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Identification of the Phenolic Components of Collard Greens, Kale, and Chinese Broccoli

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An LC-MS profiling method was used for a comprehensive study of the phenolic components of collard greens, kale, and Chinese broccoli, three *Brassica* green leafy vegetables. This study led to the identification of 45 flavonoids and 13 hydroxycinnamic acid derivatives in the three vegetables. Most of the identifications were based on comparison of compounds previously reported in the literature for *Brassica* vegetables. The results indicate that the three materials have very similar phenolic component profiles. For each, kaempferol glycosides and acylgentiobiosides were the major phenolic compounds, quercetin glycosides were minor compounds, and most of the flavononol glycosides existed in their acylated forms. In addition, each of the materials contained caffeoyl-, *p*-coumaroyl-, and feruloylquinic acid monomers with a 3-position derivative as the dominant isomer. This is the first report for most of these phenolics in collard greens and Chinese broccoli and for >20 of them in kale.

KEYWORDS: Collard greens; kale; Chinese broccoli; *Brassica* vegetables; flavonoids; hydroxycinnamates; LC-DAD-ESI/MS analysis

INTRODUCTION

Vegetables from the *Brassica* genus (Cruciferae or Brassicaceae family) are some of the most consumed vegetables in the world. Among them, broccoli, Brussels sprouts, cabbages, Savoy cabbage, cauliflower, kohlrabi, collard greens, kale, and Chinese broccoli (or Chinese kale; kai lan in Chinese) are from the varieties of *Brassica oleracea* L. and the remaining vegetables are from varieties of *Brassica rapa*, *Brassica juncea*, or other *Brassica* species (*1*−*3*). Botanically, collard greens, kale, and Chinese broccoli are the green leaves or leaf blades with thick stems of *B. oleracea* L. var. *acephala* (or *B. oleracea* L. var. *viridis*), *B. oleracea* L. var. *acephala* (both from the variety of acephala group), and *B. oleracea* var. *alboglabra* (L.H. Bailey) Musil (i.e., the variety of alboglabra group, or *B. alboglabra*), respectively. All are common *Brassica* vegetables in the United States, China, and other Asian countries (*1*−*3*).

Epidemiological studies have identified the ability of *Brassica* vegetables to reduce the risk of cardiovascular disease and some types of cancers, especially cancers of the gastrointestinal tract. Biological studies have shown that the glucosinolates, flavonoids, and other phenolic compounds in *Brassica* vegetables have antioxidant and free radical scavenging properties (*3*−*9*). For this reason, many of the *Brassica* vegetables have been the subject of chemical and biological studies.

Studies of the phenolic components of *Brassica* vegetables have identified several cinnamic acid−gentiobiosides, more than 40 glycosylated flavonols (kaempferol, quercetin, and isorhamnetin), and some hydroxycinamic acid derivatives (*10*−*31*). Thus far, the phenolic components of the enriched fractions of broccoli and cauliflower, Tronchuda cabbage, mizuna [*B. rapa* L. subsp. *nipposinica* (L.H. Bailey)], turnip top, pak choi (bok choy), Chinese leaf mustard, and some of their cultivars have been analyzed by LC-MS (*10*−*31*). A recent study of curly kale (*B. oleracea* L. Convar. *acephala* var. *sabellica*) using LC-MS^n identified 23 flavonoids and 9 phenolic acid derivatives (*32*). Anthocyanins have been reported in the colored *Brassica* vegetables, such as red cabbages (*30*,*31*) and purple cauliflower (*9*). Of the brassica flavonoids, more than 10 flavonol polyglucosides were isolated and their structures identified by NMR analysis (*10*−*15*), and the remaining compounds were identified using LC-UV−tandem MS technology (*18*−*27*, *32*).

The purpose of this study was to comprehensively survey the phenolic components of collard greens, kale, and Chinese broccoli and to compare the three vegetables. Data were collected using a standardized profiling method based on liquid chromatography with diode array and electrospray ionization/mass spectrometry (LC-DAD-ESI/MS) detection (*33*). The *Brassica* vegetables were purchased at a local grocery store. The emphasis of the study was on the identification of the phenolic compounds; there was no quantification, and individual varieties were not identified. This is the first report of most of these phenolic compounds in collard greens and Chinese broccoli.

MATERIALS AND METHODS

Standards and Other Chemicals. Quercetin dihydrate, kaempferol, and chlorogenic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, and isorhamnetin,

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were purchased from Extrasynthese (Genay, Cedex, France). 3- and 4-caffeoylquinic acids were prepared by the isomerization of chlorogenic acid and separated by C18 column chromatography (*33*, *34*). HPLC grade methanol, acetonitrile, formic acid, acetic acid, and NaOH were purchased from VWR International, Inc. (Clarksburg, MD). HPLC water was prepared from distilled water using a Milli-Q system (Millipore Laboratory, Bedford, MA).

Plant Materials. Samples of collard greens, kale, Chinese broccoli, broccoli, and white cabbage were purchased in local food stores in Maryland and dried at room temperature in a fume hood for approximately 5 days. The dried materials were powdered and passed through 20 mesh sieves prior to extraction.

Plant Extracts. Powdered samples (250 mg) were extracted with 5.00 mL of methanol/water (60:40, v/v) using sonication with an FS30 Ultrasonic sonicator (40 kHz, 100 W) (Fisher Scientific, Pittsburgh, PA) for 60 min at room temperature (< 35 °C at the end of 60 min). The slurry mixture was centrifuged at 2500 rpm for 15 min (IEC Clinical Centrifuge, Damon/IEC Division, Needham, MA). The supernatant was filtered through a 17 mm (0.45 μm) PVDF syringe filter (VWR Scientific, Seattle, WA), and 50 μL of the extract (or extract 1) was used for each HPLC injection (*33*).

Acid-Hydrolyzed Extracts. The filtered extracts (0.50 mL) were mixed with concentrated HCl (37%, 0.1 mL) and heated in a capped tube at 85 °C for 2 h. Then, 0.40 mL of methanol was added to the mixture, and the solution was sonicated for 10 min. The solution was refiltered prior to HPLC injection (*33*).

Alkali-Hydrolyzed Extracts. The filtered extracts (2.00 mL) were dried, and the residue was mixed with 0.30 mL of 2 N NaOH and kept at room temperature under a N_2 atmosphere for 18 h. Then, 0.10 mL of HCl (37%) was added to the reaction mixture to bring the pH to 1.0, and 0.60 mL of MeOH was added. The solution was refiltered prior to HPLC injection (*33*).

LC-DAD and ESI-MSD Conditions. The LC-DAD-ESI/MS system used consisted of a quaternary pump with a vacuum degasser, a thermostated column compartment, an autosampler, a diode array detector (DAD), and a single-quadrupole mass spectrometer (MSD) from Agilent Technologies (Palo Alto, CA). A 250 mm × 4.6 mm i.d., 5 μm, Symmetry C18 column with a 20 mm × 3.9 mm i.d., 5 μm, Symmetry Sentry guard column (Waters Corp., Milford, MA) was used at flow rate of 1.0 mL/min. The column oven temperature was set at 25 °C. The mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient increased linearly from 10% B (v/v) at 0 min to 26% B at 40 min, to 65% B at 70 min, to 100% B at 71 min, and was held at 100% B to 75 min. The DAD was set at 350, 310, and 270 nm to for real-time display of the peak intensities. UV spectra were recorded from 190 to 650 nm for plant component identification. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative ionization (PI and NI) modes at low and high fragmentation voltages (100 and 250 V) over the range of m/z 100−2000. A drying gas flow of 13 L/min, a drying gas temperature of 350 °C, a nebulizer pressure of 50 psi, and capillary voltages of 4000 V for PI and 3500 V for NI were used. The LC system was directly coupled to the MSD without stream splitting (*33*).

Modified LC conditions and NI fragmentation voltages (300−350 V) were used as indicated in the text to separate overlapped peaks and to produce more abundant fragments for some compounds. Negative ionization−selective ion monitoring (NI-SIM, at 100 V) was also used to detect hydroxycinnamoyl derivatives with quinic acid, malic acid, and glucose. The SIM analyses were performed on separate injections to enhance the signal-to-noise ratio. The NI ions at m/z 353, 337, 367, 383, and 397 [for caffeoyl-, *p*-coumaroyl-, feruloyl-, hydroxyferuloyl-, and sinapoylquinic acids (*23*, *24*, *34*)], m/z 279, 295, 309, 325, and 339 [their analogues consisted of malic acid (*23*, *24*)], and m/z 325, 341, 355, 371, and 385 [glucosides of hydroxycinnamic acids (*23*, *24*)] were monitored.

RESULTS AND DISCUSSION

The samples for this study were collected at local grocery stores and were not intended to constitute a statistical sampling of *Brassica* vegetables in the market place. The specific varieties were not known. Samples were dried at room temperature, which undoubtedly led to some deterioration of some of the phenolic compounds (*35*), as compared to more rigorous approaches for both identification and quantitative determination for which the harvested samples are flash frozen and lyophilized (*23*, *29*). Because the purpose of the study was intended to identify, not quantify, the phenolic compounds, these losses were deemed to be acceptable. Previous studies have shown that while some deterioration may occur, it was never sufficient to render the compounds undetectable (*35*).

Identification of O-Glycosylated Flavonols. The structures of the phenolic compounds commonly found in *Brassica* vegetables are shown **Figure 1**. HPLC chromatograms (350 nm) of collard greens, kale, Chinese broccoli, and their alkali-hydrolyzed extracts are shown in **Figure 2**. The retention times (t_R), wavelengths of maximum absorbance (λ_{max}), protonated/deprotonated molecules ($[M + H]^+/[M - H]^-$), diagnostic fragments produced with the high fragmentation voltage, and tentative identification of compounds are listed in **Table 1**. Many *Brassica* phenolic compounds, including the acylated compounds, have been previously reported in other *Brassica* vegetables (cabbage, bok choy, and broccoli) (*18*−*25*, *27*, *32*) and were available as reference plant materials. This permitted tentative, but very reliable, identification of many of the phenolic compounds despite the lack of standards.

Table 1 shows that of the 45 flavonol glycosides detected in the extracts, 15 of them were nonacylated glycosides (peaks 1A, 1B, 2A, 2B, 2C, 2D, 13C, 17A, 18, 20, 30, 31A, 31B, 31C, and 32). Multiple compounds with indistinguishable retention times are designated by a letter following the peak number. Six of the compounds (peaks 1A, 1B, 2A, 2B, 2C, and 2 D) were separated with a modified mobile phase of 4−14% B in 40 min and 26% B at 70 min. The retention times were as follows: 1A, 22.6 min; 1B, 24.2 min; 2A, 24.7 min; 2B, 25.4 min; 2C, 26.0 min; and 2D, 29.1 min (chromatograms not shown). The peak areas were in the order 2B > 2D > 1A > 2A > 2C > 1B in the aqueous methanol extracts.

Kaempferol and quercetin were the only tetra- and pentahydroxyflavones in the acid-hydrolyzed extracts, indicating that their glycosides were the main flavonoids in these vegetables. Isorhamnetin was the only other flavonol to form glycosides (minor peaks 18, 30, and 31B) observed in these three *Brassica* vegetables. Kaempferol 3-*O*-glycosides and 3-*O*-glycoside-7-*O*-glycosides had characteristic UV absorption maxima at 266 and 348 nm, and quercetin glycosides had peaks at 256, 266, and 354 nm. However, the 7-*O*-glycosides of kaempferol had UV maxima at 264 and 366 nm (*33*, *36*) and were readily distinguished by their different retention times. The retention time of the nonacylated glycosides of kaempferol and quercetin, with glycosyls at both the 3- and 7-positions, ranged from 6 to 15 min, whereas the retention times for glycosides at either the 3- or 7-position, but not both, ranged from 17 to 40 min.

Previous studies have shown that the preferential fragmentation of flavonol 3-*O*-, 7-*O*-diglycosides was at position 7 for negative ionization (*19*, *22*, *28*). In the present study, quercetin 3-*O*-diglucoside-7-*O*-rhamnoside in watercress (*18*) was used to check the fragmentation pathway (spectrum not shown) and indicated that the rhamnosyl at the 7-position was lost first. This observation is consistent with the results from previous studies on flavonoids in *Brassica* vegetables (*18*−*20*, *22*−*28*, *32*) and was key to identifying the 3-*O*-glycoside-7-*O*-glycosides of kaempferol (or quercetin) with one or two glucosyls at the 7-position.

As an example, peaks 2A and 2D had the same molecular ($[M + H]^+/[M - H]^-$, at m/z 935/933, $[M + Na]^+$ at m/z 957) and aglycone (m/z 287/285) ions, suggesting that they were isomeric

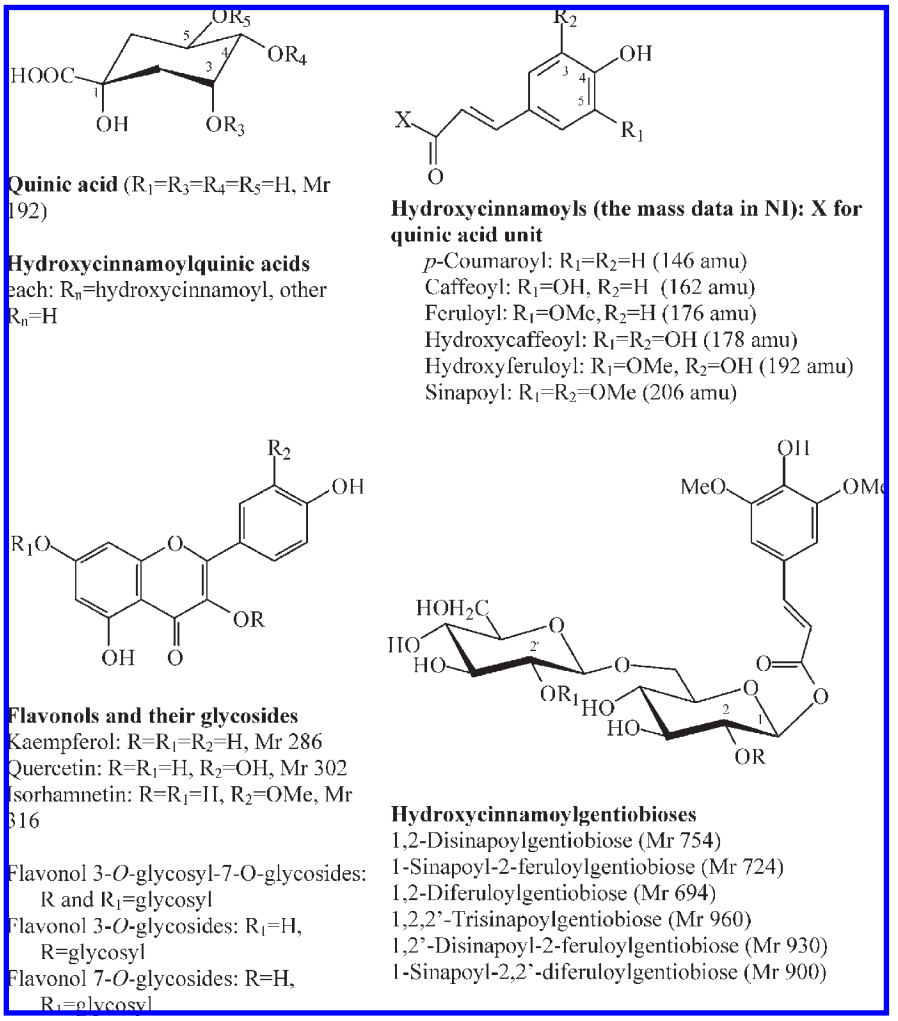

Figure 1. Structure skeletons of hydroxycinnamoylquinic acid derivatives and flavonoids existing in *Brassica* vegetables.

kaempferol glycosides with a total four glucosyls at the 7- and 3-positions. They were easily distinguished, however, by the difference in counts for their largest diagnostic fragments formed by the loss of their glucosyl(s) at the 7-position. Peak 2A had its largest fragment $[M - H - glc]^-$ at m/z 771, indicating only one glucosyl at the 7-position. This peak was identified as kaempferol 3-*O*-triglucoside-7-*O*-glucoside. Its other diagnostic fragments, formed by the sequential loss of glucosyls, are listed in **Table 1** and shown in **Figure 3A**. Unlike peak 2A, peak 2D had its largest fragment $[M - H - 2 \times glc]^-$ at m/z 609 (**Figure 3B**), indicating the existence of two glucosyls at the 7-position. This compound was identified as kaempferol 3-*O*-diglucoside-7-*O*-diglucoside.

Figure 2 shows that the remaining 30 flavonoids were acylated glycosides of kaempferol and quercetin and that they were converted to their parent glycosides after alkali hydrolysis. Peaks for nonacylated flavonoids, especially those of kampferol 3-*O*-triglucoside-7-*O*-diglucoside, kampferol 3-*O*-triglucoside-7-*O*-glucoside, kampferol 3-*O*-diglucoside-7-*O*-glucoside, kampferol 3-*O*-diglucoside-7-*O*-diglucoside, quercetin 3-*O*-diglucoside-7-*O*-glucoside, and quercetin 3-*O*-diglucoside-7-*O*-diglucoside significantly increased in the alkali-hydrolyzed extracts (compare **Figure 2B** to **Figure 2A**, **Figure 2D** to **Figure 2C**, and **Figure 2F** to **Figure 2E**).

The acylated flavonoid glycosides can be easily recognized from the wavelength of their UV maxima and the enhanced molecular weight of the parent ion. For example, a UV maximum at 310−312 nm and a molecular mass 146 amu greater than that of the parent glycoside suggested the presence of *p*-coumaroyl. The caffeoyl-, feruloyl-, hydroxyferuloyl-, sinapoyl-, and disinapoyl-glycosides had UV maxima at 330−336 nm and molecular mass increases of 162, 176, 192, 206, and 412 amu, respectively. The phenolic acids produced with alkaline hydrolysis were labeled peaks A-1 to A-5 (**Figure 2B**) for caffeic, hydroxyferulic, *p*-coumaric, sinapic, and ferulic acids, respectively.

The coeluting flavonoids in peaks 13A and 13B were identified using the same logic presented in the preceding paragraph. Both had UV maxima at 266 and 332 nm, PI and NI parent ions at m/z 1111/1109, and aglycone ions at m/z 287/285. Their retention time (12.6 min) and UV data suggested they were kaempferol tetraglucosides with all of the glucosyls at the 3- and 7-positions and a feruloyl at the 3-position. They were identified as feruloyl derivatives of kaempferol 3-*O*-triglucoside-7-*O*-glucoside (peak 2A) (**Figure 3A**) and kaempferol 3-*O*-diglucoside-7-*O*-diglucoside (peak 2D) (**Figure 3B**), respectively, by their diagnostic negative fragments (**Figure 3C**).

The largest diagnostic fragment of 13A at m/z 947 ([Ma − H − glc]$^-$), from the loss of the sole glucosyl (162 amu) at the 7-position, was 176 amu larger than the largest fragment (m/z 771) of kaempferol 3-*O*-triglucoside-7-*O*-glucoside (**Figure 3A**). The next fragment, m/z 771, was produced by the loss of feruloyl (176 amu) at the 3-position. These spectral features suggested that this peak was kaempferol 3-*O*-feruloyltriglucoside-7-*O*-glucoside (peak 13A).

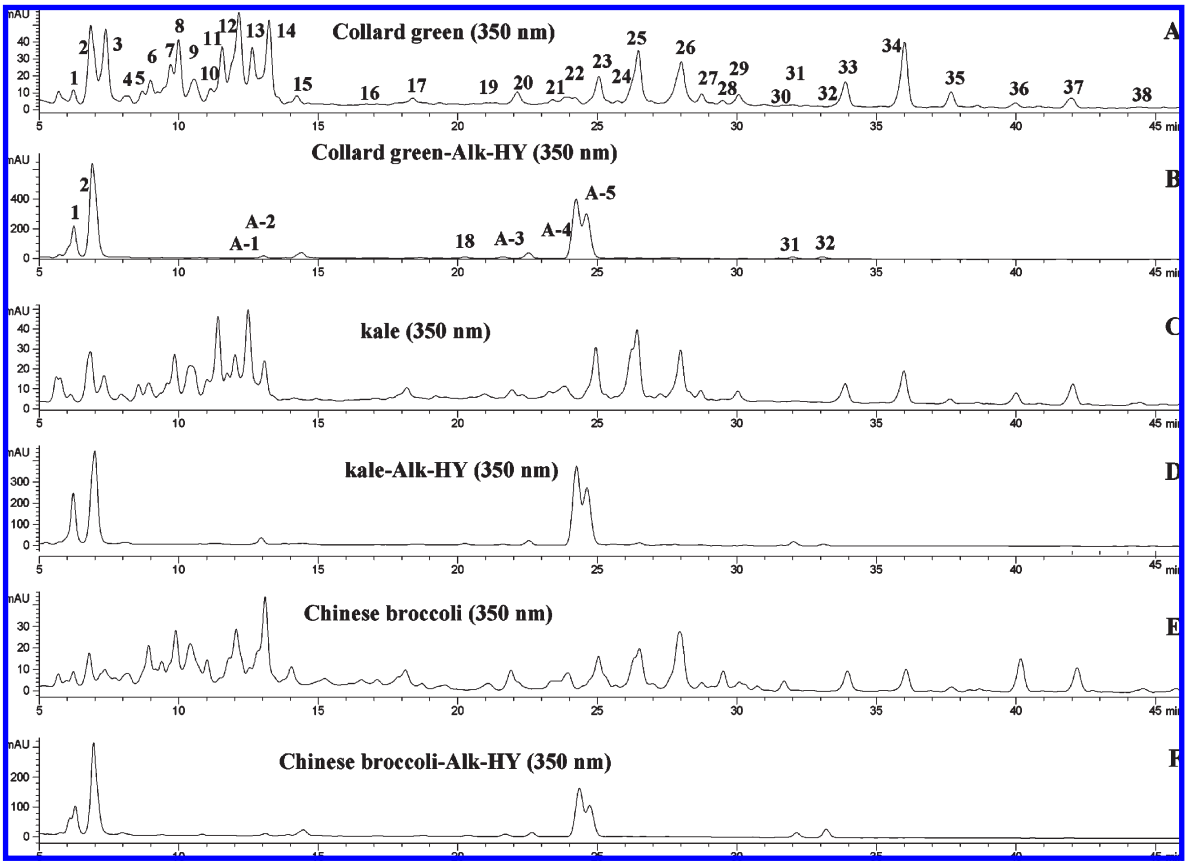

Figure 2. LC chromatograms (350 nm) of collard greens (**A**), kale (**C**), and Chinese broccoli (**E**) and their alkali-hydrolyzed extracts (**B**, **D**, and **F**, respectively).

Peak 13B was identified as kaempferol 3-*O*-feruloyldiglucoside-7-*O*-diglucoside because it has fragments at *m*/*z* 785 ([Mb − H − 2 × glc]$^-$ from the loss of the diglucosyl at the 7-position) and *m*/*z* 609 (from the loss of feruloyl at the 3-position). The latter fragment (*m*/*z* 609) was the same as the largest fragment of its parent flavonoid, kaempferol 3-*O*-diglucoside-7-*O*-diglucoside (**Figure 3B**). Assuming similar ionization potentials, the count ratios of the fragments at *m*/*z* 947 ([Ma − H − glc]$^-$) and 785 ([Mb − H − 2xglc]$^-$) (**Figure 3C**) indicate that the molar concentration ratio for peaks 13A and 13B was approximately 3:1. Their identifications were further supported by the fact that kaempferol 3-*O*-feruloyldiglucoside-7-*O*-diglucoside, kaempferol feruloyltetraglucosides, and kaempferol 3-*O*-feruloyltriglucoside-7-*O*-glucoside have been previously reported in enriched fractions of cauliflower byproducts, broccoli, Chinese leaf mustard green, and turnip tops (*19*, *23*, *25*−*27*).

Peak 9A was identified, in a manner similar to that described above, as kaempferol 3-*O*-sinapoyltriglucoside-7-*O*-diglucoside. It had a molecular ion at *m*/*z* 1301 [M − H]$^-$, diagnostic ions at *m*/*z* 977 ([M − H − 2 × glc]$^-$), *m*/*z* 771 (loss of sinapoyl, 206 amu), and *m*/*z* 609, and an aglycone ion at *m*/*z* 285 (sequential loss of glucosyls at 3-position). These data suggested that this flavonoid should be kaempferol 3-*O*-sinapoyltriglucoside-7-*O*-diglucoside. In the same manner, peaks 5B, 6, 7A, 8A, 9C, 10B, 12A, 14, 15, and 17B were identified as kaempferol 3-*O*-acyldiglucoside-7-*O*-glucosides, whereas peaks 5A, 7B, 9A, and 11A were identified as kaempferol 3-*O*-acyltriglucoside-7-*O*-glucosides and peaks 4, 9B, 9C, and 10B were identified as the acylated 3-*O*-glycoside-7-*O*-glycoside of quercetin.

The remaining eight peaks (21, 22, 23A, 24, 25, 26A, and 29) were identified as 3-*O*-diacyltriglucoside-7-*O*-diglucosides of kaempferol and quercetin. Among them, peaks 23A and 24 were isomeric kaempferol 3-*O*-feruloylhydroxyferuloyltriglucside-7-*O*-diglucoside, whereas peaks 27 and 29 were isomeric kaempferol 3-*O*-diferuloyltriglucside-7-*O*-diglucoside. The isomers arise from different positions of the acyls on the sugars. These compounds easily lost the diglucosyl from the 7-position, providing strong fragment ions at the mass corresponding to [M − H − diglucosyl]$^-$, and, in general, eluting later (from 23 to 30 min) than their monoacylated polyglycoside analogues (from 8 to 20 min). Their identification was further supported by previous reports of acylated kaempferol pentaglucosides in enriched flavonoid fractions of broccoli and cauliflower byproducts (*19*, *25*, *27*, *32*).

Polyglycosylated kaempferols and quercetins, such as quercetin 3-*O*-triglucoside-7-*O*-diglucoside (Mr 1112), were also reported in the alkali hydrolysate of curly kale (*32*) and enriched broccoli fractions (*27*). Kaempferol 3-*O*-tetraglucoside-7-*O*-diglucosides (Mr 1256) were reported in the external leaves of Tronchuda cabbage (*20*), and analogous polyglycosylated isorhamnetin were detected in the enriched broccoli fractions (*27*). However, these glycosides of kaempferol and quercetin were not clearly detected in this study using the extraction masses, solvent volumes, and injection volumes described under Materials and Methods.

The list of flavonoids identified by earlier studies on Italian kale and the kale in Maryland (*17*, *21*) has recently been expanded by a LC-MS^n study on curly kale, which identified 23 flavonoids and 9 phenolic acid derivatives (*32*). Chromatograms of the alkali-hydrolyzed extracts from the current study and the curly kale study were similar. Each showed the presence of kaempferol 3-*O*-diglucoside-7-*O*-glucoside, kaempferol 3-*O*-diglucoside-7-*O*-digucoside, quercetin 3-*O*-diglucoside-7-*O*-glucoside, and sinapic and ferulic acid as the main compounds. In all, 14 flavonoids were reported by both studies, 9 flavonoids reported for curly kale are not reported here, and more than 20 flavonoids reported here were not found in curly

Table 1. Phenolic Components of Collard Greens, Kale, and Chinese Broccoli

peak	t_R (min)	$[M + H]^+/[M - H]^-$ (*m*/*z*)	NI diagnostic ions (*m*/*z*)	UV λ_{max} (nm)	identification[a]
			45 Flavonol Glycosides		
1A[b,c]	6.1	789/787	—/625, 463, 301	256, 266, 354	Q 3-*O*-diglucoside-7-*O*-glucoside[d]
1B[b,c]	6.3	951/949	—/625, 463, 301	256, 266, 354	Q 3-*O*-diglucoside-7-*O*-diglucoside[e]
2A[b,c]	6.6	935/933	—/771, 609, 447, 285	266, 348	K 3-*O*-triglucoside-7-*O*-glucoside[e]
2B[b,c]	6.7	773/771	—/609, 447, 285	266, 348	K 3-*O*-diglucoside-7-*O*-glucoside[d]
2C[b,c]	6.8	1097/1095	—/771, 609, 447, 285	266, 348	K 3-*O*-triglucoside-7-*O*-diglucoside[e]
2D[b,c]	6.9	935/933	—/609,447,285	266, 348	K 3-*O*-diglucoside-7-*O*-diglucoside[d]
4	8.2	951/949	—/787, 625, 463, 301	256, 266, 334	Q 3-*O*-caffeoyldiglucoside-7-*O*-glucoside[d]
5A	8.6	1127/1125	—/771, 609, 285	268, 332	K 3-*O*-hydroxyferuloyldiglucoside-7-*O*-diglucoside[d]
5B	8.7	965/963	—/801, 609, 447, 285	268, 332	K 3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside
6	9.0	965/963	—/801, 609, 447, 285	268, 332	K 3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside[d]
7A	9.5	935/933	—/771, 609, 447, 285	268, 332	K 3-*O*-caffeoyldiglucoside-7-*O*-glucoside[d]
7B	9.6	1097/1095	—/771, 609, 447, 285	268, 332	K 3-*O*-caffeoyldiglucoside-7-*O*-diglucoside[d]
8A	10.0	935/933	—/771, 609, 447, 285	268, 332	K 3-*O*-caffeoyldiglucoside-7-*O*-glucoside[d]
9A	10.5	1303/1301	—/977, 771, 609, 285	nd	K 3-*O*-sinapoyltriglucoside-7-*O*-diglucoside[d]
9B	10.6	1127/1125	—/801, 625, 301	256, 266, 332	Q 3-*O*-feruloyldiglucoside-7-*O*-diglucoside[d]
9C	10.7	995/993	—/831, 625, 301	256, 266, 332	Q 3-*O*-sinapoyldiglucoside-7-*O*-glucoside
10B	11.1	965/963	—/801, 625, 301	nd	Q 3-*O*-feruloyldiglucoside-7-*O*-glucoside[d]
11A	11.6	1141/1139	—/815, 609, 447, 285	266, 332	K 3-*O*-sinapoyldiglucoside 7-*O*-diglucoside[d]
12A	12.2	979/977	—/815, 609, 449, 285	266, 332	K 3-*O*-sinapoyldiglucoside 7-*O*-glucoside
13A	12.5	1111/1109	—/947, 771, 609, 449, 285	266, 332	K 3-*O*-feruloyltriglucoside-7-*O*-glucoside
13B	12.6	1111/1109	—/785, 609, 285	nd	K 3-*O*-feruloyldiglucoside-7-*O*-diglucoside[d]
13C	12.8	611/609	—/447, 287	266, 348	K 3-*O*-glucoside-7-*O*-glucoside[d]
14	13.2	949/947	—/785, 609, 447, 285	266,332	K 3-*O*-feruloyldiglucoside-7-*O*-glucoside
15	14.2	919/917	—/755, 609, 447, 285	266, 312	K 3-*O*-*p*-coumaroyldiglucoside-7-*O*-glucoside
17A	18.4	627/625	—/463, 301	nd	Q 3-*O*-diglucoside
17B	18.7	919/917	—/755, 609, 447, 285	266, 312	K 3-*O*-*p*-coumaroyldiglucoside-7-*O*-glucoside
18	20.5	641/639	—/477, 315	256, 266sh, 352	I 3-*O*-diglucoside
19	21.0	833/831	—/625, 463, 301	nd	Q 3-*O*-sinapoyldiglucoside[d]
20	22.1	611/609	—/447, 285	266, 346	K 3-*O*-diglucoside[d]
21	23.4	1481/1479	—/1155, 625, 301	nd	Q 3-*O*-feruloylhydroxyferuloyltriglucoside-7-*O*-diglucoside
22	23.9	1495/1493	—/1169, 609, 285	266, 332	K 3-*O*-sinapoylhydroxyferuloyltriglucoside-7-*O*-diglucoside
23A	25.1	1465/1463	—/1139, 609, 285	266, 332	K 3-*O*-feruloylhydroxyferuloyltriglucoside-7-*O*- diglucoside
23B	25.2	817/815	—/609, 447, 285	266, 332	K 3-*O*-sinapoyldiglucoside
24	25.7	1465/1463	—/1139, 609, 285	266, 332	K 3-*O*-feruloylhydroxyferuloyltriglucoside-7-*O*-diglucoside
25	26.5	1509/1507	—/1183, 609, 285	266, 332	K 3-*O*-disinapoyltriglucoside-7-*O*-diglucoside[d]
26A	27.8	1479/1477	—/1153, 947, 609, 285	266, 332	K 3-*O*-sinapoylferuloytriglucoside-7-*O*-diglucoside
26B	28.0	787/785	—/609, 447, 285	nd	K 3-*O*-feruloyldiglucoside
27	28.7	1449/1447	—/1123, 947, 609, 285	266, 332	K 3-*O*-diferuloyltriglucoside-7-*O*-diglucoside
28	29.5	757/755	—/609, 449, 285	nd	K 3-*O*-*p*-coumaroylglucoside-7-*O*-glucoside
29	30.1	1449/1447	—/1123, 947, 609, 285	266, 332	K 3-*O*-diferuloyltriglucoside-7-*O*-diglucoside
30	30.6	641/639	—/477, 315	256, 266sh, 346	I *O*-diglucoside
31A	31.6	449/447	—/285	266, 348	K 3-*O*-glucoside[g]
31B	31.7	479/477	—/315	256, 266sh, 346	I *O*-glucoside[g]
31C	32.0	611/609	—/447, 285	264, 366	K 7-*O*-diglucoside
32	33.1	449/447	—/285	264, 366	K 7-*O*-glucoside
			13 Hydroxycinnamoylquinic Acids and Gentiobioses		
3[g]	7.3	—/353	—/191, 179, 135	240, 298, 328	3-caffeoylquinic acid[f]
8B	10.3	—/337	—/191, 163, 119	310	3-*p*-coumaroylquinic acid
10A[g]	11.0	—/353	—/191, 179, 135	240, 298, 328	5-caffeoylquinic acid
12B[g]	12.2	—/353	—/191, 179, 135	nd	4-caffeoylquinic acid
12C	12.3	—/367	—/191, 193, 149	nd	3-feruloylquinic acid
16	16.6	—/337	—/191, 163, 119	310	5-*p*-coumaroylquinic acid
17C	17.8	—/367	—/191, 193, 149	nd	5-feruloylquinic acid
33	34.0	777[h]/753	—/529	240, 332	1,2-disinapoylgentiobiose[d]
34	36.0	747[h]/723	—/499	240, 332	1-disinapoyl-2-feruloylgentiobiose[d]
35	37.7	717[h]/693	—/499	240, 332	1,2-diferuloylgentiobiose
36	40.0	983[h]/959	—/735	240, 332	1,2,2′-trisinapoylgentiobiose[d]
37	42.0	953[h]/929	—/705	240, 332	1,2′-disinapoyl-2-feruloylgentiobiose[d]
38	44.6	923/899	—/675	240, 332	1-sinapoyl-2,2′-diferuloylgentiobiose
			5 Hydroxycinnamic Acids in Alkali-Hydrolyzed Extract		
A-1	14.2	—/179	—/135	nd	caffeic acid
A-2	14.7	—/209	—/165	nd	hydroxyferulic acid

Table 1. Continued

peak	t_R (min)	$[M + H]^+/[M - H]^-$ (*m*/*z*)	NI diagnostic ions (*m*/*z*)	UV λ_{max} (nm)	identification[a]
A-3	21.4	—/163	—/119	310	*p*-coumaric acid
A-4	23.8	—/223	—/179	240, 298, 328	sinapic acid
A-5	24.2	—/193	—/149	240, 298, 328	ferulic acid

[a] K, kaempferol; Q, quercetin; I, isorhamnetin; nd, not determined. [b] Letters indicate compounds with the same or close retention times. [c] They were well separated with the mobile phase of 4−14% B in 40 min; the retention times are listed in the text. [d] Previously identified in curly kale (*32*). [e] Previously reported in alkali-hydrolyzed curly kale extract (*32*). [f] Just reported as caffeoylquinic acid (*32*). [g] identified with standard. [h] $[M + Na]^+$ for gentiobioses.

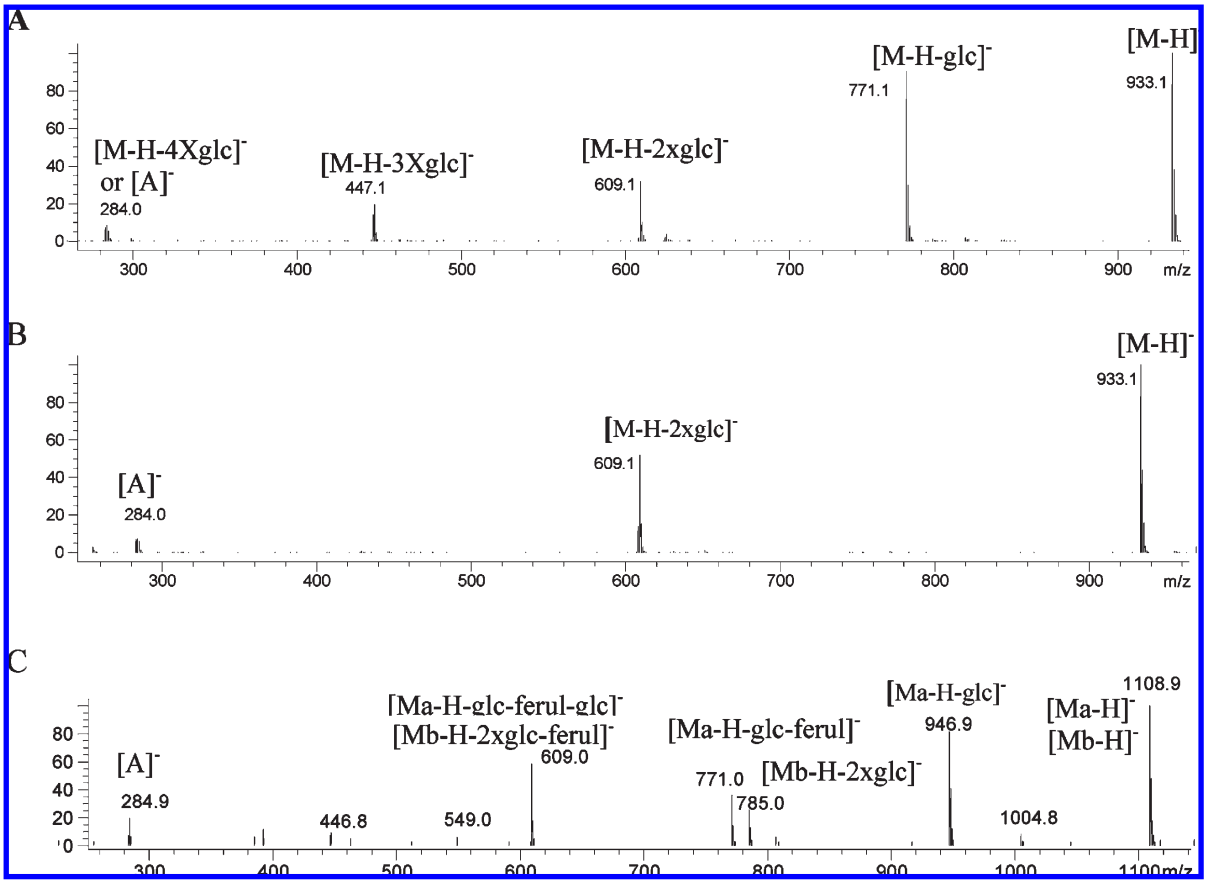

Figure 3. (**A**) Deprotonated mass spectrum of kaempferol 3-*O*-triglucoside-7-*O*-glucoside (peak 2A), obtained from negative ionization 350 V fragmentation energy TIC chromatogram. (**B**) Deprotonated mass spectrum of kaempferol 3-*O*-diglucoside-7-*O*-diglucoside (peak 2D), obtained from negative ionization 350 V fragmentation energy TIC chromatogram. (**C**) Deprotonated mass spectra of peaks 13A (kaempferol 3-*O*-sinapoyltriglucoside-7-*O*-glucoside) and 13B (kaempferol 3-*O*-sinapoyldiglucoside-7-*O*-diglucoside), obtained from negative ionization 250 V fragmentation energy TIC chromatogram.

kale. For collard greens and Chinese broccoli, most of the flavonols listed in **Table 1** are reported for the first time.

Hydroxycinnamoylquinic Acids and Gentiobioses. Six hydroxycinnamoyl gentiobioses (**Figure 1**) were previously reported (*16*−*28*) in the *Brassica* vegetables studied here. The retention times ranged from 33 to 45 min. With the exception of peak 38, their identification was made by direct comparison with hydroxycinnamoyl gentiobioses positively identified in other broccoli samples (*37*). Peak 38 (t_R 44.6 min) eluted slightly later than peak 37 (1,2′-disinapoyl-2-feruloylgentiobiose), had $[M - H]^-$ at *m*/*z* 899 and $[M + Na]^+$ ion at 923, and had the same UV maxima as peak 37. This suggested that peak 38 should be 1-sinapoyl-2,2′-diferuloylgentiobiose (**Figure 1**). This compound was previously reported in some broccoli samples as a minor component (*16*). Curly kale contained four hydroxycinnamoyl gentiobioses and three hydroxycinnamoyl triglucosides (sinapoylferuloyltriglucoside, diferuloyltriglucoside, disinapoylferuloyltriglucoside) (*32*). These triglucosides were not detected in the current study.

Derivatives formed from the interaction of hydroxycinnamic acids with quinic acid, malic acid, glucose, and benzoic acids (*29*) were previously reported in kale, pak choi, and Chinese leaf mustard and some of their cultivars (*22*−*25*, *29*, *32*). However, only some of the hydroxycinnamoylquinic acids, such as caffeoyl-, feruloyl-, and *p*-coumaroylquinic acids, were positively identified in collard greens, kale, and Chinese broccoli samples on the basis of direct comparison to standard or reference compounds (*33*, *34*). It is worth noting that the concentration of the 3-isomer was always larger than that of the 5- or 4-isomers.

The phenolic profiles of collard greens, kale, and Chinese broccoli were very similar. This was not unexpected since they are all varieties of *B. oleracea* L. The data presented here is useful for the identification of compounds in *Brassica* vegetables grown worldwide, or, conversely, for confirming the taxonomy of suspected vegetables. Further studies are warranted that more rigorously investigate specific varieties, harvest times, locations, and growing conditions.

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