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ARTICLE

Phenolic Component Profiles of Mustard Greens, Yu Choy, and 15 Other *Brassica* Vegetables

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A liquid chromatography−mass spectrometry (LC-MS) profiling method was used to characterize the phenolic components of 17 leafy vegetables from *Brassica* species other than *Brassica oleracea*. The vegetables studied were mustard green, baby mustard green, gai choy, baby gai choy, yu choy, yu choy tip, bok choy, bok choy tip, baby bok choy, bok choy sum, Taiwan bok choy, Shanghai bok choy, baby Shanghai bok choy, rapini broccoli, turnip green, napa, and baby napa. This work led to the tentative identification of 71 phenolic compounds consisting of kaempferol 3-*O*-diglucoside-7-*O*-glucoside derivatives, isorhamnetin 3-*O*-glucoside-7-*O*-glucoside hydroxycinnamoyl gentiobioses, hydroxycinnamoylmalic acids, and hydroxycinnamoylquinic acids. Ten of the compounds, 3-*O*-diacyltriglucoside-7-*O*-glucosides of kaempferol and quercetin, had not been previously reported. The phenolic component profiles of these vegetables were significantly different than those of the leafy vegetables from *B. oleracea*. This is the first comparative study of these leafy vegetables. Ten of the vegetables had never been previously studied by LC-MS.

KEYWORDS: *Brassica* vegetables; flavonoids; hydroxycinnamic acid derivatives; LC-DAD-ESI/MS analysis; phenolic component profiling

INTRODUCTION

Brassica vegetables are commonly consumed and have received considerable research attention because of their association with reduced risk for cardiovascular diseases and some cancers, especially those of the gastrointestinal tract. While primarily recognized for their sulfur compounds, that is, the glucosinolaates, they also contain numerous phenolic compounds (*1*−*6*). In a previous report, we identified the phenolic components of collard greens, kale, and Chinese broccoli, the green leafy vegetables of varieties of *Brassica oleracea* (*7*). In this study, we present the phenolic component profiles of 17 brassica vegetables, which belong to *Brassica* species other than *B. oleracea*. These vegetables are available in local food stores or local oriental food stores in the United States and are among the major brassica vegetables consumed in China and some Asian countries.

Mustard greens, baby mustard greens, Chinese leaf mustard (gai choy or gai choi), and baby gai choy are from *Brassica juncea* Coss and its varieties or cultivars. Flowering Chinese cabbage (yu choy) and yu choy tip are the edible rapes of *B. rapa* var. *oleifera* or *B. compestris*. Bok choy (or pak choi; daqingcai or dabaicai in Chinese), bok choy tip, baby bok choy (xiaoqingcai or xiaobaicai in Chinese), bok choy sum (or choy sum), Taiwan bok choy, Shanghai bok choy (Shanghai qingcai in Chinese), and baby Shanghai bok choy are the leafy stalks of *B. rapa* ssp. *chinensis* L. (Hanelt.), *B. campestris* L. ssp. *chinensis* var. *communis*, or *B. chinensis* or their cultivars. Napa (or napa cabbage, Chinese cabbage; dabaicai or huangyacai in Chinese) and baby napa are from *B. rapa* ssp. *pekinensis* or *B. pekinensis* (Lour.) Rupr. Rapini broccoli rabe (broccoli raab) is from *B. rapa* var. *ruvo*, and turnip greens are from *B. rapa* L. var. *rapa* (*8*−*10*).

Previous studies using liquid chromatography−mass spectrometry (LC-MS) have identified and quantified the phenolic components of some of the vegetables listed above, that is, turnip, pak choi, and Chinese leaf mustard and some their cultivars (*11*−*26*). However, there has been no systematic comparison of the flavonoid and hydroxycinnamic acid derivatives of these 17 green leafy brassica vegetables. In this study, we have comprehensively surveyed the phenolic components of the brassica vegetables listed above. Peaks of identified compounds were used for principal component analysis (PCA). The emphasis of this study was on the identification of the phenolic compounds and examination of the similarity of the phenolic composition of the analyzed materials.

MATERIALS AND METHODS

Flavonoid Standards and Other Chemicals. Quercetin dihydrate, kaempferol, and chlorogenic, caffeic, *p*-coumaric, ferulic, and sinapic acids were obtained from Sigma Chemical Co. (St. Louis, MO). Quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, and isorhamnetin 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 (*27*). High-performance liquid chromatography (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 Lab., Bedford, MA).

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Plant Materials. Seventeen brassica vegetables, that is, mustard greens, baby mustard greens, gai choy, baby gai choy, yu choy, yu choy tip, bok choy, baby bok choy, bok choy sum, Taiwan bok choy, Shanghai bok choy, baby Shanghai bok choy, napa and baby napa, rapini broccoli, and turnip greens, were bought in local food stores in Maryland. The specific varieties and cultivars for many of the samples were not known. These samples were not intended to constitute a statistical sampling of brassica vegetables in the market place. Samples were dried at room temperature in a hood for approximately 5 days, powdered, and passed through 20 mesh sieves prior to extraction (*7*). This drying process undoubtedly led to deterioration of some of the phenolic compounds (*28*) as compared to more rigorous approaches for quantification that flash froze the samples prior to lyophilization (*18*, *19*). Because the purpose of the study was identification, not quantification, of the phenolic compounds, these losses were deemed acceptable. Previous studies have shown that while some deterioration may occur, it was never sufficient to render the compounds undetectable (*28*). In this study, we determined that all of the phenolics observed in the flash-frozen Shanghai bok choi, baby Shanghai bok choi, gai choy, and collard greens were also observed in the air-dried samples.

Plant Extracts. The powder of each plant (250 mg) was extracted with 5.0 mL of methanol:water (60:40, v/v) using sonication with a FS30 Ultrasonic sonicator (40 kHz, 100 W) (Fisher Scientific, Pittsburgh, PA) for 60 min at room temperature (< 35 °C at the end). 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 injected into the HPLC (*7*, *27*).

Acidic Hydrolyzed Extracts. 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 filtered prior to HPLC injection (*27*).

Alkaline Hydrolyzed Extracts. 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 filtered prior to HPLC injection (*7*, *27*).

LC−Diode Array Detection (DAD) and Electrospray Ionization−Single-Quadrupole Mass Spectrometry (ESI-MSD) Conditions. The LC/DAD/MS system used consisted of a quaternary pump with a vacuum degasser, a thermostatted column compartment, an autosampler, a DAD, and a 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 to 26% B (v/v) 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, 330, 310, and 270 nm to record the peak intensities. UV spectra were recorded from 190 to 450 nm by the DAD for plant component identification. Mass spectra were simultaneously acquired using ESI in the positive and negative ionization (PI and NI) modes at low (100 V) and high fragmentation voltages (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 (*7*, *27*).

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 ions obtained with NI at m/z 353, 337, 367, 381, and 397 (for caffeoyl-, *p*-coumaroyl-, feruloyl-, hydroxyferuloyl-, and sinapoyl-quinic acids), 279, 295, 309, 325, and 339 (the related analogues consisted of malic acid), and 325, 341, 355, 369, and 385 (the related glucosides of hydroxycinnamic acids) were monitored (*7*).

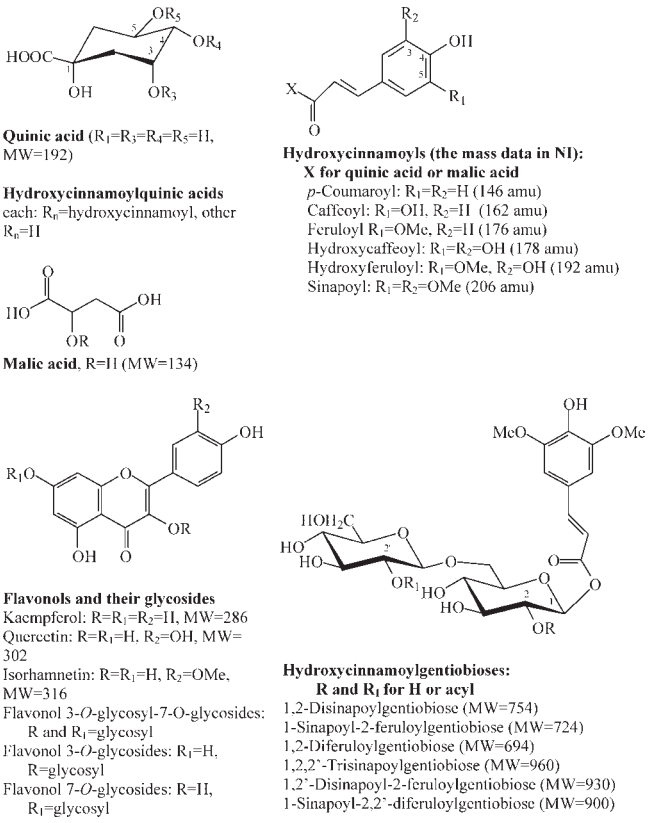

Figure 1. Structural skeletons of hydroxycinnamic acid derivatives and flavonoids found in *Brassica* vegetables.

PCA. A total of 71 phenolic compounds were identified in the 17 brassica vegetables. To elucidate the pattern of the phenolic composition of the vegetables, a 17 × 71 matrix was constructed that qualitatively identified each compound. A value of "1" was assigned if the compound was observed, and a value of "0" was assigned if the compound was not observed. A compound was omitted if it was present in each vegetable and did not contribute any information for distinguishing between vegetables, reducing the table to 17 × 45 matrix. This matrix was analyzed by PCA using Solo (eigenvector, Inc., Wenatchee, WA). Data were preprocessed by normalizing the squares of the sums of the values for each vegetable to unity. The data were then mean centered.

RESULTS AND DISCUSSION

Flavonols. Figure 1 presents the structures of the phenolic compounds commonly found in *Brassica* vegetables. **Figure 2** shows typical HPLC chromatograms of the extracts of (**A**) mustard greens, (**B**) alkali-hydrolyzed mustard greens, (**C**) yu choy, (**D**) rapini broccoli, (**E**) napa, and (**F**) Shanghai bok choy. **Table 1** provides the retention times (t_R), wavelengths of maximum absorbance (λ_{max}), protonated/deprotonated molecules ($[M + H]^+/[M - H]^-$), diagnostic fragments, and identification for each peak. Multiple compounds with indistinguishable retention times, for example 1A and 1B, are designated by a letter following the peak number. Peaks 1A, 1B, 2A, and 2B were separated with a modified mobile phase of 4−14% B in 40 min and 26% B at 70 min, and their retention times were 1A = 22.6 min, 1B = 24.2 min, 2A = 24.7 min, and 2B = 25.4 min (chromatograms not shown).

Fifty-one flavonoids, all glycosides of kaempferol, quercetin, and isrhamnetin, were detected in the 17 vegetables. Among them, 16 were nonacylated flavonoid glycosides, and 35 were acylated flavonoid glycosides. Close comparison of the retention times and UV and MS data of the chromatographic peaks acquired in this study with those acquired previously for collard

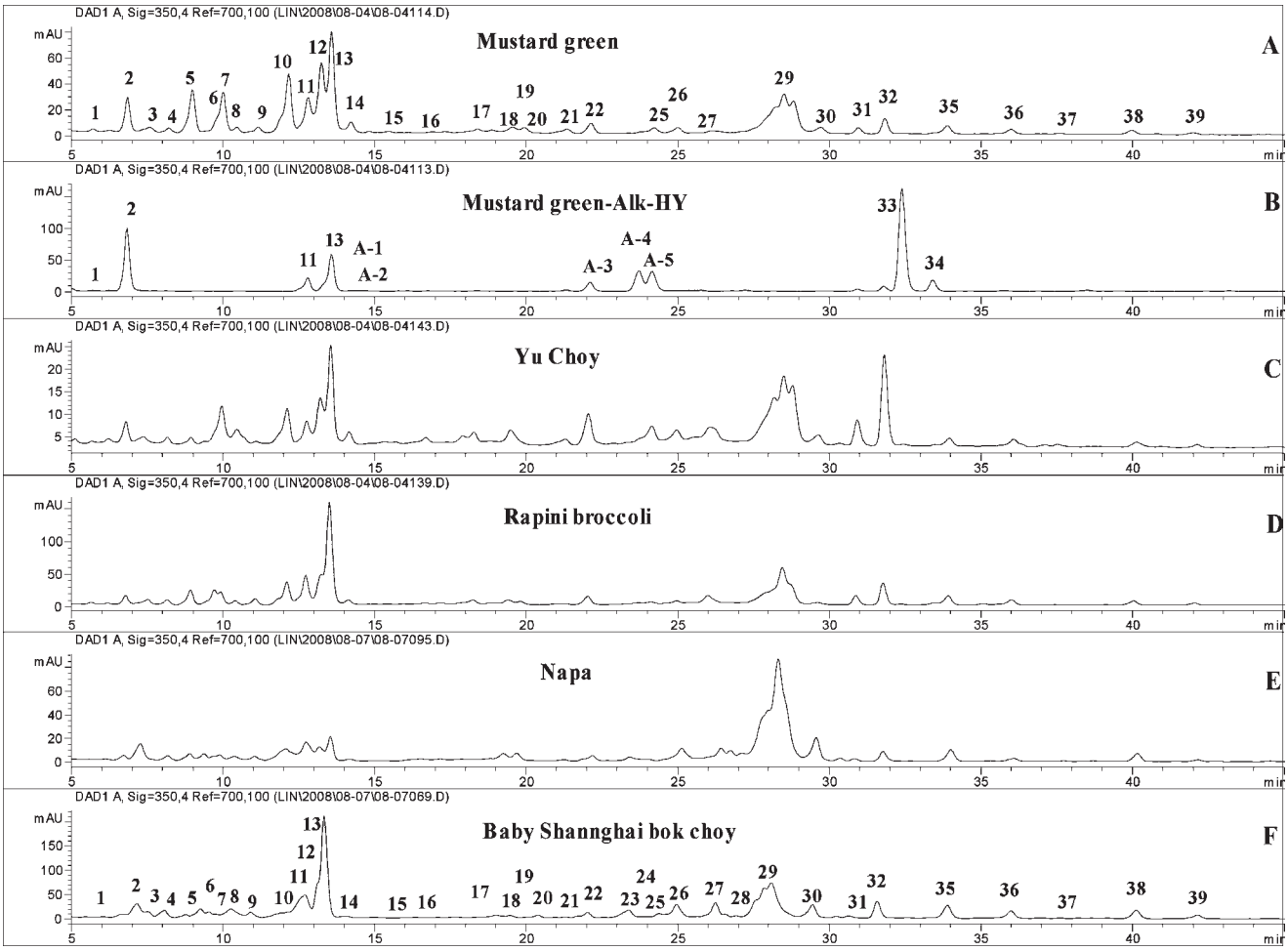

Figure 2. LC Chromatograms (350 nm) of the extracts of mustard green (**A**), mustard green alkali hydrolyzed (**B**), yu choy (**C**), rapini broccoli (**D**), napa (**E**), and baby Shanghai bok choy (**F**).

greens, kale, and Chinese broccoli (*7*) revealed that 24 of the compounds were identical. Thirty-eight of the compounds were previously reported by other researchers in bok choy, Chinese mustard greens, turnip tops, mizuna, and *B. rapa* var. *rapa* (*16*−*26*) and are denoted with a superscript "e" in **Table 1**.

Sixteen nonacylated glycosides were identified as quercetin 3-*O*-triglucoside-7-*O*-glucoside (peak 1A, only the number will be used for the rest of the text), quercetin 3-*O*-diglucoside-7-*O*-glucoside (1B), kaempferol 3-*O*-triglucoside-7-*O*-glucoside (2A), kaempferol 3-*O*-diglucoside-7-*O*-glucoside (2B), quercetin 3-*O*-glucoside-7-*O*-glucoside (7C), kaempferol 3-*O*-glucoside-7-*O*-glucoside (12B), isorhamnetin 3-*O*-glucoside-7-*O*-glucoside (13A), quercertin 3-*O*-diglucoside (17A), kaempferol dihexoside (17B), isorhamnetin 3-*O*-diglucoside (20), kaempferol 3-diglucoside (22B), quercetin 3-*O*-glucoside (27A), kaempferol 3-*O*-glucoside (31), isorhamnetin 3-*O*-glucoside (32), kaempferol 7-*O*-glucoside (33), and isorhamnetin 7-*O*-glucoside (34). Most of these identifications were confirmed by direct comparison with compounds found in collard greens, kale, and Chinese broccoli (*7*) and are denoted with a superscript "d" in **Table 1**.

Acylated flavonoid glycosides were easily identified based on the increase in mass of the parent ions and the wavelength maxima (330−336 nm) of their UV spectra. Thus, peaks 3B, 4B, 5, 6A, 7A, 8B, 9C, 10A, 11, 12C, 14, 17C, and 17 D were identified as the 3-*O*-acyldiglycoside 7-*O*-glucosides of kaempferol and quercetin, peaks 4A, 6B, 7B, 9B, and 10B were identified as kaempferol 3-*O*-acyltriglucoside-7-*O*-glucosides, and peaks 21, 25C, 26B, 29B, and 30A were identified as kaempferol 3-*O*-acyldiglucosides. Some of the peak identities (i.e., 4B, 5, 6A, 6B, 7A, 8B, 9C, 10A, 11, 12C, 14, and 26B) were also confirmed by direct comparison with compounds in collard green, kale, and Chinese broccoli (*7*).

Kaempferol *O*-acyltriglucoside-7-*O*-glucosides were detected in baby Shanghai bok choy and napa and rapini broccoli. Two of the compounds (4A and 7B) were previously reported in turnip tops (*20*), and all of them were probably present in the Chinese leaf mustard cultivar Xue Li Hong, since this cultivar contained seven kaempferol acyltetraglucosides, including three pairs of isomers (*18*). These flavonoids should be further identified as kaempferol 3-*O*-acyltriglucoside-7-*O*-glucosides. However, none of them were detected in either the Chinese mustard cultivar (Bao Bao Qing Cai) or the two bok choy cultivars (Hangzhou You Dong Er or Shanghai Qing) (*18*). Besides, the parent flavonoid, kaempferol 3-*O*-triglucoside-7-*O*-glucoside, was also reported in *B. rapa* var. rapa leave (*23*) and mizuna (*24*). It is worth mentioning that some of the peaks (e.g., 6A and 7A) have similar UV and mass spectra and are isomers with their acyls attached at different positions on the 3-*O*-glycosyl substituent.

Twelve other peaks (t_R = 22−30 min; molecular weights, 1280−1350 amu) were clearly detected in baby Shanghai bok choy and napa (**Table 1**). They were tentatively identified as the 3-*O*-diacyltriglucoside-7-*O*-glucosides of kaempferol (22A, 24, 25B, 26A, 27C, 28, 29A, and 30B) and quercetin (22C, 23A, 25A, and 25D). Their mass spectra showed that the largest diagnostic

Table 1. Phenolic Components of Mustard Green and 17 Other *Brassica* Vegetables

peak no.[a]	t_R (min)	$[M+H]^+/[M-H]^-$ (*m*/*z*)	PI/NI diagnostic ions (*m*/*z*)	UV λ_{max} (nm)	identification[b]
			51 flavonol glycosides		
1A[a,c]	5.9	951/949	−/787, 625, 463, 301	256, 266 sh, 354	Q 3-*O*-triglucoside-7-*O*-glucoside[d,e,g]
1B[a,c]	5.9	789/787	−/625, 463, 301	256, 266 sh, 354	Q 3-*O*-diglucoside-7-*O*-glucoside[d,e]
2A[a,c]	6.7	935/933	−/771, 609, 447, 285	266, 348	K 3-*O*-triglucoside-7-*O*-glucoside[d,e,g]
2B[a,c]	6.7	773/771	−/609, 447, 285	266, 348	K 3-*O*-diglucoside-7-*O*-glucoside[d,e]
3B	7.6	981/979	−/817, 787, 301	ND	Q 3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside[e]
4A	8.2	1127/1125	−/963, 935, 285	268, 332	K 3-*O*-hydroxyferuloyltriglucoside-7-*O*-glucoside[e]
4B	8.3	951/949	−/787, 625, 463, 301	256, 336	Q 3-*O*-caffeoyldiglucoside-7-*O*-glucoside[d,e]
5	8.8	965/963	−/801, 609, 447, 285	266, 334	K 3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside[d,e]
6A	9.4	935/933	−/771, 609, 449, 287	268, 332	K 3- *O*-caffeoyldiglucoside-7-*O*-glucoside[d,e]
6B	9.5	1097/1095	−/935, 773, 611, 449, 285	268, 332	K 3-*O*-caffeoyltriglucoside-7-*O*-glucoside[d,e]
7A	10.0	935/933	−/771, 609, 447, 87	266, 336	K 3-*O*-caffeoyldiglucoside-7-*O*-glucoside[d,e]
7B	10.0	1111/1109	−/947, 771, 609, 285	266, 332	K 3-*O*-feruloyltriglucoside-7-*O*-glucoside[e]
7C	10.1	627/625	465, 303/463, 301	256, 266, 354	Q 3-*O*-glucoside-7-*O*-glucoside[e]
8C	10.4	995/993	−/831, 787, 285	ND	Q 3-*O*-sinapoylldiglucoside-7-*O*-glucoside[d,e]
9A	11.1	1141/1139	−/977, 933, 773, 285	268, 332	K 3-*O*-sinapoyltriglucoside-7-*O*-glucoside[e]
9B	11.2	965/963	−/801, 625, 301	256, 336	Q 3-*O*-feruloyldiglucoside-7-*O*-glucoside[d,e]
10[b]	12.2	979/977	−/815, 771, 285	266, 334	K 3-*O*-sinapoylldiglucoside-7-*O*-glucoside[d,e]
10B	12.2	1111/1109	−/947, 771, 609, 285	266, 332	K 3-*O*-feruloyltriglucoside-7-*O*-glucoside[e]
11	12.6	949/947	−/785, 771, 285	268, 334	K 3-*O*-feruloyldiglucoside-7-*O*-glucoside[d,e]
12B	13.2	611/609	449, 287/447, 285	ND	K 3-*O*-glucoside-7-*O*-glucoside[d,e]
12C	13.2	949/947	−/785, 771, 285	268, 334	K 3-*O*-feruloyldiglucoside-7-*O*-glucoside[d,e]
13A	13.6	641/639	−/479, 315	256, 266sh, 354	I 3-*O*-glucoside-7-*O*-glucoside[e]
14	14.2	919/917	−/755, 609, 447, 285	266, 314	K 3-*O*-*p*-coumaroyldiglucoside-7-*O*-glucoside[d,e]
17A	18.4	627/625	−/463, 301	256, 266sh, 354	Q 3-*O*-diglucoside[d,e]
17B	18.4	611/609	−/449, 285	ND	K *O*-dihexoside[e]
17C	18.7	949/947	−/785, 771, 285	ND	K 3-*O*-feruloyldiglucoside-7-*O*-glucoside[c]
17D	18.7	919/917	−/755, 609, 447, 285	268, 334	K 3-*O*-*p*-coumaroyldiglucoside-7-*O*-glucoside[d,e]
20	20.3	641/639	−/477, 315	ND	I 3-*O*-diglucoside[d,e]
21	21.3	803/801	−/609, 447, 285	266, 332	K 3-*O*-hydroxyferuloyldiglucoside[d,e]
22[b]	22.0	1319/1317	−/1155, 285	266, 332	K 3-*O*-sinapoylhydroxycaffeoyltrilgucoside-7-*O*-glucoside
22B	22.1	611/609	−/477, 285	266, 346	K 3-*O*-diglucoside[d,e]
22C	22.2	1335/1333	−/1171, 625, 463, 301	ND	Q 3-*O*-dihydroxyferuoloyltriglucoside-7-*O*-glucoside
23	23.2	1305/1303	−/1141, 625, 463, 301	ND	Q 3-*O*-cafeoylhydroxyferuoloyltriglucoside-7-*O*-glucoside
24	23.7	1319/1317	−/1155, 609, 447, 285	ND	K 3-*O*-feruloylhydroxyferuoloyltriglucoside-7-*O*-glucoside
25A	24.1	1349/1347	−/1185, 625, 463, 301	256, 332	Q 3-*O*-sinapoylhydroxyferuoloyldiglucoside-7-*O*-glucoside
25B	24.1	1289/1287	−/1125, 447, 285	ND	K 3-*O*-caffeoylhydroxyferuoloyltriglucoside-7-*O*-glucoside
25C	24.2	771/771	−/609, 285	266, 332	K 3-*O*-caffeoyldiglucoside[e]
25D	24.3	1305/1303	−/1141, 625, 463, 301	ND	Q 3-*O*-cafeoylhydroxyferuoloyltriglucoside-7-*O*-glucoside
26A	24.9	1333/1331	−/1169, 285	266, 332	K 3-*O*-sinapoylhydroxyferuoloyltriglucoside-7-*O*-glucoside
26B	25.0	817/815	−/609, 447, 285	266, 332	K 3-*O*-sinapoyldiglucoside[d,e]
27A	25.9	465/463	301/−	ND	Q 3-*O*-glucoside[d—g]
27C	26.3	1303/1301	−/1139, 285	266, 332	K 3-*O*-sinapoylcaffeoyltrilucoside-7-*O*-glucoside
28	27.0	1303/1301	−/1139, 771, 609, 447, 285	266, 332	K 3-*O*-sinapoylcaffeoyltriglucoside-7-*O*-glucoside[e]
29A	28.0	1347/1345	−/1183, 771, 447, 285	266, 332	K 3-*O*-disinapoyltriglucoside-7-*O*-glucoside[e]
29B	28.3	787/785	−/609, 447, 285	ND	K 3-*O*-feruloyl diglucoside[e]
30A	29.4	757/755	−/609, 447, 285	310	K 3-*O*-*p*-coumaroyldiglucoside[e]
30B	29.4	1317/1315	−/1153, 285	266, 332	K 3-*O*-sinapoylferuloyltriglucoside-7-*O*-glucoside
31	31.1	449/447	−/285	266, 348	K 3-*O*-glucoside[d—f]
32	31.8	479/477	−/315	256, 266sh, 354	I 3-*O*-glucoside[d,e]
33	32.4	449/447	−/285	266, 366	K 7-*O*-glucoside[d,g]
34	33.4	479/477	−/315	256, 266sh, 370	I 7-*O*-glucoside[d,g]
			15 hydroxycinnamoyl-malic acids, -quinic acids, and glucoside		
3A	7.2	−/353	−/191, 179, 135	328, 298, 240	3-caffeoylquinic acid[d—f]
3C	7.7	−/341	−/163, 135	ND	caffeic acid glucoside[e]
8A	10.2	−/337	−/191, 163, 119	310	3-*p*-coumaroylquinic acid[d,e]
8B	10.3	−/353	−/191, 179, 135	ND	5-caffeoylquinic acid[d—f]
10C	12.2	−/367	−/191, 193, 149	ND	3-feruloylquinic acid[d,e]
12A	13.0	−/355	−/193, 149	ND	ferulic acid glucoside[e]
13B	13.7	−/385	−/203, 179	ND	sinapic acid glucoside[e]
15	15.6	−/337	−/191, 163, 119	ND	5-*p*-coumaroylquinic acid[d,e]
16A	16.7	−/337	−/191, 163, 119	ND	4-*p*-coumaroylquinic acid[d,e]
16B	16.8	−/367	−/191, 193, 149	ND	5-feruloylquinic acid[d,e]
18	19.6	−/295	−/179, 135, 133	328, 98, 240	caffeoylmalic acid[e]
19	20.0	−/325	−/209, 165, 133	328, 236	hydroxyferuloylmalic acid[e]

Table 1. Continued

peak no.[a]	t_R (min)	$[M+H]^+/[M-H]^-$ (*m*/*z*)	PI/NI diagnostic ions (*m*/*z*)	UV λ_{max} (nm)	identification[b]
27B	26.1	−/279	−/163, 133, 119	312	*p*-coumaroylmalic acid[e]
29C	28.5	−/339	−/223, 179, 133	240, 298sh, 328	sinapoylmalic acid[e]
29D	28.8	−309	−/193, 149, 133	240, 298sh, 328	feruloylmalic acid[e]
			five hydroxycinnamoylgentiobioses		
35	34.0	777/753	−/529	240, 332	1, 2-disinapoylgentiobiose[d,e]
36	36.0	747/723	−/499	240, 332	1-sinapoyl-2-feruloyl gentiobiose[d,e]
37	37.7	717/693	−/499	240, 332	1, 2-diferuloylgentiobiose[d,e]
38	40.0	983/959	−/735	240, 332	1, 2, 2′-trisinapoylgentiobiose[d,e]
39	42.2	953/929	−/705	240, 332	1, 2′-disinapoyl-2-feruloylgentiobiose[d,e]
			hydroxycinnamic acids in alkali-hydrolyzed extract		
A-1	14.2	−/179	−/135	ND	caffeic acid[d—f]
A-2	14.7	−/209	−/165	ND	hydroxyferulic acid[d,e]
A-3	21.4	−/163	−/119	310	*p*-coumaric acid[d—f]
A-4	23.8	−/223	−/179	240, 298, 228	sinapic acid[d—f]
A-5	24.2	−/193	−/149	240, 298, 228	ferulic acid[d—f]

[a] Letters indicate compounds with the same or a close retention time. [b] K, kaempferol; Q, quercetin; I, isorhamnetin; and ND, not determined. [c] They were well-separated with the mobile phase of 4−14% B in 40 min, and the retention times are listed in the text. [d] Previously identified in kale (*7*). [e] Previously reported in bok choy, Chinese mustard, turnip greens, and the related plants (*11*−*26*). [f] Identified with standard. [g] More clearly detected in alkali-hydrolyzed extracts.

fragments (162 amu less than that of $[M-H]^-$) were produced by the loss of the glucosyl at 7-position (**Table 1**). Of these peaks, two kaempferol glycosides (29A and 27C or 28) and one kaempferol glycoside (27C or 28) were previously reported in enriched flavonoid fractions of broccoli (*29*) and cauliflower agroindustrial byproducts (*30*). These flavonoids eluted later than their related analogues with only one acyl group (retention time, 7−13 min).

Less than 30 flavonoids have been previously reported for turnip tops, bok choy, Chinese leaf mustard and their cultivars, vegetables such as mizuna [*B. rapa* L. subsp. *nipposinica* (L. H. Bailey) Haneltand], and *B. rapa* var. *rapa* (*11*−*26*). Three kaempferol 3-*O*-acyldiglucoside-7-*O*-diglucosides and two quercetin 3-*O*-acyldiglucoside-7-*O*-diglucosides were reported in turnip tops (*20*), but none of them were detected at the injection volume and solid to extraction solvent ratio used in this study. More than 20 of the flavonoids reported in this study were not previously reported in turnip green, bok choy, Chinese mustard cultivars, or the brassica vegetables.

Hydroxycinnamoyl Gentiobioses and Other Derivatives. Like kale, collard greens, and Chinese broccoli (*7*), all 17 brassica vegetables contained five hydroxycinnamoyl gentiobioses. These compounds have been previously reported in some brassica vegetables, such as pak choi and its varieties (*17*−*19*, *21*). They were positively identified by direct comparison with those in collard greens (data not shown) (*7*).

The derivatives of hydroxycinnamic acids with quinic acid, malic acids, and glucose were previously reported in pak choi and some Chinese leaf cultivars (*17*−*19*) and were also observed in this study. All of the vegetables contained sinapoyl- and feruloylmalic as their main phenolic compnents. Some of *p*-coumaroylquinic, feruloylquinic, and caffeoylquinic acids were detected in almost all of the brassica vegetables. However, the glucosides formed with caffeic, ferulic, and sinapic acids were mainly detected in the six bok choy group vegetables. The identification of hydroxycinnamoylquinic and hydrocinnamoylmalic acids was confirmed by direct comparison to positively identified compounds in reference plant materials (*7*, *31*, *32*).

Phenolic Component Profiles of 17 *Brassica* Vegetables. Except for napa (which forms a cabbagelike head and lacks the original outside green leaves), the edible parts of each plant are the green leaves, green leaves with stalks, or young flowering shoots. Unlike *B. oleracea* (*7*) (collard greens, kale, and Chinese broccoli), all of the 17 vegetables analyzed in this study did not contain detectable amounts of 3-*O*-diglucoside-7-*O*-diglucosides or 3-*O*-triglucoside-7-*O*-diglucosides of kaempferol. With the exception of baby Shanghai bok choy and napa, they primarily contained isorhamnetin 3-*O*-glucoside-7-*O*-glucosides and kaempferol 3-*O*-diglucoside-7-*O*-glucoside and its acylated derivatives. The exceptions contained some 3-*O*-triglucoside-7-*O*-glucosides of kaempferol and quercetin. Most of the 17 vegetables also contained acylgentiobiosides, hydroxycinnamoylquinic acids, and hydroxycinnamoylmalic acids. However, hydroxycinnamoylmalic acids were not found in *B. oleracea* (*7*).

The phenolic component profiles of all 17 vegetables are summarized qualitatively in **Table 2**. There are many ways that the 71 phenolics reported in this study can vary between plants. Some can increase, some can decrease, and without knowledge of handling, storing, and growing conditions, these changes might well be artifacts. In a previous study of flavonoids in 59 fresh fruits, vegetables, and nuts, we observed relative standard deviations of 200% for the flavonoid concentrations (*33*). The most striking change that we have observed for plant foods and botanical supplements is the presence or absence of specific compounds, which tends to follow taxonomic lines. Consequently, the simplest approach was to use a binary method (0 or 1) for the presence or absence of the phenolics (**Table 2**) and to use these data as the basis for PCA.

Three groups were identified in **Table 2** (labeled I, II, and III) that had the same chromatographic pattern; that is, each peak was either present or missing for each vegetable of the group. The remaining five vegetables (gai choy, baby napa, rapini, baby Shanghai bok choy, and napa) had individual patterns. Dark boxes in **Table 2** enclose those peaks that distinguish that vegetable from the rest. For example, the boxes show that peak 3A (3-caffeoylquinic acid) is missing from group I and gai choy and is found in all of the others. Peaks 3A and 16A (4-*p*-coumaroylquinic acid) distinguished group I from the rest of the vegetables. Similarly, peak 8B (5-caffeoylquinic acid) distinguished group II, and peaks 3C (caffeic acid glucoside), 12A (ferulic acid glucoside), and 13B (sinapic acid glucoside) distinguished group III. A variety

Table 2. Phenolic Component Occurrence in 17 *Brassica* Vegetables[a]

	Group I			Group II			Group III										
Peak	MG	BMG	BGC	YC	YCT	TG	BC	BCT	BBC	BCS	TBC	SBC	GC	BN	RB	BSBC	N
1A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
2A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
3A	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1
3B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
3C	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0
4A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
6B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
7B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
7C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
8B	1	1	1	0	0	0	1	1	1	1	1	1	1	0	1	1	0
8C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
9A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
10B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
12A	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0
13B	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	1	0
15	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
16A	0	0	0	1	1	1	1	1	1	1	1	1	0	0	1	1	0
16B	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
17A	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
17B	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
17C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
17D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
22A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
22B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
22C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
25A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
25B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
25C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
25D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
26A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
26B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
27A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
27C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
29A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
29B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
30A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
30B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
34	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0

[a] BBC, baby bok choy; BC, bok choy; BCS, bok choy sum; BCT, bok choy tip; BGC, baby gai choy; BMG, baby mustard green; BN, baby napa; BSBC, baby Shanghai bok choy; GC, gai choy; MG, mustard green; N, napa; RB, rapini broccoli; SBC, Shanghai bok choy; TBC, Taiwan bok choy; TG, turnip green; YC, yu choy; and YCT, yu choy tip. Key: 1, detected; 0, not detected. Counts were detected for all of the vegetables for peaks 1B, 2B, 4B, 5, 6A 7A, 8A, 9B, 10A, 10C, 12B, 12C, 13A, 14, 18, 19, 27B, 29C, 29D, 31, 35, 36, 37, 38, and 39.

of peaks establish the individual identities of the last five vegetables.

A closer comparison of the data in **Table 2** reveals that, except for the absence of peaks 15 (5-*p*-coumaroylquinic acid) and 16B (5-feruloylquinic acid), gai choy would be a member of group I. However, a visual comparison of the patterns of the data in **Table 2** is difficult. Consequently, the data were submitted to PCA, a pattern recognition method that mathematically transforms a large number of possibly correlated variables into a smaller number of uncorrelated variables called principal components. In **Figure 3**, the score plot for the first two principal components shows that groups I and II and gai choy are very similar, and baby napa is close despite variation in their chromatographic patterns (**Table 2**). Rapini and group III are increasingly different, and napa and baby Shanghai bok choy are dramatically different.

The data in **Table 2** show that, except for baby Shanghai bok choy and baby napa, the baby or tip forms of bok choy, yu choy, gai choy, and mustard green have similar chromatographic patterns as those of their mature forms, suggesting only a minor change in phenolic composition as they mature. Baby Shanghai bok choy contained a higher amount of 3-*O*-triglucoside-7-*O*-glucosides of kaempferol and quercetin than other members of the tested vegetables. However, like all other bok choy group vegetables, baby Shanghai bok choy also contained similar nonflavonoid phenolics, such as hydroxycinnamoyl-glucoses, quinic, and malic acids. Napa (with a cabbagelike head) and baby napa (with just green leaves) had dramatically different phenolic patterns (**Table 2**). Like baby Shanghai bok choy, napa

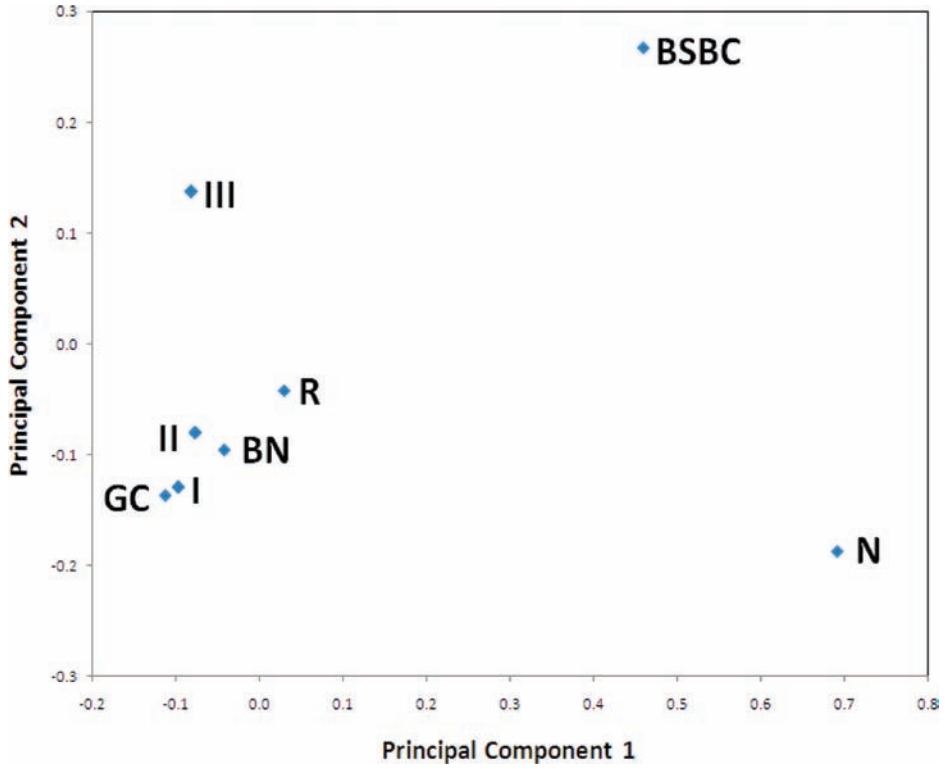

Figure 3. PCA of the phenolic component peaks of 17 *Brassica* vegetables (**Table 2**). Key: I, group I (baby gai choy, baby mustard greens, and mustard greens); II, group II (turnip greens, yu choy, and yu choy tip); III, group III (baby bok choy, bok choy, bok choy sum, bok choy tip, Shanghai bok choy, and Taiwan bok choy); BN, baby napa; BSBC, baby Shanghai bok choy; GC, gai choy; N, napa; and RB, rapini broccoli.

also contains some kaempferol 3-*O*-triglucoside-7-*O*-glucosides, while baby napa does not.

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