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TEMPERATURE AND MECHANICAL EXTRACTION EFFECT ON CHEMICAL COMPOSITIONS OF *ARGANIA SPINOSA L SEED OIL* OF EASTERN REGION OF MOROCCO

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Abstract

This study focuses on yields, chemical quality, composition, and the stability of the fatty acids of the oil extracted from *Argania spinosa L* seeds, collected from the eastern region of Morocco, influenced by the temperature of mechanical extraction methods. The results of this study revealed that *A.spinosa* is a rich source of oil. The obtained oil yields varied from 49,88±0.03 for the cold press to 55,62±0.06 for the hot press, the main fatty acids found in *Argania spinosa are* oleic acid by 37,218%±0.05 for (cold press) and 37,26%±0.18 (hot press) and linoleic acid 29,69%±0.10 for (cold press) and 32,12% ±0.11 (hot press). The oil of Argan could be classified as an oleic acid. Among the tocopherols found, a high value of γ-tocopherol has been detected in the cold press with 622,7±1,12 mg/kg, and the hot press with 610,01± 3,33mg/kg. Our results revealed the effect of temperature, that when we increase the temperature used, the yields increase also, the findings shown also that this high temperature do not affect physico-chemical proprieties, fatty acids and tocopherols.

The results of the present study reveal that the oil of *A. spinosa* could be used in cosmetics and pharmacological products.

Keywords: *Argania spinosa*, fatty acids, Tocopherols, Extraction, Oil.

1. Introduction

Argania spinosa is an endemic tree native to southwest Morocco [1] and the only representative species of the tropical Sapotaceae family in Morocco. It is widespread from sea level to 1300-1500 m altitude, and forms well developed woodlands in the plains of Essaouira and Souss near the Atlantic coast, and a rather open vegetation in the Anti-Atlas and High Atlas [2]. The Argan forest is an ecosystem in which the Argan tree occupies a predominant place. In terms of surface area, it constitutes one of the main forest formations in Morocco, where it grows over about 320.000 square miles [3]. The Argan tree is an attractive example of a woody and versatile fruit species. The fruit contains an oleaginous seed which presents an economically interesting oil, mainly extracted by traditional way, despite the recent introduction of mechanized extraction processes [4]. Argan tree has an important economic interest because its fruits provide a source of biologically active and precious edible oils that are consumed as food, used in cosmetic preparations and in traditional medicine [5]. People in Morocco traditionally use the fruits of *A. spinosa* to prepare edible oil [6]. This oil is used for many purposes. In food, argan oil has been known for centuries in Morocco where it is the basic ingredient and sometimes the exclusive source of vegetable fat in the "Amazighdiand" [7-8].In cosmetics, virgin argan oil is advocated as a moisturizing oil, acting against juvenile acne and scaly skin, skin peeling, as well as, nourishing hair [9]. Argan oil also has medicinal uses to combat rheumatism, soothe inflammation and heal scars and burns. Indeed, Argan oil is used in food and recommended in the Moroccan pharmacopoeia to treat some skin diseases [10] However, argan oil remains the main viable economic resource produced by the argan tree so far. Its worldwide marketing is currently being successfully achieved and is giving to the argan groves the necessary momentum to positively envision their rescue. It has been traditionally used in Morocco for centuries in diets and cosmetics and against skin infection. [11]. Edible argan oil is by far the most ancient type of argan oil and can be prepared following an ancestral process [10], cosmetic argan oil has been ranked the number one in the top 10 beauty ingredient list for 2009 by a major US agency (http://www.piercemattiepublicrelations.com/ beautydivision/2008/12/beauty-forecast-pierce-mattei.html (access August, 2010). The main objective of this work is to study the Temperature and the mechanical methods of extraction on the chemical composition of *Argania spinosa* collected from the eastern region of Morocco.

2. Materials and methods

2.1 Collection are:

Argania spinosa L are collected in September 2019 from Berkane (Province of Berkane), province belong to the eastern region of Morocco. The kernels were removed, dried and ground into a fine powder, then stored for the next use.

Table 1: Ecogeographic characteristics of the sampling site:

Sampling location	Region	Latitude	Longitude	Altitude (m)	MAP (mm)	MWT (°C)	MST (°C)
Berkane	Eastern	34° 55' 12 N	-2° 19' 12 O	200	287.2	7 °C	36°C

MAP: mean annual precipitation; MWT: mean of winter temperature; MST: mean of summer temperature. Source: https://fr.weatherspark.com/y/31980/Météo-habituelle-à-Smimou-Maroc.

2.2 Mechanical extraction: Hot press (120 °C) and cold press

The mechanical extraction is a biological method used to extract oil from the seeds. The seeds are placed between permeable barriers by increasing the mechanical pressure thus reducing the volume available for the seeds. In general, regardless of the seeds used, the higher the pressure, the higher the oil extraction efficiency. In an oil press brand p55/AFYACH voltage 220V/380 V electric power 1,5 kw, N° of series 0020200107 we put more than 5kg of Argan seeds, an extraction that last approximately 30 min then we filter our oil in a mechanical filter of 20 plaques and conservation at 4 until the use.

2.3 The physicochemical analysis

2.3.1 Acid index: Method ISO 660: 1996 [9.

The reference measurement of acidity was carried out by the standardized method NF EN ISO 660, in simple (repeatability verified on several samples). This is carried out by titration of the sample solubilized in an ethyl ether/ethanol mixture with an ethanolic solution of potassium hydroxide. The results are expressed in % (m/m) of oleic acid equivalent.

2.3.2 Saponification index: Method ISO 3657: 2002 [9].

Weigh to the milligram in a flat-bottomed Erlenmeyer flask, 2g of oil. Add 25ml, exactly measured, of alcoholic potash (0.5N), and bring to boiling point under a reflux condenser. It is advisable to add a boiling regulator (pumice stone, glass beads, etc.) to the Erlenmeyer flask and maintain boiling for one hour, stirring occasionally. Titrate the excess alkali in the hot soap solution with hydrochloric acid (0.5N) in the presence of phenolphthalein. Perform a blank test under the same conditions to titrate the alcoholic potash liquor.

2.3.3 Peroxide index: Method ISO 60-220: 1995 [12].

The reference peroxide value was determined by the standard method NF T 60-220, in simple (repeatability verified on several samples). The principle of this method is based on the titration by a sodium thiosulfate solution of the iodine molecules released by the oxidation of iodides by the hydroperoxides of the sample solubilized in an acetic and chloroform mixture. The results are expressed in meqO_2/kg.

2.3.4 Relative density: IUPAC Method 2.101[12].

In a clean, dry pycnometer weighed at 20°C, we introduce cooled distilled water, then weighed the pycnometer plus distilled water. The whole is brought to a temperature of 20°C. The pycnometer is emptied and dried, then it is filled with the same volume of oil cooled to 20°C and the mass of the total is determined.

2.3.5 The refractive index: Method ISO 6320: 2000 [12].

Wash the refractometer prisms with petroleum ether.

- Wipe them with a clean soft cloth.
- Then pour 2 to 3 drops of oil between the prisms.
- Then move the telescope so that the line of separation of the light and dark areas is located at the crossroads of the reticule.
- Read the refractive index of the oil at T°C=20°C.

2.4 Chromatographic analysis CPG-MS

The chromatographic analyses were carried out after transesterification by gas chromatography coupled with a mass spectrometer (SHIMADZU series GCMS-QP2010), equipped with a split/spitless injector and a column (LxDI :30m x 0.25 mm) apolar (Stationary phase: 95% dimethylpolysiloxane: 5% phenyl; Thickness; 0.25µm). The carrier gas used was the Helium.

2.5 Tocopherols:

The tocopherol analyses were performed by HPLC-FLD, (Agilent Technologies 1200 series system, Agilent Technologies) using the official method, AOCS 8-89 [13] . It was equipped with an automatic injector, on an Uptisphere 120A° NH2 column (150 mm * 3 mm, 3 µm) Interchim (Montluçon, France). The temperature was maintained at 30°C. The mobile phase was hexane/2-propanol (99:1, v/v) with a flow rate of 1 ml min-1. The different isoforms of tocopherol (α-, β-, γ- and δ-tocopherols) were estimated using an oil/hexane solution of the oils of the two extraction types *of A.Spinosa*. Tocopherols obtained from Sigma-Aldrich (Steinheim, Germany) were used as external standardization for the identification of tocopherols at 292 nm. The homologues of the tocopherols were quantified by comparing the peak response of each sample with that of the corresponding standard.

2.6 Statistical analysis

Data were statistically tested by a unidirectional analysis of variance (ANOVA) followed by a Tukey test to compare means that showed a significant variation ($P \leq 0.05$) using SPSS 20 software.

3 Results and Discussion

3.1 Yields:

The oil yields obtained from *Argania spinosa* seeds using the two extraction types are presented in (table2). The oil yields present a highly significant difference ($P < 0.05$). During our study, argan oil yields founds was 55.62±0.06% for hot press extraction and cold press extraction with 49.88±0.03%. Our results are similar to those found by many authors who worked on the Moroccan Argan oil. In fact, the maximum yield reached is 54 and 55 % [14], furthermore, Guillaume, 2018 shown that mechanical pressing of non-roasted argan seeds provides beauty oil in 40–45% yield [5]. The yield in by-products of argan fruits is largely influenced by the effect of the origin, that is due in general to the eco-geographical variations, without excluding the genetic factor.

Table 2: Oil yields extracted from *Argania spinosa* seeds using two types of mechanical Extraction

	Hot press	Cold press
Yields	55.62±0,06a%	49.88±0,03b%.

3.2 Physicochemical properties:

To control the quality of the oils, physical and chemical parameters were used. These parameters include acidity, density, refractive index, saponification index and peroxide value. The results of the quality parameters determined in the samples of organic oil and oil extracted by mechanical extraction are presented in (Table 2).The acid index analysis of *Argania spinosa* oil reveals that there is a significant difference ($P<0.05$) between

the results of the two mechanical types of extractions, that of hot press extraction with (2.4±0.09mg/KOH), and cold press with (2.409±0.05 mg/KOH). The acidity index is related to the amount of free fatty acids resulting from hydrolytic reactions of triglycerides. It is a quality criterion that informs on the conservation status of an edible oil. Our results of acid index are comparable to those of Rahmani 2005 [15] which dictates that the acid index of common virgin Argan oil is ≤ 2.5 mg/KOH. Concerning the saponification index of Argan oil, the results increase slightly in the two types of extraction (hot and cold press), 201.22 ±0.16 mg/KOH mg KOH/g and 195.45 ±0.6mg/KOH respectively. The high saponification index could due to the geographical origin of the seeds and indicates that the oils have a high amount of triglyceride content and are therefore very useful in cosmetology, Hilali et al., 2005 shown that Saponification values of Argan oils were found between 180.0 and 199.0, a larger range of value than required for virgin olive oil (between 184 and 196) [16]. Also, our results are in agreement with those of Belarbi-Benmahdi et al., 2009 with a saponification index of 193.57 mg/KOH. These results suggest to use Argan oil in the fabrication of liquid soaps and shampoos [17]. The peroxide index recorded for hot press is 5.4±0.09 Meq/Kg and 5.5±0.07 Meq/Kg for the cold press extraction. The peroxide value measures the degree of rancidity of fats due to exposure to air, which results in the formation of peroxides from unsaturated fatty acids our results shown a low value of peroxid index but our results are still below the Moroccan standard 15milieq/kg [18]. Those results are in agreement with the result of Adlouni which shows that the peroxide index for industrial extraction was 6.5 Meq/Kg while for artisanal extraction the index was 5.2 Meq/Kg.

Table 3: Physicochemical properties of *Argania spinosa* oil extracted by three methods of extraction.

	Argania spinose	
Extraction Parameters studied	**Hot press**	**Cold press**
Acid index (mg KOH / g oil)	2.411±0.09 a	2.409±0.05a
Saponification index (mg KOH / g oil)	201.22 ±0.16 a	195.45 ±0.6 b
Peroxide index 20milieq/Kg	5.4±0.09 a	5.5±0.07 a
Density (20°C)	0.905 ±0,001a	0.906±0.009. a
Refractive index (n20d °C)	1.473±0.006 a	1.473±0.009a

This low peroxide index may indicate that oxidation is relatively negligible [19]. The density is a physical index that helps to determine the purity criteria of the extracted oils. The values obtained for hot and cold extractions are 0.9±0.001 and 0.907±0.009 respectively, our results were in accordance with the CODEX STAN 210-1999 standard (Table 3). The analysis of the refractive index reveals that there is no significant difference ($P<0.05$) between the obtained value of hot press extraction 1.470±0.006 and cold press with 1.473±0.009 this little difference may be due to the experimental margin of error, our findings are the same of those reported by Hilali et al., 2007 and Charrouf and Guillaume, 1999 which were 1.4644 and 1.463 respectively [16,9].

3.3 Gas chromatography analysis

Argan oil is rich of unsaturated fatty acids (UFA), the main fatty acids are oleic acid and linoleic acid in all the types of organic extraction: cold press with (37.21%±0,05 of oleic acid and 29.69%±0.10 of linoleic acid) and hot press with (37.26%±0,08 of oleic acid and 32.12%±0,11 of linoleic acid).The saturated fatty acids (SFA) are palmitic acid with (27.16%±0,01for cold press and 22.50%±0.02 for hot press extraction), followed by stearic acid (5.92%±0,03 for cold press extraction) and arachidic acid (8.10%±0,01 for hot press extraction). Argania spinosa oil is highly unsaturated due to the dominance of UFA with (66.90% for cold press extraction, 69.38% for hot press extraction). The average SFA content for the three extraction processes (cold press and hot press) is (33.092% and 30.606%) respectively. Despite the difference of temperature used for the extractions, we found that there is no degradation of the fatty acids (C16:0, C18:1, C18:0 and C20:0) of A. spinosa oil with a slight variation in proportions (Table 4). The results show that there is a stability of saturated and unsaturated fatty acids of Argan oil collected from the eastern region of Morocco, which is in agreement with Kadda et al., 2021 shown for prickly pear seed oil that the fatty acids are stable whatever the extraction and the temperature used.[20] Comparing our results with those of other studies indicated on Moroccan oils shows that our oils are rich in oleic and linoleic acid. Many benefits of argan oil traditionally known arise from its physical and chemical properties. The argan oil food and cosmetic has the similar composition of fatty acids and is particularly rich in unsaturated fatty acids [5]. Argan oil contains between 43 and 49% oleic acid, a monounsaturated fatty acid, and 29.3 to 36% linoleic acid, a polyunsaturated fatty acid. Saturated fatty acids constitute only 16 to 20% of the composition of argan oil (palmitic acid: 11.5-15% and stearic acid 4.3-7.2%) [18]. The high oleic acid content of argan oil may contribute to its traditional indication as a cure for skin inflammation. However, several other oils are rich in oleic and/or linoleic acid but do not possess the same therapeutic effects [21]. oleic acid which has beneficial properties for the treatment of breast cancer [22], and which represents in our results about 37% of these fatty acids, gives a high nutritional value to our Argan oil extracted from the eastern region of Morocco. Thus, predominant fatty acid content analysis of Argan oils from the Admin forest (Agadir, Morocco) indicated that oleic acid varied from 41.52 to 46.73% and linoleic acid from 33.54 to 38.38% [20]. Therefore, the Argan oil of Taroudant region (Morocco), amounts are 46.8% of oleic acid and 33.8% of linoleic acid [23,24]. Some pharmacological properties of argan oil are likely to result from its high unsaturated fatty acid content. Saturated fatty acids reveal a predominance of palmitic and stearic acids in all samples studied. These findings were confirmed by those of many authors working on Moroccan oils. The high propotion of palmitic acid 20% is due to the geographical situation, which is in accordance with the results of Fellat-Zarrouk *et al*, 1987, which reports that the palmitic acid content increases when moving from the plains to the high plateaus, however, the oleic acid content increases with rainfall and the linoleic acid content increases with altitude. [25] The composition of Argan oil is very similar to that of olive oil and jujube oil, this richness of these oils in unsaturated fatty acids such as oleic and linoleic acid, proves their uses in cosmetics and nutrition. Argan oil is recommended as a moisturizing oil for the face, hands and feet. It is also used as an anti-wrinkle, against juvenile acne, against skin desquamation and for hair shine. [26]

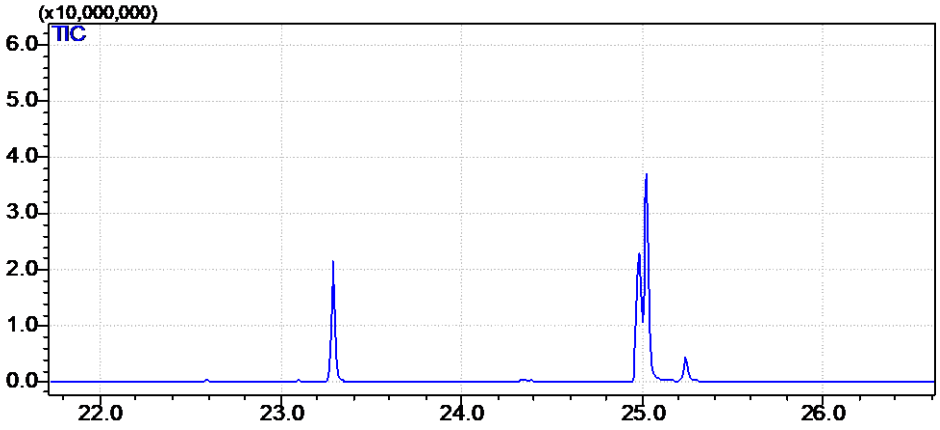


Figure2a: GC chromatogram of total lipid of *A.Spinosa*, hot press extraction

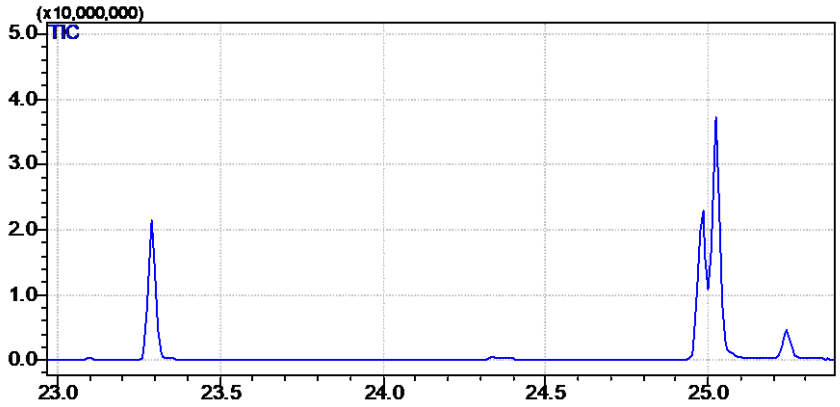
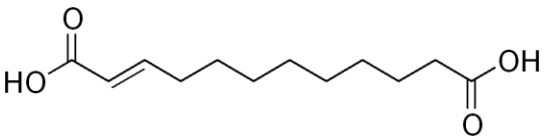
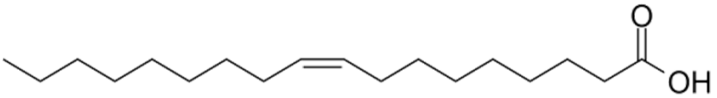


Figure2b: GC chromatogram of total lipid of *A.Spinosa*, cold press extraction



Linoleic acid



Oleic acid

Figure 3: The main unsaturated fatty acids of *Argania Spinosa* seed oil

3.4 Tocopherols:

The major tocopherol founded in *Argania spinosa* oil of eastern region of Morocco is γ-tocopherol, it represents 610.01±3.33mg/kg for hot press and 622.7 ±1.12mg/kg for cold press extraction (table 5). The α-Tocopherols with 39.55±0.01mg/kg, 35.13 ±0,03mg/kg for hot and cold press extractions respectively. Flowed by δ -tocopherol and β-tocopherols with 16.86±0.01mg/kg, 15.66 ±0.01mg/kg and 0.24±0.00mg/kg 0.21±0.01mg/kg respectively (table 5). Tocopherols are important oil components since they possess both antioxidant and vitamin action [28]. One of the specificities of Argan oil is its high content of tocopherols, γ-tocopherol being the major one [29]. Among the three forms of tocopherols it was observed that γ-tocopherol is the principal component (about 84%). This particular constituent was reported by Hensley et al

that γ-tocopherol has a good impact on human nutrition and health due to its antioxidant potential. These values confirm the richness of our oil in tocopherols in agreement with those of our Moroccan research where the concentrations vary from 629 to 660 mg / kg [7] and 637 mg / kg [16]. Argan oil also contains 600-900 mg/kg of tocopherols, of which 81-92% are γ-tocopherol. The α-, β- and δ-tocopherols represent 2.4 to 6.5%, 0.1 to 0.3% and 6.2 to 12.8% of total tocopherols, respectively [19]. Tocopherols have a strong antioxidant capacity and a powerful antiradical capacity [30-32].

Table 4: chemical composition of *Argania spinosa* Oil extracted by different mechanical extraction

	Argania spinosa		
Compounds	**Retention Time (min)**	**% Air Extraction**	
		Cold press	**Hot press**
Palmitic acid, C16 :0	23.29	27.169%±0,21a	22.50%±0,02b
Linoleic acid, C18 :2	24,99	29.690%±0,10b	32.12%±0,11a
Oleic acid, C18 :1	25.03	37.218%±0,05a	37.26%±0,08a
Stearic acid, C18:0	25,22	5.923%±0,13	_
Arachadic acid, C20:0	25.24	_	8.10%±0,01
Fatty acids	**SFA[a]**	33.092	30.06
	UFA[b]	66.908	69.38
	UFA / SFA[c]	2.02	2.30

IM: Identification Method, **MS**: Mass Spectrometry, **SFA**: Saturated Fatty Acid, **UFA**: Unsaturated fatty acid

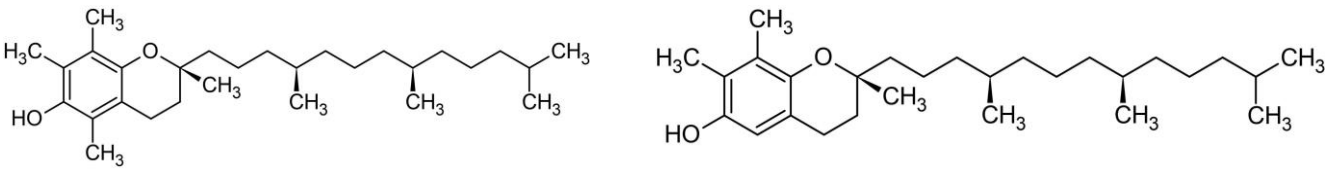


Figure 4: The main tocopherols (vitamin E) of *Argania spinosa* seed oil.

Table 5: Tocopherols composition of *Argania spinosa* for two type of extraction

Tocopherols	Hot press (120)	Cold press
α-Tocopherols mg/kg	39.55±0.01a	35.13 ±0.03b
β-tocopherol mg/kg	0.24±0.00a	0.21±0,01a
***γ*-tocopherol mg/kg**	610.01±3.33b	622.7 ±1.12a
δ -tocopherol mg/kg	16.86±0.01a	15.66 ±0.01b

4 Conclusions:

This study demonstrates that argan oil is a rich source of Saturated and Unsaturated fatty acid and tocopherols. These oils may interest the production units of food, cosmetics and pharmaceutical products. This characterization would allow all units exploiting this oil in their artisanal or industrial preparations, and their commercialization on the national and international market, either in full or by the manufacture of new products based on these Moroccan oil

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