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Physicochemical Characterizes of Argan Oil (*Argania spinosa* L.) Affected by Roasting and Pulping by Goats

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ABSTRACT

Present study investigates the influence of roasting and pulping by goats on the quality of argan oil (*Argania spinosa* L.). To carry out this work, 4 different samples selected by their method of extraction (by mechanical pressing from argan fruits pulped by goats). The study of the physicochemical characteristics of the 4 samples shows that the roasting of the almonds of the argan fruit and the pulping by the goat appear as a parameter can increase the value of the peroxide index and the acidity. It's also found that pulping by goats can decrease the value of fatty acids (linoleic $C_{18:2}$) and the percentage of triglycerides (LLL, LOO and OOO) in argan oil. The result of sterols clearly shows that roasting is reduced the total sterols. The study of the concentration of benzo-α-pyrene suggests that roasting does not produce significant amounts of benzo-α-pyrene. Also, present findings shows that roasting and pulping by goats appears as a parameter influencing the acid value of argan oil. In fact, the acidity value is higher in the argan oil samples prepared from roasted almonds and pulped by goats. Finally, the present study indicated that the high quality of argan oil can be extracted by mechanical pressing and which is not pulped by goats and, therefore, current results may support the marketing of the oil argan.

Key words: Argan oil, Physicochemical analysis, Method of extraction, Roasting, Pulping, Goats.

INTRODUCTION

Argan oil plays a very important role in the diet of indigenous populations and its residue can be considered as animal feed. It is edible oil extracted from almonds. The oil extraction rate could be increased and its quality could be improved. The extraction of argan oil by the artisanal method is done in several stages (Charrouf *et al.*, 2018). The artisanal extraction of a liter of argan oil requires 20 hours of strenuous and intense work (Haloui *et al*., 2015). Traditionally extracted argan oil goes rancid after a few months. This is caused by several factors: the premature dehulling of the fruit of the argan nut, the use of nuts rejected by goats, the precarious hygienic conditions during the extraction of artisanal oil, the packaging in bottles recycles, that's why there are big efforts have been made by our laboratory to improve the extraction of argan oil, and Thanks to mechanical extraction we can obtain two types of oil (Haloui *et al*., 2015; Khallouki *et al*., 2017).

- Edible oil, with a nutty taste, obtained by mechanical pressing of roasted almonds (AOR).
- Cosmetic oil, or virgin oil intended more for cosmetic uses, obtained from unroasted almonds (AONR).

So mechanical pressing reduces the time, and the harshness of the work also makes it possible to obtain better quality oil with a good yield. Argan oil is rich in oleic acid, which makes this oil particularly interesting in regulating cholesterol. In addition, argan oil is also rich in phytosterols which have an important activity and whose incorporation in a diet is supposed to offer the prevention of cancers (Bennani *et al.*, 2007; Drissi *et al.*, 2006). Studies also show that polyphenols and phytosterols as well as a certain number of their derivatives have anti-tumor properties (Bradford *et al*., 2007). Great efforts have been made to develop argan oil by improving extraction technology and making forest users profit from this added value by creating cooperatives in the region that produces and markets argan oil (Charrouf *et al.* 2014). Present work had repercussions in the production region, both socio-economic and environmental. The aim of this work is to study the influence of roasting and pulping of the argan fruit by goats on the physico-chemical characteristics and the chemical composition of argan oil.

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MATERIALS AND METHODS

Preparation of different samples of Argan oil

Biological material: In this work, we have selected 4 samples of argan fruits from the same region (Tamanar in south-eastern Morocco) and its different types of extraction (two samples are pulped/processed by goats). Table 1 provides information on the origin and method of extraction of each sample.

Argan oil extraction: Argan oil from 4 samples is extracted from roasted almonds and the fruits of the argan are pulped by goats) (Charrouf *et al.,* 2018): Table 1 gives information on the method of extraction and the origin of each sample of argan oil.

• AOR: Argan oil is extracted by mechanical pressing from roasted almonds (AOR).
• AOPRG: Argan oil is extracted by mechanical pressing from roasted almonds pulped by goat cheese (AORG).
• AONR: Argan oil is extracted by mechanical pressing from unroasted almonds (AONR).
• AONRG: Argan oil is extracted by mechanical pressing from unroasted almonds pulped by goats (AONRG).

Obtained oils are then analyzed at the official chemical analysis and research laboratory in Casablanca, Morocco, the physico-chemical characteristics and the chemical composition (fatty acid, sterols, triglycerides, tocopherols, benzo-a-pyrene) of all the samples are determined. The oils are analyzed according to European Norme, (1999).

Argan oil extraction

Physicochemical analyzes of oils: All analyzes were done in the Official Laboratory of Chemical Analysis and Research (LOARC) in Casablanca, Morocco. Determination of acidity (Européenne Norme, 1999), the peroxide value (Lagardere, 2004), the refractive index (ISO, NFEN- 2000) of the absorbance in the ultraviolet (Normalisation, 2002), the saponification number (Normalsation, 2002), the unsaponifiable content (Sahu *et al*., 2018) were measured according to the standardized methods of reference.

Determination of composition and nature in total sterols: Reference ISO 6799 (Aïssi *et al.,* 2009).

Operating mode: Weigh 2.5 g of argan oil and put into a 20 ml flask. 25 ml of a solution of potassium hydroxide (1N of ethanol) is added. The flask is heated under reflux for 30 minutes until the solution becomes clear.

Then, 25 ml of distilled water is added to stop the reaction. The extraction of the un-saponifiable is carried out using 75 ml of hexane or petroleum ether. The organic phase is subjected to a series of washing with 15 ml of mixture (water/ethanol 95°) (90/10) in a separatory funnel.

The hexane phase is transferred from the top of the ampoule into a 100 ml flask. After evaporation of the solvent using a rotary evaporator, the unsaponifiable material is recovered. The unsaponifiable agent, diluted with 300 µl of hexane or petroleum ether, is filtered on a silica column (25 cm × 4 mm). The HPLC device is equipped with a 205 nm-254 nm UV detector. The eluent is an isooctane/isopropanol (99/1) mixture whose flow rate is 1.2 ml/min. The duration of the analysis is 15 min, the sterol fraction recovered according to standard NF 12228 May 1999, is evaporated to dryness. The sterols are converted to silylated derivatives (TMS) using a mixture of pyridine, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS), (9/1/1), (v/v/v). The pyridine is evaporated to dryness and the silylated derivative is diluted with 60 µl of heptane or hexane. The TMS sterols are analyzed by gas chromatography (GC) on an apolar column (Chroma pack) (30m × 0.32mm, DI: 0.25µm, phase: CPSIL8CB). The HP Hewlett Packard 6890 GC Series Chromatograph is equipped with FID detector (T°: 300°C). The carrier gas is nitrogen and its flow rateis 1 ml/min (P.E: 8.6 bar). The analysis is performed in temperature programming (200°C up to 270°C with a speed of 10°C/min and an isotherm at 270°C for 35 min).

Analysis of cis fatty acids: Reference: NF ISO 5509 COFRAC code: CC30 (Normalization, 2015).

Operating mode: The test sample of argan oil 1g is supplemented with 0.5 ml of methanolic KOH for HPLC (minimum 98%) and 10 ml of methanol in a 100 ml flask. The mixture is refluxed for 15 minutes until the solution is clear. Then 1 ml of heptane is added to the reaction mixture after cooling.

The heptanic phase containing the methyl esters is transferred to a test tube and then a solution of sodium carbonate Na_2CO_3 is added. This neutralizes all free acids by giving sodium salts with a release of carbon dioxide. The methyl esters, which are in the organic phase, are removed using a 2 ml cone pipette and placed in a test tube. The methyl esters undergo a series of washing 20 ml are taken from the esters, which are placed in a tube of nominal capacity of 2 ml and then filled with heptane. The fatty acid methyl esters are analyzed by GC gas chromatography. The HP Hewlett Packard 6890 GC Series GC chromatograph is equipped with a divider (T: 240°C) and a FID (T: 260°C) injector. The carrier gas is nitrogen (PE: 12.4 bar). The analysis is carried out in temperature programming (140°C to 200°C with a speed of 10°C / min and an isotherm at 200°C for 40 min) on a capillary column (polyethylene glycol) (30 m × 0.32 mm, DI: 0.25 µm).

Tocopherols analyses: (Lara-Ortega *et al.,* 2017).

Operating mode

In a 25 ml volumetric flask, 2 g of argan oil was diluted with 2,2,4-trimethyl pentane. The test sample is added to 2, 2, 4-trimethyl pentane up to the mark, then mixed thoroughly.

The tocopherols are analyzed by HPLC, on a silica column (25 cm × 4 mm), according to the AOCS method, official method CE8-89 revised 1990 updated 1992. The SHIMADZU brand device is equipped with a fluorimetric detector (excitation wavelength 290 nm - emission wavelength 330 nm). The elution is carried out with a mixture (isooctane/isopropanol) (99/1) with a flow rate of 1.2 ml/min during the analysis time (20 min).

Triglyceride analysis: Reference: IUPAC No. 2.0 324 (Brand *et al.,* 2014).

Operating mode: To 0.15 g of the argan oil are added 0.5 ml of hexane and 15 ml of a mixture of hexane/diethyl ether (87/13). This solution is poured into a supelco brand cartridge with 0.5 g of silica gel previously activated with

hexane. The triglyceride fraction is thus separated from the diglycerides and monoglycerides. It is recovered in a 100 ml flask. It is subjected to analysis after evaporation of the solvent and dilution with 1.5 ml of acetone. The triglycerides are analyzed by HPLC on a reverse phase C18 column (250 mm × 4.6 mm, Φ silica 5 µm), according to IUPAC Method No. 2.0324. The HPLC apparatus is equipped with an HP refractometric detector 10 47A. Elution is carried out with a mixture (acetonitrile/acetone) (v/v) with a flow rate of 0.5 ml/min during the analysis time (90 min).

Determination of the percentage of benzo-α-pyrene $C_{20}H_{12}$: Official method reference CD21-91 (Hu *et al.,* 2010).

Column preparation: Suspend 22 g of alumina in 10% water. After deposit, add 5 g of anhydrous sodium sulfate.

Sample preparation: Weigh 2 g of argan oil in a 20 ml volumetric flask. It is completed with petroleum ether with a judge line (solution S1). At the head of the column, 2 ml of the dilute solution (S1) are introduced and elution is carried out with 20 ml of petroleum ether. The latter is eliminated with a flow rate of 1 ml / min (10 drops / 15s). Then, elute with 60 ml of petroleum ether at a flow rate of 1 ml / min, in a 100 ml flask. The recovered solution is concentrated to dryness, then taken up with 2 ml of petroleum ether in a tube with a nominal capacity of 2 ml, then again concentrated to dryness.

80 µl of tetrahydrofuran (THF) are then added. The percentage of benzo-α-pyrene is obtained and determined according to the AOCS method, official method CD 21-91. revised 1992 reapproved 1993. Benzopyrene is analyzed by HPLC on a column of C18 grafted silica (25 cm × 4.6 mm). The HPLC device is equipped with a fluorimetric detector in wavelength programming (280 nm-462 nm). The elution is carried out with a mixture (acetonitrile / water) (50/50) (v / v), and acetonitrile, the flow rate of the elution is 1.2 ml / min throughout the analysis.

RESULTS

Analysis of physico-chemical characteristics

Table 2 shows the results of the acidity value, the unsaponifiable level, the saponification index, the peroxide index and the specific extinction values at 270 nm (k270). All the observed acidity values of the four samples are less than 1.40%. This result shows that argan oil is characterized by a low acidity compared to other vegetable oils (acidity of olive oil ≤ 2%) (Hilali *et al.,* 2005).

The acidity of samples 2 and 4 (0.89% and 0.85% respectively) is higher compared to other samples such as 1 and 3 (0.53%, 043% respectively) (samples 2 and 4 are plumped up by goats). These results suggest that the extraction method (pulping by goats) may influence the acidity values of argan oil.

Roasting also appears as a parameter influencing the acidity value of argan oil. Indeed, the acidity value of the samples of oils prepared from non-roasted almonds is uniformly lower than those of oils prepared from roasted almonds (example of the 3 against 1 sample). As a result, the difference in acidity observed between the samples is likely related to the technology of extracting argan oil.

The unsaponifiable rate of argan oil is less than 0.81% and the saponification index of argan oil was found between 187.2 and 197.3 (Brajol, 2014) (Table 2). The specific extinction of argan oil was determined at 270 nm. In general, the values found are higher than that of olive oil, they vary between 0.335 and 0.605 for argan oil.

The results of the peroxide index of the 4 samples of argan oil. For all samples, a peroxide index lower than that required for virgin olive oil was observed. The peroxide index of sample 2 is higher compared to sample 1 (sample 3 versus sample 4) (Figure 1). Indeed. This result clearly indicates that some components of argan oil are extremely sensitive to oxidation. The high peroxide content is observed for the samples. This is probably related to the method of extraction, hygienic conditions and extraction. The determination of the peroxide index seems to be a critical measure for the evaluation of the quality of argan oil.

Analysis of fatty acids

The fatty acid composition of the different oils was determined after methylation of the oil and analysis of the methyl esters by gas chromatography on a capillary column. Table 3 groups together the results obtained for the 4 samples.

Argan oil contains 80% unsaturated fatty acids. It is of the oleic–linoleic type and contains between 29 to 35% of essential fatty acids: linoleic acid (29 to 34%) (Vitamin F) (Hilali *et al.,* 2020). This acid is said to be essential because it cannot be synthesized by the body and must be provided by food (Rahmani, 2005).

Unsaturated fatty acids play an essential role in the prevention of cardiovascular disease and the omega 6 family (such as linoleic acid) is essential for the growth of the child (Lapillonne 2007). Its oleic acid content makes argan oil particularly interesting in regulating cholesterol.

The other fatty acids present are: myristic acid $C_{14:0}$ (0.10 to 0.15%), palmitic $C_{16:0}$ (11 to 13%) and stearic $C_{18:0}$ (5 to 7%). The percentage of linolenic acid ($C_{18:3}$) in argan oil does not exceed 0.1%. Sample 1 contains a higher percentage of linoleic acid ($C_{18:2}$) (33%) compared to Sample 2 (30%) (The oil in Sample 2 is extracted from pulped almonds by goats). We found the same result for samples 3 and 4 (Fig. 2).

Nonadecanoic acid ($C_{19:1}$) only appeared in oils extracted from almonds pulped by goats. So, we can use the percentage of nonadecanoic acid ($C_{19:1}$) as a marker to control the fruits of the argan which are pulped or not pulped by the animal. These results agree with those reported in the literature (Hilali *et al.,* 2005).

Triglyceride analysis

The triglycerides of the different argan oil samples analyzed by high performance liquid chromatography are grouped in Table 4.

Table 1: Origin and method of extraction of the four samples

Sample	Procedure	Origin
1	Roasted almond extracted by mechanical press (AOR)	Tamanar Southeast of Morocco
2	Roasted almond pulped by goats cheese and extracted by mechanical pressing (AORG)	Tamanar Southeast of Morocco
3	Unroasted almond extracted by mechanical press (AONR)	Tamanar Southeast of Morocco
4	Unroasted almond pulped by goats cheese and extracted by mechanical pressing (AONRG)	Tamanar Southeast of Morocco

Table 2: The main physicochemical constants of the 4 samples of argan oil studied

Samples	1	2	3	4
Acidity in %	0.53	0.89	0.43	0.85
Unsaponifiable rate in %	0.65	0.81	0.79	0.64
Saponification index	197.3	197.2	187.2	192.4
Peroxide index in meq of O_2 / kg	1.10	1.25	0.80	0.90
Specific extinction at 270 nm (k270).	0.368	0.605	0.355	0.391

Table 3: Fatty acid composition of samples 1 to 4 (in%)

Samples	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{19:1}$	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$
1	0.12	0.04	12.26	0.04	0.07	5.19	47.63	33.24	0.08	-	0.36	0.43	0.13
2	0.11	0.04	12.91	0.07	0.07	6.26	48.73	30.10	0.07	0,08	0.45	0.48	0.08
3	0.10	0.04	12.07	0.01	-	6.01	48.23	32.10	0.08	-	0.43	0.50	0.12
4	0.12	0.04	12.49	0.08	0.08	5.93	48.13	31.33	0.04	0,09	0.42	0.48	0.15

Table 4: Triglyceride composition of samples 1 to 4 (in%)

Samples	LLL	LLO	LLP	LOO	LOP	PPL	OOO	POO	OPP	LPS	PPP	SOO	SOP
1	7.28	13.26	5.77	15.66	13.41	2.05	14.12	16.17	4.38	0.37	-	5.11	2.24
2	6.00	16.67	4.35	14.01	13.27	1.87	12.71	15.57	3.89	0.21	-	4.48	2.21
3	7.35	13.79	6.17	16.12	13.92	1.76	14.22	16.02	3.63	0.24	0.18	4.61	1.34
4	6.47	12.71	6.10	15.19	13.94	2.17	13.39	16.02	4.40	0.42	-	4.71	2.33

LLL: trilinoleoylglycerol, LLO: linoleoyl-linoleoyl-oleoylglycerol, LLP: linoleoyl-linoleoyl-palmitoylglycerol, LOO: linoleoyl-oleoyl-oleoylglycerol, LOP: linoleoyl-oleoyl-palmitoylglycerol, PPL: palmitoyl-plamitoyl-linoleoylglycerol, OOO: trioleoylglycerol, POO: palmitoyl-oleoyl-oleoylglycerol, OPP: oleoyl-palmitoyl-palmitoylglycerol, LPS: linoleoyl-palmitoyl-stearoylglycerol, SOO: stearoyl-oleoyl-oleoylglycerol, and SOP: stearoyl-oleoylpalmitoylglycerol.

Table 5: Composition in sterols of samples 1 to 4 (mg / 100g).

Samples	Campest	Stigma 8,22	Spinast.	Schott.	Stigma 7,24	Total
1	0.28	3.37	36.96	47.20	5.04	164.5
2	0.16	4.08	36.11	46.03	4.48	186.5
3	0.18	4.67	36.17	44.65	6.54	167.1
4	0.14	3.01	38.54	47.43	4.67	216.8

Campest: Δ5-campesterol; Stigma 8.22: Stigmasta-8,22-diene-3β-ol; Spinast .: spinasterol; Schott: schottenol; Stigma 7.24: stigmasta-7.24-diene-3β-ol.

Table 6: Composition of tocopherols in samples 1 to 4 (mg / kg).

Samples	γ-tocophérol	δ-tocophérol	α-tocophérol	β-tocophérol	Total
1	562.6	47.7	29.4	-	639.7
2	619.1	50.2	29.6	-	698.9
3	681.6	53.8	34.4	1.4	771.2
4	599.3	46.4	33.0	-	678.7

Table 7: Results of benzo-α-pyrene in ppb conent in argan oil samples

Samples	1	2	3	4
Benzo-α-pyrene bin pp	0.07	0.08	0.08	0.06

Analysis of the triglyceride fraction of argan oil by HPLC allowed the separation of the individual triglycerides. We note the predominance of triglycerides, LLO, LOO, LOP, OOO and POO. It is also noted that the oleic and linoleic acids occupy most of the Sn-2 position. Our results are in agreement with data from the literature (Gharby *et al* 2013) which indicate that the triglycerides LLL, LLO, LOO, LOP, OOO and POO are predominant in argan oil. From these results we found that sample 2 which was extracted from roasted almonds and pulped by goat has a low percentage of triglycerides LLL, LOO and OOO compared to sample 1 (figure 3). The result of triglyceride agrees with the fatty acid result which indicates that the sample extracted from the almonds pulped by the goat contains a low percentage of linoleic acid ($C_{18:2}$).

Analysis of sterols

The sterol composition of the various argan oil samples was determined by gas chromatography after silylation of the sterol fraction. The latter is obtained by fractionation of the unsaponifiable of argan oil by HPLC on a normal phase. This analysis was carried out in the presence of an internal witness: 0.2% α-cholestanol in chloroform. The different sterols encountered were identified by gas chromatography coupled to mass spectrometry and by comparison with data from the literature (Gharby *et al*., 2013; Hilali *et al*., 2020) Their individual and total dosage was possible by GPC using an internal standard: α-cholestanol 0.2% in chloroform. Table 5 summarizes the results obtained for the 4 selected samples. The total sterol content of all samples of argan oil ranges from 164 to 216 mg / 100g of fat. This is not negligible compared to other seed and olive oils.

The sterolic composition is in accordance with data from the literature (Hilali *et al.*, 2007). They are essentially Δ-7-stigmasterols. The main products are schottenol (or Δ-7-stigmasterol) and spinasterol. Their proportion varies respectively between 44 and 47%, and 36 and 38%. Schottenol and spinasterol are rarely found in vegetable oils and are characteristic of this oil. Two minority sterols were identified on the basis of their mass spectrum obtained by GC / MS and by comparison with data from the literature (Hilali *et al.*, 2007). These are stigmast-8,22-diene and stigmasta-7,24-28-diene (or Δ-7- avenasterol). Their proportion varies between 3% and 6.5% of the mixture of total sterols. We found that the percentage of

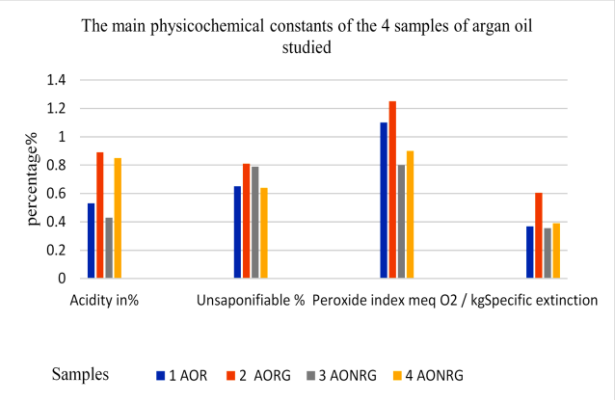

Fig. 1: The main physicochemical constants of the 4 samples of argan oil studied.

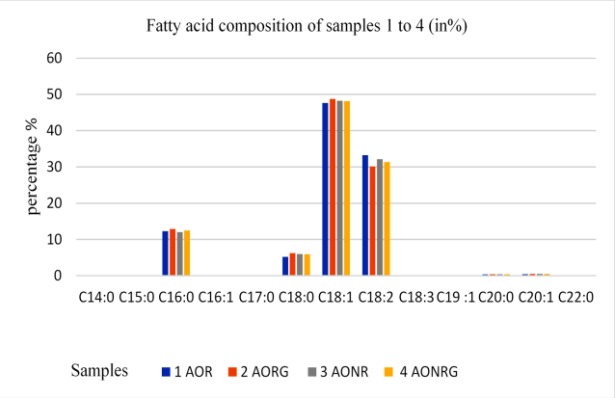

Fig. 2: Fatty acid composition of samples 1 to 4 (in%).

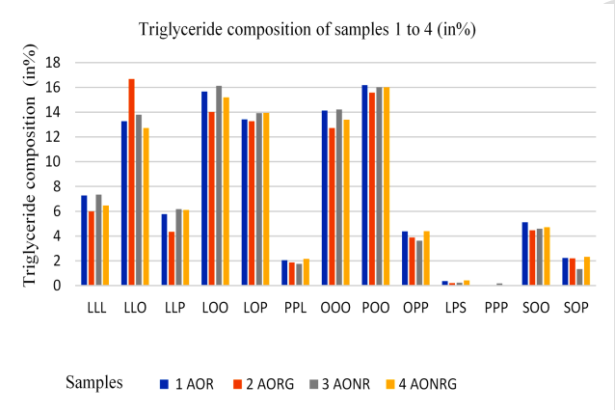

Fig. 3: Triglyceride composition of samples 1 to 4 (in%).

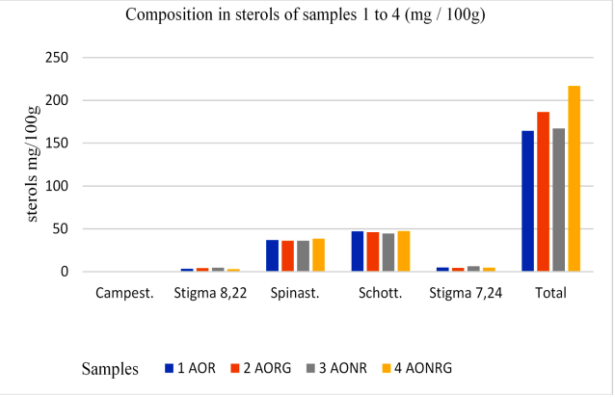

Fig. 4: Composition in sterols of samples 1 to 4 (mg / 100g).

total sterols is higher for the sample extracted from unroasted almonds (2 against 1; 4 against 3) (figure 4), so this result clearly explains that roasting is decreased total sterols.

Analysis of tocopherols

The tocopherols were analyzed by HPLC on a column in the normal phase, directly from vegetable oil without saponification. They were identified by comparison of their chromatogram with controls injected under the same conditions. Their dosage was possible by the use of α-tocopherol. The results obtained are grouped in Table 6.

Argan oil is richer in tocopherol (639 to 771 mg / kg) than olive oil (50 to 150 mg / kg). Tocopherols have vitamin E activity. This vitamin is a powerful antioxidant that captures free radicals and neutralizes destructive oxidation (Nkhili, 2009). Tocopherols are natural antioxidants; gamma tocopherol has the highest antioxidant power. Rich in gamma tocopherol, argan oil is a valuable nutraceutical.

Tocopherols (vitamin E) and polyphenols are natural antioxidants. These play an essential role in the prevention of several diseases (Giglio *et al*., 2018), because they are anti-free radicals. B-tocopherol has been detected as a trace in argan oil. No major significant variation was observed between samples. This demonstrated that roasting and pulping by the goat cannot influence the tocopherol compositions.

Determination of the percentage of benzo-α-pyrene

Roasting almonds from the argan tree could produce benzo-α-pyrene (Table 7) (Muyela *et al*., 2012), which is a carcinogenic chemical (Tryndyak *et al*., 2018). This is why we have studied the benzo-α-pyrene content in a few samples in order to assess the influence of the roasting step on the chemical composition in the oil.

For this analysis, we compared two oils produced from unroasted almonds (sample 3 and 4) and oil produced from roasted almonds (sample 1 and 2). Similar concentrations of benzo-α-pyrene were found for sample 1, 2, 3 and 4 (0.06±0.02 ppb, however below the 2-ppb regulation) suggesting that roasting does not produce significant amounts of benzo-α-pyrene.

DISCUSSION

As part of the development of argan oil, we conducted a comparative study of the different physico-chemical parameters of argan oil according to its mode of extraction. To carry out this work, we selected 4 samples of argan fruit located in the Tamanar region of Essaouira province in southeastern Morocco and extracted in different ways (by mechanical pressing but from pulped argan fruit or not plumped by goats). The study of the physicochemical characteristics shows that all the acidity values of argan oils are less than 1%. Present study shows that roasting and pulping by goats appears as a parameter influencing the acid value of argan oil. In fact, the acidity value is higher in the argan oil samples prepared from roasted almonds and pulped by goats. This result suggests that the extraction method may influence the acidity values. The results concerning the analysis of the saponification index and the unsaponifiable level of argan oil show no significant

significant variation between the samples. Analysis of the peroxide index shows that the samples of argan oil extracted from roasted almonds and which those pulped by goats have a higher peroxide content compared to the other samples. The determination of the peroxide index seems to be a critical measure for the evaluation of the quality of argan oil. Analysis of fatty acids shows that argan oil contains 80% unsaturated fatty acids. It is of the oleic – linoleic type and contains between 29 to 35% of essential fatty acids: linoleic acid (29 to 34%) (Vitamin F). Its oleic acid content makes this oil particularly interesting in regulating cholesterol. The fatty acid composition shows that pulping by goats can decrease the percentage of linoleic acid ($C_{18:2}$) (Hilali *et al*., 2020). Sterol analysis shows that the total sterol levels of argan oil vary between 164 to 216 mg / 100g of fat. (Olive 98-184, hazelnut 75-195). The sterolic composition is in accordance with the data in the literature. They are essentially Δ-7-stigmasterols. The main products are schottenol (or Δ-7-stigmasterol) and spinasterol. Their proportions vary respectively between 44 to 47% and between 36 to 38%. It is noted that schottenol and spinasterol, which are very rare in vegetable oils, can be a parameter for the detection of adulteration of this oil. Two minority sterols have been identified in argan oil. These are stigmast-8,22-diene and stigmasta-7,24-28-diene (or Δ-7- avenasterol), their proportion varies between 3% and 6.5%. From the sterol result we found that the percentage of total sterols is higher for the sample extracted from the unroasted almonds (2 against 1; 4 against 3), so this result clearly shows that the roast is poor total sterols.

Argan oil is richer in tocopherols (639 to 771 mg / kg) than olive oil (50 to 150 mg / kg) and hazelnut oil (300 to 550 mg / kg). B-tocopherol has been detected as a trace in argan oil (Haloui *et al*., 2008). The tocopherol result shows no significant significant variation between samples. This demonstrated that roasting and pulping by the goat cannot influence the tocopherol compositions. Analysis of the triglyceride fraction of argan oil allowed the separation of individual triglycerides. We note the predominance of triglycerides LLO, LOO, LOP, OOO and POO in argan oil. These triglycerides represent approximately 73% of each fraction of triglycerides in argan oil (El Abbassi *et al*., 2014). The triglyceride result shows that roasting and pulping by goats can decrease the percentage of LLL, LOO and OOO triglycerides in argan oil. Study of the concentration of benzo-α-pyrene suggests that roasting does not produce significant amounts of benzo-α-pyrene. Our study has shown that the mode of extraction can influence the chemical composition of the oil.

Conclusions

This study shows that roasting almonds from the argan fruit and pulping by goats appear as a parameter can increase the acidity value and the peroxide index, because the present work shows that the value of acidity of samples 2 and 4 (0.89% and 0.85% respectively) is higher compared to other samples such as 1 and 3 (0.53%, 043% respectively) (samples 2 and 4 are plumped up by goats). The peroxide index of sample 2 (1,25 meq of O_2 / kg) is higher compared to sample 1 (1,10 meq of O_2 / kg) (sample 3 versus sample 4). Analysis of fatty acids and triglycerides show that pulping by goats can decrease the percentage of linoleic acid ($C_{18:2}$) (sample 2 (30,10%) against sample 1 (33,24%)) and the percentage of LLL, LOO and OOO triglycerides in argan oil. From the sterol result we found that the percentage of total sterols is higher in the sample extracted from the unroasted almonds (sample 2 (186,5 mg/100g) against 1 (164,5 mg/100g); sample 4 (216,8 mg/100g) against sample 3 (176,1 mg/100g)), so this result clearly shows that the roasting is decreased total sterols. Study of the concentration of benzo-α-pyrene suggests that roasting does not produce significant amounts of benzo-α-pyrene. Our study has shown that roasting and pulping of argan fruit by goats can influence the chemical composition of the argan oil, and the result of this work can enhance the argan products for in the end this work can support the marketing of argan oil worldwide and preservation.

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REFERENCES

Aïssi VM, Soumanou MM, Tchobo FP *et al*., 2009. Etude comparative de la qualité des huiles végétales alimentaires raffinées en usage au Bénin. Bulletin d'Informations de la Société Ouest Africaine de Chimie, 06: 25-37.

Barjol JL, 2014. L'économie mondiale de l'huile d'olive. OCL, 21: 502.

Bennani H, Drissi A, Giton F, *et al*., 2007. Antiproliferative effect of polyphenols and sterols of virgin argan oil on human prostate cancer cell lines. Cancer Detec Prev, 31: 64-69.

Bradford PG and Awad AB, 2007. Phytosterols as anticancer compounds. Mol Nutr Food Res, 51: 161-170.

Brand WA, Coplen TB, Vogl J, *et al*., 2014. Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report). Pure Appl Chem, 86: 425-467.

Charrouf Z and Guillaume D, 2014. Argan oil, the 35-years-of-research product. Eur J Lipid Sci Technol, 116: 1316-1321.

Charrouf Z and Guillaume D, 2018. The argan oil project: going from utopia to reality in 20 years. OCL, 25: 209.

Drissi A, Bennani H, Giton F, *et al*., 2006. Tocopherols and saponins derived from Argania spinosa exert an antiproliferative effect on human prostate cancer. Cancer Invest, 24: 588-592.

El Abbassi A, Khalid N, Zbakh H *et al*., 2014. Physicochemical characteristics, nutritional properties, and health benefits of argan oil: a review. Crit Rev Food Sci Nutr, 54: 1401-1414.

Européenne, Norme, 1999. Corps gras d'origines animale et végétale-Détermination de l'indice d'acide et de l'acidité. Norme Française NF EN ISO, 660: 60-204.

Gharby S, Harhar H, Kartah BE, *et al*., 2013. Can fruit-form be a marker for argan oil production? Nat Prod Comm, 8: 1.

Giglio RV, Patti AM, Cicero AF, *et al*., 2018. Polyphenols: Potential use in the prevention and treatment of cardiovascular diseases. Current Pharm Design, 24: 239-258.

Haloui RB, Zekhnini A and Hatimi A, 2015. Effects of extraction methods on chemical composition and oxidative stability of Argan oil. J Chem Pharmac Res, 7: 518-524.

Haloui RB, Zekhnini A and Hatimi A, 2008. Comparative study on fatty acid and tocopherol composition in argan oils extracted from fruits of different forms, Acta Botanica Gallica, 155: 301-305.

Hilali M, El Monfalouti H and Kartah BE, 2020. Evaluation of the chemical composition of Argan (*Argania spinosa* L.) oil according to its extraction method. Online J Anim Feed Res, 10: 111-118.

Hilali M, Charrouf Z, Soulhi AEA, *et al*., 2007. Detection of argan oil adulteration using quantitative campesterol GC analysis. J Am Oil Chem Soc, 84: 761-764.

Hilali M, Charrouf Z, Soulhi AE, *et al*., 2005. Influence of origin and extraction method on argan oil physico-chemical characteristics and composition. J Agric Food Chem, 53: 2081-2087.

Hu QJ, Shen LW, Qi M, *et al*., 2010. Determination of 3, 4-benzo (a) Pyrene in meatproducts by high performance liquid chromatography. Food Res Develop, 2010: 6.

ISO, NFEN, 6320. 2000.Corps gras d'origine animale et végétale–Détermination de l'indice de réfraction, Morroco.

Khallouki F, Eddouks M, Mourad A, *et al*., 2017. Ethnobotanic, ethnopharmacologic aspects and new phytochemical insights into Moroccan Argan Fruits. Int J Mol Sci, 11: 2277.

Lagardere L, Lechat H and Lacoste F, 2004. Détermination de l'acidité et de l'indice de peroxyde dans les huiles d'olive vierges et dans les huiles raffinées par spectrométrie proche infrarouge à transformée de Fourier. Oléagineux, Corps Gras, Lipides, 11: 70-75.

Lara-Ortega FJ, Gilbert-López B, García-Reyes JF, *et al*., 2017. Fast automated determination of total tocopherol content in virgin olive oil using a single multicommuted luminescent flow method. Food Analy Meth, 10: 2125-2131.

Muyela B, Shitandi A and Ngure R, 2012. Determination of benzo [a] pyrene. Levels in smoked and oil fried Lates niloticus. Int Food Res J, 19: 4.

Nkhili EZ, 2009. Polyphénols de l'Alimentation: Extraction, Interactions avec les ions du Fer et du Cuivre, Oxydation et Pouvoir antioxydant. Université Cadi Ayyad-Marrakech. Avialable online: https://www6.paca.inra.fr/sqpov/content/download/3329/33139/version/1/file/Th%C3%A8se%20Nkhili%20Ezzohra%202009.pdf [in french].

Normalisation, Organisation Internationale, *et al.*, 2015. Corps gras d'origines animale et végétale. Détermination des esters de chloropropanediols (MCPD) et d'acides gras et des esters de glycidol et d'acides gras par CPG/SM. Pt. 1: Méthode par transestérification alcaline rapide et mesure pour le 3-MCPD et par mesure différentielle pour le glycidol. http://agris.fao.org/agris-search/search.do?recordID=XF 2016002599.

Normalisation OI, 2002. Corps gras d'origines animale et vegetale. Determination de l'alcalinite. http://agris.fao.org/agris-search/search.do?recordID=XF 2015039826.

Rahmani M, 2005. Composition chimique de l'huile d'argan «vierge». Cahiers Agric, 14: 461-465.

Sahu S, Ghosh M and Bhattacharyya DK, 2018. Isolation of the unsaponifiable matter (squalene, phytosterols, tocopherols, γ-oryzanol and fatty alcohols) from a fatty acid distillate of rice bran oil. Grasas y Aceites, 69: 262.

Tryndyak V, Kindrat I, Dreval K, *et al*., 2018. Effect of aflatoxin B1, benzo [a] pyrene, and methapyrilene on transcriptomic and epigenetic alterations in human liver HepaRG cells. Food Chem Toxicol, 121: 214-223.