Oxidative Stability of Argan Oils Extracted from an agroecological Plantation and Two Natural Forest Stands

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ABSTRACT

Argan oil is produced from the kernels of argan tree (*Argania spinosa* (L.) Skeels), endemic tree in the southwest of Morocco. Argan oil is known for its unique chemical composition responsible of its several beneficial, nutritional, therapeutic and cosmetic effects. This study concerned three oils, two originated from natural forest stands in the regions of Essaouira (**EAO**) and Taroudant (**TAO**), and one from an agroecological plantation in Casablanca (**CAO**). The main objective of this study is to evaluate and compare the shelf-life of freshly extracted argan oils after two and three years of storage, and the influence of storage period, light, and oxygen. The assessment was carried out by determining chemical properties (Acidity, peroxide value, specific absorbances K232 and K270, tocopherol content). The tree studied oils are stable to oxidative stability after two years storage in darkness, but after three years, (**CAO**) and (**TAO**) preserved there extra virgin label, compared to (**EAO**) that lost it. In addition, this study confirmed the powerful effect of light on the oxidative stability of oils during storage.

Keywords: Argan oil, extra virgin oil, storage, peroxidation, plantation.

1. Introduction

Argan oil is the extraction product of the nuts from the fruit of the argan tree (*Argania spinosa* (L.) Skeels). The argan forest was nominated a UNESCO biosphere reserve in 1998 **[1]**. This multipurpose tree is characterized by socio-economic and ecological interest, as it is defined an agro-sylvo-pastoral specie.

Argan oil is known for its nutritional, therapeutic and cosmetic properties. Numerous studies shown its interest in the prevention of cardiovascular disease in favor of its hypocholesterolemic, hypolipedimiant, and antioxidant properties **[2-5]**. In addition, this oil is endowed by moisturizing, regenerating, and enhancing skin elasticity properties **[6, 7]**. This is attributed to its specific composition characterized mainly by polyunsaturated fatty acids (essential fatty acids) and (tocopherols) **[8]**. The high quality and stability of argan oil are controlled by several parameters, including fruit quality, methods, kernels roasting, extraction method, and storage conditions **[9-10]**. Oxidation is the major factor affecting oil quality during storage, since it causes loss of oil nutritional quality and the development of an unnatural flavor which makes food that contain lipids unappealing for consumers. Additionally, some toxic compounds are formed during oil oxidation **[11]**. Edible argan oil is prepared by pressing roasted kernels while cosmetic argan oil is producer from unroasted ones. Edible argan oil is characterized by its hazelnut taste assured by the volatile compounds formed during kernel roasting **[12]**. These volatile compounds promote the preservation of edible argan oil whose estimated shelf life is an average of two years **[10, 13]**. The moisture content in cosmetic argan oil is much more important which decreases its shelf life **[10]**.

Oxidative stability of fats and oils is affected by several factors as well as fatty acid composition and contents of minor components for instance antioxidants namely tocopherols, carotenoids, etc. Additionally, different external factors affect the oil's oxidative stability, including exposure to atmospheric oxygen, light and temperature **[14]**. A more accurate assessment of oxidative stability requires consideration of also the total trans fatty acid content (which indicates previous thermal damage to the fat or oil) as well as the total antioxidant content **[15]**. The aim of this study was to provide a comparison between the oxidative stability of argan oil from an argan tree plantation outside its natural area and oils from natural forest stands in

southwestern Morocco two and three years of storage in different storage conditions.

2. Material and Methods

2.1. Plant material

Argan fruits were collected in October 2018 from spontaneously growing forest trees in the prefectures of Taroudant and Essaouira, located in southwest Morocco and from, and agroecological plantation of argan trees in Casablanca. The sun-dried fruits were then crushed, and the almonds were extracted in May 2019 by cold mechanical pressure from unroasted argan kernels.

2.2.Samples preparation and storage conditions

Three samples of argan oil were prepared by pressing unroasted kernels. Two samples of argan oil originated from natural forest stands in the region of Essaouira and Taroudant, and one from an argan tree plantation in Casablanca. Argan oils were stocked in 50 ml tubes. The samples were classified into 3 groups according to the storage conditions **(Figure 1)**.

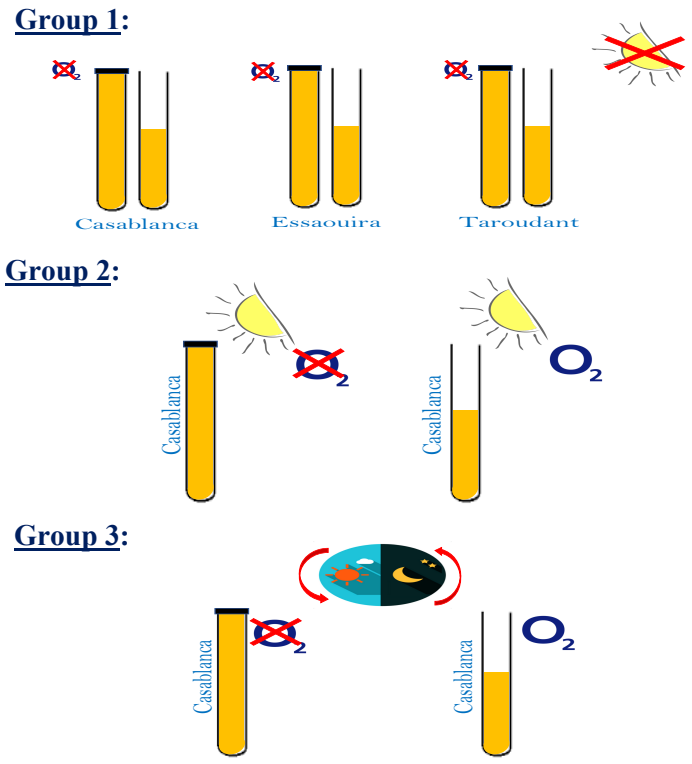

Figure 1: Storage conditions of the studied argan oils.

2.3. Oils analysis

Physiochemical parameters which include acidity, peroxide value and ultraviolet absorption (K_{270} and K_{232}) were determined according to the International Standard Organization ISO 660 **[16]**, ISO 3960 **[17]**, and International Oleic Council standard **[18]**, respectively. Results of acidity are expressed as a percentage of oleic acid and those of peroxide value are expressed in meq O2/kg. Ultraviolet absorption was measured at 232nm, 266nm, 270nm and 274nm using double beam measured at 232 nm, 266 nm, 270 nm, and 274 nm using double beam UV–Vis Spec-trophotometer (6850 UV/Vis. Spectrophotometer-JENWAY). The tocopherol content was determined by HPLC using Shimadzu instruments equipped with a C18-Varian column (250 mm × 4.6 mm × 5 μm). Detection was achieved using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330nm). The tocopherols content was determined using calibration factors determined from standard solutions using the International Standard Organization method ISO 9936 **[19]**. The initial analysis was produced directly after extraction (2019), then after two and three years of storage (2021 and 2022 respectively).

2.4. Statistical Analysis

Reported values in the tables are the means ± SE of three replications. The significance level was set at $p < 0.05$. Separation of means was performed by Bonferroni and Tukey's test at the 0.05 significance level. All statistical analyses were carried out using SPSS Statistics Software (IBM SPSS Statistics 25.0) **[20]**.

3. Results

3.1. Initial physicochemical parameters of the studied oils samples

Table 1.

Physicochemical parameters of argan oils from 1) Casablanca (CAO), 2) Essaouira (EAO) and 3) Taroudant (TAO).

Samples	% Acidity [1]	Peroxide Value $meqO_2/Kg$	OD at 232 nm [1] (K232 nm)	OD at 270 nm [1] (K270 nm)
CAO	0.37 ± 0.02 [a]	0	1.37 ± 0.06 [b]	0.19 ± 0.00 [b]
TAO	0.25 ± 0.04 [b]	0	1.70 ± 0.00 [a]	0.20 ± 0.00 [b]
EAO	0.2 ± 0.03 [c]	0	1.50 ± 0.01 [a,b]	0.34 ± 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.

Initial analysis of physicochemical parameters of the three oils (Casablanca, Essaouira and Taroudant) revealed that the highest level of acidity had been registered for Casablanca argan oil **(CAO)** compared to those of Essaouira (**EAO**) and Taroudant (**TAO**) (0.37%, 0.25% and 0.2% respectively) **(Table I)**. In terms of peroxide value, 0 meqO_2/Kg of hydroperoxides is detected in all three oils (**CAO**), **(EAO)** and (**TAO**) **(Table I)**. Concerning the evaluation of the spectrophotometric characteristics, the three oils show close values of ultraviolet absorbance at 232nm and 270nm (1.37 and 0.19 ($\Delta K = 0.00$), 1.50 and 0.34 ($\Delta K = 0.00$) and 1.70 and 0.19 ($\Delta K = 0.00$), respectively) **(Table I)**.

3.2. Influence of some storage conditions on the oxidative stability of argan oil, a two- and three-year study

3.2.1. Acidity analysis

Table 2.

Acidity analysis in (%) of argan oils from 1) Casablanca **(CAO)**, 2) Essaouira **(EAO)** and 3) Taroudant **(TAO)**, stored in different storage conditions: (**G1**: stored in the shelter of the light), (**G2**: stored at room temperature and exposed to daylight to continuous) and (**G3**: stored in a light/ dark cycle). Mean values ± standard deviation of the values (two replicate) are presented.

Sample	Initial	2 years	3 years
G1-CAO	0.37±0.02 ^{a,A}	0.40±0.00 $^{a}{}_{\alpha,A}$	0.78±0.00 ^{a,C}
G1-TAO	0.25±0.04 ^{b,A}	0.36±0.00 $^{a}{}_{\gamma,B}$	0.54±0.14 ^{b,C}
G1-EAO	0.2±0.03 ^{c,A}	0.84±0.00 $^{b}{}_{\delta,B}$	1.57±0.03 ^{c,C}
G1-CAO+O_2		0.50±0.00 $^{b}{}_{\alpha}$	
G1-TAO+O_2		0.33±0.00 $^{a}{}_{\gamma}$	
G1-EAO+O_2		0.36±0.00 $^{c}{}_{\delta}$	
G2-CAO		0.45±0.00 $^{c}{}_{\alpha}$	
G2-CAO+O_2		-	
G3-CAO		0.42±0.00 $^{a}{}_{\alpha}$	
G3-CAO+O_2		-	

Means ± standard deviation (n = 2). Values with different capital letters in each row, and values with different small letters in each column (Initial, 3year), are significantly different ($P < 0.05$). For 2 year analysis, values with the same symbol (α, γ, δ) and different small letters in each column are significantly different.

Free acidity of (**G1**-CAO) increased after two years of storage without reaching significance, but it increased significantly after 3 years of storage (0.37±0.02, 0.40±0.00

and 0.78±0.00, respectively). Values of free acidity for the three samples (**G1-**CAO), (**G1-**CAO+O_2), and (**G2-**CAO) after two years storage are significantly different (0.40±0.00, 0.50±0.00 and 0.45±0.00, respectively). Conversely, the difference between the free acidity of (**G1-**CAO), and (**G3-**CAO) stored in a light/ dark cycle is not significant (0.40±0.00, 0.42±0.00, respectively) **(Table 2)**.

Regarding Taroudant Argan Oil **(TAO)**, its acidity increased significantly after two and three years of storage in the shelter of light with absence of oxygen (**G1-**TAO) (0.25±0.04, 0.36±0.00 and 0.54±0.14, respectively). Non-significant difference was detected between free acidity of (**G1-**TAO) protected from sunlight with presence of oxygen and (**G1-**TAO+O_2) stored for two years (0.36±0.00, 0.33±0.00, respectively).

Initial acidity of Essaouira Argan Oil (**G1-**EAO) was significantly increased after two and three years of storage in the shelter of the light (0.2±0.03, 0.84±0.00 and 1.57±0.03, respectively).

3.2.2. Peroxide value analysis

The results of the analyses of the three oils **(Table 3)** show a statistically significant increase of the peroxide value after two and three years of storage in different conditions (**G1, G2** and **G3**). The obtained results revealed that (**G2-**CAO) stored at room temperature and exposed to daylight to continuous was significantly richer in hydroperoxydes with 25.6±0,00 meqO2/Kg compared to (**G1-**CAO) and (**G3-**CAO), which had 1.0±0.28 and 7.2±0,00 meqO2/Kg.

Table 3.
Peroxide value analysis in (meqO2/Kg) of argan oils from 1) Casablanca **(CAO)**, 2) Essaouira **(EAO)** and 3) Taroudant **(TAO)**, stored in different storage conditions: (**G1**: stored in the shelter of the light), (**G2**: stored at room temperature and exposed to daylight to continuous) and (**G3**: stored in a light/ dark cycle). Mean values ± standard deviation of the values (two replicate) are presented.

Sample	Initial	2 year	3year
G1-CAO	0 ^{A}	1.0±0.28 $^{a}{}_{\alpha'}{}^{B}$	2.20±0.28 $^{a,}{}_{C}$
G1-TAO	0 ^{A}	3.2±0.00 $^{a}{}_{\gamma'}{}^{B}$	9.97±0.53 $^{b,}{}_{B}$
G1-EAO	0 ^{A}	1.6±0.00 $^{a}{}_{\delta'}{}^{B}$	3.99±0.14 $^{c,}{}_{B}$
G1-CAO+O_2		1.2±0.00 $^{a}{}_{\alpha}$	
G1-TAO+O_2		18.60±0.00 $^{a}{}_{\gamma}$	
G1-EAO+O_2		-	
G2-CAO		25.6±0.00 $^{b}{}_{\alpha}$	
G2-CAO+O_2		26.20±0.00 $^{b}{}_{\alpha}$	
G3-CAO		7.2±0.00 $^{c}{}_{\alpha}$	
G3-CAO+O_2		-	

Means ± standard deviation (n = 2). Values with different capital letters in each row, and values with different small letters in each column (Initial, 3year), are significantly different ($P < 0.05$). For 2 year analysis, values with the same symbol (α, γ, δ) and different small letters in each column are significantly different

3.2.3. Ultraviolet absorption analysis

**K_{232} analysis*

After two years of storage in shelter of light and presence or absence of O_2, (**TAO**) increased significantly (**Table 4**). Compared to (**EAO**) which increased without reaching the significance. The K_{232} of (**CAO**) stored in shelter of light and absence of O_2 (**G1-**CAO), increased with none significance compared to (**G1-**CAO+O_2) which increased significantly from 1.37 to 1,65. In addition, K_{232} increased significantly from 1.37 to 2.57 for the samples of **(G2)** (**Table 4**). The same for the samples stored in a light/ dark cycle **(G3)** there K_{232} increased significantly. The difference between all samples of **(CAO)** of **(G1** & **G3)** was statically significant. The difference between (**G2-**CAO) and (**G2-**CAO+O_2) was not significant. After three years of storage, K_{232} of all analysed samples increased significantly.

Table 4.
Ultraviolet absorption analysis of argan oils from 1) Casablanca **(CAO)**, 2) Essaouira **(EAO)** and 3) Taroudant **(TAO)**, stored in different storage conditions: (**G1**: stored in the shelter of the light), (**G2**: stored at room temperature and exposed to daylight to continuous) and (**G3**: stored in a light/ dark cycle). Mean values ± standard deviation of the values (two replicate) are presented.

Sample	**Initial**		**2 year**		**3year**	
	K232nm	**K270nm**	**K232nm**	**K270nm**	**K232nm**	**K270nm**
G1-CAO	1.37±0,06	0.19±0,00	1.50±0.00	0.24±0.00	1.90±0.00	0.43±0.00
G1-TAO	1.70±0,00	0.20±0,00	2.16±0.00	0.19±0.00	3.56±0.12	0.23±0.00
G1-EAO	1.50±0,01	0.34±0,00	1.44±0.00	0.18±0.00	1.93±0.00	0.25±0.00
G1-CAO+O_2			1.65±0.00	0.16±0.00	3.25±0.00	0.26±0.00
G1-TAO+O_2			2.56±0.00	0.20±0.00	-	-
G1-EAO+O_2			1.50±0.00	0.22±0.00	1.98±0.00	0.43±0.00
G2-CAO			2.57±0.00	0.19±0.00	-	-
G2-CAO+O_2			2.57±0.03	0.21±0.00	-	-
G3-CAO			1.80±0.00	0.37±0.00	-	-
G3-CAO+O_2			1.66±0.00	0.16±0.00	3.17±0.00	0.20±0.00

K_{270} analysis

The study of variation of K_{270} for the three oils stored in different conditions after two and three years, revealed that the values of K_{270} decreased without reaching the significance (**Table 4**).

3.2.4. Tocopherols analysis

The tocopherols initial assay results presented in table 5 indicated that (**TAO**) and (**EAO**) were significantly richer in gamma-tocopherols (87.15%±0.06 and 87.88%±0.06) compared to (**CAO**) (90.53%± 0.22). Moreover, (**TAO**) was significantly less rich in total tocopherols, compared to **(CAO)** and **(EAO)** (749.43%±0.04, 758.47%±5.65 and 662.80%±3.06 respectively).

Table 5.
Tocopherols analysis of argan oils from 1) Casablanca **(CAO)**, 2) Essaouira **(EAO)** and 3) Taroudant **(TAO)**.

Tocopherols Composition [1]	**G1**-CAO	**G1**-TAO	**G1**-EAO
α tocopherols %	4.24 ± 0.05 [a]	7.04 ± 0.11 [a]	4.49 ± 0.21 [a]
γ tocopherols %	90.53 ± 0.22 [a]	87.15 ± 0.06 [b]	87.88 ± 0.06 [c]
δ 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]

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.

The second tocopherols assay results show in table 6 revealed that the tocopherols composition of all analysed samples decreased significantly after two years of storage in different conditions (**G1, G2** and **G3**). The percentage of decrees was in the average of 50% and 98%.

Table 6.
Tocopherols analysis of argan oils from 1) Casablanca **(CAO)**, 2) Essaouira **(EAO)** and 3) Taroudant **(TAO)**, stored in different storage conditions: (**G1**: stored in the shelter of the light), (**G2**: stored at room temperature and exposed to daylight to continuous) and (**G3**: stored in a light/ dark cycle). Mean values ± standard deviation of the values (two replicate) are presented.

Sample	**2year**			
	α tocopherols %	***γ tocopherols %***	***δ tocopherols %***	***Total tocopherols (mg/Kg)***
G1-CAO	0.99%	26.71%	2.38%	325.29
G1-TAO	1.84%	25.41%	1.76%	265.30
G1-EAO	1.03%	28.17%	1.63%	352.56
G1-CAO+O_2	0.95%	24.55%	0.95%	302.49
G1-TAO+O_2	1.74%	26.06%	1.56%	279.03
G1-EAO+O_2	0.98%	27.21%	2.25%	305.95
G2-CAO	0.80%	20.30%	2.19%	280.86
G2-CAO+O_2	0.75%	27.78%	2.31%	275.37
G2-CAO	0.93%	22.70%	1.58%	261.25
G3-CAO+O_2	1.18%	27.44%	1.74%	342.13

4. Discussion

According to the recommendations of the Moroccan standard N.M. 08.5.090, all fresh argan oil samples showed states factory physicochemical characteristics to be classified as extra virgin argan oil **[21]**. These results were in accordance with those reported in previous study established by Mechqoq in 2021 **[22]**.

Acidity of (**CAO**) after two years of storage increased for (**G1-**CAO), (**G2-**CAO) and (**G3-**CAO) preserving there extra virgin label. Acidity of (**G1-**CAO+O_2), (**G1-**TAO), (**G1-**EAO), (**G1-**TAO+O_2) and (**G1-**EAO+O_2) stored in darkness increased without reaching the 0,8 limit, preserving there extra virgin label too. Similarly, to the results to the finding of the study released by Gharby et al. (2011) on three

marketed types of edible argan oil whose acidity increase after two years of storage **[23]**. However, the increase rate seems to be light dependent the oxidation variation observed between light-protected and unprotected samples was significant.

The 3-years acidity of (**G1-**CAO) and (**G1-**TAO) increased to 0.78±0.00 and 0.54±0.14, respectively without reaching the limit 0.8, preserved there extra virgin label whereas that of (**G1-**EAO) reached the 0.8 limit, lost its extra virgin label.

Rancidity is mostly caused by peroxides, which are the primary byproducts of oxidative reactions. Peroxides formation is generally promoted by high temperatures and light and it has a substantial impact on the shelf life of oils as well as their acceptance by consumers. In our study for (**G1-**CAO), (**G1-**TAO), (**G1-**EAO), and (**G1-**CAO+O_2) stored in darkness for two years, the peroxide value remained below the 15meqO_2/Kg oil limit, as well (**G3-**CAO). Contrary, the peroxide value of (**G1-**TAO+O_2) well exceed the limit after two years storage. These results revealed that **(CAO)** stored in darkness was markedly more stable than the **(TAO)** and **(EAO)**. The initial peroxide value of (**G2-**CAO) and (**G2-**CAO+O_2) exposed to daylight to continuous crossed after two years the limit of 15meqO_2/Kg oil fixed for the extra virgin argan oil label **[21]**. After three years storage in darkness, argan oil behaved generally similarly but the increase of the peroxide value was lower than that observed for (**G2-**CAO) and (**G2-**CAO+O_2) who registered the highest peroxide value after two years storage (25.6±0.00 and 26.20±0.00 meqO2/Kg, respectively). This result proves that the formation of hydroperoxides is largely promoted by light. In the literature the powerful influence of lighting conditions of storage on the oxidative stability of argan oil was approved **[23, 24]**.

Shelf life of studied argan oil was also evaluated by measuring specific extinction at wavelengths 232nm (K_{232}) and 270nm (K_{270}). UV absorption at λ 232nm (K_{232}) is an indicator of primary oxidation products formation **[9]**. Moroccan regulation has defined the maximum value for K_{232} less than 2.5**[21]**. After two years, the samples (**G2-**CAO) and (**G2-**CAO+O_2) exposed to daylight to continuous crossed, and the sample (**G1-**TAO+O_2) stored in darkness crossed the limit 2.5. This result attest the strong influence of light on the quality and stability of argan oil. The

measured values of K_{232} of (**G1-**CAO), (**G1-**EAO) and (**G1-**EAO+O_2) after three years of storage in darkness, increase but still within the acceptance limits. These results attest the strength oxidative stability of (**CAO**) and (**EAO**) compared to (**TAO**). K_{270} values are related to the content of secondary oxidation products **[25]**. According to the Moroccan standard N.M. 08.5.090 good quality argan oil should present a maximum value of 3.35 for K_{270} **[21]**. All analysed oils remained within the acceptance limits, below 0.35. Only K_{270} of (**G3-**CAO) stored in a light/ dark cycle crossed the limit value of 0.35 after two years to reach 0.37±0.00, which can be attributed to the important formation of secondary oxidation products due to the lighting effect. Our results attest the decomposition of the formed hydroperoxides in the studied argan oils, to unsaturated secondary oxidation products **[26]**, and that (**G3-**CAO) present the fattest decomposition rate.

Tocopherol compounds are one of the most important characteristics of argan oil. The composition of tocopherols is closely related to stability, cosmetic properties, and nutritional properties of argan oil. This can be used to determine whether the oil is adulterated **[27]**. In our study the storage of argan oil for two years in different conditions (**G1, G2** and **G3**) resulted in a dramatic decrease of tocopherol concentration for the three studied oils. These results are in good harmony with several published studies **[8, 21, 24]**.

5. Conclusion

This study has clearly demonstrated that the argan trees grown outside their biotope in the region of Casablanca can produce high quality argan oil which was classified as an extra virgin oil. Moreover, our results designate that argan oils extracted from argan nuts collected from a plantation in Casablanca and two forest stands of argan trees growing naturally in their native environment of the south-west of Morocco in the regions of Essaouira and Taroudant, are markedly stable to oxidative stability after two years storage in darkness. In the other hand, our results attest that argan oils originated from Casablanca and Essaouira are more stable to oxidative stability after three years of storage in darkness, compared to the argan oil extracted from the nuts of argan trees growing naturally in the region of Taroudant. Moreover, this study has shown that light is the major factor

influenced the oxidative stability of argan oil during storage. The findings are of direct positive impact on development both in the ecological and socio-economic fields. Additionally, this paper suggests that the plantation of argan tree outside its native environment can be selected as a real opportunity to achieve sustainability and protection of this natural resource.

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