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Effect of filtration on virgin argan oil: Quality and Stability

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Abstract

The quality, composition and oxidative stability of argan oils from unroasted kernels were evaluated before and after filtering the cold-pressed oils. Filtering leads to significant changes in the content of free fatty acids, the peroxide value, the total content of phospholipids and the oxidative stability measured by the Rancimat test at 110°C. The high level of tocopherols, fatty acid and sterols composition were not significantly influenced by filtrations but it contributes to the oxidative stability of the oils. The removal of phospholipids as well as other antioxidant active compounds, not measured by the DPPH seems to be responsible for the effect on the oxidative stability by filtration.

Keywords: Argan oil, Filtration, Quality, Stability, Antioxidant Activity

1. Introduction

Argan oil is extracted from the kernels of fruits from the argan-tree (Argania spinosa (L.) Skeels; Sapotaceae) a tree exclusively endemic to Morocco. The worldwide commercial success of argan oil is currently impacting the seed-oil market, and in Morocco, the local economy and the sustainable development of the argan forest [1,2]. Because of its high level of unsaturated fatty acids and antioxidants, argan oil is particularly valued in the alimentary and nutraceutical domains [3-6]. Indeed, edible argan oil, the basic ingredient of the "Amazigh diet" [7]. The antibacterial properties of the oil extracted from the kernels of the argan were studied [8]. Is a culinary ethnic food endowed with heart-protecting properties [9]. Edible argan oil is prepared by cold-pressing of roasted argan kernels. In parallel, beauty-grade argan oil is extracted from unroasted kernels [10].

Virgin edible argan oil extraction requires at least six steps: fruit collection, sun-drying, dehulling, nut breaking (or kernel collection), kernel roasting, and cold-pressing. Each processing step dramatically influences the resulting oil in terms of quantity and quality.

Oil filtration is sometimes added to remove large amounts of plant material coming into the oil during extraction but also gums and waxes. The filtration procedure is especially important as a final step of the elaboration process. During filtering, quantitative and qualitative changes take place, especially on minor components.

Several studies Show that, most oil mills store virgin oils in tanks without filtering the oil before packaging, because it is empirically thought by the producers that filtered virgin oils are less stable and therefore more susceptible to alteration [11].

So far, a clear understanding of the effects of argan oil filtration on its potential nutritional properties, sensory attributes, and commercial grading is still lacking. The main objective of this study was to investigate the

influence of filtration on the stability and quality of virgin argan oil. The evaluation of phospholipids, tocopherol, antioxidant activity (as measured by 2,2-diphenyl-1-(2,4,6-trinitrophenyl (DPPH)), oxidative stability (as measured by the Rancimat test at 110°C) and oxidative state (free fatty acids, peroxide value and spectroscopic indexes at 232 and 270 nm) were studied for argan oils obtained from dried fruits before and after filtering.

2. Matériels and Méthodes

2.1. Chemicals

DPPH• (2,2-diphenyl-1-picryhydrazyl radicals), EDTA, luminol (3-aminophtalylhydrazine), boric acid and Trolox were purchased from Sigma Chemical Co. (Taufkirchen, Germany). Cobalt (II) [CoCl2·6H2O] and methanol used were from Readel de Haen (Seelze, Germany).

2.2. Sample collection

Argan fruits were collected in Tighanimine / Drargua (Province of Agadir Idaoutanane, Morocco) in 2013. Fruits were sun-dried for two weeks and then mechanically dehulled (SMIR Technotour, Agadir, Morocco). Argan nuts were manually opened using the traditional two stone methods to get the kernels.

2.3. Oil extraction

Argan oil is virgin oil extracted by cold-pressing unroasted kernels, Oil extraction was carried out mechanically using a Komet DD 85 G press (IBG Monforts Oekotec GmbH & Co. KG, Mönchengladbach, Germany).

2.4. Oil filtration

Two types of argan oil were analysed. Argan oil from unroasted kernels, mechanically extracted with filtration (Mod. FO-30 SMIR, Filter plate 20 x20cm) and argan oil from unroasted kernels, mechanically extracted without filtration. From each oil type, 100 mL of oil were prepared and stored in glass bottles at 4°C.

2.5. Physical and chemical oil parameters

Content of free fatty acids, peroxide value (PV), extinction coefficients (K232 and K270), and iodine value were determined according to the AOCS methods Ca 5a-40, Cd 8b-90, Ch 5-91, Cc 7-25, Cd 1c-85, respectively (AOCS., 1998). The content of free fatty acid was expressed as percent of oleic acid. PV was expressed as milliequivalent of active oxygen per kilogram of oil (meq O2/kg oil), and extinction coefficient (K232 and K270) was expressed as the specific extinction of a 1% (w/v) solution of oil in cyclo hexane in 1 cm cell path length, using a CARY 100 Varian UV spectrometer.

2.6. Oxidative stability

The oxidative stability was determined with the 743 Rancimat apparatus (Metrohm AG, Herisau, Switzerland), an instrument for automatic determination of the oxidation stability of oils and fats. The stability was measured by the oxidative-induction time (OIT) using 3.5 g of oil. The temperature was set at 110 ◦C, and the purified air flow passing through at a rate of 10 L/h. During the oxidation process volatile acids were formed, transferred with the air flow into the distilled water and then measured conductometrically. The induction period is defined as the time necessary to reach the inflection point of the conductivity curve.

2.7. Tocopherol composition

For the determination of tocopherols, a solution of 250 mg of oil in 25 ml of n-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm), and a D-2500 integration system. The samples in the amount of 20 µLwere injected

with a Merck 655-A40 autosampler onto a Diol phase HPLC column 25 cm × 4.6 mm ID (Merck, Darmstadt, Germany) using a flow rate of 1.3 mL/min. The mobile phase used was n-heptane/tert-butyl methyl ether (99 + 1, v/v) [12].

2.8. Antioxydant activity

The antioxidant activity of the oils was measured by the DPPH free radical assay. In brief, a methanolic solution (50 μL) of the extract at five different concentrations was added to 1.95 mL of DPPH• solution (6×10−5M in methanol). The decrease in the absorbance at 515 nm was determined using an HP 8452A diode-array spectrophotometer until the reaction reached the steady state in the dark. The remaining DPPH• concentration in the reaction medium was calculated from the calibration curve.

The percentage of remaining DPPH• was calculated as follows:

%DPPH• remaining = [DPPH•]T [DPPH•]T=0 where [DPPH•]T is the concentration of DPPH• at the time of steady state and [DPPH•]T=0 is the concentration of DPPH• at zero time. These values were plotted against mg of extract/mg DPPH• to show the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50% (EC50) using the exponential curve. Results were expressed as standard equivalents using quercetin and Trolox on the basis of the EC50 value where the lower its value, the more efficient the antioxidant [13].

2.9. Fatty acid composition

The fatty acid composition was determined following the ISO standard (ISO 5509:2000). Briefly, one drop of the oil was dissolved in 1 ml of n-heptane, 50 μg of sodium methylate was added, and the tube was closed and agitated vigorously for 1 min at room temperature. After the addition of 100 μl of water, the tube was centrifuged at 4500 g for 10 min and the lower aqueous phase was removed. Then, 50 μl of HCl (1 mol with methylorange) was added, the solution was shortly mixed, and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure; Merck, Darmstadt, Germany) was added, and after centrifugation at 4500 g for 10 min, the top n-heptane phase was transferred to a vial and injected in a Varian 5890 gas chromatograph with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 μm). The temperature program was as follows: from 155°C; heated to 220°C (1.5°C/ min), 10 min isotherm; injector 250°C, detector 250°C; carrier gas 36 cm/s hydrogen; split ratio 1:50; detector gas 30 ml/min hydrogen; 300 ml/min air and 30 ml/min nitrogen; manual injection volume less than 1 μl. The peak areas were computed by the integration software, and the percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalisation.

2.10. Sterol composition

Was determined using the International Standard Organisation method. Sterol composition was determined after trimethylsilylation of the crude sterol fraction using a Varian 3800 instrument equipped with a VF-1 ms column (30 m & 0.25 mm i.d.) and using helium (flow rate 1.6 mL/mn) as carrier gas. Column temperature was isothermal at 270 °C, injector and detector temperature was 300 °C. Injected quantity was 1uL for each analysis. Data were processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA).

Each method was carried out in triplicate for each sample. The mean values are given in the tables, without the standard deviations, because such values would represent only the deviation of the method and not the variation of the respective sample.

2.11. Statistical Analysis

Values reported in tables are the means ± SD of three replications. The significance level was set at P=0.05. Separation of means was performed by Tukey's test at the 0.05 significance level.

3 - Results and Discussion

3.1. Free fatty acids content

For all oils tested, the content of free fatty acids (FFA) was below 0.8%. This value is the limit for extra virgin argan oil Comparable to the categories described for argan oil (SNIMA 2003), argan oils can be classified as 'extra virgin' (FFA <0.8%) and 'virgin' (FFA <1.5%). No significant difference was observed between the FFA content of cold-pressed unfiltered and filtered samples ($p > 0.05$) (Table 1). After filtration a slight, but not significant increase was observed which is in contrary with the results described by Fregapa for the filtration of the olive oil [14].

Table 1: Quality of Physical characteristics of crude cold-pressed unfiltered and filtered argan oils: FFA, UV absorption, peroxide value (PV). Phospholipid, Tocopherols and DPPH assay

	Argan oil, filtered	**Argan oil, unfiltered**
FFA (%)	0.33± 0.01 [a]	0.21± 0.01 [a]
K_{232} (–)	1.22±0.03 [a]	1.21±0.06 [a]
K270 (–)	0.22±0.01 [a]	0.22±0.01[a]
PV [m_{eq} O_2/kg oil]	1.19± 0.3[a]	0.51± 0.3 [b]
Phospholipids [mg/kg oil]	143.8± 3.7 [a]	221.2±3.3[b]
Consumption of DPPH [mg/L])	298.31±0.07[a]	288.86±0.01[a]
*** OSI at _110°C (h)**	13.8 ±0.3[a]	15.1 ±0.4[b]

*different letters in the same row at mean values indicate significant differences ($p< 0.05$) as analyzed by Duncan test.

3.2. UV absorption

The absorption at 232 and 270 nm, respectively, are linked with the formation of conjugated dienes and trienes during lipid oxidation. Conjugated dienes are formed during primary oxidation, causing an increase of K232 values, while K270 is increased during secondary oxidation, due to the formation of conjugated trienes as well as carbonylic compounds.

The K values found for the samples were in the range of extra virgin argan oil (Table 1) showing that these oils were not deteriorated by oxidation, possibly due to its high content in oleic acid (58%) and high level of tocopherols [15]. Filtering did not have any significant effect on the K-values showing that oxidized compounds were not removed by filtration.

3.2. Peroxide value

Peroxides are primary oxidation products that lead to rancidity. Therefore, their formation dramatically impacts oil shelf life and consumer acceptance.

Peroxide values of filtered and unfiltered argan oils were found to be below 2.0 meq O2/kg oil, well below the maximum peroxide value of 15 meq O2/kg oil defined for the extra virgin argan oil [16].

Filtration led to a slight increase of the peroxide value with 1.2 meq O2/kg oil in comparison to 0.5 meg O2/kg for unfiltered oil (Table 1). This result corroborates the finding by Fragapa [14]. Who affirmed that filtration caused an increase in the rate of peroxide formation for the olive oil.

3.3. Phospholipids

Little is known about this class of compounds in argan oils, found that the major compounds are phosphatidhylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine [17].

The phosphorus content of filtered and unfiltered argan oils was determined by spectrophotometric measurement of the yellow phosphovanadomolybdic complex that represents the carryover of phospholipids.

Significant differences ($p<0.05$) in the phosphorus content of the filtered and unfiltered oils were observed (Table 1).
The unfiltered argan oil showed a higher phospholipid content than filtered ones with values of 221 and 143 mg/kg, respectively. This finding confirms that phospholipids were reduced by filtration as shown by Koidis [18].

3.4. Tocopherols

Tocopherols, belonging to the vitamin-E-active compounds, are a group of lipid soluble components which naturally occur in oilseeds in four various forms (α-, β-, γ- and δ-tocopherol). Tocopherols are valued for their vitamin E activity and their antioxidant properties to protect polyunsaturated fatty acids against oxidation. Thus the level of tocopherols in seed oils is extremely important for the stability of the oils. The nature and content of tocopherols are characteristic for different oilseeds and relies on the geographical area, plant cultivar, seasonal effects, the quantity of polyunsaturated fatty acid, oil processing, and storage conditions [19].
The total content of tocopherols was slightly lower in argan oil after filtration (657.59 mg/kg to 676.12 mg/kg), but this difference was not significant ($P<0.05$) showing that filtration has no effect on the content of tocopherols.
Among the tocopherols identified, γ-tocopherol was the most abundant and comprised approximately 96–98% of the total tocopherols. α-tocopherol is recommended for human consumption and it has been suggested to possess a higher biologically activity than other tocopherols. The α-tocopherol contents of the samples were lower than the values reported for Moroccan olive oil by Gharby [17]. Only trace amounts of δ-tocopherol were present. . The presence of β-tocopherol reported earlier by Gharby [17] was not confirmed in the present study. These variations can be attributed to differences in the method of lipid extraction and genetic variations (plant cultivar, variety grown).

3.5. Fatty acid composition

The argan oil containing 80% unsaturated fatty acids (Table 2) which are composed on the one hand, the majority of compounds such as oleic acid and linoleic acid, and on the other hand, a compound minority, linolenic acid less than 0.4% [15].. This oil also contains 20% saturated fatty acids are: Palmitic acid C16: 0 (11.5 to 15%) and stearic acid C 18: 0 (4.3 to 7.2%). The effect of the filtration performed on the various samples, showed no significant change in the fatty acid levels (Table 2). This proves that filtration does not change the fatty acid composition

3.6. Sterol composition

Sterols found in argan oil are considered to be important nutrients for human health [14].Results of the sterol analysis are presented as a percentage of the total sterol content Table 2. Campesterol was consistently found in very low concentration. This parameter is essential since low campesterol content is a marker to detect adulteration of argan oil with other common, cheap vegetable oils [15]. Therefore, this method remains perfectly useable, even with argan oil prepared from a single form of fruit.
The effect of the filtration performed on the various samples, showed no significant change in the sterol levels (Table 2). This proves that filtration does not change the sterol composition.

3.7. DPPH assay

The DPPH free radical assay is a suitable indicator for the antioxidant activity of compounds or mixture of compounds because the strong absorption band at about 250 nm from the DPPH free radical changes to 520 nm after addition of an antioxidant compound changing the color from deep violet to pale yellow. In the present investigation the antioxidant activity of the different oils was calculated as amount of α-tocopherol in mg/L

necessary to reduce the DPPH concentration for 50%. The lower the amount of extract necessary to reduce the DPPH frees radical concentration the higher the antioxidant activity.
The results for the antioxidant activity of filtered and unfiltered argan oil measured by the DPPH assay showed no significant difference ($p > 0.05$) indicating that removal of solid materials from the oil by filtration has no effect on the amount of antioxidant active compounds. This result is in agreement with the result for amount of tocopherols which also showed no significant influence by filtration.

Table 2: Chemical Composition of crude cold-pressed unfiltered and filtered argan oils: Tocopherols, Fatty acid and sterol composition.

	Argan oil, filtered	**Argan oil, unfiltered**
Total Tocopherol [mg/100g]	657.7 [a]	676.1 [a]
- **α-Tocopherol**	12.6±1.6 [a]	13.9±1.3[a]
- **γ –Tocopherol**	633.1±23.4[b]	659.7±27.2[a]
- **δ-Tocopherol**	11.2±1.3[a]	12.1±0.9[a]
Fatty Acids (%)		
- **Palmitic acid**	13,19 ± 0,06 [a]	13,22± 0,08 [a]
- **Stearic acid**	5,42 ± 0,10 [a]	5,86 ± 0,12 [a]
- **Oleic acid**	46,86± 0,11 [a]	46,90± 0,09 [b]
- **Linoleic acid**	32,02 ± 0,10 [a]	31,93 ± 0,11 [b]
- **Linolenic acid**	0,30 ± 0,04 [a]	0,36 ± 0,07 [a]
Sterols (% of total sterols)		
- **Campesterol**	0,19±0.04 [a]	0,12±0.02 [a]
- **Stigmasterol**	0,11±0.04 [a]	0,08±0.02 [a]
- **Stigmastadiène**	4,57±0.08 [a]	4,45±0.06 [b]
- **Spinastérol**	38,47±0.94 [b]	38,39±0.90 [a]
- **Schotténol**	44,02±1.01	44,05±0.99 [b]
- **Δ7-Avénastérol**	3,52±0.12 [a]	3,12±0.10 [a]

*different letters in the same row at mean values indicate significant differences ($p< 0.05$) as analyzed by Duncan test.

3.8 Oxidative Stability:
The oxidative stability reflects the susceptibility of fats and oils to oxidative degradation. It is influenced by the content of unsaturated fatty acids and bioactive constituents such as sterols and tocopherols which can act as pro- and antioxidants. Lipid oxidation causes a reduction of the nutritive value and functional properties of food products [20].
The results of Rancimat test show that unfiltered argan oil proved to be significantly more stable (15.1 h) against oxidation than the filtered one (13.8 h). The reason for this significant difference between the oils might be due to different amounts of minor constituents such as phospholipid or other antioxidant active compounds. Although the tocopherol content also contributes to the oxidative stability of argan oil, it is not the reason for differences between filtered and unfiltered oil since the investigation showed no significant effect of filtration on the tocopherols. Also the DPPH assay revealed no significant effect of filtration on the antioxidant activity of both oils. Thus, the higher amount of phospholipids in unfiltered argan oil and other antioxidant active

compounds not measured by the DPPH assay seem to be responsible for the significant difference of the oxidative stability measured by the Rancimat test.
In general, according to these results and results published by Harhar [15].argan oils are very stable against oxidative deterioration. It might be due to the presence of γ-tocopherol which has been suggested to possess a higher antioxidant capacity as compared to the other tocopherol homologues. Also the presence of high amounts of phospholipids might have an effect. The removal of suspended solids by filtration results in a reduction of the shelf life of argan oil [14] demonstrated that when the suspended materials were totally eliminated by filtration, the susceptibility to oxidative degradation was higher in filtered olive oils than unfiltered ones. Furthermore, the suspended solids are related with hydrophilic phenols and it is well-known their antioxidant properties.

Conclusion

The present investigation shows that filtration has a significant negative effect on the oxidative stability of argan oil resulting from the removal of antioxidant active compounds others than tocopherols from the oil. Also the peroxide value increased slightly by filtration. Thus from a quality point of view solid material from seed material seems to improve argan oil and increases the oxidative stability. In a further investigation it has to be proofed whether the solid material contributes to an improvement of the quality of argan oil during storage.

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