Food Chemistry 112 (2009) 851–857

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Monomeric phenols of cashew apple (*Anacardium occidentale* L.)

Laetitia Michodjehoun-Mestres [a], Jean-Marc Souquet [b], Hélène Fulcrand [b], Claire Bouchut [b], Max Reynes [a], Jean-Marc Brillouet [a,b,*]

[a] *UMR "QUALISUD", Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Département PERSYST, TA50/16, F-34398 Montpellier cedex 5, France*
[b] *UMR 1083 "Sciences pour l'Œnologie", INRA, Université Montpellier 1, F-34000 Montpellier, France*

ARTICLE INFO

Article history:
Received 14 March 2008
Received in revised form 6 May 2008
Accepted 24 June 2008

Keywords:
Anacardium occidentale
Anacardiaceae
Cashew apple
Pseudo fruit
Monomeric phenols
Skin
Flesh
Flavonol glycosides
Anthocyanidin glycosides
HPLC-DAD/ESI-MS

ABSTRACT

Monomeric phenols were extracted by acetone/water (60:40) from the skin and flesh of four cashew apple genotypes from Brazil and Bénin (West Africa), purified by absorption chromatography and subjected to HPLC-DAD/ESI-MS analysis. Skins were found much richer than fleshes in simple phenolics. Flavonol glycosides were dominant with myricetin and quercetin hexosides (2 of each), pentosides (3 of each), and rhamnosides as major compounds. Anthocyanidin glycosides were detected in skins from the two scarlet and orange pigmented genotypes as peonidin, petunidin and cyanidin 3-*O*-hexosides, and were absent from fleshes.

1. Introduction

The cashew (*Anacardium occidentale* L.) belongs to the *Anacardiaceae* family which includes the mango, *Mangifera indica* L., and the *Spondias* genus (Purseglove, 1974). It is a native of tropical America from Mexico to Peru and Brazil and also of the West Indies. It has become naturalised in many tropical countries such as Vietnam, India, Nigeria, Tanzania, Ivory Coast, Mozambique, and Bénin.

It is a spreading evergreen tree bearing, at season, fruits, the cashew nut (achene), a highly priced material. The kidney-shaped cashew nut, the true fruit, is embedded in a fleshy swollen pedicel, receptacle and disc, called the cashew apple (Purseglove, 1974). The cashew apple, a pseudo fruit, is nutritious, juicy and astringent (Jayalekshmy & John, 2004). Apart from the usual presence of sugars, organic acids and fibres, a typical characteristic of cashew apple is its richness in vitamin C (e.g., four times higher than sweet orange) (Akinwale, 2000). The cashew apple has, depending on cultivars, a shiny, red, orange or yellow skin (Moura et al., 2001; Sastry, Lakshminarayana, Satyavathi, Pruthi, & Siddappa, 1962) (see Fig. 1); it shows a pear-shaped or pseudo-cylindrical structure.

While the nut is a high-added value food, the cashew apple is often considered as a by-product with a world production estimated in 2006 at 30 million metric tons (Anonymous, 2007), beyond that of pineapple and mango. Part of the cashew apple production is wasted, left to rot under trees after nut harvest; however, another part is processed into juice, candy etc. (Akinwale, 2000; Sastry, Lakshminarayana, Satyavathi, Pruthi, & Siddappa, 1962). Since it contains at maturity stage, i.e. when nuts are collected, some phenolics, derived products exhibit an acrid taste which limits their use and export (Akinwale & Aladesua, 1999; da Silveria Agostini-Costa, Lima, & Lima, 2003).

Data on cashew apple monomeric phenolics are very scarce. To our knowledge, the main source of information is the work of Satyanarayana, Mythirayee, Krishnamurty, and Madhavakrishna (1978) who extracted, purified and characterised three flavonols from an Indian variety, i.e. quercetin 3-*O*-galactoside, myricetin, and quercetin. Two Brazilian teams colorimetrically measured yellow flavonoids and anthocyanins (de Abreu, 2007; Moura et al., 2001) without further characterisation.

Thus, since these pseudo fruits might be a source of antioxidants, the object of the present work was to describe into more

* Corresponding author. Address: UMR "QUALISUD", Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Département PERSYST, TA50/16, F-34398 Montpellier cedex 5, France. Tel.: +33 6 5042 4655; fax: +33 4 6761 4433.
E-mail address: brillouet@cirad.fr (J.-M. Brillouet).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.06.056

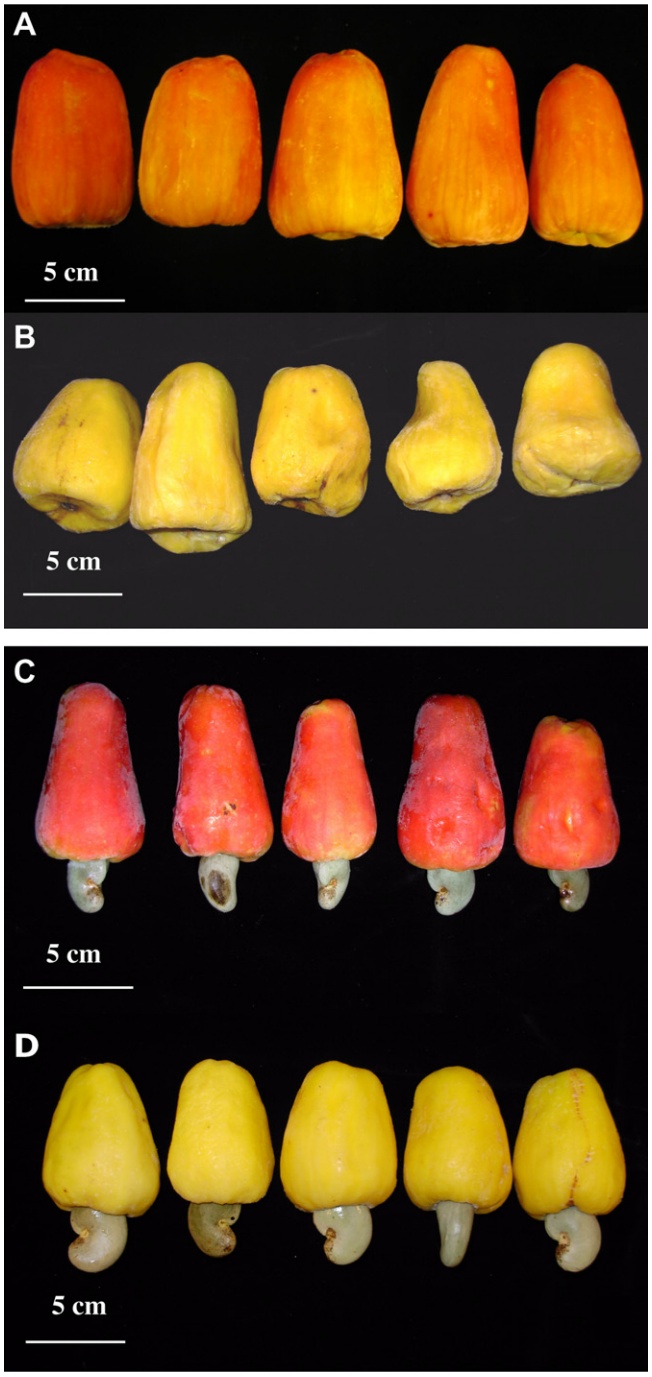

Fig. 1. Samples of cashew apples [A – clone CCP 76; B – clone Embrapa 50; collected in Brazil (November 2006)] and [C – variety Parakou Rouge and D – variety Parakou Jaune; collected in Bénin, West Africa (February 2006)].

details the monomeric phenols of cashew apples originating from Brazil and Bénin (West Africa).

2. Materials and methods

2.1. Plant materials

Two batches of cashew apples (clones CCP 76 and Embrapa 50) (20 kg each) were harvested in November 2006 at EMBRAPA Paraipaba Experimental Station (Ceará State, Brazil), immediately frozen, and air-freighted to our laboratory. Two batches of cashew apples with cashew nuts still attached (see Fig. 1) (varieties Parakou Rouge and Parakou Jaune) (20 kg each) were collected in February 2006 from orchards in the Parakou District (Bénin, West Africa), and treated as above; nuts were removed before analysis. All cashew apples were at the mature stage. Ten frozen cashew apples of each batch were randomly chosen; they were, being still frozen, rapidly, carefully, and entirely peeled by gently grating the epidermis with a razor blade; skin pieces (~0.1–0.2 mm maximum thickness) were immediately dipped in liquid nitrogen to prevent oxidation, and pulverised to a very fine powder with a pestle in a mortar. Similarly, frozen flesh was rapidly cut into bits (1×1 cm), and ground in liquid nitrogen as above. Half of the milled skin and flesh batches was freeze-dried, while the other half was kept fresh; both batches were stored at −80 °C under nitrogen before analyses.

2.2. Flavonoid standards and reagents

All flavonoid standards were of the best available quality. Cyanidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, myricetin 3-*O*-rhamnoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, kaempferol 3-*O*-glucoside, quercetin, myricetin, and (−)-epigallocatechin were purchased from Extrasynthèse (Genay, France). Gallic and ellagic acids were from Fluka (Switzerland). All other reagents and solvents were of analytical grade.

2.3. Physico-chemical analyses

Mass (g) was measured for each apple with a Sartorius balance (precision 0.1 g). Size of apples was measured with a calliper-square (Mitutoyo Digimatic model CD-15B). Dry matter was measured by heating overnight at 60 °C then for 24 h in a vacuum oven at 55 °C. Total soluble solids (°Brix) were measured at room temperature using an Abbe refractometer with a measuring range of 0−30 °Brix. Titratable acidity was estimated by titration with 0.1 M NaOH up to pH 8.1 using phenolphthalein as indicator (results were expressed as mg malic acid equivalents/100 g fresh weight).

2.4. Extraction and removal of polymeric phenols

Phenolics were extracted from fresh skin and flesh powders (~400 mg and ~1500 mg, respectively) in an acetone/water mixture (60:40, v/v) (40 ml and 120 ml, respectively); after homogenisation with an Ultra Turrax, the slurry was stirred for 60 min at 20 °C, then filtered on a Whatman filter paper (no. 1). Monomeric phenols (phenolic acids and their conjugates, anthocyanidin glycosides, and flavonols and their glycosides) were obtained according to Souquet, Labarbe, Le Guernevé, Cheynier, and Moutounet (2000). Briefly, extract was brought to dryness under vacuum and the dry residue was dissolved in 2 ml of an ethanol/water/trifluoroacetic acid mixture (55:45:0.05, v/v/v). After rinsing with 2×2 ml of the same mixture, extract and washings were gathered, then passed at 1 ml min^{-1} on a (12×1 cm i.d.) column packed with Toyopearl TSK-HW 50 F (Tosohaas, Japan) equilibrated in the same solvent mixture. Monomeric phenolic compounds were eluted with 50 ml of the above solvent mixture. The extract was brought to dryness in a rotary evaporator and kept at −80 °C under nitrogen.

2.5. HPLC-DAD analysis

HPLC analysis of phenolic acids and conjugates, flavanols, anthocyanidin glycosides, and flavonols and flavonol glycosides, was performed using an Agilent 1100 separation system (Agilent Technologies, Waldbronn, Germany) including a quaternary pump

coupled to a diode array detector and controlled by Chemstation A.10.02 software. Separations were achieved using a (250 × 4.6 mm i.d.) Modulocart QS-Lichrospher 5 μm ODS2 column (Interchim, Montluçon, France) with a guard column, operated at 30 °C. Mobile phase consisted of water/formic acid (98:2, v/v) (eluant A) and water/acetonitrile/formic acid (18:80:2, v/v/v) (eluant B). Flow rate was 0.5 ml min^{-1}. The elution program was as follows: 5–10% B (0–4 min); 10–16% B (4–8 min); 16–25% B (8–45 min); 25–35% B (45–55 min); 35–80% B (55–72 min); 80–100% B (72–75 min); 100–5% B (75–80 min). Triplicate samples were injected at a level of 10 μl. The column effluent was monitored from 230 to 600 nm. Quantification was achieved by injection of solutions of known concentrations of gallic acid, epigallocatechin, *p*-coumaric acid, peonidin 3-*O*-glucoside, myricetin 3-*O*-rhamnoside, quercetin 3-*O*-rhamnoside, kaempferol 3-*O*-glucoside, myricetin, and quercetin; when phenolic standards were available, compounds were expressed as such; otherwise they were expressed as mg equivalents/100 g fresh weight (see Table 3).

2.6. HPLC-DAD/ESI-MS analysis

Separations were performed on a (250 × 4.6 mm i.d.) Modulocart QS-Lichrospher 5 μm ODS2 column (Interchim) with a guard column, operated at 30 °C. Mobile phase consisted of water/formic acid (99.9:0.1, v/v) (eluant A) and water/acetonitrile/formic acid (18.0:81.9:0.1, v/v/v) (eluant B). Flow rate was 0.5 ml min^{-1}. The elution program was the same as above. The column eluate was then split and 0.25 ml min^{-1} was directed to an LCQ ion trap spectrometer fitted with an electrospray interface (Thermo Finnigan, San Jose, USA). Experiments were conducted in both negative and positive modes. Scan range was 100–2000 a.m.u. and scan rate 1 scan/s. The desolvation temperatures were 250 and 300 °C in the positive and negative ion modes, respectively. High spray voltage was set at 4000 V (positive) and 3500 V (negative) ion modes. Nitrogen was used as the dry gas at a flow of 5 for the auxiliary gas and 55 for the sheat gas. Identifications were achieved on the basis of the ion molecular masses and UV–visible spectra.

3. Results and discussion

3.1. Physico-chemical characterisation of cashew apples

Data are shown in Table 1. As observed by Nigerian authors (Akinwale & Aladesua, 1999), the weight of local African varieties from Bénin was found to be approximately twice lower than that of Brazilian clones. pH, titratable acidity, and soluble solids were found in ranges similar to those observed on Brazilian and Nigerian types, African varieties being, however, found more acid than Brazilian clones.

After hand dissection, the cashew apple flesh (f) and skin (s) masses were as follows (in g): Embrapa 50 – f98.4 ± 1.2, s3.8 ± 0.2; CCP 76 – f144.8 ± 8.4, s10.5 ± 0.1; Parakou Rouge – f75.3 ± 7.2, s5.9 ± 0.1; Parakou Jaune – f58.8 ± 4.9, s3.5 ± 0.5.

Table 1
Physico and physicochemical characteristics of cashew apples from Brazil and Bénin (n = 10)

	Clones and varieties			
	Embrapa 50	CCP76	Parakou Rouge	Parakou Jaune
Mass (g)	102.2 ± 12.2	155.3 ± 6.9	81.2 ± 5.4	73.3 ± 3.5
Length (cm)	8.2 ± 0.8	8.0 ± 0.4	8.5 ± 0.6	7.1 ± 0.1
Basal diameter (cm)	5.4 ± 0.8	6.2 ± 0.3	4.5 ± 0.5	4.1 ± 0.3
Apical diameter (cm)	3.3 ± 0.3	4.5 ± 0.3	2.6 ± 0.2	2.9 ± 0.4
Color	Lemon yellow	Orange	Scarlet	Lemon yellow
Morphology	Pseudocylindrical to pear-shaped	Pseudocylindrical to pear-shaped	Pseudoconical	Pear-shaped
Soluble solids (°Brix)	11.8 ± 0.6	11.2 ± 0.6	11.2 ± 0.2	13.5 ± 0.1
pH	4.60 ± 0.28	4.00 ± 0.19	4.02 ± 0.23	3.85 ± 0.22
Titratable acidity (as % malic acid)	0.3 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	1.1 ± 0.1

3.2. Identification and quantification of phenolic acid conjugates, anthocyanidin glycosides, flavonols and their glycosides

Fig. 2 shows, as an example, the HPLC-UV–vis chromatograms of a purified phenolic extract obtained from the skin of the Parakou Rouge variety (not all peaks are numbered). It must be said that, apart anthocyanidin glycosides which are only present in significant amounts in skin from Parakou Rouge variety and clone CCP 76, all other peaks were systematically encountered in various absolute and relative proportions in most extracts, being sometimes absent from flesh extracts. Identification of individual compounds was achieved by comparison of retention times, photodiode array UV–vis spectra, and HPLC-DAD/ESI-MS spectrometric data (Table 2) in comparison with authentic standards. It must be mentioned that, amongst the 61 peaks observed at various wavelengths (280, 320, 360, and 520 nm), only 39 were formally or tentatively identified.

3.2.1. Phenolic acids and flavanols

Only four phenolic acids were observed in both skins and flesh, gallic acid (peak no. 5; λ_{max} at 271 nm), two *p*-coumaric acid conjugates (nos. 12 and 13; λ_{max} at 315 and 310 nm), and ellagic acid (peak no. 30a; $[M-H]^-$ at m/z 301). Satyanarayana et al. (1978) also observed gallic acid in an Indian cashew apple variety; however, contrary to these authors, we have not detected protocatechuic and *p*-hydroxybenzoic acids. Unidentified flavanol peaks were observed amongst which peaks no. 6 (λ_{max} at 274 nm with a spectrum shape identical to epigallocatechin, and no. 8 (λ_{max} at 276 nm resembling to epigallocatechin gallate). It must be said that neither known monomeric flavan-3-ols nor were observed oligomeric proanthocyanidins.

3.2.2. Anthocyanidin glycosides

Four anthocyanidin glycosides (peaks nos. 15, 19, 22, 27) were detected at 520 nm in skins from clone CCP 76 and Parakou Rouge variety (Fig. 1); they exhibited typical anthocyanin spectra with λ_{max} at 517, 522, 514, and 500 nm, respectively (Table 2). They were present at trace levels in skins of Parakou Jaune variety and clone Embrapa 50 and absent in the flesh of the four studied apples. These anthocyanins showed M^+ at m/z 449, 479, 463, and 447, respectively, with their aglycon ions M^+ at m/z 287, 317, 301, and 285 indicating that they are cyanidin, petunidin, peonidin, and a dihydroxy-methoxyflavylium monohexosides as shown by the loss of a sugar moiety with 162 units. Peaks nos. 15, 19, and 22 were not glucosides as checked by co-injection with authentic standards; according to their elution order compared to glucosides (Yi, Akoh, Fischer, & Krewer, 2006), they are likely 3-*O*-galactosides as could be peak no. 27. Although anthocyanins were colorimetrically measured in nine Brazilian clones (de Abreu, 2007; Moura et al., 2001), they were not identified.

3.2.3. Flavonols and flavonol glycosides

Peaks nos. 23 and 25 had a λ_{max} at 357 nm (Table 2); in HPLC-DAD/ESI-MS analysis, they showed deprotonated molecular ions $[M-H]^-$ at m/z 479 with deprotonated aglycon ion at m/z 317 (loss of a sugar moiety of 162 units), indicating that they are myricetin

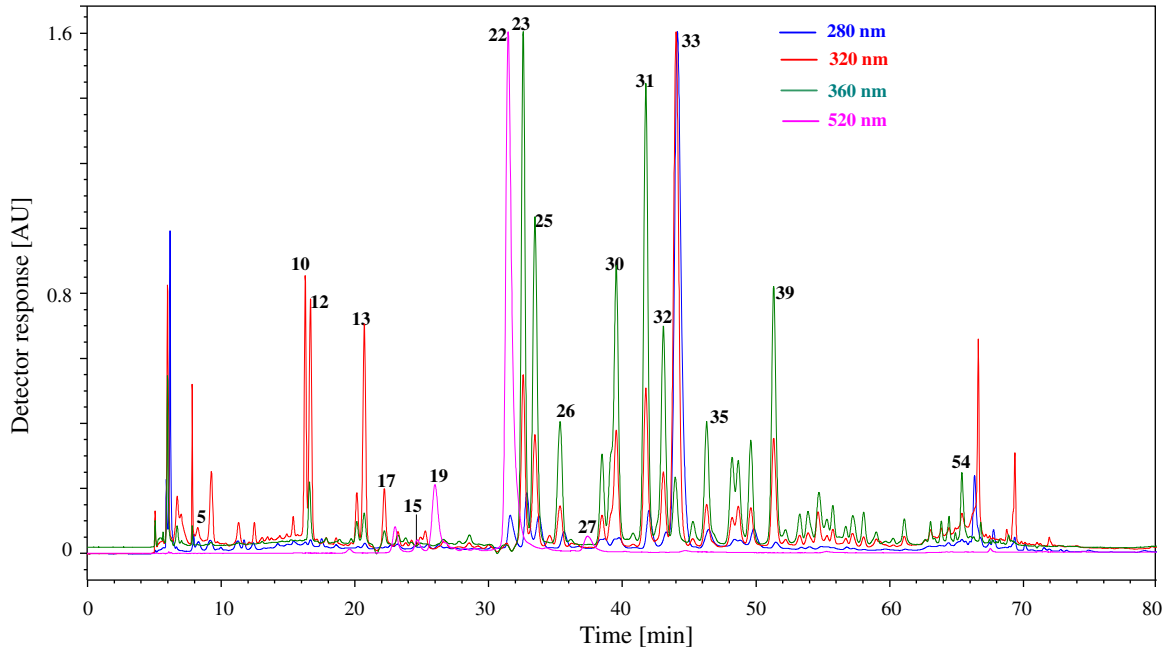

Fig. 2. HPLC chromatograms of skin extract of cashew apple (Parakou Rouge variety). (5) gallic acid; (12, 13) *p*-coumaric acid conjugates; (15) cyanidin hexoside; (19) petunidin hexoside; (22) peonidin hexoside; (23) myricetin hexoside; (25) myricetin hexoside; (26) myricetin pentoside; (27) unknown anthocyanidin hexoside; (30) myricetin 3-*O*-rhamnoside; (31) quercetin 3-*O*-galactoside; (32) quercetin 3-*O*-glucoside; (35) quercetin pentoside; (39) quercetin 3-*O*-rhamnoside; (54) quercetin. Some unknown peaks are also numbered (10, 17, and 33). Peak heights were normalised at 1.6 AU at λ = 280 nm.

monohexosides (Myr-hex$_{1,2}$), possibly according to their elution times, 3-*O*-galactoside and 3-*O*-glucoside. Peaks nos. 26, 28, and 29 had a λ_{max} at 357-358 nm; they showed in HPLC-DAD/ESI-MS analysis deprotonated molecular ions $[M-H]^-$ at *m*/*z* 449 with deprotonated aglycon ions at *m*/*z* 317 (loss of a sugar moiety of 132 units), indicating that they are myricetin monopentosides (Myr-pent$_{3,4,5}$): they could be arabinosides and xylosides (positions C3, C5, or C7). Peak no. 30 exhibited a λ_{max} at 350 nm; HPLC-DAD/ESI-MS analysis revealed a deprotonated molecular ion $[M-H]^-$ at *m*/*z* 463 with its deprotonated aglycon ion at *m*/*z* 317 (loss of a sugar moiety of 146 units), showing, after co-injection with an authentic standard, that it is myricetin 3-*O*-rhamnoside (Myr-rha = myricitrin). Peaks nos. 31 and 32 showed very similar spectra with a λ_{max} at 354 nm; both showed deprotonated molecular ions $[M-H]^-$ at *m*/*z* 463 with deprotonated aglycon ions at *m*/*z* 301 (loss of a sugar moiety of 162 units), indicating that they are quercetin monohexosides (Quer-hex$_{1,2}$). They were shown, by co-injection with authentic standards, to be quercetin 3-*O*-galactoside (=hyperoside=hyperin) and quercetin 3-*O*-glucoside (=isoquercitrin). Peaks nos. 35, 36, and 38 had λ_{max} at 354, 355, and 353 nm, respectively, and were shown to be quercetin monopentosides (Quer-pent$_{3,4,5}$) by the presence of deprotonated molecular ions $[M-H]^-$ at *m*/*z* 433 with their deprotonated aglycon ions at *m*/*z* 301 (loss of a sugar moiety of 132 units): they could be arabinosides or xylosides. Peak no. 39 showed a λ_{max} at 348 nm and a deprotonated molecular ion $[M-H]^-$ at *m*/*z* 447 with its deprotonated aglycon ion at *m*/*z* 301 (loss of a sugar moiety of 146 units), showing, after co-injection with an authentic standard, that it is quercetin 3-*O*-rhamnoside (Quer-rha = quercitrin). Two unknown quercetin conjugates were observed (peaks nos. 41 and 50; λ_{max} at 356-354 nm) with respective deprotonated molecular ions $[M-H]^-$ at *m*/*z* 585 and 571 and a deprotonated aglycon ion at *m*/*z* 301. One unknown myricetin conjugate was seen (peak no. 42; λ_{max} at 357 nm) with deprotonated molecular ion $[M-H]^-$ at *m*/*z* 601 and deprotonated aglycon ion at *m*/*z* 317. The non-aglycon moiety shared by peaks nos. 41 and 42 has a Mw of 302 (i.e. 284 + 18); thus, these two peaks could be quercetin and myricetin galloyl pentosides. It must be mentioned that a new myricetin 3-*O*-α-L-(2′-galloyl) arabinopyranoside was also isolated from roots and aerial parts of *Limonium gmelinii* (Korul'kina et al., 2004). Peak no. 48, a flavonol hexoside (λ_{max} at 358 nm; deprotonated molecular ion $[M-H]^-$ at *m*/*z* 493; aglycon moiety $[M-H]^-$ at *m*/*z* 331; late elution time), could be a myricetin monomethylether hexoside (Braca, Bilia, Mendez, & Morelli, 2001). To our knowledge, apart from hyperoside, which was observed in an Indian cashew apple variety (Satyanarayana et al., 1978), other flavonol glycosides had not been previously mentioned. Peaks no. 43 and 54 were identified as myricetin and quercetin, respectively, with λ_{max} at 374 and 371 nm, after co-injection with authentic standards ($[M-H]^-$ at *m*/*z* 317 and 301, respectively); they had been already found in an Indian cashew apple variety (Satyanarayana et al., 1978). Numerous other compounds were tentatively identified as flavonol conjugates on the basis of their UV–visible spectra (ranges of λ_{max} at 256 → 267 nm, 289 sh → 319 sh nm, 346 → 360 nm).

3.2.4. Unknown (no. 33)

It is worth to mention the presence in all samples of peak no. 33 which exhibited a strong absorption at 284 nm (no shoulder). It was 3–4 times more concentrated in skins than in fleshes (peak surface basis at 280 nm). It had a pH-dependent chromatographic behaviour, since having injected in HPLC an extract with the usual gradient but with eluant A containing 0.1% formic acid (pH 2.69) instead of 2% (pH 2.00), its elution time shifted from 44.0 min to 49.8 min while elution times of other compounds, except anthocyanidin glycosides, remained unchanged. Its identification will be achieved, after semi-preparative HPLC purification, in the near future.

3.2.5. Quantitative aspects

Phenolic acids and their conjugates, anthocyanidin glycosides, and flavonols and their glycosides were measured in skin and flesh of cashew apples and data are presented in Table 3. Concentration ranges, with their corresponding relative standard deviation ranges were as follows: 0.01–0.10 mg/100 g (20–40%);

Table 2
HPLC retention times, spectral characteristics, and HPLC-ESI/MS data[a] of compounds encountered in cashew apples

Peak[b]	t_R (min)	Compound	λ_{max} (nm)	Molecular Ion $[M+H]^+$	Aglycon $[A+H]^+$	Molecular Ion $[M-H]^-$	Aglycon $[A-H]^-$
1	6.0	Unknown	252, 256 sh				
2	6.7	Unknown	248, 305				
3	7.8	Unknown	242, 300				
4	9.3	Unknown	276				
5	11.3	Gallic acid	271				
6	11.7	Unknown flavanol	274				
7	12.5	Unknown	282				
8	15.4	Unknown flavanol	276				
9	16.3	Unknown	298				
10	16.7	Unknown	300				
11	20.1	Unknown	286, 326 sh				
12	20.7	*p*-Coumaric acid conjugate	296 sh, 315				
13	22.2	*p*-Coumaric acid conjugate	290 sh, 310				
14	22.6	Unknown	272 sh, 278, 288 sh				
15	23.3	Cyanidin hexoside[c]	281, 290 sh, 331 sh, 379 sh, 435 sh, 517	449[d]	287[d]		
16	24.9	Unknown	282, 340 sh				
17	25.2	Unknown	292, 315 sh				
18	25.3	Unknown	292,313				
19	26.3	Petunidin hexoside[c]	278, 298 sh, 349, 428 sh, 522	479[d]	317[d]		
20	26.8	Unknown	279, 326 sh				
21	31.3	Unknown flavanol	276				
22	31.6	Peonidin hexoside[c]	281, 330 sh, 376 sh, 433 sh, 514	463[d]	301[d]		
23	32.6	Myricetin hexoside	258, 302 sh, 357	481	319	479	317
24	33.2	Unknown	274, 354				
25	33.5	Myricetin hexoside	256, 304 sh, 357	481	319	479	317
26	35.3	Myricetin pentoside	263, 302 sh, 357	451	319	449	316,317
27	37.7	Unknown anthocyanidin hexoside[c]	279, 333, 426 sh, 505	447[d]	285[d]		
28	38.5	Myricetin pentoside	262, 300, 357	451	319	449	316,317
29	39.1	Myricetin pentoside	262, 297 sh, 306 sh, 358	451	319	449	316,317
30	39.6	Myricetin 3-*O*-rhamnoside	260, 302 sh, 350	465	319	463	316,317
30a	40.7	Ellagic acid	253, 308 sh, 352 sh, 368				301
31	41.8	Quercetin 3-*O*-galactoside	256, 265 sh, 298 sh, 354	465	303	463	300, 301
32	43.0	Quercetin 3-*O*-glucoside	256, 266 sh, 302 sh, 354	465	303	463	300, 301
33	44.0	Unknown	284 (strong absorption, no shoulder)				
34	45.3	Unknown flavonol conjugate	260 sh, 268, 300 sh, 360				
35	46.3	Quercetin pentoside	257 sh, 266, 290 sh, 354	435	303	433	300, 301
36	48.2	Quercetin pentoside	256, 266 sh, 300 sh, 355	435	303	433	300, 301
37	48.7	Kaempferol 3-*O*-galactoside	266, 296 sh, 347			447	285
38	49.6	Quercetin pentoside	256 sh, 265, 290 sh, 353			433	300, 301
38a	51.1	Kaempferol 3-*O*-glucoside	265, 297 sh, 347			447	285
39	51.3	Quercetin 3-*O*-rhamnoside	257, 265 sh, 298 sh, 348	449	303	447	300, 301
40	52.2	Unknown	267, 298 sh, 348				
41	53.3	Quercetin galloyl pentoside[e]	267, 298 sh, 356	587	303	585	301
41a	53.6	Kaempferol pentoside	262, 295 sh, 347			417	285
42	53.9	Myricetin galloyl pentoside[e]	266, 298 sh, 357	603	319	601	317
43	54.7	Myricetin	252, 304 sh, 374		319		317
44	55.3	Unknown flavonol conjugate	267, 291 sh, 320 sh, 350				
45	55.7	Unknown flavonol conjugate	266, 299 sh, 348				
46	56.7	Kaempferol hexoside	270, 348			447	285
47	57.2	Kaempferol pentoside	262, 297 sh, 319 sh, 350			417	285
48	58.0	Myricetin monomethylether hexoside[e]	262, 300 sh, 358			493	331
49	59.0	Unknown flavonol conjugate	263, 349				
50	61.1	Unknown quercetin conjugate	266, 294 sh, 354	573	303	571	301
51	63.1	Unknown flavonol conjugate	266, 299 sh, 346				
52	63.8	Unknown flavonol conjugate	256, 319 sh, 351				
53	64.4	Unknown flavonol conjugate	256, 316 sh, 355				
54	65.4	Quercetin	256,271 sh,304sh, 371		303		301
55	66.3	Unknown	276				
56		Unknown flavonol conjugate	265, 290 sh, 360				
57		Unknown flavonol conjugate	264, 289 sh, 358				
58		Unknown	245, 312				

[a] When no HPLC-ESI/MS data are mentioned, it means that compounds gave no response in both positive and negative modes or that MS spectra were not exploitable.
[b] Only peaks in bold were quantified (see Table 3).
[c] λ_{max} (nm) determined along HPLC analyses, *i.e.* in 2% formic acid (pH 2.00).
[d] In the case of anthocyanidin glycosides, molecular ions and aglycons are given as $[M]^+$.
[e] Tentatively identified.

0.10–0.50 mg/100 g (10–30%); 0.50–5.00 mg/100 g (5–15%); 5.00–35.00 mg/100 g (2–5%).

Skins from the four studied apples were 15−20 times richer in total phenolic compounds than their fleshes, reaching ~30 → 110 and 2 → 5 mg/100 g fresh weight, respectively. A preferential location of monomeric phenols in skin was also observed in apples from temperate climate, e.g. in Granny Smith skin, total monomeric phenolic compounds reached 1750 mg/100 g fw, while they

Table 3
Concentrations of phenolics in skin and flesh of cashew apples (mg/100 g fresh weight)

Peak	t_R (min)	Compound	Clones and varieties							
			Embrapa 50		CCP 76		Parakou Rouge		Parakou Jaune	
			Skin	Flesh	Skin	Flesh	Skin	Flesh	Skin	Flesh
5	11.3	Gallic acid	1.60	0.49	1.22	0.34	0.42	0.17	0.58	0.22
6	11.7	Unknown flavanol	3.40	1.58	2.21	0.94	0.71	0.51	0.97	0.83
7	12.3	Unknown[a]	0.48	0.13	0.25	0.03	0.66	0.15	0.50	0.24
8	15.4	Unknown flavanol	1.21	0.23	0.40	0.31	0.33	0.19	0.32	0.18
9	16.3	Unknown[a]	2.03	1.43	0.39	0.11	0.31	0.05	0.38	0.14
10	16.7	Unknown[a]	1.34	0.30	0.78	0.20	1.39	0.24	2.74	0.26
12	20.7	*p*-Coumaric acid conjugate[b]	0.64	0.14	0.10	0.02	0.50	0.04	1.65	0.13
13	22.2	*p*-Coumaric acid conjugate[b]	0.19	0.05	–	–	0.12	0.02	1.52	0.05
15	23.3	Cyanidin hexoside[c]	–[g]	– –[h]	0.02	– –	1.22	– –	–	– –
19	26.3	Petunidin hexoside[c]	0.05	– –	0.04	– –	4.46	– –	0.03	– –
22	31.6	Peonidin hexoside[c]	0.12	– –	0.71	– –	33.40	– –	0.15	– –
23	32.6	Myricetin hexoside[d]	2.81	0.10	7.05	0.10	17.91	0.04	11.98	0.04
25	33.5	Myricetin hexoside[d]	2.14	0.08	3.53	0.05	10.73	0.03	6.93	0.04
26	35.3	Myricetin pentoside[d]	0.86	0.02	1.53	0.02	5.73	–	3.27	–
27	37.7	Unknown anthocyanidin hexoside[c]	–	– –	0.09	– –	1.37	– –	–	– –
28	38.5	Myricetin pentoside[d]	0.51	0.01	2.50	–	2.73	_	1.49	–
29	39.1	Myricetin pentoside[d]	0.48	0.01	2.45	–	2.56	_	1.72	–
30	39.6	Myricetin 3-*O*-rhamnoside	1.63	0.07	5.61	0.09	3.01	_	2.00	0.02
31	41.8	Quercetin 3-*O*-galactoside	2.31	0.05	9.39	0.06	10.90	0.01	3.55	–
32	43.0	Quercetin 3-*O*-glucoside	1.18	0.01	2.67	0.01	3.80	–	1.06	–
34	45.3	Unknown flavonol conjugate[e]	0.35	0.01	0.17	–	0.42	0.01	0.14	–
35	46.3	Quercetin pentoside[e]	0.66	–	2.09	0.01	3.54	–	1.19	–
36	48.2	Quercetin pentoside[e]	0.45	–	1.70	–	1.85	–	0.50	–
37	48.7	Kaempferol 3-*O*-galactosidef[f]	0.51	–	1.70	–	1.11	–	1.10	–
38	49.6	Quercetin pentoside[e]	0.50	–	2.45	0.01	2.05	_	0.87	–
39	51.3	Quercetin 3-*O*-rhamnoside	1.29	0.03	4.58	0.04	1.67	–	0.68	–
41	53.3	Quercetin galloyl pentoside	–	– –	–	– –	–	– –	–	– –
42	53.9	Myricetin galloyl pentoside	–	– –	–	– –	–	– –	–	– –
43	54.7	Myricetin	0.16	–	0.23	–	0.91	–	0.51	–
44	55.3	Unknown flavonol conjugate[e]	0.05	–	0.15	–	0.43	–	0.25	–
46	56.7	Kaempferol hexoside[f]	0.07	–	0.10	–	0.52	–	0.38	–
47	57.2	Kaempferol pentoside[f]	0.16	–	0.19	–	0.44	–	0.43	–
49	59.0	Unknown flavonol conjugate[e]	0.03	–	0.11	–	–	–	–	–
50	61.1	Unknown quercetin conjugate[e]	–	– –	–	– –	–	– –	–	– –
52	63.8	Unknown flavonol conjugate[e]	– –	–	0.03	–	–	–	–	–
53	64.4	Unknown flavonol conjugate	0.05	–	0.15	–	0.29	–	–	–
54	65.4	Quercetin	0.15	–	0.73	–	0.85	–	0.35	–
56	66.6	Unknown flavonol conjugate[e]	– –	–	0.12	–	0.46	–	–	–
57	68.1	Unknown flavonol conjugate[e]	– –	–	0.32	–	–	–	–	–
		Total phenolic acids and conjugates	2.43	0.68	1.32	0.36	1.04	0.23	3.75	0.40
		Total flavanols	8.46	3.67	4.03	1.59	3.40	1.14	4.91	1.65
		Total anthocyanidin glycosides	0.17	– –	0.86	– –	37.46	– –	0.18	– –
		Total flavonols and flavonol glycosides	16.35	0.39	49.55	0.39	71.91	0.16	38.40	0.10
		Total monomeric phenolics	27.41	4.74	55.76	2.34	113.81	1.53	47.24	2.15

[a] Expressed as epigallocatechin (280 nm).
[b] Expressed as *p*-coumaric acid (320 nm).
[c] Expressed as peonidin 3-*O*-glucoside (520 nm).
[d] Expressed as myricetin 3-O-rhamnoside (360 nm).
[e] Expressed as quercetin 3-O-rhamnoside (360 nm).
[f] Expressed as kaempferol 3-O-glucoside (360 nm).
[g] <0.02 mg/100 g for anthocyanidin glycosides and 0.01 mg/100 g for other compounds.
[h] Not detected.

were present at 22 mg/100 g in flesh (Pérez-Ilzarbe, Hernández, & Estrella, 1991).

Gallic acid and an unknown flavanol (peak no. 6) were measured in 1/2 concentration ratios in skins from the four apples. Anthocyanidin glycosides were observed in skins from pigmented apples at a level, for CCP 76, of 0.06 mg/100 g (whole apple; concentration calculated from the skin and flesh mass distribution), a value extremely different from the 37 mg/100 g and the 9 mg/100 g obtained for the same clone (de Abreu, 2007; Moura et al., 2001).

African apples were found to be richer in skin flavonols and flavonol glycosides than Brazilian ones, while the reverse situation was found in the flesh. Taking into account the apple skin and flesh mass distributions, total flavonols and flavonol glycosides in our unpeeled cashew apples were as follows: Embrapa 50, CCP 76, Parakou Rouge, and Parakou Jaune (1.00, 3.72, 4.36, and 1.40 mg/100 g); our values are close to previously published unpeeled cashew apple flavonol contents [addition of the three flavonols purified by Satyanarayana et al. (1978) gave 1.3 mg/100 g; a triplicate determination on a local Brazilian variety gave 3 mg/100 g (Mélo, de Lima, Maciel, Caetano, & Leal, 2006)]. Thus, as stated by Satyanarayana et al. (1978), cashew apple can be seen as poor in these components. Very different values were formerly released: 105 mg/100 g [average content in yellow flavonoids (i.e. flavonols and their glycosides) from nine Brazilian clones] (Moura et al., 2001) and 40 mg/100 g for both Embrapa 50 and CCP 76 clones (de Abreu, 2007).

It is worth to mention that cashew apples, whatever their origin, show in their skin and flesh a constant relative distribution pattern for three myricetin and five quercetin glycosides (average molar ratios): myricetin series – Myr-hex$_1$/Myr-hex$_2$/Myr-pent$_1$ = 1/0.61/0.30; quercetin series – Quer-hex$_1$/Quer-hex$_2$/Quer-pent$_1$/Quer-pent$_2$/Quer-pent$_3$ = 1/0.36/0.31/0.18/0.25. Myricitrin (Myr-rha) and quercitrin (Quer-rha) behave differently: they are undersynthesized with regards to Myr-hex$_1$ and Quer-hex$_1$, respectively, in African apples (0.17/1 and 0.18/1) compared to Brazilian ones (0.71/1 and 0.54/1). It must be noted that the existence of the above six quercetin glycosides was already mentioned in apple juice (Schieber, Keller, & Carle, 2001) and in *Vaccinium angustifolium* extracts (Harris et al., 2007).

Acknowledgements

Thanks are due to Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA, Brazil) for providing Brazilian cashew apples; thanks are also due to orchard owners from the Parakou District (Bénin). Thanks are also due to Dr. G. Mazerolles and E. Meudec (UMR "Science pour l'Œnologie", Plate-forme Polyphenols, INRA, Montpellier, France) for their help. This work was financially supported by European Union (PAVUC project, INCO 015279).

References

Akinwale, T. O. (2000). Cashew apple juice: Its use in fortifying the nutritional quality of some tropical fruits. *European Food Research Technology, 211*, 205–207.

Akinwale, T. O., & Aladesua, O. O. (1999). Comparative study of the physico-chemical properties and the effect of different techniques on the quality of cashew juice from Brazilian and local varieties. *Nigerian Journal of Tree Crop Research, 3*, 60–66.

Anonymous (2007). Statistical Databases (FAOSTAT), FAO, Roma, Italy.

Braca, A., Bilia, A. R., Mendez, J., & Morelli, I. (2001). Myricetin glycosides from *Licania densiflora*. *Fitoterapia, 72*, 182–185.

da Silveria Agostini-Costa, T., Lima, A., & Lima, M. V. (2003). Determinacão de tanino em pedúnculo de caju: método da vanilina versus método do butanol ácido. *Quimica Nova, 6*, 763–765.

de Abreu, C. R. A. (2007). Qualidade e atividade antioxidante total de pedúnculos de clones comerciais de cajueiro añao precoce. *M.Sc. Universidade Federal do Ceará* (Fortaleza, Brazil) (pp. 1–111).

Harris, C. S., Burt, A. J., Saleem, A., Mai Le, P., Martineau, L. C., Haddad, P. S., et al. (2007). A single HPLC-PAD-APCI/MS method for the quantitative comparison of phenolic compounds found in leaf, stem, root and fruit extracts of *Vaccinium angustifolium*. *Phytochemical Analysis, 18*, 161–169.

Jayalekshmy, V. G., & John, P. S. (2004). "Sago" – A natural product for cashew apple juice clarification. *Journal of Tropical Agriculture, 42*, 1–2.

Korul'kina, L. M., Shul'ts, E. E., Zhusupova, G. E., Abilov, Zh. A., Erzhanov, K. B., & Chaudri, M. I. (2004). Biologically active compounds from *Limonium gmelinii* and *L. Popovii* I.. *Chemistry of Natural Compounds, 40*, 465–471.

Mélo, E. A., de Lima, V. L. A. G., Maciel, M. I. S., Caetano, A. C. S., & Leal, F. L. L. (2006). Polyphenol, ascorbic acid and total carotenoid contents in common fruits and vegetables. *Brazilian Journal of Food Technology, 9*, 89–94.

Moura, C. F. H., Alves, R. E., Inneco, R., Filgueiras, H. A. C., Mosca, J. L., & Pinto, S. A. A. (2001). Características físicas de pendúnculos de cajueiro para comercialização in natura. *Revista Brasileira Fruticoltora*, 537–540.

Pérez-Ilzarbe, J., Hernández, T., & Estrella, I. (1991). Phenolic compounds in apples: Varietal differences. *Z. Lebensm. Unters. Forsch., 192*, 551–554.

Purseglove, J. W. (1974). *Anacardiaceae*. In J. W. Purseglove (Ed.), *Tropical crops dicotyledons* (pp. 8–32). London, Great Britain: Longman Group Ltd.

Sastry, L. V. L., Lakshminarayana, Setty, Satyavathi, V. K., Pruthi, J. S., & Siddappa, G. S. (1962). Polyphenol constituents in cashew apple juice as influenced by region, strain and selection. *Indian Journal of Applied Chemistry, 25*, 119–122.

Satyanarayana, D., Mythirayee, C., Krishnamurty, V., & Madhavakrishna, W. (1978). Studies on the polyphenols of cashew apple. *Leather Science, 25*, 51–54.

Schieber, A., Keller, P., & Carle, R. (2001). Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. *Journal of Chromatography A, 910*, 265–273.

Souquet, J.-M., Labarbe, B., Le Guernevé, C., Cheynier, V., & Moutounet, M. (2000). Phenolic composition of grape stems. *Journal of Agricultural and Food Chemistry, 48*, 1076–1080.

Yi, W., Akoh, C. C., Fischer, J., & Krewer, G. (2006). Absorption of anthocyanins from blueberry extracts by caco-2 human intestinal cell monolayers. *Journal of Agricultural and Food Chemistry, 54*, 5651–5658.