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Effect of Ripening Degree of Argane Fruit on the Phenolic Composition and Antioxidant Activity of the fruit Pulp, Kernel and Oil

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Abstract

The quality of the vegetal oil depends to the quality of the fruit used during the extraction. Ripening of the fruit is one of the most important processes associated with metabolic changes in the fruit. These changes include variations in the fatty acid profile, fluctuations in the level of phenolic compounds. In the present study, we investigated the effect of ripening on the characteristics of Argane fruits including the variation of total phenol and flavonoid present in the pulp of the fruit, the kernel and the oil as well as their antioxidant potential. The rate of total phenol and flavonoid increases upon ripeness process in fruit parts and its oil; however, it decreases again in latest stages of ripeness. When compared to kernel extract and fruit pulp, oil extract with a ripeness index of 2.07 displays the highest inhibition of 2.2-diphenyl-1-picrylhydrazyl (DPPH). Moreover, it was revealed that the oil and kernel extract gave higher reducing power measured with the FRAP assay, It increases along the ripping process starting from ripeness index (RI) = 2.34 till 3.17.These results can be considered for a better use of fruit pulp press-cakes and oil as nutraceuticals or cosmetics. The study also helps to determine the best harvesting time in order to get a oil a high quality rich in phenolic compounds, and a large antioxidant potential which seems to be at ripeness index higher than 2.07 and lower than 3.07

Keywords: Pulp, Kernel Argane Fruit, Argan Oil, Phenolic Compounds, Antioxidant potential, Ripeness stage

1.Introduction

The Argane tree (*Argania spinosa* L. *Skeels*) belongs to the Sapotaceae family. Locally, it plays essential ecological and economical roles in south-western Morocco; it is also perfectly adapted to semi-arid environment. Its fruit is a drupe with an average weight from 5 to 20 g [1]. The mesocarp or pulp surrounds the epicarp or nut which contains between 1 to 3 seeds. The latter is the most important component for its oil wealth yet it does not represent more than 10% of the dry fruit weight. Traditionally, Argane oil is prepared by Argane forest dwellers that manually collect the fruit and depulped to get Argane nuts whose shell is broken subsequently. The Argane kernels are amassed, then eventually roasted; next they are mechanically cold-pressed to afford virgin Argane oil for beauty (unroasted kernels) or edible oil (roasted kernels) grade [2,3]. Each oil type possesses its own set of pharmacological properties [4]. The by-products engendered during the oil preparation process are the nut-shell, the fruit pulp and the press-cake. Two types of press-cake exist since kernels can be roasted or not. Fruit pulp and press-cakes that are palatable to cattle are used as cheap protein-rich material in all animal farms; the shell is commonly simply recycled as fuel. This product can also be recycled as cattle food thanks to its high energetic value [5]. It is mainly composed of carbohydrates, proteins, and polyphenols. Phenolic compounds are plant secondary metabolites. They play important roles in disease resistance [6,7], protection against pests and species dissemination. The importance of these compounds is related with their antioxidant activity which represents a important concern in benefits of health promotion [8] and they can prevent atherosclerosis as well as cancer progression [9,10].

Virgin Argane oil is important dietary oil, rich in natural antioxidants [11]. These substances have a remarkable pharmacological effect coupled with their low toxicity. Studying polyphenol content in Argane oil by-product seems, therefore, to be particularly engaging in further support, and would inevitably reinforce sustainable development of the Argane forest. The fruits ripening stage is one of the most important features associated with changes in the quality of fruit compound [12,13]. While ripening, the fruits are subject to several metabolic processes 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 the obtained product. Chlorophyll and carotenoid pigments are examples of compounds involved in this phenomenon, as well as polyphenol and fatty acid compositions. [14]

The purpose of this work is to quantify, on the same fruit batch, the total phenolic and total flavonoid content of the Argane-fruit pulp, Argane oil and kernel in different stage of fruits ripeness. The antioxidant activity is evaluated with 2.20-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays.

2. Materials and Methods

2.1. Samples; Samples pre-treatment

Argane fruits were collected in Guelmime area (south of Agadir, Souss-Massa-Drâa region). Care was taken to collect a representative sample, taking into account variable stages of maturity within each tree (based on fruit color) Harvest dates were March, April, May 2017. After collection, fruits were manually depulped. To obtain pastes, to the pulp is crushed using a blender; crack shells manually; then, separate and crush kernels. After that, the oil is prepared by cold extraction.

2.2. Color

We used a colorimeter to assess the fruits' color and applied the Hunter colorimetric system (L*, lightness; a*, redness; b*, yellowness) [24]. The measurement was made on 20 fruit samples.

2.3. Ripeness Index

The ripeness index (RI) was determined according to [25]. Accordingly, the empirical procedure consists in distributing randomly taken sample of 100 Argane fruits in 8 groups according to the skin colors: bright green (group N =0), green with yellow spots (group N = 1), green with red spots (group N =2), yellow green spot (group N=3), yellow (group N=4), yellow with brown spots (group N=5), brown with yellow spots (group N=6), brown (group N=7).With (a) to (h) being the number of fruits in each category, the IR. is:

$$RI = (a \times 0 + b \times 1 + c \times 2 + d \times 3 + e \times 4 + f \times 5 + g \times 6 + h \times 7) \div 100$$

Length and width of fruits are measured using a caliper on a sample of 20 fruits taken randomly. The 20 fruits are then weighed, one by one, using a precision balance. The averages of the weighing are calculated for each sample.

2.4. Determination of fat content

A dried sample (10g) was submitted to Soxhlet extraction, using hexane as solvent. After solvent evaporation, the flask containing fat was dried at 40°C, and reweighed. All the measurements were repeated on triplicate samples; the data reported are the mean of them.

2.5. Determination of water content

A fruit sample (100-200 g) was weighed and then dried overnight at 50°C until weight stabilization, on leaving the oven the samples were weighed (weight dry). The humidity in% (m / m) was calculated by the following equation:

$$humidity\% \left(\frac{m}{m}\right) = \frac{fresh\ weight - dry\ weight}{fresh} \times 100$$

2.6. Extraction of phenolic compounds

5 g of samples (fresh paste pulp, powder of kernels and oil) was extracted according to the method reported [26].Three successive hexane extractions (3-50 mL) applied allowed removal of most lipids. The phenolic compounds were extracted by a mixture methanol: water (80:20, v/v) after three extractions (3-50 mL), the final extract was evaporated and stored at 4 ° C until performing analysis [19].

2.7. Determination of total Phenols

Total phenols were determined by using the Folin-Ciocalteu reagent according to the method of Singleton [27]. Appropriate dilutions were used for each extract. Briefly, a 50 µL aliquot of the extracts was assayed with 250 µL of Folin reagent and 500 µL of sodium carbonate (20%, w/v). The mixture was vortexed and diluted with water to a final volume of 5 mL. After incubation for 30 min at room temperature, the absorbance was read at 765 nm. Total phenols were expressed on a dry weight basis as gallic acid equivalents (GAE), using a calibration curve of a freshly prepared gallic acid solution. For the gallic acid, the curve absorbance versus concentration is described by the equation y = 0.0037x + 0, 0871 (R^2 = 0.9477).

2.8. Determination of total flavonoids

The crude extracts of pulp, kernels and oil were used for the determination of total flavonoids. Each crude extract of pulp, kernels and oil (4 mg) was diluted with methanol (4 mL). Each crude sample (250 µL) was taken in a separate test tube. Then 125 µL of water and 75 µL of sodium nitrate solution were added to each test tube. The mixture was kept at room temperature for 6 min and then added aluminum chloride (150 µL) to each test tube and kept for another 2 h in a dark

place. All the working test tubes were diluted with sodium hydroxide (500 µL) and water (500 µL). The absorbance was measured by UV-visible spectrophotometer at fixed wavelength 510 nm. Finally, total flavonoids content of the crude extract samples was calculated by using established formula. [28]. Total flavonoid were expressed on a dry weight basis as catechic acid equivalents (CE), using a calibration curve of a freshly prepared catechic acid solution. For the catechic acid, the curve absorbance versus concentration is described by the equation y = 0,0024x + 0,2665 (R^2 = 0,9815).

2.9. Determination of DPPH radical scavenging activity (RSA)

Hydrogen-donating ability of the crude extract and radical scavenging activity (RSA) of Argane fruit parts and oil were investigated using the DPPH• (2,2-diphenyl-1-picrylhydrazyl (6 105 M in ethanol) radical-scavenging assay (RSA). A dilution series was prepared of each sample(60., 80., 100., 200. and 250µg/ml) 50 µl of phenolic extracts are added to 2 ml of DPPH the mixture kept in the dark for 1 h and the absorbance reading at 517 nm. [29]. The inhibition percentage (IP) of the DPPH• by the extracts was calculated according the formula

%IP= [(A0min – A60min)/A0min] × 100 where A0min is the absorbance of the control at t = 0 min, and A60min is the absorbance of the samples at 60 min. (gallic acid, α tocopherol, tannic acid) are used as stallions. The inhibitory concentration (IC_{50}), efficient concentration (EC_{50}) and antiradical power (ARP) were estimated and calculated as described by [23].

2.10. Determination of reducing power

The reducing power of samples was determined via the method of Jadouali *et al* [30], with few modifications. Samples with different concentrations A dilution series is prepared of each sample (60., 80., 100., 200. And 250µg/mL) were mixed with 0.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferricyanide (w/v). The mixture was incubated for 20 min at 50°C. After incubation, 2 ml of 10% TCA (w/v) was added to the mixture, followed by 10 min of centrifugation at 650 rpm. The upper layer (0.5 ml) was mixed with 0.5 ml of deionized water and 0.1 ml of 0.1% ferric chloride (w/v), and the absorbance of the resultant solution was measured at 700 nm.

2.11. Statistical analysis

Values reported in tables and figures are the means ± 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:

3.1 Characterization of Argane fruits

Table 1 shows the composition, the physical parameters of Argane fruits and the correlation between RI and the other characteristics. The fruits used for this study had the following RI: 1.58; 2.34; 3.07; 4.1; 4.89, monitoring the RI showed that the ripeness of Argane fruits increases with advancing date. Argane tree fruit is the source of Argane oil, the fruit is consisted of different components: a pulp, a shell and kernels that are inside the shell. The study of changes in weight, size and water content of fruits show that these parameters decrease significantly with the maturity of fruits; this decrease due to the fact that ripe fruits passed a long time in trees and that they were subject to continuous exposure to temperature. As a result, the whole fruit loses large amount of water ending in a reduction of weight, size and water [15]. The interaction between these parameters and ripeness index can be illustrated by analyzing the relationship between these two settings .In this respect, there is a higher and positive correlation between the ripeness index and the characteristics of Argane

fruits. Differences in fat content, measured on a dry basis Increases significantly with the age of fruit [16].The correlation coefficients between the chemicophysical parameters of the Argane fruits showed that (RI) was highly correlated to the colorimetric values: RI vs. L (R^2= 0. 8971, $P \leq 0.05$), RI vs. a (R^2= 0. 8662, $P \leq 0.05$),
RI vs. b (R^2= 0. 7132, $P \leq 0.05$).

Table 1: Composition and Physical Parameters of Different Samples of fruit in different stage of ripeness

Sample	RI	FAT %	Weight/g	Water %	L	a	b	l/cm	W/cm
S1	1.58	38±0.14^{a}	10.33±1.6^{a}	51.8±0.01^{a}	61.27±0.14^{a}	-2.63±2.83^{a}	41.63±2.8^{a}	3.4±0.3^{a}	2.26±0.2^{a}
S2	2.34	42.5±0.28^{b}	9.53±1.01^{ab}	51.6±0.28^{a}	59.97±1.4^{a}	-2.36±0^{a}	40.46±2.8^{a}	3.13±0.11^{ab}	2± 0.1^{ab}
S3	3.07	45.5±0.02^{c}	8.733±1,44^{ab}	44.5±0.4^{b}	58.2±2.8^{a}	4.02±2.12^{a}	45.87±2.12^{a}	2.7±0.26^{b}	1.93±0.05^{abc}
S4	4.1	51.5±0.14^{d}	6.83±0.3^{b}	44.06±0.5^{b}	42.25±3.5^{b}	4.04±0.02^{a}	25.39±2 .97^{b}	2.76±0.15^{ab}	1.4±0.36^{c}
S5	4.89	55.5±0.42^{e}	6.13±1.5^{b}	38.6±0.07^{c}	38.8±1.4^{b}	12.8±1.41^{b}	21.13±2.12^{b}	2.66±0.2^{b}	1.56±1.15^{bc}
	R^2	0.998	0.9865	0.9019	0.8971	0.8662	0.7132	0.7814	0.8507

Values are expressed as means±SD, Values followed by different letters (a, b, c, d,) in the same row are significantly different at P≤0,05; CI=95% R^2:Correlation; l:length; W:width; RI:ripeness index; (L): lightness; (a): redness; (b):yellowness, n =3

3.2. Phenolic and flavonoid compounds in pulp, kernel and Argane oil during ripening

Phenolic compounds are responsible of the nutritional and sensory quality of extra-virgin Argane oil (EVAO). The composition of phenolic compounds in EVAO is related to the initial content of phenolic compounds in the Argane fruit tissues as well as the activity of enzymes acting on these compounds during the industrial process to the oil production. [17]. Quantitative analysis of different phenolic extracts of pulp, kernel and Argane oil during the maturity of fruit table 2 shows that the t extract of the pulp is the richest in phenolic compounds followed by the extract of the kernel then the oil extract total phenol content expressed in mg as levels of gallic acid/g. This difference between the quantity of phenolic compounds between fruit and oil is explained by that during crushing and malaxation in industrial-scale extraction systems, only 0.3%–1.5% of available phenols in fruits were transferred to the oil, whereas the rest ended up in wastes [17]. The reduction of phenolics compounds in the ripe pulp kernel fruits and oil cannot be ascribed to the simple effect of dilution because of the activity of hydrolytic enzymes which increases with ripening. This phenomenon has been observed in many fruits [22]. Flavonoid has very important and very considerable biological properties. Indeed, many subsequent studies have proven that the flavonoid has the capacity to neutralize different types of free radicals. We may claim that these are particularly significant antioxidants and antimicrobials [23]. The quantity of total flavonoid (table 2) changes in the same way that polyphenols increase between (RI=2, 34 .RI=3, 07) and decrease again at the end of maturity. The total phenol increase significantly until they reach a maximum at RI between (2.34 and 3.07); and then decreased at the end of

maturity (RI = 4.89) both in the fruit and the oil. These results match those observed in olive [18, 14, 19]. This can be explained by the fact that the accumulation of polyphenols and other molecules have a hydroxyl group and aromatic nuclei at the beginning of maturation of fruit; they are also responsible for the bitterness of the unripe fruit. This bitter taste is meant to protect the fruit against the herbivorous enemy who would nibble the fruit at an early stage. Also, it serves as Pathogen Defense: mainly molds bacteria Phytopathogens; and as a Protection against ultraviolet radiation; [20,21].

Table 2 : Phenolic and Flavonoid content in Pulp, Kernel,Oil at different stages of Argane Fruits Ripeness

	RI									
	1.58		2.34		3.07		4.1		4.89	
Samples	TP(mg EAG/g)	TF (mg of CE/g)	TP	TF	TP	TF	TP	TF	TP	TF
P	81.05±1.14^{c}	1.93±0,01^{ab}	83.75±0.9^{c}	1.72±0.14^{ab}	100.5±0.7^{d}	2.14±0.02^{a}	90.2±0.35^{b}	1.53±0.1^{a}^{b}	74.28±0.4^{d}	1.52±0.02^{ab}
K	53.4±0.28^{f}	1.81±0.35^{ab}	58.4±0.5^{e}	1.8±0.28^{ab}	70.7±0.35^{d}	1.41±0.2^{a}^{b}	30.48±0.5^{h}	1.66±0.2^{a}^{b}	30.7±0.7^{h}	1.49±0.04^{ab}
O	41.8±2.12^{g}	1.07±0.7^{ab}	42.3±0.4^{g}	1±0.14^{b}	50.5±1.7^{f}	1.08±0.2^{a}^{b}	25.9±0.14^{i}	1.5±0.35^{ab}	21.3±0.04^{j}	1.17±0.01^{ab}

Values are expressed as means±SD, Values followed by different letters (a, b, c, d,e..i) in the same row are significantly different at P≤0,05 IC=95% P: Pulp ; K: Kernel; O : oil ; TP: Total Phenolic ; TF: Total Flavonoid ;mg GAE/mg of powder crude extract.; mg of CE/g of dry plant material. RI: ripeness index; n=3

3.3. Antioxidant potential of pulp kernel and Argane oil extracts during ripening

3.3.1. Antiradical activity

The DPPH radical scavenging effect for all of the ethanol/water extracts is shown in table 3. All extracts exhibited an antioxidant activity at all maturation stages. The lower antioxidant capacities were found in the ripe samples; with high IC_{50} values (2,81±0,04 in pulp ,1,58±0,17 in kernel and 1,68 ±0,28 in oil). This is probably due to the simple effect of dilution of antioxidant compounds during increased activity of the hydrolytic enzymes with maturation [22, 8]. The obtained extract of fruits that have RI between 1,57and 3,07 showed the highest antioxidant activity, with a lower IC_{50} ,a lower EC_{50} and a large ARP. The results reveal that the extracts have a strong antioxidant activity present in oil followed by kernel and then pulp. The lowest IC_{50}(0, 54±0.5), EC_{50} = 23,4 and ARP= 4.27 present in the extract of oil with RI= 2.34; this extract shows antioxidant activity almost similar to that of tannic acid and very low contribution to gallic acid. Values are expressed as means±SD, P: Pulp ; K: Kernel; O : oil ; EC_{50}=efficient concentration; antiradical power= ARP; IC_{50}= concentration inhibit 50% RI=Ripeness Index ; n=3

Table 3: Antiradical activities of the ethanol extracts of pulp kernel Argane oil and synthetic antioxidants at different ripeness stage

RI	Samples	IC_{50}mg/mL	EC_{50} (mg/mg)	ARP
1.58	P	2.18±0.16	94.7	1.05
	K	0.95±0.7	41.3	2.42
	O	0.98±0.09	42.6	2.34
2.34	P	0.85±0.01	36.9	2.71
	K	0.95±0.04	41.3	2.42
	O	0.54±0.5	23.4	4.27
3.07	P	1.15±0.2	50	2
	K	0.995±0.02	43	2.32
	O	1±0.19	43.4	2.3
4.1	P	2.81±0.04	122	0.8
	K	1.4±0.2	60.8	1.64
	O	1.02±0.07	44.3	2.25
4.89	P	2.2±0.7	95.65	1.04
	K	1.58±0.17	68.6	1.45
	O	1.68±0,28	73.04	1.28
IC_{50} Gallic Acid		0.015±0.007	0.65	153.8
IC_{50} α Tocopherol		0.15±0	6.5	15.3
IC_{50} Tanin Acid		0.48±0.01	20.8	4.8

3.3.2. Ferric reducing antioxidant power

The table 4 shows the total antioxidant capacities of the extracts determined as (FRAP) assay. The reducing power increased in a concentration-dependent manner in all the samples, the highest reducer power is found in the oil followed by kernel then the pulp and increases with the maturity of the fruits beginning the RI = 2.34 up to 3.07 after is starting to decrease from RI = 4.1

4. Conclusion

To conclude, this work is a report on the quantification and characterization of phenolic, flavonoid and a study the antioxidant potential of pulp, kernel, and oil during the ripening of Argane fruits. Our results show that the total phenol and total flavonoid content increase with ripeness and decrease again in the last stage the maturity of fruits in the different part of fruit and in oil , the potential antioxidant effect is the highest in early and intermediate fruits especially in the oil. Based on our results it appeared that the ripening stages have a significant effect on the character of the fruit and on the quantity of polyphenol and flavonoid present in the pulp, kernel and oil as well as their reducing power. These results can be considered for a better use of fruit pulp and press-cakes as a nutraceutical or in cosmetics. At the same time, this study helps in determining the best ripening stages for having a good oil quality rich in phenolic compounds with good antioxidant potential; that is, to choose early fruits green fruits and ripe yellow fruits.

Table 4: The total antioxidant capacities of the extracts determined as ferric reducing antioxidant power (FRAP) at different stage of ripeness

Values are expressed as means±SD, P: Pulp; K: Kernel; O: oil ; C: concentration ; RI: ripness index ; n=3

RI	1.58			2.34			3.07			4.1			4.89		
C µg/mL	P	K	O	P	K	O	P	K	O	P	K	O	P	K	O
60	0.1525±0.0007	0.1945±0.007	0.101±0.001	0.1545±0.002	0.175±0.03	0.1065±0.006	0.1595±0.007	0.141±0.001	0.112±0.001	0.1515±0.002	0.174±0.01	0.101±0.001	0.149±0.001	0.1535±0.002	0.106±0.005
80	0.1555±0.0007	0.205±0.002	0.1145±0	0.1575±0.002	0.1945±0.007	0.115±0.001	0.1635±0.002	0.1925±0.004	0.115±0.001	0.158±0	0.232±0.005	0.117±0.001	0.156±0.001	0.1615±0.002	0.1595±0.05
100	0.158±0.002	0.2635±0.05	0.116±0.002	0.1635±0.002	0.204±0.004	0.1215±0.004	0.165±0.001	0.203±0.002	0.1365±0.002	0.161±0.001	0.282±0.06	0.1195±0.007	0.1605±0.002	0.1635±0.001	0.184±0.09
200	0.167±0.002	0.3165±0.002	0.133±0	0.169±0.0014	0.195±0.035	0.14±0.009	0.1725±0.003	0.21±0.014	0.1435±0.004	0.162±0.005	0.3405±0.017	0.143±0.005	0.166±0.001	0.172±0.002	0.149±0.001
250	0.1695±0.007	0.3815±0.002	0.1295±0.004	0.1735±0.002	0.406±0.033	0.8555±0.04	0.179±0.001	0.5535±0.002	0.8825±0.004	0.1675±0.003	0.849±0.09	0.809± 0.05	0.166±0.001	0.5905±0.088	0.705±0.02

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