

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

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Abstract – The argan tree is an endemic plant of Morocco that plays a great socio-economical and ecological impact in the south of the country. This plant is well known for the oil extracted from the almond, characterized by high nutritional value and its large spectrum of uses. This study aims to evaluate the chemical composition and the physicochemical parameters of fixed oils produced by the argan trees introduced in Oujda City and to compare them with other oils produced by the same trees in their natural biotope in the northeast and southwest of Morocco. The obtained results indicated that the oil yield varies between 57.65% and 47.60%. The investigation of the chemical composition using HPLC-DAD showed the presence of three types of tocopherols: α -tocopherols (9.7%), δ -tocopherols (6.6%), and γ -tocopherols (83.6%), the total content of tocopherols vary between 323.86 and 553.12 mg/kg. While, the methyl esters analysis using GC-MS showed the presence of 4 main fatty acids: oleic acid (55.40–41.14%), linoleic acid (36.92–26.75%), palmitic acid (18.69–9.97%) and stearic acid (12.09–3.68). The physicochemical parameters (the free acidity, the peroxide value, and the specific extinction) indicated that the different tested oils are characterized by a good oil quality according to the Moroccan standard concerning argan oil (NM 08.5.090). The obtained results indicated that the introduced argan in Oujda City showed a quality and a chemical composition that was comparable to that produced in the natural area of the argan tree, which shows the plasticity the plant under different climatic and edaphic conditions. These results emphasize the encouragement of the introduction of this species at a high level in eastern Morocco.

Keywords: argan oil / introduced argan tree / chemical composition / physicochemical parameters / eastern Morocco

Résumé – **Caractérisation chimique des huiles produites par quelques génotypes natifs et introduits de l'arganier au Maroc oriental par HPLC-DAD/GC-MS, et évaluation de leurs paramètres physico-chimiques.** L'arganier est une plante endémique du Maroc qui joue un grand rôle socio-économique et écologique dans le sud-ouest du pays. Cette plante est bien connue pour l'huile extraite de ses amandes, qui est caractérisée par une valeur nutritionnelle élevée et une multitude d'utilisations. Cette étude vise à évaluer la composition chimique et les paramètres de qualité physico-chimiques des huiles fixes produites par les arganiers introduits dans la ville d'Oujda et à les comparer avec d'autres huiles produites par les mêmes arbres dans leur biotope naturel au nord-est et au sud-ouest du Maroc. Les résultats obtenus indiquent que le rendement en huile varie entre 57,65 et 47,60 %. L'étude de la composition chimique par HPLC-DAD a montré la présence de trois types de tocophérols : α -tocophérols (9,7 %), δ -tocophérols (6,6 %), et γ -tocophérols (83,6 %), la teneur totale en tocophérols varie entre 323,86 et 553,12 mg/kg. Tandis que l'analyse des esters méthyliques par GC-MS a montré la présence de 4 principaux acides gras : l'acide

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oléique (55,40–41,14%), l'acide linoléique (36,92–26,75%), l'acide palmitique (18,69–9,97%) et l'acide stéarique (12,09–3,68%). Les paramètres physico-chimiques (l'acidité libre, l'indice de peroxyde, et l'extinction spécifique) montrent que les différentes huiles testées sont caractérisées par une bonne qualité d'huile selon la norme marocaine concernant l'huile d'argan (NM 08.5.090). Les résultats obtenus indiquent que l'huile produite par l'arganier introduit dans la ville d'Oujda présente une qualité et une composition chimique comparables à celles produites par l'arganier dans son biotope naturel, ce qui montre la plasticité de cette plante face aux différentes conditions climatiques et édaphiques. Ces résultats encouragent l'introduction de cette espèce à un niveau élevé dans le Maroc oriental.

Mots clés : huile d'argan / arganier introduit / composition chimique / paramètres physico-chimiques / Maroc oriental

1 Introduction

Argan tree scientifically named *Argania spinosa* is an endemic plant of Morocco, belonging to the Sapotaceae family (Rammal *et al.*, 2009). This native species is concentrated mainly in the southwest and exists as a relic in the northeast and the west of the country (Tazi *et al.*, 2003; Faouzi *et al.*, 2014; Moukrim *et al.*, 2018). Side to side with holm oak and cedar, the argan constitute an essential species of the Moroccan forest (Chaussod *et al.*, 2005), and the vast area covered by argan trees in the south of Morocco was designated a UNESCO biosphere reserve in 1998 (Mohammed *et al.*, 2021). This tree is endowed with a great socio-economical and ecological impact (Bellefontaine *et al.*, 2010). The argan oil is considered among the main product obtained from the plant kernels and is well known worldwide for its nutritional value (Mechqoq *et al.*, 2021b), which gives the oil a large use not only for its nutritional value but also for its various health benefits (Boukhobza and Pichon-Prun, 1988). Traditionally, the oil constitutes the main element in the different gastronomic preparations of the Amazigh population living in the southwest of Morocco. In cosmetics, the oil is used as a moisturizer and for the care of different body parts and hair (Charrouf *et al.*, 2002; Moukal, 2004; Charrouf and Guillaume 2010). Recently, the argan oil was found to be endowed with important biological activities especially, antidiabetic (Daoudi *et al.*, 2020; Kamal *et al.*, 2021), anticancer (Driissi *et al.*, 2006), and anti-inflammatory activity (Kamal *et al.*, 2019). These different virtues and benefits of argan oil could be attributed to its richness in tocopherols, unsaturated fatty acids (oleic acid), and phenolic compounds (Marfil *et al.*, 2011). The chemical composition of the argan oil is essentially formed by two fractions, a saponified fraction which represents 99% and is characterized by the presence of a high amount of unsaturated fatty acids such as oleic acid (45–50%) and linoleic acid (30–35%) (El Kharrassi *et al.*, 2018; Zaaboul *et al.*, 2018). While, the unsaponified fraction represents 1% and is formed by chemical components such as polyphenols, sterols, and tocopherols (Charrouf and Guillaume, 2008). The global demand for the oil increases and this is due to the different virtues and nutritional quality, but parallelly this species knows a regression, despite its high tolerance to the extreme climate as well as its plasticity towards the climatic conditions. This is caused mainly by the climatic changes, overgrazing as well as overexploitation, which implies the conservation of the population of the argan tree by increasing the reforestation and introduction of this species at a large scale.

This work is a preliminary study which aims to characterize the phytochemical variability and to evaluate the physicochemical properties of oils obtained from argan different genotypes introduced to Oujda City in comparison to some native trees of eastern Morocco (Beni-Znassen Mountain range) and those southwest of Morocco (Agadir).

2 Material and methods

2.1 Plant material

The planted argan trees in Oujda City are resulting from seeds propagation that was raised in the nursery of the faculty of sciences, Oujda until their suitability for transplantation (2 years). Then they were planted in 2011 in the different green spaces of the same faculty, and in Oujda City. The introduced argan trees are named as follow (O-1, O-2, O-3, O-4, O-5, O-6, O-7, O-8). The fruits were harvested during the maturation process of each genotype, and this during May and June 2020. The geographic location is depicted in Figure 1.

Six genotypes representing the argan grove of Beni-Znassen Mountains that were named as follow (CH-1, CH-2, CH-3, CH-4, CH-5, CH-6). The fruits were harvested in June 2020 and the geographic location of the studied genotypes is depicted in Figure 2.

Finally, two genotypes originating from the southwest of Morocco particularly located at the anti-atlas, near Taroudant City at the following location (AG-1: 30.6337755, –8.8271660), and (AG-2: 30.6322814, –8.8283253) the fruits were collected in June 2020.

The results drafted in Table 1 show some morphological characteristics of the studied genotypes such as the shape of the tree and the fruits.

2.2 Sampling plan

The sampling plan adopted is based on several criteria such as production, the stability of the production and rentability. A total of 100 genotypes originating from Oujda and Chouihya were followed up during 3 years and the interesting genotypes were adopted for the evaluation of their oils physicochemical parameters. The two genotypes from Agadir were chosen randomly just for the comparison.

2.3 Climatic characteristics

The climatic characteristics of the different regions are represented in Table 2. The climatic parameters collected

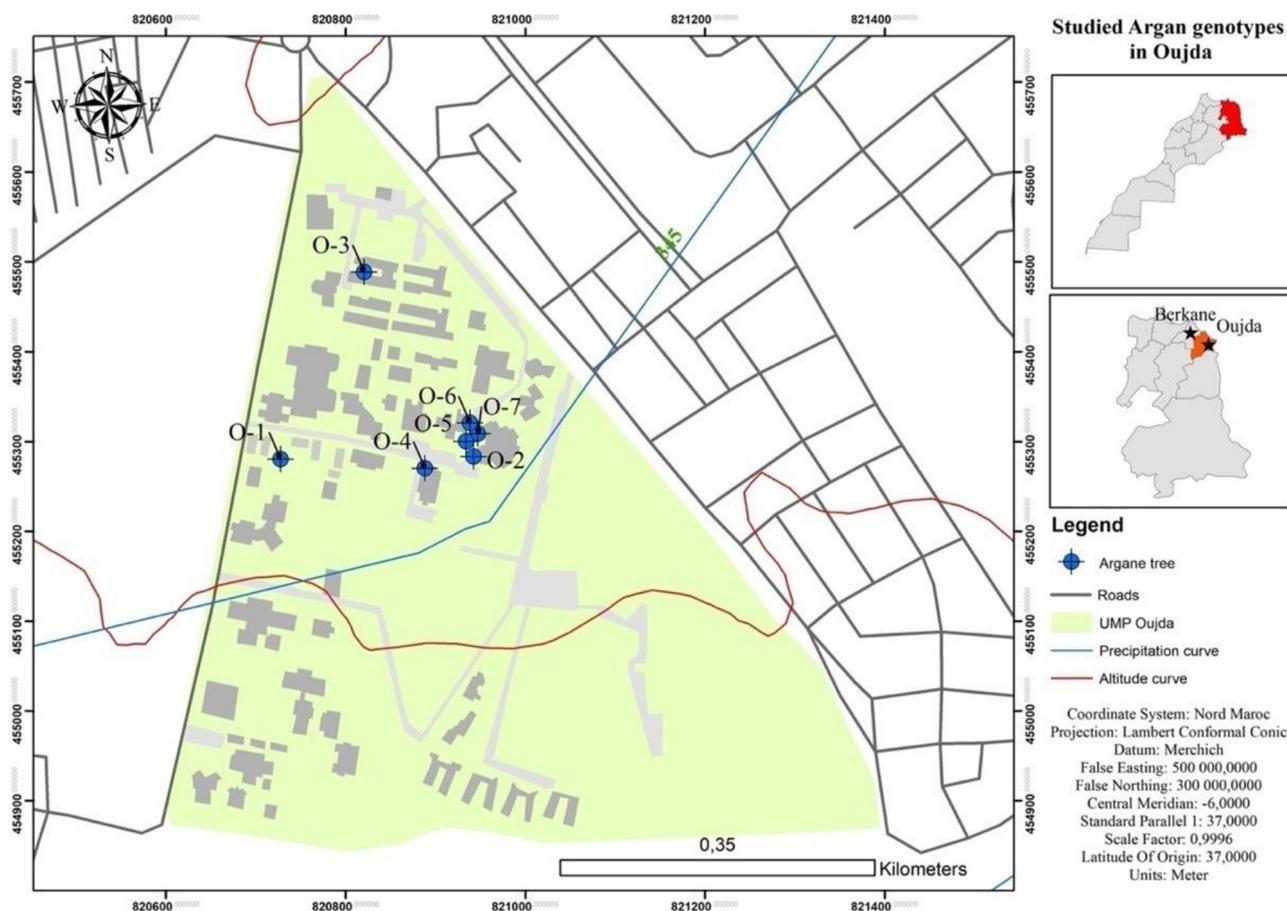


Fig. 1. Location of studied *Argania spinosa* L. Skeels tree in Oujda.

represent the average minimum, maximum temperature, annual precipitation, altitude and bioclimatic stage in the regions concerned by the study (*climatic data was obtained from Terra climate dataset which provides a climate and climatic water balance data for global terrestrial surfaces from 1958 to present*) (Abatzoglou *et al.*, 2018).

2.4 Preparation and extraction of the argan fixed oil

2.4.1 Harvesting process and almonds preparation

The mature fresh fruits of the *A. spinosa* were harvested periodically during their maturity period (May and June 2020) with about 3 kg of fresh fruits from each tree. After that, the different collected fruits were kept in a dark room to be naturally dried for approximately 10 days. Finally, the fully dried fruits were subject to followed pulping, and to crushing to extract the almonds.

2.4.2 Oil extraction

Before proceeding with the oil extraction, the almonds were put in the oven for 24 h at a temperature of 35 °C until a total dryness. After that, 50 g of the dried kernels were turned into a fine powder that was subject to a chemical extraction in a Soxhlet apparatus using n-hexane (250 ml) as an extraction solvent. The

extraction process lasted about 3 h until total exhaustion of the plant material. The rotary evaporator was used for the total elimination of the extraction solvent. Once recovered, the oils were put in opaque glass bottles of 30 ml and stored at −20 °C until further use. All experiments were carried out in triplicate and data was expressed as the mean ± SD.

2.5 Tocopherols quantification using HPLC-DAD

The different tocopherols isomers (α , δ , γ) were separated and identified using the methods described by Ben Moumen *et al.* (2015). The oils were dissolved in hexane (0.5/0.5 W/W) and was analyzed using an HPLC apparatus (Shimadzu LC-6AD system) equipped with a DAD detector. The tocopherols were separated using Uptisphere 120 Å NH₂ column (150 × 3 mm, 3 μm) Interchim (Montluçon, France), the solvent system used was hexane/2-propanol (99:1, V/V) eluted at a flow rate of 1 mL·min^{−1}, and the injection volume was 20 μL. The identification was carried out using commercial standards for tocopherols (Sigma-Aldrich, St-Louis, USA) at 292, 296, and 298 nm. The tocopherol concentration was calculated from the external calibration curve with commercial tocopherols obtained from Sigma-Aldrich (St-Louis, MO, USA). All experiments were carried out in triplicate and data was expressed as the mean ± SD.

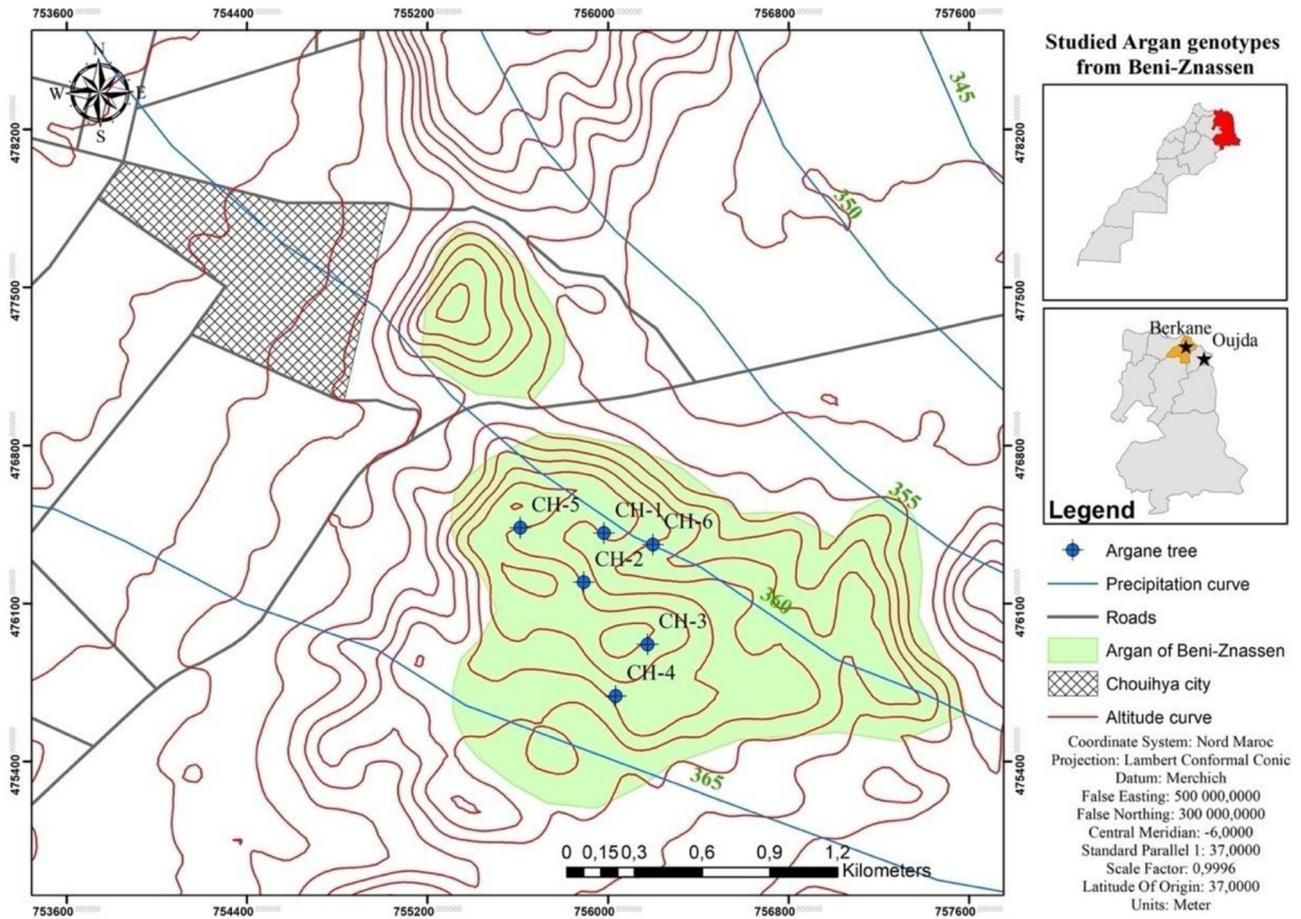


Fig. 2. Geographical location of studied *A. spinosa* L. Skeels tree in the west of Beni-Znassen Mountains.

Table 1. Morphological characteristics of the studied genotypes.

Origin	Genotypes	Age (years)	Shape	Fruits form
Oujda	O-1	10	Spreading	Spherical
	O-2	10	Upright	Oval
	O-3	10	Upright	Oval
	O-4	10	Drooping	Oval
	O-5	10	Drooping	Spherical
	O-6	10	Spreading	Oval
	O-7	10	Upright	Spherical
	O-8	10	Upright	Spherical
Chouihya	CH-1	> 50	Spreading	Oval
	CH-2	> 50	Drooping	Spherical
	CH-3	> 50	Drooping	Oval
	CH-4	> 50	Spreading	Oval
	CH-5	> 50	Spreading	Fisiform
	CH-6	> 50	Spreading	Oval
Agadir	AG-1	> 50	Spreading	Fusifform
	AG-2	> 50	Spreading	Oval

Table 2. Climatic characteristics of the study areas.

Locations	Year	TMAX (degC)	TMIN (degC)	TAVE (degC)	PPT (mm)	ALT (m)	BIOCLIMAT
Oujda	2020	23.9	11.2	17.6	230.6	618	Semi-arid (cool Winter)
Chouihya	2020	24.1	13.8	19.01	233.3	265	Arid (hot Winter)
Anti-Atlas	2020	26.1	13.7	19.9	139	839	Arid (hot Winter)

2.6 Qualitative and semi-quantitative Fatty acid analysis using GC-MS

The methyl ester preparation was assessed following the protocol of the standard NF T60-233. The esterified hexane extracts were the subject of chemical analysis using the Gaz Chromatography (Shimadzu GC-2010) supplied with a fused-silica capillary column that is directly related to mass spectrometer detector (GC-MS-QP2010). After the helium pressure was adjusted to 100 kPa. This was followed by a regulation of the oven temperature of 50 °C (maintained for 1 minute) initially, followed by a gradient of 10 °C/min up to 250 °C (maintained for 1 minute). Concerning the qualitative and semi-quantitative, 1 µl was taken from the original samples, then diluted in hexane (50 mg/g), and finally injected in split mode (split ratio = 50–80). The mass spectra were recorded at 70 eV (electron impact ionization mode) with an *m/z* range of 40–350 a.m.u. (rate and solvent delays were 5 s/scan and 4.5 minutes, respectively). The identification of the different obtained peaks was accomplished by the comparison of the obtained MS data with that stored at the library of the National Institute of Standards and Technology (NIST147). LabSolutions (version 2.5) was used for data collection and processing. All experiments were carried out in triplicate and data was expressed as the mean ± SD.

2.7 Physicochemical analysis

The acid value and peroxide value were determined according to the Codex Standard 210-1999 ([Alimentarius Codex, 1999](#)). Specific extinction was determined according to the official analytical method (ISO 3656:2002) ([Organisation Internationale de Normalisation, 2002](#)). All experiments were carried out in triplicate and data was expressed as the mean ± SD.

2.8 Statistical analysis

The results obtained were subjected to descriptive statistical analysis and an analysis of the variance (ANOVA), using the software “SPSS for Windows version 23” followed by the Tukey test with *post hoc* multiple comparisons threshold of 5%. The oil content and the different chemicals components such as fatty acids and tocopherols contents were subjected to hierarchical cluster analysis (HCA) and principal component analysis (PCA) using the same software. In the case of HCA, the dendrogram (tree) was produced using Ward’s method of hierarchical clustering with squared Euclidean distance between oil samples. All experiments were carried out in triplicate, and data were expressed as the mean ± SD.

Table 3. Oil content of argan seeds of the studied genotypes cultivated in three regions of Morocco.

Origin	Genotypes	Oil content (%)
Oujda	<i>O-1</i>	55.16 ± 1.10 ^g
	<i>O-2</i>	53.53 ± 0.62 ^{ef}
	<i>O-3</i>	48.23 ± 0.88 ^{ab}
	<i>O-4</i>	47.60 ± 0.55 ^a
	<i>O-5</i>	53.70 ± 0.61 ^f
	<i>O-6</i>	48.26 ± 0.56 ^{ab}
	<i>O-7</i>	53.85 ± 0.84 ^f
	<i>O-8</i>	51.86 ± 0.81 ^d
Chouihya	<i>CH-1</i>	52.00 ± 0.86 ^d
	<i>CH-2</i>	57.65 ± 0.50 ^h
	<i>CH-3</i>	53.78 ± 0.45 ^f
	<i>CH-4</i>	49.40 ± 0.47 ^{bc}
	<i>CH-5</i>	48.59 ± 0.55 ^{abc}
	<i>CH-6</i>	49.69 ± 0.78 ^c
Agadir	<i>AG-1</i>	50.29 ± 0.47 ^d
	<i>AG-2</i>	52.43 ± 0.49 ^{de}

Values followed by different letters are significantly different ($P < 0.05$).

Values with the same letters indicate no significant difference ($P > 0.05$).

3 Results

3.1 Oil content

The oil content of the different samples is depicted in [Table 3](#), and it varies between 47.61%, and 57.65% according to the genotypes and the origins. The genotype named CH-2 was found to have the highest oil content, while the O-6 Oujda genotype recorded the lowest oil content of about 48.23%.

Regarding the average of the oil content for each region, it was registered 51.85% for the genotypes originated from Chouihya followed by those from Oujda with a value of 51.52%. Finally, the Agadir genotypes oil content was 51.36%.

The statistical analysis of the variance showed the existence of a significant difference between the different genotypes (< 0.05), while no significant difference was noticed between the regions ($p > 0.05$).

3.2 Physicochemical parameters

The spectrophotometric examination allowed us to calculate the specific extinction at 230 and 270 nm, and also

Table 4. Specific extinction, free acidity (%) and peroxide index (Meq O₂/kg) of argan seeds oil of the studied genotypes cultivated in three regions of Morocco.

Genotype	K232	K270	Δ K	Free acidity (%)	Peroxide index
O1	1.10 ± 0.24 ^{ab}	0.11 ± 0.00 ^a	0.00 ± 0.00 ^a	0.27 ± 0.00 ^c	3.30 ± 0.15 ^f
O2	0.95 ± 0.04 ^a	0.11 ± 0.00 ^a	0.00 ± 0.00 ^a	0.29 ± 0.00 ^c	2.29 ± 0.1 ^{de}
O3	0.95 ± 0.01 ^a	0.19 ± 0.00 ^{bc}	0.00 ± 0.00 ^a	0.12 ± 0.01 ^a	1.42 ± 0.10 ^{ab}
O4	0.98 ± 0.06 ^a	0.10 ± 0.00 ^a	0.00 ± 0.00 ^a	0.27 ± 0.00 ^c	2.60 ± 0.07 ^e
O5	0.88 ± 0.08 ^a	0.09 ± 0.00 ^a	0.00 ± 0.00 ^a	0.55 ± 0.02 ^e	1.19 ± 0.06 ^a
O6	1.00 ± 0.05 ^a	0.21 ± 0.00 ^{bc}	0.00 ± 0.00 ^a	0.54 ± 0.02 ^{ef}	1.07 ± 0.04 ^a
O7	0.95 ± 0.04 ^a	0.11 ± 0.00 ^a	0.00 ± 0.00 ^a	0.15 ± 0.00 ^b	1.58 ± 0.06 ^{bcd}
O8	0.99 ± 0.05 ^a	0.17 ± 0.00 ^b	0.00 ± 0.00 ^a	0.40 ± 0.00 ^d	1.15 ± 0.10 ^a
CH1	1.03 ± 0.08 ^a	0.18 ± 0.01 ^{bc}	0.00 ± 0.00 ^a	0.54 ± 0.00 ^e	1.50 ± 0.08 ^{abc}
CH2	0.88 ± 0.04 ^a	0.17 ± 0.00 ^b	0.00 ± 0.00 ^a	0.53 ± 0.00 ^e	1.32 ± 0.20 ^{ab}
CH3	1.00 ± 0.04 ^a	0.19 ± 0.00 ^{bc}	0.00 ± 0.00 ^a	0.40 ± 0.00 ^d	1.45 ± 0.14 ^{ab}
CH4	1.14 ± 0.01 ^{ab}	0.16 ± 0.00 ^b	0.00 ± 0.00 ^a	0.51 ± 0.00 ^e	1.36 ± 0.05 ^{ab}
CH5	1.04 ± 0.06 ^a	0.21 ± 0.00 ^{cd}	0.00 ± 0.00 ^a	0.54 ± 0.01 ^e	2.17 ± 0.09 ^{de}
CH6	0.92 ± 0.04 ^a	0.17 ± 0.00 ^b	0.01 ± 0.00 ^a	0.54 ± 0.01 ^e	2.07 ± 0.12 ^{cde}
AG1	1.36 ± 0.06 ^b	0.12 ± 0.00 ^a	0.00 ± 0.00 ^a	0.64 ± 0.01 ^f	1.27 ± 0.09 ^{ab}
AG2	1.03 ± 0.13 ^a	0.23 ± 0.02 ^d	0.01 ± 0.00 ^a	0.54 ± 0.00 ^e	1.49 ± 0.15 ^{abc}

Values followed by different letters are significantly different ($P < 0.05$).

Values with the same letters indicate no significant difference ($P > 0.05$).

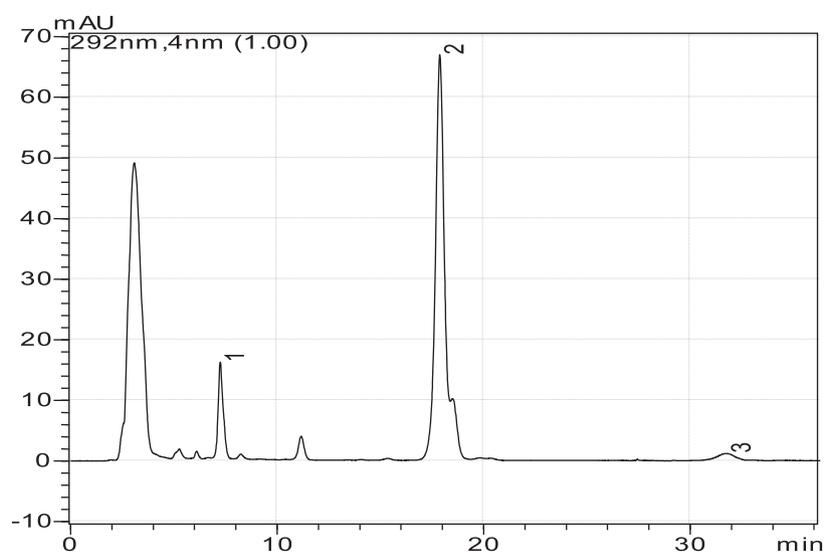


Fig. 3. Example of the chromatogram of the *A. spinosa* seeds oil tocopherols obtained using the HPLC-DAD. (1: α-tocopherol; 2: γ-tocopherol; 3: δ-tocopherol).

to calculate ΔK of the oils produced by the different genotypes. The obtained results are depicted in Table 4.

Concerning the specific extinction at 232 nm, the results showed a maximum of 1.362 for AG-1 and a minimum of 0.882 for CH-2. Concerning the specific extinction at 272 nm the values were ranging from 0.09 (O-5) to 0.226 (AG-2). Finally, the ΔK corresponding to the difference of the specific extinction registered a maximum value of 0.01 by AG-2 and CH-6 genotypes.

Regarding the free fatty acids content, it was found to vary between 0.12% (O-3) to 0.64% (AG-1). As depicted in Table 3, the values obtained for the peroxide index were ranging from 1.07 recorded by O-6 and 3.30 meq O₂/kg by O-1.

The statistical analysis indicated the existence of a significant difference between the different studied genotypes. It was also showed the existence of homogenous subgroups ($p < 0.05$), but these values are still under the threshold established by the Moroccan standard concerning argan oil (NM 08.5.090) (Moroccan Standard, 2003).

Table 5. Tocopherols content (mg/kg) of different *A. spinosa* seeds oil of the studied genotypes cultivated in three regions of Morocco.

Genotypes	α -tocopherol (tr:10.5)	γ -tocopherol (tr:18.9)	δ -tocopherol (tr:22.5)	Total tocopherols
O1	41.83 \pm 0.39 ^{cde}	447.66 \pm 4.54 ^{gh}	37.74 \pm 1.53 ^f	527.24 \pm 6.48 ^{def}
O2	48.34 \pm 0.36 ^{ef}	427.17 \pm 10.01 ^{fgh}	27.63 \pm 0.21 ^c	503.14 \pm 9.43 ^{de}
O3	25.72 \pm 0.6 ^a	285.66 \pm 18.00 ^a	43.57 \pm 1.67 ^g	354.96 \pm 20.29 ^a
O4	48.13 \pm 0.57 ^{ef}	247.25 \pm 10.61 ^a	28.47 \pm 0.17 ^{cd}	323.86 \pm 11.37 ^a
O5	44.35 \pm 0.12 ^{de}	404.19 \pm 5.54 ^{def}	45.09 \pm 0.69 ^g	493.64 \pm 6.36 ^{cde}
O6	39.81 \pm 0.01 ^{cd}	435.26 \pm 14.73 ^{fgh}	34.14 \pm 0.77 ^{ef}	509.22 \pm 13.97 ^{def}
O7	44.37 \pm 1.50 ^{de}	442.58 \pm 6.23 ^{fgh}	19.72 \pm 0.68 ^a	506.69 \pm 8.43 ^{d^{ef}}
O8	44.53 \pm 1.05 ^{de}	346.46 \pm 14.18 ^{bc}	36.27 \pm 1.41 ^{ef}	427.26 \pm 11.71 ^b
CH1	57.69 \pm 3.01 ^{gh}	446.03 \pm 14.79 ^{gh}	28.22 \pm 1.55 ^c	531.94 \pm 19.37 ^{ef}
CH2	44.92 \pm 1.43 ^{de}	366.88 \pm 9.02 ^{bcd}	18.62 \pm 1.54 ^a	430.42 \pm 11.99 ^b
CH3	46.8 \pm 0.27 ^e	380.08 \pm 3.21 ^{bcd^e}	27.35 \pm 0.06 ^c	454.24 \pm 3.55 ^{bc}
CH4	63.7 \pm 2.15 ^h	465.04 \pm 24.33 ^h	24.36 \pm 1.98 ^{bc}	553.12 \pm 28.47 ^f
CH5	36.34 \pm 2.17 ^b	416.65 \pm 3.36 ^{efg}	27.65 \pm 1.46 ^c	480.64 \pm 6.99 ^{cd}
CH6	37.17 \pm 2.37 ^{bc}	382.15 \pm 28.59 ^{cde}	32.4 \pm 1.81 ^{de}	451.74 \pm 32.78 ^{bc}
AG1	42.59 \pm 4.19 ^{cde}	373.73 \pm 6.7 ^{bcd}	36.65 \pm 0.83 ^f	452.99 \pm 11.73 ^{bc}
AG2	54.47 \pm 3.51 ^{fg}	341.61 \pm 1.513 ^b	21.41 \pm 2.56 ^{abc}	417.5 \pm 7.59 ^b

Values followed by different letters are significantly different ($P < 0.05$).

Values with the same letters indicate no significant difference ($P > 0.05$).

3.3 Tocopherols content

The HPLC-DAD analysis showed that argan oils resulting from different genotypes of the three studied regions are rich in tocopherols and are composed mainly of 3 isomers (Fig. 3 and Tab. 5). γ -tocopherols are found to be the most abundant, followed by α -tocopherols, and δ -tocopherols.

The total tocopherols content varies between 553.12 \pm 28.47 mg/kg and 323.86 \pm 11.37 mg/kg. Concerning the quantity of the different isomers. As noted, the γ -tocopherols have the largest part and they vary between 465.04 \pm 23.33 mg/kg and 247.25 \pm 1.61 mg/kg followed by the α -tocopherols with a quantity that varies between 63.7 \pm 2.15 mg/kg and 25.72 \pm 0.60 mg/kg. Finally, the δ -tocopherols had the smallest amount of all identified tocopherols and vary among 45.09 \pm 0.69 and 18.62 \pm 1.54 mg/kg.

Concerning the genotype, the CH-4 showed the highest content of total tocopherols, α -tocopherols, and γ -tocopherols. While O-5 was the richest one with the δ -tocopherols. On the other hand, 5 introduced genotypes have shown higher total tocopherols content, which was superior to 500 mg/kg, (O-1; O-2; O-6; O-7).

The ANOVA variance analysis indicated the existence of a statistical difference between the different genotypes ($p < 0.05$). On the other hand, *post hoc* multiple comparisons allowed the differentiation of homogenous subgroups.

3.4 Fatty acids composition

The GC-MS analysis drafted in Table 6 revealed the existence of four main fatty acids in the different analyzed samples (palmitic acid, linoleic acid, oleic acid, and stearic acid).

The oleic acid was the most dominant compound with a percentage ranging from 41.14% to 55.40% followed by the

linoleic acid that varies between 26.75% and 36.92%. While the palmitic acid percentage varies between 9.97% and 18.69%. Finally, the stearic acid was less found in the different samples with a percentage of 3.68% to 12.09%. On the other side, the richness of the oil samples with oleic and linoleic acids is translated by the increase of the unsaturated fatty acid percentage, which is over 70% for all tested samples with a maximum value of 83.88%. Regarding the saturated fatty acids, the percentage was ranging between 16.16% and 25.79%.

The genotype CH-2 has recorded the highest percentage of oleic acid, while the highest rate of linoleic acid was recorded in O-6, O-8, and O-7 genotypes. In addition, the genotypes named O-7, and O-4 had respectively the highest amounts with the palmitic acid rate. Finally, the highest percentage of palmitic acid was registered by the genotype named AG-1.

The statistical analysis demonstrated the existence of a significant difference between the different studied genotypes ($p < 0.5$). Also, the different genotypes were classed into subgroups.

3.5 PCA and HCA analysis

The different identified chemical compounds using the GC-MS were subject to hierarchical cluster (HCA) followed by principal component analysis (PCA) to determine the existing relation between the different chemicals parameters, and to illustrate the existing chemical variability between all studied samples.

The results drawn in Figures 4A and 4B revealed that the (PC1) and (PC2) represented 52.28% of the total variance. The first component (PC1) accounted for 32.63% of the total variance and was correlated positively with the γ -tocopherols, total tocopherols, α -tocopherols, linoleic acid, and oil content, while it was found to be correlated negatively with the

Table 6. Main fatty acids composition of argan seeds oil of the studied genotypes cultivated in three regions of Morocco (% of the main fatty acids).

	Palmitic acid	Linoleic acid	Oleic acid	Stearic acid	UFA	SFA
O-1	14.41 ± 0.01 ^f	30.54 ± 0.01 ^f	49.31 ± 0.02 ^k	5.73 ± 0.02 ^d	79.85 ± 0.03 ⁱ	20.14 ± 0.03 ^d
O-2	14.50 ± 0.10 ^f	30.95 ± 0.05 ^g	48.12 ± 0.05 ^j	6.41 ± 0.10 ^f	79.07 ± 0.00 ^{gh}	20.92 ± 0.004 ^{ef}
O-3	13.91 ± 0.03 ^e	31.23 ± 0.05 ^h	47.91 ± 0.01 ^j	6.93 ± 0.06 ^g	79.15 ± 0.05 ^h	20.84 ± 0.01 ^e
O-4	17.69 ± 0.03 ^j	30.58 ± 0.05 ^f	45.67 ± 0.07 ^d	6.039 ± 0.03 ^e	76.26 ± 0.03 ^c	23.73 ± 0.03 ^j
O-5	17.03 ± 0.33 ⁱ	30.51 ± 0.13 ^f	46.67 ± 0.25 ^{fg}	5.77 ± 0.13 ^d	77.18 ± 0.21 ^e	22.81 ± 0.21 ^h
O-6	13.08 ± 0.06 ^c	36.92 ± 0.03 ^k	44.86 ± 0.08 ^c	5.11 ± 0.05 ^b	81.79 ± 0.1 ^j	18.20 ± 0.10 ^c
O-7	18.69 ± 0.02 ^k	32.06 ± 0.00 ^j	43.81 ± 0.02 ^b	5.42 ± 0.04 ^c	75.88 ± 0.02 ^b	24.11 ± 0.02 ^k
O-8	15.72 ± 0.06 ^g	35.91 ± 0.05 ^k	41.14 ± 0.05 ^a	7.21 ± 0.00 ^{hi}	77.05 ± 0.07 ^e	22.94 ± 0.07 ^h
CH-1	9.97 ± 0.04 ^a	31.38 ± 0.07 ^h	51.58 ± 0.11 ^k	7.04 ± 0.04 ^{gh}	82.97 ± 0.08 ^k	17.02 ± 0.08 ^b
CH-2	12.42 ± 0.05 ^b	28.47 ± 0.04 ^b	55.40 ± 0.02 ^m	3.68 ± 0.05 ^a	83.88 ± 0.05 ^k	16.11 ± 0.05 ^a
CH-3	16.96 ± 0.04 ⁱ	29.76 ± 0.03 ^e	46.17 ± 0.02 ^e	7.09 ± 0.07 ^{gh}	75.93 ± 0.02 ^b	24.06 ± 0.02 ^k
CH-4	15.70 ± 0.04 ^g	29.45 ± 0.01 ^d	49.43 ± 0.00 ^k	5.41 ± 0.05 ^c	78.88 ± 0.01 ^g	21.11 ± 0.01 ^f
CH-5	14.29 ± 0.03 ^f	31.68 ± 0.05 ⁱ	46.42 ± 0.02 ^{ef}	7.59 ± 0.04 ^k	78.10 ± 0.07 ^f	21.89 ± 0.07 ^g
CH-6	16.52 ± 0.04 ^h	29.25 ± 0.02 ^c	46.72 ± 0.10 ^g	7.50 ± 0.06 ^{jk}	75.97 ± 0.10 ^b	24.02 ± 0.10 ^k
AG-1	13.58 ± 0.02 ^d	26.75 ± 0.12 ^a	47.57 ± 0.07 ⁱ	12.09 ± 0.05 ^l	74.32 ± 0.05 ^a	25.67 ± 0.05 ^l
AG-2	15.94 ± 0.06 ^g	29.64 ± 0.04 ^{de}	47.04 ± 0.11 ^h	7.36 ± 0.05 ^{ij}	76.68 ± 0.08 ^d	23.31 ± 0.08 ⁱ

UFA: Unsaturated fatty acids SFA: saturated fatty acid.

Values followed by different letters are significantly different ($P < 0.05$) values with same letters indicate no significant difference ($P > 0.05$).

δ -tocopherols and the palmitic acid content. On the other side, the second principal component (PC2) accounted for 19.65% was found to be correlated positively with linoleic acid, γ -tocopherols. While correlated negatively with stearic and oleic acids.

Regarding the genotypes distribution (Fig. 4), the O-6 and O-5 were positively aligned to the axis of the second component, which is strongly correlated to linoleic acid and δ -tocopherols, these genotypes were found to be the richest in linoleic acid and δ -tocopherols. On the counterpart, the genotypes AG-1, AG-2, and CH-3 were the richest in stearic acid.

The genotypes CH-1, O-2, O-1, CH-4, and CH-2 were positively aligned to the axis of the first component, which highlights their richness in α -tocopherols, total tocopherols; oleic acid or high oil content, and low content of palmitic and stearic acids. On the other hand, the genotypes O-3, and O-4 are negatively aligned to the axis of the first component and are characterized by low oil content, as well as a content of total tocopherols and γ -tocopherols that is very low.

The dendrogram drafted in Figure 5 produced by hierarchical cluster analysis based on Euclidean distances shows that the different genotypes can be classified into 6 main clusters (at a distance of 5 units), which shows that there is a significant difference in the chemical composition of *A. Spinosa* genotypes. The samples linked by short distances are more similar than those connected by large ones.

The first cluster is represented by 4 genotypes (O-1, O-2, CH-4, and CH-1). These genotypes are characterized by an interesting oil content (up to 55%) as well as a high content of α -tocopherols (≤ 67 mg/kg), γ -tocopherols (> 400 mg/kg) and important total-tocopherols content (> 520 mg/kg). Finally, their oleic acid content was high (48–51%).

CH-2 sample was found to construct the second cluster. This latter is characterized by a very high oil content (57%) as well as a very high rate of oleic acid (55%) against a moderate content of tocopherols-totals (< 500 mg/kg) with the lowest rate of linoleic acid and stearic acid respectively 28% and 3%. On the other hand, the third cluster is represented by 3 genotypes (O-5, O-7, and CH-5), which are characterized by a high percentage of palmitic acid (up to 17%) and a moderate to a high content of total tocopherols (480 to 500 mg/kg).

The fourth cluster is composed of 2 genotypes from Oujda (O-6, O-8), these genotypes have the highest values of linoleic acid (35–36%). The two genotypes constituting the fifth group (O-4, O-3) are characterized by a very low oil content ($< 49\%$) and a very low content of γ -tocopherols (< 300 mg/kg), as well as a high percentage of stearic acid.

Regarding the sixth cluster, which includes 4 genotypes (CH-3, CH-6, AG-1, and AG-2), this cluster is characterized by a low level of linoleic acid (29%) and a content of total tocopherols below 400 mg/kg.

Finally, the HCA analysis has identified similar genotypes of the 2 regions where the argan tree grows wild (Cluster 6: CH-3, CH-6, AG-1, AG-2), And 2 introduced genotypes (O-1, O-2,) was found to have a comparable composition and oil content to the argan grown in Beni-Znassen (CH-1, CH-4) and have been grouped in the same cluster. While the genotype CH-2 was found to have the best oil and oleic acid yield.

3.6 Statistical correlation

Table 7 presents the statistical correlation between the fatty acids, and tocopherols found in the chemical composition of the oil, and the climatic parameters recorded during the 6 months before fruits harvesting of the three studied areas

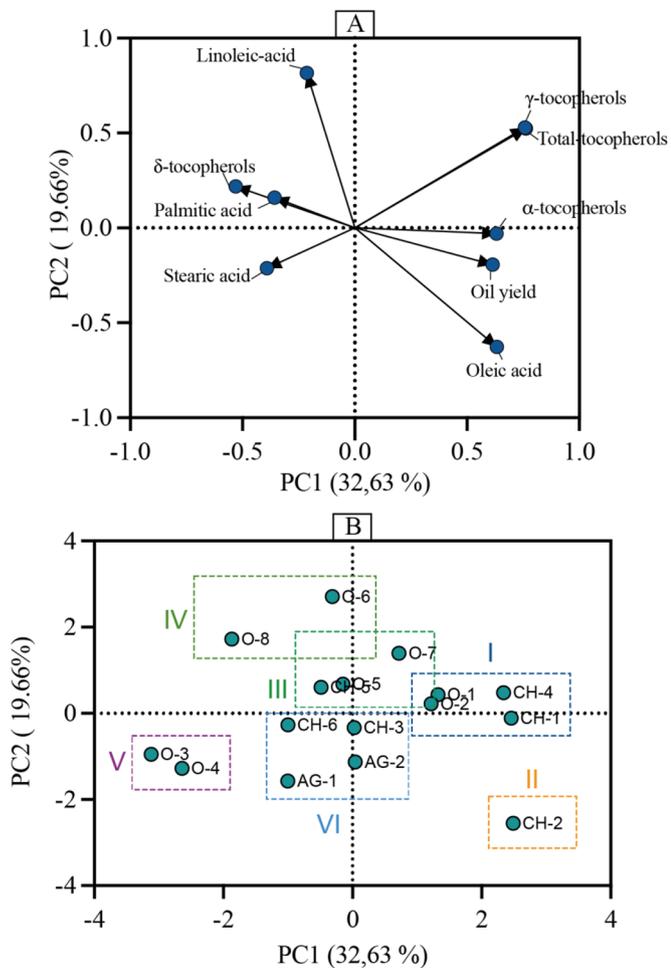


Fig. 4. Graphical representation of principal component analysis of chemical composition argan oil. (A) 2D distribution of variables and (B) 2D genotypes distribution.

(Oujda, Beni-Znassen, Anti-atlas), also the altitude was taken into consideration.

The collected results indicated the existence of a statistical correlation between almost all of the studied parameters.

The T max registered correlated negatively with the δ-tocopherol content, total tocopherols content ($p < 0.05$), and the linoleic acid ($p < 0.01$), and found to correlate positively with the percentage of stearic acid ($p < 0.01$). On the other side, the T min noted correlated negatively with the δ-tocopherol content, and also with the percentage of the linoleic acid ($p < 0.01$), and positively with the Oleic acid ($p < 0.01$) and the α-tocopherols ($p < 0.05$). Concerning the precipitations (PPT), a positive correlation was observed with the γ-tocopherols, δ-tocopherols, total tocopherols content ($p < 0.05$), and the linoleic acid ($p < 0.01$), on the other part, a negative correlation was observed with the stearic acid ($p < 0.01$). The mean temperature (TAVE) correlated negatively with the δ-tocopherols content, the linoleic acid content, and positively with the oleic and stearic acids ($p < 0.01$).

Regarding the altitude, a low correlation with the different compounds except for a positive correlation with linoleic acid and a negative one with oleic acid ($p < 0.01$), which indicated

that the linoleic acid percentage increases with the increase of the altitude, while the oleic acid decreases with the increase of this latter.

4 Discussion

In the present study, we have proceeded to an evaluation of the physicochemical quality and chemical composition of the different oils produced by the different genotypes in three regions of Morocco.

Regarding the physio-chemical quality of the extracted oils, it was observed that there is a significant difference between the genotypes, this finding was reported by several studies that indicated that the physicochemical quality of the oil varies according to several factors such as genotype, origin, harvest date, and extraction temperature (Seiquer *et al.*, 2015; El Kharrassi *et al.*, 2018; Mechqoq *et al.*, 2021b; Ouchbani *et al.*, 2021). Concerning the specific extinction (232 nm, 272, and ΔK) and peroxide index, results registered by the different samples were inferior to the limit fixed by the national norm related to the argan extra virgin oil (NM 08.5.090) and the same for the acidity index (Moroccan Standard, 2003; Rahmani, 2005). These different parameters enable the measurement of the primary and secondary oxidation compounds such as the conjugated dienes and trienes (Ouchbani *et al.*, 2021), and the presence of these elements in high quantities prove the low quality of the samples and it is translated by a low aptitude for conservation (Boulfane *et al.*, 2015).

Concerning the fatty acids profile, the GC-MS analysis of the methyl esters indicated the presence of four principal fatty acids in the different tested samples. These compounds are mainly predominated with oleic acid, followed by linoleic, palmitic acids, and finally the stearic acid. These results were in accordance with those reported in previous studies (Gharby *et al.*, 2011; Taribak *et al.*, 2013; El Kharrassi *et al.*, 2018; Zaaboul *et al.*, 2018), who have confirmed that oleic acid is a fatty acid abundant in the argan oil (43.1–57.0%) followed by linoleic acid (29.30–36.00%), palmitic acid (5.00–15.0%) and finally the stearic acid (4.30–12%). Other fatty acids were detected as a trace by Kharbach *et al.* (2018, 2019) such as linolenic acid, arachidic acid, and myristic acid. On the other side, our results are in contradiction with the results obtained by El Adib *et al.* (2015) who have found that the level of linoleic acid is higher than that of oleic acid.

The PCA showed that the oleic acid correlated negatively with the linoleic and palmitic acids, which means that the elevation of the first is related to the decrease of the second or the opposite. These results are in accordance with the results published by Chergui *et al.* (2021), who have demonstrated that the level of oleic acid increases according to the maturity of the fruits. The difference in fatty acid composition observed between the different genotypes could be explained by a difference in fruits maturity. In fact, Chergui *et al.* (2021) have observed a difference in the precocity of flowering and therefore in the ripening of the fruits. On the other hand, this finding seems to be natural regarding the biosynthesis way of the long-chain fatty acids in the oleaginous plants, which starts with palmitic acid converted to stearic acid under the effect of elongation. After that the stearic acid is converted to oleic acid

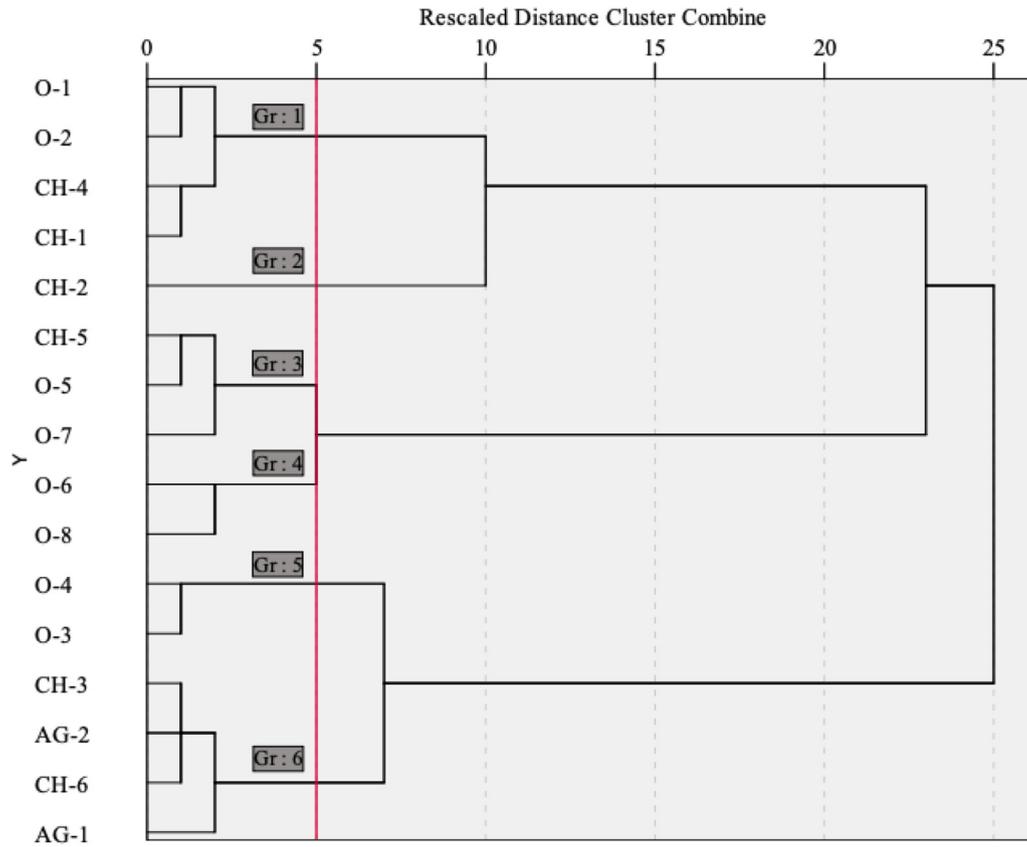


Fig. 5. Dendrogram of different argan genotypes produced by the hierarchical cluster analysis.

Table 7. Correlation matrix between the climatic and chemical settings.

	α -tocopherols	γ -tocopherols	δ -tocopherols	Total-tocopherols	Palmitic acid	Linoleic acid	Oleic acid	Stearic acid	Unsaturated	Saturated
TMAX	-0.026	-0.345	-0.344*	-0.366*	-0.052	-0.435**	0.003	0.667**	-0.398**	0.398**
TMIN	0.311*	0.09	-0.48**	0.073	-0.288	-0.529**	0.482**	0.23	0.081	-0.0814
PPT	0.024	0.344*	0.346*	0.364*	0.0548	0.438**	-0.006	-0.667**	0.397**	-0.397**
TAVE	0.264	-0.028	-0.528**	-0.049	-0.269	-0.599**	0.424**	0.410**	-0.053	0.0532
Altitude	-0.196	-0.06	0.304	-0.049	0.265	0.405**	-0.394**	-0.186	-0.091	0.091

Values followed by * $P < 0.05$ and ** $P < 0.01$.

using $\Delta 9$ -desaturase and finally using $\Delta 12$ -desaturase the oleic acid is converted to linoleic acid. All this means that the increase of each fatty acid implies the decrease of its precursor (Metougui, 2017).

The tocopherols exist naturally in four forms α , β , γ , δ -tocopherols, and are characterized by a high antioxidant activities which play a crucial role in the anti-stress mechanisms of the plant, meanwhile the presence of these compounds contribute to the conservation of the oil (Rey *et al.*, 2021), and the preservation of the integrity of the cell membrane and its components against various types of stress (Sadiq *et al.*, 2019).

Argan oil is very well known for its richness in tocopherols, especially the γ -tocopherols (Charrouf and

Guillaume, 2008). According to the obtained results, the γ -tocopherols were the most abundant followed by α -tocopherols, and finally the δ -tocopherols. These obtained results are in accordance with the majority of the published studies (Hilali *et al.*, 2005; Matthäus *et al.*, 2010; Gharby *et al.*, 2021; Mouahid *et al.*, 2021). On the other hand, the β -tocopherols were noted to be present by El Kharrassi *et al.* (2018) as a trace (0.12%).

On the other hand, the total tocopherols content was ranging from 323.86 and 553.12 mg/kg according to the genotype and the origin. These results were in accordance with the results of Matthäus *et al.* (2010) and Hilali *et al.* (2005) who have indicated that total tocopherols content varies between 334.7 and 457.1 mg/kg and between 400 and

775 mg/kg, respectively. Mouahid *et al.* (2021) have demonstrated also that the total tocopherols content of the argan oils extracted using the Soxhlet apparatus were ranging from 412 to 553 mg/kg.

However, it was lower than that mentioned in Gharby *et al.* (2021)'s study, which has indicated that argan oil tocopherols content was ranging between 649 and 766 mg/kg and that of Taribak *et al.* (2013) with total tocopherols content of 637 mg/kg. This difference could be due to, the method of extraction used, kernels pretreatment (Mouahid *et al.*, 2021) and genotypes effect. This indicates that different settings could influence the total tocopherols content such as high temperature and intense light (Spicher *et al.*, 2016).

The variance analysis showed the existence of a significant difference between the tested samples, which indicated that the argan oil is endowed with an important diversity which was similar to the results obtained by Aithammou *et al.* (2019) and Ait Abd *et al.* (2013) who have mentioned that the argan tree is characterized by a very high genetic diversity and that the effect of the clone is very marked on the chemical characteristics of oils produced.

The genotype effect is not only responsible for the chemical composition but also the origin and the climatic conditions are influencing the chemical composition of the oil (El Monfalouti *et al.*, 2020). Our results indicated that the climatic parameters correlated significantly with the chemical composition. Similarly, Nerd *et al.* (1994) evaluated the chemical characteristics of argan oil produced in two desert locations in Israel, as well as the work of El Monfalouti *et al.* (2020) who have mentioned that the climate has affected the amount of triglycerides while the other parameters have not presented any difference between regions. In the same context Elgadi *et al.* (2021a) have demonstrated that the fatty acids composition was influenced by the altitude and latitude, a high percentage of the linoleic acid was a marker of coastal argan oil, while the high content of palmitic acid was considered to be the marker of continental argan oil. On the other hand Elgadi *et al.* (2021b) have shown that the tocopherols content of argan oil is strongly influenced by origin, the distance from the coast, and altitude. Finally, Kharbach *et al.* (2018, 2019) have allowed a geographical classification of argan oils according to chemical composition.

5 Conclusion

As a preliminary study, our results indicated that the trees introduced in Oujda City have shown a chemical composition similar to that of argan oils produced in its natural domain (Agadir and Chouihya). The majority of argan trees introduced in Oujda City have shown an important quantity of total tocopherols content higher than 500 mg/kg, as well as a rate of oleic acid up to 49.31%, and unsaturated fatty acid (81.79%). This evokes the plasticity of the argan tree to the climatic conditions of Oujda City. On the other hand, our study has showed that the argan tree is characterized by a significant variability, which can be exploited in the selection of subjects according to their areas of use. In the same sense, the adaptation of the argan tree to other regions differing in their environmental conditions will undoubtedly help to contribute to the protection and sustainability of the argan grove in

Morocco. Finally, further studies are needed to confirm these results, such as the chemical composition stability over the years, the effect of the climatic change and the interaction between genotype and environment.

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