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Characterization of gallotannins and benzophenone derivatives from mango (*Mangifera indica* L. cv. 'Tommy Atkins') peels, pulp and kernels by high-performance liquid chromatography/electrospray ionization mass spectrometry

Nicolai Berardini, Reinhold Carle and Andreas Schieber*

Institute of Food Technology, Section Plant Foodstuff Technology, Hohenheim University, August-von-Hartmann-Strasse 3, D-70599 Stuttgart, Germany

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Polyphenolics were extracted from peels, pulp and kernels of mango fruits (*Mangifera indica* L. cv. 'Tommy Atkins') and characterized by high-performance liquid chromatography/electrospray ionization mass spectrometry. In the peel 18 gallotannins and five benzophenone derivatives were detected which were tentatively identified as galloylated maclurin and iriflophenone glucosides. Twenty-one and eight gallotannins were found in the kernels and pulp, respectively, whereas no evidence for the presence of benzophenone derivatives was obtained. Gallotannins quantified by the rhodanine assay amounted to 1.4 mg/g dm in the peels (expressed as gallic acid), while only small amounts (0.2 mg/g dm) were found in the pulp. In contrast, mango kernels contained 15.5 mg/g dm and thus proved to be a rich source of gallotannins. Copyright © 2004 John Wiley & Sons, Ltd.

Extracts of mangos (*Mangifera indica* L., Anacardiaceae) are widespread herbal drugs in traditional medicine. In India seed extracts have been applied as an anti-diarrhoeal.[1] Extracts of the stem bark have been used for the treatment of diseases such as diarrhoea and cutaneous infections.[2] Very recent investigations have shown that these extracts exhibit antioxidative, immunomodulatory, analgesic, anti-inflammatory effects, and also inhibit macrophage activity.[3–7] The xanthone *C*-glycoside mangiferin has been demonstrated to be the predominant constituent of mango stem bark extracts, displaying a multitude of pharmacological effects. Gallotannins, which have been detected in mango stem bark, pulp, kernel and leaves,[8–13] are also likely candidates contributing to the described effects. However, information on their structures is rather limited and studies carried out so far date back to the 1960s and 1970s. More recent investigations have demonstrated that the tannins obtained from mango leaves are a mixture of penta- to undecagalloylglucoses with a 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose core.[14] Apart from gallotannins, the presence of several galloyl and *p*-hydroxybenzoyl esters of benzophenone *C*-glycosides has been reported. Maclurin 3-*C*-*β*-D-glucoside, maclurin 3-*C*-(6″-*O*-*p*-hydroxybenzoyl)-*β*-D-glucoside, maclurin 3-*C*-(2″-*O*-galloyl-6″-*O*-*p*-hydroxybenzoyl)-*β*-D-glucoside, maclurin 3-*C*-(2″-*O*-*p*-hydroxybenzoyl-6″-*O*-galloyl)-*β*-D-glucoside, maclurin 3-*C*-(2″,3″,6″-tri-*O*-galloyl)-*β*-D-glucoside, iriflophenone 3-*C*-(2″,6″-di-*O*-galloyl)-*β*-D-glucoside and iriflophenone 3-*C*-(2″,3″,6″-tri-*O*-galloyl)-*β*-D-glucoside have been isolated and characterized by NMR spetroscopy. The structures of maclurin and iriflophenone are given in Fig. 1. Maclurin 3-*C*-*β*-D-glucoside is considered a key intermediate in the biosynthesis of mangiferin and isomangiferin.

In a previous study the presence of a broad pattern of phenolic compounds, especially of flavonol glycosides, in mango puree[15] was demonstrated for the first time. Further investigations revealed that peels from the cultivar 'Tommy Atkins' are a promising source of flavonol and xanthone glycosides.[16] In continuation of these studies, gallotannins extracted from peels, pulp and kernels of this cultivar were characterized by high-performance liquid chromatography/electrospray ionization mass spectrometry (HPLC/ESI-MS). In addition, several galloyl esters of benzophenone *C*-glucosides so far not reported in mango fruits were detected in peel extracts and characterized by their fragmentation pattern.

**Correspondence to*: A. Schieber, Institute of Food Technology, Section Plant Foodstuff Technology, Hohenheim University, August-von-Hartmann-Strasse 3, D-70599 Stuttgart, Germany.
E-mail: Schieber@uni-hohenheim.de
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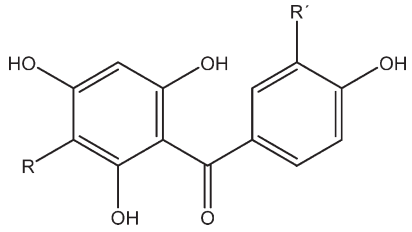

Figure 1. Structures of galloyl esters of maclurin (R′=OH) and iriflophenone (R′=H). R = galloyl-glucoside moiety.

EXPERMIENTAL

Solvents and reagents

Gallic acid was obtained from Sigma (Steinheim, Germany). Maclurin standard used for identification purposes with LC/MS was purchased from Extrasynthese (Lyon, France). Rhodanine was obtained from Fluka (Steinheim, Germany). All other reagents and solvents used were purchased from VWR (Darmstadt, Germany) and were of analytical or gradient grade. Deionized water was used throughout.

Sample preperation

According to Schieber *et al*.,[16] mature Brazilian mango fruits (cv. 'Tommy Atkins') obtained from the local market were washed and the peels were removed from the pulp with a stainless steel knife, immediately lyophilized, and finely ground using a S1/2 ball mill (Retsch, Haan, Germany). The pulp was lyophilized and ground in a mortar. After manual dehusking of the seed coat, the kernels were homogenized with a Grindomix GM 200 knife mill (Retsch). For the quantification of the tannins using the rhodanine assay the homogenized kernels were lyophilized.

Aliquots of 2.5 g of the lyophilized peels and 5.0 g of the lyophilized pulp, respectively, were mixed with 50 mL of aqueous acetone (80%, v/v) and 0.5 g of ascorbic acid in an amber glass round-bottomed flask. The mixture was extracted in a nitrogen atmosphere for 3 h under stirring at ambient temperature. The extract was centrifuged (10 min, 3480 *g*), and the residue was extracted with 50 mL of aqueous acetone for 10 min. The supernatants were combined and organic solvent was evaporated *in vacuo* at 30°C. The residual aqueous solution was transferred to a graduated flask and made up to 25 mL with deionized water and filtered through a fluted filter. The filtrates were evaporated to dryness *in vacuo* at 30°C and the residues were dissolved in 2.5 mL of aqueous methanol (20%, v/v). The samples were membrane-filtered (0.2 µm, Ziemer, Langerwehe, Germany) and used for LC/MS. 10.0 g of the homogenized kernels were defatted by triple extraction with 50 mL of hexane for 15 min under stirring. The extracts were centrifuged (10 min, 3480 *g*) and the supernatants were discarded. The residues were extracted with aqueous acetone as described above, without the described last concentration step.

LC/MS analyses

LC/MS analyses were performed using an Agilent (Waldbronn, Germany) HPLC series 1100 system equipped with ChemStation software, a model G1379A degasser, a model G1312A binary gradient pump, a model G1313A autosampler, a model G1316A column oven, and a model G1315B diode-array detector. The HPLC system was connected in series with a Bruker (Bremen, Germany) Esquire 3000+ ion trap mass spectrometer fitted with an ESI source. The column used was a 150 × 3.0 mm i.d., 4 µm Synergi Hydro-RP (Phenomenex, Torrance, USA), with a 4.0 × 2.0 mm i.d. C18 ODS guard column, operated at 25°C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and of 0.5% acetic acid in water/acetonitrile (50:50, v/v; eluent B). The flow rate was 0.5 mL/min and the gradient program was optimized as follows: 20% B to 35% B (25 min), 35% B to 40% B (25 min), 40% B to 80% B (20 min), 80% B (2 min), 80% B to 20% B (0.5 min). The injection volume was 4 µL for the peel and kernel samples and 50 µL for the pulp samples. Monitoring was performed at 280 nm and UV/Vis spectra were recorded from 200–600 nm (peak width 0.2 min). Negative ion mass spectra of the column eluate were recorded in the range *m/z* 50–2000 at a scan speed of 13 000 Th/s (peak width 0.6 Th, FWHM). Nitrogen was used as the drying gas at a flow rate of 10.0 L/min and at a pressure of 50 psi. The nebulizer temperature was set at 365°C. Collision-induced dissociation (CID) spectra were obtained with a fragmentation amplitude of 1.0 V using helium as the collision gas (1.2×10^{-5} mbar). Fragmentation experiments were performed in Auto-MS mode. If no unambiguous results were obtained using this method, the experiments were repeated using manual isolation and fragmentation of ions.

Quantification of hydrolyzable tannins

The quantification of hydrolyzable tannins was carried out as described by Inoue and Hagerman.[17] For this purpose, 1 g of the lyophilized peel and pulp and 0.3 g of the lyophilized kernel, respectively, were extracted for 30 min with 1 mL of aqueous acetone (70%, v/v) in a sonicator. The extracts were vacuum filtered through an Econo-Pac column (BioRad, Munich, Germany) filled with glass wool into a screw-cap test tube. The column was washed with 5 mL of 2 N H_2SO_4. The test tube was flushed with nitrogen and sealed tightly. After heating at 100°C for 26 h, the samples were diluted to 50 mL with water, an aliquot of 1 mL was pipetted in a 25 mL graduated flask, and 1.5 mL of 0.667% methanolic rhodanine solution were added. After 5 min, 1 mL of 0.5 N aqueous potassium hydroxide solution was added. After 2.5 min the graduated flask was made up with water, and after another 10 min the absorbance at 520 nm was recorded using a Lambda 20 spectrometer (Perkin Elmer, Überlingen, Germany). The amount of free gallic acid was determined in the same way but without hydrolysis.

RESULTS AND DISCUSSION

LC/MS analysis of mango peel polyphenolics

The separation of polyphenolics in mango peel extracts is shown in Fig. 2. Among the large number of phenolic compounds, 33 could be characterized as benzophenone derivatives (1, 2, 5, 7, 11), flavonols (12–14, 16–18), xanthones (3, 6, 10) and gallotannins (4, 8, 9, 15, 19–32) by their UV and mass spectrometric data, which are given in Table 1.

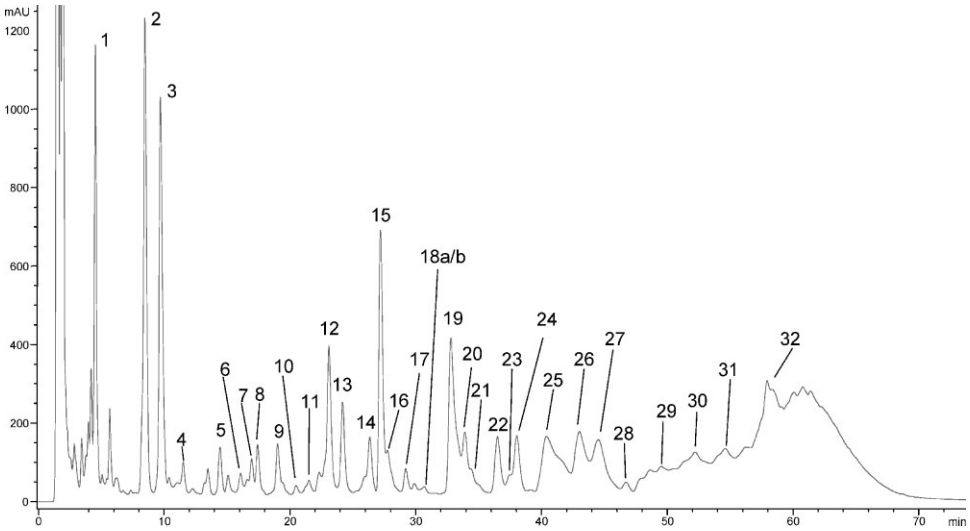

Figure 2. Separation of benzophenone derivatives and gallotannins in mango peels by HPLC (280 nm). Peak assignment: (1) maclurin mono-*O*-galloyl-glucoside, (2) maclurin di-*O*-galloyl-glucoside, (3) mangiferin, (4) tetra-*O*-galloyl-glucose, (5) iriflophenone di-*O*-galloyl-glucoside, (6) mangiferin gallate, (7) maclurin tri-*O*-galloyl-glucoside, (8) + (9) tetra-*O*-galloyl-glucose, (10) isomangiferin gallate, (11) maclurin tri-*O*-galloyl-glucose, (12) quercetin 3-*O*-galactoside, (13) quercetin 3-*O*-glucoside, (14) quercetin 3-*O*-xyloside, (15) penta-*O*-galloyl-glucose, (16) quercetin 3-*O*-arabinopyranoside, (17) quercetin 3-*O*-arabinofuranoside, (18a) quercetin 3-*O*-rhamnoside, (18b) kaempferol 3-*O*-glucoside, (19)–(24) hexa-*O*-galloyl-glucose, (25)–(28) hepta-*O*-galloyl-glucose, (29)–(31) octa-*O*-galloyl-glucose, (32) nona-*O*-galloyl-glucose.

Table 1. UV spectra and characteristic ions of benzophenone derivatives and gallotannins extracted from peels of *Mangifera indica* L. cv. 'Tommy Atkins'[a]

Peak	Identity	HPLC-DAD λ_{max} [nm]	$[M-H]^-$ *m/z*	HPLC-ESI(-)-MS^n experiment *m/z* (% base peak)
1	maclurin mono-*O*-galloyl-glucoside	231, 283, 322sh	575	-MS^2 [575]: 465 (26), 455 (22), 439 (23), 423 (21), 327 (19), 313 (22), 303 (100), 285 (28), 261 (21), 193 (29) - MS^3 [575 → 303]: 193 (100), 167 (23), 165 (12) - MS^4 [575 → 303 → 193]: 165 (100), 149 (86)
2	maclurin di-*O*-galloyl-glucoside	233, 280, 322sh	727	- MS^2 [727]: 576 (55), 575 (100), 557 (10), 466 (10), 465 (20) - MS^3 [727 → 575]: 531 (18), 485 (100), 465 (31), 439 (64), 423 (22), 405 (49), 369 (24), 333 (27), 315 (33), 313 (28) - MS^4 [727 → 575 → 485]: 333 (100) - MS^5 [727 → 575 → 485 → 333]: 223 (100)
3	mangiferin		421	
4	tetra-*O*-galloyl-glucose	230, 278	787	- MS^2 [787]: 635 (100), 617 (17), 465 (11) - MS^3 [787 → 635]: 465 (100) - MS^4 [787 → 635 → 465]: 313 (100), 169 (76)
5	iriflophenone di-*O*-galloyl-glucoside	231, 281, 323sh	711	- MS^2 [711]: 560 (47), 559 (100), 390 (12), 389 (26) - MS^3 [711 → 559]: 469 (20), 439 (12), 407 (30), 389 (100), 317 (13), 299 (11) - MS^4 [711 → 559 → 389]: 360 (100), 353 (48), 345 (56), 299 (90) - MS^5 [711 → 559 → 389 → 299]: 205 (100)
6	mangiferin gallate		573	
7	maclurin tri-*O*-galloyl-glucoside	230, 281, 322sh	879	- MS^2 [879]: 727 (100) - MS^3 [879 → 727]: 575 (100), 485 (59), 465 (11), 333 (11), 315 (16) - MS^4 [879 → 727 → 575]: 485 (100), 405 (73), 287 (38) - MS^5 [879 → 727 → 575 → 485]: 349 (39), 333 (81), 315 (100) - MS^6 [879 → 727 → 575 → 485 → 315]: 205 (100)
8	tetra-*O*-galloyl-glucose	230, 278	787	- MS^2 [787]: 635 (100), 617 (20) - MS^3 [787 → 635]: 617 (55), 483 (61), 465 (100), 423 (23) - MS^4 [787 → 635 → 465]: 313 (100)

Continues

Table 1. Continued

Peak	Identity	HPLC-DAD λ_{max} [nm]	$[M-H]^-$ m/z	HPLC-ESI(-)-MS^n experiment m/z (% base peak)
9	tetra-*O*-galloyl-glucose	230, 278	787	- MS^2 [787]: 635 (25), 617 (100) - MS^3 [787 → 617]: 573 (96), 465 (100), 447 (39), 403 (23) - MS^4 [787 → 617 → 465]: 465 (47), 313 (100)
10	isomangiferin gallate		573	
11	maclurin tri-*O*-galloyl-glucoside	231, 281, 325sh	879	- MS^2 [879]: 727 (100) - MS^3 [879 → 727]: 591 (29), 575 (100), 485 (20), 405 (23) - MS^4 [879 → 727 → 575]: 485 (56), 405 (100), 315 (54), 219 (49) - MS^5 [879 → 727 → 575 → 405]: 369 (60), 315 (100) - MS^6 [879 → 727 → 575 → 405 → 315]: 205 (100)
12	quercetin 3-*O*-galactoside		463	
13	quercetin 3-*O*-glucoside		463	
14	quercetin 3-*O*-xyloside		433	
15	penta-*O*-galloyl-glucose	231, 279	939	- MS^2 [939]: 787 (12), 769 (100), 617 (12) - MS^3 [939 → 769]: 725 (13), 617 (100), 601 (37), 599 (36) - MS^4 [939 → 769 → 617]: 617 (33), 465 (100)
16	quercetin 3-*O*-arabinopyranoside		433	
17	quercetin 3-*O*-arabinofuranoside		433	
18a	quercetin 3-*O*-rhamnoside		447	
18b	kaempferol 3-*O*-glucoside		447	
19	hexa-*O*-galloyl-glucose	230, 279	1091	- MS^2 [1091]: 939 (100) - MS^3 [1091 → 939]: 787 (19), 769 (100), 617 (35) - MS^4 [1091 → 939 → 769]: 725 (10), 617 (100), 601 (38), 599 (35), 369 (29)
20	hexa-*O*-galloyl-glucose	230, 278	1091	- MS^2 [1091]: 939 (100) - MS^3 [1091 → 939]: 769 (100), 617 (11) - MS^4 [1091 → 939 → 769]: 617 (100), 601 (18), 599 (18), 446 (13) 431 (14)
21	hexa-*O*-galloyl-glucose	231, 280	1091	- MS^2 [1091]: 939 (100) - MS^3 [1091 → 939]: 787 (15), 769 (100), 635 (20), 601 (28) - MS^4 [1091 → 939 → 769]: 725 (23), 617 (65)
22	hexa-*O*-galloyl-glucose	229, 278	1091	- MS^2 [1091]: 939 (100) - MS^3 [1091 → 939]: 769 (100), 599 (12) - MS^4 [1091 → 939 → 769]: 617 (100)
23	hexa-*O*-galloyl-glucose	230, 277	1091	- MS^2 [1091]: 939 (100) - MS^3 [1091 → 939]: 787 (45), 769 (100), 617 (10) - MS^4 [1091 → 939 → 769]: 725 (36), 617 (100), 429 (27), 271 (12)
24	hexa-*O*-galloyl-glucose	229, 279	1091	- MS^2 [1091]: 939 (100) - MS^3 [1091 → 939]: 787 (12), 769 (100), 617 (19) - MS^4 [1091 → 939 → 769]: 617 (100), 601 (53), 429 (13)
25	hepta-*O*-galloyl-glucose	229, 277	1243	- MS^2 [1243]: 1091 (93), 939 (100) - MS^3 [1243 → 939]: 769 (100) - MS^4 [1243 → 939 → 769]: 725 (84), 617 (100), 601 (24)
26	hepta-*O*-galloyl-glucose	229, 277	1243	- MS^2 [1243]: 1091 (83), 939 (100) - MS^3 [1243 → 939]: 769 (100), 617 (14), 601 (13), 447 (12) - MS^4 [1243 → 939 → 769]: 617 (174), 429 (100)
27	hepta-*O*-galloyl-glucose	230, 279	1243	- MS^2 [1243]: 1091 (100), 939 (95) - MS^3 [1243 → 939]: 787 (18), 769 (100) - MS^4 [1243 → 939 → 769]: 617 (100), 571 (25)
28	hepta-*O*-galloyl-glucose	229, 279	1243	- MS^2 [1243]: 1091 (65), 939 (100), 769 (11) - MS^3 [1243 → 939]: 769 (100), 617 (17) - MS^4 [1243 → 939 → 769]: 617 (100), 601 (43)
29	octa-*O*-galloyl-glucose	229, 278	1395	- MS^2 [1395]: 1243 (76), 1091 (100) - MS^3 [1395 → 1091]: 939 (100) - MS^4 [1395 → 1091 → 939]: 769 (100)
30	octa-*O*-galloyl-glucose	229, 278	1395	- MS^2 [1395]: 1243 (58), 1091 (100), 939 (18) - MS^3 [1395 → 1091]: 939 (100) - MS^4 [1395 → 1091 → 939]: 769 (100)
31	octa-*O*-galloyl-glucose	230, 278	1395	- MS^2 [1395]: 1243 (53), 1091 (100) - MS^3 [1395 → 1091]: 939 (100) - MS^4 [1395 → 1091 → 939]: 769 (100)
32	nona-*O*-galloyl-glucose	229, 276	1547	- MS^2 [1547]: 1541 (29), 1395 (100), 1243 (32), 1091 (36) - MS^3 [1547 → 1395]: 1091 (100)
Std	maclurin	234, 300, 316	261	- MS^2 [261]: 151 (100), 107 (17) - MS^3 [261 → 151]: 107 (100)

[a] Data of maclurin reference (Std) is also included.

Benzophenone derivatives

Compound 1 showed a $[M-H]^-$ ion of *m/z* 575 (Table 1) and a loss of 272 Da in the MS/MS experiment, resulting in the formation of a prominent fragment ion of *m/z* 303. The MS^3 experiment yielded a fragment of *m/z* 193 (loss of 110 Da) as the predominant ion which decarboxylated (loss of 44 Da) in the MS^4 experiment. This fragmentation behavior is typical of *C*-glycosides which, in contrast to *O*-glycosides, do not generate abundant aglycone ions but show losses of 120 and 90 Da, corresponding to cross-ring cleavage of the sugar moiety (Fig. 3).[18,19] Therefore, the loss of 272 Da ($[M-H-120-152]^-$) observed for compound 1 is indicative of the presence of a galloylated benzophenone *C*-glycoside, especially since in the MS/MS experiment another fragment (*m/z* 423) corresponding to the loss of a galloyl moiety was also detected. Since the maclurin reference substance showed a loss of 110 Da in the MS^2 experiment followed by a decarboxylation in the MS^3 event (Fig. 4), compound 1 could be tentatively identified as a maclurin *O*-galloyl-glucoside. This pathway has recently been described for the flavonoid luteolin.[19,20] Several galloyl esters of benzophenone *C*-glucosides have been identified in mango leaves,[14] but, to the best of our knowledge, this is the first report of galloylated benzophenone derivatives in mango peels. These findings provide evidence that in the biosynthesis of mangiferin and isomangiferin, galloylation takes place prior to cyclization of benzophenones.

Compound 2 showed a UV spectrum identical to compound 1 and a $[M-H]^-$ ion of *m/z* 727. The loss of a galloyl moiety (152 Da) yielded a product ion at *m/z* 575 in the MS/MS experiment. CID of this fragment resulted in the loss of 90 Da. In the MS^4 experiment the loss of a second galloyl moiety was observed. The resulting fragment with *m/z* 333 provided a product ion of *m/z* 223, corresponding to a loss of 110 Da, that has also been observed with compound 1 and the maclurin reference. Therefore, compound 2 was assigned to a maclurin di-*O*-galloyl-glucoside.

The UV spectrum of compound 5 proved to be identical to those of compounds 1 and 2. CID of the $[M-H]^-$ ion of *m/z* 711 showed the loss of a galloyl moiety (152 Da), yielding a prominent fragment of *m/z* 559. Further fragmentation caused the loss of a gallic acid (170 Da) which has previously been observed for gallotannins.[21,22] Since CID of the fragment of *m/z* 360 did not provide any useful information, further fragmentation experiments were carried out on the *m/z* 299 ion (loss of 90 Da) with a relative intensity of 90%. The loss of 94 Da indicated that compound 5 is not a maclurin but an iriflophenone derivative. Based on these findings, compound 5 was assigned to iriflophenone di-*O*-galloyl-glucoside. Iriflophenone di- and tri-*O*-galloyl-glucosides have previously been found in mango leaves but have not been detected in the fruits.[14]

Compounds 7 and 11 both showed an $[M-H]^-$ ion of *m/z* 879. Their UV spectra were identical to the benzophenone ester spectra described above. In the MS^2 and MS^3 experiments the loss of a galloyl moiety (152 Da) could be observed for both compounds. CID of the fragment with *m/z* 575 resulted in the formation of two prominent fragments (*m/z* 485 and 405), however, of different relative intensities. While *m/z* 485 (loss of 90 Da) was the predominant fragment in the case of compound 7, *m/z* 405 proved to be the most intense fragment of compound 11 and corresponded to the loss of a gallic acid moiety (170 Da). In the MS^5 and MS^6 experiments identical predominant fragments (*m/z* 315 and 205) were observed for both compounds. Taking together these findings, compounds 7 and 11 were both assigned to maclurin tri-*O*-galloyl-glucosides.

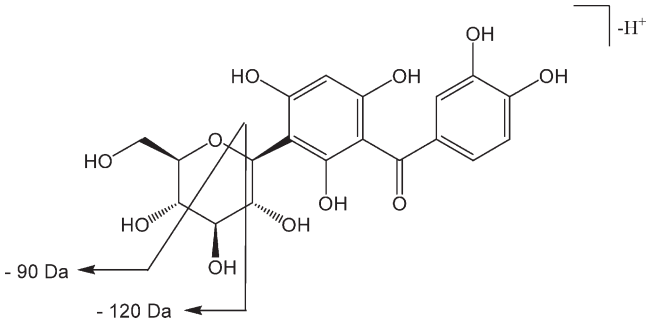

Figure 3. Postulated fragmentation pathways of maclurin *C*-glycosides.

Flavonols and xanthones

Since the characterization of flavonol 3-*O*-glycosides and xanthone *C*-glucosides in mango peels has recently been reported in detail,[16] UV and mass spectrometric data are not given in Table 1. Mangiferin (3), mangiferin gallate (6), isomangiferin gallate (10), quercetin (Q) 3-*O*-galactoside (12), Q 3-*O*-glucoside (13), Q 3-*O*-xyloside (14), Q 3-*O*-arabinopyranoside (16), Q 3-*O*-arabinofuranoside (17), Q 3-*O*-rhamnoside (18a), and kaempferol 3-*O*-glucoside (18b) could unambiguously be identified.

Gallotannins

Compounds 4, 8, 9, 15 and 19–32 showed nearly identical UV spectra which are very similar to that of gallic acid.

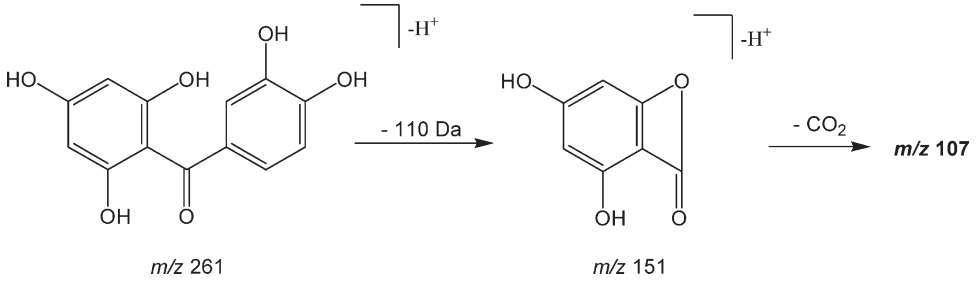

Figure 4. Postulated fragmentation pathway of maclurin.

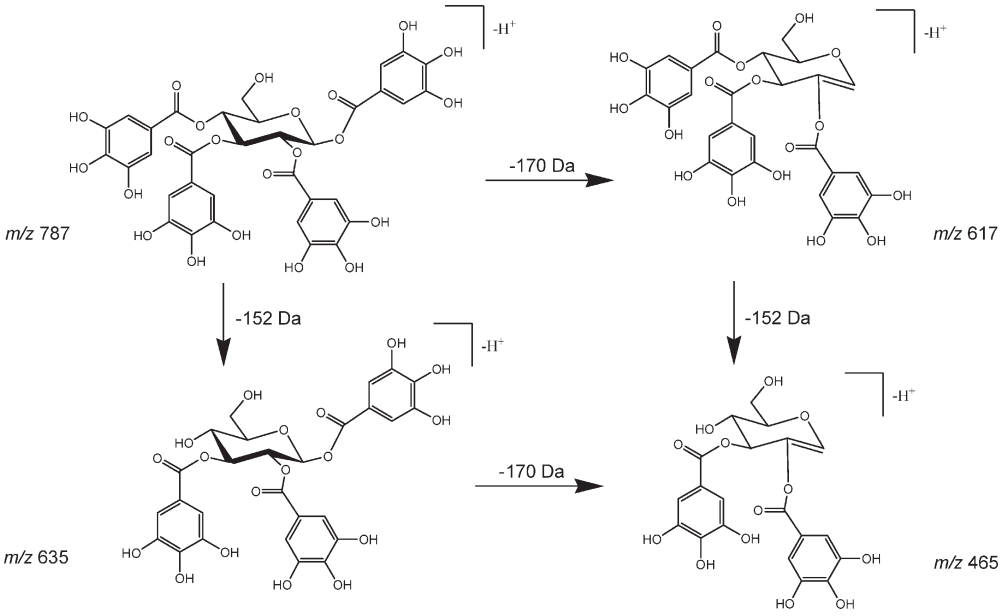

Figure 5. Postulated fragmentation pathway of tetra-*O*-galloyl-glucose.

Compounds 4, 8 and 9 all provided $[M-H]^-$ ions of *m/z* 787 and fragments of *m/z* 635 and 617 in the MS^2 experiment caused by the loss of a galloyl moiety (152 Da) and gallic acid (170 Da), respectively. In contrast to compounds 4 and 8, however, *m/z* 617 was the most intense fragment of compound 9. Since MS^3 and MS^4 experiments revealed the loss of two further gallic acid and galloyl moieties, compounds 4, 8 and 9 were identified as tetra-*O*-galloyl-glucoses. Postulated fragmentation pathways are exemplified in Fig. 5.[21,22] In the same way, compound 15 could be assigned to penta-*O*-galloyl-glucose, while compounds 19–24 were identified as hexa-*O*-galloyl-glucoses. Remarkably, the latter compounds did not show the loss of 170 Da (gallic acid) in the MS^2 experiment, confirming previous reports that galloyl residues attached to the penta-*O*-galloyl-glucose core via *meta*-depside bonds are more susceptible to cleavage than those directly linked to the glucose core.[22] Compounds 25–32 displayed identical fragmentation patterns of gallotannins and were identified as hepta-, octa- and nona-*O*-galloyl-glucoses by their $[M-H]^-$ ions of *m/z* 1243, 1395 and 1547.

LC/MS analysis of mango kernel polyphenolics

The separation of polyphenolics extracted from mango kernels is shown in Fig. 6. All compounds were identified as gallotannins consisting of glucose and four to nine gallic acid moieties. Their UV and mass spectrometric data are given in Table 2. It is of particular interest that the elution profile of the gallotannins shown in Fig. 6 is almost identical to that reported by Kabuki *et al.*,[23] who investigated the antimicrobial properties of several fractions; however, they did not elucidate the structure of the bioactive constituents. Taking together their findings and the results of our studies, it is concluded that the antimicrobial activities observed by Kabuki *et al.*[23] may be attributed to gallotannins.

LC/MS analysis of mango pulp polyphenolics

Our preliminary investigations had already shown that in contrast to mango peels the pulp is a poor source of polyphenolics. As can be seen from Table 3, eight compounds were characterized as galloyl-glucoses by their UV and mass spectra. Based on the fragmentation patterns already discussed above, compounds 1–8 could be identified as tetra-, penta-, hexa- and hepta-*O*-galloyl-glucoses, respectively.

Rhodanine assay of gallotannins

Owing to their structural variability and the lack of reference substances, the quantification of gallotannins is difficult. A common way to estimate the amount of gallotannins is the rhodanine assay. After acidic hydrolysis, gallic acid released from the tannins reacts with rhodanine to yield a red complex which can be determined spectrophotometrically at 520 nm.[17] Free gallic acid present in the samples is determined in the same way and subtracted. In the peels, 1.4 mg/g dry matter gallotannins, expressed as gallic acid, were found, while only 0.2 mg/g was determined in the pulp. In contrast, the kernels proved to be a very rich source of gallotannins, amounting to 15.5 mg/g dry matter. Contents reported so far ranged from 0.2–5.7% on a dry matter basis;[10,24–27] however, due to the different assays used for quantification, data are difficult to compare.

CONCLUSIONS

Using ESI-LC/MS^n, a large number of individual gallotannins and several benzophenone *C*-glycosides were characterized in mango fruits for the first time. The results obtained in the present study demonstrate that by-products of mango processing are a rich source of polyphenols which may be used as functional food ingredients, as antimicrobials or as antioxidants. Since mango kernel fat has already been approved as a cocoa butter substitute and the peels are known to contain a high-value pectin, the recovery of polyphenols from seeds and peels would allow further valorization of by-products. Apart from their use in food and feed, pharmaceutical applications may also be taken into consideration. From a phytochemical point of view, the detection

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Table 2. UV spectra and characteristic ions of gallotannins extracted from kernels of *Mangifera indica* L. cv. 'Tommy Atkins'

Peak	Identity	HPLC-DAD λ_{max} [nm]	$[M-H]^-$ m/z	HPLC-ESI(-)-MS^n experiment m/z (% base peak)
1	tetra-*O*-galloyl-glucose	230, 278	787	- MS^2 [787]: 636 (12), 635 (100), 617 (16) - MS^3 [787 → 635]: 483 (100) - MS^4 [787 → 635 → 483]: 313 (100)
2	tetra-*O*-galloyl-glucose	230, 281	787	- MS^2 [787]: 635 (25), 618 (19), 617 (100) - MS^3 [787 → 617]: 465 (100) - MS^4 [787 → 617 → 465]: 313 (100), 295 (53)
3	tetra-*O*-galloyl-glucose	230, 277	787	- MS^2 [787]: 635 (100), 617 (30), 483 (15) - MS^3 [787 → 635]: 617 (47), 483 (712), 465 (100), 423 (43), 313 (32) - MS^4 [787 → 635 → 465]: 313 (100)
4	tetra-*O*-galloyl-glucose	230, 278	787	- MS^2 [787]: 635 (16), 617 (100) - MS^3 [787 → 617]: 465 (100) - MS^4 [787 → 617 → 465]: 313 (100)
5	tetra-*O*-galloyl-glucose	229, 279	787	- MS^2 [787]: 636 (22), 635 (67), 618 (15), 617 (100) - MS^3 [787 → 617]: 617 (45), 466 (47), 465 (100) - MS^4 [787 → 617 → 465]: 313 (100)
6	penta-*O*-galloyl-glucose	229, 279	939	- MS^2 [939]: 787 (100) - MS^3 [939 → 787]: 635 (16), 617 (100) - MS^4 [939 → 787 → 617]: 465 (100)
7	tetra-*O*-galloyl-glucose	229, 278	787	- MS^2 [787]: 618 (34), 617 (100) - MS^3 [787 → 617]: 617 (77), 466 (53), 465 (100) - MS^4 [787 → 617 → 465]: 313 (100)
8	penta-*O*-galloyl-glucose	229, 281	939	- MS^2 [939]: 787 (100) - MS^3 [939 → 787]: 635 (100) - MS^4 [939 → 787 → 635]: 483 (76), 465 (100), 295 (63)
9	penta-*O*-galloyl-glucose	231, 280	939	- MS^2 [939]: 770 (31), 769 (100) - MS^3 [939 → 787]: 617 (100), 601 (23) - MS^4 [939 → 787 → 617]: 465 (100), 447 (97)
10	hexa-*O*-galloyl-glucose	231, 278	1091	- MS^2 [1091]: 940 (19), 939 (100) - MS^3 [1091 → 939]: 770 (13), 769 (100), 617 (12) - MS^4 [1091 → 939 → 769]: 617 (100)
11	hexa-*O*-galloyl-glucose	230, 279	1091	- MS^2 [1091]: 940 (19), 939 (100) - MS^3 [1091 → 939]: 770 (19), 769 (100), 617 (15) - MS^4 [1091 → 939 → 769]: 617 (100)
12	hexa-*O*-galloyl-glucose	230, 279	1091	- MS^2 [1091]: 940 (15), 939 (100) - MS^3 [1091 → 939]: 770 (15), 769 (100), 617 (25) - MS^4 [1091 → 939 → 769]: 617 (100), 431 (36)
13	hexa-*O*-galloyl-glucose	230, 280	1091	- MS^2 [1091]: 940 (10), 939 (100), 787 (16) - MS^3 [1091 → 939]: 787 (66), 769 (100), 617 (25) - MS^4 [1091 → 939 → 769]: 617 (100), 235 (59)
14	hexa-*O*-galloyl-glucose	230, 280	1091	- MS^2 [1091]: 940 (15), 939 (100) - MS^3 [1091 → 939]: 770 (11), 769 (100) - MS^4 [1091 → 939 → 769]: 617 (100)
15	hepta-*O*-galloyl-glucose	230, 277	1243	- MS^2 [1243]: 1091 (71), 939 (100) - MS^3 [1243 → 939]: 787 (22), 770 (10), 769 (100), 617 (30) - MS^4 [1243 → 939 → 769]: 617 (100)
16	hepta-*O*-galloyl-glucose	230, 277	1243	- MS^2 [1243]: 1091 (81), 939 (100) - MS^3 [1243 → 939]: 787 (17), 769 (100), 617 (11) - MS^4 [1243 → 939 → 769]: 617 (100)
17	hepta-*O*-galloyl-glucose	230, 278	1243	- MS^2 [1243]: 1091 (69), 939 (100) - MS^3 [1243 → 939]: 787 (16), 769 (100) - MS^4 [1243 → 939 → 769]: 617 (100)
18	hepta-*O*-galloyl-glucose	230, 278	1243	- MS^2 [1243]: 1091 (73), 939 (100) - MS^3 [1243 → 939]: 769 (100) - MS^4 [1243 → 939 → 769]: 617 (100)
19	hepta-*O*-galloyl-glucose	230, 279	1243	- MS^2 [1243]: 1091 (64), 939 (100) - MS^3 [1243 → 939]: 787 (17), 770 (16), 769 (100) - MS^4 [1243 → 939 → 769]: 617 (100)
20	octa-*O*-galloyl-glucose	230, 278	1395	- MS^2 [1395]: 1243 (66), 1091 (100), 939 (100) - MS^3 [1395 → 939]: 787 (17), 769 (100), 617 (12) - MS^4 [1395 → 939 → 769]: 617 (100)
21	nona-*O*-galloyl-glucose	230, 276	1547	- MS^2 [1547]: 1397 (42), 1396 (31), 1395 (82), 1245 (34), 1244 (34), 1243 (100), 1093 (13), 1092 (17), 1091 (45), 939 (22) - MS^3 [1547 → 1243]: 1091 (63), 939 (100) - MS^4 [1547 → 1243 → 939]: 788 (10), 770 (10), 769 (100)

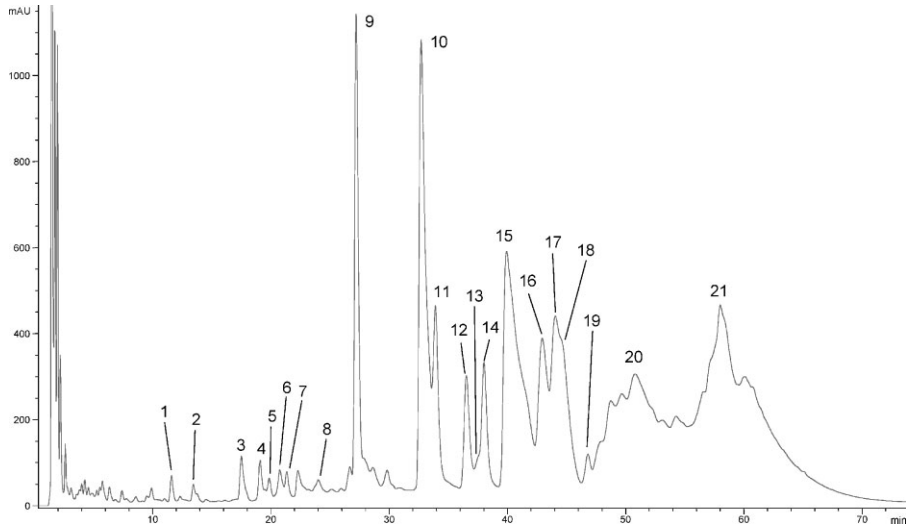

Figure 6. Separation of gallotannins in mango kernels by HPLC (280 nm). Peak assignment: (1)–(5) tetra-*O*-galloyl-glucose, (6) penta-*O*-galloyl-glucose, (7) tetra-*O*-galloyl-glucose, (8) + (9) penta-*O*-galloyl-glucose, (10)–(14) hexa-*O*-galloyl-glucose, (15)–(19) hepta-*O*-galloyl-glucose, (20) octa-*O*-galloyl-glucose, (21) nona-*O*-galloyl-glucose.

Table 3. UV spectra and characteristic ions of gallotannins extracted from the pulp of *Mangifera indica* L. cv. 'Tommy Atkins'

Peak	Identity	HPLC-DAD λ_{max} [nm]	$[M-H]^-$ *m/z*	HPLC-ESI(-)-MS^n experiment *m/z* (% base peak)
1	tetra-*O*-galloyl-glucose	231, 280	787	- MS^2 [787]: 635 (13), 618 (17), 617 (100)
2	penta-*O*-galloyl-glucose	230, 279	939	- MS^2 [939]: 787 (15), 770 (25), 769 (100), 617 (12) - MS^3 [939 → 769]: 617 (100) - MS^4 [939 → 769 → 617]: 465 (100)
3	hexa-*O*-galloyl-glucose	230, 278	1091	- MS^2 [1091]: 940 (19), 939 (100) - MS^3 [1091 → 939]: 787 (17), 769 (100) - MS^4 [1091 → 939 → 769]: 617 (100)
4	hexa-*O*-galloyl-glucose	230, 278	1091	- MS^2 [1091]: 940 (11), 939 (100) - MS^3 [1091 → 939]: 788 (15), 787 (32), 770 (15), 769 (100) - MS^4 [1091 → 939 → 769]: 617 (100), 599 (72)
5	hexa-*O*-galloyl-glucose	230, 281	1091	- MS^2 [1091]: 940 (19), 939 (100) - MS^3 [1091 → 939]: 787 (18), 769 (100), 617 (13) - MS^4 [1091 → 939 → 769]: 617 (100)
6	hepta-*O*-galloyl-glucose	230, 277	1243	- MS^2 [1243]: 1091 (80), 939 (100) - MS^3 [1243 → 939]: 769 (100), 617 (21)
7	hepta-*O*-galloyl-glucose	230, 277	1243	- MS^2 [1243]: 1091 (64), 939 (100) - MS^3 [1243 → 939]: 769 (100) - MS^4 [1243 → 939 → 769]: 617 (100)
8	hepta-*O*-galloyl-glucose	230, 279	1243	- MS^2 [1243]: 1091 (91), 939 (100) - MS^3 [1243 → 939]: 769 (100), 617 (13) - MS^4 [1243 → 939 → 769]: 617 (100)

of galloylated maclurin and iriflophenone *C*-glycosides may also be helpful in understanding the biosynthetic pathways of xanthone derivatives occurring in mango.

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