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Colorimetric Evaluation of Phenolic Content and GC-MS Characterization of Phenolic Composition of Alimentary and Cosmetic Argan Oil and Press Cake

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The global phenolic content of argan oil and press cake samples (alimentary and cosmetic) was evaluated using the Folin−Ciocalteu colorimetric method and the phenolic composition of argan oil (alimentary and cosmetic) and press cake (alimentary) samples were analyzed by GC-MS after extraction with 80:20 (v/v) methanol:water and silylation. Identification of chromatographic peaks was made by mass selective detection. Nineteen simple phenols were detected, 16 in press cake, 6 in the alimentary oil, and 7 in the cosmetic oil, among which 15 compounds [3-hydroxypyridine (3-pyridinol), 6-methyl-3-hydroxypyridine, catechol, resorcinol, 4-hydroxybenzyl alcohol, vanillin, 4-hydroxyphenylacetic acid, vanillyl alcohol, 3,4-dihydroxybenzyl alcohol, 4-hydroxy-3-methoxyphenethyl alcohol, methyl 3,4-dihydroxybenzoate, hydroxytyrosol, protocatechuic acid, epicatechin, and catechin] were identified for the first time in such materials.

KEYWORDS: Argan oils; *Argania spinosa*; plant phenols; GC-MS

INTRODUCTION

Argania spinosa (L.) Skeels (also known as *Sideroxylon spinosum* L.) belongs to the Sapotaceae family and is endemic to southwestern Morocco, where it grows over about 320 000 square miles (*1*). The argan tree has had an essential ecological function for centuries. It protects the soil against heavy rain or wind-induced erosion and maintains soil fertility. The tree plays a crucial role in the rural and urban economy. The fruits contain almonds that are used to prepare an edible oil, and the leaves are utilized to feed cattle. In addition to its alimentary utilization, the oil is also traditionally used as a cosmetic to treat light skin damage (*1*). It is also used in rheumatology and prescribed against various pathologies because of the antioxidant, hepatoprotective, and anticholesterolemic activities supposedly attributed to some of its constituents (*1*−*5*). A program aimed at increasing the industrial value of *A. spinosa* is currently being carried out in Morocco (*2*).

Triglycerides constitute about 99% of the oil material. The major triglycerides include three oleic acid residues (O,O,O); two oleic and one linoleic (O,O,L); two linoleic and one oleic (L,L,O); one palmitic, one oleic, and one linoleic (P,O,L); or one palmitic and two oleic (P,O,O) acid residues (*6*, *7*). Unsaponifiable matter contains carotenes (37%), tocopherols (8%), triterpene alcohols (20%), sterols (29%), and xantophyls (5%) (*9*). The argan oil is about twice richer in tocopherol than olive oil (*8*) (620 mg/kg vs 314 mg/kg) with the following distribution: α-tocopherol (35−46 mg/kg), δ-tocopherol (111−122 mg/kg), and γ-tocopherol (480−504 mg/kg) (*4*). The presence of these antioxidant tocopherols in relatively significant quantities is probably responsible for the good preservation qualities of argan oil (*4*, *5*). Several additional triterpenoid alcohols have also been isolated from the unsaponifiable matter. These are butyrospermol, tirucallol, β-amyrin, lupeol, 24-methylene cycloartanol, citrostadienol, and cycloeucalenol (*9*). Four sterols have been found in argan oil (*9*) with the following relative distribution: schottenol (48%), spinasterol (44%), stigmasterol (4%), and Δ^7-avenasterol (4%). The phenolic compounds isolated from an argan oil collected in Ouled Taïma (Agadir region) and extracted using hexane were caffeic acid and oleuropein (*10*, *11*), but the compounds isolated from an argan oil extracted using unprecise oil/water separation technique and collected in Tamanar (Essaouira region) were 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, vanillic acid, syringic acid, 4-*O*-glycosylated ferulic acid, and tyrosol (*4*).

The press cake can also be recycled as cattle food due to its high energetic value. It is mainly composed of glucides, proteins,

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Table 1. Collection Time (h) and Quantity (g) of Alimentary Oils (CK), Cosmetic Oils (CO), Traditional Oils (TO) (Isolated after Different Hours of the Press Process), Alimentary Oil Filtrates (FCK), and Cosmetic Oil Filtrates (FCO) from *Argania spinosa*

oil sample designation	collection time	quantity of oil
CK-1	1:00	15.7454
CK-2	7:00	14.8022
CK-3	9:30	14.9301
CK-4	10:00	15.7509
FCK-1	1:00	15.0113
CO-1	1:00	15.1582
CO-2	7:00	16.0526
CO-3	9:30	15.6491
CO-4	10:00	15.4991
FCO-1	1:00	15.7625
TO-1	2:00	15.6491
TO-2	2:00	15.1582

Table 2. Quantity (g) of Alimentary (TCK), Cosmetic (TCO), and Traditional (TTO) Press Cakes of *Argania spinosa,* Directly Isolated (Humid) and Lyophilized (Dry)

sample designation	press cake	quantity of press cake (humid)	quantity of press cake (dry)
TCK-1	alimentary	18.025	17.754
TCO-2	cosmetic	18.236	17.853
TTO-1	traditional	18.860	15.960
TTO-2	traditional	17.625	14.945

and saponins. Seven saponins have been isolated from the aqueous alcoholic extract, including five new ones, arganine A, B, and D−F, and two known ones, arganine C, previously isolated from *Crossopteryx febrifuga* (*12*), and misaponin A, isolated from *Madhuca longifolia* (L.) Macbride (Sapotaceae) (*13*). All these saponins are bidesmosidic, and their aglycone is either protobassic acid or 16-α-hydroxyprotobassic acid, both belonging to the Δ-12 oleanane family (*14*). Recently published work (*15*) has given data on the influence of geography and the extraction method over the chemical composition of argan oils. Whereas results for acid and saponification values, the composition of sterols, and the fatty acid content are given, no data are presented for the phenolic composition. The objective of this study was to evaluate quantitatively the phenolic content in argan oils produced from different process types (i.e., cosmetic, alimentary, and traditional) and in the corresponding press cakes. An exhaustive qualitative and quantitative GC-MS-based characterization of the phenolic composition was carried out on selected samples.

MATERIALS AND METHODS

Plant Material. All oils and press cakes of *Argania spinosa* (L.) Skeels were collected from Tamanar (Essaouira region) in Morocco. The plant material was identified by Z.C. Argan fruits were harvested in the summer of 2001. Traditional argan oil was prepared per the methods of Guillaume (*1*). The cosmetic and alimentary argan oils were prepared from unroasted and roasted argan seeds, respectively, using a vegetable oil expeller according to the method of Hilali et al. (*15*). The cosmetic and alimentary oils and the alimentary press cake used for both quantitative colorimetric evaluation and identification of phenolic composition, as well as the 12 oil samples (5 cosmetic, 5 alimentary, and 2 traditional) taken at different times of the extraction process (i.e., continuous pressure process) and the 4 press cake samples (1 alimentary, 1 cosmetic, and 2 traditional) used for the colorimetric evaluation of the total phenolics were donated by Z.C. (see **Tables 1** and **2**).

Extraction of Phenolic Compounds from Oils. The extraction procedure described by Montedoro et al. (*16*) was used: the extraction of phenolic compounds from cosmetic (152 g) and alimentary (147.9 g) oils were performed by adding 30 mL of CH_3OH-H_2O (80:20) and hexane (30 mL), mixing with vortex agitation for 2 min, and further centrifuging at 5000*g* for 10 min. This extraction with hexane was repeated three times. The two phases were separated by removing the methanolic layer with a Pasteur pipet. The methanolic layer was then concentrated under vacuum at 30 °C until it reached a syrupy consistency. This layer was dissolved in acetonitrile (10 mL) and washed twice with hexane (20 mL), and the resulting syrup was concentrated under vacuum at 38 °C to give a residue that was dissolved in MeOH (1 mL). This methanolic solution was submitted to chromatographic and spectroscopic analyses and evaluation of total phenols.

Extraction of Phenolic Compounds from the Press Cake. The extraction of phenolic compounds from the press cake (303.9 g) was performed by using 200 mL of $MeOH-H_2O$ (80:20) and vigorous shaking for 5 h, after which time the material was filtrated under vacuum and the aqueous methanolic extract was concentrated under vacuum at 38 °C until it reached a syrupy consistency; this syrup was then subjected to freeze-drying to furnish a residue of 51.57 g. A portion (2 g) was added to 15 mL of $MeOH-H_2O$ (80:20) and washed three times with hexane (10 mL), and the aqueous methanolic layer was concentrated under vacuum at 38 °C until it reached a syrupy consistency. This syrup was dissolved in water (10 mL) and extracted three times with butanol (10 mL). The aqueous layer was evaporated under vacuum at 38 °C, and the residue was dissolved in methanol (4 mL) and filtrated. The filtrate was submitted to chromatographic and spectroscopic analyses and evaluation of total phenols.

Reference Compounds. Standard compounds were obtained from Sigma-Aldrich: benzoic acid, catechol, vanillin, *trans*-cinnamic acid, 2,6-di-*tert*-butylphenol, tyrosol, 4-hydroxyphenyl acetic acid, vanillic acid, vanillyl alcohol, 4-hydroxy-3-methoxyphenethyl alcohol, syringic acid, protocatechuic acid, catechin, epicatechin, *p*-hydroxy benzoic acid; from Acros Organics: 3-hydroxypyridine; from Lancaster: resorcinol, methyl 3,4-dihydroxybenzoate, 6-methyl-3-hydroxy pyridine; from Fluka: 4-hydroxybenzyl alcohol. Hydroxytyrosol was prepared in our laboratory according to a new method we recently developed for large-scale production of this powerful antioxidant (*17*), and the 3,4-dihydroxybenzyl alcohol was obtained by reduction of 3,4-dihydroxybenzaldehyde with $NaBH_4$ in MeOH. δ-Tocopherol and γ-tocopherol were kindly supplied by the Institut des Corps Gras (ITERG, France).

Colorimetric Evaluation of Total Phenols. The concentration of total phenols in the methanolic extract solutions was estimated with the Folin−Ciocalteu reagent using gallic acid as a calibration standard (*16*). The procedure consisted of diluting an aliquot of the extract solution (0.1 mL) in water (5 mL) followed by the addition of the Folin−Ciocalteu reagent (0.5 mL). After 3 min, 1 mL of an aqueous Na_2CO_3 solution (35 g/L) was added, mixed with a vortex, and allowed to stand at room temperature for 1 h. Absorbance was measured after 1 h at 725 nm against a blank.

Trimethylsilylation of Phenols. An aliquot of the methanolic extract solution (0.8 mL) was evaporated until dryness under vacuum at 38 °C, and the residue was derivatized by adding *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide) (BSTFA Supelco, 150 μL) at room temperature for 30 min.

Solubility Control of Catechin and Epicatechin in Butanol−H_2O. Epicatechin (2 mg) and catechin (1 mg) were dissolved in 4 mL of H_2O and extracted three times with 2 mL of butanol. Both butanolic solutions were evaporated under vacuum at 38 °C. The residues were analyzed by high-performance liquid chromatography (HPLC).

High-Performance Liquid Chromatography. The HPLC system consisted of a SpectraSystem set composed of an AS3000 autosampler, a SCM1000 pump, and a 996 photodiode array detector operated with the Chromquest software and using a C18 Beckman column (250 mm × 4.6 mm i.d., 5 μm). The optimum efficiency of separation was achieved with a mobile phase composed of a mixture of solvents A and B [A, H_2O-HCO_2H (95:5); B, $MeOH-HCO_2H$ (95:5)] utilizing the following linear gradient over a total run time of 55 min: 0−20% solvent B in 20 min, 20−100% solvent B in 25 min, 100% solvent B maintained until completion of the run (i.e., 10 min), and linearly returning to 0% solvent B in 4 min. Other parameters adopted were as

follows: injection volume, 10 μL; column temperature, ambient; flow rate, 1.0 mL/min; detection wavelength, 280 nm.

GC-MS Identification and Quantification. Analysis was carried out on a Finnigan Trace GC Ultra equipped with an on-line injection system and a mass-selective detector Finnigan Trace DSQ. A capillary column CP-Sil-8 CB (30 m length; 0.25 mm i.d.; 0.25 μm film thickness) was used. The carrier gas was helium. Injector and detector temperatures were set at 250 °C. The oven temperature program was as follows: from 70 to 135 °C at 2 °C/min, for 10 min at 135 °C, from 135 to 220 °C at 4 °C/min, for 10 min at 220 °C, from 220 to 270 °C at 3.5 °C/min, and for 20 min at 270 °C; the transfer line temperature was held at 300 °C. An aliquot of 2.0 μL of the silylated extract solution in BSTFA was injected. Identification of phenolic compounds was carried out by comparison of their retention time and MS spectra with those obtained from the corresponding standards and data from the NIST MS library 2.0. The quantification was expressed in milligrams per kilogram and was determined against calibration curves established using vanillic acid and syringic acid in the 0−14 ppm range.

RESULTS AND DISCUSION

The detection of phenolic compounds in argan oils and related press cakes can be considered as an analysis challenge because of their very low levels that preclude their detection by LC-MS in the methanolic extracts using the methodologies developed for such compounds in olive oil (*19*). Extracted phenolic compounds were silylated prior to GC-MS analysis, as suggested by Khallouki et al (*4*). All resulting chromatograms (alimentary oil, cosmetic oil, and press cakes) were very complex due to the presence of a large number of peaks (**Figure 1**). Among all the peaks, phenolic compounds were among the lowest ones in intensity. The only phenolic compounds found in argan oils by Chimi et al. (*10*) were caffeic acid and oleuropeine. Khallouki et al. (*4*) reported the presence of 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, vanillic acid, syringic acid, tyrosol, and ferulic acid under its 4-*O*-glycosylated form. In this study, 16 phenolic compounds were identified in press cake, 6 in the alimentary oil, and 7 in the cosmetic oil (**Table 3**). Benzoic and cinnamic acids were the only nonphenolic aromatic compounds detected. The tocopherols found in both the alimentary and cosmetic oil samples (2.4 and 0.1 mg/kg, respectively) are in fact residual amounts that have escaped elimination by the hexane extraction. The observation of the prevalence of γ-tocopherol over δ-tocopherol (2.2 vs 0.2 mg/kg in argan oil samples) is in agreement with literature precedents (*4*, *5*−*16*). Among the phenols identified by Khallouki et al. (*4*), only vanillic acid and tyrosol were present in the phenolic extracts of the cosmetic oil and press cake, while tyrosol was the only one in the alimentary oil. Surprisingly, none of the phenols reported by Chimi et al. (*10*) were identified in our samples. These analytical variations are attributed to the significant dependence of argan oil composition on the collection sites [Agadir region (*10*) vs Essaouira region (*4*)] and on the extraction technique used, although very few details were given in the protocol followed [hexane extraction (*10*) and mechanical extraction (*4*)].

Catechin and epicatechin were identified both in press cake and in the cosmetic oil, epicatechin being the most abundant phenol in press cake (110 mg/kg). Regarding the solubility of these two molecules, one could have thought that they would have been extracted by butanol. However, HPLC analysis of the butanol extract showed a total absence of catechin and epicatechin. We thus performed a test of partitionning on pure catechin and epicatechin between water and butanol. Only two-thirds of the initial amounts of both flavanols were extracted by butanol. The fact that these flavanols remained entirely in the aqueous extract of the press cake could be due to a saturation of the butanol layer with protein material and saponins (*14*).

The samples analyzed by GC-MS were also evaluated for their total phenolic content using the Folin−Ciocalteu reagent. The results are shown in **Table 4**. The concentrations found in press cake and the alimentary oil are approximately twice the level of the total concentration of individual phenolic compounds as determined by GC-MS analysis (**Table 3**), whereas it is an order of magnitude higher for the cosmetic oil. This difference is simply due to the fact that the Folin−Ciocalteu method quantifies all phenolic compounds, whereas GC analysis only takes into account low molecular mass and volatile compounds. Furthermore, other factors such as a different response factor for each compound, partial silylation, and coelution with other compounds, especially in the chromatographic zones 2−6 (**Figure 1**) could also introduce some discrepancies between the two methods. The alimentary and cosmetics oils obtained at different times during the pressure process (hours) were then analyzed with the Folin−Ciocalteu reagent. The results showed that the phenolic concentration regularly decreased for the cosmetic and alimentary oil grade (**Table 5**). This trend can find a simple explanation in the fact that the pressed oils are not filtered. Therefore, the amount of residual press cake, richer in phenolic compounds and remaining in suspension in the oil, will decrease over time of application of the pressure, consequently also the amount of phenols. The oils analyzed by GC-MS showed Folin−Ciocalteu values (**Table 4**) similar to those of the second oil sample set (**Table 5**), the cosmetic oils showing the smallest concentrations. In any event, this study confirms that argan oils contain low concentrations of phenolic compounds, whatever the geographical origin and the extraction process used. However, these concentrations are enhanced when the argan nuts are roasted prior pressing, as it can be noticed from our results on the alimentary oil analysis (**Table 5**). Press cake samples all retained much larger quantities of phenolic compounds. **Table 6** shows concentrations varying from about 25 mg/kg for a press cake obtained after cosmetic oil extraction up to 286 mg/kg for a press cake obtained after traditional oil extraction (*1*). These values are fairly low as compared to those obtained with the different press cake samples also used for the GC-MS analysis (up to 482.6 mg/kg, **Table 4**). The smallest value of total phenolics (i.e. 25.9 mg/kg, **Table 6**) is for a cosmetic press cake sample that was particularly dark in color, probably an indication of some significant oxidative degradation.

In conclusion, this analytical study of different argan oil samples (traditional, cosmetic, and alimentary grades) and their corresponding press cakes showed that their content of phenolic compounds is relatively low as determined by GC-MS analysis of their silylated methanolic extracts and evaluation of their total phenolic content by the Folin−Ciocalteu method. Furthermore, the argan oil phenolic composition appeared to be highly dependent on technical extraction factors, as well as handling and storage factors, as alluded to from the apparent oxidative degradation that some samples underwent, with variations observed both qualitatively and quantitatively. Differences between results of this work and those in the literature can also be attributed to the influence of geographical location of argan tree, as it has been recently demonstrated by Charrouf and coworkers (*15*).

The influence of the extraction process used can be clearly pointed out not only from the analysis of the oil samples but also from their corresponding press cakes at different pressure times. The press cakes retained the highest amount of phenolics,

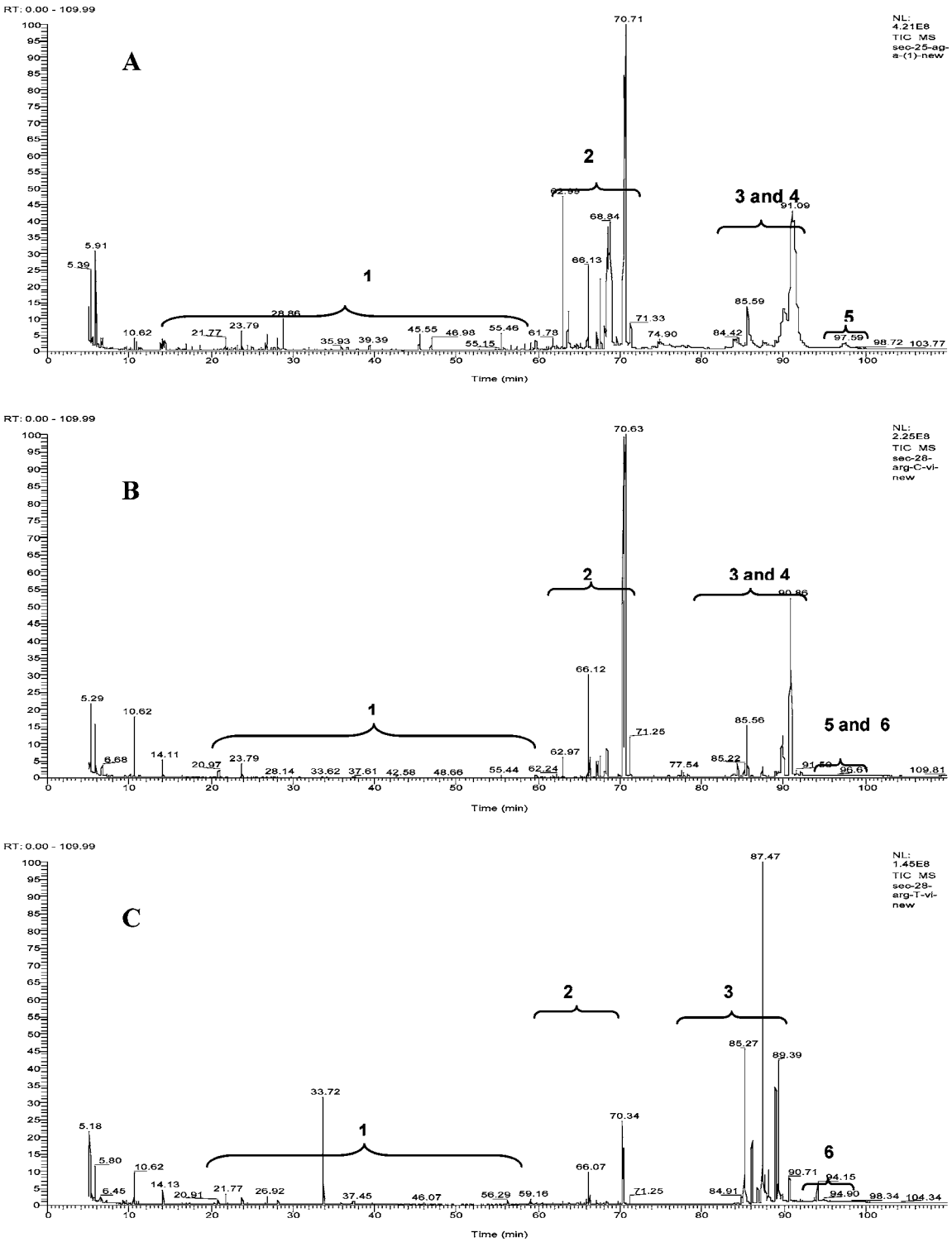

Figure 1. GC-MS chromatograms: A, alimentary oil; B, cosmetic oil; C, press cake from *Argania spinosa*. Legend: 1, phenolic TMS derivatives zone; 2, fatty acid ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$) TMS derivatives and monosaccharides zone; 3, disaccharide TMS derivatives; 4, monoglyceride TMS derivatives zone; 5, tocopherol TMS derivatives zone; 6, flavonoid TMS derivatives zone.

as evidenced in this work for the first time. Their concentration of phenolics is however not as high (i.e., from about 25 to 483 mg/kg by the weight of the dried material) as that found in olive oil press cakes in which it can reach up to 1 wt % of the dried material (*20*). This study only constitutes a preliminary assessment of the complete phenolic profile of argan oils and press cakes, and the general observations made here will need to be firmly established from the analysis of a larger and more diverse collection of samples. Nevertheless, it should be pointed out that 9 phenols (i.e., 3-hydroxypyridine (3-pyridinol), 6-methyl-3-hydroxypyridine, catechol, resorcinol, 4-hydroxybenzyl alcohol, vanillyl alcohol, 4-hydroxy-3-methoxyphenethyl alcohol, epicatechin, and catechin) out of the 11different phenols detected here by GC-MS analysis were identified in alimentary and cosmetic argan oils for the first time. The analysis of the press cake revealed 16 phenols, among which 6 new ones not present

Table 3. Concentration (mg/kg of the TMS Derivatives) of the Compounds (Name and Molecular Weight) Identified (Retention Times, min) in Cosmetic Oil, Alimentary Oil, and Press Cake

compound name	retention time	MW	press cake alimentary	alimentary oil	cosmetic oil
3-hydroxypyridine (3-pyridinol)	13.88	167		0.9	
6-methyl-3-hydroxypyridine	17.63	181		0.8	
catechol	25.26	254	1.4	0.3	<0.1
resorcinol	30.83	254	1.3	<0.1	
4-hydroxy benzyl alcohol	37.22	268	8.6	<0.1	
vanillin	38.41	224	1.1		
tyrosol	41.49	282	6.2	0.1	<0.1
p-hydroxybenzoic acid	46.07	282	14.1		
(4-hydroxyphenyl)acetic acid	47.39	296	1.0		
vanillyl alcohol	47.8	298	3.6		<0.1
3,4-dihydroxy benzyl alcohol	53.08	356	0.9		
4-hydroxy-3-methoxyphenethyl alcohol	53.26	312			<0.1
methyl 3,4-dihydroxybenzoate	53.81	312	1.6		
vanillic acid	56.29	312	16.3		<0.1
hydroxytyrosol	56.65	370	0.9		
protocatechuic acid	59.03	370	15.2		
syringic acid	61.8	342	6.6		
epicatechin	94.15	650	110.1		0.2
catechin	94.9	650	11.0		0.1
TOTAL			201.2	2.3	0.3

Table 4. Content of Total Phenolic in Oils and Press Cake from the *Argania spinosa* (Expressed as Milligrams of Gallic Acid per Kilogram of Argan Oil or Press Cake)

samples	total phenolic content
press cake alimentary	482.6
alimentary oil	13.2
cosmetic oil	3.1

Table 5. Content of Total Phenolics in Alimentary (CK), Cosmetic (CO), Traditional (TO), and Filtrate (FCK, FCO) Oils from *Argania spinosa* (Expressed as Milligrams of Gallic Acid per Kilogram of Oil)

sample designation	oil grade	collection time	total phenolic content
CK-1	alimentary	1:00	14.1
CK-2	alimentary	7:00	13.8
CK-3	alimentary	9:30	12.2
CK-4	alimentary	10:00	12.1
FCK-1	alimentary (filter)	1:00	12.0
CO-1	cosmetic	1:00	4.4
CO-2	cosmetic	7:00	3.8
CO-3	cosmetic	9:30	1.3
CO-4	cosmetic	10:00	1.5
FCO-1	cosmetic (filter)	1:00	0.8
TO-1	traditional	2:00	11.8
TO-2	traditional	2:00	23.1

Table 6. Content of Total Phenolics in Press Cake (TCK Alimentary, TCO Cosmetic, TTO Traditional) from the *Argania Spinosa.* (Expressed as Milligrams of Gallic Acid per Kilogram of Press Cake)

sample designation	press cake grade	total phenolic content (humid press cake)	total phenolic content (dry press cake)
TCK-1	alimentary	173.4	176.0
TCO-2	cosmetic	25.4	25.9
TTO-1	traditional	127.2	150.4
TTO-2	traditional	243.0	286.6

in oils were identified (vanillin, 4-hydroxyphenylacetic acid, 3,4-dihydroxybenzyl alcohol, methyl 3,4-dihydroxybenzoate, hydroxytyrosol, and protocatechuic acid).

Finally, the higher amount of phenolics found in press cake as compared to that present in the oils may contribute to the overall nutritional quality of such a byproduct used in animal feed and may raise additional interests in the development of novel applications of argan oil press cakes in cosmetic and nutraceutic products.

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