

Fruits maturity effect on the Argan oil amount, quality and chemical composition

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Abstract

In order to show the level maturity effect of the fruit on the quantity, quality and chemical composition of the Argan oil, we used an extracted oil from fruits at three different levels of maturity; the too rip (P), the rip (I) and unripe (T) fruits. The sampling trees were from Taroudant province forest, in Southwest Morocco. The results showed that the ripeness is a factor determining the quality of oil ; indeed, the amount of oil, acidity index, the K230 and K227 increased with fruit ripeness but it was not significant for K270 ; the compound of fatty acids increased in the (I) level, and decreased in the (P) one. The sterols showed a significant increase of spinasterol and schottenol with increasing maturity level, however, the delta 7-avenasterol content decreased with it, meanwhile, the campesterol content varied with fruits maturity but not significantly. These results can be considered for a better valorization and use of Argan products, hence better management of the Argan tree and therefore its sustainability. Indeed, determining the appropriate time to collect fruits according to their levels of maturity for various types of wanted oil.

1. Introduction

Argania Spinosa (L.) Skeels is an endemic tree of Southwest Morocco; it ranks third nationally after the oak and cedar [1]. It is a tree that can live up to 250 years. The current geographical area of the Argan tree covers over 870.000 ha versus 1500000 ha in the beginning of the twentieth century [2] mainly in the Southwest of Morocco. It's extended from Safi at the North to Saharan fringe in the South. This area is one of the most remarkable parts of Moroccan territory, both by its flora as by its vegetation. The expansion of the Argan tree accounts over shaft 20 millions.

Argania spinosa (L.) plays an important and irreplaceable socio-economic and ecological role [3-4]. The direct economic role is reflected in the production of Argan oil. The "virgin" Argan oil intended for food and cosmetics derived from the Argan seeds. Oil extraction is mainly made with water by hands, despite a recent introduction of mechanization process [5].

The fruiting process of Argan tree presents an important variability [6-7]. Three types of fruiting Argan trees can be distinguished in Argan field: early fruiting, late fruiting and intermediate one that has an extended fruiting. The productivity of Argan is variable. It's improved by a rainfall of at least 100 mm/year, maximum in autumn and mild temperatures (23-25°C). The fruits weights vary significantly under the effects of genotype, and environmental interaction [8]. The fruit maturity will appear by changing the color from green to yellow or brown, and their size varies from one to five centimeters [7]. Flowering time can be narrow or wide depending on the genotype and the cycle of maturation. The fruit can be in different forma depending on the tree genotype. It can be long, oblong, and spherical [7] which reflected on the oil quality and variability [9]. The quality of virgin oil depends on the quality of the raw material used for production [10]. And physiological changes, directly linked to the age of the fruit matures and occurred with a change in the oil quality [11]

The fruiting period of Argan tree can be narrow or wide, and the cycle of maturation of the fruit can be long or short [9], which is reflected on the quality variability of the produced oil, while the quality virgin oil depends on

the quality of the raw material used for production [10]. The fruits ripening stage is one of the most important factor associated with the quality evaluation of oil [11-12]. Argan oil is excellent cosmetic and dietetic value food oil [13-14]. Several factors could influence the virgin Argan oil quality since the quality and the method of collecting the fruit to the oil extraction, and, several suitable operating conditions to optimize production at different scales can exist.

In addition to that, and during the fruit ripening, several metabolic processes take place in fruits with subsequent variations on the chemical structure and concentration of some compounds. These changes are reflected in the quality grade, sensorial characteristics, oxidative stability and nutritional value of oil [15].

In this context the aim of this study is to investigate the effect of fruit maturity stage on the quantity and the quality of Argan oil including oxidative stability, composition of fatty acid and sterols for a better product then a better valorization of Argan oil.

2. Experimental

2.1. Sampling site

The Argan fruits were collected from five trees at 3/06/2014 in the Taroudant province (Figure 1.). Each tree contained three types of fruits according to their levels of maturity; the trees references localization on table 1. To limit the heterogeneity of plant material, the sampled and studied trees for their morphological and morpho-physiological showed three stages of fruit ripening on the same tree (Figure 2) : old or over mature fruits (brown color, represented by P), mature fruits (yellow represented by I) and late or unripe fruits, (green color represented by T).

Table 1. Localization of the sampled trees

Tree's number	Geographical parameters		
	Altitude in (m)	North	West
T1	323	30°32'56.7"	08°51'33.3"
T2	316	30°32'56.5"	08°51'33.7"
T3	311	30°32'55.4"	08°51'34.1"
T4	312	30°32'55.2"	08°51'34.7"
T5	313	30°32'54.2"	08°51'37.7"



Figure 1: Location of the sampling site at Taroudant province

2.2. Samples preparation and oil extraction

Fruits were collected (Figure 1), dried at 25°C, pulped, crushed at the end of the oil extraction. Extraction was performed with a soxhlet. Twenty grams of ground kernels were placed in a Soxhlet apparatus and extracted with 150 mL of hexane for 8 h[16]. Then it was removed under vacuum using a rotary evaporator. The samples

were kept at a temperature of 40 ° C until the total elimination of hexane traces, and then the resulted oil was stored at 4 ° C until analyses.



Figure 2 : Argan fruits in different stages of maturity to rip (Brown), rip or mature (yellow) and unripe (green) Collected from the same tree

2.3. Determination of free acidity:

Titrateable acidity was determined using the International Standard Organisation method [17]. (10 g) of oil was dissolved in 80 ml neutralised taste ethanol(96°).Two drops of phenolphthalein (1% in ethanol) was added to the solution. The solution was then titrated with 0.1N sodium hydroxide (NaOH), previously standardised against hydrochloric acid (HCl). The volume of titrating was recorded and the results calculated as a percentage of the oil (expressed as oleic acid).

2.4. Specific ultraviolet absorbance:

Ultraviolet absorption was determined using the International Standard Organisation method [18].Oil (0.25g) was weighed into a 25 ml volumetric flask and made to volume with cyclohexane. The absorbance of the oil sample was measured on a double beam spectrophotometer, using cyclohexane in 1 cm cellpath length, at 232 and 270 nm.

2.5. Analytical determination

2.5.1. Fatty acids composition

Fatty acid composition was determined using the International Standard Organisation method [19]. Fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 60 mg oil and 3 ml of hexane with 0.3 ml of 2 N methanolic potassium hydroxide.They were analyzed by gas chromatograph (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 were 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.

2.5.2. Sterol composition

Sterol composition was determined using the International Standard Organisation method [20]. To 5 g of argan oil, 500 mL of chloroform was added. Saponification was performed using 2N ethanolic KOH solution. After 1h of boiling, 100 mL of water was added and extraction of unsaponifiable matter was carried out using 200 mL of Et2O. The organic solution was collected, evaporated, and 20 mg of unsaponifiable matter was dissolved in 0.5 mL of chloroform then chromatographed on a silica gel plate eluted with a mixture of n-hexane and diethylether (65:35 v/v). Then the plate was sprayed with a solution of 2,7-dichlorofluorescein (0.2% in EtOH), and the band corresponding to sterols carefully removed. The silicagel recovered from the plate was suspended in chloroform and filtered through a paper filter. The solvent was evaporated under N2 and the sterol composition determined after trimethylsilylation of the crude sterol fraction. Trimethylsilylated derivatives were analyzed by GC using a Varian 3800 instrument equipped with a VF-1ms column (30 m, 0.25 mm i.d.) using helium (flow rate 1.6 mL/min) as carrier gas. Column temperature was isothermal at 270°C; injector and detector temperature was 300°C. Injected quantity was 1 mL for each analysis. Data were processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA).

2.6. Statistical analysis.

Values reported in tables and figures are the means \pm SE of two replications. The significance level was set at $P \leq 0.05$. Separation of means was performed by Tukey's test at the 0.05 significance level.

3. Results and discussion

The flowering-fruiting cycle lasts for a period of 9-16 months depending on the trees [18]. The fruits maturity can be grouped or spread [9].

To limit the heterogeneity of plant material, five trees that had the three levels of maturity at the same time and the same tree from the same site were selected and their fruits were collected. The sampling site was Taroudant province in the South-West of Morocco. Table 1, gave the geographical parameters of sampling site and geographical localization of sampling trees. The collecting fruits with three level of maturity from the same tree; too ripe fruits called (P), ripe fruits called (I) and no rip fruits called (T), a sample is represented in figure 2.

3.1. Amount of oil, acidity percentage and ultraviolet specific extinctions K232 and K270

3.1.1. Quantity parameter; percentage of oil :

The oil quantity was significantly affected by fruits maturity; it's varied from 38% to 54%, the lowest percents were in (T) levels (the too ripe fruits) and the maximal percents were in (P) ones (the no ripe fruits), significantly increase was observed with increasing maturity for the five trees (table 2), a significant variability was also observed between trees. These changes in the oil % were in concordance with other works [21-22]. Another study on fruit maturity showed that the amount of oil increased over time, and decreased at the end of the ripening fruits [23].

Table 2: Effect of stage maturity on the oil %, acidity and absorbance's (K232 and K270)

Trees	MD	Oil %	Acidity %	K232	K270
T1	P	42.5 \pm 0.01 ^g	1.3 \pm 0.02 ^a	1.54 \pm 0.02 ^a	0.24 \pm 0.028 ^a
	I	40 \pm 0.1 ^h	0.9 \pm 0.014 ^b	1.5 \pm 0.028 ^{ab}	0.22 \pm 0.04 ^a
	T	38 \pm 0.2 ⁱ	0.7 \pm 0.02 ^{cd}	1.2 \pm 0.04 ^d	0.16 \pm 0.042 ^a
T2	P	54.5 \pm 0.02 ^b	0.18 \pm 0.028 ^f	1.43 \pm 0 ^{ab}	0.15 \pm 0.07 ^a
	I	51.5 \pm 0.1 ^c	0.16 \pm 0 ^f	1.43 \pm 0.01 ^{ab}	0.15 \pm 0.02 ^a
	T	50.4 \pm 0.04 ^{de}	0.16 \pm 0.04 ^f	1.4 \pm 0.05 ^{abc}	0.13 \pm 0.028 ^a
T3	P	55.5 \pm 0.02 ^a	1.2 \pm 0.02 ^a	1.52 \pm 0.05 ^a	0.14 \pm 0.05 ^a
	I	51.5 \pm 0.01 ^c	1.2 \pm 0.01 ^a	1.5 \pm 0.014 ^{ab}	0.13 \pm 0.04 ^a
	T	50 \pm 0.28 ^e	0.8 \pm 0.042 ^{bc}	1.48 \pm 0.07 ^{ab}	0.11 \pm 0.01 ^a
T4	P	50.5 \pm 0.1 ^{de}	0.6 \pm 0.02 ^{de}	1.52 \pm 0.028 ^a	0.22 \pm 0.028 ^a
	I	50.5 \pm 0 ^{de}	0.5 \pm 0.14 ^e	1.48 \pm 0.07 ^{ab}	0.2 \pm 0 ^a
	T	45.5 \pm 0.2 ⁱ	0.3 \pm 0.04 ^f	1.4 \pm 0.05 ^{ab}	0.15 \pm 0.04 ^a
T5	P	50.8 \pm 0.01 ^d	0.96 \pm 0.042 ^b	1.4 \pm 0.014 ^{ab}	0.18 \pm 0.05 ^a
	I	50.5 \pm 0.04 ^{de}	0.96 \pm 0 ^b	1.34 \pm 0.02 ^{bcd}	0.14 \pm 0.05 ^a
	T	45.5 \pm 0 ^f	0.12 \pm 0.01 ^f	1.3 \pm 0.01 ^{cd}	0.16 \pm 0.04 ^a

Data are expressed as mean values \pm SD (n= 5). Content oil expressed as % dry matter, Values followed by different letters (a, b, c, d, e, f) in the same row are significantly different at $P \leq 0,05$

3.1.2. Quality parameters: percentage of Acidity and K232, K270:

Oil acidity is one of the main procedures that allow the valuation of the oil quality. Fatty acids are present in Argan oil as 98-99% [24], the dosage of the hydrolysis of these frees fatty acids give an idea about the progress of the oil degradation. The results of the analysis of this parameter showed that the percentage of acidity increased significantly with ripeness within the tree, for all trees. It's varied from 0,1 to 1,3%, the lowest % was for no ripe fruits oil (T) and the highest one for the too rip fruits oil (P) (table 2); This change can be explained by the lipase reaction during the ripening process [25].

Measurements of absorbance at specific UV wavelengths are used to provide information on the quality of Argan oil. In fact, the specific extinction coefficient at 232 nm is related to the primary oxidation of oil and it is an indication of conjugation of polyunsaturated fatty acids; whereas K270 is an indication of carbonylic compounds (aldehyds and ketones) in fruits and is related to the secondary oxidation products [26].

K₂₃₂ significantly increased in the oil rip fruits such an increase indicates the occurrence of significant oxidative processes impacting the unsaturated fatty acid chains contained in [26]. The same case of K₂₇₀ specific absorption, it didn't increase significantly with maturity but these results could be explained by the fruit exposure to light and to the area which promoted oxidation [26]. All the obtained results of quality parameters Argan oils stayed within the accepted range for oil [27]. They belonged to the 'extra virgin and virgin oil category.

3.2. Fatty acids composition

Fatty acids (FA) composition is an essential indicator of the nutritional value [26]. Argan oil is particularly rich in unsaturated fatty acids. It's oleic and linoleic acid levels are generally between 42 and 47%, and between 31 and 35%, respectively. In addition, Argan oil also contains two saturated fatty acids: palmitic acid (12–14%) and stearic acid (5–7%) [28]. Our studied on cosmetic Argan oil consisted of 12.9% palmitic, 4.9% stearic, 46.8% oleic, and 33.8% linoleic acids. Edible Argan oil consisted of 12.5% palmitic, 5.1% stearic, 46.7% oleic, and 34.4% linoleic acids. Those fatty acids give the Argan oil an important nutritional and dietary value, and justify its use in the prevention of cardiovascular disease, drying and the physiological aging of the skin. The fatty acid composition of the Argan oil is therefore very different from those of the olive oil containing 75% of oleic acid [30].

The results in (table 3) (Figure3) showed the trend of the major saturated fatty acids and unsaturated Argan oil depending on the stage of maturity, in order to simplified the analysis and discussion of results, all trees were confounded (each value was the mean of one of the three stage in the five sampling trees). All the data found were in agreement with the values of Moroccan standards of virgin Argan oil [27].

Table 3. Effect of maturity degree on fatty acids composition

Trees	MD	C16 :0	C16 :1	C18:0	C18 :1	C18 :2	C18 :3
T1	P	13.32± 0.04 ^{de}	0.00 ^b ±0.00	6.15± 0.05 ^{ab}	45.83± 0.02 ^e	32.44± 0.04 ^e	0.00 ^b ±0.00
	I	13.69± 0.05 ^{bc}	0.23± 0.01 ^a	6.54± 0.01 ^a	46.44± 0.04 ^d	33.45± 0.01 ^d	0.1± 0 ^a
	T	13.47± 0.01 ^{dc}	0.29± 0.05 ^a	6.31± 0.01 ^{ab}	46.99± 0.01 ^c	31.61± 0.01 ^g	0.13± 0.04 ^a
T2	P	13.01± 0.01 ^f	0.1± 0 ^a	5.46± 0.04 ^{de}	45.83± 0.02 ^e	32.53± 0.14	0.08± 0.01 ^a
	I	13.9± 0.04 ^b	0.18± 0.04 ^a	6.41± 0.01 ^{ab}	46.44± 0.01 ^d	34.32± 0.01 ^c	0.15± 0.05 ^a
	T	13.08± 0.01 ^f	0.16± 0.01 ^a	5.91± 0.01 ^{bcd}	46.58± 0.01 ^d	32.02± 0.02 ^f	0.11± 0.01 ^a
T3	P	13.31± 0.02 ^a	0.16± 0.01 ^a	5.2± 0.01 ^{ef}	42.66± 0.04 ^f	36.61± 0.02 ^b	0.00 ^b ±0.00
	I	13.62 ± 0.14 ^c	0.00 ^b ±0.00	5.27± 0.02 ^{ef}	43.47± 0.01 ^g	37.07± 0.03 ^a	0.08± 0.01 ^a
	T	13.05± 0.01 ^f	0.00 ^b ±0.00	4.85± 0.28 ^f	44.75± 0.01 ^h	32.41± 0.02 ^e	0.15± 0.04 ^a
T4	P	12.71± 0.01 ^g	0.00 ^b ±0.00	6.07± 0.04 ^{abc}	51.58± 0.01 ^a	27.88± 0.01 ⁱ	0.00 ^b ±0.00
	I	13.2± 0.14 ^{ef}	0.13± 0.04 ^a	5.59± 0.01 ^{cde}	52.19± 0.01 ^b	29.44± 0.01 ^h	0.00 ^b ±0.00
	T	12.97± 0.01 ^f	0.13± 0.01 ^a	6.1± 0.02 ^{abc}	51.7± 0.14 ^b	27.64± 0.01 ^j	0.1± 0.02 ^a
T5	P	13.32± 0.02 ^{de}	0.00 ^b ±0.00	6.15± 0.14 ^{ab}	45.83± 0.05 ^e	32.44± 0.02 ^e	0.00 ^b ±0.00
	I	13.69± 0.01 ^{cd}	0.23± 0.01 ^a	6.54± 0.4 ^{ab}	46.44± 0.28 ^d	33.45 ± 0.01 ^d	0.1± 0 ^a
	T	13.47± 0.01 ^{bc}	0.29± 0.05 ^a	6.31± 0.01 ^{ab}	46.99± 0.01 ^c	31.61± 0.01 ^g	0.13± 0.04 ^a
SNIMA Standards EVAO		11,5-15	≤0.2	4.3-7.2	43.0- 49.1	29.3-36	≤0.3

Results were expressed as % of total fatty acid fraction. Data are expressed as mean values ±SD (n= 2). Values followed by different letters

Oleic and linoleic acid contents changed high significantly with the fruits ripeness. Those compound increased in the (I) degree and showed a small reduction in (P) degree. This reduction can be explained by the implication of unsaturated acids in addition to oleic acid during the fruits maturity process into the composition of phospholipids that form the structure of the newly formed cell membranes [23].

Linolenic acid did not change during different stages of maturity, there always existed at minima values. It's a long chain polyunsaturated acid which lead to radical reactions [31]. Indeed, linolenic acid has an oxidation rate 25 times higher than oleic acid and 2 times more than linoleic acid [32]. Furthermore, palmitic and stearic acids decreased little significantly with fruit ripening. This decrease was explained by a dilution effect because the absolute amount was constant [29]. A few significant variations between palmitic acid and stearic acid were observed mainly in the oil of rip fruits (I) and the no rip fruits (T) stages, which was in accordance with the results found for olive oil [33].

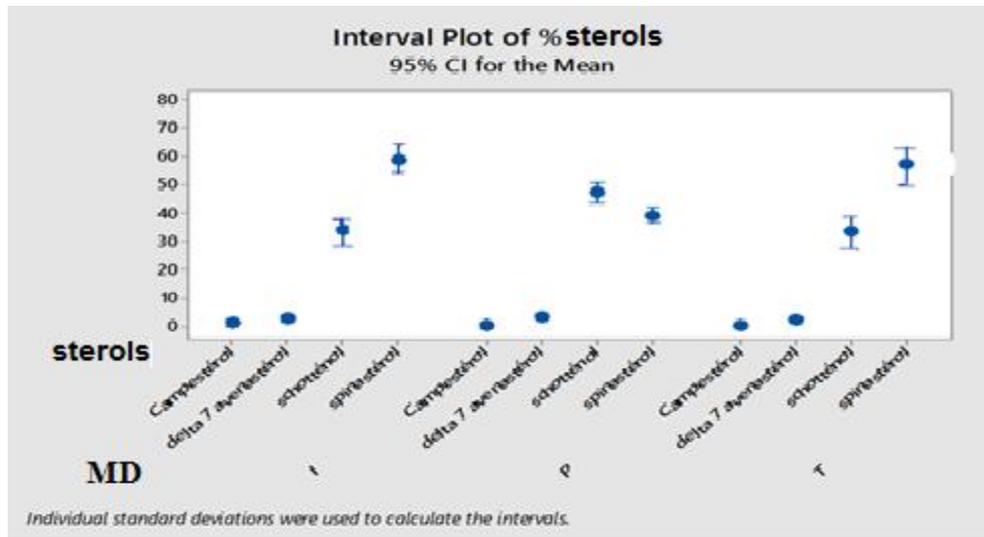


Figure 3: Effect of maturity level on the fatty acid composition of Argan oil (all trees confounded)
 MD: Maturity Degree; I : Rip fruits oil; P: Too rip fruits oil; T: Not rip fruits oil

3.3. Sterols content

Vegetable oils contain phytosterols (on average from 0.1 to 0.5%) [34]. For Argan oil, it contains an original sterol fraction; the delta 7 sterols that is important in unripe and in ripe fruits (table 4) (figure4). The Argan oil is composed primarily of spinasterol and schottenol [35]. These micronutrients could contribute to the prevention and fight against the threat of metabolic dysregulation, mainly in the cardiovascular and neurodegenerative disease [36]. They are also known to reduce effectively the cholesterol and LDL cholesterol concentrations in blood serum [37].

Table 4: Sterols content by maturity level with all trees confused

MD	Campesterol	Spinasterol	Schottenol	Delta7
P	0.34 ^a	38.90 ^c	47.58 ^c	2.78 ^a
I	0.37 ^a	37.70 ^b	43.22 ^b	3.32 ^b
T	0.34 ^a	36.54 ^a	42.9 ^a	3.52 ^c
LSD 0.05	0.07	0.196	0.123	0.112

Values from the same column, followed by the same letter were not significant at $\alpha=0,05$.
 LSD is the Least Significant Differences; MD: Maturity degree.

The results of the phytosterols contents showed that they were present in all stages of fruit development. However, the content of the spinasterol and the schottenol increased significantly with the fruit maturity, confirming that spinasterol and schottenol are influenced by the stage of fruit ripening. On the other hand, we note that the content of delta-7 avenasterol significantly decreased with maturity what confirmed the results of other authors [38]. Figure 4 represents the oil sterols contents by stage maturity with all trees confused. The variability observed in all analyzed parameters between trees (tables 2, 3 and 5) must be deepened in study by the determination of other oil contents as antioxidants and vitamins and used for selecting performed material in a breeding program of Argan.

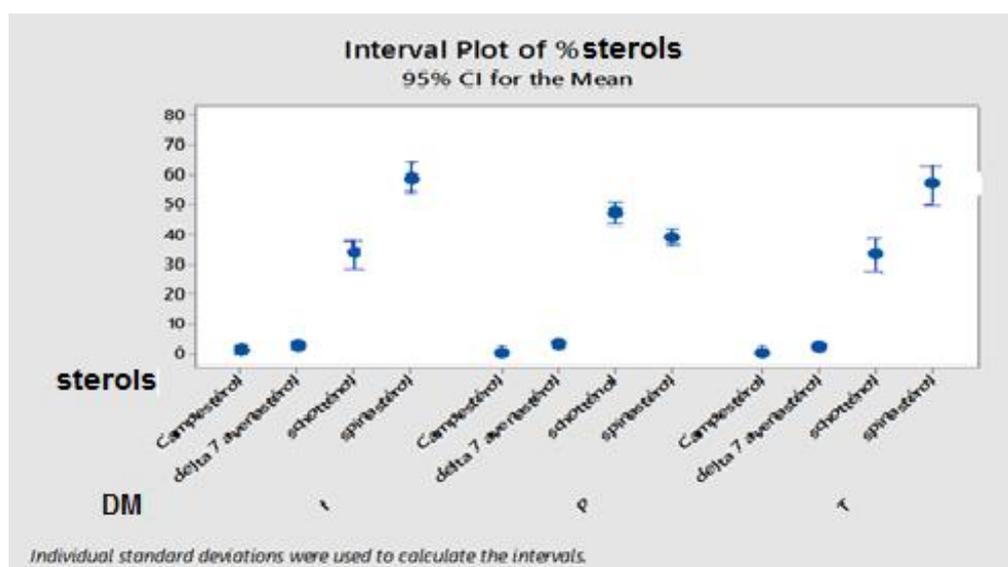


Figure 4: Sterols content by maturity level (with all trees confounded)
 DM: Degree of Maturity; I : Rip fruits oil; P: Too rip fruits oil; T: Not rip fruits oil

Table 5: Sterols content by tree with confused all maturity levels

Trees	Sterols content			
	Campesterol	Spinasterol	Schottenol	Avenasterol
T1	0.35 ^{ab}	36.76 ^c	48.28 ^c	3.4 ^b
T2	0.33 ^{ab}	38.10 ^d	44.1 ^b	3.03 ^a
T3	0.45 ^b	42.72 ^e	39.03 ^a	3.16 ^a
T4	0.26 ^a	35.86 ^b	44.3 ^c	3.03 ^a
T5	0.35 ^{ab}	35.13 ^a	47.11 ^d	3.4 ^b
LSD0,05	0.1002	0.254	0.158	0.144

Values from the same column, followed by the same letter were not significant at $\alpha=0,05$.
 LSD is the Least Significant Differences

Conclusion

After the results of our study, we can conclude that ripeness level is a factor determining the quality and quantity of Argan oil. The results showed that the amount of oil were maximum in the too rip fruits but the acidity and K230 increased lightly with the ripeness. Modification of those components can be commercially relevant as they reflected the oil stability.

The fruits maturation also influenced the compound of fatty acid and sterols; the total fatty acids changed significantly with the ripeness, it increased in the mature fruits, and decreased with maturity in the too rip fruits. About the profile of spinasterol and schottenol the results showed that they increased, while the delta 7 avenasterol decreased with maturity.

Finally, and based on our results it appeared that the best ripening stages for having a good oil quality, with the highest stability values, a high oil yield and good characteristics concerning quality parameters and compounds of nutrients were the no mature (green fruits) and the mature fruits (yellow fruits). These results can be considered for a better use of Argan oil, and better management of the Argan tree and therefore its sustainability. The differences found between the trees can be used in an Argan breeding program.

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