

Comparative Study on Output and Chemical Composition of Argan Oils Extracted from Fruits of Different Regions in the Southwest of Morocco

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Abstract

The argan oil was extracted from the kernels of oval-shaped fruits, collected from three different regions in the Southwest of Morocco (Ait Melloul, Argana and Tamanar). The output of oil extraction was similar in the fruits of the three regions (about 58%), in which the kernels weight was considered. However a significant difference between the three types of oils was noted when the extraction yield was related to nuts weight. In fact, the oil production was superior in the Ait Melloul fruits (12.22 kg/100 kg) than in those of Tamanar (10.12 kg/100 kg) and Argana (7.69 kg/100 kg). The concentration of saturated fatty acids (Palmitic and stearic acid) was similar in the three oils. Moreover, significant differences were noted in concentrations of unsaturated oleic and linoleic acids. In Argana oil, the oleic acid concentration was higher (49.23%; $p < 0.05$) than in Tamanar oil (42.78%; $p < 0.05$). However, a greater content of linoleic acid was found in the oil extracted from Tamanar fruits (37.45% $p < 0.05$) compared to that obtained from Argana fruits (30.87%; $p < 0.05$). In addition, the study showed differences on the antioxidants composition in the three oils. The content of tocopherols was higher in the oil of Ait Melloul (1410.95 mg/kg) than in those of Argana (1102.02 mg/kg) and Tamanar (910.45 mg/kg). Regarding to the phospholipids, the value was higher in Argana (0.56%) than in Tamanar (0.41%) and Ait Melloul (0.29%).

Keywords: Argan oil, fatty acids, fruits, output, phospholipids, tocopherols

1. Introduction

The oil extracted from fruits of the argan tree (*Argania spinosa* L., Sapotaceae) constitutes an important source of fatty food in the southwest of Morocco (Collier and Lemaire, 1974). In this region which is known for its mediterranean climate, argan tree is widespread and its distribution covers approximately 828 300 ha (Emberger, 1925). The climatic conditions are different between the main sites of argan tree, particularly as regards the altitude (which ranges from 5 to up of 1300 m), the rainfall (100 to 500 mm) and the temperature (22 to 38 °C). Previous studies showed that the content and the chemical composition of oil in oilseeds are affected by the geographical location and climatic conditions, especially temperature and rainfall (Johansson et al., 1997; Onemli, 2012; Arslan et al., 2013). In the case of the soya and sunflower, it was reported that the content of oleic acids (monounsaturated) increased in warm regions compared to cool regions (Kandil et al., 1990). In addition, the oil content in the sunflower seeds increased with the decrease of the temperature and the opposite occurred with the soya seeds (Wolf et al., 1982). The aim of this study was to compare the extraction output and the chemical composition (fatty acids, tocopherols and phospholipids) of argan oil obtained from fruits of three regions: Ait Melloul, Argana and Tamanar.

2. Materials and methods

2.1 Fruit sampling

Fruit samples of the argan tree were collected from three regions of the southwest of Morocco:

- Ait Melloul, located at 12.5 Km from the sea and 16 km southeast of Agadir town. Its altitude is about 35 m. The average annual rainfall is approximately 230 mm.
- Argana, situated on the hillside (South) of the occidental High Atlas at 60 km from the sea and at 620 m of altitude. The average annual precipitation is 180 mm.
- Tamanar, located at 70 km in the North of Agadir city at an altitude of 255 m. The average annual rainfall is 240 mm.

According to Emberger (1955), the bioclimate is arid in Argana and Aït Melloul and semi-arid in Tamanar.

2.2 Oil extraction

The oval-shaped fruits coming from the three regions were carefully dried in the open air, and then their nuts were isolated. The oil extraction was done using a chemical method. Indeed, kernels were finely ground in a mortar and were then placed in a Soxhlet for a first oil extraction with hexane for four hours. Afterwards, the kernels were ground again and put back in the Soxhlet for other two extractions of two hours each. Another chemical extraction of oil with Folch method (Folch et al., 1957) was done using a Chloroform/Methanol (2/1) mixture to measure the phospholipids.

2.3 Fatty acid composition

After its extraction, argan oil was methylated with Boron trifluoride (BF₃). Three extractions with hexane were then made to obtain fatty acid methyl esters. These esters were evaporated under nitrogen conditions and 50 µg 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). After their separation in the column, fatty acids were detected using a flame ionization detector at 250 °C. The identification of fatty acids was made by comparing their equivalent chain length with a standard mixture of fatty acids. The pressure of vector gas (H₂) was 1 bar.

2.4 Tocopherols content

Tocopherols composition of argan oil was performed with the HPLC method. The chromatographer comprised a Jasco 880-PU pump, a Jasco 875-UV spectrophotometer detector and a Varian 4400 integrator. Argan oil was diluted with 100% methanol and injected into a C18-grafted silica column (length 25 cm; diameter 3 µm). The tocopherol content in argan oil was determined in comparison to a standard mixture.

2.5 Phospholipids quantification

The extracted oil by Folch method was used for phospholipids quantification. A sample of oil was injected in silica column chromatograph (Sep-Pack) for lipid separation (Juaneda and Rocquelin, 1985). Triglycerids, glycolipids and phospholipids were eluted using chloroform, acetone and methanol, respectively. The phospholipids concentration was determined by measuring the phosphorus content according to the method of Ames (1966). A quantity of 100 µL of distilled water and 600 µL of 10% of magnesium nitrate were added to the phospholipids samples. Samples were transformed to white deposits by mineralization with a Bunsen burner. Chlorhydric acid 0.5 mol.L⁻¹ (300 µL) was added and the samples were incubated in a water bath at 100 °C for 15 min and then cooled to ambient temperature. A mixture of 700 µL of 10% of ascorbic acid and ammonium molybdate (2.1 g in 500 ml of sulfuric acid) was added, and then samples were incubated at 45 °C for 20 min. Phosphorous concentrations were determined by measuring their absorbance at 820 nm.

2.6 Statistical analyses

Each value corresponds to the mean of four replicates. Mean values were subjected to one-way analysis of variance. A multiple comparison test, with the least significant difference test ($p < 0.05$), was also used.

3. Results and discussion

The output of oil extraction (Table 1) was similar for the fruits of the three regions (about 58%) in which kernel weight was considered. It was slightly higher than those reported by other authors using the same extraction method (Charrouf and Guillaume, 1999 ; Rahmani, 2005). However, significant differences between the three regions were recorded when the quantity of extracted oil was related to nut weight. The value obtained for Aït Melloul fruits (12.22 kg/100 kg) was superior to those found for Tamanar and Argana fruits (10.12 kg/100 kg and 7.69 kg/100 kg respectively). This result can be explained by differences of the ratio between kernel weight (Kw) and nut weight (Nw) for the fruits of the three regions (Table 1). In fact, the Argana fruits were biggest than those collected in Aït Melloul region, but the kernel weight was similar for the two types of fruits. The Kw/Nw ratio for Aït Melloul fruits (0.209) was obviously higher than the one of Tamanar (0.170) and Argana (0.138). According to previous studies, the variation in the fruit size could be due to several factors such as the climatic conditions, the year of collecting fruit, the age and the size of the plant (Cherry et al., 1985; Zunzunegui et al, 2010). In the case of Argan tree, the fruits characteristics (Shape, size and weight) were associated to the climatic conditions and the geographical location (Ferradous et al.,1996 ; Bani-Aameur et al., 1999; Bani-Aameur and Ferradous , 2001).

Table1: Biometric measurements and extraction output related to the regions.

Regions		Aït Melloul	Argana	Tamanar
*Kw (g)		0.372	0.405	0.280
Nw (g)		1.775	2.927	1.640
Kw/Nw		0.209 a	0.138 c	0.170 b
Output	% of kernels	58.25 a	59.25 a	55.75 a
	Per 100Kg of nuts	12.22 a	7.69 c	10.12 b

*Kw: Kernel weight ; Nw: Nut weight

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

The content of saturated fatty acids was similar for the fruits of the three regions, whereas significant differences were noted between the concentrations of oleic and linoleic acids in the three regions (Table 2). In Argana oil, the oleic acid concentration (49.23%) was higher than in Tamanar oil (42.78%). In contrast, the linoleic acid content was significantly higher in the oil of Tamanar (37.45%) in comparison to Argana oil (30.87%). These variations affected the global rate of mono- and polyunsaturated fatty acids. Indeed, the lower concentration of monounsaturated acids (43.52%) and the greater content of polyunsaturated acids (37.58%) were found in Tamanar fruits. These inverse variations in fatty acids composition permitted to obtain a similar degree of unsaturation in the three types of oils. The obtained differences in the oleic and the linoleic acids concentrations would be due to the variations of climatic conditions (Temperature, rainfall and drought degree) between the three regions. Several studies on vegetable oils have reported a high content of oleic acid in warm region oils in comparison to cool region oils, whereas the linoleic acid concentration increased in cool region (Johansson et al., 1997 ; Izquierdo et al., 2002 ; Onemli, 2012). So, the arid climate of Argana region may explain the high content of oleic acid compared to Tamanar where the climate was classified as semi-arid. However, the concentrations reported in the present study corresponded to the scale of values reported by different authors (Maurin , 1992 ; Yaghmur et al., 2001 ; Drissi et al., 2004 ; El Monfalouti et al., 2010) and also to those fixed by the Moroccan norms (NM 08.5.090) (Rahmani, 2005). So, we can consider that the profile of unsaturated fatty acids in argan oil is comparable for the three regions.

Table 2: Fatty acids composition of argan oil in relation to the regions.

Fatty acids (%)	Aït Melloul	Argana	Tamanar
14:0	0.15	0.15	0.12
15:0	0.05	0.05	0.05
16:0	13.15 a	12.43 b	12.87 ab
16:1n-7	0.13	0.14	0.14
17:0	0.09	0.10	0.10
18:0	5.56 a	5.36 a	5.23 a
18:1n-9	47.17 a	49.23 a	42.78 b
18:2n-6	32.40 b	30.87 c	37.45 a
18:3n-3	0.12	0.12	0.11
20:0	0.40	0.35	0.37
20:1n-9	0.37	0.38	0.34
22:0	0.11	0.11	0.13
22:1n-9	0.01	0.01	0.01
24:0	0.01	0.01	0.01
Saturated	19.61 a	18.89 a	18.36 a
Monounsaturated	47.84 a	49.86 a	43.52 b
Polyunsaturated	32.52 b	31.06 c	37.56 a

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

In addition, our study showed a relative lack in linolenic acid (essential fatty acid of the n-3 family) in the three types of the argan oil. This result confirmed reports of other authors (Maurin, 1992; Rezanka and Rezankova, 1999; Yaghmur et al., 2001; Rahmani, 2005). All this demonstrates that the nutritional value of the argan oil

would be constituted by its richness in linoleic acid, essential fatty acid of the n-6 family, and oleic acid of the n-9 family. On another hand, these two fatty acids were known to play an evident role in the prevention of cardiovascular and cancer diseases (Khallouki et al., 2003 ; Drissi et al., 2004, Mekhfi et al., 2012).

The tocopherols contents were also significantly different in the three types of oil (Table 3) with 1410.90, 1101.60, and 893.83 mg of total tocopherols per kg of oil extracted from Aït Melloul, Argana and Tamanar fruits, respectively. The γ -tocopherol represented the principal form with 64.50%, 55.20% and 54.60% respectively for Aït Melloul, Argana and Tamanar. It was followed by the δ -tocopherol (25.66%, 32.40% and 33.07% respectively for Aït Melloul, Argana and Tamanar) and the α -tocopherol which represented 11.93% for Aït Melloul, 12.44% for Argana and 12.31% for Tamanar. The contents of γ -tocopherol and α -tocopherol varied in the same way than the total tocopherols. However, the concentrations of δ -tocopherol were lower for Tamanar.

Our results were in agreement with the values reported in the literature on the total tocopherol content in argan oil, that was ranging between 600 and 1500 mg/kg of oil (Khallouki et al., 2003 ; Rahmani 2005 ; Belcadi et al., 2008). Also, studies on many vegetable oils (argan, colza, olive, soya, and sunflower) reported the effect of environment, the ripening of the fruits and the genetic variability on tocopherols concentration (Kandil et al., 1990; Almonor et al., 1998; Yoshida et al., 2003, Harhar et al., 2014).

In the present study, the tocopherols were mainly constituted by the γ form, followed by the δ form. Relatively, low concentrations of α -tocopherol (12% of total tocopherols) were found in the three types of oils. These findings confirmed the report described by Khallouki et al. (2003) and Rahmani (2005) and demonstrated that argan oil did not permit an important intake of vitamin E. However, the high antioxidant power of γ - and δ -tocopherol in vegetable oils contributed to protect unsaturated fatty acids towards oxidation reactions (Eskin et al., 1996 ; Braunrath et al, 2010). As the argan oil extracted from Aït Melloul fruits contained the highest tocopherols concentration, this oil would be better protected and its nutritional qualities would be longer conserved than those of the other regions.

Table 3: Tocopherols and phospholipids composition of argan oil in relation to the regions

Compounds		Aït Melloul	Argana	Tamanar
Tocopherols (mg/Kg)	α -tocopherol	168,45 a	137,15 b	112,15 c
	γ -tocopherol	910.33 a	607,92 b	497,13 c
	δ -tocopherol	362,17 a	356,95 a	301,17 b
	Total	1410.95 a	1102.02 b	910,45 c
Phospholipids (%)		0.29 c	0.56 a	0.41 b

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

For phospholipids quantification, results revealed significant differences between the three regions. In fact, a greatest concentration was found in Argana oil (0.56%) and a lower content was obtained in Aït Melloul oil (0.29%). The amount of extracted phospholipids obtained in the present study was higher than that found for peanut (0.14%) and sunflower (0.18% to 0.25%) oils, similar compared to that extracted from olive oil (0.41 to 0.70%) and lower than that found for soya oil (1.5 to 2.5%) (Viedermann, 1981; Salunkhe et al., 1992). Our results showed that phospholipids content are also affected by climatic variations of the three regions. These results constitute a new finding for argan oil. Such as phospholipids are known for their antioxidant activity, their presence would offer protection to polyunsaturated fatty acids in the oil towards oxidation reactions (Hildebrand et al., 1984 ; Miraliakbari and Shahidi, 2008). Also, according to several authors, the presence of phospholipids increased the antioxidant activity of tocopherol (Hildebrand et al., 1984 ; Judde et al., 2003 ; Hidalgo et al., 2006).

In conclusion, the present study reported a high argan oil production, related to nut weight, for Aït Melloul region located at low altitude and for which the rainfall was important. For antioxidant composition, the oil of this region had greatest tocopherols content and low phospholipids concentration. However, the output extraction from kernels and the fatty acids composition were similar despite the origin of the fruits.

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