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Metal Content and Physicochemical Parameters Used as Quality Criteria in Virgin Argan Oil: Influence of the Extraction Method

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Metal content was determined in 26 samples of virgin argan oil from Morocco. An ETA-AAS with previous sample dilution with MIBK technique was used. In oil obtained by traditional method, Fe ranged from 0.8 to 4.0 mg/kg, Cu from 160.4 to 695.7 μg/kg, Cr from 10.3 to 55.3 μg/kg, Mn from 18.1 to 70.8 μg/kg, and Pb from 28.5 to 450.0 μg/kg. In oil obtained by a half-industrialized method, Fe ranged from 0.8 to 1.7 mg/kg, Cu from 158.4 to 385.0 μg/kg, Cr from 10.0 to 48.1 μg/kg, Mn from 15.0 to 68.5 μg/kg, and Pb from 32.0 to 100.0 μg/kg. Acidity value, peroxide index, K_{270} and K_{232}, humidity and sludge volatile, and insoluble sludges in petroleum ether were also determined. A high variability in these quality parameters and a decrease of the quality in the oils obtained by the traditional method were observed.

KEYWORDS: Virgin argan oil; *Argania spinosa;* metal content; ETA-AAS

INTRODUCTION

Virgin argan oil is harvested from the fruits of the argan tree [*Argania spinosa* (L.) Skeels], an endemic species from southwestern Morocco, where it plays major ecologic and socioeconomic roles, surviving in extreme drought and poor soil, and provides environmental protection by slowing desert progression (*1*). The tree bears a plum-sized fruit, which has a stone-like structure, containing one to three kernels (nuts) with high oil content. About 4000 tons of argan oil are produced each year in Morocco. This oil is a rich source of linoleic and oleic acids, with mean contents of 35−38 and 45%, respectively. The presence of minor compounds such as tocopherols, polyphenols, sterols, carotenoids, xanthophylls, and squalene contributes to better oil preservation and its dietetic value (*2, 3*). Virgin argan oil presents a delicate flavor and is currently used as a culinary oil in this region; it represents 25% of the fat in the local diet (*4*). In addition, it is prescribed in traditional medicine for its cosmetic, bactericide, and fungicide properties (*4*).

Virgin argan oil is currently under investigation to improve its economic and environmental role and its nutritional value. Recent studies also suggest that this oil may have a relevant role in disease prevention. Several authors have pointed out hypolipidemic, hypocholesterolemic, and antihypertensive effects in rats and its relation to cancer prevention (*3–7*). However, definitive data on metal content and physicochemical properties are sparse, and wide data variability is observed (*8, 9*). A Moroccan Normative (N.M. 08.5.090) was performed in 2003 to define the quality specifications of virgin argan oil and its classification in different categories; extra virgin argan oil is the highest quality level (*10*). As well as other organizations related to edible oil quality such as the International Oil Council (*11*), this normative includes limits for several metals.

The oil extraction method from nuts is complex and has considerable influence on its physicochemical composition, nutritional value, and sensorial properties (*8*). At this moment, two methods are used to extract virgin argan oil: the traditional method (hand pressed) and a half-industrialized method (mechanical cold-pressed). The traditional method is usually carried out by indigenous women for home self-consumption. The fruits of the tree are harvested and allowed to dry in the sun before the pericarp is removed. The nuts are broken with rocks and the kernels are air-dried in clay containers and slowly roasted. The roasted kernels are crushed and kneaded into a paste or dough with hot water. The resulting oil/water mixture is separated (*12*). Traditional oil extraction is frequently achieved

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in unsatisfactory sanitary conditions. Because of this, several cooperatives aimed at producing and commercializing quality certified virgin argan oil use a half-industrialized method with mechanical cold-pressing without water addition (*12*). This is the most important difference between the two methods.

Lipid oxidation is a major deteriorative reaction affecting edible oils and fats and consequently of primary concern to processors and consumers. Unsaturated lipids are particularly susceptible to oxidation during processing and storage via autoxidation and photosensitized oxidation. The most common mechanism of oxidation is a free radical chain reaction (*13*). This process is retarded by antioxidant compounds and accelerated by prooxidants such as trace metals. In addition, the lipid oxidation process is related to several oil physicochemical parameters and sensorial oil properties.

The analytical technique most widely used for metal determination in edible oils is electrothermal atomization−atomic absorption spectroscopy (ETA-AAS). This technique gives reliable results, and the sample preparation process could be minimized (*14–17*).

The aim of this work is to evaluate the influence of the extraction process on the virgin argan oil quality. For this purpose, we determined prooxidant metals (Cu, Fe, Cr, and Mn) that accelerated the oil oxidation process and the presence of Pb as a relevant toxic element to human health. We used ETA-AAS as the analytical technique with prior oil dilution with methyl isobutyl ketone (MIBK). We also determined the most important physicochemical oil quality criteria and the statistical correlations between metals and these parameters.

MATERIALS AND METHODS

Sample Collection. A total of 26 samples of virgin argan oil from southwestern Morocco were analyzed. The argan oil samples were taken randomly at different locations. Among them, 11 samples were obtained by half-industrialized method and are commercially available. The other samples were obtained by traditional method and were purchased in local markets. Samples were correctly fitted and kept fresh at 4 °C. Preliminary assays established the appropriate amount of sample for analysis to ensure homogeneity between samples, and they were representative.

Apparatus. For metal determination, we used a Perkin-Elmer 1100B double-beam atomic absorption spectrometer equipped with deuterium-arc-background correction (Perkin-Elmer, Norwalk, CT) and a Perkin-Elmer HGA-700 graphite furnace atomizer. Pyrolytically coated graphite tubes (ref B013-5653) and pyrolytic graphite platforms (ref B012-1092) were obtained from Perkin-Elmer. The K_{270} and K_{232} extinction coefficients were measured using a Perkin-Elmer Lambda 2 UV spectrophotometer. A Selecta stove (Selecta S.A., Barcelona, Spain) was used in humidity and sludge determinations.

Reagents. Standard solutions of Fe, Cu, Cr, Mn, and Pb dissolved in oil (100 ppm) (CertiPUR, Merck, Darmstadt, Germany) were used and diluted as necessary to obtain working standards. Bidistilled deionized water from a Milli-Q system (Millipore, Milford, MA) was used. Argon of 99.999% purity (SEO Barcelona, Spain) at 300 mL/min flow was used as internal gas, in the atomic absorption spectrometer. MIBK, egg lecithin, ethanol, diethyl ether, ethanolic potassium, 0.1 N hydroxide solution, sodium thiosulfate solution, potassium iodide, trichloromethane, acetic acid, cyclohexane, petroleum ether 40−60 °C, hexane, and aluminum oxide were purchased from Merck. All reagents were of analytical reagent grade or higher purity.

Material. To decrease the risk of contamination, all glassware and plastic-ware was nitric acid-washed and rinsed several times with bidistilled deionized water.

Analytical Procedures. Fe, Cu, Cr, Mn, and Pb were directly determined in the argan oil samples previously dissolved in MIBK by ETA-AAS. A portion of 1.0 g of previously filtered argan oil was dissolved in MIBK to a final volume of 10 mL. The optimized instrumental conditions for each element are summarized in **Table 1**. All determinations were done in triplicate. The analytical characteristics of the method applied in each case were evaluated, and results are summarized in **Table 2**. The detection limit was calculated according to IUPAC rules and the analytical sensitivity estimated as the mass of analyte that produced 0.0044 absorbance unit (*18, 19*). Precision was evaluated by performing 10 determinations on 5 different samples chosen at random. The accuracy of the method was checked by recovery assays of known amounts of analyte added to a vegetable oil of low metal content, which was obtained by dissolving 1 part of refined oil in 3 parts of hexane and then eluting with 5 parts of hexane through a column of aluminum oxide, which was previously activated by heating in an oven at 150 °C for 14 h. Twice the recommended mass of aluminum oxide was used. Hexane of the eluate was eliminated by stripping under reduced pressure (*14*). Prior to using the external standard method, we checked that there were no matrix interferences after applying the standard addition method to several samples. To check the similarity of slopes, Student's test was applied (*20*). The similarity of the slopes was accepted ($p > 0.05$) and, therefore, the parallelism between calibration lines revealed the lack of this type of interference.

Table 1. Instrumental Conditions for Fe, Cu, Cr, Mn, and Pb Determination in Virgin Argan Oil by ETA-AAS

	Fe	Cu	Cr	Mn	Pb
wavelength (nm)	248.3	324.7	357.8	279.5	283.3
intensity lamp (mA)	35	15	16	35	12
slit width (nm)	0.2	0.7	0.7	0.2	0.7
matrix modifier					egg lecithin
sample volume	20	20	20	10	10
graphite furnace program					
dry temperature (°C)	150	200	150	110	150
mineralization (°C)	1000	1100	1000	1000	500
atomization (°C)	2400	2400	2400	1900	2100

Acidity, peroxide value, humidity and volatile sludges, insoluble sludges in petroleum ether, and K_{270} and K_{232} extinction coefficients were determined according to the analytical methods described in Regulation EEC/2568/91 of the European Union Commission for olive oil (*21*). All determinations were done in triplicate.

Statistical Analysis. The different parameters we determined in virgin argan oil were studied as statistical variables. Each value of the variables corresponds to the average value of three independent measurements of a sample. Variability in each parameter and between both oil groups (traditional and half-industrialized oil extraction methods) was analyzed. Nonparametric tests were used because no variable was considered to be statistically normal (standard skewness or standard curtosis out of the range of −2 to 2 or P value of chi-squared normality test ≤ 0.05). Possible correlations between metals and between metal content and several quality parameters were also studied; P values of ≤ 0.05 were considered to be statistically significant. Statistical analysis was performed by using the Statgraphics Plus package (*22*).

RESULTS AND DISCUSSION

Fe, Cu, Cr, Mn, and Pb Contents in Virgin Argan Oil. We report here a rapid analytical method for the determination of these metals, useful for routine analysis. Sample preparation was carried out by simply diluting oil with MIBK prior to detection by ETA-AAS. Methods requiring a pretreatment of the samples to destroy the organic matrix involve certain manipulations and the subsequent risk of sample contamination and/or analyte loss (*17*). The analytical characteristics of the method demonstrate its validity (**Table 2**). On the other hand, the Cr and Mn analysis in foods is difficult because of their low concentrations and problems in their collection, storage, processing, and final determination without outside contamination. Sample preparation is a critical stage in the analysis. For all of the elements considered in this study, the use of the L'vov platform avoided dispersion of the sample inside the tube,

Table 2. Analytical Characteristics for the Method Used To Determine Fe, Cu, Cr, Mn, and Pb in Virgin Argan Oil by ETA-AAS

	detection limit[a] (pg)	sensitivity (pg)	recovery[b] (%)	precision[c] (%)	external standard/standard addition slope ratio
Fe	85.0	8.8	98.50 ± 0.6	4.2−4.5	0.980−1.050
Cu	26.0	4.5	98.70 ± 0.5	3.0−3.2	0.990−1.100
Cr	1.0	3.0	98.15 ± 0.20	3.5−4.2	0.995−1.100
Mn	2.0	3.5	98.70 ± 0.65	3.4−3.7	0.995−1.000
Pb	4.0	10.0	98.80 ± 0.80	2.8−3.6	0.990−1.100

[a] Confidence level of 99.9%. [b] Mean ± SD at 95% CI interval about the mean ($n = 10$). [c] Relative standard deviation (%) for 10 replicate determinations in each of 5 different samples.

Table 3. Fe, Cu, Cr, Mn, and Pb in Virgin Argan Oil Obtained by Traditional Extraction Method[a]

sample	procedence	Fe (mg/kg)	Cu (μg/kg)	Cr (μg/kg)	Mn (μg/kg)	Pb (μg/kg)
1	Rabat	0.9 ± 0.1	190.6 ± 2.0	12.7 ± 0.0	30.2 ± 0.0	28.5 ± 0.2
2	Rabat	0.9 ± 0.1	292.5 ± 3.0	28.0 ± 0.1	40.2 ± 0.1	30.2 ± 0.2
3	Fez	3.1 ± 0.9	513.6 ± 2.5	31.1 ± 0.1	48.4 ± 0.1	106.3 ± 0.3
4	Tanger	0.8 ± 0.1	185.0 ± 1.5	25.7 ± 0.1	25.0 ± 0.0	52.2 ± 0.5
5	Tanger	0.8 ± 0.0	175.7 ± 2.0	12.0 ± 0.0	26.0 ± 0.0	107.5 ± 2.0
6	Rabat	2.5 ± 0.7	410.4 ± 3.2	31.1 ± 0.1	38.5 ± 0.1	305.0 ± 2.2
7	Essaouira	0.9 ± 0.1	165.4 ± 0.6	11.5 ± 0.0	18.3 ± 0.0	35.6 ± 0.2
8	Essaouira	1.0 ± 0.1	170.5 ± 0.8	10.3 ± 0.0	25.2 ± 0.0	45.5 ± 0.3
9	Essaouira	2.8 ± 0.1	300.1 ± 2.8	12.0 ± 0.0	45.0 ± 0.1	105.2 ± 0.2
10	Tanger	0.9 ± 0.1	160.4 ± 0.4	15.6 ± 0.0	21.0 ± 0.0	29.0 ± 0.1
11	Agadir	0.8 ± 0.0	180.2 ± 0.6	10.8 ± 0.0	18.1 ± 0.0	52.0 ± 0.2
12	Agadir	0.9 ± 0.1	280.5 ± 0.8	35.0 ± 0.1	55.3 ± 0.1	30.8 ± 0.2
13	Bosocto	4.0 ± 0.1	695.7 ± 0.9	55.3 ± 0.2	70.8 ± 0.3	450.0 ± 2.0
14	Tiznit	0.8 ± 0.0	180.5 ± 0.4	38.1 ± 0.1	40.3 ± 0.1	38.2 ± 0.7
15	Essaouira	0.9 ± 0.1	190.0 ± 0.6	22.0 ± 0.1	20.3 ± 0.1	28.5 ± 0.1

[a] Results are expressed as mean ± standard deviation of three replicates of each sample.

Table 4. Fe, Cu, Cr, Mn, and Pb in Virgin Argan Oil Obtained by Half-Industrialized Extraction Method[a]

sample	procedence	Fe (mg/kg)	Cu (μg/kg)	Cr (μg/kg)	Mn (μg/kg)	Pb (μg/kg)
16	Casablanca	1.0 ± 0.1	165.4 ± 0.6	11.5 ± 0.0	18.3 ± 0.0	35.6 ± 0.2
17	Rabat	0.8 ± 0.1	160.0 ± 0.5	17.4 ± 0.1	20.3 ± 0.0	80.1 ± 0.1
18	Dakhala-Agadir	0.9 ± 0.1	170.6 ± 0.8	32.0 ± 0.1	42.7 ± 0.1	32.0 ± 0.3
19	Casablanca	1.5 ± 0.1	385.0 ± 0.9	48.1 ± 0.1	68.5 ± 0.1	80.4 ± 0.4
20	Essaouira	0.8 ± 0.0	176.8 ± 0.4	25.2 ± 0.1	35.0 ± 0.1	100.0 ± 0.3
21	Essaouira	0.9 ± 0.1	282.6 ± 0.7	31.1 ± 0.1	48.5 ± 0.1	40.0 ± 0.3
22	Casablanca	1.7 ± 0.1	158.4 ± 0.5	12.1 ± 0.1	15.0 ± 0.1	90.1 ± 0.3
23	Casablanca	0.9 ± 0.1	290.7 ± 0.6	13.0 ± 0.0	25.5 ± 0.1	45.6 ± 0.3
24	Dakhla-Agadir	0.9 ± 0.1	167.4 ± 0.4	12.6 ± 0.0	20.2 ± 0.0	95.7 ± 0.4
25	Essaouira	0.9 ± 0.1	280.0 ± 0.4	10.0 ± 0.0	21.1 ± 0.0	48.6 ± 0.3
26	Essaouira	0.9 ± 0.0	278.6 ± 0.7	13.1 ± 0.0	15.5 ± 0.0	52.8 ± 0.5

[a] Results are expressed as mean ± standard deviation of three replicates of each sample.

improved reproducibility of measurements, and led to a complete mineralization of samples with low thermal conductivity, such as oils, thus creating a more uniform temperature. It was also shown that when the tube was used without a platform, the final result was affected by the proportion of oil in the solution injected into the furnace. When the L'vov platform was used, the calibration lines by external standards and standard addition method had the same slope, which shows that the matrix effects have been minimized.

We determined the Fe, Cu, Mn, Cr, and Pb contents in the 26 samples of virgin argan oil. Results are summarized in **Tables 3** and **4**. In addition, the procedence description and the extraction method applied to obtain each sample are specified.

Fe concentrations in argan oil obtained by traditional method ranged from 0.8 to 4.0 mg/kg (**Table 3**), whereas in the samples obtained by the half-industrialized method, Fe content oscillated between 0.8 and 1.7 mg/kg (**Table 4**). Statistically significant differences at a 95% confidence level were found between both oil groups (*P* value for Cochran's test is 0.0001 and for Bartlett's test is 0.0003). The variability of Fe is lower in the samples obtained by the half-industrialized extraction method, with a coefficient of variation (CV) of 30.8%; in the samples obtained by traditional method the CV is 70.3%. The most elevated levels were detected in samples 13 (4.0 mg/kg) and 3 (3.2 mg/kg), both obtained by the traditional method; these samples exceed the maximum limit of 3.0 mg/kg set down by the International Oil Council (COI) in olive oils (virgin olive oil, olive oil, and olive-residue oil) (*11*) and by the Moroccan Normative in argan virgin oil (*10*). The FAO/WHO Codex Alimentarius Comission established maximum limits of 5 and 2.5 mg/kg of Fe in virgin and refined oils, respectively (*23*). The results that we obtained are similar to those reported by other authors (**Table 5**). Usually Fe is present in edible oils as a result of processing and storage contaminations. Several papers have described the deleterious effects of Fe on the flavor and oxidative stability of oils (*13, 14, 24*).

Copper concentrations in the samples obtained by traditional method ranged from 160.4 to 695.7 μg/kg (**Table 3**), whereas in the samples obtained by the half-industrialized method, they

Table 5. Fe, Cu, Mn, Cr, and Pb Levels in Edible Oils According to Data Reported by Other Authors

edible oil	Fe (mg/kg)	Cu (μg/kg)	Mn (μg/kg)	Cr (μg/kg)	Pb (μg/kg)	ref
virgin oilve oil	0.48	48.18	13.21−242.46	nd[a]−41.51	44.29	
olive oil	0.44	45.16	16.11−174.18	nd−31.38	11.52	
olive residue oil	0.706	47.40	13.90−293.35	20.14−26.15	142.05	
olive oil	0.75	18.29		44.17		
olive oil refined	1.27					*25*
olive oil	1.32−2.92					*25*
soybean oil	1.74					*25*
peanut oil	1.08					*25*
virgin olive oil	0.893		nd−25.2	116.49−437.4		*27*
sunflower oil	15.0−15.8	<4				*28*
sesame seed oil	14.40−15.2	510−590				*28*
hazelnut oil	15.4−15.5	48−540				*28*
soya oil	23.1−23.5	<4				*28*
kernel oil from *Canarium album* L. (Chinese olive)	124	90	25			
olive residue oil			13.90−296.35	20.14−26.15		*29*
virgin olive oil			13.21−242.46	nd−41.510		*29*
olive oil			16.11−174.18	nd−31.380		*29*
sunflower oil					nd−167.58	*30*
extra virgin olive oil				21.25		*16*
virgin oilive oil				19.12		*16*
sunflower oil	15.3	<4				*28*
sesame seed oil	14.8	560				*28*
hazelnut oil	15.4	500				*28*
soya oil	23.3	<4				*28*
olive oil	15.6	40				*28*
olive oil				<1−31	5−85	
sunflower oil					nd	*14*
olive oil	0.12-.015	73−86		nd		*14*
olive oil	0.083−1.795	2.1−209.0		11.0−40.3	nd−271.7	*7*
soybean oil					18−86	*23*

[a] nd, not detectable.

oscillated between 158.4 and 385.0 μg/kg (**Table 4**). The most elevated levels were detected in several samples of argan oil obtained by traditional method (samples 13 and 3). No statistically significant differences between the two groups at a 95% confidence level were observed (*P* value for Cochran's test is 0.025 but for Bartlett's test is 0.333). The variability of Cu is lower in the samples obtained by the half-industrialized extraction method (CV = 33.8%) than in the samples obtained by traditional method (CV = 54.4%). All of the samples exceed the maximum limit of 0.1 mg/kg set down by the International Oil Council (COI) (*11*) and the Moroccan Normative (*10*). In general, Cu data that we obtained are higher that those reported by other authors (**Table 5**). These data clearly demonstrate the differences in the metal concentrations of edible oils. The determination of Cu became one of the routine determinations performed during the quality control of oils (*23, 25*). The Cu presence in argan oil could be due to different factors such as soil composition, influence, and contaminations during extraction and conservation process. Copper acts as a prooxidant in the catalytic oxidation of the hydroperoxides of oils, in the presence of oxygen, originating ketones and aldehydes that change the taste of oils and new radicals that continue the oxidation process (*25*). Several studies demonstrated that the catalytic effect of Cu is greater than that of Fe. For example, Romano et al. (*24*) estimated that the catalytic effect of Cu(II) was greater than that of Fe(III) on the kinetic oxidation of the soybean oil in various experimental conditions. The autoxidative capacity of Cu has been demonstrated at levels lower than 30 ppb (*26*).

Chromium levels in the samples of argan oil we analyzed ranged from 10.3 to 55.3 μg/kg (CV = 54.8%) in oil obtained by the traditional method and from 10.0 to 48.1 μg/kg (CV = 59.1%) in samples obtained by the half-industrialized method (**Tables 3** and **4**, respectively). These data revealed no statistically significant differences between variability in both oil groups (*P* value for Cochran's test is 0.825 and for Bartlett's test is 0.828) or between medians (*P* value for Mann−Whitney's test is 0.603). Our data are similar to data obtained by other authors, but a wide variability is observed in the different edible oils (**Table 5**). Technology used in oil processing can increase the natural levels of Cr in raw material due to the transfer of the element from apparatus, utensils, containers (i.e., ceramic containers), and packages; this metal is widely used in the food industry, especially in stainless steel (*16*).

Manganese levels in the samples of argan oil we analyzed varied from 18.1 to 70.8 μg/kg (CV = 40.7%) in argan oil obtained by the traditional method and from 15.0 to 68.5 μg/kg (CV = 56.4%) in the samples obtained by the half-industrialized method (**Tables 3** and **4**, respectively). As for Cr and Cu, no statistically significant differences between the two oil groups were detected. There is no statistical difference of variability for the two methods or statistical differences between means (*P* value for Cochran's test is 0.528 and for Bartlett's test is 0.533; *P* value for Mann−Whitney's test is 0.157). Oil processing can increase the natural levels of Mn in raw material, but the influence of the soil Mn levels could be more relevant. Our data are similar to or lower than results obtained by other authors (**Table 5**) and do not exceed the concentrations that are considered as critical levels in the oil oxidation process. A Mn concentration near 0.6 ppm induces a decrease of 50% in the oil resistence to oxidation process; its catalytic activity oscillates between those of Cu and Fe.

Lead presence was detected in all samples we analyzed. In argan oil obtained by traditional method, Pb levels varied between 28.5 and 450.0 μg/kg. In argan oil obtained by the half-industrialized method, Pb levels varied between 32.0 and 100.0 μg/kg. The results obtained are summarized in **Tables 3** and **4**, respectively. We observed a high variability in the Pb content inside each of the oil groups, and the most elevated concentrations were found in argan oil obtained by the traditional method. The variability of Pb is lower in the samples obtained

Table 6. Statistically Significant Correlations between Metals in Virgin Argan Oil

	Cu	Fe	Mn	Pb
Cr	$r = 0.643, P < 0.001$	$r = 0.439, P < 0.05$	$r = 0.858, P < 0.001$	$r = 0.488, P < 0.05$
Cu		$r = 0.853, P < 0.001$	$r = 0.721, P < 0.001$	$r = 0.752, P < 0.001$
Fe			$r = 0.576, P < 0.01$	$r = 0.798, P < 0.001$
Mn				$r = 0.456, P < 0.05$

Table 7. Quality Criteria Parameters of the Analyzed Virgin Argan Oil Samples

	traditional method ($n = 15$)		half-industrialized method ($n = 11$)	
	mean	range	mean	range
acidity value (% oleic acid)	1.7	0.2−6.5	0.6	0.1−2.0
peroxide index (mequiv of O_2/kg)	3.7	1.2−10.9	4.4	1.0−7.9
K_{270}	0.329	0.137−1.023	0.297	0.220−0.559
K_{232}	1.586	0.683−3.020	1.573	0.170−2.085
humidity + volatile sludges (%)	0.09	0.02−0.4	0.06	0.03−0.08
insoluble sludges in petroleum ether (%)	0.19	0.02−0.94	0.20	0.03−0.30

by the half-industrialized method (CV = 40.4%) than in traditional method (CV = 125.6%). A total of five samples obtained by the traditional method and only one sample obtained by the half-industrialized method exceed the maximum limit of 0.1 ppm in edible oils allowed by Moroccan Normative (*10*) and by the Codex Alimentarius Comission (*23*). **Table 5** summarizes data reported by other authors, and in general our results are higher than these data. Lead presence in argan oil could be due to Pb content in soils (natural concentrations and for any added substances such as fertilizers, irrigation water, air pollution,...). Lead persists in the environment and has a long biological half-life. Plant absorption processes are influenced by pH, soil characteristics, the plant species involved, and the presence of other elements. However, the influence of food technology on Pb contamination has been widely described and is an additional factor that needs to be considered. Therefore, a deeper industrialization of the process, eliminating the use of stones and similar materials (i.e., hand broken, ceramic containers, metallic utensils,...) could surely contribute to reduce Pb presence in argan oil.

We studied the possible linear correlations between metals (**Table 6**) and observed that Cu and Cr have a moderate relationship, that there are many coefficients from 0.7 to 0.8 so there is a bigger correlation, and the strongest relationships are found between Fe and Cu and between Mn and Cr. These elements are usually associated in equipment and materials used in food technology. It is also remarkable that all of the correlations are positive so all of the relationships are direct, so that when one parameter increases so does the other one.

Quality Criteria Parameters in Virgin Argan Oil. We determined in all of the samples the acidity value, peroxide index, K_{270} and K_{232} extinction coefficients, humidity and volatile sludges, and insoluble sludges in petroleum ether. The data we obtained are summarized in **Table 7**. In samples obtained by the traditional method, acidity values fluctuated between 0.2 and 6.5% (expressed as oleic acid). In samples obtained by the half-industrialized method, acidity values varied between 0.1 and 2.0% (expressed as oleic acid). A total of eight samples of oil obtained by traditional method and two samples of oil obtained by half-industrialized method exceed the limit established in Moroccan Normative of 1.0% (expressed as oleic acid) for extra virgin argan oil (*10*). The elaboration method, hygienic conditions, and storage conditions of the seed can influence the acidity value (*8*). Nut roasting also appears to be a parameter that influences the acidity value. According to Hilali et al. (*8*), the acidity value of virgin argan oil samples prepared from roasted nuts was consistently lower than those of oils prepared from nonroasted nuts. In our study, the samples prepared from nonroasted nuts (samples 11 and 14) show the highest acidity values (2.8 and 6.5%, respectively). Both samples were obtained by the traditional method (hand extraction).

Classical chemical parameters used to determine the extent of oxidation in edible oils are peroxide value and spectrophotometric absorption at 232 and 270 nm, for initial stages of oxidation. In the samples we analyzed, peroxide index ranged from 1 to 11 mequiv of O_2/kg in oils obtained by the traditional extraction method and from 1 to 8 mequiv of O_2/kg in oils obtained by the half-industrialized method; all values are lower than the maximum limit of 20 mequiv of O_2/kg established for extra virgin argan oil in the Moroccan Normative (*10*). A variation coefficient of 61.1% indicates the high variability of the argan oil under obtaining conditions and conservation. Some argan oil components are highly sensitive to oxidation, but several studies have shown that virgin argan oil is less sensitive to oxidation than virgin olive oil. This stability has been attributed to the presence of tocopherols and polyphenols (*1*).

For the traditional method, extinction coefficients K_{270} and K_{232} oscillate between 0.137 and 1.023 and between 0.683 and 3.020, respectively. A total of seven samples exceed the maximum limit established for extra virgin argan oil ($K_{270} \leq 0.250$) according to the Moroccan Normative (*10*). For the half-industrialized method, K_{270} and K_{232} oscillate between 0.220 and 0.559 and between 0.170 and 2.085, respectively. A total of seven samples exceed the maximum limit established in the Moroccan Normative for extra virgin argan oil (*10*). We observed that the variability of both parameters (K_{270} and K_{232}) is significantly different depending on the oil extraction method. These specific extinction indices also serve as purity criteria, because the oils with industrial treatments, such as the application of high temperatures, have increased conjugated trienes.

In relation to humidity and volatile sludges, only one sample (sample 12) among the total we analyzed, obtained by the traditional method, exceeded the maximum limit of 0.2% established for extra virgin argan oil according to the Moroccan Normative (*10*). The variability of this parameter is again lower in the samples obtained by the half-industrialized extraction method (CV = 30.6%) than by the traditional method (CV = 104.7%). As a consequence, we detected statistically significant differences between both oil groups. There is more water left in the oil obtained by the traditional extraction method. For insoluble sludges in petroleum, two samples (traditional method) and three samples (half-industrialized method) exceed the

maximum limit of 0.3% stablished for extra virgin argan oil in the Moroccan Normative (*10*).

For the total samples we analyzed ($n = 26$), it can be remarked that some parameters have large dispersion. The most scattered parameters are acidity, humidity and volatile sludges, insoluble sludge in petroleum ether, and lead content. In fact, we detected some outliers for several parameters in the box and shisker plot. We give special importance to the fact that several samples (*6, 8, 9, 11*) are outliers for many parameters. In particular, sample 13 is an outlier for most metals (Cu, Fe, and Pb) and sample 6 for Pb. It is notable that all of the mentioned samples are obtained by the traditional extraction method. On the other hand, nonstatistically significant correlations between metal content and the quality parameters that we determined were observed.

The data we obtained indicated that an improvement technology with a more industrialized process and a correct production control could increase the virgin argan oil quality and preserve its chemical composition, flavor, and nutritional value. In addition, it could preserve the health of consumers by reducing the presence of toxic metals.

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LITERATURE CITED

(1) Khallouki, F.; Younos, C.; Soulimani, R.; Oster, T.; Charrouf, Z.; Spiegelhalder, B.; Bartsch, H.; Owen, R. W. Consumption of argan oil in Morocco with its unique profile of fatty acids, tocopherols, squalene, sterols and phenolic compounds should confer valuable cancer chemopreventive effects. *Eur. J. Cancer Prev.* **2003**, *12*, 67–75.

(2) Cherki, M.; Berrougui, H.; Drissi, A.; Adlouni, A.; Khalil, A. Argan oil: which benefits on cardiovascular diseases. *Pharmacol. Res.* **2006**, *54*, 1–5.

(3) Bennani, H.; Drissi, A.; Giton, F.; Kheuang, L.; Fiet, J.; Adlouni, A. Antiproliferative effect of polyphenols and sterols of virgin argan oil on human prostate cancer cell lines. *Cancer Detec. Prev.* **2007**, *31*, 64–69.

(4) Berrougui, H.; Ettaib, A.; Herrera, M. D.; Álvarez de Sotomayor, M.; Bennani-Kabchi, N.; Hmamouchi, M. Hypolipidemic and hypocholesterolemic effect of argan oil (*Argania spinosa*) in Meriones shawi rats. *Ethnopharmacology* **2003**, *89*, 15–18.

(5) Berrougui, B.; Cloutier, M.; Isabelle, M.; Khalil, A. Phenolic-extract from argan oil (*Argania spinosa* L.) inhibits human low-density lipoprotein (LDL) oxidation and enhances cholesterol efflux from human THP-1 macrophages. *Atherosclerosis* **2006**, *184*, 389–396.

(6) Drissi, A.; Girona, J.; Cherki, M.; Godàs, G.; Derouiche, A.; El-Messal, M.; Saile, R.; Kettani, A.; Solà, R.; Masana, L.; Adolouni, A. Evidence of hypolipemiant and antioxidant properties of argan oil derived from the argan tree (*Argania spinosa*). *Clin. Nutr.* **2004**, *23*, 1159–1166.

(7) Derouiche, A.; Cherki, M.; Drissi, A.; Bamou, Y.; El Mescal, M.; Idrissi-Oudghiri, A.; Lecerf, J. M.; Adlouni, A. Nutritional intervention study with argan oil in man: effects on lipids and apolipoproteins. *Ann. Nutr. Metabol.* **2005**, *49*, 196–210.

(8) Hilali, M.; Charrouf, Z.; Soulhi, A. E.; Hachimi, L.; Guillaume, D. Influence of origin and extraction method on argan oil physicochemical characteristics and composition. *J. Agric. Food Chem.* **2005**, *53*, 2081–2087.

(9) Rahmani, M. The chemical composition of "virgin" argan oil. *Cah. Etudes Rech. Fr.* **2005**, *14*, 461–465.

(10) Norme Marocaine 08.5.090. Ministry of Industry, Trade, Energy and Mines. Rabat, 2002.

(11) COI (International Oil Council). Applicable trade regulations to olive oil and olive residue oil. N° 3/ Rev 2; 24 November, 2006.

(12) Khallouki, F.; Spiegelhalder, B.; Bartsch, H.; Owen, R. W. Secondary metabolites of the argan tree (Morocco) may have disease prevention properties. *African J. Biotechnol* **2005**, *4*, 381–388.

(13) Choe, E.; Min, D. B. Mechanisms and factors for edible oil oxidation. *Compreh. Rev. Food Sci. Food Saf.* **2006**, *5*, 169–186.

(14) Martin-Polvillo, M.; Albi, T.; Guinda, A. Determination of trace elements in edible vegetable oils by atomic absorption spectrophotometry. *J. Am. Oil Chem. Soc.* **1994**, *71*, 347–353.

(15) Karadjova, I.; Zachariadis, J.; Boskou, G.; Stratis, J. Electrothermal atomic absorption spectrometric determination of aluminium, cadmium, copper, iron, manganese, nickel and lead in olive oils. *J. Anal. At. Spectrom.* **1998**, *13*, 201–204.

(16) Lendínez, E.; Lorenzo, M. L.; Cabrera, C.; López, M. C. Chromium in basic foods of the Spanish diet: seafood, cereals, vegetables, olive oils and dairy products. *Sci. Total Environ.* **2001**, *278*, 183–189.

(17) Matos, M. N.; Campos, R. C. Determination of copper and nickel in vegetable oils by direct sampling graphite furnace atomic absorption spectrometry. *Talanta* **2006**, *70*, 929–932.

(18) Horwitz, W.; Albert, R.; Deutsch, M. J.; Thompson, J. N. Precision parameters of methods of analysis required for nutrition labelling. *J. AOAC Int.* **1990**, *73*, 661–680.

(19) Smart, N. A. Interlaboratory comparability of accuracy in residue analysis. *Anal. Proc.* **1993**, *30*, 75–77.

(20) Cuadros, L.; Garcia, A. M.; Alés, F.; Jiménez, C.; Roman, M. Validation of an analytical instrumental method by standard addition methodology. *J. AOAC Int.* **1995**, *78*, 471–476.

(21) 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. *Off. J. Eur. Communities* **2003**, *248*, 1−109.

(22) Statgraphics Plus, version 4.1, Statistical Graphics Corp., Copyright 1994−1999.

(23) FAO/WHO Food Standards Program, Codex Alimentarius Comission 14th Session, 1981, rev. 2003.

(24) Romano, R.; Riccio, F.; Borriello, I.; Toraldo, G. Catalytic effect of Cu(II) and Fe(III) on kinetic oxidation of fatty substances: the soybean oil case. *Riv. Ital. Sostanze Grasse* **2007**, *84*, 25–32.

(25) Pinto, P.; Saraiva, M. L.; Lima, J. L. A flow sampling strategy for the analysis of oil samples without pre-treatment in a sequential injection analysis system. *Anal. Chim. Acta* **2006**, *555*, 377–383.

(26) Wong, K. H.; Fung, Y. S.; Fung, K. W. Determination of trace amounts of copper in palm oil by differential-pulse-anodic-stripping voltammetry. *Analyst* **1980**, *105*, 30–36.

(27) Benincasa, Z.; Lewis, J.; Perri, E.; Sindona, G.; Tagarelli, A. Determination of trace element in Italian virgin olive oils and their characterization according to geographical origin by statistical analysis. *Anal. Chim. Acta* **2007**, *585*, 366–370.

(28) Cindric, I. J.; Zeiner, M.; Steffan, I. Trace elemental characterization of edible oils by ICP-AES and GFAAS. *Microchem. J.* **2007**, *85*, 136–139.

(29) Roca, A; Cabrera, C.; Lorenzo, M. L.; López, M. C. Levels of calcium, magnesium, manganese, zinc, selenium and chromium in olive oils produced in Andalusia. *Int. J. Fats Oils* **2000**, *6*, 393–399.

(30) Roca, A.; Cabrera, C.; Lorenzo, M. L.; López, M. C. Lead and cadmium content in sunflower oil. *Int. J. Fats Oils* **2001**, *52*, 229–234.

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