Research Article

Quality of Cosmetic Argan Oil Extracted by Supercritical Fluid Extraction from *Argania spinosa* L.

Chouaa Taribak,¹ Lourdes Casas,² Casimiro Mantell,² Zoubaida Elfadli,¹
Rédouane E. Metni,¹ and Enrique J. Martínez de la Ossa²

¹ Department of Chemical Engineering, Faculty of Science, University Abdel Malek Essaadi of Tétouan, 93002 Tétouan, Morocco

² Department of Chemical Engineering and Food Technology, Faculty of Science, International Agri-food Campus of Excellence, University of Cadiz, 11510 Puerto Real, Cadiz, Spain

Correspondence should be addressed to Lourdes Casas; lourdes.casas@uca.es

Received 15 January 2013; Revised 8 March 2013; Accepted 6 April 2013

Academic Editor: Marleny D. A. Saldaña

Copyright © 2013 Chouaa Taribak et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Argan oil has been extracted using supercritical CO₂. The influence of the variables pressure (100, 200, 300, and 400 bar) and temperature (35, 45, 55°C) was investigated. The best extraction yields were achieved at a temperature of 45°C and a pressure of 400 bar. The argan oil extracts were characterized in terms of acid, peroxide and iodine values, total tocopherol, carotene, and fatty acids content. Significant compositional differences were not observed between the oil samples obtained using different pressures and temperatures. The antioxidant capacity of the argan oil samples was high in comparison to those of walnut, almond, hazelnut, and peanut oils and comparable to that of pistachio oil. The physicochemical parameters of the extracted oils obtained by SFE, Soxhlet, and traditional methods are comparable. The technique used for oil processing does not therefore markedly alter the quality of argan oil.

1. Introduction

Argania Spinosa L. (Sapotaceae) is an endemic tree from southwestern Morocco, where it is the third most common tree. The oleaginous fruits of the argan tree furnish edible and marketable oil known as “argan oil”, which provides up to 25% of the daily lipid diet for the local population and 9% of the annual oil production in Morocco [1].

Generally, this oil is rich in unsaturated fatty acids (80%), principally oleic, and linoleic acids (44.8 and 33.7%, resp.). Interestingly, the unsaponifiable fraction (1% of the oil constituents) of argan oil is mainly rich in antioxidant compounds such as tocopherols, which is present in a higher proportion compared to olive oil (637 mg/kg versus 258 mg/kg, resp.) and especially in its γ -isoform (75%) (Table 1) [2]. Moreover, this nonglyceric fraction is rich in phenolic compounds, principally ferulic and syringic acids (3147 and 37 μ g/kg, resp.), which are absent in olive oil. Also, it is rich in some sterols such as schottenol (1420 mg/kg) and spinasterol (1150 mg/kg). These two families of sterols, known for their anticancer properties, are rarely encountered in other

vegetable oils. Argan oil also contains a nonnegligible proportion of squalene, another anticancerous product (3140 mg/kg versus 4990 mg/kg in olive oil). These compounds prevent oxidation, contributing to the stability of the oil.

By far the main traditional use of argan oil is for nutritional purposes. Natives either eat the oil directly on toasted bread, generally for breakfast, or use it for cooking.

As a cosmetic product, the oil is traditionally used to cure all kinds of skin pimples and, more particularly, juvenile acne and chicken pox pustules [3]. Argan oil is also recommended to alleviate dry skin conditions and to slow down the appearance of wrinkles. This oil is also used in rheumatology. In this latter field, as well as in the cosmetic area, argan oil is used as a skin lotion and is applied on the area to be treated.

In addition, and in a similar way to olive oil, argan oil is also administered orally, and it is traditionally prescribed as a choleric, hepatoprotective agent and in cases of hypercholesterolemia and atherosclerosis.

The method employed to extract the oil from nuts is complex and has a considerable influence on the physicochemical composition, nutritional value, and sensorial properties of

TABLE 1: Chemical composition of argan oil and olive oil [2].

	Argan oil	Olive oil
Tocopherols (mg/kg oil)		
γ -Tocopherols	480 $\pm$ 7	26 $\pm$ 1
α -Tocopherol	35 $\pm$ 1	190 $\pm$ 1
δ -Tocopherol	122 $\pm$ 10	42 $\pm$ 2
Total	637 $\pm$ 18	258 $\pm$ 3
Sterols (mg/100 g oil)		
Schottenol	142 $\pm$ 11	nd
Spinasterol	115 $\pm$ 7	nd
δ^{8-22} Stigmastadiene-3 β -ol	9 $\pm$ 1	nd
β -Sitosterol	nd	156 $\pm$ 3
Campesterol	nd	12 $\pm$ 1
Stigmasterol	nd	nd
Others	29 $\pm$ 1	151 $\pm$ 10
Phenolic compounds (μ g/kg oil)		
Vanillic acid	67 $\pm$ 3	359 $\pm$ 7
Syringic acid	37 $\pm$ 5	0
Ferulic acid	3147 $\pm$ 20	51 $\pm$ 2
Tyrosol	12 $\pm$ 1	19,573 $\pm$ 37
Others	0	773,000 $\pm$ 53
Total	3263 $\pm$ 29	792,983 $\pm$ 99
Squalene (mg/100 g oil)	314 $\pm$ 1	499

nd: not detected.

the oil [4]. At present, two methods are used to extract argan oil for nutritional purposes: the traditional method (hand pressed) and a semi-industrial method (mechanical cold-pressed) [5].

For many years, argan oil has been prepared exclusively by Berber women according to an ancestral multistep process [6]. Between May and August, fallen ripe fruit is collected in the argan forest. The fruit is then sun-dried for a few days, and the dried peel is removed manually to give argan nuts. An average of 100 kg of dried-fruit and 15 hours (per person) is necessary to obtain 60 kg of argan nuts. Argan nuts are then crushed between two stones, and the white kernels are collected. From 60 kg of argan nuts, only 6.5 kg of kernels are collected. To prepare edible argan oil, kernels have to be roasted for a few minutes, but overheating should be avoided since it has a negative impact on the final oil taste. The roasted kernels are subsequently crushed using a millstone to give a brownish viscous liquid that is mixed with water. This dough is hand-malaxed for several minutes, and it slowly becomes solid and releases an emulsion from which the argan oil is finally decanted [7].

Traditional oil extraction is frequently carried out in unsatisfactory sanitary conditions. As a result, several cooperatives aim to produce and commercialize quality-certified virgin argan oil using a semi-industrial method with mechanical cold-pressing without the addition of water [10]. This is the most important difference between the two methods outlined above. All other steps remain unchanged and the oil is obtained in about 45% yield (calculated from the kernels), and, once the kernels have been obtained, only half an hour

is needed to obtain 1 L of oil, that is, a shorter time than that required for the traditionally obtained oil [11].

For industrial or laboratory purposes, argan oil can be extracted from ground kernels using any volatile lipophilic solvent. The solvent is evaporated to give the oil in 50–55% yield. However, this type of extraction furnishes oil with unsatisfactory organoleptic properties compared to the traditional or press-extracted oil. As a consequence, this technique is exclusively used to prepare argan oil for cosmetic purposes [3].

Another product that is also used exclusively for cosmetic purposes is the so-called “enriched argan oil”, which can be prepared by flash distillation, under reduced pressure at 270°C, of the crude oil previously obtained by pressexttraction. The level of unsaponifiable matter contained in this oil is three times higher than the value observed for the press-extracted oil.

Supercritical fluid extraction (SFE) is considered to be an environmentally benign alternative to the conventional extraction of triglycerides. SFE has been successfully used to obtain oil from seeds of apricot [12, 13], palm [14, 15], canola [16, 17], rape [18], soybean [19, 20], sunflower [21–23], jojoba [24], sesame [25–27], celery [28], parsley [29], neem [30], amaranth [31], borage [32], flax [33], and grape [34]. Oil has also been extracted from nuts such as acorn [35], walnut [36–38], almond [39], and pistachio [40].

In this paper we present a study into the extraction of argan oil using supercritical CO₂ for cosmetics uses. CO₂ is the most widely used supercritical fluid because it is nontoxic, nonflammable and is available at low cost and with a high degree of purity. Furthermore, the use of CO₂ is acceptable in the food and pharmaceutical industries. Despite the numerous studies on this kind of supercritical extraction, literature the concerning the extraction of argan seeds is lacking in terms of SFE. The quality of the oil obtained is also compared with other examples reported in the literature.

2. Material and Methods

2.1. Samples and Chemicals. Argan seeds were used in this study. The fruit was collected in May 2011 in Morocco. The fruit was sun-dried for seven days to constant weight, and the dried peel was manually removed to provide the argan nuts. The argan seeds were ground to obtain the appropriate particle size distribution (mean size 0.8 mm).

Carbon dioxide (99.995%) was supplied by Abello-Linde S.A. (Barcelona, Spain). 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH), methyl ester standards of fatty acids, β -carotene, and tocopherol were supplied by Sigma-Aldrich (Steinheim, Germany) and the other reagents were supplied by Panreac.

2.2. Extraction of Argan Oil. The oil extractions at high pressure were carried out in equipment supplied by Thar Technology (Pittsburgh, PA, USA, model SF100) provided with an extraction vessel (capacity of 100 mL) and a pump with a maximum flow rate of 50 g/min of carbon dioxide. The extraction temperature was controlled with a thermostated jacket. The cyclonic separator allowed periodic discharge of

FIGURE 1: Schematic diagram of the equipment.

the extracted material during the SFE process. The extraction system is represented in Figure 1.

The operating methodology involved loading the extraction vessel with approximately 15 g of the sample, which had previously been homogenized in order to maintain a constant apparent density in all experiments of 520 Kg/m³. Since the apparent density of all samples was approximately constant, porosities were also constant and equal to

$$\varepsilon = 1 - \frac{\rho_{ap}}{\rho_r} = 0.68, \quad (1)$$

where ε is porosities, ρ_{ap} is apparent density, and ρ_r is real density.

The extracts were collected in a cyclonic separator and transferred to glass bottles, which were stored at 4°C with the exclusion of light.

The experiments on each sample were carried out in duplicate in order to evaluate the variability of the measurements. Experiments were carried out at different temperatures and pressures. The measured flow rate for the supercritical fluids was 20 g/min for 3 hours.

The oil extraction was also carried out using a Soxhlet extraction system with hexane as solvent. An extraction time of 8 hours was chosen. After extraction, the extracts were evaporated on a rotary evaporator (Laborota 4001, Germany) at 40°C, and the samples were stored at 4°C with the exclusion of light.

2.3. Analytical Methods. Refractive index and density were carried out according to the reported AOCS methods [41].

2.3.1. Acid Value. The acid value of each oil obtained under different conditions was analyzed according to UNE-55011. Acidity index is the mass, in mg, of potassium hydroxide that is necessary to neutralize free fatty acid present in 1 g of sample. It is usual to represent this value as percentage of oleic acid, which is the most abundant of the fatty acids [41].

2.3.2. Peroxide Value. Peroxide value is related to hydroperoxides in terms of milliequivalents per kg of oil. These hydroperoxides oxidize potassium iodide under standard conditions [41]. Peroxide value determinations were carried

out by iodine titration against $\text{Na}_2\text{S}_2\text{O}_3$ with starch indicator in the second stage. An $\text{Na}_2\text{S}_2\text{O}_3$ concentration of 0.01 eq L^{-1} was used. The blank was taken into account in all titrations.

2.3.3. Iodine Value. One of the most useful parameters for the characterization of oils and fats is the iodine number or value, which is a measure of unsaturation. The iodine value is defined as grams of I_2 added across the multiple bonds of a 100 g sample.

The iodine value is determined using classical titration methods [41]. In these methods, a reagent containing iodine is added to an oil sample and the excess iodine is titrated with a standard sodium thiosulfate solution. The official AOAC methods for the determination of iodine value of oil involve the use of Wijs iodine monochloride [41].

2.3.4. Unsaponifiable Matters. The term “unsaponifiable matter” is applied to the substances nonvolatile at $100\text{--}105^\circ\text{C}$ obtained by extraction with an organic solvent from the substance to be examined after it has been saponified. Unsaponifiable matters were carried out according to the reported AOCS methods [41].

2.3.5. Composition of Fatty Acids. Fatty acid compositions were determined by gas chromatography (GC) on an Agilent Technologies model 6890N chromatograph with a TR-CN100 capillary column (60 m length $\times$ 0.25 mm internal diameter $\times$ 0.20 μm thickness) and a flame ionization detector. The injector and detector temperatures were 280°C and 260°C , respectively. The oven temperature was 185°C . The carrier gas was hydrogen at the rate of 38.02 cm/s, and air and hydrogen were used as auxiliary gases.

A preparation step was required prior to the introduction of the oil into the GC for the individual determination of fatty acid composition. The extracts obtained were treated to convert them into the corresponding fatty acid methyl ester (FAME) [42].

2.3.6. Total Tocopherol Content. High-performance liquid chromatography (HPLC) analysis of the total tocopherol present in the extracts was performed using an Agilent Technologies 1100 Series chromatograph. The elution solvent was methanol at a flow rate of 1.0 mL/min and the column used was C_{18} Hypersil ODS (250 $\times$ 4.6 mm) (5 μm particle size) (Supelco). The compounds were detected using a UV-Vis detector at a wavelength of 280 nm. The peak was identified by comparison of retention time with the commercial standards (Sigma), and the compound was quantified by means of calibration curve.

The experiments for each extraction were carried out in triplicate in order to evaluate the variability of the measurements. The results are shown as the average of all the independent analyses with a reproducibility of approximately 8% CV (coefficient of variation).

2.3.7. β -Carotene Content. β -Carotene content was determined using the same HPLC system as for tocopherol content. The elution solvent used was acetonitrile: methanol: water (90:8:2) at 1.0 mL/min and the column was a C_{18}

Hypersil ODS (250 $\times$ 4.6 mm) (5 μm particle size) (Supelco). The compounds were detected using a UV-V that is a detector at a wavelength of 450 nm. The peak was identified by comparison of retention time with the commercial standard (Sigma) and was quantified by means of calibration curve.

The experiments for each extraction were carried out in triplicate, and the results are shown as the average of all the independent analyses with a reproducibility of approximately 6% CV (coefficient of variation).

2.4. Antioxidant Assay with DPPH. The antioxidant activities were determined using DPPH as a free radical. Different concentrations were tested (expressed as mg of extract/mg DPPH) for each set of extraction conditions. Extract solution in ethyl acetate (0.1 mL) was added to a $6 \times 10^{-5} \text{ mol/L}$ DPPH solution (3.9 mL). The decrease in absorbance was determined at 515 nm at different times until the reaction had “reached a plateau”. The initial DPPH concentration (C_{DPPH}) in the reaction medium was calculated from a calibration curve with the following equation:

$$\text{Abs}_{(515 \text{ nm})} = -0.0065 \times 29.3112 (C_{\text{DPPH}}) \quad (2)$$

as determined by linear regression ($r = 0.9999$).

For each set of extraction conditions a plot of % remaining DPPH versus time (min) was generated. These graphs were used to determine the percentage of DPPH remaining at the steady state and the values were transferred to another graph showing the percentage of residual DPPH at the steady state as a function of the weight ratio of antioxidant to DPPH. Antiradical activity was defined as the amount of antioxidant required to decrease the initial DPPH concentration by 50% [Efficient Concentration = EC_{50} (mg oil/mg DPPH)] [43].

The experiments were carried out in triplicate in order to evaluate the variability of the measurements.

3. Results and Discussion

3.1. Supercritical Fluid Extraction (SFE). The extraction yields expressed as g of extracted oil/g of seeds are represented in Figure 2. The effect of pressure and temperature on the supercritical CO_2 extraction yield was studied at 35, 45, and 55°C and 100, 200, 300, and 400 bar. The extraction yield was found to vary significantly with temperature and pressure. Similar yields have been reported for the SFE of several oils from palm [15], sunflower [23] and celery seeds [28], and from acorns [35]. As expected, for a given temperature the yield increased with pressure due to the increase in the density of CO_2 . For a given pressure, the extraction yield using supercritical CO_2 decreased as temperature increased, an effect that is attributed to the decrease in the CO_2 density, which dominates over the increase in the solute vapour pressure for pressures up to 200 bar. A similar variation has been reported for CO_2 SFE of seeds and nuts by several authors [23, 28, 30, 31, 35]. At 300 bar the extraction yield was independent of the temperature. There was some compensation between the decrease in the supercritical carbon dioxide density and the increase in vapour pressure of the compounds as the temperature increased. At higher pressures (400 bar),

FIGURE 2: Extraction yield of argan oil at different pressures and temperatures.

TABLE 2: Physical properties of argan oil obtained by SFE and Soxhlet extraction. Also appear the properties of commercial oil.

	SFE	Soxhlet	Commercial oil
Refractive index	1.4731	1.4719	1.4730
Density g cm ⁻³	0.9170	0.9158	0.9166

the effect of temperature on density is less pronounced and the solute vapour pressure effect dominates, leading to oil solubility and an increase in the yield with temperature. At 400 bar and 55°C the decrease in the diffusivity leads to a reduction in the interaction between the supercritical fluid and the solute contained within the matrix and this in turn leads to a decrease in the yield of the extraction process.

The extraction yields obtained by SFE at 45°C and 400 bar were higher (48%) than that obtained in the semi-industrial method with mechanical cold-pressing without water (45%) and far higher than those obtained by traditional methods. Unfortunately, the traditional method is very slow (for a single person 58 hours of work is necessary to obtain 2–2.5 L of oil).

The extraction yield in liquid hexane (Soxhlet) was 52% (w/w). In comparison, the yield in the supercritical fluid extraction was 48% for the experiment conducted at 45°C and 400 bar. Assuming that the oil extraction using hexane is complete, the value obtained by SFE represents 92% of the maximum value. It is important to bear in mind, however, that the extract obtained with the organic solvent has unsatisfactory organoleptic properties [3]. Consequently, SFE can be considered as a good alternative to conventional liquid extraction.

The extraction yields obtained at different temperatures and pressure were statistically analyzed. Regression analysis was performed on the experimental data and the coefficients of the model were evaluated for significance. The Pareto diagram for the analysis of the experimental design is shown in Figure 3. The effect of extraction pressure was highly significant on the extraction yield of compounds, and the effect of temperature, the crossed interaction pressure-temperature, and square pressure were also significant.

The relationship between temperature and pressure for the extraction yield of argan oil is represented by (3) as follows:

$$Y = 30.63 + 18.17P - 3.93T - 6.09P^2 + 5.58PT + 0.31T^2, \quad (3)$$

where Y represents extraction yield; P the pressure (bar), and T the temperature (°C).

In order to achieve complete extraction of the substances in question, a relatively long extraction time was used (3 h) and the low flow rate for the supercritical fluids was 1,2 kg/h. Therefore, the amount of CO₂ consumed in each test was of 3.6 kg of CO₂.

3.2. Properties of the Oil. Some physical properties of argan oil obtained by SFE (400 bar and 45°C) and Soxhlet extraction are shown in Table 2. In the same table also appear physical properties of commercial argan oil obtained by first extraction, cold pressing and commercialized by Greenpharma. The refractive index of commercial argan oil is similar to SFE and slightly higher than that obtained by Soxhlet. These results are similar to those reported by other authors [4]. The density of SFE is higher than commercial argan oil and Soxhlet extraction. This is due to the high selectivity of CO₂ in triglycerides.

Some chemical parameters of freshly prepared argan oil samples obtained with different extraction conditions are shown in Table 3. All fresh argan oil samples had the required physicochemical properties to be “edible grade” as defined by the recommendations of the official argan oil guidelines [44].

Acid value is a measure of free fatty acids and is usually considered to be one of the main parameters that reflect the quality of oil and degree of refining, as well as the changes in quality during storage. The presence of free fatty acid in the oil is not desirable and it can also give rise to undesired saponification reactions. It can be seen from the results in Table 3 that there is no difference between the acid values of the oils obtained under the extraction conditions studied. All acidity values were found to be lower than 0.6 mg/g, where an acidity value of less than 2 mg/g is required for virgin olive oil [45].

Peroxide value is another important factor to characterize the quality of oils and it appears to be an indicator of the lipid oxidation and deterioration of oil properties. The oxidative stability of argan oil has been attributed to its high content in tocopherols and carotenes [46]. In a similar way to the acid value, the peroxide values determined for the oils extracted under different conditions are very similar.

Another one of the most useful parameters for the characterization of oils is the iodine value, which is a measure of unsaturation. In this case the results obtained for the oil extracted by the Soxhlet technique are lower than those obtained by SFE.

Unsaponifiable matters were found to be lower than 1.23% (unsaponifiable matter of virgin olive oil has to be lower than 1.5 [45]). The lower unsaponifiable matter was found to be Soxhlet hexane-extraction (0.48%). Several authors have been found that extraction technology influences the quantity of unsaponifiable matters in argan oil. The unsaponifiable matter contains carotenes (37%), tocopherols (8%), triterpene alcohols (20%), sterols (20%), and xanthophylls (5%) [2].

TABLE 3: Chemical characteristics of argan oil obtained by SFE (pressure/temperature) and Soxhlet extraction.

	100/35	200/35	200/45	200/55	300/35	300/45	300/55	400/35	400/45	400/55	Soxhlet
Acid value (mg/g) (± 0.1)	0.4	0.3	0.4	0.3	0.4	0.5	0.3	0.5	0.4	0.3	0.4
Peroxide value (Meq/Kg) (± 0.1)	0.7	0.5	0.8	0.8	0.8	0.7	0.9	0.5	0.8	0.9	1.0
Iodine value (g I ₂ /100 g oil) (± 0.8)	97.7	93.1	98.5	95.1	95.7	97.3	92.9	96.6	92.1	95.0	93.4
Unsaponifiable matters (%) (± 0.3)	0.93	0.98	1.02	1.10	1.10	1.20	1.19	1.20	1.23	1.21	0.48

FIGURE 3: Pareto diagram and estimated extraction yields using empirical correlation (temperature °C, pressure bar).

TABLE 4: Fatty acid compositions of extracts obtained.

Fatty acid	Fatty acid composition GC area (%)		
	SFE CO ₂	Soxhlet	Traditionally extracted oil ^a
Myristic (C14:0)	0.3–0.4	0.3–0.4	0.42 $\pm$ 0.01
Palmitic (C16:0)	10.4–14.3	11.3–12.6	13.49 $\pm$ 0.05
Stearic (C18:0)	4.5–6.7	6.7–8.7	5.40 $\pm$ 0.08
Oleic (C18:1)	45.4–49.0	45.2–46.5	41.19 $\pm$ 0.15
Linoleic (C18:2)	29.2–34.8	32.4–33.2	38.90 $\pm$ 0.22
Linolenic (C18:3)	1.6–1.7	0.6–0.7	Not detected

^aFrom Benzaria et al. [8].

The four parameters described above have similar values to those reported by other authors [46]. Hilali et al. [47] collected twenty one samples of argan oil with different geographical origins and/or obtained by different extraction methods (traditional, press-extracted, or hexane-extracted) and their physicochemical properties were analyzed. The

TABLE 5: Total tocopherol and β -carotene contents for the oil samples.

	100 bar	200 bar	300 bar	400 bar	Soxhlet
Total tocopherol content in mg/kg					
35°C	691.9	639.7	608.5	671.9	
45°C	689.2	615.2	586.9	598.3	642.3
55°C	645.3	698.8	611.1	603.8	
β -Carotene content in mg/Kg					
35°C	20.3	20.1	17.8	20.3	
45°C	20.1	20.1	18.2	17.1	16.4
55°C	20.8	21.5	18.5	18.7	

acid, peroxide and unsaponifiable matter values obtained in this work are similar to those obtained in this paper [47]. The sample traditionally prepared by Hilali et al. [47] using certified argan nuts and sanitary condition presents 1.40, 0.5 and 0.68 of acid, peroxide, and unsaponifiable matter values. These results and the obtained in this work at different extraction condition of pressure and temperature present similar characteristics than required for virgin olive oil [44]. Therefore, the SFE of argan oil with CO₂ provides extracts with similar properties to obtain by traditional extraction methods.

The total fatty acids compositions of the extracts obtained in this study were determined by GC. Significant variations were not observed between the samples extracted at different pressures and temperatures, with oleic and linoleic acids consistently making up 80% of the fatty acids fraction. The results shown in Table 4 are the average values of all determinations. The chromatograms obtained from commercial oil and SFE are included in Figure 4. The compositions of both samples are similar.

Significant differences were not observed in the compositions of the oils obtained in this study using supercritical CO₂ and the oil obtained by Soxhlet extraction with hexane. The oils contain mainly oleic acid (45.4–49.0%), linoleic acid (29.2–34.8%), and palmitic (10.4–14.3%) acids.

For the sake of comparison, the results obtained using traditional extraction methods are also shown in Table 4. The amounts of fatty acids obtained in this study are similar to those reported by Benzaria et al. [8]. The results clearly show that the oil processing does not markedly affect the oil fatty acid composition.

The contents of total tocopherols and β -carotene in the oil extracts, as determined by HPLC, are shown in Table 5. All total tocopherol values were between 589.9 and 698.8 mg/Kg of the oil sample, and the β -carotene values are between 17

FIGURE 4: Chromatograms obtained from (a) Commercial oil, (b) FSC oil extracted to 45°C and 400 bar.

TABLE 6: Antioxidant capacity of oils measured by the DPPH method (EC_{50} values, mg/mg DPPH).

	100 bar	200 bar	300 bar	400 bar	Soxhlet	Commercial oil
35°C	253 ± 17	277 ± 20	357 ± 19	283 ± 18		
45°C	—	325 ± 15	360 ± 21	356 ± 20	299 ± 20	407 ± 16
55°C	—	251 ± 16	353 ± 20	359 ± 19		
	Walnut oil ^b	Almond oil ^b	Hazelnut oil ^b	Peanut oil ^b		Pistachio oil ^b
	1514.3 ± 70.2	712.2 ± 36	478.5 ± 8.6	1395.9 ± 99.7		377.9 ± 31.8

^bFrom Pérez-Jiménez et al. [9].

and 21 mg/Kg. These results are similar to those reported by other authors [46, 47].

3.3. Antioxidant Activities of the Extracts. The antiradical activities of the oils obtained at different pressures and temperatures were assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The DPPH assay is a quick, reliable, and reproducible method to assess the antiradical activities of oil. This method depends on the reduction of the purple DPPH to give a yellow-coloured diphenyl picrylhydrazine and the remaining DPPH.

The antioxidant capacity data for oils obtained at different pressures and temperatures are shown in Table 6. The same table also shown the antioxidant capacity of different oils: walnut, almond, hazelnut, peanut, and pistachio [9]. The antioxidant capacity data were not measured for oil samples obtained at 45°C/100 bar or 55°C/100 bar due to the low yields obtained.

The EC_{50} values for all oil samples are in the range 251–369 mg/mg DPPH. The EC_{50} value of the sample obtained by Soxhlet extraction (299 ± 20 mg/mg DPPH) is similar to that found for the SFE sample. The EC_{50} values are low in comparison to those determined for other oils reported in the literature (see Table 6). A lower EC_{50} value means that the sample has a higher antioxidant capacity. As a result, it is possible to infer that the antioxidant capacity of argan oil is higher than those of walnut, almond, hazelnut, and peanut oils and comparable to that of pistachio oil. The EC_{50} of commercial argan oil is the highest values of all.

Of all the pressures and temperatures investigated, the best result (lowest EC_{50}) was obtained at 55°C/200 bar, although the conditions 35°C/400 bar also gave good results and the extraction yield was also 27% higher than that

obtained at 55°C/200 bar. On the basis of these results the latter conditions should be selected for the SFE of argan oil.

The high antioxidant capacity can be attributed to present a high content on potent phenolic compounds, mainly ferulic acid known by their potent antioxidant properties. Tocopherols, also, are important components of oil since they possess both antioxidant and vitamin action. One of the characteristics of argan oil is its high content of tocopherols. Indeed, tocopherol levels are at least four times higher in argan oil than in olive oil and two times higher than in hazelnut oil [1]. In general, the antioxidant activity of extracts can be correlated with their phenolic and tocopherols contents. Nonetheless, the activity of each compounds and the content of each one in the extract can influence the antioxidant activity too.

Extracts obtained at 300 bar at the three temperatures studied (Table 5) showed the lowest levels of total tocopherols, which correspond to the highest EC_{50} value. However, the extracts obtained at 200 bar/55°C and 400 bar/35°C showed the highest levels of total tocopherols, which correspond to the lowest EC_{50} value.

4. Conclusions

Supercritical CO₂ has proven to be effective in the extraction of oil from *Argania spinosa* L. The highest extraction yield was obtained at 45°C and a pressure of 400 bar. The extraction yields increase with pressure for a given temperature and decrease as the temperature increases for pressures up to 200 bar.

Significant variations were not observed between the physicochemical parameters of freshly obtained argan oil under different extraction conditions of pressure and temperature. The quality of the oil also did not vary significantly

on employing SFE, traditional press-extraction or hexane-extraction, systems. Therefore our study provides an evidence that the quality of argan oil extracted by supercritical fluid from *Argania spinosa* L. is acceptable as this technology preserves the chemical composition of the oil.

The antioxidant capacity of the argan oil obtained by SFE is high compared with walnut, almond, hazelnut, and peanut oils and is comparable to that determined for pistachio oil. A relationship was found between total tocopherol contents and EC₅₀ values.

References

- [1] M. Cherki, H. Berrougui, A. Drissi, A. Adlouni, and A. Khalil, "Argan oil: which benefits on cardiovascular diseases?" *Pharmacological Research*, vol. 54, no. 1, pp. 1–5, 2006.
- [2] F. Khallouki, C. Younos, R. Soulimani et al., "Consumption of argan oil (Morocco) with its unique profile of fatty acids, tocopherols, squalene, sterols and phenolic compounds should confer valuable cancer chemopreventive effects," *European Journal of Cancer Prevention*, vol. 12, no. 1, pp. 67–75, 2003.
- [3] Z. Charrouf and D. Guillaume, "Ethnoeconomical, ethnomedical, and phytochemical study of *Argania spinosa* (L.) Skeels," *Journal of Ethnopharmacology*, vol. 67, no. 1, pp. 7–14, 1999.
- [4] M. Hilali, Z. Charrouf, A. E. A. Soulhi, L. Hachimi, and D. Guillaume, "Influence of origin and extraction method on argan oil physico-chemical characteristics and composition," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 6, pp. 2081–2087, 2005.
- [5] R. Marfil, C. Cabrera-Vique, R. Giménez, P. R. Bouzas, O. Martínez, and J. A. Sánchez, "Metal content and physicochemical parameters used as quality criteria in virgin argan oil: influence of the extraction method," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 16, pp. 7279–7284, 2008.
- [6] Z. Charrouf, D. Guillaume, and A. Driouich, "The argan tree, an asset for Morocco," *Biofutur*, no. 220, pp. 54–56, 2002.
- [7] B. Matthäus, D. Guillaume, S. Gharby, A. Haddad, H. Harhar, and Z. Charrouf, "Effect of processing on the quality of edible argan oil," *Food Chemistry*, vol. 120, no. 2, pp. 426–432, 2010.
- [8] A. Benzaria, N. Meskini, M. Dubois et al., "Effect of dietary argan oil on fatty acid composition, proliferation, and phospholipase D activity of rat thymocytes," *Nutrition*, vol. 22, no. 6, pp. 628–637, 2006.
- [9] J. Pérez-Jiménez, S. Arranz, M. Tabernero et al., "Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of results," *Food Research International*, vol. 41, no. 3, pp. 274–285, 2008.
- [10] F. Khallouki, B. Spiegelhalter, H. Bartsch, and R. W. Owen, "Secondary metabolites of the argan tree (Morocco) may have disease prevention properties," *African Journal of Biotechnology*, vol. 4, no. 5, pp. 381–388, 2005.
- [11] Z. Charrouf, A. El Kabouss, R. Nouaim, Y. Bensouda, and R. Yaméogo, "Etude de la composition chimique de l'huile d'Argan en fonction de son mode d'extraction," *Al Biruniya Reviews in Marine Pharmacology*, vol. 13, pp. 35–39, 1997.
- [12] S. G. Özkal, M. E. Yener, and L. Bayindirli, "Response surfaces of apricot kernel oil yield in supercritical carbon dioxide," *LWT*, vol. 38, no. 6, pp. 611–616, 2005.
- [13] S. G. Özkal, M. E. Yener, and L. Bayindirli, "Mass transfer modeling of apricot kernel oil extraction with supercritical carbon dioxide," *The Journal of Supercritical Fluids*, vol. 35, no. 2, pp. 119–127, 2005.
- [14] I. S. Md Zaidul, N. A. Nik Norulaini, and A. K. Mohd Omar, "Separation/fractionation of triglycerides in terms of fatty acid constituents in palm kernel oil using supercritical CO₂," *Journal of the Science of Food and Agriculture*, vol. 86, no. 7, pp. 1138–1145, 2006.
- [15] I. S. M. Zaidul, N. A. Nik Norulaini, A. K. Mohd Omar, and R. L. Smith, "Supercritical carbon dioxide (SC-CO₂) extraction of palm kernel oil from palm kernel," *Journal of Food Engineering*, vol. 79, no. 3, pp. 1007–1014, 2007.
- [16] F. Temelli, "Extraction of triglycerides and phospholipids from canola with supercritical carbon dioxide and ethanol," *Journal of Food Science*, vol. 57, no. 2, pp. 440–457, 1992.
- [17] N. T. Dunford and F. Temelli, "Extraction conditions and moisture content of canola flakes as related to lipid composition of supercritical CO₂ extracts," *Journal of Food Science*, vol. 62, no. 1, pp. 155–159, 1997.
- [18] D. F. G. Walker, K. D. Bartle, and A. A. Clifford, "Determination of the oil content of rapeseed by supercritical fluid extraction," *The Analyst*, pp. 1471–1474, 1994.
- [19] E. Reverchon and L. S. Osséo, "Comparison of processes for the supercritical carbon dioxide extraction of oil from soybean seeds," *Journal of the American Oil Chemists' Society*, vol. 71, no. 9, pp. 1007–1012, 1994.
- [20] L. Brühl and B. Matthäus, "Extraction of oilseeds by SFE—a comparison with other methods for the determination of the oil content," *Fresenius' Journal of Analytical Chemistry*, vol. 364, no. 7, pp. 631–634, 1999.
- [21] L. Calvo, M. J. Cocero, and J. M. Díez, "Oxidative stability of sunflower oil extracted with supercritical carbon dioxide," *Journal of the American Oil Chemists' Society*, vol. 71, no. 11, pp. 1251–1254, 1994.
- [22] M. J. Cocero and L. Calvo, "Supercritical fluid extraction of sunflower seed oil with CO₂—ethanol mixtures," *Journal of the American Oil Chemists' Society*, vol. 73, no. 11, pp. 1573–1578, 1996.
- [23] U. Salgin, O. Döker, and A. Çalimli, "Extraction of sunflower oil with supercritical CO₂: experiments and modeling," *Journal of Supercritical Fluids*, vol. 38, no. 3, pp. 326–331, 2006.
- [24] U. Salgin, "Extraction of jojoba seed oil using supercritical CO₂+ethanol mixture in green and high-tech separation process," *The Journal of Supercritical Fluids*, vol. 39, no. 3, pp. 330–337, 2007.
- [25] M. Namiki, Y. Fukuda, Y. Takei, K. Namiki, and Y. Koizumi, "Changes in functional factors of sesame seed and oil during various types of processing, in: bioactive compounds in foods," *ACS Symposium Series*, vol. 816, pp. 85–104, 2002.
- [26] Q. Hu, J. Xu, S. Chen, and F. Yang, "Antioxidant activity of extracts of black sesame seed (*Sesamum indicum* L.) by supercritical carbon dioxide extraction," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 4, pp. 943–947, 2004.
- [27] J. Xu, S. Chen, and Q. Hu, "Antioxidant activity of brown pigment and extracts from black sesame seed (*Sesamum indicum* L.)," *Food Chemistry*, vol. 91, no. 1, pp. 79–83, 2005.
- [28] I. Papamichail, V. Louli, and K. Magoulas, "Supercritical fluid extraction of celery seed oil," *The Journal of Supercritical Fluids*, vol. 18, no. 3, pp. 213–226, 2000.
- [29] V. Louli, G. Folas, E. Voutsas, and K. Magoulas, "Extraction of parsley seed oil by supercritical CO₂," *The Journal of Supercritical Fluids*, vol. 30, no. 2, pp. 163–174, 2004.

- [30] P. Tonthubthimthong, S. Chuaprasert, P. Douglas, and W. Luewisutthichat, "Supercritical CO₂ extraction of nimbin from neem seeds—an experimental study," *Journal of Food Engineering*, vol. 47, no. 4, pp. 289–293, 2000.
- [31] D. Westerman, R. C. D. Santos, J. A. Bosley, J. S. Rogers, and B. Al-Duri, "Extraction of Amaranth seed oil by supercritical carbon dioxide," *The Journal of Supercritical Fluids*, vol. 37, no. 1, pp. 38–52, 2006.
- [32] T. Lu, F. Gaspar, R. Marriott et al., "Extraction of borage seed oil by compressed CO₂: effect of extraction parameters and modelling," *The Journal of Supercritical Fluids*, vol. 41, no. 1, pp. 68–73, 2007.
- [33] B. Bozan and F. Temelli, "Supercritical CO₂ extraction of flaxseed," *Journal of the American Oil Chemists' Society*, vol. 79, no. 3, pp. 231–235, 2002.
- [34] T. H. J. Beveridge, B. Girard, T. Kopp, and J. C. G. Drover, "Yield and composition of grape seed oils extracted by supercritical carbon dioxide and petroleum ether: varietal effects," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 5, pp. 1799–1804, 2005.
- [35] M. G. Bernardo-Gil, I. M. G. Lopes, M. Casquilho, M. A. Ribeiro, M. M. Esquivel, and J. Empis, "Supercritical carbon dioxide extraction of acorn oil," *The Journal of Supercritical Fluids*, vol. 40, no. 3, pp. 344–348, 2007.
- [36] R. Oliveira, M. F. Rodrigues, and M. G. Bernardo-Gil, "Characterization and supercritical carbon dioxide extraction of walnut oil," *Journal of the American Oil Chemists' Society*, vol. 79, no. 3, pp. 225–230, 2002.
- [37] T. D. Crowe and P. J. White, "Oxidation, flavor, and texture of walnuts reduced in fat content by supercritical carbon dioxide," *Journal of the American Oil Chemists' Society*, vol. 80, no. 6, pp. 569–574, 2003.
- [38] S. Salgin and U. Salgin, "Supercritical fluid extraction of walnut kernel oil," *European Journal of Lipid Science and Technology*, vol. 108, no. 7, pp. 577–582, 2006.
- [39] C. Marrone, M. Poletto, E. Reverchon, and A. Stassi, "Almond oil extraction by supercritical CO₂: experiments and modelling," *Chemical Engineering Science*, vol. 53, no. 21, pp. 3711–3718, 1998.
- [40] T. K. Palazoglu and M. O. Balaban, "Supercritical CO₂ extraction of lipids from roasted pistachio nuts," *Transactions of the American Society of Agricultural Engineers*, vol. 41, no. 3, pp. 679–684, 1998.
- [41] K. Helrich, *Official Methods of Analysis of the AOAC*, AOAC International, Arlington, Va, USA, 15th edition, 1990.
- [42] E. Hernández-Martín and C. Otero, "Different enzyme requirements for the synthesis of biodiesel: novozym 435 and Lipozyme TL IM," *Bioresource Technology*, vol. 99, no. 2, pp. 277–286, 2008.
- [43] J. C. Espín, C. Soler-Rivas, and H. J. Wichers, "Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 3, pp. 648–656, 2000.
- [44] Service de normalisation industrielle (Snima), Corps gras d'origine animale et végétale—Huiles d'argane, Spécifications, Norme Marocaine NM 08.5.090. Rabat, Morocco, 2003.
- [45] Commission of the European Communities, "Regulation 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis," *Official Journal of the European Communities*, vol. 248, pp. 1–83, 1991.
- [46] S. Gharby, H. Harhar, D. Guillaume, A. Haddad, B. Matthäus, and Z. Charrouf, "Oxidative stability of edible argan oil: a two-year study," *LWT*, vol. 44, no. 1, pp. 1–8, 2011.
- [47] M. Hilali, Z. Charrouf, A. E. A. Soulhi, L. Hachimi, and D. Guillaume, "Influence of origin and extraction method on argan oil physico-chemical characteristics and composition," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 6, pp. 2081–2087, 2005.