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# Profiling Polyphenols in Five *Brassica* Species Microgreens by UHPLC-PDA-ESI/HRMS$^n$

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**ABSTRACT:** *Brassica* vegetables are known to contain relatively high concentrations of bioactive compounds associated with human health. A comprehensive profiling of polyphenols from five *Brassica* species microgreens was conducted using ultrahigh-performance liquid chromatography photodiode array high-resolution multistage mass spectrometry (UHPLC-PDA-ESI/HRMS$^n$). A total of 164 polyphenols including 30 anthocyanins, 105 flavonol glycosides, and 29 hydroxycinnamic acid and hydroxybenzoic acid derivatives were putatively identified.The putative identifications were based on UHPLC-HRMS$^n$ analysis using retention times, elution orders, UV−vis and high-resolution mass spectra, and an in-house polyphenol database as well as literature comparisons. This study showed that these five *Brassica* species microgreens could be considered as good sources of food polyphenols.

**KEYWORDS:** *microgreens, Brassicaceae, acylated cyanidin 3-sophroside-5-mono- and diglucosides, acylated flavonol glycosides, hydroxycinnamic acid derivatives, UHPLC-PDA-ESI/HRMS$^n$*

## INTRODUCTION

Microgreens are young edible greens produced from vegetables, herbs, or other plants, ranging in size from 5 to 10 cm long including stem and cotyledons (seed-leaves). They are popular for their pretty colors, intense flavors, delicate textures, and relatively high nutritional contents.[1] The entire plant (seedling) is harvested at the ground level when cotyledon or seed-leaves have fully expanded and before true leaves have fully emerged.

The Brassicaceae offer some of the most commonly consumed vegetables worldwide, which can be grown as microgreens. Five *Brassica* vegetables commonly found in the U.S. marketplace are red cabbage (*Brassica oleracea* var. *capitata*), purple kohlrabi (*B. oleracea* var. *gongylodes*), red and purple mustards (*Brassica juncea*), and mizuna (*Brassica rapa* var. *nipposinica* or *B. juncea* var. *japonica*). *Brassica* vegetables are known to be rich sources of ascorbic acid, carotenoids, glucosinolates, polyphenols, and tocopherols,[2−4] which have human-health beneficial attributes reportedly involved in preventing cardiovascular diseases and some types of cancers.[5−8]

Previous studies have tentatively identified phenolic compounds from 22 mature-leaf *Brassica* vegetables,[9−12] and phenolic compounds have been found in tronchuda cabbage (*B. oleracea* var. *costata*) seeds,[13] mature leaves,[14] and internodal shoots and roots.[15,16] Twelve specific phenolic compounds have been profiled in 2−12-day-old seedlings possessing both seed-leaves and true leaves. The aim of the present study was to characterize and quantify the naturally occurring polyphenols in five commonly consumed *Brassica* species (mizuna, red cabbage, purple kohlrabi, red mustard, and purple mustard) at their microgreen growth stage. The analyses of their native polyphenols and flavonol aglycones were performed using state-of-the-art analytical tools: ultrahigh-performance liquid chromatography photodiode array high-resolution multistage mass spectrometry (UHPLC-PDA-ESI/HRMS/MS$^n$). Results showed that *Brassica* microgreens contained notable levels of hydroxycinamic acids and may contain different compounds from their true leaves. Totals of 30 anthocyanins, 105 flavonol glycosides, and 29 hydroxycinnamic acid and hydroxylbenzoic acid derivatives were tentatively identified. This is the first known reported study of polyphenol compounds in vegetables at the cotyledonary leaf (microgreen) stage of growth of an array of *Brassica* microgreens.

## MATERIALS AND METHODS

**Chemicals.** Formic acid, HPLC grade methanol, and acetonitrile were purchased from VWR International, Inc. (Clarksburg, MD, USA). HPLC grade water was prepared from distilled water using a Milli-Q system (Millipore Laboratory, Bedford, MA, USA).

**Plant Materials and Sample Preparation.** Five *Brassica* species, at the microgreen growth stage, were obtained from Sun Growers Organic Distributors, Inc. (San Diego, CA, USA). All of the fresh samples were lyophilized and then powdered. Powdered samples (100 mg) were extracted with 5.00 mL of methanol/water (60:40, v/v) using sonication for 60 min at room temperature and then centrifuged

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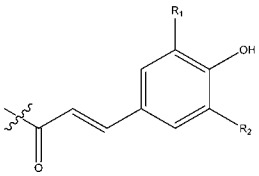
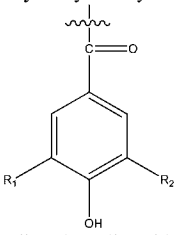
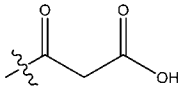
Table 1. Typical Substitutional Groups and Common Neutral Losses of Polyphenols in Five *Brassica* Species Microgreens

| Substitutional groups | Name | Neutral Loss in HRMS |
|---|---|---|
| mono-saccharides | pentose (xylose, arabinose) | 132.0422 ($C_5H_8O_4$) |
| | methyl-pentose (rhamnose) | 146.0579 ($C_6H_{10}O_4$) |
| | hexose (glucose, galactose) | 162.0528 ($C_6H_{10}O_5$) |
| di-saccharides | sophorose=2-β-D-glucopyranosyl-D-glucose<br>gentiobiose=6-β-D-glucopyranosyl-D-glucose | 324.1056 ($C_{12}H_{20}O_{10}$) |
| tri-saccharides | sophorotriose (2'''- β-D-glucopyranosyl-2''-β-D-glucopyranosyl-D-glucose) gentiotriose (6'''- β-D-glucopyranosyl-6''-β-D-glucopyranosyl-D-glucose) | 486.1584 ($C_{18}H_{30}O_{15}$) |
| hydroxycinnamoyls | *p*-coumaroyl $R_1$=H $R_2$=H | 146.0347 ($C_9H_6O_2$) |
| | caffeoyl $R_1$=OH $R_2$=H | 162.0317 ($C_9H_6O_3$) |
|  | feruloyl $R_1$=$OCH_3$ $R_2$=H | 176.0473 ($C_{10}H_8O_3$) |
| | sinapoyl $R_1$=$OCH_3$ $R_2$=$OCH_3$ | 206.0579 ($C_{11}H_{10}O_4$) |
| hydroxybenzoyls | *p*-hydroxybenzoyl $R_1$=H $R_2$=H | 120.0211 ($C_7H_4O_2$) |
|  | galloyl $R_1$= OH $R_2$=OH | 152.0109 ($C_7H_4O_4$) |
| di-carboxylic acid acyls | malonyl | |
|  | | 86.0004 ($C_3H_2O_3$) |

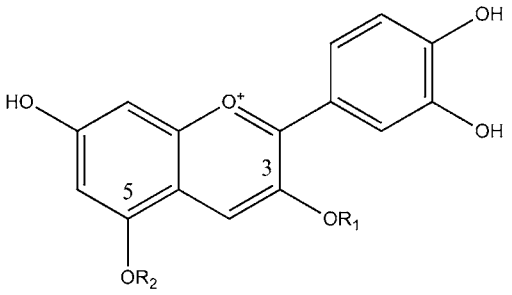
at 1000*g* for 15 min (IEC Clinical Centrifuge, Damon/IEC Division, Needham, MA, USA). The supernatant was filtered through a 17 mm (0.45 μm) PVDF syringe filter (VWR Scientific, Seattle, WA, USA), and 10 μL of the extract was used for each HPLC injection.

**UHPLC-PDA-ESI/HRMS/MS$^n$ Conditions.** The UHPLC-HRMS system used consisted of an LTQ Orbitrap XL mass spectrometer with an Accela 1250 binary pump, a PAL HTC Accela TMO autosampler, a PDA detector (ThermoFisher Scientific, San Jose, CA, USA), and a G1316A column compartment (Agilent, Palo Alto, CA, USA). Separation was carried out on a Hypersil Gold AQ RP-C18 UHPLC column (200 mm × 2.1 mm i.d., 1.9 μm, ThermoFisher Scientific) with an UltraShield precolumn filter (Analytical Scientific Instruments, Richmond, CA, USA) at a flow rate of 0.3 mL/min. The mobile phase consisted of a combination of A (0.1% formic acid in water, v/v) and B (0.1% formic acid in acetonitrile, v/v). The linear gradient was from 4 to 20% B (v/v) at 40 min, to 35% B at 60 min, and to 100% B at 61 min and held at 100% B to 65 min. The PDA was set at 520, 330, and 280 nm to record the peaks, and UV−vis spectra were recorded from 200 to 700 nm.

Both positive and negative ionization modes were used, and the conditions were set as follows: sheath gas at 70 (arbitrary units), auxiliary and sweep gases at 15 (arbitrary units), spray voltage at 4.8 kV, capillary temperature at 300 °C, capillary voltage at 15 V, and tube lens at 70 V. The mass range was from 100 to 2000 amu with a resolution of 15000, FTMS AGC target at 2e5, FT-MS/MS AGC target at 1e5, isolation width of 1.5 amu, and maximum ion injection time of 500 ms. The most intense ion was selected for the data-dependent scan to offer their $MS^2$ to $MS^5$ product ions, respectively, with a normalization collision energy at 35%.

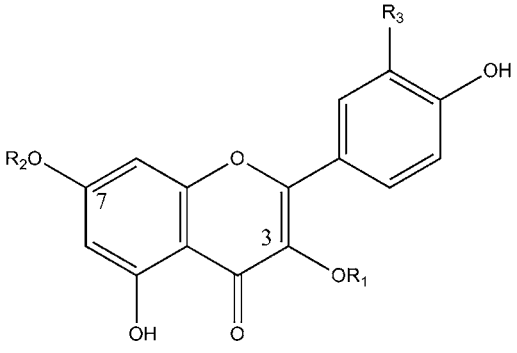
## RESULTS AND DISCUSSION

**Strategies for Systematic Identification of Polyphenols from Microgreen *Brassica*.** Brassicaceae polyphenol composition has been extensively investigated. The main flavonols in *Brassica* vegetables are the *O*-glycosides of quercetin, kaempferol, and isorhamnetin.[2,17−22] The sugar moiety found in *Brassica* vegetables is glucose, occurring as



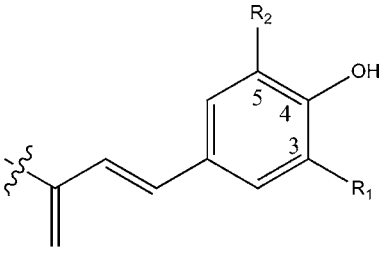
**Anthocyanins**

$R_1$ = sophorosyl, diglucosyl or triglucosyl with acyls
$R_2$ = glucosyl, glucosyl with malonyl



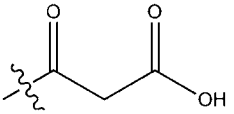
**Flavonols and Flavonol glycosides**

Kaempferol $R_1$=$R_2$=$R_3$=H ( MW, 286 Da )
Quercetin $R_1$=$R_2$=H, $R_3$=OH (MW, 302 Da)
Isorhamnetin $R_1$=$R_2$=H, $R_3$=$OCH_3$ (MW, 316 Da)
Flavonol glycosides $R_1$ and/or $R_2$ = glycosyls



**Hydroxycinnamoyls**

*p*-Coumaroyl $R_1$=$R_2$=H
Caffeoyl $R_1$=OH, $R_2$=H
Feruloyl $R_1$=$OCH_3$, $R_2$=H
Hydroxyferuloyl $R_1$=$OCH_3$, $R_2$=OH
Sinapoyl $R_1$=$R_2$=$OCH_3$
$R_1$ and/or $R_2$ = glycosyls



Malonyl

**Figure 1.** Basic chemical structures identified from five *Brassica* species microgreens.

mono-, di-, tri-, tetra-, and pentaglucosides.[17−23] They are also commonly found acylated by different hydroxycinnamic acids. Anthocyanins are another main class of flavonoid found in *Brassica* vegetables, and cyanidin is the most common anthocyanidin in colored-leaf *Brassica* vegetables.[2,24] Hydroxycinnamic acids (C6−C3) are phenolic acids characterized in *Brassica* vegetables with the most common ones being *p*-coumaric, caffeic, sinapic, and ferulic acids, often found in conjugation with sugars or other hydroxycinnamic acids.[2,17−19,21,22]

The five *Brassica* species microgreen phenolic compounds exhibit absorbance maxima at three wavelengths (280 nm for flavonols and flavonol glycosides, 320 nm for hydroxycinnamic acid derivatives, and 520 nm for anthocyanins).[2,17−19,21,22]

HRMS was used for the determination of chemical formulas. Neutral loss information from MS was used for identification of sugar moiety and acyl groups. In MS analysis, cleavage of the first glycosidic linkage is expected to take place at the *O*-glycosidic bond at the 7-position of the flavonols and at the 5-position of the anthocyanins, leading to the fragmentations $[(M - H) - 162]^-$ for monohexosides and $[(M - H) - 324]^-$ for dihexosides.[23,25,26] The remaining glucose moieties of the flavonoid molecule are expected to be linked to the hydroxyl group at the 3-position of the aglycone. The disaccharide moieties of the flavonoids in *Brassica* species are mainly sophorosides.[2] The MS fragmentation behavior can be used for the determination of interglucoside linkage, and neutral losses of 180, 162, and 120 amu indicate a sophoroside with a 1→2 interglucoside linkage, whereas loss of 324 amu, and in some cases low abundance of 162 amu, corresponds to a diglucoside with a 1→6 linkage such as gentiobioside.[27] The saccharides (mono-, di-, trisaccharides) and acyl groups of flavonol glycoside and their possible neutral losses in CID MS/MS analysis are listed in Table 1, and the basic structures of the phenolic compounds found in these five *Brassica* species microgreens are shown in Figure 1.

**Anthocyanins.** Among the five *Brassica* species microgreens, red cabbage, red mustard, purple mustard, and purple kohlrabi have red to purple seed-leaves. UHPLC chromatograms at 520 nm revealed 30 different anthocyanins are likely responsible for this coloration (Figure 2). The retention times ($t_R$), HRMS masses $[M]^+$, molecular formulas, errors (ppm) between theoretical and measured values, and major $MS^2$ and $MS^3$ product ions are summarized in Table 2.

In these five *Brassica* species microgreens, only cyanidin (Cy) derivatives were found, which is in accordance with the other studies on *Brassica* species.[24,28−30] The anthocyanins found in red cabbage microgreens were Cy 3-diglucoside-5-glucoside derivatives acylated with different hydroxycinnamic acids at the diglucosyl moiety in the 3-position. High-resolution mass spectroscopic analysis with multistage mass fragmentation was used as an important tool for anthocyanin characterization. Among the 30 Cy glycosides found in red cabbage, red mustard, purple mustard, and purple kohlarabi microgreens,

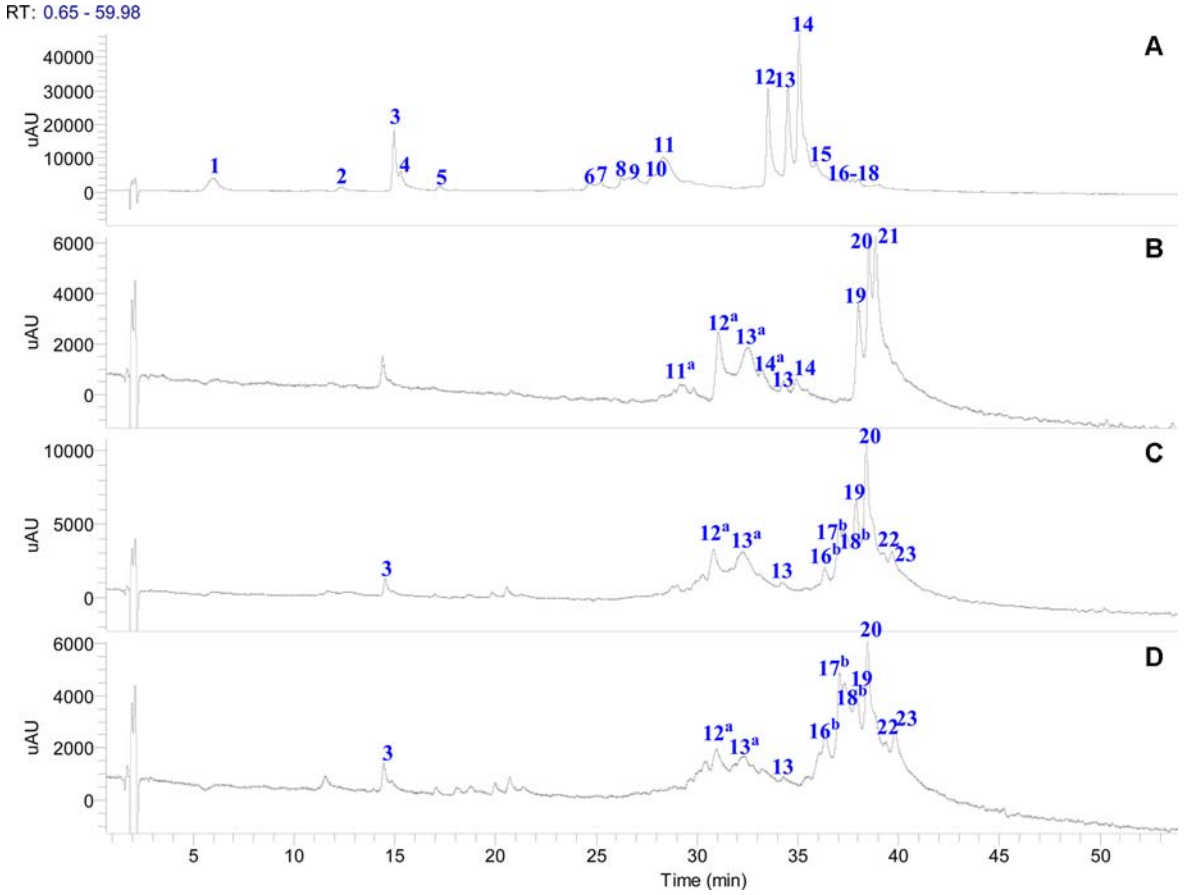



**Figure 2.** UHPLC chromatogram from four *Brassica* species microgreens, red cabbage (A), purple kohlrabi (B), red mustard (C), and purple mustard (D), under 520 nm.

peak 1 at $m/z$ 773.2106 ($C_{33}H_{41}O_{21}$, −1.36 mmu) was the lowest molecular weight anthocyanin, and losses of three hexosyl units were observed in $MS^2$ spectra, suggesting Cy 3-diglucoside-5-glucoside, a typical compound reported in red cabbage. The major acylated anthocyanins were Cy 3-diglucoside-5-glucoside derivatives with various acylated groups, for example, coumaroyl, feruloyl, and sinapoyl connected to the diglucoside. The MS/MS of most of the molecular ions of acylated anthocyanins gave the major product ions at $m/z$ 449, a Cy 5-glucoside residue, and at $m/z$ 611, a Cy 3-diglucoside residue. The MS/MS fragments of the acylated anthocyanins allow for a rough determination of the location of the acylating groups. Peaks 12, 13, and 14 are the major anthocyanins in microgreen red cabbage, and they were identified as cyanidin 3-diferuloyl-sophoroside-5-glucoside, Cy 3-(sinapoyl)(sinapoyl)sophoroside-5-glucoside, and Cy 3-(sinapoyl)(feruloyl)sophoroside-5-glucoside, respectively. Using peak 12 as an example, HRMS gave the $[M]^+$ ion at 1125.3070, corresponding to the formula of $C_{53}H_{57}O_{27}$. Fragmentation of the ion at $m/z$ 1125 in positive mode produced ions at $m/z$ 963 by loss of a glucosyl residue (162 amu) from the 5-position. The ion at $m/z$ 449 was produced by a total loss of 676 amu, corresponding to a diferuloyl-diglucosyl residue (176 + 176 + 324 amu), from the terminal 3-position.

In a previous study of purple kohlrabi, 12 anthocyanins have been identified. The major ones are Cy 3-(feruloyl)(sinapoyl) diglucoside-5-glucoside, Cy 3-(feruloyl) diglucoside-5-glucoside, and Cy 3-(sinapoyl)(sinapoyl) diglucoside-5-glucoside.[31] In our study, acylated anthocyanins with one malonyl group attached to the hexose of C-5 and other aromatic groups (caffeic, *p*-coumaric, sinapic, or ferulic acid) attached to the C-3 glycosidic substituent were found. In the $MS^2$ spectra, the fragment ions at ($m/z$ 1023, 993, and 963), with the two acyl groups attached to the dihexose of C-3, are usually observed as the base peak. This fragmentation pattern was evidenced with most anthocyanins analyzed and led to the tentative identification of Cy 3-(feruloyl)(feruloyl)diglucoside-5-(malonyl)-glucoside ($m/z$ 1211, peak 19), Cy 3-*O*-(sinapoyl)-(feruloyl)diglucoside-5-*O*-(malonyl)glucoside ($m/z$ 1241, peak 20), and Cy 3-*O*-(sinapoyl)(sinapoyl)diglucoside-5-*O*-(malonyl)glucoside ($m/z$ 1271, peak 21). Peaks $11^a$−$14^a$ were identified as Cy 3-*p*-(coumaroyl)sophoroside-5-(malonyl)-glucoside, cy 3-*O*-(*p*-coumaroyl)(sinapoyl) diglucoside-5-*O*-(malonyl) glucoside, Cy 3-*O*-(feruloyl)glucoside-5-*O*-(malonyl) glucoside, and Cy 3-*O*-(sinapoyl)glucoside-5-*O*-(malonyl)-glucoside in red mustard microgreens.[10] Peaks 19 and 20 were two major anthocyanins identified in red and purple mustard. Peaks $16^b$, $17^b$, and $18^b$ were identified as Cy 3-(sinapoyl)-(coumaroyl)triglucoside-5-(malonyl)glucoside, Cy 3-(caffeoyl)-(sinapoyl)(xylosyl)glucoside-5-(malonyl)glucoside and Cy 3-(coumaroyl)(sinapoyl)diglucoside-5-(malonyl)glucoside, respectively.

***O*-Glycosylated Flavonols and Their Acylated Derivatives.** Acylated flavonoid glycosides were easily identified on the basis of the increased mass of the parent ions and the wavelength maxima (330−336 nm) of their UV spectra (Figure 3). According to the $MS^n$ ($n$ = 2−5) data, the aglycones of the flavonol glycosides were quercetin (Qn), kaempferol (Km), and

**Table 2. UHPLC-HRMS Data of Anthocyanins from Five *Brassica* Species Microgreens: Red Cabbage, Red Mustard, Purple Mustard, Mizuna, and Purple Kohlrabi**

| peak | $t_R$ (min) | $[M]^+$ | formula | error (mmu) | major and important $MS^2$ ions | major $MS^3$ ion | tentative identification |
|---|---|---|---|---|---|---|---|
| 1 | 5.97 | 773.2106 | $C_{33}H_{41}O_{21}$ | −1.36 | 611 (29), 449 (40), 287 (100) | 287 (100) | Cy 3-diglucoside-5-glucoside[a] |
| 2 | 12.34 | 965.2528 | $C_{43}H_{49}O_{25}$ | −2.12 | 803 (100), 641 (20), 287 (60) | 287 (100) | Cy 3-hydroxyferuloyl-5-glucoside[a] |
| 3 | 14.98 | 979.2699 | $C_{44}H_{51}O_{25}$ | −1.53 | 817 (71), 449 (46), 287 (100) | 287 (100) | Cy 3-(sinapoyl)-diglucoside-5-glucosides[a] |
| 4 | 15.29 | 979.2708 | $C_{44}H_{51}O_{25}$ | −0.61 | 817 (82), 449 (52), 287 (100) | 287 (100) | Cy 3-(sinapoyl)-diglucoside-5-glucoside[a] |
| 5 | 17.21 | 1141.3246 | $C_{50}H_{61}O_{30}$ | 0.34 | 979 (100), 449 (54) | 287 (100) | Cy 3-(glucopyranosyl-sinapoyl) diglucoside-5-glucoside[a] |
| 6 | 24.63 | 919.249 | $C_{42}H_{47}O_{23}$ | −1.38 | 757 (100), 449 (19), 287 (50) | 287 (100) | Cy 3-(coumaroyl)sophoroside-5-glucoside[a] |
| 7 | 25.23 | 1287.3597 | $C_{59}H_{67}O_{32}$ | −1.01 | 1125 (100), 449 (6) | 963 (100), | Cy 3-(glucosyl)(sinapoyl)(*p*-coumaroyl) sophorside-5-glucoside[a] |
| 8 | 26.31 | 1317.369 | $C_{60}H_{69}O_{33}$ | −1.94 | 1185 (100), 1155 (35), 449 (2) | 1023 (100), 449 (3) | Cy 3-(glucosyl)(sinapoyl)(feruloyl) sophorside-5-glucoside[a] |
| 9 | 26.97 | 919.249 | $C_{42}H_{47}O_{23}$ | −1.38 | 757 (100), 449 (19), 287 (50) | 287 (100) | Cy 3-(coumaroyl)sophoroside-5-glucoside[a] |
| 10 | 27.71 | 949.2602 | $C_{43}H_{49}O_{24}$ | −0.66 | 787 (100), 449 (18), 287 (49) | 287 (100) | Cy 3-(feruloyl)sophoroside-5-glucoside[a] |
| 11 | 28.31 | 1141.3016 | $C_{53}H_{57}O_{28}$ | −1.30 | 979 (100), 449 (11) | 287 (100) | Cy 3-(caffeoyl)(sinapoyl)diglucoside-5-glucoside |
| 11a | 29.14 | 1005.2492 | $C_{45}H_{49}O_{26}$ | −1.46 | 757 (22), 535 (100), 491 (10), 287 (73) | 287 (100) | Cy 3-(coumaroyl)sophoroside-5-(malonyl)glucoside |
| 12 | 33.37 | 1125.307 | $C_{53}H_{57}O_{27}$ | −1.04 | 963 (100), 449 (13) | 287 (100) | Cy 3-diferuloylsophoroside-5-glucoside[a] |
| 12a | 31.07 | 1211.3088 | $C_{56}H_{59}O_{30}$ | 0.23 | 963 (100), 535 (81), 521 (9) | 287 (100) | Cy 3-(coumaroyl)(sinapoyl)diglucoside-5-(malonyl)glucoside[a] |
| 13 | 34.50 | 1125.307 | $C_{53}H_{57}O_{27}$ | −1.04 | 963 (100), 449 (13) | 287 (100) | Cy 3-diferuloylsophoroside-5-glucoside[a] |
| 13a | 32.56 | 1035.2599 | $C_{46}H_{51}O_{27}$ | −1.32 | 992 (7), 787 (40), 780 (5), 535 (100), 492 (12), 449 (6), 287 (5) | 287 (100) | Cy 3-(feruloyl)glucoside-5-(malonyl)-glucoside[a] |
| 14 | 35.07 | 1155.3192 | $C_{54}H_{59}O_{28}$ | 0.40 | 993 (100), 449 (9) | 287 (100) | Cy 3-sinapoylferuloylsophoroside-5-glucoside[a] |
| 14a | 33.21 | 1065.2702 | $C_{47}H_{53}O_{28}$ | −1.59 | 817 (73), 535 (100), 492 (2), 449 (3) | 287 (100) | Cy 3-(sinapoyl)glucoside-5-(malonyl)-glucoside[a] |
| 15 | 35.91 | 1155.3192 | $C_{54}H_{59}O_{28}$ | 0.40 | 993 (100), 449 (9) | 287 (100) | Cy 3-(sinapoyl)(feruloyl)sophoroside-5-glucoside[a] |
| 16 | 37.14 | 1185.3298 | $C_{55}H_{61}O_{29}$ | 0.50 | 1023 (100), 449 (10) | 287 (100) | Cy 3-(sinapoyl)(sinapoyl)sophoroside-5-glucoside[a] |
| 16b | 36.34 | 1373.3585 | $C_{62}H_{69}O_{35}$ | −2.89 | 963 (100), 697 (66), 653 (28) | 287 (100) | Cy 3-(sinapoyl)(coumaroyl)triglucoside-5-(malonyl)-glucoside[a] |
| 17 | 37.55 | 1155.3192 | $C_{54}H_{59}O_{28}$ | 0.40 | 993 (100), 449 (9) | 287 (100) | Cy 3-(sinapoyl)(feruloyl)sophoroside-5-glucoside[a] |
| 17b | 37.08 | 1197.2902 | $C_{55}H_{57}O_{30}$ | −2.72 | 949 (18), 860 (3), 535 (100), 517 (3), 491 (9) | 287 (100) | Cy 3-(caffeoyl)(sinapoyl)(xylosyl) glucoside-5-(malonyl)glucoside[a] |
| 18 | 37.99 | 1185.3298 | $C_{55}H_{61}O_{29}$ | 0.49 | 1023 (100), 449 (10) | 287 (100) | Cy 3-(sinapoyl)(sinapoyl)sophoroside-5-glucoside[a] |
| 18b | 37.37 | 1227.3008 | $C_{56}H_{59}O_{31}$ | −2.68 | 979 (82), 535 (100), 491 (10) | 287 (100) | Cy 3-(*p*-coumaroyl)(sinapoyl)diglucoside-5-*O*-(malonyl)glucoside |
| 19 | 38.00 | 1211.3082 | $C_{56}H_{59}O_{30}$ | −1.46 | 963 (91), 535 (100), 491 (3) | 287 (100) | Cy 3-(feruloyl)(feruloyl)diglucoside-5-(malonyl)glucoside[a] |
| 20 | 38.56 | 1241.3192 | $C_{57}H_{61}O_{31}$ | −1.03 | 1206 (15), 1198 (30), 993 (100), 535 (88), 449 (8) | 287 (100) | Cy 3-(sinapoyl)(feruloyl)diglucoside-5-(malonyl)glucoside[a] |
| 21 | 38.85 | 1271.3296 | $C_{58}H_{63}O_{32}$ | −0.10 | 1023 (100), 535 (51), 491 (7) | 287 (100) | Cy 3-(sinapoyl)(sinapoyl)diglucoside-5-(malonyl)glucoside[a] |
| 22 | 39.35 | 1241.3190 | $C_{57}H_{61}O_{31}$ | −0.13 | 993 (100), 535 (70), 492 (13) | 287 (100) | Cy 3-(sinapoyl)(feruloyl)diglucoside-5-(malonyl)glucoside[a] |
| 23 | 39.81 | 1211.3078 | $C_{56}H_{59}O_{30}$ | −0.77 | 963 (86), 535 (100) | 287 (100) | Cy 3-(*p*-coumaroyl)(sinapoyl)diglucoside-5-(malonyl)glucoside[a] |

[a]Compared with literature data; Cy, cyanidin.

isorhamnetin (Is). Using the strategy described previously, 105 flavonol glycosides were characterized in five microgreens vegetables (Figure 3). Among them, 18 were nonacylated flavonoid glycosides and 87 were acylated flavonoid glycosides. The compound distribution in these five microgreens is shown in Table 3. Qn 3-sophoroside-7-glucoside, Qn 3-hydroxyferuloylsophoroside-7-glucoside, Km 3-hydroxyferuloylsophoroside-7-glucoside, Km 3-sinapoylsophoroside-7-glucoside, and Is 3-caffeoylsophoroside-7-glucoside are common peaks in all five *Brassica* species microgreens. Is 3-*O*-glucoside, Qn 3,7-di-*O*-glucoside, Km 3-*p*-coumaroyldiglucoside, Qn 3-caffeoylsophoroside, Qn 3-feruloylsophoroside, Qn 3-feruloylsophoroside-7-glucoside, and Km 3-sinapoylsophoroside were found only in mizuna microgreens, whereas Km 3-sinapoylsophoroside-7-glucoside and Qn 3-sinapoylsophorotrioside were found only in purple kohlrabi. Red cabbage microgreens had Km 3-*p*-coumaroylsophorotrioside, Km 3-*p*-coumaroylsophoroside-7-diglucoside, Km 3-hydroxyferuloylsophorotrioside-7-glucoside, Km 3-disinapoyldiglucoside-7-glucoside, Km 3-sinapoylferuloylsophoroside-7-glucoside, and Qn 3-disinapoylsophorotrio-

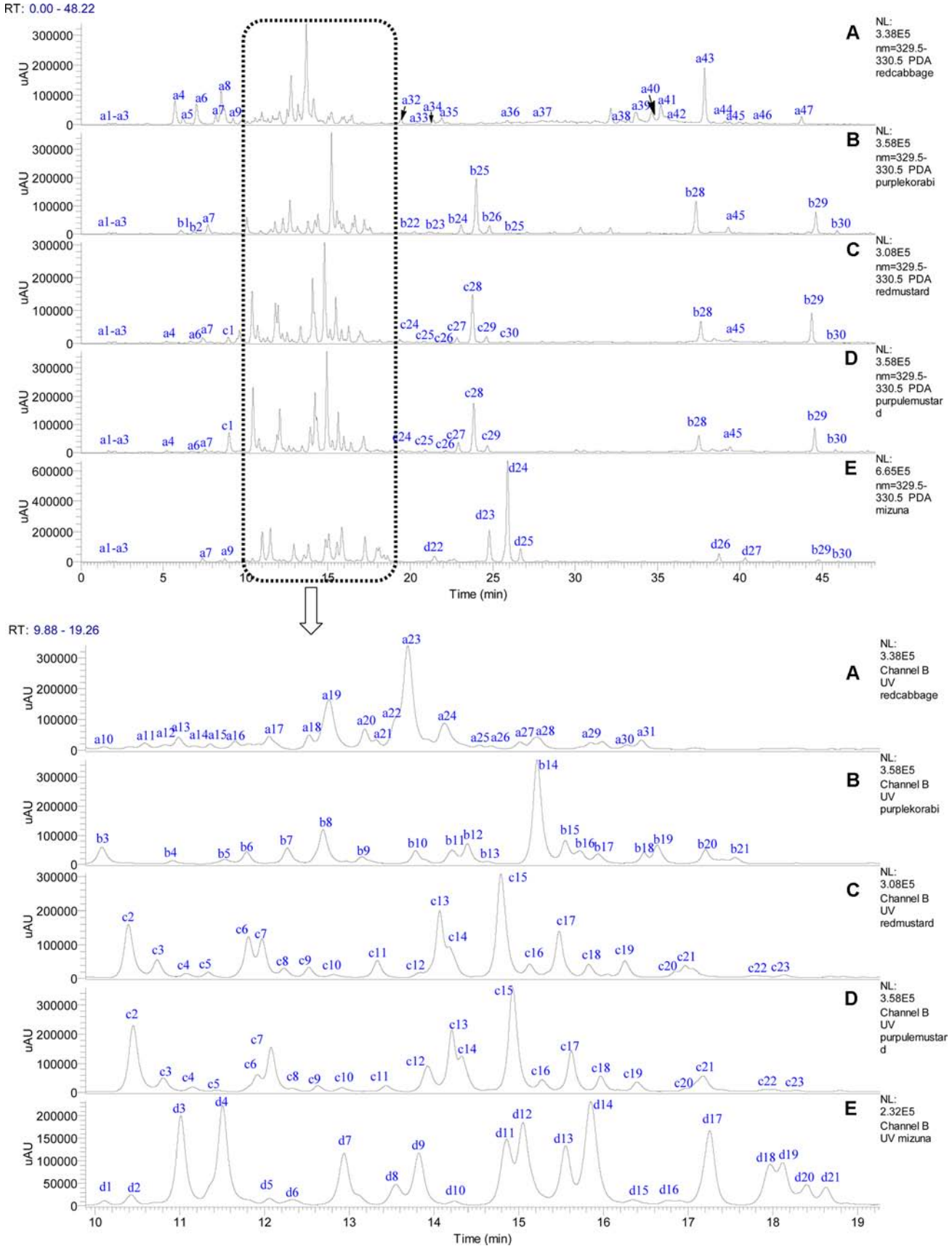



**Figure 3.** UHPLC chromatogram of five *Brassica* species microgreens, red cabbage (A), purple kohlrabi (B), red mustard (C), purple mustard (D), and mizuna (E), under 330 nm.

**Table 3. UHPLC-HRMS Data of flavonol Glycosides and Derivatives of Hydroxycinnamic Acids and Hydroxybenzoic Acids from Five *Brassica* Species Microgreens: Red Cabbage, Red Mustard, Purple Mustard, Mizuna, and Purple Kohlrabi**

| peak | $t_R$(min) | $[M-H]^-$ | formula | error (mmu) | major and important $MS^2$ ions | $MS^3$ ion | tentative identification[a] |
|---|---|---|---|---|---|---|---|
| a1 | 1.66 | 133.0143 | $C_4H_5O_5$ | 0.40 | 24 (83), 153 (100) | 115 (100) | malic acid[b] |
| a2 | 1.98 | 191.0192 | $C_6H_7O_7$ | −0.53 | 173 (22), 111 (100) | 67 (100) | citric acid[b] |
| a3 | 3.37 | 205.0349 | $C_7H_9O_7$ | −0.48 | 173 (61), 159 (6), 143 (11), 111 (100) | | methyl citric acid |
| b1 | 5.56 | 503.1398 | $C_{21}H_{27}O_{14}$ | −0.83 | 341 (100), 179 (9) | 179 (100) | courmaroyl-diglucoside |
| a4 | 5.71 | 355.1029 | $C_{16}H_{19}O_9$ | −1.56 | 217 (59), 193 (100), 175 (40) | 134 (100) | feruloyl-glucose |
| b2 | 5.93 | 353.0870 | $C_{16}H_{17}O_9$ | −0.80 | 191 (100), 179 (43), 135 (8) | 173 (100) | caffeoyl-quinic acid |
| 5a | 6.12 | 299.0768 | $C_{13}H_{15}O_8$ | −0.44 | 239 (90), 179 (71), 137 (100) | | salicyloyl-glucose[b] |
| a6 | 6.21 | 547.1671 | $C_{23}H_{31}O_{15}$ | 0.47 | 223 (100) | 208 (100) | sinapoyl-gentiobiose[b] |
| a7 | 7.18 | 447.0557 | $C_{20}H_{15}O_{12}$ | −1.12 | 357 (38), 275 (55), 259 (100) | 139 (100) | rhamnosyl-ellagic acid |
| a8 | 8.32 | 787.1942 | $C_{33}H_{39}O_{22}$ | 1.45 | 625 (100) | 300 (100) | Qn 3-diglucoside-7-glucoside[b] |
| a9 | 9.20 | 787.1920 | $C_{33}H_{39}O_{22}$ | −1.85 | 625 (100) | 300 (100) | Qn 3-diglucoside-7-glucoside[b] |
| c1 | 9.68 | 933.2486 | $C_{39}H_{49}O_{26}$ | −0.79 | 771 (100) | 591 (100) | Km 3-sophorotrioside-7-glucoside |
| b3 | 10.06 | 787.1916 | $C_{33}H_{39}O_{22}$ | −2.25 | 625 (100) | 300 (100) | Qn 3-sophoroside-7-glucoside[b] |
| d1 | 10.09 | 845.2113 | $C_{39}H_{41}O_{21}$ | −3.28 | 683 (100), 477 (15), 315 (6) | 353 (100) | Is 3-sinapoylglucoside-7-glucoside[b] |
| a10 | 10.11 | 771.1978 | $C_{33}H_{39}O_{21}$ | −1.13 | 609 (100) | 285 (100) | Km 3-sophoroside-7-glucoside[b] |
| d2 | 10.43 | 817.2015 | $C_{34}H_{41}O_{23}$ | −2.91 | 609 (100), 447 (34) | 447 (100) | Km 3-diglucoside-7-glucoside with HCOOH |
| c2 | 10.45 | 1141.2889 | $C_{49}H_{57}O_{31}$ | 1.07 | 979 (100), 949 (93), 787 (72) | 787 (100) | Qn 3-hydroxyferuloylsophorotioside-7-glucoside[b] |
| a11 | 10.59 | 979.2349 | $C_{43}H_{47}O_{26}$ | −1.23 | 817 (98), 787 (100), 625 (59) | 625 (100) | Qn 3 hydroxyferuloylsophoroside-7-glucoside[b] |
| c3 | 10.80 | 1111.2760 | $C_{48}H_{55}O_{30}$ | −2.13 | 949 (100), 787 (30) | 787 (100) | Qn 3-caffeoylsophorotrioside-7-glucoside[b] |
| a12 | 10.82 | 979.2333 | $C_{43}H_{47}O_{26}$ | −2.80 | 817 (98), 787 (100), 625 (59) | 625 (100) | Qn 3 hydroxyferuloylsophoroside-7-glucoside[b] |
| b4 | 10.92 | 787.1906 | $C_{33}H_{39}O_{22}$ | −3.25 | 625 (100) | 300 (100) | Qn 3-sophoroside-7-glucoside[b] |
| d3 | 10.97 | 979.2359 | $C_{43}H_{47}O_{26}$ | −0.20 | 817 (92), 787 (100), 625 (51) | 625 (100) | Qn 3 hydroxyferuloylsophoroside-7-glucoside[b] |
| a13 | 10.99 | 949.2256 | $C_{42}H_{45}O_{25}$ | 0.06 | 787 (100), 625 (22) | 625 (100) | Qn 3-caffeoylsophoroside-7-glucoside[b] |
| c4 | 11.11 | 1111.2749 | $C_{48}H_{55}O_{30}$ | −3.46 | 949 (100), 787 (29) | 787 (100) | Qn 3-caffeoylsophorotrioside-7-glucoside[b] |
| a14 | 11.12 | 949.2231 | $C_{42}H_{45}O_{25}$ | −2.44 | 787 (100), 625 (20) | 625 (100) | Qn 3-caffeoylsophoroside-7-glucoside[b] |
| c5 | 11.31 | 1111.2762 | $C_{48}H_{55}O_{30}$ | −2.16 | 949 (100), 787 (29) | 787 (100) | Qn 3-caffeoylsophorotrioside-7-glucoside[b] |
| a15 | 11.36 | 1111.2780 | $C_{48}H_{55}O_{30}$ | −0.36 | 949 (100), 787 (30) | 787 (100) | Qn 3-caffeoylsophorotrioside-7-glucoside[b] |
| d4 | 11.47 | 949.2234 | $C_{42}H_{45}O_{25}$ | −2.14 | 787 (100), 625 (20) | 625 (100) | Qn 3-caffeoylsophoroside-7-glucoside[b] |
| b5 | 11.53 | 609.1447 | $C_{27}H_{29}O_{16}$ | −1.41 | 489 (7), 447 (100), 285 (10) | 285 (100) | Km 3-diglucoside |
| a16 | 11.65 | 1111.2761 | $C_{48}H_{55}O_{30}$ | −2.26 | 949 (100), 788 (34), 625 (36) | 625 (100) | Qn 3-caffeoylsophorotrioside-7-glucoside[b] |
| b6 | 11.81 | 771.1976 | $C_{33}H_{39}O_{21}$ | −1.33 | 609 (100) | 285 (100) | Km 3-sophoroside-7-glucoside[b] |
| c6 | 11.92 | 787.1942 | $C_{33}H_{39}O_{22}$ | 1.45 | 625 (100) | 300 (100) | Qn 3-sophoroside-7-glucoside[b] |
| a17 | 12.05 | 963.2385 | $C_{43}H_{47}O_{25}$ | −2.69 | 801 (100), 609 (2) | 609 (100) | Km 3-hydroxyferuloylsophoroside-7-glucoside[b] |
| d5 | 12.07 | 1111.2766 | $C_{48}H_{55}O_{30}$ | −1.76 | 949 (100), 787 (38) | | Qn 3-caffeoylsophorotrioside-7-glucoside[b] |
| c7 | 12.08 | 979.2349 | $C_{43}H_{47}O_{26}$ | −1.23 | 817 (98), 787 (100), 625 (59) | 625 (100) | Qn 3 hydroxyferuloylsophoroside-7-glucoside[b] |
| b7 | 12.25 | 979.2329 | $C_{43}H_{47}O_{26}$ | −3.21 | 817 (95), 787 (100), 625 (55) | 625 (100) | Qn 3 hydroxyferuloylsophoroside-7-glucoside[b] |
| c8 | 12.28 | 1125.2937 | $C_{49}H_{57}O_{30}$ | −0.28 | 963 (100) | 771 (100) | Km 3-hydroxyferuloylsophorotrioside-7-glucoside[b] |
| d6 | 12.37 | 949.2234 | $C_{42}H_{45}O_{25}$ | −2.14 | 787 (100), 625 (20) | 625 (100) | Qn 3-caffeoylsophoroside-7-glucoside[b] |
| a18 | 12.53 | 977.2541 | $C_{44}H_{49}O_{25}$ | −2.80 | 831 (43), 771 (100), 625 (21) | 301 (100) | Qn 3 sophoroside-7-sinapoylrhamoside |
| c9 | 12.63 | 1095.2826 | $C_{48}H_{55}O_{29}$ | −0.28 | 975 (2), 933 (100), 809 (7) | 771 (100) | Km 3-caffeoylsophorotrioside-7-glucoside[b] |
| b8 | 12.69 | 949.2236 | $C_{42}H_{45}O_{25}$ | −1.94 | 787 (100), 625 (20) | 625 (100) | Qn 3-caffeoylsophoroside-7-glucoside[b] |
| a19 | 12.72 | 933.2289 | $C_{42}H_{45}O_{24}$ | −1.73 | 771 (100) | 609 (100) | Km 3-caffeoyldiglucoside-7-glucoside |
| c10 | 12.91 | 547.1671 | $C_{23}H_{31}O_{15}$ | 0.47 | 223 (100) | 208 (100) | sinapoylgentiobiose[b] |
| d7 | 12.94 | 963.2381 | $C_{43}H_{47}O_{25}$ | −3.09 | 801 (100) | 609 (100) | Km 3-hydroxyferuloylsophoroside-7-glucoside[b] |
| b8 | 13.15 | 1111.2760 | $C_{48}H_{55}O_{30}$ | −2.13 | 949 (100), 787 (30) | 787 (100) | Qn 3-caffeoylsophorotrioside-7-glucoside[b] |


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**Table 3. continued**

| peak | $t_R$(min) | $[M - H]^-$ | formula | error (mmu) | major and important $MS^2$ ions | $MS^3$ ion | tentative identification[a] |
|---|---|---|---|---|---|---|---|
| a20 | 13.18 | 1095.2797 | $C_{48}H_{55}O_{29}$ | −3.42 | 949 (84), 933 (100), 787 (45) | 787 (100) | Qn 3-*p*-coumaroyltriglucoside-7-glucoside |
| a21 | 13.32 | 1095.2826 | $C_{48}H_{55}O_{29}$ | −0.28 | 975 (2), 933 (100), 809 (7) | 771 (100) | Km 3-caffeoylsophorotrioside-7-glucoside[b] |
| c11 | 13.44 | 625.1410 | $C_{27}H_{29}O_{17}$ | −0.04 | 463 (8), 343 (16), 301 (100) | 179 (100) | Qn diglucoside[b] |
| a22 | 13.52 | 1155.3022 | $C_{50}H_{59}O_{31}$ | −2.38 | 993 (100), 950 (41), 787 (39) | | Qn 3-sinapoyltriglucoside-7-glucoside |
| d8 | 13.58 | 961.2592 | $C_{44}H_{49}O_{24}$ | −2.84 | 623 (72), 609 (100), 592 (27) | 257 (100) | Km 3-sophoroside-7-sinapoylrhamnoside |
| a23 | 13.69 | 993.2493 | $C_{44}H_{49}O_{26}$ | −2.45 | 801 (13), 787 (100) | 607 (100) | Qn 3-sinapoylsophoroside-7-glucoside[b] |
| d9 | 13.77 | 933.2275 | $C_{42}H_{45}O_{24}$ | −3.13 | 771 (100) | 609 (100) | Km 3-caffeoyldiglucoside-7-glucoside |
| b10 | 13.78 | 963.2391 | $C_{43}H_{47}O_{25}$ | −2.09 | 801 (100) | 609 (100) | Km 3-hydroxyferuloylsophoroside-7-glucoside[b] |
| c12 | 13.92 | 355.1029 | $C_{16}H_{19}O_9$ | −1.56 | 217 (59), 193 (100), 175 (40) | 134 (100) | feruloylglucose[b] |
| a24 | 14.12 | 1125.2937 | $C_{49}H_{57}O_{30}$ | −0.28 | 963 (100) | 771 (100) | Km 3-hydroxyferuloylsophorotrioside-7-glucoside[b] |
| b11 | 14.21 | 355.1029 | $C_{16}H_{19}O_9$ | −1.56 | 217 (59), 193 (100), 175 (40) | 134 (100) | feruloylglucose[b] |
| d10 | 14.21 | 933.2283 | $C_{42}H_{45}O_{24}$ | −2.24 | 787 (10), 771 (100), 625 (11) | 625 (100) | Km 3-caffeoyldiglucoside-7-glucoside |
| c13 | 14.21 | 1155.3028 | $C_{50}H_{59}O_{31}$ | −1.78 | 993 (100), 950 (29), 788 (30) | | Qn 3-sinapoyltriglucoside-7-glucoside |
| c14 | 14.33 | 1155.3023 | $C_{50}H_{59}O_{31}$ | −2.28 | 993 (100), 950 (29), 788 (30) | | Qn 3-sinapoyltriglucoside-7-glucoside |
| b12 | 14.39 | 933.2306 | $C_{42}H_{45}O_{24}$ | −0.03 | 787 (14), 771 (100), 625 (11) | 625 (100), 607 (8) | Qn 3-*p*-coumaroyldiglucoside-7-glucoside[b] |
| a25 | 14.52 | 1095.2810 | $C_{48}H_{55}O_{29}$ | −2.45 | 949 (100), 933 (38), 771 (62), 625 (40) | | Km 3-caffeoyl-triglucoside-7-glucoside |
| b13 | 14.61 | 933.2280 | $C_{42}H_{45}O_{24}$ | −2.63 | 771 (100) | 609 (100) | Km 3-caffeoyl-diglucoside-7-glucoside |
| a26 | 14.67 | 1095.2811 | $C_{48}H_{55}O_{29}$ | −2.35 | 949 (100), 933 (38), 932 (6), 787 (6), 771 (62) | | Km 3-caffeoyl-triglucoside-7-glucoside |
| d11 | 14.89 | 385.1137 | $C_{17}H_{21}O_{10}$ | −0.32 | 247 (52), 223 (100), 205 (55) | 164 (100) | sinapic acid-glucose |
| c15 | 14.91 | 993.2486 | $C_{44}H_{49}O_{26}$ | −3.15 | 831 (99), 787 (100), 769 (6), 625 (44) | 625 (100) | Qn 3-sinapoylsophorotrioside[b] |
| a27 | 15.01 | 1139.3093 | $C_{50}H_{59}O_{30}$ | −0.32 | 977 (100) | 771 (100) | Km 3-sinapoylsophorotrioside-7-glucoside[b] |
| d12 | 15.04 | 993.2481 | $C_{44}H_{49}O_{26}$ | −3.65 | 831 (100), 787 (94), 769 (6), 625 (45) | | Qn 3-sinapoyldiglucoside-7-glucoside |
| a28 | 15.21 | 977.2535 | $C_{44}H_{49}O_{25}$ | −2.24 | 815 (100), 609 (3) | 609 (100) | Km 3-sinapoylsophoroside-7-glucoside[b] |
| b14 | 15.22 | 993.2496 | $C_{44}H_{49}O_{26}$ | −2.15 | 831 (99), 787 (100), 769 (6), 625 (44) | 625 (100) | Qn 3-sinapoyltriglucoside |
| c16 | 15.27 | 963.2387 | $C_{43}H_{47}O_{25}$ | −2.49 | 801 (100), 609 (2) | 609 (100) | Km 3-hydroxyferuloylsophoroside-7-glucoside[b] |
| b15 | 15.55 | 963.2381 | $C_{43}H_{47}O_{25}$ | −3.09 | 801 (100), 787 (45), 625 (26) | 625 (100) | Km 3-hydroxyferuloylsophoroside-7-glucoside[b] |
| d13 | 15.55 | 963.2374 | $C_{43}H_{47}O_{25}$ | −3.79 | 801 (100), 787 (47), 625 (25) | | Km 3-hydroxyferuloylsophoroside-7-glucoside[b] |
| c17 | 15.63 | 1139.3103 | $C_{50}H_{59}O_{30}$ | 0.56 | 977 (100), 771 (3) | 771 (100) | Km 3-sinapoylsophorotrioside-7-glucoside[b] |
| b16 | 15.72 | 963.2391 | $C_{43}H_{47}O_{25}$ | −2.09 | 801 (100), 787 (45), 625 (26) | 625 (100) | Km 3-hydroxyferuloylsophoroside-7-glucoside[b] |
| d14 | 15.82 | 933.2283 | $C_{42}H_{45}O_{24}$ | −2.33 | 787 (10), 771 (100), 625 (11) | 625 (100) | Km 3-caffeoyldiglucoside-7-glucoside |
| a29 | 15.87 | 947.2429 | $C_{43}H_{47}O_{24}$ | −2.28 | 827 (2), 785 (100), 609 (2) | 609 (100) | Km 3-feruloylsophoroside-7-glucoside[b] |
| b17 | 15.93 | 933.2280 | $C_{42}H_{45}O_{24}$ | −2.63 | 788 (10), 771 (100), 625 (11) | 625 (100) | Km 3-caffeoyldiglucoside-7-glucoside |
| c18 | 15.93 | 1109.2946 | $C_{49}H_{57}O_{29}$ | −4.06 | 947 (100) | 771 (100) | Km 3-feruloylsophorotrioside-7-glucoside |
| a30 | 16.28 | 917.2318 | $C_{42}H_{45}O_{23}$ | −4.26 | 755 (100) | 609 (100) | Km 3-*p*-coumaroylsophoroside-7-glucoside[b] |
| d15 | 16.36 | 1095.2805 | $C_{48}H_{55}O_{29}$ | −2.95 | 933 (100), 787 (28) | | Km 3-caffeoyltriglucoside-7-glucoside |
| c19 | 16.39 | 977.2535 | $C_{44}H_{49}O_{25}$ | −2.24 | 815 (100), 609 (3) | 609 (100) | Km 3-sinapoylsophoroside-7-glucoside[b] |
| a31 | 16.47 | 1079.2852 | $C_{48}H_{55}O_{28}$ | −3.33 | 755 (100), 609 (12) | 609 (100) | Km 3-*p*-coumaroylsophoroside-7-diglucoside |
| b18 | 16.48 | 1139.3065 | $C_{50}H_{59}O_{30}$ | −3.16 | 977 (100) | 771 (100) | Km 3-sinapoylsophorotrioside-7-glucoside[b] |
| b19 | 16.63 | 977.2542 | $C_{44}H_{49}O_{25}$ | −2.64 | 815 (100) | 609 (100) | Km 3-sinapoylsophoroside-7-glucoside[b] |
| d16 | 16.76 | 609.1441 | $C_{27}H_{29}O_{16}$ | −2.01 | 489 (13), 447 (100), 285 (19) | 284 (100) | Km 3-glucoside-7-glucoside[c] |
| c20 | 16.93 | 947.2429 | $C_{43}H_{47}O_{24}$ | −2.28 | 827 (2), 785 (100), 609 (2) | 609 (100) | Km 3-feruloylsophoroside-7-glucoside[b] |
| c21 | 17.17 | 639.1566 | $C_{28}H_{31}O_{17}$ | 2.08 | 519 (10), 477 (100), 315 (12) | 314 (100) | Is 3-glucoside-7-glucoside[b] |
| b20 | 17.20 | 947.2439 | $C_{43}H_{47}O_{24}$ | −2.38 | 785 (100) | 609 (100) | Km 3-feruloylsophoroside-7-glucoside[b] |
| d17 | 17.25 | 977.2535 | $C_{44}H_{49}O_{25}$ | −3.34 | 815 (100), 771 (10) | 609 (100) | Km 3-sinapoylsophoroside-7-glucoside[b] |
| b21 | 17.55 | 917.2328 | $C_{42}H_{45}O_{23}$ | −2.91 | 755 (100) | 609 (100) | Km 3-*p*-coumaroylsophoroside-7-glucoside |
| d18 | 17.97 | 947.2429 | $C_{43}H_{47}O_{24}$ | −3.38 | 785 (100) | 609 (100) | Km 3-feruloylsophoroside-7-glucoside[b] |
| c22 | 18.03 | 551.1753 | $C_{26}H_{31}O_{13}$ | −1.71 | 389 (100), 341 (6) | 341 (100) | ferulic acid-rhamnosylglucose with a 48 amu group |


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**Table 3. continued**

| peak | $t_R$(min) | $[M-H]^-$ | formula | error (mmu) | major and important $MS^2$ ions | $MS^3$ ion | tentative identification[a] |
|---|---|---|---|---|---|---|---|
| d19 | 18.12 | 947.2449 | $C_{43}H_{47}O_{24}$ | −1.38 | 785 (100) | 609 (100) | Km 3-feruloylsophoroside-7-glucoside[b] |
| c23 | 18.28 | 993.2473 | $C_{44}H_{49}O_{26}$ | −4.49 | 801 (13), 787 (100) | 607 (100) | Qn 3-sinapoylsophoroside-7-glucoside[b] |
| d20 | 18.36 | 639.1548 | $C_{28}H_{31}O_{17}$ | −1.87 | 519 (11), 477 (100), 315 (12) | 314 (100) | Is 3-glucoside-7-glucoside |
| d21 | 18.63 | 917.2330 | $C_{42}H_{45}O_{23}$ | −2.71 | 755 (100) | 609 (100) | Km 3-*p*-coumaroyldiglucoside-7-glucoside |
| b22 | 19.13 | 625.1382 | $C_{27}H_{29}O_{17}$ | −2.82 | 505 (21), 463 (37), 445 (55), 301 (60), 300 (100) | | Qn 3-diglucoside |
| a32 | 19.40 | 935.2444 | $C_{42}H_{47}O_{24}$ | −1.88 | 773 (100), 755 (29), 663 (52), 285 (30) | 285 (100) | Km aglycone with 7 glucoside and 3 acyl glucosyls |
| a33 | 20.43 | 625.1414 | $C_{27}H_{29}O_{17}$ | 0.60 | 505 (18), 463 (17), 445 (54), 300 (100) | 271 (100) | Qn 7-sophoroside[b] |
| b23 | 21.28 | 639.1548 | $C_{28}H_{31}O_{17}$ | −2.9 | 315 (100), 300 (16) | | Is 3-diglucoside |
| a34 | 21.40 | 965.2516 | $C_{49}H_{41}O_{21}$ | 1.02 | 803 (100), 785 (24), 693 (48), 667 (9), 285 (21) | 285 (100) | Km 3-caffeoyldiglucoside-7-glucoside |
| d22 | 21.48 | 933.2271 | $C_{42}H_{45}O_{24}$ | −3.53 | 787 (10), 771 (100), 625 (11) | 625 (100) | Km 3-caffeoyldiglucoside-7-glucoside |
| a35 | 21.90 | 935.2436 | $C_{42}H_{47}O_{24}$ | −2.68 | 773 (100), 756 (31), 663 (55), 637 (10), 285 (24) | 285 (100) | Km aglycone with 7 glucoside and 3 acyl glucosyls |
| c24 | 22.16 | 831.1997 | $C_{38}H_{39}O_{21}$ | 0.93 | 625 (100) | 300 (100) | Qn 3-sinapoylsophoroside[b] |
| b24 | 23.13 | 193.0506 | $C_{10}H_9O_4$ | −0.03 | 178 (19), 149 (50), 134 (100) | 106 (100) | ferulic acid[c] |
| b25 | 24.01 | 223.0607 | $C_{11}H_{11}O_5$ | −2.23 | 208 (8), 179 (11), 164 (100) | 149 (100) | sinapic acid[c] |
| d23 | 24.79 | 193.0502 | $C_{10}H_9O_4$ | −0.43 | 178 (25), 149 (55), 134 (100) | 106 (100) | ferulic acid[c] |
| b26 | 24.92 | 593.1503 | $C_{27}H_{29}O_{15}$ | −1.51 | 447 (100) | 284 (100) | Km 3-glucoside-7-rhanmoside |
| d24 | 25.86 | 223.0607 | $C_{11}H_{11}O_5$ | −0.50 | 208 (8), 179 (11), 164 (100) | 149 (100) | sinapic acid isomer |
| b27 | 26.17 | 977.2536 | $C_{44}H_{49}O_{25}$ | −3.24 | 815 (100), 653 (14) | 653 (100) | Km 3-sinapoylsophoroside-7-glucoside[b] |
| d25 | 26.69 | 223.0607 | $C_{11}H_{11}O_5$ | −2.23 | 208 (8), 179 (11), 164 (100) | 149 (100) | sinapic acid isomer |
| a36 | 32.77 | 753.2253 | $C_{34}H_{41}O_{19}$ | 0.73 | 529 (100) | 205 (100) | disinapoylgentiobiose[b] |
| a37 | 33.68 | 1123.2886 | $C_{53}H_{55}O_{27}$ | −4.47 | 961 (100), 755 (20) | 755 (100) | Km 3-hydroxyferuloylsophorotrioside-7-glucoside[b] |
| a38 | 34.63 | 1153.2981 | $C_{54}H_{57}O_{28}$ | 1.32 | 991 (100), 785 (20) | 785 (100) | Km 3-sinapoylferuloylsophoroside-7-glucoside[b] |
| a39 | 35.20 | 1183.3081 | $C_{55}H_{59}O_{29}$ | −5.62 | 1021 (100), 816 (19) | 815 (100) | Km 3-disinapoyldiglucoside-7-glucoside |
| a40 | 35.52 | 1183.3086 | $C_{55}H_{59}O_{29}$ | −5.20 | 977 (22), 959 (7), 815 (100), 609 (14), 591 (7) | 609 (100) | Km 3-sinapoyldiglucoside-7-sinapoylglucoside |
| b28 | 37.34 | 753.2253 | $C_{34}H_{41}O_{19}$ | 0.73 | 529 (100) | 205 (100) | disinapoylgentiobiose[b] |
| a41 | 37.86 | 753.2253 | $C_{34}H_{41}O_{19}$ | 0.73 | 529 (100) | 205 (100) | disinapoylgentiobiose[b] |
| d26 | 38.81 | 753.2258 | $C_{34}H_{41}O_{19}$ | 1.39 | 529 (100) | 223 (100) | disinapoylgentiobiose[b] |
| a42 | 39.11 | 723.2144 | $C_{33}H_{39}O_{18}$ | 0.29 | 529 (100), 499 (21) | 223 (100) | sinapoyl-feruloylgentiobiose[b] |
| a43 | 39.35 | 723.2125 | $C_{33}H_{39}O_{18}$ | −1.69 | 529 (100), 499 (21) | 223 (100) | sinapoyl-feruloylgentiobiose[b] |
| a44 | 39.99 | 1199.3057 | $C_{55}H_{59}O_{30}$ | −3.96 | 993 (100, −206), 787 (12) | 787 (100) | Qn 3-disinapoylsophorotrioside |
| d27 | 40.41 | 723.2120 | $C_{33}H_{39}O_{18}$ | −2.19 | 529 (100), 499 (21) | 223 (100) | sinapoyl-feruloylgentiobiose |
| a45 | 43.75 | 959.2830 | $C_{45}H_{51}O_{23}$ | 0.35 | 735 (100), 529 (7), 511 (11) | 529 (100) | trisinapoylgentionbiose[b] |
| a47 | 44.67 | 959.2798 | $C_{45}H_{51}O_{23}$ | −2.87 | 735 (100), 529 (10), 511 (13) | 223 (100) | trisinapoylgentionbiose[b] |
| b30 | 45.98 | 929.2695 | $C_{44}H_{49}O_{22}$ | −2.63 | 705 (100), 511 (6) | 499 (100) | feruloyl-disinapoyl-gentionbiose |

[a]Km, kaempferol; Qn, quercetin; Is, isorhamnetin. [b]Identified with literature data. [c]Identified with reference standards.

side, which were also found in mature red cabbage. Km 3-sophorotrioside-7-glucoside, Qn 3-caffeoylsophorotrioside-7-glucoside, and Qn 3-hydroxyferuloylsophorotioside-7-glucoside existed only in microgreens of red mustard and purple mustard.

Using MS analysis of peak a19, as an example, the deprotonated molecular ion at $m/z$ 933 ($C_{42}H_{45}O_{24}$) lost a hexosyl group from position 7, giving the product ion at $m/z$ 771. The $MS^3$ product ion revealed a loss of 162 amu, corresponding to a caffeoyl group, and a loss of dihexoxyl group at the 3-position (324 amu), leading to the Km aglycone ($m/z$ 285). Thus, peak a19 was tentatively identified as Km 3-caffeoyldiglucoside-7-glucoside. Peak b13 also exhibited the deprotonated ion at $m/z$ 933 but showed different fragmentation pathways. During the MS fragmentation of peak 18a, loss of 162 amu, corresponding to a hexosyl moiety at the terminal 7-position, was observed. Further fragmentation of the acylated ion, $m/z$ 625, gave the loss of *p*-coumaroyl group and the loss of a dihexosyl group, producing the Qn aglycone ion ($m/z$ 301). Thus, peak b13 was assigned as Qn 3-*p*-coumaroyldiglucoside-7-glucoside. Using this strategy, the remaining flavonols were identified on the basis of HRMS, MS fragmentation pattern, UV maxima, and retention times as flavonols, previously characterized in the five *Brassica* species microgreens.

**Derivatives of Hydroxycinnamic Acids and Hydroxybenzoic acids.** Hydroxycinnamic acids and hydroxybenzoic acids are considered nonflavonoid phenolics and are characterized by their C6−C3 and C6−C structures, respectively. Most of the hydroxycinnamic acids and hydroxybenzoic acid derivatives detected in mature vegetables[17−19,21] were also detected in our five *Brassica* species microgreens. However, our five *Brassica* species microgreens contained a greater variety and higher concentrations of cinnamic acids than their mature leaf counterparts. The retention times, HRMS molecular ions $[M-H]^-$, diagnostic $MS^2$ and $MS^3$ product ions, UV $\lambda_{max}$, and identification of the hydroxycinnamates, arranged by molecular

weight, are listed in Table 3. Their peaks are eluted with the flavanol glycoside peaks, as shown in Figure 3. The hydroxycinnamic acids, hydoxycinnamoylquinic acids, hydroxycinnamoylmalic acids, and hydroxycinnamoyl saccharides with one to three glucosides were identified using reference compounds (designated by [c]) or from the literature (designated by [b]). Sixteen of the hydroxycinnamoylsaccharides were formed from di- or triglucoses, mainly gentiobiose, with one to three hydroxycinnamoyl units. By direct comparison with reference compounds in mustard greens, peaks a36, a41, b28, and d26 (Figure 3) were identified as disinapoylgentiobioses. Peaks a4, b11, and c12 were identified as feruloyl-glucosides. Peaks a42, d27, and a43 were identified as sinapoyl-feuloylgentiobioses. Peaks a47 and b30, identified as trisinapoylgentionbiose and feruloyl-disinapoyl-gentionbiose, are peaks common to microgreens of mizuna, purple kohlrabi, red mustard, and purple mustard. Peak d11 is found only in mizuna and was tentatively identified as sinapic acid-glucose.

Other organic acids, such as caffeoylquinic acid, ferulic acid, sinapic acid, citric acid, malic acid, and caffeoylquinic acid, are organic acids common in these five microgreens. There were a number of organic acid isomers found in the five *Brassisa* microgreens, and identification was based on their similar $MS^2$ and $MS^3$ spectra. However, they exhibited different retention times based on species. For example, peaks a42, a43, and d27 all had the same $[M - H]^-$ at $m/z$ 723. HRMS measurements suggested the formula $C_{33}H_{39}O_{18}$, with the main $MS^2$ product ion at $m/z$ 529 (M − 194, neutral loss of ferulic acid) and the main $MS^3$ product ion at $m/z$ 223 (sinapic acid). These compounds were identified as sinapoyl-ferulic acid and its isomers. Similarly, peaks a36, b28, and a41 ($[M - H]^-$ at $m/z$ 753, with a main $MS^2$ product ion at 529 and main $MS^3$ product ions at 205) were identified as disinapoylgentiobiose and its isomers.

In summary, this is the first study characterizing phenolic profiles specifically in *Brassica* species microgreens. A total of 165 phenolic compounds were tentatively identified using complementary information from UHPLC-PDA-$HRMS^n$ in negative and positive modes, revealing a large number of highly glycosylated and acylated quercetin, kaempferol, and cyanidin aglycones and complex hydroxycinnamic and benzoic acids. The results showed that the *Brassica* species microgreens tended to have more complex polyphenol profiles and to contain more varieties of polyphenols compared to their mature plant counterparts. Thus, *Brassica* species microgreens could be considered a good source for polyphenols. This compositional study should serve as reference base for these five *Brassica* species microgreens and enhance their value to health agencies and consumers.

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