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Identification of Flavonol and Xanthone Glycosides from Mango (*Mangifera indica* L. Cv. "Tommy Atkins") Peels by High-Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry

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Flavonol *O*- and xanthone *C*-glycosides were extracted from mango (*Mangifera indica* L. cv. "Tommy Atkins") peels and characterized by high-performance liquid chromatography-electrospray ionization mass spectrometry. Among the fourteen compounds analyzed, seven quercetin *O*-glycosides, one kaempferol *O*-glycoside, and four xanthone *C*-glycosides were found. On the basis of their fragmentation pattern, the latter were identified as mangiferin and isomangiferin and their respective galloyl derivatives. A flavonol hexoside with *m*/*z* 477 was tentatively identified as a rhamnetin glycoside, which to the best of our knowledge, has not yet been reported in mango peels. The results obtained in the present study confirm that peels originating from mango fruit processing are a promising source of phenolic compounds that might be recovered and used as natural antioxidants or functional food ingredients.

KEYWORDS: Mango; *Mangifera indica* (L.); peels; flavonols; xanthones; HPLC-MS/MS

INTRODUCTION

Mango (*Mangifera indica* L., Anacardiaceae) is one of the most important tropical fruits. Mango and mango products such as puree, nectar, leather, chutneys, pickles, and canned slices experience worldwide popularity and have also gained increasing importance in the European market (*1*). Major byproducts of mango processing are peels and seeds, amounting for 35 and 60% of the total fruit weight, respectively (*2*). Because these byproducts represent a serious disposal problem, various attempts at utilizing seed kernels and peels have been made in the past decades. While a number of investigations have been conducted on the composition and possible utilization of mango seed kernels (*3*−*7*), studies on peels are scarce.

Srirangarajan and Shrikhande (*8*) investigated the chemical and physical characteristics of mango peel pectin and suggested its commercial exploitation. Sudhakar and Maini (*9*) developed a standardized method for the recovery of pectin from "Totapuri" mango peels. In more recent investigations, mango peels were reported to be a good source of dietary fiber containing large amounts of total extractable polyphenolics (*2*). This high content of polyphenolics was reflected by high antioxidative activity in in vitro studies (*10*, *11*). Because total extractable polyphenolics were determined by the Folin-Ciocalteu assay, no conclusions could be drawn as to their chemical structures. Furthermore, owing to their lack of selectivity, spectrophotometric methods tend to overestimate the phenolic content (*12*).

In a previous study, the presence of a broad pattern of phenolic compounds, especially of flavonol glycosides, in mango puree concentrate was demonstrated for the first time (*13*). It was assumed that these phenolics may in part originate from the peel, because mango puree is prepared from both peeled and unpeeled fruits, and especially because total polyphenolics are higher in the peel than in the pulp at all stages of mango fruit development (*14*). However, because the sample examined was a commercial puree concentrate, the origin of the polyphenolics could not be conclusively elucidated.

In continuation of our investigations on the recovery of natural food ingredients from byproducts of fruit processing (*15*, *16*), polyphenolics were extracted from mango peels and characterized by HPLC with diode array and mass spectrometric detection.

MATERIALS AND METHODS

Standards. Standards used for identification purposes with HPLC and MS were as follows: quercetin 3-*O*-xyloside, quercetin 3-*O*-arabinofuranoside (Plantech, Reading, UK); quercetin, quercetin 3-*O*-arabinopyranoside, quercetin 3-*O*-arabinoglucoside (Roth, Karlsruhe, Germany); mangiferin [2-*C*-*β*-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone], quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, kaempferol 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside, rhamnetin (Extrasynthese, Lyon, France).

Sample Preparation. Mature Peruvian mango fruits of the cultivar Tommy Atkins were obtained from the local market. The peels were removed from the flesh with a stainless steel knife, immediately lyophilized, and finely ground using an S1/2 ball mill (Retsch, Haan,

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Germany). Aliquots of 2.5 g of the lyophilizate were weighed into an amber glass round-bottomed flask. After addition of 0.5 g of ascorbic acid, the flask was flushed with nitrogen and the mixture extracted with 50 mL of aqueous acetone (80%, v/v) for 3 h under stirring. The extract was centrifuged (10 min, 4000 rpm), and the residue extracted with 50 mL of aqueous acetone for 10 min. The organic solvent was removed from the combined supernatants by evaporation in vacuo at 30 °C. The aqueous solution was transferred into a graduated flask and made up to 50 mL with deionized water. After microfiltration (5 μm), aliquots of 20 mL were used for further purification. Polyamide CC6 (2 g, 0.05−0.16 mm) (Macherey-Nagel, Dueren, Germany) was filled into an Econo-Pac column (BioRad, Munich, Germany) and successively conditioned with 25 mL methanol and 50 mL deionized water prior to application of the peel extract to the column. After washing with water (50 mL), the polyphenolic fraction was recovered by elution with methanol (100 mL). The eluate was evaporated to dryness, and the residue was dissolved in 0.5 mL of aqueous methanol. The solution was membrane-filtered (0.45 μm, Whatman Inc., Clifton, NJ) and used for HPLC.

HPLC Analysis. The separation of phenolic compounds was performed using an Agilent HPLC, series 1100 (Agilent, Waldbronn, Germany) equipped with ChemStation software, a model G1322A degasser, a model G1312A binary gradient pump, a model G1329/1330A thermoautosampler, a model G1316A column oven, and a model G1315A diode array detector. The column used was a 150 × 3.0-mm i.d., 4-μm C18 Hydro-Synergi (Phenomenex, Torrance, CA), with a 4.0 × 2.0-mm i.d. C18 ODS guard column, operated at a temperature of 25 °C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and of 0.5% acetic acid in water and acetonitrile (50:50, v/v; eluent B). The gradient program was as follows: 0−25% B (15 min), 25−30% B (35 min), 30−80% B (10 min), 80−100% B (5 min), 100−0% B (0.5 min). The injection volume for all samples was 4 μL. Simultaneous monitoring was performed at 320 nm (xanthones), and 370 nm (flavonols) at a flow rate of 0.6 mL/min. Spectra were recorded from 200 to 600 nm (peak width 0.2 min, data rate 1.25/s).

LC-MS Analyses. LC-MS analyses were performed with the same HPLC system as described above connected in series with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an ESI source. Negative ion mass spectra of the column eluate were recorded in the range *m*/*z* 50−1000. Nitrogen was used as the dry gas at a flow rate of 10.0 L/min and at a pressure of 60.0 psi. The nebulizer temperature was set at 365 °C. Collision-induced dissociation spectra were obtained with a fragmentation amplitude of 1.2 V (MS/MS) and 1.5 V (MS>2) for flavonoids, 1.2 V (MS/MS) and 1.7 V (MS>2) for xanthones, 1.2 V (MS/MS and MS^3) and 1.7 V (MS^4) for xanthone gallates, 1.5 V (MS/MS) for flavonoid aglycones. Helium was used as the collision gas (1.2×10^{-5} mbar). Quercetin 3-*O*-galactoside was used for the optimization of ionization parameters.

RESULTS AND DISCUSSION

Extraction and Purification of Phenolic Compounds. In our previous study, polyphenolics were extracted from a commercial mango puree concentrate with organic solvents and fractionated on Sephadex LH20. The eleven fractions obtained after elution with water−methanol mixtures were analyzed for their phenolic compounds, yielding phenolic acids, mangiferin, flavonol glycosides, and a gallotannin (*13*). However, this procedure proved to be time consuming, tedious, and hardly applicable to routine analysis of a great number of samples. Therefore, phenolic compounds from mango peels were purified by solid-phase extraction using polyamide. Hydrolyzable tannins were not considered in the present study because their isolation and characterization is currently underway and will be reported in a separate communication.

HPLC Analysis of Xanthone and Flavonol Glycosides. A stationary phase with hydrophilic endcapping was used for the analysis of phenolic compounds. Such types of phases have been developed especially for the separation of very polar analytes that are not sufficiently retained on conventional reverse phase systems. They have been demonstrated to be highly suitable for the determination of phenolic compounds from apple and pear (*17*, *18*) and strawberry (*19*). As can be seen from **Figure 1**, about twenty major compounds were separated and detected at 370 nm, fourteen of which could be characterized and identified.

Compound **1** was readily identified as mangiferin (**Figure 2**), a xanthone *C*-glycoside occurring in a variety of plants (*20*). Compound **2** displayed identical UV and mass spectrometric data and was therefore assigned to isomangiferin (**Figure 2**). Mass spectrometric analysis of both compounds showed a pseudomolecular ion of *m*/*z* 421 and a loss of 120 amu (atom mass units) in the MS^2 event (**Table 1**), a fragmentation behavior typical of *C*-glycosides. In contrast to flavonoid *O*-glycosides, *C*-glycosides do not generate abundant aglycone ions (*21*).

While no evidence for the presence of homomangiferin was obtained, two compounds (**3** and **4**) also displaying xanthone UV spectra were detected. Mass spectrometric analysis revealed a pseudomolecular ion of *m*/*z* 573. In the MS^2 event, a loss of 152 amu indicative of a galloyl moiety was observed. Because further fragmentation (MS^3, MS^4) was almost identical to mangiferin and isomangiferin, it is concluded that these compounds represent galloylated xanthone *C*-glycosides. The occurrence of mangiferin 6′-*O*-gallate has been described in mango leaves, but no evidence was obtained for the presence of its isomer (*22*). Because the nongalloylated xanthone glycosides elute in the order mangiferin−isomangiferin, it is reasonable to assume that mangiferin 6′-*O*-gallate elutes prior to the isomangiferin derivative. Mangiferin has been shown to be the predominant constituent of mango stem bark aqueous decoctions traditionally used as a nutritional supplement in Cuba (*23*). Furthermore, there is ample evidence that mangiferin displays a multitude of pharmacological effects such as hypolipidemic (*24*), antidiabetic (*25*), antioxidant (*26*,*27*), hepatoprotective (*28*), as well as immunomodulatory, antiviral, and antitumor (*29*−*31*) activities. Very recently, inhibitory effects in bowel carcinogenesis of male F344 rats have also been described (*32*). Therefore, extracts from mango peels represent a valuable source of mangiferin and further xanthone glycosides.

Compounds **5**−**11** were identified as quercetin *O*-glycosides (**Figure 3**) based on their UV and mass spectra and by comparison with standard substances (**Table 1**). Compound **14** represented the quercetin aglycone. It is interesting to note that the profile of flavonol glycosides obtained in the present study is almost identical to that found in mango puree concentrate in previous investigations (*13*), with quercetin 3-*O*-galactoside and quercetin 3-*O*-glucoside being the predominant compounds. However, at that time, only part of these flavonol glycosides could be identified, owing to the lack of reference samples. Improved methods and the availability of a greater range of standard substances now allowed the unambiguous identification of most of the flavonol glycosides extracted from mango peels.

Consistent with the results of a very recent study on the collision-induced dissociation of flavonoid glycosides (*33*), homolytic and heterolytic cleavage was observed under the mass spectrometric conditions applied. In most cases, quercetin glycosides produced a Y_0^- ion at *m*/*z* 301 as the predominant fragment caused by heterolytic cleavage. The formation of the radical aglycone as the main fragment was observed only for compounds **5** and **9**. The data given in **Table 1** for the MS^3 event include fragments recorded for both pathways.

Compound **5** showed a pseudomolecular ion of *m*/*z* 595 identical to quercetin 3-*O*-arabinoglucoside (peltatoside), how-

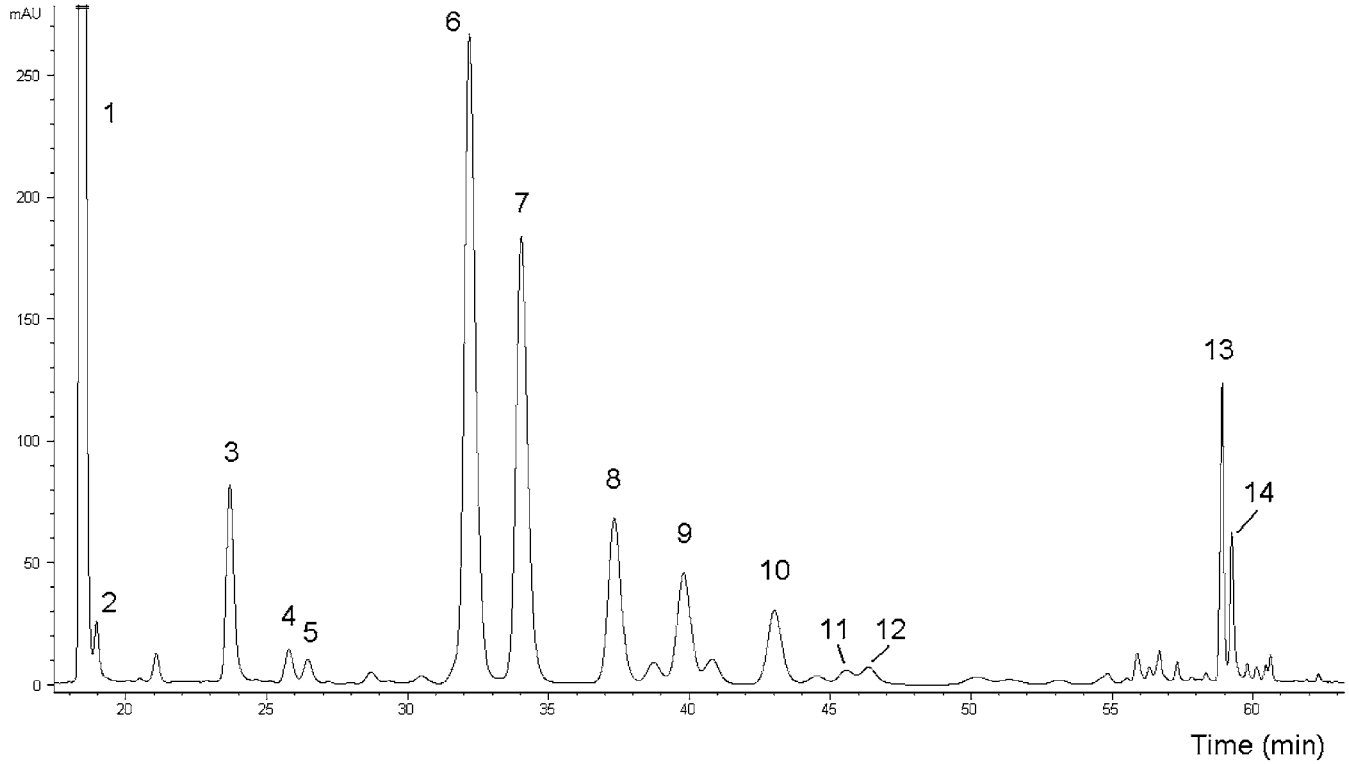
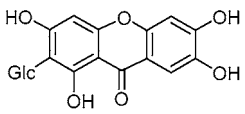


Figure 1. Separation of xanthone *C*- and flavonol *O*-glycosides by high-performance liquid chromatography (370 nm). Peak assignment: (1) Mangiferin, (2) isomangiferin, (3) and (4) mangiferin gallate and isomangiferin gallate (tentatively identified), (5) quercetin 3-*O*-diglycoside, (6) quercetin 3-*O*-galactoside, (7) quercetin 3-*O*-glucoside, (8) quercetin 3-*O*-xyloside, (9) quercetin 3-*O*-arabinopyranoside, (10) quercetin 3-*O*-arabinofuranoside, (11) quercetin 3-*O*-rhamnoside, (12) kaempferol 3-*O*-glucoside, (13) rhamnetin 3-*O*-glycoside, (14) quercetin (aglycone).



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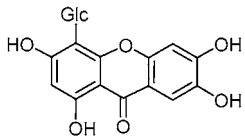

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Figure 2. Structures of the xanthone *C*-glycosides mangiferin (1) and isomangiferin (2).

ever, it did not coelute with the reference substance. It is therefore concluded that **5** or the reference represents an isomer of peltatoside differing in conformation or linkage of the saccharide moiety. A similar problem has been encountered in a recent study of quercetin glycosides in apple pomace (*18*).

Compounds **6**, **7**, and **8** were identified as quercetin 3-*O*-galactoside (hyperoside), 3-*O*-glucoside (isoquercitrin) and 3-*O*-xyloside (reynoutrin), respectively. Mass spectrometric characterization of compounds **9** and **10** provided evidence for the presence of further two quercetin pentosides with *m*/*z* 433 as the pseudomolecular ion. Spiking with reference substances allowed their identification as quercetin 3-*O*-arabinopyranoside (guajaverin) and quercetin 3-*O*-arabinofuranoside (avicularin). Both quercetin glycosides have also been found in apples (*18*), however, to our knowledge, this is the first report on their simultaneous occurrence in mango.

Compound **11** provided a pseudomolecular ion of *m*/*z* 447 and an aglycone fragment of *m*/*z* 301 and could therefore be readily identified as quercetin attached to a desoxy hexose. Comparison of retention time, UV spectra, and mass spectrometric data of the reference substance revealed the presence of quercetin 3-*O*-rhamnoside (quercitrin).

Compound **12** was the only kaempferol glycoside detected in the mango peel extract. It showed a pseudomolecular ion of *m*/*z* 447 and prominent ions of the kaempferol aglycone of *m*/*z* 285 (Y_0-) and *m*/*z* 284 ($Y_0-H)-$. Because it coeluted with the reference, it was identified as kaempferol 3-*O*-glucoside (astragalin) (**Figure 3**).

Compound **13** showed a pseudomolecular ion of *m*/*z* 477 and a prominent aglycone fragment of *m*/*z* 315. From these data and the UV spectrum it could be deduced that **13** represented either isorhamnetin or rhamnetin attached to a hexose. Both compounds are methoxylated flavonoids that only differ in the position of the methyl group. Whereas rhamnetin is methoxylated in position 7 of the A-ring (**Figure 3**), the methoxyl group of isorhamnetin is localized in position 3′ of the flavonoid B-ring. Recently, Justesen (*34*) reported that methoxylated flavonoid aglycones can be easily distinguished by means of mass spectrometry because of their different fragmentation profiles. Later, this strategy was used for the identification of isorhamnetin glycosides in apples (*35*). In the present study, fragmentation of compound **13** was compared with that of isorhamnetin 3-*O*-glucoside and of the rhamnetin aglycone. The latter compound was not available in glycosidic form. From **Table 1** it can be seen that isorhamnetin 3-*O*-glucoside generated a prominent ion of *m*/*z* 300 in the MS^3 event, whereas in the case of compound **13,** the most abundant ion was *m*/*z* 165, which exactly matched the fragmentation profile of the rhamnetin aglycone in the MS^2 event. According to Justesen (*34*), the formation of an A-ring fragment of *m*/*z* 165 as the most prominent fragment is a peculiarity of rhamnetin. Therefore, ample evidence for the first proof of a rhamnetin glycoside

Table 1. UV Spectra and Characteristic Ions of Xanthone and Flavonol Glycosides 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	mangiferin	241, 258, 275sh, 318, 366	421	–MS^2 [421]: 403 (22), 331 (99), 301 (100) –MS^3 [421 → 301]: 273 (88), 258 (100)
2	isomangiferin	241sh, 256, 275sh, 317, 365	421	–MS^2 [421]: 403 (10), 331 (90), 301 (100) –MS^3 [421 → 301]: 273 (73), 258 (100)
3	mangiferin gallate[b]	241, 258, 275sh, 318, 366	573	–MS^2 [573]: 421 (100), 403 (10), 331 (11), 301 (14) –MS^3 [573 → 421]: 331 (40), 301 (100) –MS^4 [573 → 421 → 301]: 273 (100), 258 (30)
4	isomangiferin gallate[b]	241sh, 256, 275sh, 317, 366	573	–MS^2 [573]: 421 (100), 403 (38), 331 (19), 301 (22), 283 (76), 259 (23) –MS^3 [573 → 421]: 331 (32), 301 (100) –MS^4 [573 → 421 → 301]: 273 (75), 258 (100)
5	quercetin 3-*O*-diglycoside	232, 256, 266sh, 296sh, 355	595	–MS^2 [595]: 301 (51), 300 (100), 271 (19) –MS^3 [595 → 300]: 271 (100), 255 (51) –MS^3 [595 → 301]: 272 (53), 256 (51), 179 (80), 151 (100)
6	quercetin 3-*O*-galactoside	232, 256, 266sh, 296sh, 353	463	–MS^2 [463]: 301 (100), 300 (34) –MS^3 [463 → 300]: 271 (100), 255 (64) –MS^3 [463 → 301]: 179 (79), 151 (100)
7	quercetin 3-*O*-glucoside	232, 256, 266sh, 296sh, 353	463	–MS^2 [463]: 301 (100), 300 (22) –MS^3 [463 → 300]: 271 (100), 255 (65) –MS^3 [463 → 301]: 179 (94), 151 (100)
8	quercetin 3-*O*-xyloside	232, 256, 266sh, 296sh, 354	433	–MS^2 [433]: 301 (100), 300 (18) –MS^3 [433 → 300]: 271 (100), 255 (47) –MS^3 [433 → 301]: 179 (97), 151 (100)
9	quercetin 3-*O*-arabinopyranoside	231, 256, 266sh, 296sh, 354	433	–MS^2 [433]: 301 (77), 300 (100) –MS^3 [463 → 300]: 271 (100), 255 (49) –MS^3 [433 → 301]: 179 (100), 151 (81)
10	quercetin 3-*O*-arabinofuranoside	230, 257, 266sh, 296sh, 352	433	–MS^2 [433]: 301 (100), 300 (11) –MS^3 [463 → 300]: 300 (21), 271 (100), 255 (69) –MS^3 [433 → 301]: 179 (98), 151 (100)
11	quercetin 3-*O*-rhamnoside	231, 256, 266sh, 296sh, 350	447	–MS^2 [447]: 301 (100), 300 (32) –MS^3 [463 → 300]: 271 (100), 255 (54) –MS^3 [447 → 301]: 179 (100), 151 (90)
12	kaempferol 3-*O*-glucoside	232, 265, 294sh, 347	447	–MS^2 [447]: 447 (22), 285 (94), 284 (100), 255 (31) –MS^3 [447 → 284]: 255 (100) –MS^3 [447 → 285]: 267 (34), 257 (100), 256 (64), 241 (31), 229 (51), 213 (30), 163 (28)
13	rhamnetin-hexoside	231, 257, 355	477	–MS^2 [477]: 315 (100), 314 (29) –MS^3 [477 → 314]: 299 (100) –MS^3 [477 → 315]: 300 (23), 193 (25), 165 (100)
14	quercetin	230, 255, 266sh, 302sh, 371	301	–MS^2 [301]: 179 (89), 151 (100)
Std	mangiferin	240, 258, 275sh, 318, 366	421	–MS^2 [421]: 403 (16), 331 (100), 301 (98) –MS^3 [421 → 301]: 273 (67), 272 (18), 258 (100)
Std	quercetin 3-*O*-arabino-glucoside	231, 256, 266sh, 296sh, 355	595	–MS^2 [595]: 301 (100), 300 (28), 271 (10) –MS^3 [595 → 300]: 271 (100), 255 (63) –MS^3 [595 → 301]: 179 (96), 151 (100)
Std	quercetin 3-*O*-galactoside	232, 256, 266sh, 296sh, 353	463	–MS^2 [463]: 301 (100), 300 (31) –MS^3 [463 → 300]: 271 (100), 255 (50) –MS^3 [463 → 301]: 179 (92), 151 (100)
Std	kaempferol 3-*O*-glucoside	232, 265, 294sh, 347	447	–MS^2 [447]: 447 (35), 285 (84), 284 (100), 255 (33) –MS^3 [447 → 284]: 255 (100) –MS^3 [447 → 285]: 267 (32), 257 (100), 256 (69), 241 (21), 229 (44), 213 (30), 197 (19), 163 (23)
Std	isorhamnetin 3-*O*-glucoside	255, 265sh, 297sh, 354	477	–MS^2 [477]: 477 (76), 357 (18), 315 (37), 314 (100) –MS^3 [477 → 314]: 286 (27), 285 (100), 271 (93), 243 (23) –MS^3 [477 → 315]: 300 (100)
Std	rhamnetin	230, 256, 370	315	–MS^2 [315]: 300 (26), 193 (37), 165 (100)

[a] Data of selected reference compounds (std) are also included. [b] Tentatively identified.

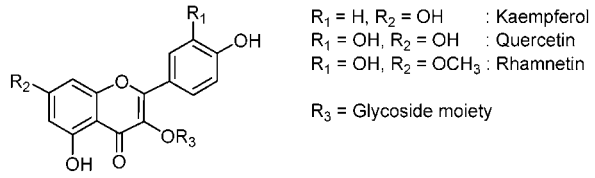

Figure 3. General structure and substitution pattern of flavonol glycosides detected in mango peels.

in mango peels is provided. Currently, methods for the preparative isolation and structure elucidation of compound **13** are being developed.

The results obtained in the present study demonstrate that the profile of xanthone and flavonol glycosides in mango peels is even more complex than known so far. These compounds might contribute to the antioxidant activity that has been shown for mango peel dietary fiber (*10*, *11*). The results are also interesting from a technological point of view. Mango puree concentrate, which represents an important intermediate for the production of mango beverages and mango leather, is prepared from both peeled and unpeeled fruits, depending on the cultivar used. Analysis of the phenolic profile of mango puree would allow a clear statement whether fruits were peeled prior to processing. This is particularly important because the peels might contain allergenic alkylresorcinols which act as fungitoxic compounds and may originate from the latex (*36*) but have also been detected in peels of unripe mango fruits (*37*).

We have recently established a new process for the combined recovery of pectin and polyphenolics from apple pomace. The

process comprises extraction of the dried pomace with diluted mineral acids and adsorption of phenolic compounds to a styrene-divinylbenzene copolymerizate that has been approved for food use by the FDA (*38*). Because mango peels are not only rich in polyphenolics but also contain a high quality pectin with a high degree of esterification (about 75%), this process may also be adopted to the recovery of phenolic compounds, thus adding value to mango fruit processing. Currently, there is an increasing tendency to maximize juice yields by the use of pectolytic and cellulolytic enzymes (*39*). However, apple pomace resulting from mash liquefaction cannot be exploited for the recovery of pectin because the polysaccharides are partly depolymerized. On a long-term basis, enzymatic liquefaction of fruits might lead to scarcity of apple pomace as a raw material, and alternative sources of pectins are urgently needed. Because mango peels are available in large amounts and have been shown to contain high-quality pectin with a high degree of esterification (*9*) as well as dietary fiber (*2*), they are promising sources of valuable compounds and should be strongly considered for exploitation. To evaluate their potential as well as possible risks, economically relevant mango cultivars are currently being screened for their pectin contents and quality and for their phenolic profile and contents, including hydrolyzable tannins and alkylresorcinols.

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