MALDI-TOF MS Analysis of Food Flavonol Glycosides

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a new technique that is having a great impact on food analysis. This study is the first to demonstrate the use of MALDI-TOF MS to identify flavonol glycosides in food samples. 2′,4′,6′-Trihydroxyacetophenone was chosen as the best matrix because it worked for crude sample extracts and ionized flavonol glycosides in both positive and negative MALDI-TOF MS modes. In the positive mode, multiple ion forms were observed for flavonol glycosides, including $[M + H]^+$, $[M + Na]^+$, $[M + K]^+$, and $[M - H + Na + K]^+$, with further fragmentation through loss of glycosidic residues. The negative mode for all flavonol glycosides resulted in $[M - H]^-$ ion formation without detectable fragmentation. The multiple ions in the positive mode gave more information on individual flavonol glycoside structures than the negative mode. Flavonol glycosides showed similar intensities or responses in the positive mode, while kaempferol glycosides exhibited much less response than quercetin glycosides in negative mode.

Keywords: *Onion; green tea; kaempferol; quercetin; myricetin; isorhamnetin*

INTRODUCTION

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. More than 2000 chemically distinct flavonoids have been reported, and each fits into one of several classes. In a dietary context, the most significant are the flavonols (Figure 1), especially quercetin and kaempferol, which are found at high concentrations in onions, tea, and apples. The flavonols are almost entirely found in glycosylated forms, with one, two, or three carbohydrates attached to flavonol hydroxyl groups (Williamson et al., 1998). Epidemiological studies have shown that flavonols have anticarcinogenic and antioxidant properties (Formica and Regelson, 1995; Rhodes and Price, 1996; Lean et al., 1999).

Because of the importance of flavonol glycosides, many techniques have been used to identify and quantify these compounds. Early investigations on flavonol glycosides were carried out using paper chromatography and thin-layer chromatography (Roberts et al., 1956; Oshima and Nakabayashi, 1953a,b). In the early 1990s, high-performance liquid chromatography (HPLC) with photodiode array detection was used to isolate and quantify flavonol glycosides in tea (McDowell et al., 1990; Bailey et al., 1990; Finger et al., 1991). ^1H and ^{13}C NMR spectroscopy were the most powerful techniques for determination of flavonol glycoside molecular structure (Finger et al., 1991). The coupling of HPLC and mass spectrometry methods, such as electrospray, thermospray, or fast-atom bombardment, has been widely used to provide molecular weight and characteristic fragment ions for structural elucidation of flavonol glycosides (Finger et al., 1991; Bailey et al., 1994; Kiehne and Engelhardt, 1996; Sägesser and Deinzer, 1996; Price et al., 1997).

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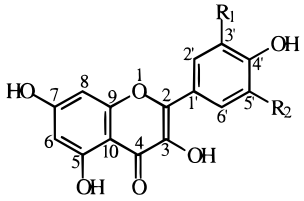

Figure 1. Structures of flavonols. Kaempferol: $R_1 = R_2 = H$. Quercetin: $R_1 = OH$, $R_2 = H$. Myricetin: $R_1 = R_2 = OH$. Isorhamnetin: $R_1 = OCH_3$, $R_2 = H$.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was first introduced in 1987 and originally developed for large biomolecules (Karas et al., 1987). MALDI-TOF MS has advantages over other methodologies including speed of analysis, high sensitivity, wide applicability combined with a good tolerance toward contaminants, and the ability to analyze complex mixtures (Karas, 1996). However, simple MALDI-TOF MS instruments cannot tell the difference between isomers, which have identical mass. The potential application of MALDI-TOF MS in food systems allows for analysis of most molecules. Presently, MALDI-TOF MS food applications are limited (Sporns and Wang, 1998). MALDI-TOF MS has been reported for both qualitative and quantitative analysis of anthocyanins, which are structurally very similar to flavonols, in several important foods (Wang and Sporns, 1999). This study presents the use of MALDI-TOF MS to study food flavonol glycosides.

MATERIALS AND METHODS

Materials and Reagents. Yellow onion bulbs (*Allium cepa* L.) and green tea were purchased from local markets in Edmonton, Alberta, Canada. Isoquercitrin (quercetin 3-glucoside), kaempferol 3-glucoside, and kaempferol 3-rutinoside were obtained from Extrasynthese S.A. (Genay Cedex, France). Rutin (quercetin 3-rutinoside), 4-hydroxy-α-cyanocinnamic acid (HCCA), *N*-*t*-Boc-Met-Asp-Phe amide, and *N*-*t*-Boc-Trp-

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Met-Asp-Phe amide were purchased from Sigma Chemical Co. (St. Louis, MO). 1-Kestose and nystose were a gift from Dr. A. Ohta (Nutritional Science Center, Meiji Seika Kaisha, Japan). 2′,4′,6′-Trihydroxyacetophenone monohydrate (THAP), 2-(4-hydroxyphenylazo)benzoic acid (HABA), 3-aminoquinoline (3-AQ), *trans*-3-indoleacrylic acid (IDA), and 2,5-dihydroxybenzoic acid (DHB) were obtained from Aldrich Chemical Co. (Milwaukee, WI). All water used was double deionized (Milli-Q water purification system, Millipore Corp., Bedford, MA). A common solvent used was aqueous 70% methanol.

Extraction of Flavonol Glycosides from Yellow Onion and Green Tea. Fresh yellow onion was pealed to remove the dry outer layers and then chopped using a food processor (Braun, UK 100, type 4259, Germany) for 5 min. The mixed sample was freeze-dried. The dried sample (5 g) was extracted with 100 mL of 70% methanol for 30 min with stirring and then filtered through Whatman No. 4 filter paper. Methanol was removed from the filtrate using a Büchi Rotavapor 461 water bath (Brinkmann Instruments Ltd., Mississauga, Ontario, Canada) in vacuo with a bath temperature of 35 °C. Methanol-free filtrate (10 mL) was loaded onto a Sep-pak C18 cartridge (Waters Corp., Milford, MA) at a flow rate of about 1 mL/min, washed with 10 mL of water three times, and eluted with 2 mL of 70% methanol. Green tea (4 g) was extracted with 100 mL of hot water (80 °C), stirred for 15 min, and then filtered through glass wool. The purification of flavonol glycosides using a Sep-pak C18 cartridge was the same as the onion sample, except that 2 mL of filtrate was loaded on the Sep-pak C18 cartridge and the final eluant was 2 mL of 0.01 M NaCl in 70% methanol. The yellowish flavonol glycoside extracts were kept in a refrigerator until used.

MALDI-TOF MS. MALDI-TOF MS was performed using a Proflex III in linear mode (Bruker Analytical Systems Inc., Billerica, MA). Flavonol glycosides cocrystallized with matrixes on the probe were ionized by a nitrogen laser pulse (337 nm) and accelerated under 20 kV with time-delayed extraction before entering the time-of-flight mass spectrometer. For THAP, HABA, 3-AQ, DHB, and HCCA, the preparation of matrix and sample was the same as previously reported (Wang et al., 1999). For IDA, the matrix solution contained 10 mg/mL IDA in 1:1 acetone/water. The ratio of matrix solution and sample was 1:1. For all matrixes, either 1 μL of sample solution or a 2 μL mixture of matrix and sample was applied to a MALDI-TOF MS probe and air-dried. When flavonol glycosides were identified after HPLC separation, 0.5 μL of THAP (saturated in acetone) was applied on the probe first and air-dried. Then 2 μL of the preparative HPLC fraction was spotted on the THAP crystals and further air-dried. MALDI-TOF MS was attenuated (the lower the attenuation, the higher the laser strength) to obtain the best signal-to-noise ratio and isotopic resolution for the matrix used. Two point external calibrations were performed using $[\text{kestose} + K]^+$ (exact isotopic mass = 543.13) and $[\text{nystose} + K]^+$ (exact isotopic mass = 705.19) for positive MALDI-TOF MS mode, and [*N*-*t*-Boc-Met-Asp-Phe amide $- H]^-$ (exact isotopic mass = 509.21) and [*N*-*t*-Boc-Trp-Met-Asp-Phe amide $- H]^-$ (exact isotopic mass = 695.29) for the negative mode. The mass accuracy was below 500 ppm. Each spectrum represents the sum of 60 laser pulses.

Preparative HPLC. The preparative HPLC system consisted of a Varian VISTA 5500 HPLC (Varian Canada Inc., Mississauga, Ontario, Canada), a Varian 9090 Auto sampler, and a Spectro Monitor III UV detector (LDC/MILTON ROY, Riviera Beach, FL). The system was equipped with a 75 × 4.5 mm preinjection C18 saturator column containing silica-based packing (12 μm) and a 50 × 4.6 mm guard column containing Supelco LC-18 reversed-phase packing (20−40 μm, Supelco, Bellefonte, PA). Flavonol glycosides were separated on a Supelcosil SPLC-18-DB 250 × 10 mm (5 μm) preparative reversed-phase column (Supelco, Bellefonte, PA). The solvents used were 2% (v/v) aqueous acetic acid (solvent A) and acetonitrile (solvent B). The flow rate was at 5 mL/min, with a linear gradient profile consisting of solvent A with the following proportions (v/v) of solvent B: 0 min, 15% B; 0−26 min, 15−18% B; 26−30 min, 18−31% B; 30−32 min, 31% B; 32−33 min, 31−15% B; 33−34 min, 15% B. Detection was at 354 nm, and the total run time was 38 min. Sample extracts (500 μL) were injected into the HPLC for preparative collection. A Shimadzu CLASS-VP chromatography data system (Shimadzu Scientific Instruments Inc., Columbia, MD) was used to monitor the eluted peaks, and the flavonol glycoside fractions of interest were collected for MALDI-TOF MS analysis.

RESULTS AND DISCUSSION

For analysis of any compound in food samples using MALDI-TOF MS, several factors, including the selection of matrixes, preparation of matrix and sample, signal-to-noise ratio, and spot-to-spot repeatability, must be considered.

Selection of MALDI-TOF MS Matrixes for Flavonol Glycosides. Six common matrixes were examined for desorption and ionization of flavonol glycosides. Of these matrixes, 4-hydroxy-α-cyanocinnamic acid (HCCA), 2′,4′,6′-trihydroxyacetophenone monohydrate (THAP), 2-(4-hydroxyphenylazo)benzoic acid (HABA), *trans*-3-indoleacrylic acid (IDA), and 2,5-dihydroxybenzoic acid (DHB) produced good quality spectra in MALDI-TOF MS positive mode for flavonol glycosides. THAP and IDA were also good matrixes for producing negative ions with flavonol glycosides. 3-Aminoquinoline (3-AQ) was not a suitable matrix since matrix and sample did not cocrystallize properly, although noticeable (but poor) analyte signal was still observed. Among all the matrixes studied, THAP was chosen as ideal for flavonol glycosides for further study in terms of the ease of preparation of matrix and sample, tolerance to contaminates, good spot-to-spot repeatability, and ability to generate both positive and negative analyte ions. All figures presented in this paper are spectra using THAP as matrix.

Laser strength used for desorption and ionization of flavonol glycosides was matrix related. HCCA, HABA, and IDA can desorb and ionize flavonol glycosides at very low laser strength, while THAP and DHB required higher laser strength. Generally, laser strength was selected based on good signal-to-noise ratio and the best resolution of analytes of interest (Wang et al., 1999). In all experiments, isotopic resolution was achieved in both positive and negative modes for all matrixes. The best spectra were those that reduced fragmentation to a minimum while maintaining isotopic resolution.

Ionization of Flavonol Glycosides. When analyzed by MALDI-TOF MS in the positive mode, flavonol glycosides showed ion forms of protonated $[M + H]^+$, single alkali metal adducts $[M + Na]^+$ or $[M + K]^+$, and double alkali metal adduct $[M - H + Na + K]^+$ ions (Figure 2). Ionization of flavonol glycosides was mainly influenced by matrixes. HCCA and HABA generated alkali adduct ions only, whereas DHB, IDA, and THAP produced both protonated and alkali adduct ions. Compared to flavonol monoglycosides, the di- or triglycosides exhibited high affinity for alkali metals (Figure 3). Furthermore, the relative amount of $[M + Na]^+$ vs $[M + H]^+$ was correlated to the concentration of alkali metals in a sample. By addition of an alkali metal (0.01 M NaCl) in the sample solution, $[M + H]^+$ ion formation decreased while $[M + Na]^+$ ion intensity increased. Alkali adduct ions could be exchanged by adding a relatively high concentration (0.01 M) of various alkali metal salts (Figure 4).

In MALDI-TOF MS negative mode, flavonol glycosides were ionized through deprotonation. Only one

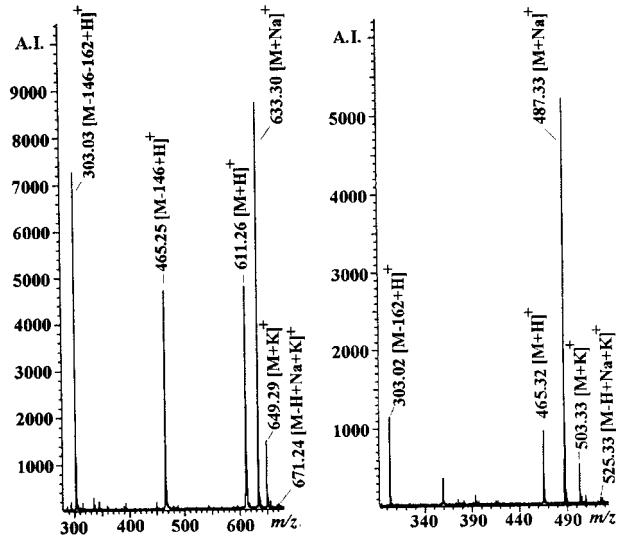

Figure 2. MALDI-TOF MS positive ion spectra of flavonol glycoside standards. Left: rutin (1.5×10^{-3} M in 70% methanol). Right: isoquercitrin (1.5×10^{-3} M in 70% methanol).

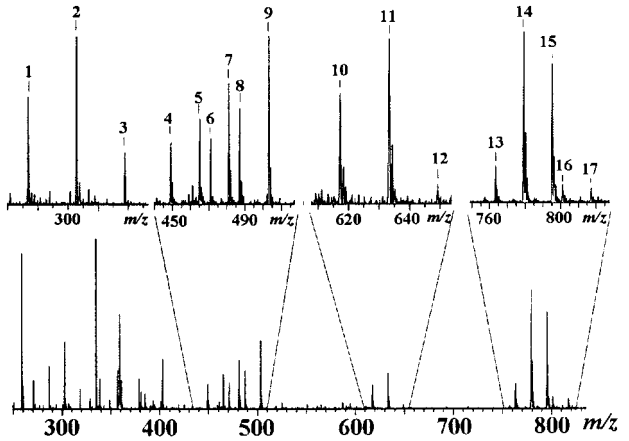

Figure 3. MALDI-TOF MS positive ion spectrum of flavonol glycosides from green tea. 1: 286.83 [kaempferol + H]$^+$. 2: 302.88 [quercetin + H]$^+$. 3: 318.93 [myricetin + H]$^+$. 4: 449.15 [kaempferol 3-glycoside + H]$^+$. 5: 465.15 [quercetin 3-glycoside + H]$^+$. 6: 471.20 [kaempferol 3-glycoside + Na]$^+$. 7: 481.20 [myricetin 3-glycoside + H]$^+$. 8: 487.20 [quercetin 3-glycoside + Na]$^+$. 9: 503.20 [myricetin 3-glycoside + Na]$^+$. 10: 617.28 [kaempferol 3-rutinoside + Na]$^+$. 11: 633.33 [quercetin 3-rutinoside + Na]$^+$. 12: 649.25 [myricetin 3-rutinoside + Na]$^+$. 13: 763.31 unknown (possibly [kaempferol 3-glucosylrhamnosylrhamnoside + Na]$^+$). 14: 779.29 [kaempferol 3-glucosylrhamnosylglycoside + Na]$^+$. 15: 795.27 [quercetin 3-glucosylrhamnosylglycoside + Na]$^+$. 16: 801.27 [kaempferol 3-glucosylrhamnosylglycoside − H + 2Na]$^+$. 17: 817.28 [quercetin 3-glucosylrhamnosylglycoside − H + 2Na]$^+$.

molecular negative ion form (Figure 5) was observed. Generally, the intensity or abundance of molecular ions in the negative mode was much less (four times or more) than in the positive mode. The laser strength used for desorption and ionization was slightly higher in the negative mode than in the positive mode.

Fragmentation of Flavonol Glycosides. In MALDI-TOF MS positive mode, all the flavonol glycosides exhibited fragmentation with loss of their carbohydrate residues. Figure 2 shows that rutin fragmented and produced [M − 146 + H]$^+$ ions at *m*/*z* 465 with loss of the rhamnose residue and [M − 146 − 162 + H]$^+$ ions at *m*/*z* 303 with loss of the rutinose residue (6-*O*-α-L-rhamnosyl-D-glucose). Isoquercitrin generated fragment

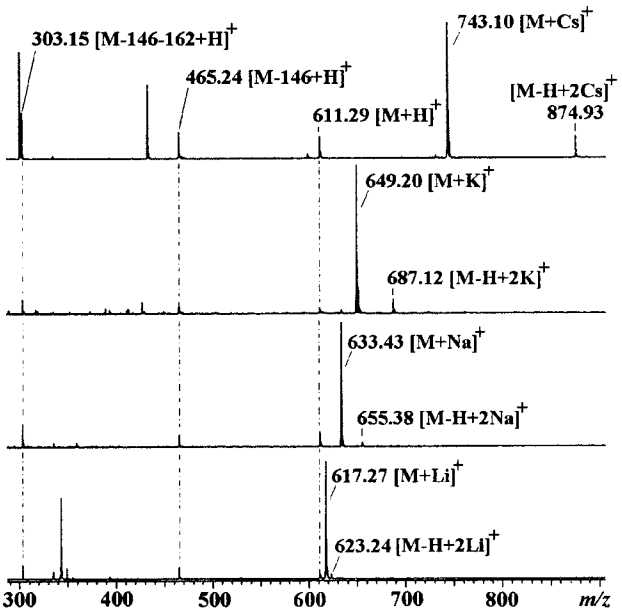

Figure 4. MALDI-TOF MS positive ion spectra of rutin (1.5×10^{-3} M dissolved in 0.01 M alkali 70% methanol) with different alkali adduct ions (Li^+, Na^+, K^+, and Cs^+). Note that all the other peaks are matrix peaks by fragmentation.

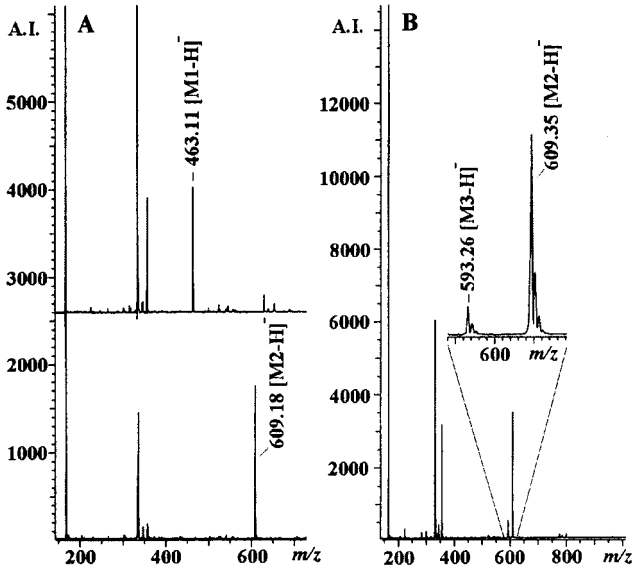

Figure 5. MALDI-TOF MS negative ion spectra of flavonol glycoside standards. M1: isoquercitrin. M2: rutin. M3: kaempferol 3-rutinoside. A: The spectra from bottom to top are rutin (1.5×10^{-3} M in 70% methanol) and isoquercitrin (1.5×10^{-3} M in 70% methanol). B: A mixture of rutin (4.2×10^{-4} M in 0.01 M NaCl in 70% methanol) and kaempferol 3-rutinoside (4.2×10^{-4} M in 0.01 M NaCl in 70% methanol). Ions at masses less than 400 are from the THAP matrix.

ions [M − 162 + H]$^+$ at *m*/*z* 303 resulting from loss of the glucose residue. No noticeable negative mode fragment ions were observed (Figure 5). The ionization forms and fragmentation patterns of flavonol glycosides in MALDI-TOF MS are similar to those observed in electrospray mass spectrometry, except for the low responses in the negative mode (Sägesser and Deinzer, 1996). The fragmentation patterns and ion forms of flavonol glycosides in MALDI-TOF MS provide characteristic information for structural elucidation of flavonol glycosides, which is important for their identification.

The relative fragmentation of flavonol glycosides was examined in a quantitative manner (Table 1). Flavonol

Table 1. Fragmentation and Responses of Flavonol Glycosides in MALDI-TOF MS[a]

MALDI-TOF MS	mixture of flavonol glycosides[b]	[Kae 3-Rut[c] − 146 + H]$^+$/ [Kae 3-Rut[c] + H]$^+$	[rutin − 146 + H]$^+$/ [rutin + H]$^+$	ratios of fragment ions[e] over parent ions[f] (%) kaempferol	quercetin	kaempferol/ quercetin	response ratios of all ions kaempferol/ quercetin
		1	2	3	4	5	6
positive mode	Kae 3-Rut[c] + rutin	1.05 (0.24)	0.94 (0.14)	39.3 (14.7)	39.7 (11.6)	0.97 (0.08)	1.25 (0.08)
	Kae 3-Glu[d] + isoquercitrin			37.0 (8.0)	37.3 (9.6)	1.00 (0.07)	1.24 (0.06)
	Kae 3-Glu[d] + rutin		0.96 (0.06)	29.5 (16.9)	29.3 (17.9)	1.03 (0.10)	1.02 (0.10)
	Kae 3-Rut[c] + isoquercitrin	1.03 (0.15)		20.3 (7.4)	24.8 (9.8)	0.82 (0.10)	1.53 (0.05)
	Kae 3-Rut[c] rutin	0.97 (0.14)	1.01 (0.18)	31.2 (14.8)	16.4 (5.2)		
negative mode	Kae 3-Glu[d] + isoquercitrin + Kae 3-Rut[c] + rutin						0.14 (0.02) 0.17 (0.03)

[a] All data are means of five replicates, and numbers in parentheses indicate the standard deviation of these five replicates ($n = 5$). [b] The concentration of individual flavonol glycoside standards was 1.5×10^{-3} M in methanol (stock solution). Any mixture of flavonol glycosides was made by mixing equal amounts (v/v) from stock solution. Final solution was made by mixing 70 μL of flavonol standard solution and 30 μL of 0.033 M NaCl. THAP as matrix. [c] Kaempferol 3-rutinoside. [d] Kaempferol 3-glucoside. [e] Ions from flavonol glycosides with loss of their carbohydrate residues. [f] Total of unfragmented protonated and alkali adduct molecular ions.

glycosides with a different aglycon, for example, kaempferol or quercetin, were chosen to prepare the sample so that molecular or fragment ions would not overlap. The fragmentation of individual flavonol glycosides was calculated on the basis of all the fragment ions (loss of carbohydrates) to their parent ions (total of unfragmented protonated and alkali metal adduct ions) in terms of percentage observed in a single MALDI-TOF MS sample or MALDI-TOF MS spectrum (Table 1, data columns 3 and 4). The fragmentation of each flavonol glycoside varied widely from sample to sample as indicated by the large standard deviation (Table 1, data columns 3 and 4). However, within any single MALDI-TOF MS sample, all the flavonol glycosides exhibited a similar fragmentation percentage. This similarity was shown by the ratios of fragment ions of kaempferol glycosides to those of quercetin glycosides, which were close to 1 (Table 1, data column 5). That is, for individual MALDI-TOF MS spectra the ratios of fragment ions to parent ions were very consistent and similar for different flavonol glycosides. With further investigation on rutin, it was found that the formation of $[M - 146 + H]^+$ ions was linearly proportional to $[M + H]^+$ ions in an equal amount (slope = 0.95, $R^2 = 0.98$) for varying concentrations of rutin. This relationship was also evident since ratios of $[M - 146 + H]^+$ to $[M + H]^+$ were close to 1 even in a mixture of flavonol glycosides (Table 1, data columns 1 and 2). The above quantitative information about fragment ions is useful for proper evaluation of the amount of monoglycosides in a mixture, since fragment ions from the diglycosides, e.g., $[M - 146 + H]^+$ ions at *m/z* 465 from rutin (quercetin 3-rutinoside), would overlap the monoglycosides with the same aglycon, e.g., $[M + H]^+$ ions at *m/z* 465 of isoquercitrin (quercetin 3-glucoside). In general, it seems that fragmentation patterns and amounts are predictable for any defined sample preparation method. Therefore, any flavonol glycoside MALDI-TOF MS spectrum should provide both qualitative and quantitative information about the flavonol glycosides in a sample.

Responses of Flavonol Glycosides in MALDI-TOF MS. Generally, the intensity (total of all ions) or response of flavonol glycosides in MALDI-TOF MS positive mode was linearly correlated to their molar ratios in the sample. This linearity (slope = 1.24, $R^2 =$ 0.98) was determined by choosing kaempferol 3-rutinoside (ranging from 8.4×10^{-5} to 4.2×10^{-4} M) as an analyte while using rutin (quercetin 3-rutinoside, 4.2×10^{-4} M) as internal standard. Extensive studies showed that the responses of kaempferol and quercetin glycosides were all similar and the ratios of the intensity of kaempferol glycosides over quercetin glycosides were close to 1 (Table 1, data column 6). However, in MALDI-TOF MS negative mode, kaempferol glycosides exhibited one-fifth or less the responses of quercetin glycosides (Figure 5B; Table 1, data column 6).

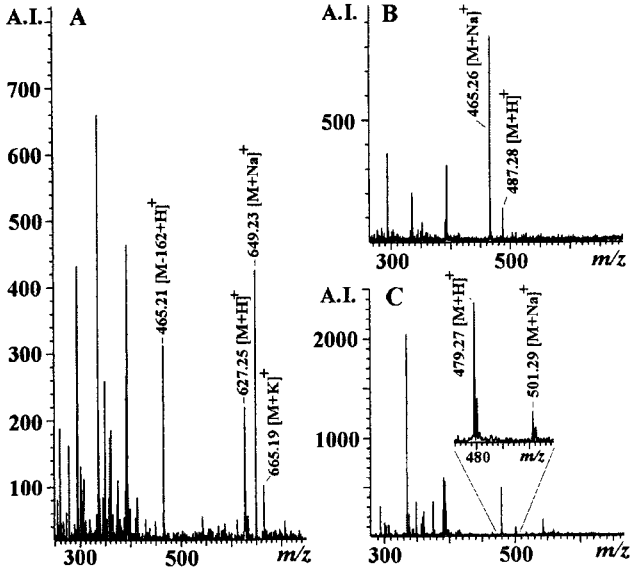

Figure 6. MALDI-TOF MS positive ion spectra of flavonol glycosides (from yellow onion) after HPLC separation. A: Quercetin 3,4′-diglucoside. B: Quercetin 4′-glucoside. C: Isorhamnetin 4′-glucoside.

In general, because MALDI-TOF MS positive mode can generate abundant ions and the spectra provided more structural information, subsequent studies on flavonol glycosides in food samples were carried out in the positive mode.

MALDI-TOF MS Identification of Flavonol Glycosides in Yellow Onion Extracts. Onions primarily contain two or three quercetin conjugates, quercetin 3,4′-diglucoside, the 4′-glucoside (Price et al., 1997), and isorhamnetin 4′-glucoside (Park and Lee, 1996). After

Table 2. MALDI-TOF MS Identification of Flavonol Glycosides in Green Tea

		mass of MALDI-TOF MS detected ions after HPLC separation						
flavonol glycosides[a]	theor mass	$[M+H]^+$	$[M+Na]^+$	$[M+K]^+$	$[M-146+H]^+$	$[M-162+H]^+$	$[M-308+H]^+$	$[M-470+H]^+$
myricetin 3-rhamnosylglucoside	626							
myricetin 3-galactoside	480	481	503			319		
myricetin 3-glucoside[b]	480	481	503			319		
quercetin 3-glucosylrhamnosylgalactoside[c]	772	773	795	811			465	303[d]
quercetin 3-glucosylrhamnosylglucoside	772	773	795	811			465	303[d]
kaempferol 3-glucosylrhamnosylgalactoside	756	757	779	795			449	287[d]
quercetin 3-rutinoside	610	611	633		465			303[d]
quercetin 3-galactoside	464	465	487			303[d]		
quercetin 3-glucoside	464	465	487			303[d]		
kaempferol 3-glucosylrhamnosylglucoside	756	757	779	795			449	303[d]
kaempferol 3-galactoside	448	449	471			287[d]		
kaempferol 3-rhamnosylglucoside	594							
quercetin 3-rhamnoside	448							
kaempferol 3-glucoside	448	449	471			287[d]		

[a] Based on the HPLC retention time order. [b] Shown in Figure 6A. [c] Shown in Figure 6B. [d] THAP also produced fragment ions at *m/z* 287 and 303.

HPLC separation, three major peaks, at 7.7, 25.9, and 32.1 min, were collected and analyzed by MALDI-TOF MS. The characteristic ions, observed at *m/z* 465, 627, 649, and 665 ($[M-162+H]^+$, $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$, respectively), were assigned to quercetin 3,4′-diglucoside (HPLC peak at 7.7 min), at *m/z* 465 and 487 ($[M+H]^+$ and $[M+Na]^+$, respectively) for 4′-glucoside (HPLC peak at 25.9 min), and at *m/z* 479 and 501 ($[M+H]^+$ and $[M+Na]^+$, respectively) for isorhamnetin 4′-glucoside (HPLC peak at 32.1 min) (Figure 6). The fragmentation patterns were as expected. The monoglycosides produced fragment ions at *m/z* 303 or 317 ($[M-162+H]^+$), and the diglucosides generated fragment ions at *m/z* 465 ($[M-162+H]^+$) and 303 ($[M-162-162+H]^+$). THAP also generated matrix ion peaks at *m/z* 303 and 287. The intensities of matrix ions were effected by the concentration of flavonol glycosides. High concentrations of flavonol glycosides suppressed the matrix ions in the *m/z* 200 and 400 region (Figure 2). Therefore, the peak at *m/z* 303 (Figure 2) is definitely from the fragmentation of the flavonol glycosides. However, at lower flavonol glycoside concentrations, both flavonol glycoside and THAP fragmentation can contribute to ion peaks at *m/z* 303 and 287.

MALDI-TOF MS Identification of Flavonol Glycosides in Green Tea Extracts. Flavonol glycosides present in green tea are kaempferol, quercetin, and myricetin mono-, di-, and triglycosides. They are listed in Table 2 using the HPLC retention time order according to Bailey et al. (1990), Engelhardt et al. (1992), and McDowell et al. (1995). Figure 3 shows the MALDI-TOF MS spectrum of flavonol glycosides in a green tea extract. Because flavonol glycosides were prepared with an excess of sodium ions (0.01 M NaCl in 70% methanol), the di- and triglycosides were predominately ionized in the form of sodium adduct ions, while monoglycosides were ionized as both protonated and sodium adduct ions. Since only very small amounts or no noticeable $[M+H]^+$ ions were observed for flavonol diglycoside (masses between 590 and 650) or triglycoside (masses between 740 and 820), the amounts of the fragment ions $[M-\text{glycoside}+H]^+$ at *m/z* 465 or 449 were assumed to be insignificant. The MALDI-TOF MS profiles in the monoglycoside region (masses between 400 and 500) should represent the monoglycosides in the original sample. The masses of flavonol glycosides were determined in eleven HPLC fractions by MALDI-TOF MS (two of them given in Figure 7). The MALDI-TOF MS spectra provided the characteristic molecular or fragment ions for all these HPLC fractions, which were then assigned as flavonol glycosides in green tea (Table 2). However, flavonol triglycosides, such as quercetin 3-glucosylrhamnosylgalactoside, did not exhibit fragment ions $[M-162+H]^+$ at *m/z* 611 with loss of the glucose residue. This may be because of the low concentration of the triglycoside in the HPLC fraction or the easy cleavage of glycosidic linkage between rhamnose and galactose, since mass at *m/z* 465 (loss of glucosylrhamnose residue) was observed (Figure 7B).

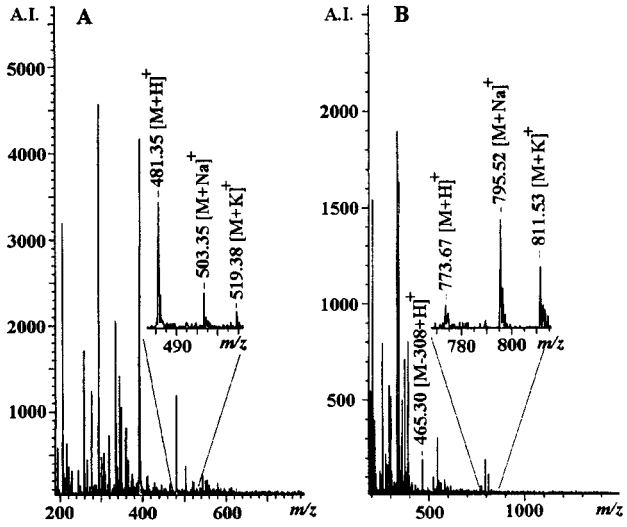

Figure 7. MALDI-TOF MS positive ion spectra of flavonol glycosides (from green tea) after HPLC separation. A: myricetin 3-glucoside. B: quercetin 3-glucosylrhamnosylgalactoside.

In conclusion, MALDI-TOF MS is a valuable rapid technique for identification of flavonol glycosides, even in complex mixtures. The fragment ions of flavonol glycosides provided characteristic information for structural elucidation of flavonol glycosides. Fragmentation and amounts of flavonol glycosides were predictable in a MALDI-TOF MS sample. MALDI-TOF MS spectra, therefore, could provide the flavonol glycoside profiles for food samples and also be used to identify flavonol glycosides in conjunction with other separation techniques such as HPLC. It is likely that MALDI-TOF MS has the potential to analyze flavonol glycosides in other foods and that these spectral ion patterns could be used for identification and quality control.

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