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Oxidative Stability of Virgin and Refined Argan [*Argania spinosa* L. (Skeels)] Oil under Accelerated Aging Conditions and Shelf-Life Prediction at Room Temperature: A Comparative Study

Rabha Aissa [1], Abderrahim Asbbane [2], Samira Oubannin [2], Laila Bijla [2], Zahra Bousaid [2], Otmane Hallouch [2], Said El Harkaoui [3], Bertrand Matthäus [3], El Hassan Sakar [4,*] and Saïd Gharby [2,*]

1 Laboratory of Engineering Sciences and Energy Management (LASIME), Superior School of Technology, Ibn Zohr University, Agadir 80000, Morocco
2 Biotechnology, Analytical Sciences, and Quality Control Team, Polydisciplinary Faculty of Taroudant, Ibn Zohr University, Agadir 80000, Morocco; hallouch.o@gmail.com (O.H.)
3 Department of Safety and Quality of Cereals, Max Rubner-Institut, Working Group for Lipid Research, Schützenberg 12, D-32756 Detmold, Germany
4 Laboratory of Biology, Ecology, and Health, FS, Abdelamlek Essaadi University, Tetouan 93002, Morocco
* Correspondence: e.sakar@uae.ac.ma or sakar.statistics@gmail.com (E.H.S.); s.gharby@yahoo.fr (S.G.)

Abstract: Argan kernels, fruits regurgitated by goats, are 30% cheaper than the regular kernels used to prepare food argan oil. The use of such argan kernels as a cosmetic ingredient, after refining, is thus economically attractive. The oxidative stability of argan oil prepared from sub-quality kernels is not known. In the present paper, the physicochemical quality, oxidative stability indices, and bioactive compounds of refined argan oil (RAO) obtained from sub-quality kernels and virgin argan oil (VAO) were compared and evaluated over a period of storage of 12 weeks at 60 °C. Quality parameters consisted of free fatty acids (FFAs), peroxide value (PV), *p*-anisidine value (p-AV), UV extinction coefficients (K232 and K270), total oxidation, iodine and saponification values, induction time, fatty acid composition, and tocopherol content. Our outcomes reveal that the combined effects of refining and storage generally resulted in high values of the routinely measured quality indices, including FFA, p-AV, K270, K232, and PV. Likewise, refining reduced the levels of individual tocopherols and unsaturated fatty acids (USFAs) but increased saturated fatty acids (SFAs). Similar trends were observed under storage with decreased levels of tocopherols and high SFA for both RAO and VAO. Storage also resulted in an increased level of USFAs in the case of RAO but not in VAO. The obtained results show that RAO was more sensitive to oxidation than VAO. At room temperature, RAO had a shorter induction time of six months, implying that RAO will have a shorter shelf life compared to VAR. Thus, such instability means that the refining process for argan oil must be carried out with great care, and this oil needs to be protected once refined.

Keywords: fatty acids; oxidative stability; Rancimat; refining; virgin argan oil

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1. Introduction

Virgin argan oil (VAO) is one of the newest international vegetable oils. VAO is extracted from the fruits of the argan tree (*Argania spinosa* (L.) Skeels) [1]. This tree is mainly grown in Morocco for its socioeconomic and environmental importance. One of the greatest economic interests is the production of VAO. It is obtained solely by mechanical cold extraction. There are two types of VAO on the market: edible and cosmetic oil [2]. Cosmetic oil is prepared from unroasted kernels, while food argan oil is prepared from roasted kernels. Edible argan oil has a very pale gold color and a slightly bitter taste [1]. VAO only undergoes mechanical extraction, decantation, as well as filtration. These can be performed without a refining process, which results in oil highly appreciated for its high

nutritional value, health benefits, and unique organoleptic properties. Indeed, VAO is rich in bioactive molecules such as sterols, squalene, tocopherols, polyphenols, carotenoids, simple triterpenes, CoQ10, and melatonin, as well as volatile compounds [3]. The concomitant presence of such compounds (unsaponifiable matter) is behind its cardioprotective, nutritional, pharmacological, and antioxidant properties [4]. Indeed, tocopherols and polyphenols are known to have a powerful antioxidant activity [5]. VAO is rich in oleic acid (43–49 g/100 g), a monounsaturated fatty acid from the omega-9 family [6,7]. VAO also has a meaningful amount of linoleic acid (29–37 g/100 g), a polyunsaturated fatty acid belonging to the omega-6 family. The high level of unsaturated fatty acid content in argan oil is linked with various pharmacological properties. Like VAO, refined argan oil (RAO) is obtained by cold pressing for the preparation of an argan oil with certified quality. Once extracted, this oil undergoes a physical refining process. RAO is widely used as a preservative in the cosmetics industry [2]. This requires odorless, limpid, neutral in taste, and colorless oils. The main objectives of refining are to obtain a good quality and an acceptable aspect such as lighter odor and color. However, the drawback is that most of the refining processes also remove the substances contributing to argan oil's biological properties. This directly impacts the safety and the stability of refined oils. Moreover, generally, the physical refining is a two-stage process. The first one is bleaching, which is one of the most important steps in the refining process and it is a complex physico-chemical process [8]. The objective of bleaching is to reduce the level of colored pigments (chlorophylls and carotenoids). It also further removes residue traces of phosphatide, soap, phospholipid contaminants, lipid peroxidation products, and other bioactive molecules like phytosterols [8,9] and tocopherols [10]. In fact, several studies confirm a significant decrease in phytosterol content during the bleaching process, notably in olive oil [11], corn oil [9,12], sunflower oil, soybean oil, and rapeseed oil [13]. Total phytosterol content reduction is likely due to the hydrolysis of sterol esters during the bleaching step [9]. A similar trend was outlined for tocopherol in refined oils from the highly oleic safflower oil [14], olive oil [11], soybean, and rapeseed oils [10].

Secondly, deodorization is the final step through which volatile components, carotenoids, free fatty acids, as well as contaminants (pesticides, light polycyclic aromatic hydrocarbons) are removed [8,11,15,16]. Deodorization also removes residues of mineral oil saturated hydrocarbons (MOSHs) and mineral oil aromatic hydrocarbons (MOAHs) [17]. On the other hand, deodorization also has negative effects. Among them, important bioactive molecules such as squalene, sterols, polyphenols, and tocopherols may be removed in this step [17]. Likewise is the destruction of some essential nutrients and unwanted side reactions like cis-trans-isomerization [8].

As far as we know, no information is available on the effect of the physical refining process on the chemical composition, oxidative stability, and shelf-life parameters of argan oil, hence the originality of the present research. This aimed at investigating the effects of physical refining (namely, bleaching and deodorization) on the physicochemical properties, oxidation stability measured at 60 °C, and shelf life of VAO and RAO measured at room temperature.

2. Materials and Methods

2.1. Materials and Experimental Design

The RAO samples (extracted from sub-quality argan kernels, regurgitated by goats) investigated in this study were provided by Arganisme SARL (Essaouira, Morocco) and the VAO samples were acquired from the Tighanimine cooperative. In fact, while RAO and VAO came from two providers, they were obtained by the same process of extraction, and the argan kernels came from the same geographic area. For each batch, some argan oil samples were directly analyzed. Then, RAO and VAO were distributed in bottles of 30 mL and placed in a Memmert UF110 plus oven (Memmert GmbH, Schwabach, Germany) equipped with a Kimo KTT310-RF thermostat with a constant temperature (60 ± 1 °C). Basic quality indices (free fatty acids, peroxide value, *p*-anisidine value, and specific UV

extinction coefficients (K232 and K270)) were measured every 2 weeks for 12 weeks. Fatty acid composition was determined after 4, 8, and 12 weeks and the tocopherol content was evaluated twice, at the beginning as well as at the end of the experiment.

2.2. Standards and Reagents

The tocopherols were identified using α-, δ-, and γ-tocopherol reference standards (chromatographic purity 97.6–99.6%, Merck KGaA, Darmstadt, Germany) and quantified through external calibration with standard solutions. The remaining reagents (cyclohexane, isooctane, isopropanol and heptane) were of analytical grade, acquired from a professional lab (Casablanca, Morocco).

2.3. Analytical Methods

2.3.1. Quality Indices

Basic quality indices, namely, free fatty acid (FFA) content, peroxide (PV), *p*-anisidine value (p-AV), and UV specific extinction coefficients (K232 and K270) were determined according to the official analytical methods [18–21]. FFA and PV are expressed as % oleic acid and milli-equivalents of active molecular oxygen per kilogram of oil (mEq O_2/kg), respectively. K232 and K270 are expressed as the specific extinctions of a 1% (*w*/*v*) solution of cyclohexane–argan oil measured in a 1 cm cuvette, using a SCILOGEX SP- UV1100 spectrometer (Scilogex, Rocky Hill, CT 06067, USA). To evaluate p-AV, a solution of oil in iso-octane was reacted with *p*-anisidine standard in glacial acetic acid, producing yellowish reaction products. The mixture absorbance was then read at 350 nm in a cuvette of 1 cm, using a SCILOGEX SP- UV1100 spectrometer (Scilogex, Rocky Hill, CT 06067, USA). The *p*-anisidine value was calculated using the following formula:

$$\text{p-AV} = \frac{25 \times (1.2 \times Abs_2 - Abs_1)}{m}$$

where, p-AV = *p*-anisidine value; Abs_1 = absorbance of the lipid solution after reaction with isooctane solution; Abs_2 = absorbance of the lipid solution after reaction with *p*-anisidine solution; *m* = mass of the sample (g).

2.3.2. Fatty Acid Composition Determination and Iodine Value

Fatty acid (FA) profiling was evaluated following the official analytical method ISO 12966-2:2017. FAs were then converted into their corresponding methyl esters and injected into a gas chromatograph (Agilent 6890; Santa Clara, CA 95051, USA) on a CPWAX 52CB column (30 m × 0.25 mm i.d., 0.25 µm film thickness) coupled to a flame ionization detector (GC-FID). Helium (He) was used as a carrier gas (flow rate was 1 mL/min), and through a flame ionization detector (FID) 1 µL of each sample was injected at 220 °C (split ratio of 1/50). Oven temperature was programmed at 180 °C for 5 min and was raised to 220 °C with a slope of 15 °C /min. Detection was performed at 230 °C. Results were presented as the relative percentage of each individual fatty acid peak area [22]. The iodine value (*IV*) was calculated based on the percentage of unsaturated fatty acids, as outlined in the following equation [23]:

$$IV = (\%C16:1 \times 1.001) + (\%C18:1 \times 0.899) + (\%C18:2 \times 1.814) + (\%C18:3 \times 2.737)$$

2.3.3. Saponification Value

The determined saponification value (*SV*) is derived from the composition of fatty acids using the equation below [24]. To begin with, the fractional molecular weight of each fatty acid in the sample needs to be ascertained by multiplying the percentage of the fatty acid (divided by 100) by its molecular weight. The mean molecular weight is then calculated as the sum of the fractional weights of all fatty acids present in the sample.

$$SV \text{ (mg KOH per g of oil)} = 3 \times n \times 56 \times 1000$$

where,

$$n = 1/MT_G \text{ and } M_{TG} = (mean\ molecular\ weight \times 3) + 92.09 - (3 \times 18).$$

2.3.4. Tocopherol Content Determination

Briefly, a solution of 250 mg of oil dissolved in 25 mL of n-heptane was prepared. Then, 20 µL was injected into a High-Performance Liquid Chromatograph according to the official analytical method ISO 9936:2016 using Shimadzu equipment with a C18-Varian column (5 µm particle size, L × I.D. 25 cm × 4 mm; Varian Inc., Middelburg, The Netherlands). A fluorescence detector (Excitation wavelength was 290 nm, detection wavelength was 330 nm) was involved. Eluent was a 99:1 isooctane/isopropanol (*v*/*v*) mixture (flow rate of 1.2 mL/min). Identification of different tocopherols was made based on retention time and quantification via external standards. Results were presented as mg (of tocopherols) per kg of oil.

2.3.5. Rancimat Test

Oxidative stability (OS) was evaluated following the ISO official analytical method (ISO 6886:2016) [25]. In brief, OS was expressed as oxidative induction time (in hours) measured through the Rancimat test (Metrohm, Herisau, Switzerland, Figure 1). To determine each sample's shelf life, 3 g of pressed argan oil was introduced into the reaction vessel, which in turn was placed into the heating block at different temperatures (373, 383, 393, and 403 °K) with an airflow of 20 L/h. Volatile compounds released during the degradation process were collected in a receiving flask filled with 60 mL of distilled water. The conductivity of this solution was measured and recorded. The resulting curves were evaluated automatically by the Rancimat software. The shelf life of both kinds of argan oil was determined by the extrapolation method at an ambient storage temperature of 298 °K (25 °C). With regard to OS, induction times were determined at 393 °K (120 °C).

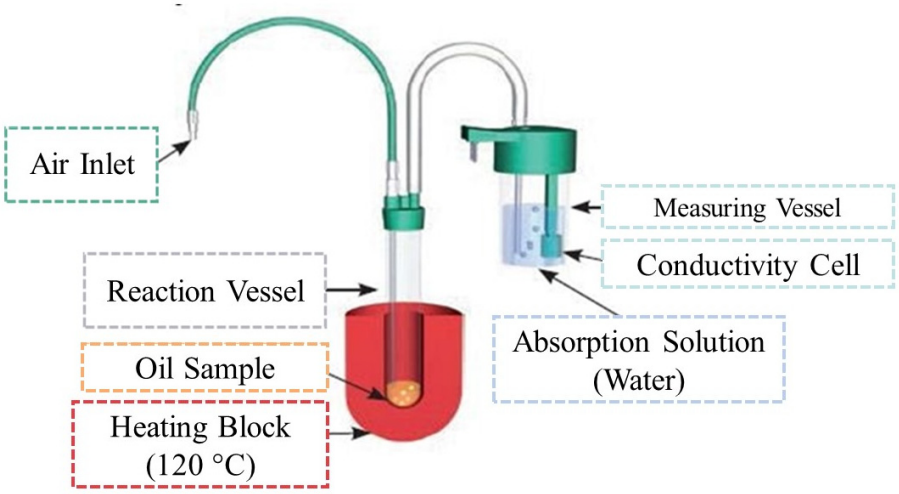

Figure 1. Principle of the Rancimat method.

2.4. Statistical Analysis

The results are reported as mean values ± SD (n = 3, repeatability). The significance of the differences among mean values was determined by Tukey's test at the 0.05 significance level.

3. Results and Discussion

3.1. Free Fatty Acid Content

Free fatty acids are an estimation of the fatty acids separated from the triacylglycerol molecules [26]. The percentage of free fatty acids is related to the predominant fatty acid for

each oil, which in this case was oleic acid. The initial FFA content of VAO was calculated to be 0.76 g oleic acid/100 g oil (Figure 2). During 12 weeks of storage at 60 °C, a gradual and almost linear increase in FFA content of VAO was noticed, indicating hydrolysis of triacylglycerols. The hydrolysis continued to increase steadily and the FFA content of VAO finally reached 1.91 g oleic acid/100 g oil after 12 weeks at 60 °C. While RAO had a higher initial FFA content than VAO (0.95 g oleic acid/100 g oil), the FFA content increased at an accelerated rate until the 8th week, when the increase was slowed down to reach a maximum value of 1.72 g oleic acid/100 g oil. This small difference between the FFA content of VAO and RAO after heat treatment may be due to the refining process, which removes a significant amount of the FFA by neutralization and deodorization [27], and consequently an extension of the conservation properties of argan oil.

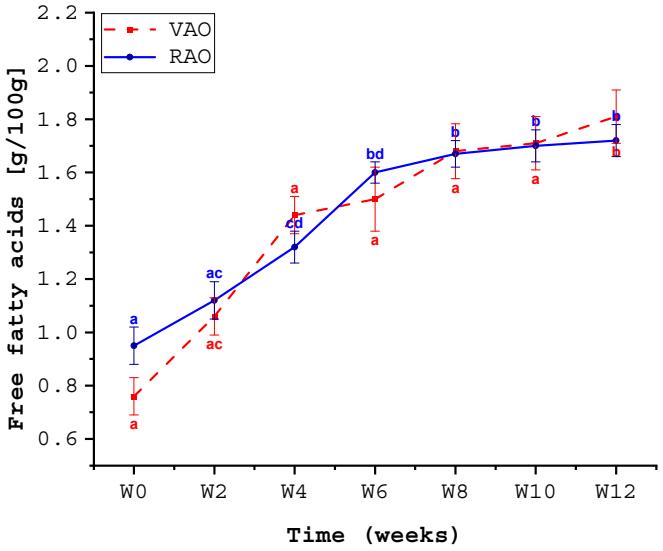

Figure 2. Free fatty acid content of argan oils (VAO and RAO). RAO: Refined argan oil and VAO: Virgin argan oil. Values are mean ± SD (n = 3).

3.2. Primary Oxidation

The content of primary oxidation products in both types of argan oil was investigated by examining the peroxide value (PV) and the specific coefficient extinction at λ232 [28].

PV is a qualitative test, useful for a satisfactory sensitivity to the early stages of oxidative deterioration. Peroxides are the intermediate oxidation products of vegetable oils that lead to the formation of a complex mixture of volatile compounds such as aldehydes, ketones, and hydrocarbons responsible for the deterioration of oil organoleptic properties. VAO and RAO had initial PV values of 2.95 mEq O_2/kg oil and 3.37 mEq O_2/kg oil, respectively (Figure 3). Hydroperoxide formation in VAO reached a maximum value of 76.59 mEq O_2/kg after 8 weeks (Figure 3). After this period, the decomposition of peroxides into secondary oxidation products probably took over the peroxide formation process, resulting in a decrease in the PV (21.87 mEq O_2/kg). The highest PV in the RAO was recorded after 6 weeks of storage (44.83 mEq O_2/kg, Figure 3). This reduction in RAO was observed to be much stronger than that of VAO. However, the observed decrease in the PV of RAO can be interpreted as a removal of substances inhibiting the decomposition of hydroperoxides, such as tocopherols, carotenoids, and polyphenols. Moreover, even though hydroperoxide formation was slower in RAO, both oils showed a similar peroxide state after 12 weeks at 60 °C. Although refining leads to kinetics changes in hydroperoxide formation and decomposition, both types of oils reached a similar final peroxidation state after a certain time calculated at 12 weeks. This could be ascribed to the availability of the same oxygen amount inside the bottles. Similar results were found by Gharby et al. [11], who reported that primary oxidation products can be reduced during the refining process.

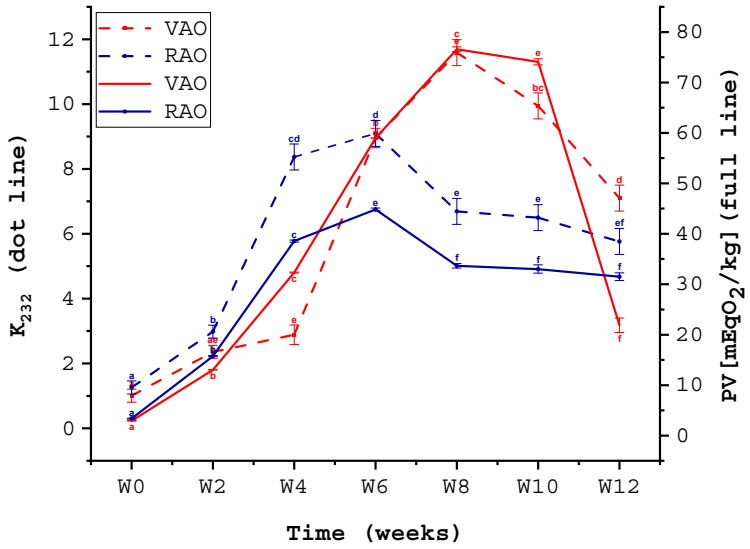

Figure 3. Peroxide value (PV) and specific extinction (K232) of argan oils (VAO and RAO). RAO: Refined argan oil and VAO: Virgin argan oil. Values mean ± SD (n = 3). Mean values with different letters are significantly different at $p < 0.05$, letters are the comparison between storage periods for the same samples.

The specific extinction coefficient measured at λ = 232 nm is a very important indicator of the degradation degree of an oil in its initial stage [29]. The initial K232 of RAO was slightly higher than that of VAO (Figure 3), suggesting the possibility of the rapid formation of primary oxidation products that went unnoticed during the determination of PV. These extinction values confirmed the presence of similar amounts of primary oxidation products in VAO and RAO, as already deduced from the PV, and indicate that refining removed a large proportion of these primary oxidation products. For RAO, the K232 suggests that the primary oxidation products started to accumulate significantly after 6 weeks of storage at 60 °C before peaking after 6 weeks and then decomposed. VAO peaked during the 8th week and then declined very remarkably after the 10th week.

3.3. Secondary Oxidation

Argan oil can be rancid or possess poor organoleptic properties without having a high primary oxidation product content. This is a reason why the determination of secondary oxidation products is important. Secondary oxidation product content is usually assessed by examining the *p*-anisidine value and the specific extinction coefficient K270. It is evidenced that p-AV is considered a more accurate indicator of oxidation [29]. The results of the p-AV revealed a significantly different increase between RAO and VAO. The initial p-AV of RAO was determined to be 0.73, which was not significantly different from that of the VAO (0.53, Figure 4). The p-AV of the VAO remained moderately low for the first 4 weeks. After this period, it increased slowly but steadily to reach 11.03 after 12 weeks. This change parallels that of the PV, which can be explained by the immediate transformation of the primary oxidation products into secondary oxidation products. It is noteworthy that the peroxide value of RAO started to decrease from the 6th week onwards (Figure 3). In contrast, the p-AV of RAO increased rapidly to record a maximum value of 17.21. Secondary oxidation product formation continued to progress for both types of oil over the 12 weeks, suggesting the lack of stabilization of hydroperoxides, especially for RAO. This difference in content between RAO and VAO can be detected by the presence of a class of compounds such as tocopherols, carotenoids, and polyphenols in VAO that would slow down the formation of secondary oxidation products. Several studies from the literature have confirmed the role of antioxidants in protecting oils. Recently, in a study conducted on 33 rapeseed oil samples subjected to microwave and conventional heating, Yan et al. [30] reported strong negative correlations between the oxidation degree on one

hand and the polyphenol, tocopherol, phytosterol, and squalene contents on the other hand. Likewise, oxidation positively correlated with the unsaturation degree and water content but did not correlate with the contents of chlorophylls and β-carotene. Unfortunately, these compounds would be unevenly removed during the refining steps, thus allowing a faster oxidation of RAO.

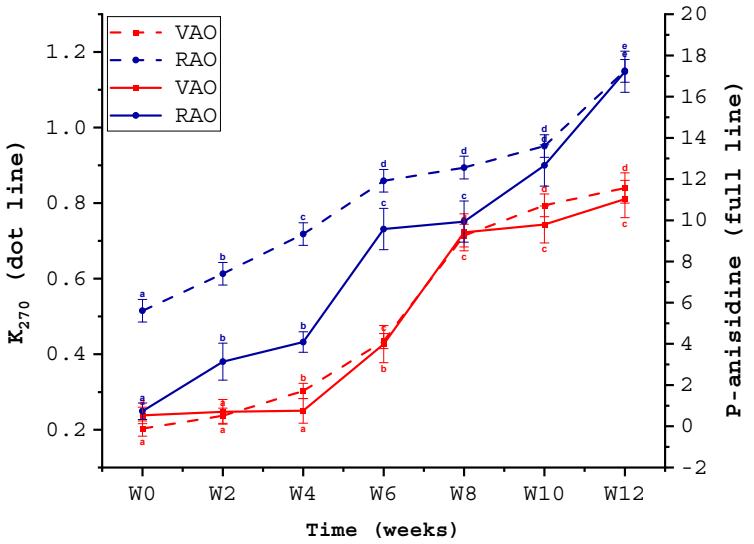

Figure 4. *p*-anisidine value (p-AV) and specific extinction (K270) of argan oils (VAO and RAO). RAO: Refined argan oil and VAO: Virgin argan oil. Values are mean ± SD (n = 3). Mean values with different letters are significantly different at $p < 0.05$, letters are the comparison between storage periods for the same samples.

The specific extinction coefficient K270 is another important index used to assess secondary oxidation products [31]. K270 complements *p*-anisidine since the latter does not detect ketones [29]. The increase in K270 values was different for each kind of oil. The increase in K270 value for VAO was significantly lower (from 0.20 to 0.84) than that of RAO (from 0.52 to 1.15, Figure 4). In fact, the secondary oxidation products detected by specific extinction at 270 started to appear from the first week for both oils. These results confirm those found previously in the *p*-anisidine value.

3.4. Variations in Chemical Composition

Argan oil has a high content of fatty acids, mainly unsaturated fatty acids and antioxidants, namely, tocopherols, making it important to evaluate the influence of argan oil refining on these two compounds during the storage period at 60 °C.

3.4.1. Variation in Fatty Acid Content, Iodine Value, and Saponification Value

Because most of argan oil's therapeutic properties are linked to its high unsaturated fatty acid content, we determined several parameters, including the fatty acid composition, iodine value, and saponification value, in oil samples after every month. Fatty acid profiling is an important factor in the oil's nutritional value. In addition, it is noteworthy that the stability of oil depends strongly upon the unsaturated fatty acid composition [1]. In fact, argan oil is composed of about 80% unsaturated fatty acids, which makes it highly susceptible to oxidation processes during processing and storage due to their exposure to extrinsic factors, including high temperature, light, and oxygen accessibility [32]. That is why we decided to examine the variation in fatty acids during storage at 60 °C. More recently, Vidal et al. [32] studied the quality and chemical stability of long-term stored cold-pressed cake lipids (soy, canola, and sunflower) before and after thermomechanical processing (extrusion). According to the same authors, extrusion did not modify lipid quality just after processing. However, lipid stability was significantly decreased after

one year because of the degradation of their polyunsaturated acyl groups and fatty acids on one hand, and the increase in primary and secondary oxidation products on the other hand. FA composition was determined every 4 weeks during 12 weeks of storage at 60 °C (Table 1). As shown in Table 1, both argan oils (VAO and RAO) showed the expected fatty acid composition [33–35]. Furthermore, the initial fatty acid composition of VAO and RAO showed no significant difference, suggesting that the refining process does not influence the relative percentage of argan oil fatty acid profiles. In fact, during 12 weeks of storage at 60 °C, no significant changes were observed in terms of relative fatty acid content. Generally, the fatty acid composition did not change, even during the storage period, as already found by several previous studies confirming that the refining process has no significant effects on the relative percentage in olive oil, hazelnut oil [36], peanut oil [37], and kenaf seed oil [38].

Table 1. Fatty acid composition (g/100 g) of argan oils (VAO and RAO). SFA *: Saturated fatty acids, USFA **: Unsaturated fatty acids, RAO: Refined argan oil, VAO: Virgin argan oil, and W: Week.

	W0		W4		W8		W12		
	RAO	**VAO**	**RAO**	**VAO**	**RAO**	**VAO**	**RAO**	**VAO**	**NORM [7]**
C14:0 (Myristic acid)	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	≤0.2
C16:0 (Palmitic acid)	12.5 ^{a} ± 0.2	12.3 ^{a} ± 0.2	12.3 ^{a} ± 0.3	12.4 ^{a} ± 0.2	12.7 ^{a} ± 0.1	12.6 ^{a} ± 0.2	12.7 ^{a} ± 0.1	12.7 ^{a} ± 0.1	11.5–15.0
C16:1 (Palmitoleic acid)	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	0.1 ^{a} ± 0.0	≤0.2
C18:0 (Stearic acid)	6.01 ^{a} ± 0.1	5.8 ^{a} ± 0.0	6.2 ^{a} ± 0.1	5.8 ^{a} ± 0.0	6.0 ^{a} ± 0.0	5.8 ^{a} ± 0.0	5.9 ^{a} ± 0.1	5.8 ^{a} ± 0.0	4.3–7.2
C18:1 (Oleic acid)	47.6 ^{a} ± 0.1	47.8 ^{a} ± 0.1	48.0 ^{a} ± 0.1	47.8 ^{a} ± 0.1	48.0 ^{a} ± 0.1	47.67 ^{a} ± 0.1	48.5 ^{a} ± 0.1	48.5 ^{a} ± 0.1	43.0–49.1
C18:2 (Linoleic acid)	30.2 ^{a} ± 0.1	32.2 ^{a} ± 0.1	30.1 ^{a} ± 0.1	32.1 ^{a} ± 0.1	29.2 ^{a} ± 0.1	31.5 ^{a} ± 0.1	29.56 ^{a} ± 0.1	31.2 ^{a} ± 0.1	29.3–36.0
C18:3 (Linolenic acid)	0.14 ^{a} ± 0.1	0.27 ^{a} ± 0.1	0.15 ^{a} ± 0.1	0.22 ^{a} ± 0.1	0.25 ^{a} ± 0.1	0.12 ^{a} ± 0.1	0.24 ^{a} ± 0.1	0.2 ^{a} ± 0.1	≤0.3
C20:0 (Arachidic acid)	0.4 ^{a} ± 0.1	0.4 ^{a} ± 0.0	0.4 ^{a} ± 0.0	0.4 ^{b} ± 0.1	0.4 ^{a} ± 0.0	0.4 ^{a} ± 0.0	0.4 ^{a} ± 0.0	0.2 ^{b} ± 0.0	≤0.5
SFA *	19.01	18.6	19	19	19.2	18.9	19.1	18.8	-
USFA **	78.17	80.37	78.35	77.55	77.55	79.39	78.4	80	-
IV (g_{I2}/100 g)	98.06	102.22	98.26	101.90	96.91	100.42	97.98	100.85	91–110
SV (mg KOH per g of oil)	198.11	194.38	197.48	194.49	198.73	195.72	197.25	194.80	189–199.1

Values are mean ± SD (n = 3). Mean values followed by the same letter within a line are not significantly different at $p < 0.05$.

In order to have a better idea of the unsaturated fatty acid content in the two oils, the iodine value was calculated. This parameter is another quality index used to rate the degree of unsaturation of fatty acids. As observed for fatty acids, no significant variations in iodine value were observed for both oils (VAO and RAO) during 12 weeks of accelerated aging. In fact, the iodine value recorded by VAO passed from 102.22 to 100.85 g I_2/100 g oil, while the RAO values passed from 98.06 in the first week to 97.98 g I_2/100 g oil. This result suggests that the unsaturation of fatty acid distribution was not influenced by this treatment. Also, our findings confirm those found previously in the fatty acid composition.

Concerning the saponification value, a slight variation was observed for VAO and RAO during 12 weeks at 60 °C, but the saponification value calculated after three months was consistently found between 189 and 199, as required by the official norm [7].

3.4.2. Tocopherols

Tocopherols belong to the vitamin E family and have a strong antioxidant activity [39]. Tocopherols are widely used for their technological properties, as they limit the oxidation of fats, thus preventing their rancidity and ensuring better preservation [40]. The tocopherol content was assessed at the beginning and the end of the storage period (Table 2). According to published literature, both argan oils have a high content of tocopherols, more precisely, γ-tocopherol [1]. The VAO showed an initial tocopherol content of 430 mg/kg oil. After 12 weeks of accelerated aging, this value decreased to 44 mg/kg oil. A similar behavior was presented by RAO, which recorded a total tocopherol content of 312 mg/kg oil at the beginning and after 12 weeks. This value decreased to a minimum at the end (23 mg/kg oil). Therefore, it is clear that exposure to a high temperature or a long storage period is

detrimental to the oxidative quality of argan oil. Also. the tocopherol content of RAO was lower than that of VAO. Moreover, the initial content of VAO was 430 mg/kg oil, while RAO was calculated to be 312 mg/kg oil. Several studies confirm a significant decrease in tocopherol content during the refining process, notably in olive oil, corn oil [12], palm oil [41], soybean oil [13], sunflower oil [14], and rapeseed oil [42].

Table 2. Tocopherol content (mg/kg) of argan oils (VAO and RAO). RAO: Refined argan oil, VAO: Virgin argan oil, and W: Week.

	RAO		VAO	
	W0	**W12**	**W0**	**W12**
α-Tocopherol	$35^{a} \pm 1$	<LQ	$37^{c} \pm 0$	<LQ
γ-Tocopherol	$262^{a} \pm 2$	$15^{b} \pm 2$	$369^{c} \pm 1$	$33^{d} \pm 0$
δ- Tocopherol	$15^{a} \pm 1$	$8^{b} \pm 0$	$24^{c} \pm 2$	$11^{d} \pm 1$
Total Tocopherols	$312^{a} \pm 2$	$23^{b} \pm 1$	$430^{c} \pm 0$	$44^{d} \pm 1$

Values are mean ± SD (n = 3). Mean values followed by the same letter within a line are not significantly different at $p < 0.05$. LQ = Limit of Quantification.

3.5. Rancimat Test

Another parameter index used to evaluate the stability of VAO and RAO is the induction period (IP) using the Rancimat test. It is an instrument for the automatic measurement of the oxidation stability of oils based on the conductivity of secondary oxidation volatile compounds, which subsequently allows the measurement of the induction period, which in turn reflects the stability of vegetable oils [43]. The initial stability of RAO and VAO at 120 °C (393 °K) was 4.2 ± 1.5 h and 6.5 ± 1.5 h, respectively. The argan oil induction is higher than that of sunflower oil (1.7 h), soybean oil (2.6 h), and cactus seed oil (3.2 h) [44]. This difference is based on the fatty acid profile of argan oil and its content of tocopherols. The difference between the mean value Rancimat induction period of VAO and RAO was 2 h (Figure 5). After 12 weeks of storage at 60 °C, the IP of both argan oils dramatically decreased to reach the lowest values, 1 h and 0.8 h for VAO and RAO, respectively (Figure 6).

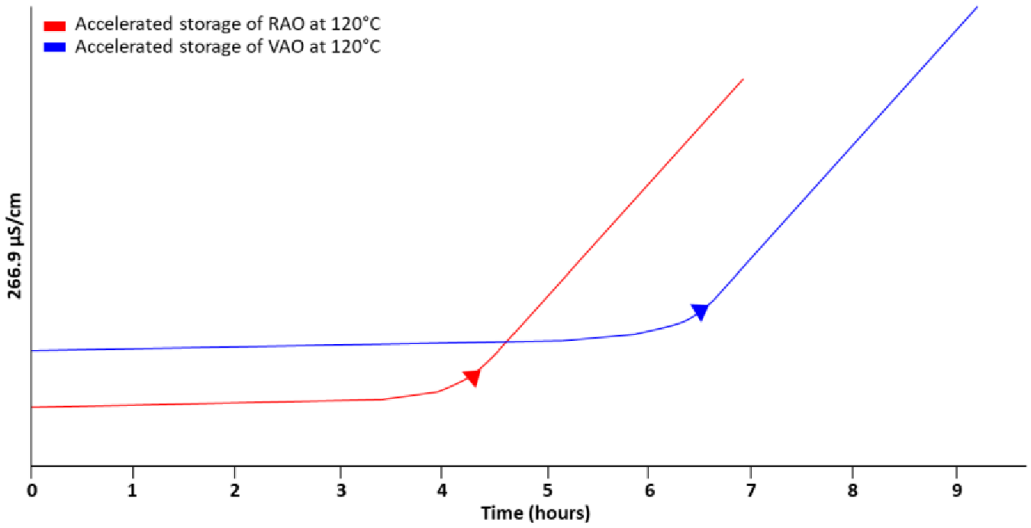

Figure 5. Rancimat induction period of VAO and RAO at 120 °C (393 °K). RAO: Refined argan oil and VAO: Virgin argan oil. Values are mean ± SD (n = 3).

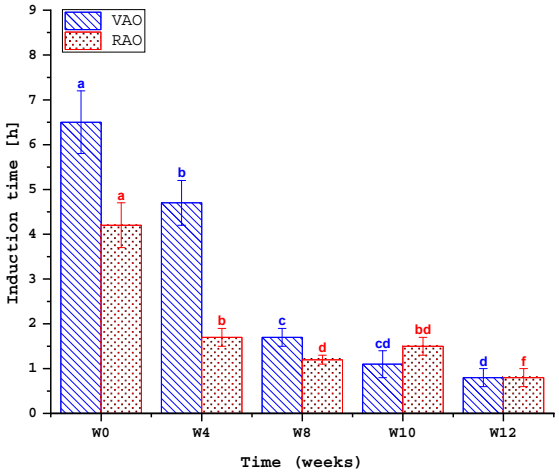

Figure 6. Rancimat induction period (h) of argan oils (VAO and RAO). RAO: Refined argan oil, VAO: Virgin argan oil, and W: Week. Values are mean ± SD (n = 3). Mean values with different letters are significantly different at $p < 0.05$, letters are the comparison between storage periods for the same samples.

3.6. Shelf-Life Prediction

Argan oil shelf life was evaluated through the accelerated Rancimat test method. This is an easy test method to quickly determine the oxidative stability of oil and fats, which can also provide important information with respect to the oxidative state like shelf life in real-time conditions [45]. The induction time was determined at different temperatures, 373, 383, 393, and 403 K, by plotting the logarithm of the times against temperature. The oil shelf life can be estimated at room temperatures (298 °K, Figure 7). Induction time was extrapolated at room temperature (25 °C) to predict the shelf life of both oils using Rancimat induction times [46]. The induction time provides an indication of how long the oils will take to become oxidized. The induction time is also an indicator to evaluate the oil's relative oxidative stability. The accelerated testing may then be extrapolated to real-time conditions [46]. The shelf life of RAO and VAR was determined at 25 °C (by extrapolation, Figure 7). At room temperature, RAO was found to possess a shorter induction time of 6 months, indicating that RAO will have a shorter shelf life compared to VAR, with an anticipated time span of usability of about 11 months when extrapolated to 25 °C. Such shelf-life estimations are in line with the other results found, especially the tocopherol profile, which showed a significant decrease after the refining process.

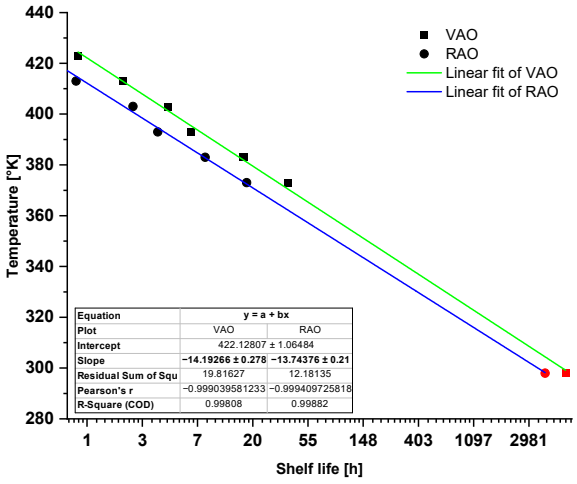

Figure 7. Extrapolation at 25 °C (298 K) of the shelf life of VAO and RAO. RAO: Refined argan oil and VAO: Virgin argan oil. Values are presented as mean ± SD (n = 3).

4. Conclusions

Generally, the heating process impacted the quality of both VAO and RAO oils. Considering the oxidative state of both oils after heating, the Rancimat test showed the highest oxidative stability of VAO when compared to RAO during storage. Such instability means that the argan oil refining process must be carried out with great care and this oil needs to be protected once refined. It was also found that high levels of antioxidant compounds in VAO had a positive effect on the oxidative stability during heating. Such antioxidant compounds, including tocopherols in the VAO, may protect the oil from oxidative deterioration, which is considered as an important factor in the oil stability. Further investigations are needed with the aim of optimizing refining and storage conditions while maintaining a good oxidative stability. Keeping in mind the short shelf life of RAO, special attention should be paid to storage and packaging.

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