The relationship between fatty acid, phenolic composition and antioxidant activity in argane oil: effect of ripeness

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Abstract— this study aims to evaluate the impact of maturity on the composition of argane oil, and the evaluation of the antiradical activity of these oils. Also, we studied the correlation between maturity and fatty acid composition, and polyphenol. We study the relationship between the level of fatty acids and polyphenol, present in argane oil at different levels of maturity. Further analysis of the correlation between the oil composition and the antiradical activity was examined. The results show that the composition of polyphenols and fatty acids is correlated with the age of fruit. The study of the antiradical activity shows a decrease in the ripest fruits. A strong positive correlation between the level of fatty acids and the amount of polyphenol at different stages of maturity of the fruit; and a very weak correlation appear between the flavonoids and fatty acids in the different stages of maturity. In addition, the analysis of the correlation between polyphenols, flavonoids and the antiradical activity shows that the antiradical activity is strongly correlated with the amount of polyphenol. On the other hand, the level of flavonoid not correlated with the antioxidant activity. These results provide information about the anticipated harvest-date with the purpose of having extra virgin oil that bears high nutritional and economic value, rich in antioxidant and essential fatty acid and hold the best oxidative shelf-life of the extra virgin Argane oil.

Index Terms— Argane Oil, Correlation, Fatty acid, Phenolic compound, activity antioxidant

1 INTRODUCTION

The Extra Virgin Argane Oil (EVAO) is the Moroccan noble product with special dietary and organoleptic characteristics is extracted from the kernels of fruits from the argan-tree. The Argane tree (Argania spinosa (L) Skeels) is a tree exclusively endemic to Morocco and is a vital resource of Morocco nicknamed "the tree of life" [1]. , Argane oil was used for centuries by the Berbers of the Atlas as well for its culinary properties as therapeutic and cosmetic, with more than 80% unsaturated fatty acid of the oleic-linoleic type.

Virgin edible argane oil extraction requires at least six steps: fruit collection, sun-drying, dehulling, nut breaking (or kernel collection), kernel roasting, and cold-pressing. Each processing step dramatically influences the resulting oil in terms of quantity and quality.
However, the techniques used to extract Argane oil and the state of the fruit used as well as its origin can affect the physicochemical characteristics of the extracted Argane oil [2].
The importance of the extraction method on the oil quality is already particularly well documented [2 ; 3]. The influence of fruit quality and drying-time on argan oil physicochemical parameters and quality, respectively, has also been recently reported [2 ; 3; 4]. The Origin of Virgin Argane Oil's High Oxidative Stability Unraveled.

There are different commercial categories of Virgin Argane oil (VAO) depending on their quality) obtained by purely physical means and with the physicochemical and sensorial parameters characteristic of healthy fruit and Virgin Argane oil (VAO) "delicate" also obtained by purely physical means but whose physicochemical and/or sensorial parameters of the fruits in perfect health. This last category is unfit for consumption. [5]

EVAO with its slight nutty flavor gradually shows up on European tables. In fact, it has become one of the most expensive edible oils in the world. It is even more expensive as a cosmetic product and is holding several cosmetic patents both in the USA and Europe [6]. Thanks to these special characteristics, demands of EVAO were going up continuously. EVAO'richness with a significant unsaturated fraction of omega-6 and omega 3 series, as well as with an important fraction of antioxidant compound makes it very useful in culinary treatments in addition to ensuring a good food intake. These nutritional properties are not only provided by fatty acid (FA) profile and high monounsaturated/saturated fatty acid ratio (MUFA/SFA), but also by antioxidant content, particularly that of phenols which is of assistance in the prevention of diseases [7] stability against rancidity and oxidative processes [8].

Stability of oil is affected by Such factors are: industrial processing including environmental factors during the process, contact with light beams, the level of oxygen present during

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storage, the presence of oxidation catalyst metals, the quality of fruit used during the extraction, the moisture surrounding the seeds, and the amount of heat to which the seed is exposed during roasting. [9]. The most unsaturated oils are oxidized more rapidly than the less unsaturated oils [10, 11]. Indeed, linolenic acid (C18: 3) tends to oxidize faster than linoleic acid (C18: 2). In addition, the more fatty acids contain double bonds the more they are sensitive to oxidation.

Any substance capable of suppressing, delaying or preventing the oxidation process is called an antioxidant. Thus, the antioxidant is valuable in increasing the shelf life of a food and reducing losses of vitamins and essential fatty acids. One of the most important substances of these categories is Phenol. The phenols can act as "scavengers". They can capture free radicals, in combination with peroxyl and alkoxyl radicals, and can also chelate trace metals. This property seems to be more efficient in the decarboxymethyl (DOA) and aldehydic (AOA) forms of oleuropein aglycone compared with hydroxytyrosol [12]. The phenol or Primary antioxidants, also known as antiradicals, are molecules capable of blocking lipid radicals L°, LO° and LOO° by transfer of an H°: LOO° + AOH LOOH + AO° The antioxidant then becomes itself a carrier of a radical, but unlike lipid radicals, it is not very reactive, which stops the radical propagation. This group of antiradicals is composed almost exclusively of phenolic compounds because of the great stability brought by their aromatic cycle [13].

The composition of the vegetal oil is different according to several intrinsic and extrinsic factors and the maturity of fruit is one of the main parameters that influences the quality of the fruit's composites and automatically the quality of the final product which is the oil. [14]

The purpose of this study is to determine the effect of ripeness on the composition of Argane oil and to study of the correlation between the ripeness and composition of Argane Oil; along with an examination of the relationship between the level of fatty acids and the polyphenol, flavonoid present in Argane oil in different degrees of maturity. More than that, we took a look into the relationship between the antioxidant activity and the rate of polyphenol, flavonoid with progression of the fruit maturity

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2 PROCEDURES

2.1 Sample collection

Argane fruits were collected in Taroudant region (Province in South of Morocco). Sampling was done on different dates March, April, May, June, and July. Sample swas taking into account variable stages of maturity within each tree (based on fruit pulp color).the unripe fruit with bright green color, green with yellow spots, the ripe fruit with color yellow and yellow with brown spots and the over ripe fruit with brown color. An aliquot of 100 fruits was taken from each fruit sample in order to determine its ripening index (RI) [15].The Lipid Matrices is prepared by cold pressing in the laboratory and the obtained oil was stored at temperature

2.2 Oil analyze

The phenolic compounds were extracted by a liquid liquid extraction and were performed by methanol water (80:20, v/v) (3_50 mL). After 3 successive washing with hexane (3_50 mL) after three extractions, the final extract was evaporated and stored at 4 ° C until performing analysis. [16]. Using the Folin-Ciocalteu reagent the total polyphenol is determined according to the method of [17], and the absorbance was read at 765 nm. The Total phenols were expressed on a dry weight basis as gallic acid equivalents (GAE)

The determination of total flavonoid content is sorted out according to to the method reported by [18]. The absorbance was measured by UV-visible spectrophotometer at fixed wavelength 510. The total flavonoid expressed on a dry weight basis as catechic acid equivalents (CE)

The antiradical activity of different samples (methanolic extracts) were investigated using the DPPH• (2,2-diphenyl-1-picrylhydrazyl radical-scavenging assay (RSA). The result was estimated according to the method reported by [19]

2.3 Fatty Acid Methyl Ester (FAME):

The Fatty Acid Methyl Ester was obtained of from the oil samples by shaking a solution of 60 mg oil and 3 ml of hexane with 0.3 ml of 2N methanolic potassium hydroxide. They were analyzed by (Varian CP-3800, Varian Inc.) equipped with a FID. The column used was a CP- Wax 52CB column (30 m×0.25 mm i.d.; Varian Inc., Middelburg, The Netherlands). The carrier gas was helium, and the total gas flow rate was 1 ml/min. Steps of 4 °C/min increased the initial column temperaturewas170 ° C, the final temperature 230 °C, and the temperature. The injector and detector temperature was 230°C. Data was processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA). The results were expressed as the relative percentage of each individual fatty acid (FA) presents in the sample [20]

2.4 Statistical analysis:

Statistical Analysis Values reported in tables are the means ± SD of three replications. Mean values obtained for the variables studied in the different groups were compared by a one-way ANOVA. Separation of means was performed by Tukey"s test at the 0.05 significance level. . A Pearson (r) corre-

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lation analysis and a linear regression were applied to examine the possible relationships between the variables.

3. Result and Discussion

3.1 Characterization of Phenolic and fatty acid Compounds in Argane oil in different ripeness stage

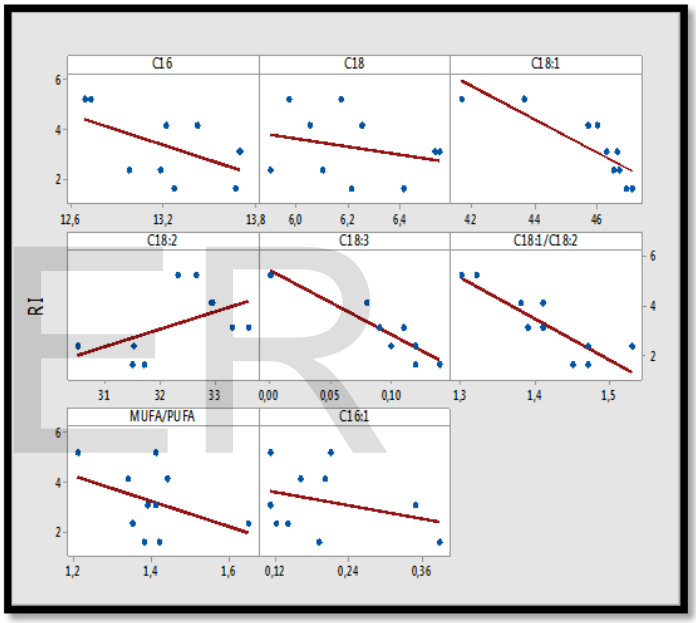
The composition and quality of EVAO depends of the compounds present in the fruit tissues. FA and phenolic compounds are important components for being beneficial to human nutrition and even to oil storage. These components are sensitive and degrading either during oil preparation, during storage or under the effect of enzymatic activity [21]

The values of the different RI of the samples of the fruits studied are (1.58, 2.3, 3.17, 4.5, 5.19) it increases with the change of color of the fruit pulp from green color to color Brown. Table 1 shows the effect of the stage of maturity on acid content of total polyphenols, total flavonoids and the antiradical power (ARP) of these components in argan oil. Table 2 shows the results of the study of the Correlation between the different RI maturity indices studied and the parameters analyzed in argane oil. While Figures 1, 2, 3 show the distribution of the different scatter plots and the equation of regression between the different (RI) fruits and the various components studied in addition to the antiradical power, in the EVAO.

Fatty acids as well as major fatty acids are (palmitic (C16: 0), stearic (C18: 0), oleic (C18: 1), linoleic (C18: 2), linolenic (C18: 3). the appearance of fatty acids shows that there is no great variation during maturation processes. In fact, saturated fatty acids (palmitic, stearic) accumulate in ripe fruits (RI = 3.17) and decrease slightly in the more mature fruits (RI = 5.19) this decrease may be due to the dilution effect (Gutierrez et al., 1999), a high Pearson coefficient is observed between RI and the palmitic acid content (r = -0.565) On the other hand, a weak correlation with RI (r = -0.264) for stearic acid.

During fruit ripening, the content of oleic (C18: 1) and linoleic (C18: 2) acids changes with the maturity of the fruits. Increased in ripe fruits (RI = 2,3, RI = 3,17), and a slight reduction at the end of lipogenesis in advanced fruit ripening (RI = 5,19). As a result, the observed changes in the ratio (C18: 1 / C18: 2), from the first harvest to the last harvest, show a downward trend during the ripening process, which is also confirmed by a good correlation negative (r = -0.870) between this ratio and the RI of argan oils. As a result, the ratio between monounsaturated and polyunsaturated fatty acids (MUFA / PUFA) tended to decrease during the maturation process, this reduction being explained by the fact that unsaturated fatty acids in addition to oleic acid during the maturation process they enter into the composition of phospholipids that form the structure of newly formed cell membranes. [22].

The amount of phenolic compounds in the oil is an important factor when evaluating its quality, as natural phenols improves its resistance to oxidation. These compounds, though minor in quantity, are nutritionally important, providing the oil with important organoleptic and nutritional characteristics giving them a creamy and melting texture with a specific flavor. The biological properties of oils, especially in unrefined oils (olive oil, argane virgin), During fruit ripening The quantitative analysis of the different phenolic extracts shows that (tab 1) the level of polyphenol and flavonoid compounds increases in ripe fruits (RI = 1.58, RI = 3.17) and its concentration increases, decreased with the physiological development of the fruit (RI = 4.4, RI = 5.19). With a strong negative correlation (tab2, fig 2), between tau polyphenols and IR (r = -0.789), a very weak correlation between RI and flavonoids (tab 2, fig 2) with a Pearson coefficient of the order of (r = 0.037). These results coincide with those observed in olive oil [23, 24] .This decrease is explained by the simple effect of dilution during the increased activity of hydrolytic enzymes. With maturation, a phenomenon observed in many fruits [25].



	Equation	R2 %
C18 :1	RI= 33,74-0,6671 C18 :1	72,8
C18 :2	RI= -19,97+0,7190 C18 :2	26,6
C16 :1	RI=4,109 - 4,310 C16 :1	9,9
C18 :3	RI= 5,43-25,88 C18 :3	85,8
C16	RI=30,12-2,027 C16	31,9
C18	RI=13,09- 1,58 C18	7
MUFA /PUFA	RI=10,4-5,1 MUFA /PUFA	17,2
C18 :1/C18 :2	RI=26,72-16,61C18 :1/C18 :2	75,6

Figure 1: The relationship between fatty acid composition (FA) and maturity index (RI) in Argane oil

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TABLE 1 : EFFECT OF MATURITY ON DIFFERENT PARAMETERS ANALYSIS IN ARGANE OIL

Dates	march	april	may	june
	july			
RI	1,58	2,3	3,17	4,5	5,19
C18 :1	46,44±0,2a	46,58±0,14a	46,99±0,14a	45,83±0,18a	42,66±1,41b
C18 :2	31,61±0,14bc	31,02±0,7c	33,45±0,21a	32,94±0,01ab	32,48±0,23ab
C16 :1	0,29±0,14a	0,13±0,01a	0,23±0,17a	0,18±0,02a	0,16±0,07a
C18 :3	0,13±0,01a	0,11±0,014ab	0,1±0,001ab	0,08±0,00b	0,00±0,00 c
C16	13,47±0,28a	13,08±0,14ab	13,69±0,007a	13,32±0,14ab	12,71±0,028b
C18	6,31±0,14ab	6±0,141b	6,54±0,01a	6,15±0,14ab	6,07±0,14ab
MUFA /PUFA	1,47±0,02a	1,5±0,21a	1,4±0,014a	1,39±0,07a	1,31±0,14a
C18 :1/C18 :2	1,46	1,5	1,4	1,39	1,31
PT	41,8±0,14b	42,3±0,17b	50,5±0,212a	25,9±0,01c	21,3±0,28d
FT	1,17±0,01a	1,2±0,35a	1,28±0,01a	1,5±0,14a	1,07±0,07a
ARP	1,12±0,1	1,85±0,014	1,2±0,14	0,98±0,01	0,6±0,15

Values are expressed as means (SD), values followed by different letters (a, b, c, d) are significantly different at P≤0.05; IC = 95%; ; RI: maturity index C16: 0 palmitic, C16: 1 palmitoleic, C18: 0 stearic, C18: 1 / C18: 2: ratio of oleic acid to linoleic acid PT: total polyphenols; FT: total flavonoid; ARP: the antiradical power C18: 2 linoleic, C18: 3 linolenic, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

TABLE 2: CORRELATION BETWEEN THE DIFFERENT PARAMETERS ANALYZED BETWEEN RI AND ARP IN ARGANE OIL

	RI	ARP
RI	1	-0,655*
ARP	-0,655*	1
PT	-0,789*	0,494
FT	0,037	-0,349
C18 :1	-0,853*	_____
C18 :2	0,516	_____
C16 :1	-0,314	
C18 :3	-0,926*	_____
C16	-0,565	_____
C18	-0,264	_____
MUFA /PUFA	-0,415	_____
C18 :1/C18 :2	-0,870*	_____

3.2 The antioxidant activity and the phenolic composition of argane oil

Polyphenols are bioactive molecules with antioxidant activity present in unrefined vegetable oils. They are responsible for preventing the auto-oxidation of unsaturated fatty acids, which make this oil particularly resistant to oxidation during storage. In addition, the higher levels of phenolic substances present in Vegetable oil can improve its nutritional properties and contribute to well-known positive effects on health. ARP is the analytical method applied to phenolic extract to evaluate oxidative phenomena, as shown by (tab1, fig2), the antioxidant activity indicated by the ARP index seems to follow a trend of decreasing trend, from its high value from 1.12 at the first stage of maturity to 0.6 at the date of the last harvest with an increase of 1.85 for fruits with an IR = 2.3. This trend was corroborated by the negative correlation (r = -0.6550). Antioxidant activity as measured by the reduction of DPPH has been proposed as an index to differentiate extra virgin oils [26]. This trend is explained by the loss of natural antioxidants (phenols), as indicated previously. These results are in agreement with those of other authors [23].

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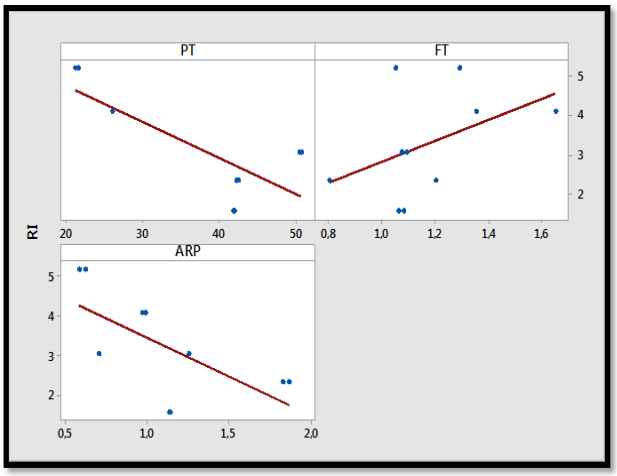
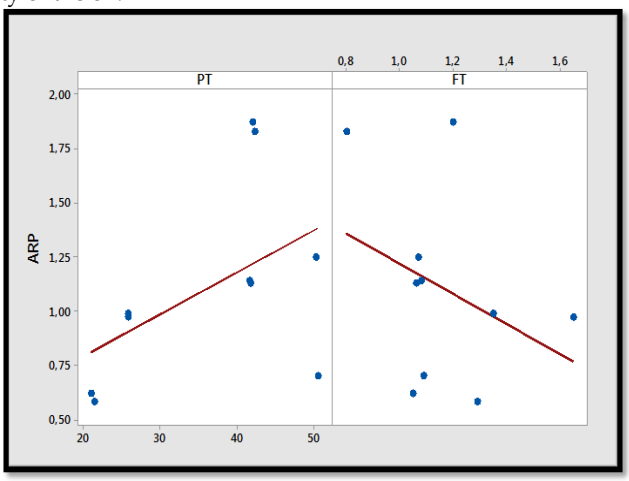


	equation	R2%
ARP	RI= 5,413-1,946 ARP	42,8
PT	RI= 6,591-0,0917 PT	62,2
FT	RI=0,163+2,657 FT	20,2

Figure 2: The Relationship between RI and Polyphenol (PT), Flavonoids (FT), ARP in Argane Oil

In fact, analysis of statistical data, highlighting positive correlations between the ARP and PT values (r = 0.494) and a negative correlation with the FTs (r = -0.349) (tab2, fig 3). These results are confirmed by several studies on olive oil (Gomez-Rico et al 2007, Baccouri et al, 2008) The reduction of phenol compounds and antioxidant activity during ripening directly affects the stability and the quality of the oil.



	Equation	R²%
PT	ARP= 0,4055+0,01932 PT	24,4
FT	ARP= 1,916-0,6946 FT	12,2

Figure 3: The relationship between ARP and (PT) and (FT)

3.3 The relationship between fatty acids and polyphenol compounds in EVAO at different stages of maturity:

The stability of extra-virgin oils due to several factors intervenes to promote or otherwise slow down the oxidation reactions of the oil. Storage conditions such as heat and light will of course increase the rate of auto-oxidation. But this depends primarily on the fatty acid composition of the oil, in particular PUFA, and its composition of pro-oxidant minor compounds such as phenolic compounds. ; The content of these compounds depends on the cultivar, the stage of fruit ripening, agroclimatic conditions and fruit growing techniques. [27, 28] Extra Virgin Argan Oil (EVAO) is considered one of the best oils for its organoleptic nutritional characteristics, its oxidation stability and its chemical composition. It is practically the best vegetable oil with olive oil that can be consumed directly in their raw state and contains important nutrients. Their nutritional properties and stability are not only assured by their fatty acid profile (FA) and their high ratio of monounsaturated / saturated fatty acids (AGMI / AGS), but also by their antioxidant content [15]. The study of the correlation between polyphenols and fatty acids at different stages of maturity (fig 4) shows that saturated fatty acids (FAS) have a strong correlation with polyphenols with a (r = 0.711, P = 0.021) for C16); (r = 0.576, C18) same case for C18: 1 (r = 0.753) whereas for C16: 1, a weak correlation with polyphenol content (r = 0.261), a very weak correlation between C18: 2 and polyphenols (r = -0.092, p = 0.800), but a strong relationship between C18: 3 and the polyphenol content of the oil at different stages of maturity (r = 0.773) C18: 1 / C18: 2 (r = 0.636) MUFA / PUFA (r = 0.368) while a weak relationship between flavonoids and fatty acids (fig 5) at different stages of maturity. (C16 r = 0.446); (C18r = -0.083) (C16: 1 r = -.066) (C18: 1r = 0.332, p = 0.349) (C18: 2r = 0.189) (C18: 3r = 0.172). In contrast, the relationship between the MUFA / PUFA ratio is positively correlated with the flavonoid level (r = 0.514).
The positive correlation between polyphenol and fatty acid levels may explain the action of antioxidant polyphenols, which act as protectors of fatty acids against oxidation, because C18: 1, C18: 2 and C18: 3 oxidize at 1: 1.7: 2.3 ratio ratios during photosensitized oxidation [29] compared to 1:12:25 ratio ratios in normal autooxidation reactions [11] according to these results, the stability of oil extracted from young fruits has a good oxidation power compared to the oil extracted from older fruits, because the rate of its polyphenols decreases with the progression of the

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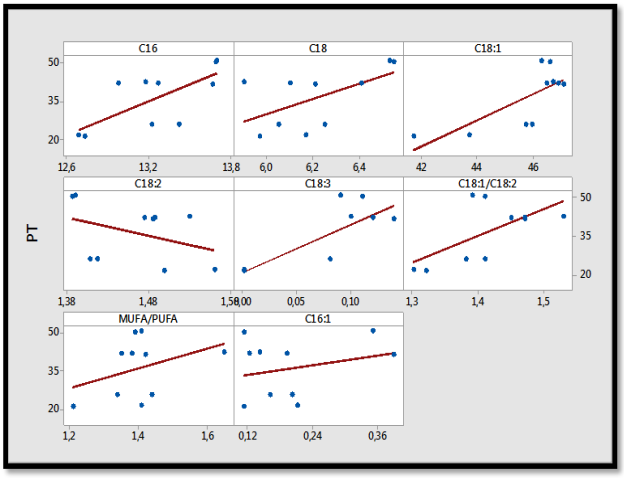
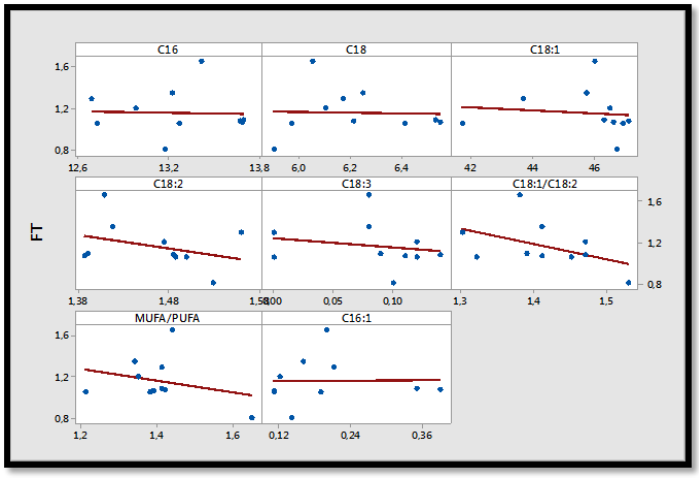
ripening of the fruit which influences the stability of the oil subsequently during storage. These results are confirmed by several authors (31,32,24).



	Equation	R^2 %
C18 :1	PT= - 194,9+5,06 C18 :1	56,7
C18 :2	PT=141-71,49 C18 :2	13,9
C16 :1	PT=30,28+30,71 C16 :1	6,8
C18 :3	PT=20,77+185,6 C18 :3	59,7
C16	PT= -254,5+21,94 C16	50,6
C18	PT= -148,1+29,68 C18	33,2
MUFA /PUFA	PT= -18,18+38,96MUFA /PUFA	13,6
C18 :1/C18 :2	PT= - 111,1+104,3C18 :1/C18 :2	40,4

Figure 4: The correlation between the phenolic composition (PT) and the fatty acid composition in Argane oil in different stages of maturity



	Equation	R^2%
C18 :1	FT=1,794-0,01378 C18 :1	1,1
C18 :2	FT=3,075- 1,305 C18 :2	12
C16 :1	FT=1,151+0,0633 C16 :1	0,1
C18 :3	FT=1,243-0,939 C18 :3	5,9
C16	FT=1,484- 0,0241 C16	0,2
C18	FT=1,378-0,0344 C18	0,1
MUFA /PUFA	FT=1,9590,568MUFA /PUFA	7,4
C18 :1/C18 :2	FT=3,299-1,511 C18 :1/C18 :2	21,9

Figure 5: The correlation between the flavonoid content (FT) and the fatty acid composition in Argane oil in the different stages of maturity

4 Conclusions:

The objective of this study is the quantification of the average fatty acid and polyphenol composition of the virgin argan oil, and the evaluation of the antioxidant activity of the latter considering the modifications during the process. ripening of the fruit. In addition to a study of correlation between the different parameters analyzed. For the purpose of determining the anticipated harvest date giving the best results in terms of composition considered and in terms of stability.

This research has shown that the composition and stability of argan oil changes during the fruit ripening process as the ratio of oleic acid / linoleic acid and phenolic compounds tends to decrease as the fruit matures. This decrease is reflected by a good linear correlation between the different oil components and the degree of maturity of the fruit.

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This work shows that the anticipated harvest date (IR between 2.3 and 3.17) has the best results in terms of all parameters considered. (Moderately ripe and immature fruits) gives a good, more stable oil in a good nutritional, sensory and commercial relationship.

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REFERENCES

[1]
[2] Hilali, M., Charrouf ,Z., El Aziz Soulhi ,A., Hachimi, L., Guillaume, D., (2005). Influence of origin and extraction method on argan oil physico-chemical characteristics and composition. J. Agric Food Chem. (53), 2081-2087
[3] Matthäus B, Guillaume D, Gharby S, Haddad A, Harhar H, Charrouf Z. (2010) Effect of processing on the quality of edible argan oil. Food Chemistry, 120, 426-432. K. Elissa, "
[4] Harhar H, Gharby S, Kartah BE, El Monfalouti H, Charrouf Z, Guillaume D. (2010) Long argan fruit drying time is detrimental for argan oil quality. Natural Product Communications, 5, 1799-1802.
[5] Rahmani, M., 2005.Composition chimique de l'huile d'argane « vierge Cahiers Agricultures vol. 14, n° 5.
[6] Aboudrare, A., T.J. Lybbert, and N. Magnan. 2009. "Le Marché de l'huile d'Argan et son impact sur les ménages et la forêt dans la région d'Essaouira " Transfert de Technologie en Agriculture 175(Avril).
[7] Servili .M (2014). The phenolic compounds: a commercial argument in the economic war to come on the quality of olive oil. OCL 21(5) D509
[8] Mateos, R.; Domı´nguez, M. M.; Espartero, J. L.; Cert, (2003). A.Antioxidant effect of phenolic compounds, R-tocopherol and other minor components in virgin olive oil. J. Agric. Food Chem., 51, 7170-7175.
[9] Velasco & Dobarganes 2002 Oxidative stability of virgin olive oil euro fed lipid 104, Issue 9-10 October 2002 Pages 661–676
[10] Pinelli P; C. Galardi ; N. Mulinacci ; F. FVincieri ; A. Cimato ; A.Romani (2003). Minor polar compound and fatty acid analyses in monocultivar virgin olive oils from Tuscany Food Chemistry Volume 80, Issue 3, March 2003, Pages 331-336
[11] Pardo, J.E., Sena, E., Cuesta, M.A., Granell, J.D., Valiente, J., Alvarez-Ortí, M., (2013).Evaluation of potential and real quality of virgin olive oil from "Campos de Hellín" (Albacete, Spain). J. Am. Oil Chem. Soc., 90, 851–862.
[12] Martín-Polvillo, M. , Márquez-Ruiz ,G.,. Dobarganes, M. C., (2004) .Oxidative stability of sunflower oils differing in unsaturation degree during long-term storage at room temperature. J. of the American Oil Chemists' Society. 81, 6, 577–583
[13] Cuvelier , M-E MAILLARD, M-N (2012). Stabilité des huiles alimentaires au cours de leur stockage OCL VOL. 19 N8 2
[14] Atifi H, Bouzoubaâ Z, Gharby S, Laknifli A, Mamouni R.(2017) Fruits maturity effect on the Argan oil amount, quality and chemical composition. Journal of Materiel and Environmental Science, 8 (2), pp. 512-519
[15] Beltrán, G., Ruano, M.Y., Jiménez, A., Uceda, M., Aguilera, M.P., (2007).Evaluation of virgin olive oil bitterness by total phenol content analysis. Eur. J. Lipid Sci. Technol. 108, 193–197.
[16] Jemai, H., Bouaziz, M., Sayadi, S., 2009. Phenolic composition, sugar contents and antioxidant activity of Tunisian sweet olive cultivar with regard to fruit ripening. J.Agric. Food Chem. 57, 2961–2968
[17] Singleton, VL., Rossi, J., (1965).A Colorimetry of total phenolic with phosphomolibdic-phosphotungstic acid reagents. J.Am. J. Enol. Vitic. 16, 144–158
[18] Jadouali, S.M., Atifi, H., Bouzoubaa, Z., Majourhat ,K., Gharby, S., Achemchem, F., Elmoslih, A., Laknifli, A., Mamouni, R., 2018. Chemical characterization, antioxidant and antibacterial activity of Moroccan Crocus sativus L petals and leaves. J. Materials and Environmental Sciences.9, 113-118
[19] Kroyer ,GTh., (2004). Red clover extract as antioxidant active and functional food ingredient. Innovative Food Science and Emerging Technologies 5 101–105 guide.J. Phytochem. 61 ,3, 339-43.
[20] ISO 5508 .,1990. Analysis by gas chromatography of methyl esters of fatty acids
[21] Talhaoui ,N., Gómez-Caravaca ,A. M., Lorenzo, L., Raúl, R., (2016) .From Olive Fruits to Olive Oil: Phenolic Compound Transfer in Six Different Olive Cultivars Grown under the Same Agronomical Conditions. Int. J. Mol. Sci. 17, 337.
[22] Sebei, K., (2005). Etude Par Couplage CGL-SM des fractions glycéridiques et non glycéridiques au cours de la lipogenèse des graines de colza (Brassica Napus L). These doctorat en biologie. Faculté Des Sciences de Tunis, 220 p.
[23] Salvador,MD., Aranda ,F., Fregapane, G., (2001). Influence of fruit ripening on Cornicabra virgin olive oil quality: a study of four crop seasons.J. Food Chem. 73,45–53.
[24] Ben Youssef, N., Zarrouk ,W., Carrasco, A., Pancorbo Ouni ,Y., Segura-Carretero, A., Fern´andez-Gutierrez, A., Daouda ,D., Zarrouka, M.,(2010) .Effect of olive ripeness on chemical properties and phenolic composition of chetoui virgin olive oil J. Sci Food Agric. 90, 199–204.
[25] Ryan, D., Antolovich, M., Prenzler, P., Robards, K., Lavee, S, (2002). Biotransformations of phenolic compounds.J. Sci Hortic. 92,147–176.
[26] Lavelli, V. Comparison of the antioxidant activities of extra virgin olive oils. J. Agric. Food Chem. 2002, 50, 7704-7708.
[27] Beltran, G., (2000). Influencia del proceso de maduraci_on del fruto deOlea europaea L. sobre las caracter_isticas fisicoqumicas de los aceites. PhD Thesis. Facultad de CC. Experimentales, Universidad de Jaen
[28] Romero-Aroca, A., (2011).Caracterización y Diferenciación de los Aceites Vírgenes de Oliva de la Comarca del Priorat (Tarragona), dentro del Mercado Global de Aceites de la Variedad "Arbequina". Ph.D. Thesis,University of Lleida, Lleida, Spain,.
[29] Yousfi, K.,Weiland, C.M., García, J.M., (2012). Effect of harvesting system and fruit cold storage on virgin olive oil chemical composition and quality of superintensive cultivated "Arbequina" olives. J. Agric. Food Chem.60, 4743–4750.
[30] Baccouri, O., Guerfel, M., Baccouri, B., Cerretani, L., Bendini, Al., Lercker, G., Zarrouk, M ., Ben Miled, D., (2008). Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. Food Chemistry .109 ,743–754
[31] Guti_errez-Rosales, F., Perdiguero, S., Guti_errez, R., Olıas, J. M., (1992). Evaluation of the bitter taste in virgin olive oil. Journal of the American Oil Chemists' Society. 69, 394–395