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Metabolic Profiling of Glucosinolates, Anthocyanins, Carotenoids, and Other Secondary Metabolites in Kohlrabi (*Brassica oleracea* var. *gongylodes*)

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ABSTRACT: We profiled and quantified glucosinolates, anthocyanins, carotenoids, and other secondary metabolites in the skin and flesh of pale green and purple kohlrabis. Analysis of these distinct kohlrabis revealed the presence of 8 glucosinolates, 12 anthocyanins, 2 carotenoids, and 7 phenylpropanoids. Glucosinolate contents varied among the different parts and types of kohlrabi. Glucoerucin contents were 4-fold higher in the flesh of purple kohlrabi than those in the skin. Among the 12 anthocyanins, cyanidin 3-(feruloyl)(sinapoyl) diglucoside-5-glucoside levels were the highest. Carotenoid levels were much higher in the skins than the flesh of both types of kohlrabi. The levels of most phenylpropanoids were higher in purple kohlrabi than in pale green ones. *trans*-Cinnamic acid content was 12.7-fold higher in the flesh of purple kohlrabi than that in the pale green ones. Thus, the amounts of glucosinolates, anthocyanins, carotenoids, and phenylpropanoids varied widely, and the variations in these compounds between the two types of kohlrabi were significant.

KEYWORDS: *anthocyanins, carotenoids, glucosinolates, phenylpropanoids, purple kohlrabi*

INTRODUCTION

The beneficial health effects of frequent vegetable consumption are well established. These benefits are not merely related to primary nutrients but also to secondary compounds, called phytochemicals. Vegetables from the Brassicaceae family are among the most commonly grown vegetables worldwide. Members of Brassicaceae, which include many important vegetable crops such as cabbage, kale, Brussels sprouts, cauliflower, broccoli, and kohlrabi, contain very potent phytochemicals, glucosinolates, and their breakdown products.[1] With its edible turnip-like swollen stem, kholrabi is a cool-weather plant. It is closest in form to wild cabbage (*Brassica oleracea* ssp. *oleracea*), the progenitor of all the *B. oleracea* varieties, and grows along the coasts of Europe and North Africa. There are purple and pale green cultivars of kohlrabi (Figure 1). Kohlrabi is more tolerant of heat and drought than most of its cabbage relatives. Glucosinolates and β-thioglucoside-*N*-hydroxysulfates (*cis*-*N*-hydroximinosulfate esters) are sulfur-rich anionic secondary metabolites derived from glucose and amino acids. They are found almost exclusively within the plant order Brassicales (which includes family Brassicaceae, Capparidaceae, and Caricaceae) but also in the genus *Drypetes* (family Euphorbiaceae). About 200 different glucosinolates are known to occur naturally in plants.[2,3] Glucosinolates are hydrolyzed into many bioactive compounds by the endogenous enzyme myrosinase (β-thioglucosidase glucohydrolase) (EC 3.2.3.1). These hydrolysis products include substituted isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidinethiones, which play roles in defense and have various biological activities related to human health.[4,5] Glucosinolates from vegetables of the Cruciferae family are associated with reduced risks of cancer of the lungs, stomach, breasts, prostate, pancreas, colon, and rectum.[6,7]

Anthocyanins are natural pigments responsible for the blue, purple, red, and orange colors in all higher plants, mostly in flowers and fruits, but also in leaves, stems, and roots. More than 500 different anthocyanins have been described in the literature.[8] Anthocyanins are bioactive food compounds that have attracted much interest due to their impact on the sensorial characteristics of food products as well their health-related properties through various biological activities.[9,10]

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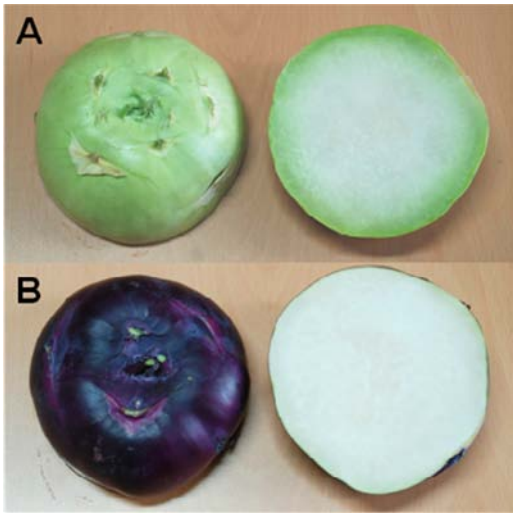

Figure 1. Photographs of two types of kohlrabi: (A) pale green kohlrabi and (B) purple kohlrabi.

Anthocyanins are thought to protect plant tissues and/or senescing autumn leaves against damaging photo-oxidative effects[11] and UV irradiation, promote pollination, and facilitate seed distribution.[12]

Carotenoids are a diverse group of more than 600 naturally occurring red, orange, and yellow pigments[13] that accumulate in the plastids of leaves, flowers, and fruits. These pigments play important roles in many physiological processes in plants. For example, some act as light absorbers in photosynthetic membranes and prevent damaging photo-oxidative processes.[14] In addition, the colors of carotenoids in flowers and fruits attract pollinators and seed-dispersal agents.[15] In humans, some carotenoids are essential nutrients, while others have protective effects against several diseases. For example, provitamin A carotenoids such as α- and β-carotene, which are precursors of vitamin A, are necessary to prevent xerophthalmia, blindness, and premature death.[16,17]

The number of documented cases of metabolic profiling of kohlrabi remains small, and only a few are published,[18−20] and to our knowledge, no previous report or publication has established for complete metabolic profiling of kohlrabi either in the skin or flesh of different cultivars. Therefore, this study focused on profiling and quantifying glucosinolates, anthocyanins, carotenoids, and other secondary metabolites between two typical types of kohlrabi.

MATERIALS AND METHODS

Plant Materials. Pale green (cultivar Winner) and purple kohlrabi (cultivar Early purple Vienna) seeds were purchased from Stokes Seeds Ltd., St. Catharines, Canada and stored at 4 °C. Kohlrabi seeds were germinated in a greenhouse, and the seedlings were transferred to the experimental farm at Chungnam National University (Daejeon, Korea). The pale green and purple kohlrabis were harvested after 10 weeks. Prior to experiments, kohlrabis were peeled manually and the epidermal tissues (skin) and the flesh were cut into small cubes. The samples were then freeze-dried at −80 °C for at least 72 h and then ground into a fine powder using a mortar and pestle for phytochemical analysis.

Glucosinolate Analysis. *Chemicals and the HPLC-MS.* High performance liquid chromatography (HPLC)-grade acetonitrile (CH_3CN) and methanol (MeOH) were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA), and sodium acetate ($CH_3COONa{\cdot}3H_2O$) was obtained from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan). Aryl sulfatase (type H-1, EC 3.1.6.1), sinigrin (2-propenyl glucosinolate), and DEAE-Sephadex A-25 were supplied by Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Glucosinolates were extracted and desulfated according to the procedure of Kim et al. (2007)[21] and ISO 9167-1 (1992).[22] Briefly, crude glucosinolates were extracted with 70% (v/v) boiling methanol (1.5 mL) from lyophilized powder (100 mg) in a water bath at 70 °C for 5 min to inactivate endomyrosinase. The mixture was centrifuged at 12,000*g* for 10 min at 4 °C, and the resultant supernatant was collected. The residue was re-extracted twice more in the same manner, and the supernatants were combined. The crude glucosinolate extract was applied to a mini-column by using a 1,000-μL pipet tip packed with DEAE-Sephadex A-25 (H^+ form by 0.5 M sodium acetate, approximately 40 mg dry wt.). Glucosinolates were then desulfated by adding aryl sulfatase solution (75 μL, 29 units) to the column. After an overnight reaction at ambient temperature, the desulfo-glucosinolates were eluted with 0.5 mL of high-purity water (×4). The solution was then filtered through a 0.45 μm hydrophilic PTFE syringe filter (Ø, 13 mm, Advantec, Tokyo, Japan) in a brown vial.

Glucosinolates in the methanolic kohlrabi extracts were characterized using an HPLC-MS system. The HPLC-MS experiment was conducted on a 4000 Q-Trap LC/MS/MS system (Applied Biosystems, Foster City, CA, USA) connected to an Agilent Technologies 1200 series HPLC (Santa Clara, CA, USA). The LC conditions were as follows: column, Inertsil ODS-3 (150 × 3.0 mm i.d., particle size, 3 μm; GL Science, Tokyo, Japan) with the Inertsil ODS-2 Cartridge guard column (10 × 2 mm i.d., 5 μm); detector wavelength, 227 nm; injection volume, 10 μL; column temperature, 40 °C; and flow rate, 0.2 mL·min^{-1}. The mobile phase consisted of water (A) and acetonitrile (B). The following solvent program was used: 7% solvent B at 0 min, 24% solvent B at 18 min kept constant until 32 min, down to 7% solvent B at 32.01 min, and then kept constant at 7% solvent B for 10 min (total 40 min). The individual glucosinolates were quantified according to their HPLC area and response factor with respect to that of an external standard (ISO 9167-1, 1992).[22] To identify individual glucosinolates, MS analysis was carried out with an ESI interface operated in the positive ion mode. The MS operating conditions were as follows: ion spray voltage, 5.5 kV; curtain gas (20 psi), nebulizing gas (50 psi), and heating gas (50 psi) were high-purity nitrogen (N_2); heating gas temperature, 550 °C; spectra range, m/z 100−800; and scan time, 4.8 s.

Anthocyanin Analysis. *Chemicals and HPLC-MS.* HPLC-grade acetonitrile (CH_3CN) was purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA), and formic acid (HCOOH) was obtained from Acros Organics (Morris Plain, NJ, USA). HPLC-grade cyanidin-3-*O*-glucoside chloride as an external standard was supplied by Fujicco Co., Ltd. (Kobe, Japan).

After 100 mg of liphophilized powder was weighted into a 2.0- mL microcentrifuge tube, anthocyanins were extracted by the addition of water (20 mL) containing 5% formic acid followed by vortexing for 1 min and sonication for 20 min at ambient temperature (modified from Tatsuzawa et al.).[23] The mixture was then centrifuged at 12,000 rpm for 13 min at 4 °C, and the supernatant was filtered through a 0.45 μm hydrophilic PTFE syringe filter (Ø, 13 mm, Advantec, Tokyo, Japan) in a brown vial and immediately stored in the refrigerator at −70 °C until HPLC analysis.

Anthocyanins were separated and quantified using an Agilent 1200 series HPLC (Santa Clara, CA, USA) coupled to a 4000 Q-Trap LC-ESI-MS/MS system (Applied Biosystems, Foster City, CA, USA) (modified from Wu and Prior).[24] Chromatographic separation was performed on an analytical Synergi 4 μm POLAR-RP 80A column (250 × 4.6 mm, i.d., particle size 4 μm; Phenomenex, Torrance, CA, USA) equipped with a Security Guard Cartridges Kit AQ C18 column (Phenomenex, Torrance, CA, USA). The column temperature was set to 40 °C, detection wavelength was 520 nm, flow rate was 1.0 mL·min^{-1}, and injection volume was 10 μL. Mobile phases A and B were 5% formic acid in water (v/v) and acetonitrile (v/v), respectively. The gradient program largely modified from that previously published[24] is described as follows: 5−10% B from 0 to 8 min; 13% B from 8 to 13 min; kept constant at 13% B until 15 min; 18% B from

15 to 18 min; kept constant at 15% B until 25 min; 18% B from 25 to 30 min, kept constant at 18% B until 35 min; 21% B from 35 to 40 min; kept constant at 21% B until 45 min; quickly decreasing to 5% B at 45.1 min; and kept constant at 5% B until 50 min. The phases were allowed to equilibrate between injections. Individual anthocyanins were qualified by comparing the HPLC peak area with that of an authentic standard (cyanidin-3-*O*-glucoside) and expressed as mg/g dry weight. For identification of individual anthocyanins, the LC eluate was introduced (0.2 mL) directly into the ESI interface with splitting at 1.0 mL·min^{-1} and analyzed in the positive ionization mode. The conditions for MS analysis were the same as those described in the Glucosinolate Analysis section, except the spectra range was m/z 100–1400. The interpretation of MS spectra were carried out in comparison with our database and previously published reports.[24,25]

Carotenoid Analysis. *Chemicals and HPLC.* Ascorbic acid and β-apo-8′-carotenal were purchased from Sigma Chemical Co. (St. Louis, MO). Lutein and β-carotene were obtained from CaroteNature (Lupsingen, Switzerland). Carotenoids were extracted from 0.1 g of kohlrabi samples with 3 mL of ethanol containing 0.1% ascorbic acid (w/v). This mixture was vortexed for 20 s and incubated in a water bath at 85 °C for 5 min. Next, 120 μL of potassium hydroxide (80% w/v) was added to saponify any potentially interfering oils. After vortexing and incubation at 85 °C for 10 min, the samples were placed on ice, and 1.5 mL of cold deionized water and 0.05 mL of β-apo-carotenal (12.5 μg·mL^{-1}) as an internal standard were added. Next, the carotenoids were extracted twice with 1.5 mL of hexane and centrifuged at 1200*g* each time to separate the layers. Then, the extracts were freeze-dried under a stream of nitrogen gas and resuspended in 50:50 (v/v) dichloromethane/methanol. The extraction method used for carotenoid analysis is slightly modified from that of a previous report.[26] For quantification purposes, calibration curves were drawn by plotting at four different concentrations of carotenoid standards according to the peak area ratios with β-apo-8′-carotenal. The linear equations and regression coefficients for lutein and β-carotene were $y = 0.1928x - 0.027$, with $r = 0.9999$, and $y = 0.1126x - 0.0212$ with 0.9985, respectively.

For HPLC analysis, the carotenoids were separated on an Agilent 1100 HPLC system with a C_{30} YMC column (250 × 4.6 mm, 3 μm; Waters Corporation, Milford, MA, USA) and detected with a photodiode array (PDA) detector at 450 nm. Solvent A consisted of methanol/water (92:8 v/v) with 10 mM ammonium acetate; solvent B consisted of 100% methyl *tert*-butyl ether (MTBE). The flow rate was maintained at 1 mL·min^{-1}, and samples were eluted using the following gradient program: 17% solvent B at 0 min; 30% solvent B at 23 min; 41% solvent B at 29 min; 70% solvent B at 35 min; 70% solvent B at 40 min; 17% solvent B at 44 min; and 17% solvent B at 55 min. The identification and peak assignment of carotenoids were primarily based on comparisons of their retention time and UV–visible spectrum data with those of standards and using previously reported guidelines.[26,27]

HPLC Analysis of Phenolic Compounds (Phenylpropanoids). Dried and ground samples (0.1 g) were extracted with 3 mL of pure methanol at 60 °C for 30 min to obtain phenolic compounds and extracted twice with 3 mL of 80% methanol for 1 h, at room temperature to obtain the remaining test compounds. The extracts were filtered through a 0.45-μm poly filter and then diluted 2-fold with methanol prior to HPLC analysis. The HPLC quantification of phenolic compounds was performed using a Futecs model NS-4000 HPLC apparatus (Daejeon, Korea). The analysis was monitored at 280 nm and performed using a C_{18} column (250 × 4.6 mm, 5 μm; RStech, Daejeon, Korea). A gradient prepared from mixtures of acetonitrile and 0.15% acetic acid was used as the mobile phase. The column was maintained at 30 °C, the flow rate was 1.0 mL/min, and the injection volume was 20 μL. The resultant concentrations of phenolic compounds were calculated using a standard curve. All samples were run in triplicate.

Statistical Analysis. All results are expressed as the mean of three biological replicates. Three subsamples were randomly generated from each harvested kohlrabi genotype, and then separate extractions on each replicate sample were conducted. Data were subjected to analysis of variance (ANOVA) with sums of squares partitioned to reflect trial effects using SAS Software, release 9.2,[28] and means were separated via Duncan Multiple Range Test ($P < 0.05$).

RESULTS AND DISCUSSION

Identification and Quantification of Glucosinolate Compounds in Different Parts of Kohlrabi Cultivars. Eight different glucosinolates including glucoraphanin, glucoalyssin, glucotropaeoline, 4-hydroxyglucobrassicin, glucoerucin, glucobrassicin 4-methoxyglucobrassicin, and neoglucobrassicin were separated and identified from kohlrabi (Table 1). Pale green and purple kohlrabis were used in the present study; both were divided into flesh and skin parts. 4-Hydroxyglucobrassicin was absent in both types of kohlrabi skin, and glucoerucin was absent in pale green kohlrabi flesh (Table 1). Glucoraphanin and 4-hydroxyglucobrassicin were the predominant glucosinolates in kohlrabi seeds, but progoitrin and glucoiberin were also expressed in measurable amounts.[29] The isothiocyaninate (ITC) profile of kohlrabi tubers was dominated by 4-methylthiobutyl ITC followed by sulforaphane, phenylethyl ITC, and allyl ITC, resulting from the hydrolysis of glucoerucin, glucoraphanin, gluconasturtiin, and sinigrin, respectively.[30] These results indicate that the glucosinolate profiles are dependent upon the kohlrabi cultivar.

Table 1. Glucosinolate Content (μmol/g Dry Wt.) in Different Parts of Kohlrabi Cultivars

	pale green kohlrabi		purple kohlrabi	
glucosinolates	skin	flesh	skin	flesh
glucoraphanin	0.66 c[a]	0.63 c	2.70 a	1.68 b
glucoalyssin	0.43 a	0.33 b	0.29 b	0.32 b
glucotropaeoline	0.94 c	4.27 a	1.24 c	1.90 b
4-hydroxyglucobrassicin	0 c	1.15 b	0 c	1.93 a
glucoerucin	0 c	0 c	2.06 b	8.08 a
glucobrassicin	0.82 c	0.43 d	1.73 b	2.91 a
4-methoxyglucobrassicin	0.44 c	0.27 c	1.05 a	0.68 b
neoglucobrassicin	3.4 a	2.62 b	3.12 a	0.74 c
total	6.84 c	11.35 b	12.67 b	18.98 a

[a]Mean values (mean of 3 replicates with three samples from each replicate) indicated by the same letter in a *row* do not differ significantly at the 5% level by Duncan's Multiple Range Test.

Analysis of the skin and flesh of pale green and purple kohlrabis revealed 6 glucosinolates from pale green kohlrabi skin, 7 from pale green kohlrabi flesh and purple kohlrabi skin, and 8 from purple kohlrabi flesh were separated (Table 1). Among them, glucoerucin was only detected in purple kohlrabi both in skin and flesh, while DS-4-hydroxyglucobrassicin was present in the flesh of both types. The levels of these glucosinolates compounds varied among the different parts and types of kohlrabi. Glucoerucin levels were 4 times higher in the flesh than the skin of purple kohlrabi. The total glucosinolates contents in the flesh of both pale green (66%) and purple kohlrabis (50%) were higher than those in their respective skins; moreover, they were higher in purple kohlrabi (85% and 67% in skin and flesh, respectively) than in pale green kohlrabi. However, the total glucosinolate contents ranged from 6.8 to 19.0 μmol/g dry weight and are lower than those in other kohlrabi cultivars including white Vienna and purple Vienna (51.6 and 45.9 μmol/g fresh weight, respectively, after converting previous data from fresh weight to dry weight assuming 5% moisture content).[21] In that case,[21] only

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Table 2. Anthocyanins Identified Only in the Skin of Purple Kohlrabi and Content (μmol/g Dry Wt.)

no.[a]	RT[b] (min)	trivial names	$[M + H]^+$ (*m/z*)	MS/MS (*m/z*)	purple kohlrabi (skin)
1	9.90	cyanidin 3-diglucoside-5-glucoside	773	611/449/287	0.20 ± 0.00
2	15.41	cyanidin 3-(sinapoyl)-diglucoside-5-glucoside	979	449/287	0.06 ± 0.00
3	17.41	cyanidin 3-(caffeoyl)(*p*-coumaroyl) diglucoside-5-glucoside	1081	449/287	0.02 ± 0.00
4	18.02	cyanidin 3-(glycopyranosyl-feruloyl) diglucoside-5-glucoside	1111	449	0.10 ± 0.00
5	23.45	cyanidin 3-(*p*-coumaroyl)(sinapoyl) triglucoside-5-glucoside	1287	287	0.03 ± 0.00
6	24.57	cyanidin 3-(feruloyl)(sinapoyl) triglucoside-5-glucoside	1317	1155/287	0.16 ± 0.00
7	27.17	cyanidin 3-(*p*-coumaroyl) diglucoside-5-glucoside	919	757/449/287	0.05 ± 0.00
8	29.12	cyanidin 3-(feruloyl) diglucoside-5-glucoside	949	787/287	0.37 ± 0.00
9	30.89	cyanidin 3-(glycopyranosyl-sinapoyl) diglucoside-5-glucoside	1141	979/287	tr[c]
10	36.07	cyanidin 3-(*p*-coumaroyl)(sinapoyl) diglucoside-5-glucoside	1125	449/287	0.07 ± 0.00
11	38.31	cyanidin 3-(feruloyl)(sinapoyl) diglucoside-5-glucoside	1155	993/449/287	0.47 ± 0.01
12	39.56	cyanidin 3-(sinapoyl)(sinapoyl) diglucoside-5-glucoside	1185	1023/287	0.21 ± 0.01
total					1.75 ± 0.03

[a]no., the elution order of anthocyanins (kohlrabi purple skin) during HPLC analysis. [b]RT, retension time. [c]tr, trace.

Table 3. Carotenoid and Phenylpropanoid Contents in Different Parts of Kohlrabi Cultivars (μg/g Dry Wt.)

		pale green kohlrabi		purple kohlrabi	
groups	compds	skin	flesh	skin	flesh
carotenoids	*β*-carotene	9.55 a[a]	1.34 c	6.22 b	0.52 d
	lutein	6.66 a	0.76 c	5.46 b	0.33 c
	total	16.21 a	2.10 c	11.68 b	0.85 c
phenylpropanoid	4-hydroxybenzoic acid	29623.44 c	47625.06 a	32604.34 b	48933.84 a
	caffeic acid	17.24 c	27.62ab	30.02 a	25.0 b
	p-coumaric acid	14.86 c	22.7 b	51.28 a	25.5 b
	benzoic acid	245.98 b	50.08 c	516.98 a	35.64 c
	trans-cinnamic acid	4.84 b	2.16 bc	0 c	27.42 a
	quercetin	83.20 b	11.52 c	108.76 a	14.48 c
	kaempferol	20.06 a	12.64 b	17.98 a	6.36 c
	total	30009.62 c	47751.78 b	33329.36 b	49068.24 a

[a]Mean values (mean of 3 replicates with three samples from each replicate) indicated by the same letter in a *row* do not differ significantly at the 5% level by Duncan's Multiple Range Test.

glucoraphanin and 4-hydroxyglucobrassicin were isolated. The results for the identity and content of GLS in kohlrabi were confirmed by the previous studies[18,19] except for glucotropaeoline and neoglucobrassicin GLS; these were identified in the skin and flesh of kohlrabi from this study. The trend of glucosinolate content was not exactly similar with previously studies in kohlrabi,[18,19] which is probably because the glucosinolates in this study were isolated from different plant parts (skin and flesh). This is the first time glucosinolates have been isolated from the skin of kohlrabi cultivars.

Identification of Anthocyanins in Kohlrabi. Analysis of the skin and flesh of both kohlrabi cultivars revealed a total of 12 anthocyanins only in the skin of purple kohlrabi (Table 2). No anthocyanins were present in the skin or flesh of pale green kohlrabi or the flesh of purple kohlrabi. The identified anthocyanins from the skin of purple kohlrabi were cyanidin glycoside acylated derivatives with different hydroxycinnamic acids such as *p*-coumaric, caffeic, ferulic, and sinapic acid. Individual anthocyanins were confirmed in accordance with previously published MS/MS data on anthocyanins in red cabbage.[31] Only cyaniding derivatives were found, which is in accordance with previously published data on anthocyanins in *Brassica* species except for kohlrabi.[32−34]

Anthocyanins in Different Parts of Kohlrabi. Among the anthocyanins detected in kohlrabi, although the spectral data identified the presence of cyanidin 3-(glycopyranosyl-sinapoyl) diglucoside-5-glucoside, it was not detected in the HPLC profile because it was only present in very small amounts (Table 2). Among the 12 anthocyanins, the levels of cyanidin 3-(feruloyl)(sinapoyl) diglucoside-5-glucoside were the highest (0.47 mg/g dry weight) followed by cyanidin 3-(feruloyl) diglucoside-5-glucoside (0.37 mg/g dry weight). The levels of cyanidin 3-(sinapoyl)(sinapoyl) diglucoside-5-glucoside (0.21 mg/g dry weight), cyanidin 3-diglucoside-5-glucoside (0.20 mg/g dry weight), and cyanidin 3-(feruloyl)(sinapoyl) triglucoside-5-glucoside (0.16 mg/g dry weight) were similar; their levels were in the middle range among anthocyanins. Only a few studies have reported on the isolation and quantification of anthocyanins in *Brassica* spp., i.e., in a common red variety of curly kale;[31] in red cabbage;[32,33] and in cauliflower.[34] This study is the first report on anthocyanins in kohlrabi.

Carotenoids in Different Parts of Kohlrabi. Analysis of the skin and flesh of both pale green and purple kohlrabi revealed 2 kinds of carotenoids: *β*-carotene and lutein (Table 3). The amounts of carotenoids were much higher in the skins of both types of kohlrabi than in their flesh. Pale green kohlrabi contained more carotenoids in both skin and flesh than purple kohlrabi. In pale green kohlrabi, the amounts of *β*-carotene were 54% and 157% higher in the skin and flesh than those of purple kohlrabi, respectively. Similarly, in pale green kohlrabi, the amounts of lutein were 22% and 130% higher in the skin and flesh than those of purple kohlrabi, respectively. No violaxanthin was present in the flesh of either kohlrabi type. Meanwhile, violaxanthin levels were 12% higher in the skin of

pale green kohlrabi than that of purple kohlrabi. In addition to glucosinolates and their breakdown products, Brassicaceae species are also rich sources of carotenoids. Great diversity and variable levels of carotenoids such as lutein, zeaxanthin, and β-carotene are reported. In addition, α- and γ-tocophierols are prevalent in brassicaceous vegetables.[35−37] Of these, α-tocopherol is generally predominant.[38] In the past, only a few studies were documented in terms of isolation and quantification of carotenoids in *Brassica* spp. especially in broccoli[35,36] and *Brassica oleracea*.[37] We reported here the isolation and quantification of carotenoids from different parts of kohlrabi cultivars.

Phenylpropanoid-Derived Compounds in Different Parts of Kohlrabi. The analysis of the skin and flesh of both pale green and purple kohlrabi revealed the presence of 7 phenylpropanoids-derived compounds: 4-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, benzoic acid, *trans*-cinnamic acid, quercetin, and kaempferol (Table 3). Among the compounds, 4-hydroxybenzoic acid levels were much higher than those of the other compounds. The 4-hydroxybenzoic acid contents in the skin and flesh of both kohlrabi types ranged from 29623.44 to 48933.84 μg/g dry wt. (Table 3). The levels of 4-hydroxybenzoic acid were higher in both the skin and flesh of purple kohlrabi than those of pale green kohlrabi. In purple kohlrabi, the levels of 4-hydroxybenzoic acid were 11% and 10% higher in the skin and flesh than those of pale green kohlrabi, respectively. After 4-hydroxybenzoic acid, the second most prevalent compound was benzoic acid. Benzoic acid levels were 2.1-fold higher in the skin of purple kohlrabi than in that of pale green kohlrabi; in contrast, in the flesh of pale green kohlrabi, benzoic acid levels were 1.4-fold higher than in that of purple kohlrabi. The quercetin contents in the skin and flesh of both kohlrabi types ranged from 11.52 to 108.76 μg/g D.W (Table 3). Both the skin and flesh of purple kohlrabi contained greater amounts of quercetin than those of pale green kohlrabi (Table 4). In both parts, purple kohlrabi contained 1.3-fold more quercetin than pale green kohlrabi. Kaempferol levels in the flesh of pale green kohlrabi were double those in the flesh of purple kohlrabi, although the variation was not substantial between the 2 types of kohlrabi. Caffeic acid levels in the skin of purple kohlrabi were 1.7-fold higher than those in pale green kohlrabi, although the variation was very low in the flesh of both types. The *p*-coumaric acid contents in the skin and flesh of both kohlrabi types ranged from 14.86 to 51.28 μg/g dry wt. (Table 3). Both the skin and flesh of purple kohlrabi contained greater amounts of *p*-coumaric acid than those of pale green kohlrabi (Table 3). The levels of *p*-coumaric acid in the skin of purple kohlrabi were 3.5-fold greater than those in the skin of pale greenkohlrabi, although the variation was very low between the flesh of these 2 types. Among the 7 compounds, *trans*-cinnamic acid levels were relatively low. No *trans*-cinnamic acid was detected in the skin of purple kohlrabi. However, *trans*-cinnamic acid levels were 12.7-fold greater in the flesh of purple kohlrabi than in the flesh of pale green kohlrabi. The phenolic compound composition may differ between cultivars, as well as among parts within the individual plant as shown in several crops like turnip greens and turnip tops,[39] pak choi, [40] and tronchuda cabbage.[41,42] These results are inconsistent with our study results where we found much variation among the cultivars and different parts with in the individual plant.

In summary, this is the first study to profile and quantify glucosinolates, anthocyanins, carotenoids, and other secondary metabolites in pale green and purple kohlrabis. In all, 8 glucosinolates, 12 anthocyanins, 2 carotenoids, and 7 phenylpropanoids were identified using pressurized fluid extraction and an HPLC coupled to a DAD detector and an ESI/Qtrap mass spectrometer. The combination of spectrometric and spectroscopic characteristics registered by these 2 detection system was crucial for the accurate identification of all chromatographic peaks. The present information regarding the identity and contents of glucosinolates, anthocyanins, carotenoids, and phenylpropanoids present in pale green and purple kohlrabis for human consumption should be useful in future databases.

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Notes

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