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Separation and characterization of phenolic compounds in argan fruit pulp using liquid chromatography–negative electrospray ionization tandem mass spectroscopy

Zoubida Charrouf [a,*], Miloudi Hilali [a], Olga Jauregui [b], Mohamed Soufiaoui [a], Dominique Guillaume [c]

[a] *Laboratoire de Chimie des Plantes et de Synthèse Organique et Bioorganique, Faculté des Sciences, Université Mohamed V-Agdal, BP 1014 RP, Rabat, Morocco*
[b] *Scientific and Technical Services, University of Barcelona, Josep Samitier 1-5, Barcelona 08028, Spain*
[c] *FRE2715, 51 rue Cognacg Jay, Reims 51100, France*

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Abstract

Liquid chromatography (LC) coupled to negative electrospray ionization (ESI) tandem mass spectrometry (MS/MS) was used for the sensitive identification of phenolic compounds in argan fruit pulp. Sixteen compounds were identified, mainly flavonoid glycosides and flavonoid aglycons.

Keywords: *Argania spinosa*; Argan tree; Flavonoid; Tandem mass spectrometry; Phenolic, Morocco

1. Introduction

The argan tree (*Argania spinosa* (L.) Skeels) of the family sapotaceae, is endemic in southwestern Morocco. Argan seed, that contains the kernels used for the preparation of the well-known edible argan oil, is covered by a milky pulp sheathed by a particularly thick peel. Argan oil is exclusively prepared in Morocco and it has for a long time only been sold on the Moroccan market. Nowadays, because of dramatic improvements brought to its preparation (Charrouf, Guillaume, & Driouich, 2002), its high dietetic value (Charrouf & Guillaume, 1999), and its general beneficial effect on human health (Charrouf & Guillaume, 1999), argan oil is found in all industrialized countries. As the pulp of the argan fruit is palatable to cattle and provides a cheap protein-rich material for goats and other farm animals, its agricultural impact is of importance. Chemical analysis of argan fruit pulp has already shown the presence of saponins (Charrouf & Guillaume, 2002a). its lipidic extract has also been analyzed (Charrouf, Fkih-Tetouani, Charrouf, Fournet, & Mouchel, 1991; Charrouf et al., 2002). However, its phenolic composition has only been superficially studied and only four compounds have been unambiguously identified so far (Chernane, Hafida, El Hadrami, & Ajana, 1999). Continuing our study on *A. spinosa* metabolites (Aloui et al., 2002; Charrouf & Guillaume, 2002a; Charrouf & Guillaume, 2002b), we describe the analysis of the phenolic fraction of the pulp of argan fruit using liquid chromatography coupled to negative electrospray ionization (ESI) tandem mass spectrometry (MS/MS) for the separation and identification, respectively.

2. Materials and methods

2.1. Chemicals

Methanol, ethyl acetate, acetonitrile, and hexane (HPLC grade) were from SDS (Peypin, France), and acetic

* Corresponding author. Tel.: +212 376 828 48; fax: +212 377 132 79.
E-mail address: zcharrouf@menara.ma (Z. Charrouf).

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acid (analytical grade) was from Merck (Darmstadt, Germany). Ultrapure water (Milli-Q, Waters, Milford, MA) was used. Phenolic standards were purchased from Extrasynthèse (Genay, France). The standard purity was above 98% and all of them were prepared as stock solutions at 1 g/L in methanol. Dilution of stock solutions with LC mobile phase furnished working standard solutions (1 mg/L).

2.2. Plant material

The fruits of *A. spinosa* were harvested at Tamanar (Essaouira, Morocco) in 2000. A voucher specimen was deposited in the scientific Institut of Rabat under N RAB 65342. The fruits were mechanically peeled and the pulp was air-dried, then powdered.

2.3. Extraction

A suspension of powdered pulp (20 g) in 150 ml of a methanol/water (4:1) solution was magnetically stirred for 30 min. The liquid phase was filtered and the solid residue again extracted twice more, each time with 100 ml of fresh methanol/water (4:1) solution. Each time, the suspension was filtered then the pooled liquid phases were concentrated by mild heating to 150 ml. The solution was then defatted three times by liquid extraction with hexane (100 ml). The combined polar phases were extracted twice with ethyl acetate. The combined organic phases were concentrated to dryness under reduced pressure. A 5 ml solution, that was kept at 4 °C until LC–MS/MS analysis, was prepared by dissolving the residue in acetonitrile. An aliquot of this solution was diluted with the initial LC mobile phase (nine times its volume) prior to its injection in the LC–MS system.

2.4. Analytical conditions

LC analyses were performed using a Perkin–Elmer series 200 quaternary pump system equipped with autosampler and UV detector. A 50 × 2.1 mm i.d., 3.5 μm, Luna C_{18} column was used for the separation. Gradient elution was performed with water/0.05% acetic acid (solvent A) and acetonitrile (solvent B) at a constant flow rate of 600 μl/min. An increasing linear gradient (v/v) of solvent B was used [*t* (min), % A]: 0, 94; 5, 83.5; 7, 82.5; 12.5, 81.5; 21, 0; 23, 94. Detection was carried out at 280 nm. A triple quadrupole mass spectrometer API 3000 (PE Sciex, Concord, Ontario, Canada) equipped with a Turbo ion spray source was used to obtain the MS/MS data. The MS/MS conditions used were adapted from previous works on the identification of phenolic compounds in vegetable residue samples (Parejo, Jauregui, Sanchez-Rabaneda, Viladomat, Bastida, & Codina, 2004; Sanchez-Rabaneda, Jauregui, Casals, Andrés-Lacueva, Izquierdo-Pulido, & Lamuela-Raventos, 2003; Sanchez-Rabaneda, Jauregui, Lamuela-Raveentos, Viladomat, Bastida, & Codina, 2004). All analyses were performed using a Turbo ion spray source in the negative mode and using as capillary voltage: −3500 V; nebulizer gas, curtain gas, drying gas and collision gas: N_2; entrance potential: 10 V; focusing potential: −200 V. The declustering potential was 60 V and the collision energy between 30 and 40 V. Full scan data acquisition was performed by scanning from *m*/*z* 100 to 800 amu in profile mode at a cycle time of 2 s with a step size of 0.1 amu and a pause between each scan of 2 ms.

3. Results and discussion

Analysis of the phenolic fraction of argan fruit pulp was performed by LC–ESI–MS/MS following our previously used (Parejo et al., 2004; Sanchez-Rabaneda et al., 2003; Sanchez-Rabaneda et al., 2004) and now well-described protocol (Cuyckens & Claeys, 2004; Cuyckens, Ma, Pocsfalvi, & Claeys, 2000). When possible, sample peaks were compared with those of available reference compounds analyzed under the same LC conditions, in order to compare their retention time and UV and mass data. The 18 compounds identified in argan fruit pulp are listed in Table 1.

3.1. Simple phenolic acid derivatives

Gallic acid (**1**) and protocatechuic acid (**2**) were directly identified by comparison of their retention time with that of standards and confirmed by MS experiments. In the MS spectrum, characteristic ion peaks at *m*/*z* 125 and 109, indicating the loss of CO_2, were observed for **1** and **2**, respectively.

3.2. Flavonoid *O*-rhamnoglucosides

The presence of flavonoid *O*-rhamnoglucosides can be revealed by MS/MS experiments in neutral loss scan mode of 308 amu, which corresponds to the fragments resulting from the neutral loss of a rhamnosylglucosyl moiety from the deprotonated molecule. Additionally, the use of MS/MS experiments allows to differentiate easily rutinosides (rhamnose 1 → 6 glucose) from neohesperidosides (rhamnose 1 → 2 glucose) by the relative intensities of the ion fragments in product ion scan mass spectrum (Sanchez-Rabaneda et al., 2003). From the pulp of argan fruit, three flavonoid *O*-rhamnoglucosides were detected, all of them belonging to the rutinoside group. These were quercetin-3-*O*-rutinoside (rutin) (**7**), rhamnetin-*O*-rutinoside (**13**), apigenin-7-*O*-rutinoside (isorhoifolin) (**4**), and hesperetin-7-*O*-rutinoside (hesperidin) (**8**). Compounds **7** and **13** were unambiguously identified by comparison of their MS spectrum and MS/MS experiments with those of literature (Hvattum & Ekeberg, 2003) and reference substances or by their LC retention time. Compound **4** was unambiguously identified by comparison of its retention time with that of a standard. Two compounds having a very close retention time (17.95 and 18.18 min) displayed mass data consistent with the structure of hesperidin or an isomer (ion fragment

Table 1
Phenolic compounds identified in argan fruit pulp

Compound (%)[a]	Peak	t_R (min)	Ions full scan MS		MS/MS experiments		
			$[M-H]^-$	Fragments	Neutral loss scan	Precursor ion scan	Product ion
Gallic acid (5.0)	1	0.82	169	125			
Protocatechuic acid (21.8)	2	1.44	153	109			
Catechin (2.8)	3	4.06	289			289	245
Isorhoifolin[b] (7.2)	4	7.13	577				
Epicatechin (14.7)	5	7.65	289			289	245
Procyanidin (2.7)	6	7.67	579			579	289, 245
Rutin (0.1)	7	10.87	609		308	609	301
Hesperidin[b] (4.5)	8	11.19 or 11.43	609	463, 301	308	609	301
Hyperoside (13.4)	9	11.46	463		162	463	301
Isoquercitrin (10)	10	11.70	463		162	463	301
Quercetin-*O*-pentose[c]	11	12.33	433		132	433	301
Naringenin-7-*O*-glucoside[c]	12	12.69	433		162	433	271
Rhamnetin-*O*-rutinoside (0.5)	13	13.37	623		308	623	315
Quercetin (1.6)	14	17.83	301			301	151, 121, 107
Luteolin (0.2)	15	17.94	285				
Naringenin (0.07)	16	18.51	271			271	119,109

[a] Calculated as the percentage of the identified compounds.
[b] Tentatively assigned.
[c] Total percentage for the two compounds – 15.3%.

at *m*/*z* 301). However, further investigations will be necessary to fully assess the exact structure of these compounds.

3.3. Flavonoid glycosides

Flavonoid *O*-glycosides, such as quercetin-3-*O*-galactoside (hyperoside) (**9**), quercetin-3-*O*-glucoside (isoquercetin) (**10**), and naringenin-7-*O*-glucoside (**12**) were identified by comparison of their retention time (11.46, 11.70, and 12.69 min, respectively) with that of reference substances and by MS/MS analysis in neutral loss of 162 amu (loss of tetrahydroxylated hexose) (Sanchez-Rabaneda et al., 2003; Sanchez-Rabaneda et al., 2004). MS/MS analysis in neutral loss of 132 amu (loss of trihydroxylated pentose) revealed the presence of one flavonoid glycoside having a pentose within its structure (**11**, retention time 12.33 min). its ion fragments were observed at *m*/*z* 433 and 301 in full scan mode. To elucidate the aglycone, a product ion scan of *m*/*z* 301 was performed. The observed fragment ions at *m*/*z* 151 and 121 evidenced that the aglycone of **11** was quercetin. From its retention time, compound **11** was identified as quercetin-3-*O*-arabinose, a compound that we already identified in a recent study (Sanchez-Rabaneda et al., 2003).

3.4. Other phenolic compounds

The flavonoid aglycones catechin (**3**), epicatechin (**5**), procyanidin (**6**), quercetin (**14**), luteolin (**15**), and naringenin (**16**) were identified by comparison with a standard. Procyanidin (**8**) was identified by product ion scan experiments.

Among the 16 compounds identified in the pulp of argan fruits, only compounds **3**, **5**, and **7** had been previously identified (Chernane et al., 1999). However, the presence of *p*-hydroxybenzoic acid (Chernane et al., 1999) could not be confirmed in this study. The strong antioxidant activity exhibited by some pulp phenolic compounds (Chu, Chang, & Hsu, 2000) justifies the study of the use of the pulp as a nutraceutical.

References

Aloui, A., Charrouf, Z., Soufiaoui, M., Carbone, V., Malorni, A., Pizza, C., et al. (2002). Triterpenoid saponins from the shells of *Argania spinosa* seeds. *Journal of Agricultural and Food Chemistry, 50*, 4600–4603.

Charrouf, Z., Fkih-Tetouani, S., Charrouf, M., Fournet, B., & Mouchel, B. (1991). Triterpènes et stérols extraits de la pulpe d'*Argania spinosa* (L.) (sapotaceae). *Plantes Médicinales et Phytothérapie, 2–3*, 112–117.

Charrouf, Z., & Guillaume, D. (1999). Ethnoeconomical, ethnomedical, and phytochemical study of *Argania spinosa* (L.) Skeels. *Journal of Ethnopharmacology, 67*, 7–14.

Charrouf, Z., & Guillaume, D. (2002a). Secondary metabolites from *Argania spinosa* (L.) Skeels. *Phytochemistry Reviews, 1*, 345–354.

Charrouf, Z., & Guillaume, D. (2002b). Chemistry of the secondary metabolites from *Argania spinosa* (L.) Skeels. *Current Topics in Phytochemistry, 5*, 99–102.

Charrouf, Z., Guillaume, D., & Driouich, A. (2002). L'arganier, un atout pour le Maroc. *Biofutur, 220*, 54–57.

Chernane, H., Hafida, A., El Hadrami, I., & Ajana, H. (1999). Composition phénolique de la pulpe des fruits d'Arganier (*Argania spinosa* L. Skeels) et relation avec leurs caractéristiques morphologiques. *Agrochimica, 43*, 137–150.

Chu, Y.-H., Chang, C.-L., & Hsu, H.-F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture, 80*, 561–566.

Cuyckens, F., & Claeys, M. (2004). Mass spectrometry in structural analysis of flavonoids. *Journal of Mass Spectromerty, 39*, 1–15.

Cuyckens, F., Ma, Y. L., Pocsfalvi, G., & Claeys, M. (2000). Tandem mass spectral strategies for the structural characterization of flavonoid glycosides. *Analysis, 28*, 888–895.

Hvattum, E., & Ekeberg, D. (2003). Study of the collision-induced radical cleavage of flavonoid glycosides using negative electrospray ionization tandem quadrupole mass spectrometry. *Journal of Mass Spectrometry, 38*, 43–49.

Parejo, I., Jauregui, O., Sanchez-Rabaneda, F., Viladomat, F., Bastida, J., & Codina, C. (2004). Separation and Characterization of phenolic compounds in fennel (*Foeniculum vulgare*) using liquid chromatography–negative electrospray ionization tandem spectrometry. *Journal of Agricultural and Food Chemistry, 52*, 3679–3687.

Sanchez-Rabaneda, F., Jauregui, O., Casals, I., Andrés-Lacueva, C., Izquierdo-Pulido, M., & Lamuela-Raventos, R. M. (2003). Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). *Journal of Mass Spectrometry, 38*, 35–42.

Sanchez-Rabaneda, F., Jauregui, O., Lamuela-Raveentos, R. M., Viladomat, F., Bastida, J., & Codina, C. (2004). Qualitative analysis of phenolic compounds in apple pomace using liquid chromatography coupled to mass spectrometry in tandem mode. *Rapid Commununication in Mass Spectrometry, 18*, 553–563.