa*. *Am. J. Food Technol.* **2007**, *2*, 679–683. [[CrossRef](#)]
- Charrouf, Z.; Guillaume, D. Huile d'argan une production devenue adulte. *Les Technol. Lab.* **2007**, *2*, 4–7.
- Boucetta, K.Q.; Charrouf, Z.; Aguenau, H.; Derouiche, A.; Bensouda, Y. The effect of dietary and/or cosmetic argan oil on postmenopausal skin elasticity. *Clin. Interv. Aging* **2015**, *10*, 339–349. [[PubMed](#)]
- DB-City: Toutes Les Infos sur Les Pays, Régions, Villes et Villages. Available online: <https://fr.db-city.com/fr.db-city.com/Maroc-Souss-Massa--Taroudannt--Tafingoult> (accessed on 2 January 2023).
- DB-City: Toutes Les Infos sur les Pays, Régions, Villes et Villages. Available online: <https://fr.db-city.com/fr.db-city.com/Maroc-Marrakech-Safi--Essaouira--Smimou> (accessed on 2 January 2023).
- Gharby, S.; Charrouf, Z. Argan Oil: Chemical Composition, Extraction Process, and Quality Control. *Front. Nutr.* **2022**, *8*, 1251. [[CrossRef](#)] [[PubMed](#)]
- Standard Method ISO 660; Animal and Vegetable Fats and Oils—Determination of Acid Value and Acidity. ISO: Geneva, France, 1996.
- ISO 3960; Corps Gras D'origines Animale et Végétale—Détermination de L'indice de Peroxyde—Détermination Avec Point D'arrêt Iodométrique. DE NORMALISATION, Organisation Internationale: Geneva, France, 2007.
- Standard Method ISO 3656: 2002; Animal and Vegetable Fats and Oils—Determination of Ultraviolet Absorbance Expressed as Specific uv Extinction. ISO: Geneva, France, 2002.
- Standard Method ISO 12966-2; Corps Gras D'origines Animale et Végétale—Chromatographie en Phase Gazeuse des Esters Méthyliques D'acides Gras—Partie 2: Préparation des Esters Méthyliques D'acides Gras. ISO: Geneva, France, 2011.
- IOC/T.20/Doc. No. 30; Determination of the Sterol Composition and Content and Alcoholic Compounds by Capillary Gas Chromatography. IOC (International Olive Council): Geneva, France, 2011.
- Standard Method ISO 9936; Animal and Vegetable Fats and Oils—Determination of Tocopherol and Tocotrienol Contents by High-Performance Liquid Chromatography. ISO: Geneva, France, 1997.

32. Méthodes Standard UICPA pour l'analyse des Huiles, Graisses et Produits Dérivés. In *Determination of Lead*, 7th ed.; 1er Supplément, 1988; p.2.632.
33. IBM Corp. *IBM SPSS Statistics for Windows*; Version 25.0; IBM Corp.: Armonk, NY, USA, 2017.
34. Moroccan Standard NM 08.5.090; Animal and Vegetable Fats and Oils—Argan Oils Specifications. SNIMA (Service de Normalisation Industrielle Marocaine): Rabat, Morocco, 2003.
35. Gharby, S.; Harhar, H.; Badr, K.; El Monfalouti, H.; Haddad, A.; Charrouf, Z. Analyse chimique et sensorielle de l'huile d'argane. *Les Technologies de Laboratoire* **2011**, *6*, 13–23.
36. Azizi, S.-E.; Dalli, M.; Mzabri, I.; Berrichi, A.; Gseyra, N. Chemical characterization of oils produced by some native and introduced genotypes of argan tree in eastern Morocco using HPLC-DAD/GC-MS, and the evaluation of their physicochemical parameters. *OCL* **2022**, *29*, 14. [[CrossRef](#)]
37. Rahmani, M. Composition chimique de l'huile d'argane «vierge». *Cah. Agric.* **2005**, *14*, 461–465.
38. Hilali, M.; Charrouf, Z.; Soulhi, A.E.A.; Hachimi, L.; Guillaume, D. Influence of origin and extraction method on argan oil physico-chemical characteristics and composition. *J. Agric. Food Chem.* **2005**, *53*, 2081–2087. [[CrossRef](#)]
39. IOC/T.15/NC No. 3/Rev. 7; Trade Standard Applying to Olive Oils and Olive Pomace Oils. IOC (International Olive Council): Geneva, France, 2013.
40. Lopera, L.; Lococo, F.; Mavrogeni, E.; Giuffrida, D.; Dugo, G. Determination of copper (II), lead (II), cadmium (II) and zinc (II) in virgin olive oils produced in Sicily and Apulia by derivative potentiometric stripping analysis. *Ital. J. Food Sci.* **2002**, *14*, 389–399.
41. Khallouki, F.; Younos, C.; Soulimani, R.; Oster, T.; Charrouf, Z.; Spiegelhalter, B.; Bartsch, H.; Owen, R.W. Consumption of argan oil (Morocco) with its unique profile of fatty acids, tocopherols, squalene, sterols and phenolic compounds should confer valuable cancer chemopreventive effects. *Eur. J. Cancer Prev.* **2003**, *12*, 67–75. [[CrossRef](#)]
42. Gharby, S.; Guillaume, D.; Elibrahimi, M.; Charrouf, Z. Physico-Chemical Properties and Sensory Analysis of Deodorized Argan Oil. *ACS Food Sci. Technol.* **2021**, *1*, 275–281. [[CrossRef](#)]
43. Zarrouk, A.; Martine, L.; Grégoire, S.; Nury, T.; Meddeb, W.; Camus, E.; Badreddine, A.; Durand, P.; Namsi, A.; Yammine, A.; et al. Profile of Fatty Acids, Tocopherols, Phytosterols and Polyphenols in Mediterranean Oils (Argan Oils, Olive Oils, Milk Thistle Seed Oils and Nigella Seed Oil) and Evaluation of their Antioxidant and Cytoprotective Activities. *Curr. Pharm. Des.* **2019**, *25*, 1791–1805. [[CrossRef](#)]
44. Fellat-Zarrouck, K. Étude de Corps Gras d'Origine Marocaine: Huile d'Olive, Adultérations Huile de Sardine, Sardina Pilchardus Huile d'Argan, Argania Spinosa. Doctoral Dissertation, Aix-Marseille 1, AV ESCAD NORMANDIE NIEMEN 13013 MARSEILLE, 1987.
45. Maurin, R.; Fellat-Zarrouck, K.; Kirs, M. Huile d'argan, Argania spinosa du Maroc, Triglycérides. In Proceedings of the Euro-Lipid Congress, Angers, France, 6–9 June 1989.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.