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Effects of extraction methods on chemical composition and oxidative stability of Argan oil

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ABSTRACT

The effect of four extraction methods on chemical composition and oxidative stability of argan oil were studied. The output was low with artisanal extraction (39%) and press (41%) compared to Soxhlet (59%) and Folch (56%) methods. The acidity, fatty acids, phospholipids and tocopherols contents were significantly influenced by the extraction procedure. Acidity and phospholipids rates were superior in the oil extracted with Folch technique. The concentration of fatty acids varied from 19.45 to 20.30%, 44.95 to 47.02% and 32.17 to 34.56% for saturated, monounsaturated and polyunsaturated fatty acids respectively. The tocopherols content was significantly higher with the methods of Folch (1256 mg/kg) and Soxhlet (1158.5 mg/kg) compared to the traditional technique (588 mg/kg) and the press (864 mg/kg).The oil extracted by Folch was more stable compared to those obtained with other extraction methods. This result was explained by the higher content of antioxidants (tocopherols and phospholipids) in the oil extracted by the Folch technique.

Key words: Argan oil-Extraction-Fatty Acids-Oxidation-Phospholipids-Tocopherols

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INTRODUCTION

Argania spinosa L. is an endemic tree of the Moroccan Southwest, which produces a seed with one to three almonds (oilseeds) used for the extraction of argan oil. The extraction is carried out by rural women in a traditional way using millstones for grinding roasted almonds. The obtained paste is mixed to small amounts of warm water in order to extract the oil. This type of extraction requires long hours to yield close to 30%. In recent years, presses have been introduced. This process significantly reduced the time of production, increased the extraction output and improved the stability of the oil [1]. As for the chemical extraction, it is industrially practiced using nonpolar organic solvents such as hexane, and a suitable stainless steel appliance [2]. The industrial argan oil is richer in unsaponifiable but its organoleptic characteristics are not appreciated by the consumer. It is primarily intended for pharmaceutical and cosmetic uses [3].

Argan oil presents a rich and varied chemical composition. The glyceridic fraction consists of 80% unsaturated fatty acids oleic-linoleic type. Saturated fatty acids, about 20%, are mainly represented by stearic and palmitic acids [4, 5]. The oil is thus characterized by a high ratio of unsaturated/saturated fatty acids recommended by nutritionists. Regarding the unsaponifiable fraction of argan oil, it is represented mainly by tocopherols, triterpene alcohols, carotenes, sterols and phospholipids [6, 7]. Some studies report that argan oil, due to its composition of fatty acids and antioxidants, has therapeutic and preventive effects against many diseases such as diabetes, hypertension , hypercholesterolemia and cancer [6, 8, 9]. However, the chemical composition of argan oil, particularly in minor compounds (unsaponifiable), may be influenced by several factors such as the phenotypic varieties of the fruit, geographical origin and extraction processes [4, 5, 7, 10, 11]. Changes in the chemical composition in minor elements, such as tocopherols, phospholipids, sterols and phenols, may have significant effects on the nutritional quality, pharmaceutical and cosmetic properties, and the stability of the oil during storage. Thus, the objective of our

work is to study the impact of the extraction method on yield, chemical composition and oxidative stability of argan oil. Four extraction protocols were compared: press, traditional and chemical extractions. In the case of the chemical method, two types of solvents were tested: an apolar solvent (hexane with Soxhlet method) and a slightly polar mixture of chloroform and methanol (Folch method). Analysis of the oil chemical composition related to the fatty acids, tocopherols and phospholipids contents. The oxidative stability was investigated using both Swift and oven tests.

EXPERIMENTAL SECTION

1 Source of fruits

Fruits were harvested in the region of Admine which is located at 20 Km from the sea and 30 Km southeast of the Agadir city. Fruits were first dried in the open air and then pulped by hand. The resulting seeds were then crushed to give kernels used for oil extraction.

2 Extractions

Four methods were used: mechanical press, artisanal and chemical extraction. For the chemical extraction, two methods were considered according to the nature of the solvent (Soxhlet and Folch). After extraction, the oil content was expressed as a percentage of the almonds weight.

2.1 Traditional Extraction

It was performed in the laboratory following the traditional process manually practiced by rural women. Almonds obtained after crushing seeds were roasted over low heat. Roasting lasted until almonds reached a brown uniform color. Roasted almonds were crushed in a rotary arm grinding stone and the obtained paste was mixed by hand by adding small quantities of warm water. The oil, immiscible with water, separated from the paste and floated to the surface. It was recovered and then filtered.

2.2 Press extraction

Unroasted almonds obtained after crushing were directly pressed to extract the oil. The machine was type screw press for extracting at a temperature not exceeding 75 °C. During extraction, the oil was separated from the meal.

2.3 Chemical Extraction using Soxhlet method

Almonds were ground in a mortar and placed in a cartridge which was introduced into the Soxhlet extractor. The flask containing hexane was heated at a temperature of 65 °C. This caused the evaporation of solvent which entered the cartridge in contact with the crushed almonds. During this contact, oil dissolved in the solvent and when the cartridge was filled, the mixture came down towards the ball. The solvent was evaporated a second time for a second cycle. The operation consisted of several cycles until complete extraction of the oil (about 5 hours). At the end, the mixture (oil and hexane) was placed in a rotary vacuum evaporator to evaporate the solvent and recover the oil.

2.4 Chemical Extraction using Folch method

The extraction was performed according to the technique of Folch et al. [12]. Two grams of ground almonds were mixed with 13 ml of methanol. After 5 minutes of stirring, 26 ml of chloroform were added and the mixture was subjected to stirring for one hour. Then, 8.5 ml of KCl (0.8%) were added and the obtained mixture was stirred for 5 min and centrifuged at 2000 rpm for 2 min at 4 °C. Then, the mixture was separated into two phases, an upper aqueous phase and a lower organic phase. The aqueous phase was aspirated and replaced with chloroform-methanol-KCl (0.8%) mixture (3:48:47, v/v/v). Afterwards, the whole was stirred for 5 min and centrifuged a second time. The Folch upper phase was removed and the operation was repeated twice. At the end, the lipid obtained was filtered, extracted with a mixture of chloroform and methanol (2:1, v/v) and evaporated to dryness using a vacuum rotary evaporator.

3. Chemical analyzes of the oil

3.1 Peroxide value (PV) [13]

To a mixture of 2 g of oil and 10 ml of chloroform were added 15 ml of acetic acid and 1 ml of potassium iodide. After stirring for one minute, the mixture was placed in the dark for 5 minutes. Then, were added 75 ml of distilled water and a small amount of starch powder as a color indicator. The titration was performed by the addition of sodium thiosulfate.

3.2 Acidity [14]

In an Erlenmeyer flask were mixed 50 ml of ethanol and 50 ml of diethyl-ether. Then 3 drops of phenolphthalein were added. The mixture was neutralized using potassium hydroxide solution (0.1 N) by adding drop wise until the color shift. A volume of 10 ml of oil was added. The mixture was decolorized due to the presence of free fatty acids

in the oil. Titration of the oil sample, dissolved in the mixture of diethyl-ether/ethanol, was carried out with an ethanolic solution of potassium hydroxide (0.1 N). The acidity was expressed as percentage of oleic acid.

3.3 Phospholipids analysis

The separation of non phosphorus lipids and phospholipids was performed using Sep-pack Silica cartridges [15]. Triglycerids, glycolipids and phospholipids were eluted using chloroform, acetone and methanol, respectively. The analysis of phospholipids was carried out by phosphorous assay using the Ames method [16]. To the sample dissolved in 100 ml of bi-distilled water were added 600 µl of a magnesium nitrate solution at 10% in ethanol (96%). Mineralization was made by heating with Bunsen burner until complete combustion. Then 300 µl of hydrochloric acid (0.5 N) were added and the mixture was heated at 100 °C for 15 min. After cooling, 700 µl of a mixture of 100 µl of ascorbic acid (10% in bi-distilled water) and 600 µl of ammonium molybdate (2.1 g in 500 of sulfuric acid N) were added. Then, the whole was heated at 45 °C for 20 min. The intensity of the coloration was measured at 820 nm.

3.4 Fatty acids analysis

The fatty acid composition of the oil was analyzed by gas chromatography after methylation with Boron trifluoride. Three extractions with hexane were made to obtain fatty acid methylic esters. These esters were evaporated under nitrogen conditions and 50 µl were directly injected into a Cpcil-88 column (length 50 m, diameter 0.25 mm) of a Carlo Erba gas chromatograph whose temperature increased from 150 to 225 °C (5 °C/min). The pressure of vector gas (H2) was 1 bar. After their separation in the column, fatty acids were detected using a flame ionization detector at 250 °C.

3.5 Tocopherols analysis

The analysis of tocopherols was performed by HPLC. The apparatus included a Jasco 880-PU pump, a Jasco 875 UV detector and a Varian spectrophotometer integrator 4400. After dilution with methanol, the samples were injected into a grafted silica C18 column (length 25 cm, diameter 3 microns). The elution solvent was 100% methanol. Determining the tocopherols content was carried out by comparison to a standard mixture.

3.6 Determination of the oxidative stability of argan oil

The oxidative stability of the oil was studied by using two methods: Swift and oven tests.

In the case of the Swift test [17], tubes containing oils (20 ml) were placed for 5 min in boiling water and then in a water bath at a temperature of 100° C. The tubes were closed with plugs of two drilled holes which were attached two pipes: one allowed bringing a quantity of air from the manifold to the sample, and the other evacuated volatiles. The air flow was adjusted to 2.33 ml per second in each tube. After a specified period, the oxidation was stopped by rapid cooling of the tubes in cold water. A sample of 0.3 g of oil from each sample was removed to measure the peroxide value. If the peroxide value was less than 75 meqO_2/kg, the time required to reach this value was estimated. At that time, the peroxide value was measured and the value found retained. After an oxidation time, another sample (0.3 g) analysis was performed to determine the second peroxide value which should not exceed 175 meqO_2/Kg. The two values (comprised between 75 and 175 meqO_2/Kg) allowed to graphically determining the oxidation life of the oil which corresponded to a peroxide value of 100 meqO_2/kg. The value of this time is regarded as a marker of resistance to autoxidation. The test was repeated three times for each extraction mode.

For the oven test, oil samples of 15 g were placed in Petri dishes. The boxes were then stored open in an oven at 65 °C (in the dark) for a period of 10 weeks. The peroxide value was measured every week.

3.7 Statistical Analysis

Values represented the average of four repetitions. The results were subjected to ANOVA single factor. The averages were compared by means of a multiple comparison test, using the least significant difference test (LSD, P = 0.05).

RESULTS AND DISCUSSION

Table 1 shows the extraction yield obtained using different extraction methods. The output, was significantly greater with both chemical methods (59% and 56% for Soxhlet and Folch respectively) compared to traditional and mechanical extractions (39 and 41% respectively). In general, the extraction yield of oil seeds depended on the plant species, the genetic and climatic variations within the same species [18]. For argan oil, our results were consistent with the literature data [4, 6] and showed that the extraction method was the main factor that could significantly influence the extraction yield expressed in respect to the kernels weight.

The effect of extraction procedure on the chemical characteristics of the oil, acidity and PV, is shown in Table 1. The acidity was significantly higher in the oil extracted with the method of Folch (1.22%) in comparison to the other methods. This could be explained by the polarity of the solvent used to extract the molecules of free fatty acids in larger quantities. This result was consistent with that obtained by Kim and Yoon on soybean oil [19]. However, free fatty acids are considered as pro-oxidants and their presence in the oil could initiate oxidation reactions [20] The acidity of the traditional oil was the lowest (0.13%), those of oils extracted by Soxhlet and press were 0.30% and 0.26% respectively. These values were lower than the upper limit set by the Moroccan standard (0.8%) for the qualitative classification of argan oil [21].

Table 1. Output and chemical characteristics of argan oil according to the extraction method

	Folch	Soxhlet	Traditional	Press
Output (%)	56.25 ± 3.53a	59.25 ± 2.82a	39.00 ± 0.86b	41.25 ± 1.89b
Acidity (%)	1.22 ± 0.10a	0.26 ± 0.05b	0.13 ± 0.05c	0.30 ± 0.03b
Peroxyde Value (meqO_2/kg)	2.45 ± 0.52a	2.16 ± 0.28ab	0.13 ± 0.05c	0.30 ± 0.03b

For each line, the values followed by the same letter are not significantly different at P = 0.05.

The PV was slightly influenced by the extraction method and ranged from 1.77 to 2.45 meqO_2/kg. In addition, the measured values in the oils from the four extraction methods were less than the upper limit recommended by the Moroccan standard (15 meqO_2/kg).

Table 2. Fatty acids content of argan oil according to the extraction method

Fatty acids	Folch	Soxhlet	Traditional	Press
14:0	0.16 ± 0.003b	0.18 ± 0.003a	0.130 ± 0.001c	0.16 ± 0.004b
15:0	0.05 ± 0.003b	0.060 ± 0.004a	0.05 ± 0.003b	0.05 ± 0.003b
16:0	13.80 ± 0.15a	12.70 ± 0.12c	12.68 ± 0.09c	13.40 ± 0.34b
16:1	0.13 ± 0.004a	0.11 ± 0.01b	0.015 ± 0.001c	0.12 ± 0.004a
17:0	0.087 ± 0.00c	0.10 ± 0.008b	0.12 ± 0.002a	0.09 ± 0.002c
18:0	5.65 ± 0.31ab	5.84 ± 0.14b	6.46 ± 0.05a	5.53 ± 0.04c
18:1	46.48 ± 0.83a	45.45 ± 0.54ab	44.52 ± 1.07b	46.05 ± 0.39a
18:2	31.71 ± 0.55c	33.70 ± 0.48a	34.07 ± 0.71a	32.65 ± 0.33b
18:3	0.16 ± 0.005a	0.16 ± 0.002b	0.15 ± 0.002b	0.13 ± 0.001c
20:00	0.33 ± 0.025b	0.39 ± 0.033a	0.35 ± 0.013b	0.36 ± 0.006ab
20:1	0.35 ± 0.062a	0.29 ± 0.067a	0.35 ± 0.017a	0.35 ± 0.01a
20:2	0.14 ± 0.002a	0.13 ± 0.004a	0.14 ± 0.003a	0.09 ± 0.017b
20:3	0.15 ± 0.003b	0.18 ± 0.005a	0.19 ± 0.003a	0.11 ± 0.01c
22:0	0.33 ± 0.025b	0.39 ± 0.033a	0.12 ± 0.008b	0.10 ± 0.002c
22:1	0.06 ± 0.009ab	0.035 ± 0.006c	0.06 ± 0.008a	0.05 ± 0.005bc
24:0	0.07 ± 0.009a	0.07 ± 0.007a	0.05 ± 0.003b	0.03 ± 0.003
SFA	20.30 ± 0.42a	19.45 ± 0.17c	19.96 ± 0.09ab	19.73 ± 0.38bc
MUFA	47.02 ± 0.82a	45.89 ± 0.54ab	44.95 ± 1.07b	46.57 ± 0.39a
PUFA	32.17 ± 0.55 b	34.18 ± 0.48a	34.56 ± 0.71a	32.98 ± 0.35b

For each line, the values followed by the same letter are not significantly different at P = 0.05. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

The fatty acid composition is shown in Table 2. The content of major fatty acids showed significant variations according to the extraction method. Indeed, palmitic acid (16: 0) was significantly higher in the oil extracted by Folch (13.8%) compared to the other extraction methods (13.40, 12.70 and 12.68% for the press, Soxhlet and traditional method respectively). For stearic acid (18: 0), the concentration was significantly lower in the oil extracted by press (5.53%). That of oleic acid (18: 1 n-9) was 44.52% in traditional oil and 46.48% in oil extracted by Folch. As for linoleic acid (18: 2 n-6), the content was significantly lower in the oil extracted by Folch technique (31.71%) and higher in the oils extracted by Soxhlet and the traditional method (33.70% and 34.07% respectively). Minor fatty acids, with a content lower than 0.4%, also showed significant variations at $p < 0.05$. This was the case of linolenic acid (18: 3 n-3) which recorded a higher content in the oil extracted by Folch and Soxhlet methods (0.16%) and less in the oil extracted by press (0.13%). The results also supported a finding that the total proportion of fatty acids of argan oil extracted with different methods ranged from 19.45 to 20.30% for saturated-, 44.95 to 47.02% for monounsaturated- and 32.17 to 34.56% for polyunsaturated fatty acids. These levels were consistent with previous reports [4, 22] and those adopted by the Moroccan standard (08.5 NM .090) defining the criteria for purity and quality of argan oil [21]. On another hand, our results showed that the four types of the oil had a low content in α-linolenic acid (from 0.13 to 0.16%), an essential fatty acid of the n-3 family. They confirmed those reported in the literature [3, 4, 5] and showed that the nutritional value of argan oil was mainly based on its high content of linoleic acid which is an essential fatty acid of the n-6 family, and oleic acid of the n-9 family. In this

regard, many studies showed the beneficial effects of these fatty acids on health including the prevention of coronary heart disease and cancer [8, 22].

Unlike fatty acids, the tocopherols content presented great variations according to the extraction method (Table 3). The rate of total tocopherols was significantly different: 1256.00, 1158.50, 864.25 and 588.25 mg/kg respectively in oils extracted by Folch, Soxhlet, press and traditional methods. These results showed that tocopherols were more extractable with the chemical techniques using organic solvents (Soxhlet and Folch). The traditional method had the lowest rate in tocopherols. Indeed, the use of water as solvent for the traditional method could affect the extraction of tocopherols in comparison to organic solvents whose ability to extract lipids is greater [23]. In addition, tocopherols are particularly sensitive to high temperatures and therefore they could destroy during roasting almonds in artisanal extraction, and heating during the extraction with press. In this regard, a destruction of tocopherols during heating vegetable oils and technological process of refining crude oils was reported [24]. Furthermore, some authors noted that roasting seeds caused loss of tocopherols. The loss increased with temperature and roasting time [25, 26, 27, 28].

Our results showed that γ-tocopherol represented the most important form of tocopherols. Thus, values of 76% (traditional method and press), 64% (Soxhlet) and 69% (Folch) were noted. Besides its antioxidant role, γ-tocopherol is regarded as a form of vitamin E with unique nutritional and metabolic characteristics that distinguish it from α-tocopherol which is the predominant and active form in the tissues. In fact, recent studies showed preventive effects against cancer and cardiovascular disease in people with higher plasma levels of γ-tocopherol in comparison to α-tocopherol [29, 30]. Thus, argan oil could be assessed as a good source of γ-tocopherol, better than conventional oils known for their richness in this molecule as rapeseed and soybean oils, and unlike olive and sunflower oils which are more important sources of α-tocopherol [31].

The content of phospholipids was also influenced by the extraction method (Table 3). It was significantly higher in the oil extracted by the Folch method (0.45%) compared to the other techniques (0.08%, 0.07% and 0.11% respectively for Soxhlet, press and traditional methods). Similarly, previous works on other oilseeds (sunflower, soybean, olive) showed that the composition of minor compounds was strongly influenced by the nature of the solvents used for extraction [19, 32, 33]. Indeed, the extraction of the phospholipids depended on the type of the solvent used in relation to their bipolar structure allowing them to be more extractable with a polar solvent (Folch method).

Table 3. Tocopherols, phospholipids and oxidative stability of argan oil according to the extraction method

	Folch	Soxhlet	Traditional	Press
α-tocopherol (mg/Kg)	158.50 ± 7.54a	133.5 ± 4.79 b	62 ± 2.45d	86.5 ± 2.081c
γ-tocopherol (mg/Kg)	870.75 ± 3.77a	740.25 ± 10.781b	450.75 ± 10.99d	658.50 ±5.97c
δ-tocopherol (mg/Kg)	226.75 ± 2.97b	284.75 ± 4.27a	75.50 ± 12.39d	119.25 ± 2.5c
Total tocopherols (mg/Kg)	1256.00 ± 8.33a	1158.5 ± 18.63b	588.25 ±19.29d	864.25 ± 5.91c
Total phospholipids (%)	0.45 ± 0.09b	0.08 ± 0.02a	0.11 ± 0.03a	0.07 ± 0.02a
Swift test (hours)	45.06 ± 0.43a	26.00 ± 0.54b	20.37 ± 0.14d	23.50 ± 0.41c

For each line, the values followed by the same letter are not significantly different at P = 0.05.

Furthermore, we noted a slight increase in the phospholipids content in the traditional oil compared to those extracted using Soxhlet and press. This could be due to the roasting almonds before extraction. Indeed, some studies reported that the brown coloration obtained by roasting seeds was due to the formation of the Maillard reaction products and the increase of phospholipids content [34, 35].

As to the oil oxidative stability, significant variations depending on the extraction method were recorded (Table 3). The accelerated oxidation test at 100 °C and the controlled flow of air sparging, indicated that the time to reach a PV of 100 meqO_2/kg in the oils extracted by Folch , Soxhlet, press and traditional method are respectively 45.06, 26.00, 23.5 and 20.37 hours. The higher stability recorded in the case of the oil extracted by Folch would be due to the protective effect of tocopherols and phospholipids which were better extracted using this method.

The oils stored at 65 °C in darkness indicated that the PV evolution presented two phases (Figure 1). The first phase corresponded to the gradual increase of this index until reaching a maximum value. The second phase was characterized by a rapid decrease of the values. Thus, the oils extracted by traditional method and press showed an increase in PV from the first days of storage. The maximum values were reached after 5 weeks for artisanal oil and 6 weeks for the oil extracted by press. The oil extracted by Soxhlet remained stable during the first three weeks. PV increased from week 4 and a maximum value of 533.33 meqO_2/kg was reached at week 8. In the case of the oil extracted by Folch, PV remained close to 10 meqO_2/kg for 8 weeks of storage. After this time a slight rise was recorded and the maximum value was reached at week 10 (166.66 meqO_2/kg). The combining of these results to the

accelerated oxidation test (Swift) demonstrated that the argan oil extracted by the traditional method had a lower oxidative stability compared to oils extracted by Folch, Soxhlet and press techniques. This low stability of the traditional oil could be related firstly to its low tocopherols content as these molecules are natural antioxidants and prevent oxidation reactions of unsaturated fatty acids [20]; and secondly to the presence of oxidation catalysts such as metals and free fatty acids. In this context, previous studies showed that the argan oil extracted by the traditional method had higher levels of metals as copper [36] and was less stable compared with that obtained by pressure [10, 37].

In our study, the highest oxidative stability was observed in the oil extracted by Folch method and whose tocopherols content was the most important. Furthermore, this technique permitted extracting more phospholipids compared with other methods. These molecules act as secondary antioxidants by chelating metals (iron, copper) and are synergists with tocopherols which are primary antioxidants [20, 38, 39]. This synergistic action was probably the origin of the stability observed in the oil extracted by the Folch method despite the high content of free fatty acids that are pro-oxidants. This finding was in agreement with results reported on soybean oil by Kim and Yoon [19]. These authors showed that the oxidative stability of the oil varied depending on the mode of extraction (Folch, Hexane and Water) and the oil extracted by the Folch method was the most stable and the richest in phospholipids.

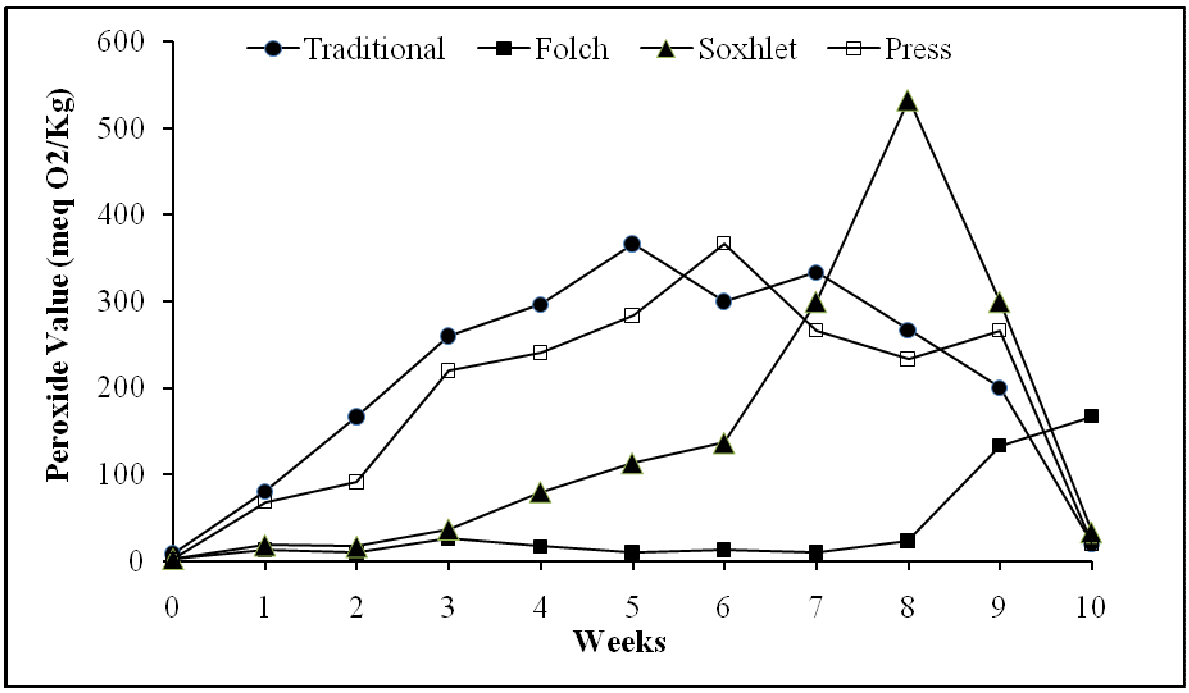

Figure 1. Evolution of peroxide value at 65 °C during 10 weeks according to the extraction met

CONCLUSION

Our results demonstrated that the composition and the stability of argan oil varied with the extraction method. The traditional oil showed a lesser quality and a low stability compared to other extraction techniques. The oil extracted by the Folch method was characterized by a high content of tocopherols and phospholipids compared to the other extraction methods. Thus, the data from this study showed the interest to optimize the extraction method in order to reduce the loss of nutrients and natural antioxidants which would better preserve the oil quality.

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