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## Research Article

# Glucosinolates, Carotenoids, and Vitamins E and K Variation from Selected Kale and Collard Cultivars

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Glucosinolates, carotenoids, and fat-soluble vitamins E and K contents were analyzed from various kale and collard cultivars at mature stage. We found a significant difference in these phytonutrients among cultivars. Among kale cultivars, “Beira” and “Olympic Red” were the highest in the total glucosinolate and “Toscana” kale was the highest in total carotenoid content. “Scarlet” kale was highest in tocopherols. For collard, total glucosinolate was the highest in “Top Bunch” while carotenoids were the highest in “Green Glaze.” An accession PI261597 was the highest in phyloquinone. In addition to the total content of each phytonutrient class, their composition differed among cultivars, indicating that each cultivar may have differential regulatory mechanisms for biosynthesis of these phytonutrients. Our result indicates that cultivar selection may play an important role in consumption of kale and collard with greater nutritional benefit. Therefore, the result of this study will provide a more thorough profile of essential and nonessential phytonutrients of kale and collard cultivars for consumers’ choice and for future research on nutritional value of these crops.

## 1. Introduction

Studies have reported inverse association between consumption of *Brassica* vegetables and the risk of cardiovascular disease and cancers, especially lung, stomach, colon, and rectal cancers due to the abundance of phytonutrients such as glucosinolates, carotenoids, and vitamins in *Brassica* vegetables [1–3]. Collard (*B. oleracea*), classified in the kale “*acephala*” group [4], and kale (*B. oleracea* or *B. napus*) are one of the frequently consumed leafy vegetables all around the world. A study from Centers for Disease Control and Prevention reported collard and kale being ranked at 10th and 15th place of “powerhouse” fruits and vegetables [5].

Glucosinolates are sulfur-containing plant secondary metabolites that are present in *Brassica* crops. The health-promoting effects of glucosinolates are in fact from their hydrolysis products, and anticarcinogenic activities of such hydrolysis products have been reported in cell culture, pre-clinical, and clinical studies [3, 6, 7]. The profile of glucosinolates of kale has been shown to vary depending on crop, cultivar, maturity, or plant tissue [8, 9]. However, the glucosinolate profile in collard has only been reported relatively

recently and there is a large variation in glucosinolate content, partially due to different cultivars investigated [10–12].

Carotenoids play an important role in plants such as photoprotection from excessive light energy and also are phytochemicals that possess potential health benefits. Consumption of carotenoids have been reported to decrease the incidence of cardiovascular disease and cancers and protect the late stage of age-related macular degeneration (AMD) [13]. Besides, certain types of carotenoids including  $\beta$ -carotene commonly are referred to as provitamin A. Green leafy vegetables such as kale and collard are considered an excellent source of dietary carotenoids. Variation of carotenoids of kale has been found to be related to environmental factors during cultivation including different exposure level to sunlight and temperature, growing seasons, and agriculture practice such as conventional or organic farming, postharvest practice, for instance, thermal or nonthermal oxidation, and different cooking methods [14, 15]. Although a few studies have been done for carotenoid variation among cultivars or effect of cultural conditions on carotenoids in kale, there are only few reports on these effects on carotenoids in collard [4].

Vitamin E, a lipid-soluble vitamin, has been reported to be associated with coronary heart disease, cancer, eye

disorders, and cognitive decline [16]. Vitamin E refers to a total of 8 compounds, 4 tocopherols and 4 tocotrienols, and  $\alpha$ -tocopherol is thought to possess the greatest vitamin E activity in many green vegetables [17, 18]. According to the USDA nutrient database [19], the content of vitamin E, analyzed as  $\alpha$ -tocopherol, in kale and collard was 1.54 and 2.26 mg per 100 g, respectively. The content of  $\alpha$ -tocopherol in *Brassica* vegetables such as broccoli, Brussel's sprout, cabbage, cauliflower, and Chinese cabbage was found to differ between cultivars or depending on cooking method [20, 21]. However, to our knowledge, this information has not yet been reported for kale or collard.

Collard and kale are an excellent source of vitamins especially vitamin K. Vitamin K has been reported to be related to blood coagulation, bone health, and cardiovascular disease [22]. The content of vitamin K in 100 grams of raw collard or kale was reported at 407  $\mu$ g and 705  $\mu$ g, respectively [19], which is higher than the adequate intake (AI) for an adult male (120  $\mu$ g) and female (90  $\mu$ g) [23]. Although leafy vegetables are the major source of vitamin K in daily diet, the factors contributing to the content of vitamin K in green vegetables are not yet well understood. A previous report on *Brassica* oil crop canola revealed that vitamin K concentration differed among cultivars or cultivated regions (North America or South America) [24]. This study suggests that the concentration of vitamin K may be cultivar-dependent, and, therefore, more commercial cultivars should be reported to improve the database.

Compared to other *Brassica* vegetables such as broccoli or cabbage, the reported cultivars from kale and collard on these compounds are relatively limited. In addition to cultivar selection, cultivation conditions such as temperature or precipitation also affect phytonutrients [14, 24]. Thus, these factors complicate direct comparison of phytonutrient composition from different studies. Therefore, the goal of this study was to provide a more comprehensive phytochemical comparison from various kale and collard cultivars. For this reason, various kale and collard cultivars were grown in a greenhouse under the same environmental conditions and analyzed for phytonutrient composition.

## 2. Materials and Methods

**2.1. Plant Materials.** Eight cultivars of kale (*B. oleracea* "Starbor," "Beira," "Scarlet," "Premier," "Olympic Red," "Toscano," and "Dwarf Siberian"; *B. napus* "Red Russian") and 4 cultivars of collard (*B. oleracea* "Champion," "Top Bunch," "Flash," and "Green Glaze") were used in this study. Seeds of "Dwarf Siberian" kale and "Green Glaze" collard were purchased from Baker Creek Heirloom Seed Company (Mansfield, MO) and Southern Exposure Seed Exchange (Mineral, VA), respectively. Seeds of the other cultivars were purchased from Johnny's Selected Seeds (Winslow, ME). A collard accession PI261597 was provided by the National Plant Genetic Resource Unit of the USDA-ARS (Cornell University, Geneva, NY). After sowing (November 6, 2015) to 36-cell plug trays filled with Sunshine LC1 professional soil mix (Sun Gro Horticulture, Agawam, MA), seedlings were grown

in a greenhouse at West Virginia University set at 24/18°C (day/night) temperature regime and 14 h/d photoperiod with supplemental high pressure sodium lighting (600W HS200 deep reflector; Hortilux, Pijnacker, The Netherlands) when the light intensity was below 50 W/m<sup>2</sup>. Thirteen days after sowing, seedlings were transplanted to a 15 cm pot and grown for additional 52 days (harvested on January 10, 2016). After transplanting, 20% Hoagland modified nutrient solution #2 (PhytoTechnology Lab., Shawnee Mission, KS) was applied weekly. Two leaves from middle of plant were harvested at commercial maturity (recommended by the seed company) from three plants with each plant as a biological replication, freeze-dried, ground into fine powder, and stored at -20°C until analysis.

**2.2. Glucosinolates Analysis.** Glucosinolates were analyzed following the method of Ku et al. [25] with slight modifications. Freeze-dried kale or collard powder (50 mg) was extracted with 0.5 mL of 70% methanol at 95°C for 10 min. Tubes were cooled on ice, and an internal standard (0.907 mM glucosinalbin, isolated from *Sinapis alba*) was added. Then, tubes were vortexed and centrifuged at 12,000  $\times$ g for 2 min at room temperature. After the supernatants were collected, pellets were reextracted as described above. A mixture of 1 M lead acetate and 1 M barium acetate (1:1, v/v) (0.15 mL) was added to the pooled extract for protein precipitation. After centrifugation at 12,000  $\times$ g for 1 min, 0.2 mL of supernatant was loaded onto a column containing DEAE Sephadex A-25 resin precharged with 1 M NaOH and 1 M pyridine acetate (GE Healthcare, Piscataway, NJ, USA). The *Helix pomatia* type 1 arylsulfatase (Sigma-Aldrich, St. Louis, MO, USA) was added to each column and incubated overnight for desulfation. Desulfoglucosinolates were eluted with 3 mL of deionized distilled water followed by filtration through a 0.2  $\mu$ m nylon filter.

Filtered sample of 2  $\mu$ L was injected into a Nexera-i, LC 2040C ultrahigh performance liquid chromatography (UHPLC) (Shimadzu, Kyoto, Japan) equipped with photo diode array detector. A 100 mm  $\times$  2.1 mm i.d., 1.8  $\mu$ m, 100 Å, Kromasil RP-C18 column was used to analyze glucosinolates (AkzoNobel, Bohus, Sweden). Deionized distilled water (mobile phase A) and 100% acetonitrile (mobile phase B) were used with the following gradient conditions: 0 min 1.5% B, 1.5 min 1.5% B, 4 min 18% B, 10 min 18% B, 10.1 min 100% B, 12 min 100% B, and 12.01 min 1.5% B with a flow rate of 0.3 mL/min. The flow rate was 0.4 mL/min and oven temperature maintained at 40°C. Glucosinolates were detected at 229 nm and quantified using an internal standard.

**2.3. Carotenoids Analysis.** Carotenoids were extracted following the method of Maurer et al. [26] with modifications. Freeze-dried powder (0.1 g) of each sample was mixed with 8.5 mL of acetone : methanol (2 : 1, v/v) containing 0.5% butylated hydroxytoluene (BHT) followed by addition of 3 mL of hexane containing 0.5% BHT in a 20 mL amber vial. Samples were sonicated for 20 min in ice water, and 8 mL of 1 M cold sodium chloride was added. Then, samples were centrifuged at 1,800 rpm for 10 min. Upper hexane layer was filtered

through a 0.2  $\mu\text{m}$  PTFE syringe filter to a HPLC amber vial. Samples were kept at  $-20^{\circ}\text{C}$  until analysis.

Carotenoids were analyzed using UHPLC (Shimadzu, Kyoto, Japan) equipped with photo diode array detector according to the method of Guzman et al. [27] with modifications. Carotenoids were separated using ACQUITY HSS T3 (2.1 mm  $\times$  100 mm, 1.8  $\mu\text{m}$ ; Waters Corp., Milford, MA). The solvent system consisted of 0.05% ammonium acetate (A) and acetonitrile/methanol/chloroform (74 : 19 : 17, v/v/v) (B) with the following gradient condition: 0 min 25% B, 0.7 min 25% B, 1 min 85% B, 4 min 100% B, 11.5 min 100% B, and 11.6 min 25% B. The flow rate was 0.4 mL/min. Injection volume was 2  $\mu\text{L}$  and oven temperature was  $40^{\circ}\text{C}$ . Carotenoids were detected at 450 nm and identified by comparing retention time and spectrum of authentic standards. A standard curve of each compound was used for quantification.

**2.4. Vitamins E and K Analyses.** Vitamins E and K were extracted from 0.3 g of freeze-dried tissue following the method of Xiao et al. [28] with modifications. Each sample was mixed with 5 mL of deionized distilled water and 8 mL of isopropanol : hexane (3 : 2, v/v) in a 20 mL amber vial. Then 40  $\mu\text{L}$  of menaquinone and 60  $\mu\text{L}$  of  $\alpha$ -tocopherol acetate were added as internal standards. Samples were centrifuged at 1,800 rpm for 10 min. Upper hexane layer was collected and the samples were reextracted with 5 mL of hexane as described above. The pooled hexane extract was completely dried under nitrogen stream and reconstituted in 1 mL of hexane. Samples (0.8 mL) were loaded to 1.5 g of prepacked Florisil (6 mL glass tubes with PTFE Frits, Restek Corp., Bellefonte, PA), preconditioned with 5 mL of 30% ethyl ether in hexane followed by 8 mL of hexane, in order to separate vitamins E and K from other nonpolar organic compounds such as chlorophylls and carotenoids. The columns were washed with 5 mL of hexane and then vitamins E and K were eluted with 10 mL of 30% ethyl ether in hexane. The solvent was completely dried under nitrogen stream and the sample was reconstituted in 300  $\mu\text{L}$  of hexane. All samples were filtered through a 0.2  $\mu\text{m}$  PTFE syringe filter to a HPLC amber vial and kept at  $-20^{\circ}\text{C}$  until analysis.

Vitamins E and K were identified using Q Exactive high-resolution quadrupole and Orbitrap LC-MS/MS (Thermo Scientific, Waltham, MA). Vitamins were identified based on fragmentation and their mass and by comparing with an authentic standard.

Vitamin quantification was done using UHPLC (Shimadzu, Kyoto, Japan) following the method of Otles and Cagindi [29] with slight modifications. A reversed-phase C18 column (Ascentis Express Biphenyl, 10 cm  $\times$  2.1 mm, 2.7  $\mu\text{m}$ ; Supelco, Bellefonte, PA) equipped with a precolumn filter was used to analyze vitamins. Deionized distilled water (mobile phase A) and mixture of acetonitrile, dichloromethane, and methanol (60 : 20 : 20, v/v/v) (mobile phase B) were used with the following gradient condition: 0 min 78% B, 3 min 78% B, 3.5 min 100% B, 5 min 100% B, and 5.6 min 78% B. The flow rate was 0.4 mL/min. Each sample was injected at 1  $\mu\text{L}$  and the oven temperature was maintained at  $30^{\circ}\text{C}$ . Vitamins E and K were detected at 292 and 248 nm, respectively. Vitamins were

quantified using an internal standard and relative response factor.

**2.5. Statistical Analysis.** All analyses were done using 3 replications. Univariate analysis of variance (ANOVA) and Tukey's HSD were performed using JMP Pro 12 (SAS Institute, Cary, NC, USA) to determine the variation of phytonutrient content among cultivars of each crop.

### 3. Results and Discussion

**3.1. Glucosinolates.** We detected a total of 12 glucosinolates (6 aliphatic, 1 aromatic, 4 indole, and 1 unknown) with different composition among cultivars. Glucoiberin, progoitrin, glucoraphanin, sinigrin, gluconapin, and glucoerucin were detected as aliphatic glucosinolates as well as an aromatic glucosinolate gluconasturtiin. Indole glucosinolates including glucobrassicin, neoglucobrassicin, 4-hydroxyglucobrassicin, and 4-methoxyglucobrassicin were also found. The total content of aliphatic and indole glucosinolates is presented in Figure 1. In general, indole glucosinolates glucobrassicin and neoglucobrassicin were detected in all cultivars used, and the content of aliphatic glucosinolates greatly differed among cultivars. For instance, glucoiberin content was up to 3.34  $\mu\text{mole/g}$  DW in "Olympic Red" kale but not detected in kale cultivars "Dwarf Siberian," "Premier," "Red Russian," "Scarlet," and "Starbor" and collards "Champion" and "Flash." The total aliphatic glucosinolate content in kale and collard was 0.04–6.55 and 0.98–7.30  $\mu\text{mole/g}$  DW, respectively, whereas the total indole glucosinolate ranged 0.55–5.06 and 2.06–9.91  $\mu\text{mole/g}$  DW in kale and collard. Indole and total glucosinolate contents were significantly higher in collard than in kale while there was no significant difference in total aliphatic glucosinolates between these two crops. Among kale cultivars, "Olympic Red" was the highest in the total aliphatic glucosinolate while the total indole glucosinolate was the highest in "Beira." Collard cultivar "Top Bunch" was the highest in both total aliphatic and indole glucosinolates. Although collard was generally consistent in the aliphatic to indole glucosinolate percentage (%) (0.2–0.7), this percentage (%) varied more greatly among kale cultivars (0.1–12.5) (data not shown). This result suggests that regulation of aliphatic and indole glucosinolates in kale might have more diverse mechanisms than in collard. Additionally, the total glucosinolate varied significantly within a crop, for instance, 0.66–8.03  $\mu\text{mole/g}$  DW in kale. This result indicates that cultivar selection plays an important role in consuming kale with greater health-promoting values that are related to glucosinolates.

Glucosinolates have been of interest in various *Brassica* crops, partially due to their potential health benefits. In fact, health-promoting values are from the hydrolysis products of glucosinolates rather than glucosinolates themselves. For instance, 3-butenyl isothiocyanate, a hydrolysis product of an aliphatic glucosinolate gluconapin, was reported to have antiproliferative activity against human prostate, lung, cervical, liver, and breast cancers, as well as human neuroblastoma and osteosarcoma cell lines [30]. Indole-3-carbinol, a

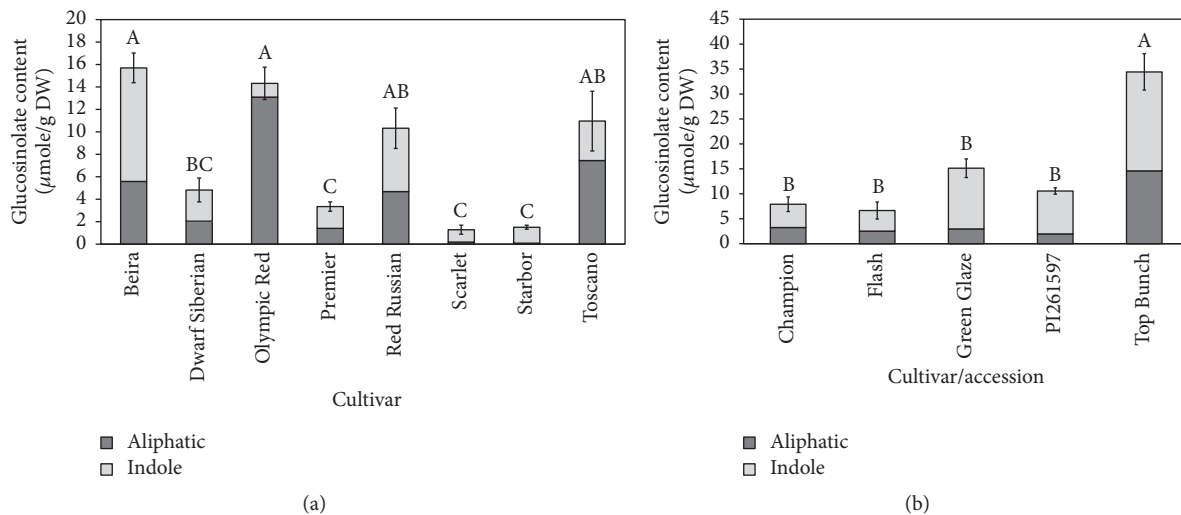


FIGURE 1: Glucosinolate composition of kale (a) and collard (b). Dark grey bars, aliphatic glucosinolates; light grey bars, indole glucosinolates. An unknown compound was included in the quantification of aliphatic glucosinolate. Data are shown as mean  $\pm$  standard error for the total glucosinolate content ( $n = 3$ ). Different letters mean significant difference in the total glucosinolate content among cultivars by Tukey's HSD at  $p \leq 0.05$ .

hydrolysis product of glucobrassicin which was one of the major glucosinolates in the kale and collard cultivars used in this study, has been reported for its beneficial effect against obesity in mice [31, 32]. Although hydrolysis products of glucosinolates, not glucosinolates, are bioactive, a positive and significant correlation was found between glucosinolate content and quinone reductase-inducing activity, an index for anticarcinogenic activity, in horseradish and arugula [25, 33]. In fact, Verhoeven et al. [1] reviewed 7 cohort studies and 87 case-control studies for association between consumption of *Brassica* vegetables and risk of cancers. The authors concluded that a high consumption of *Brassica* vegetables was inversely correlated with lower risk of cancers partially due to glucosinolates. Therefore, increasing glucosinolate content may contribute to greater health-promoting values of glucosinolate-containing foods.

In the present study, we screened glucosinolate composition of various kale and collard cultivars grown under uniform environmental conditions in a greenhouse. Glucosinolate composition in kale and collard has been reported [9, 12], but glucosinolate composition in different collard cultivars is still relatively less reported. Additionally, glucosinolate composition can be affected by various biotic and abiotic factors, complicating comparison of the result of different studies. Therefore, results for crops grown under uniform conditions are very important to better understand glucosinolate composition and how it varies among crops and cultivars. Our results will also assist breeders, growers, and consumers with selecting a crop/cultivar with greater potential health benefits related to glucosinolates and their hydrolysis products.

**3.2. Carotenoids.** We analyzed 3 carotenoids from kale and collard and carotenoid composition differed among cultivars of each crop (Table 1). However, carotenoids were not significantly different between kale and collard except for higher

lutein in kale. Neoxanthin, lutein, and  $\beta$ -carotene content in kale ranged 41.89–177.41, 18.19–712.73, and 68.25–958.93  $\mu\text{g/g}$  DW, respectively, and these carotenoids represented 9.7–23.6, 5.7–43.8, and 36.0–74.9% of the total carotenoid content, respectively (Table 1). Similar to kale, collard leaves also had lutein and  $\beta$ -carotene as the major carotenoids representing 11.8–42.0 and 47.5–74.6% of the total carotenoid, respectively. This is in agreement with the result of De Azevedo and Rodriguez-Amaya [14]. Among investigated cultivars, “Toscano” and “Green Glaze” were found to be the highest in the total carotenoid for kale and collard, respectively. Similar to our result, Kurilich et al. [34] reported variation in  $\beta$ -carotene content among cultivars of *B. oleracea* crops, including broccoli, Brussels sprout, cabbage, cauliflower, and kale. Kopsell et al. [35] reported a significant correlation between carotenoid and chlorophyll contents from 23 *B. oleracea* cultivars. In our study, the kale cultivar “Toscano,” where the leaves are dark green, contained significantly higher level of carotenoids than the other cultivars. Kopsell et al. [35] also reported that “Toscano” kale and “Calvolto Palmizio Nero,” which were the highest in chlorophyll content, contained the highest level of lutein and  $\beta$ -carotene among 23 *B. oleracea* cultivars investigated. Our result of carotenoid content was generally lower than that of Kurilich et al. [34] and Kopsell et al. [35]. This difference is possibly due to the growing condition. Our materials were grown during winter in a greenhouse, where light intensity is lower than other seasons and compared to open field. Moreover, ultraviolet (UV) light can be blocked by greenhouse covering materials.

The percentage (%) composition of each carotenoid, in particular lutein and  $\beta$ -carotene, varied among cultivars. For instance, lutein and  $\beta$ -carotene represented 41.7 and 36.0%, respectively, of the total carotenoid in the kale cultivar “Beira” but the percentage composition of these carotenoids was 5.7 and 74.8% in “Red Russian” kale (Table 1). Similarly,

TABLE 1: Carotenoid composition of freeze-dried kale and collard leaves.

Cultivar/accession	Neoxanthin	Lutein	$\beta$ -Carotene	Total
Kale				
Beira	41.9 $\pm$ 1.2b (22.3) <sup>z</sup>	78.7 $\pm$ 3.6b (41.7)	68.2 $\pm$ 5.5b (36.0)	188.8 $\pm$ 10.1b
Dwarf Siberian	47.3 $\pm$ 1.6b (18.8)	110.8 $\pm$ 10.4b (43.8)	94.2 $\pm$ 4.7b (37.4)	252.3 $\pm$ 10.0b
Olympic Red	51.6 $\pm$ 15.1b (20.3)	45.9 $\pm$ 29.3b (17.8)	159.5 $\pm$ 24.0b (61.9)	256.9 $\pm$ 24.9b
Premier	32.5 $\pm$ 9.6b (18.9)	43.8 $\pm$ 26.3b (21.9)	105.0 $\pm$ 10.1b (59.2)	181.3 $\pm$ 18.0b
Red Russian	65.6 $\pm$ 21.5b (19.5)	18.4 $\pm$ 0.4b (5.7)	240.5 $\pm$ 4.0b (74.8)	324.6 $\pm$ 20.8b
Scarlet	52.4 $\pm$ 15.3b (18.7)	18.2 $\pm$ 0.5b (6.5)	211.3 $\pm$ 19.7b (74.9)	281.8 $\pm$ 11.9b
Starbor	67.7 $\pm$ 1.7b (23.6)	19.7 $\pm$ 0.7b (6.9)	199.2 $\pm$ 8.2b (69.5)	286.6 $\pm$ 9.7b
Toscano	177.4 $\pm$ 23.7a (9.7)	712.7 $\pm$ 132.9a (38.2)	958.9 $\pm$ 138.0a (52.1)	1849.1 $\pm$ 289.9a
Collard				
Champion	46.3 $\pm$ 3.8a (18.3)	105.7 $\pm$ 4.6b (42.0)	100.8 $\pm$ 7.6b (39.8)	252.8 $\pm$ 9.7bc
Flash	47.2 $\pm$ 22.5a (9.2)	115.3 $\pm$ 51.9ab (18.0)	418.4 $\pm$ 112.9a (72.8)	580.9 $\pm$ 142.9ab
Green Glaze	86.4 $\pm$ 18.6 (10.8)	255.6 $\pm$ 24.2a (32.6)	445.2 $\pm$ 41.4a (56.7)	787.1 $\pm$ 83.5a
PI261597	28.4 $\pm$ 7.6a (15.1)	73.0 $\pm$ 29.7b (37.3)	89.7 $\pm$ 16.6b (47.5)	191.0 $\pm$ 6.1c
Top Bunch	40.5 $\pm$ 15.0a (13.6)	43.0 $\pm$ 26.1b (11.8)	246.5 $\pm$ 34.2ab (74.6)	329.9 $\pm$ 37.8bc

<sup>z</sup>Different letters indicate significant difference within a crop by Tukey's HSD at  $p \leq 0.05$ . Numbers in the bracket indicate the percentage (%) of each carotenoid to the total carotenoid content.

the percentage composition of lutein and  $\beta$ -carotene in collard ranged from 11.8 to 42.0 and from 39.8 to 74.6%, respectively, depending on cultivar. However, the percentage of neoxanthin was relatively stable compared to lutein and  $\beta$ -carotene, 9.7–23.6% and 9.2–18.3% in kale and collard, respectively. Carotenoids play an important role in protecting photosystem by dissipating excessive light energy, and biosynthesis of xanthophylls, including lutein, is thought to be regulated by light intensity and chloroplast redox status [36]. Therefore, differential carotenoid composition, especially lutein, among cultivars suggests that these cultivars might have differences in tolerance to light energy and/or mechanisms to control excessive light energy. Although factors influencing carotenoid composition in kale has been reported [14, 15], only few studies have reported variation in carotenoid in collard leaves [4].

Carotenoids have been studied for their potential health benefits. Some carotenoids such as  $\alpha$ - and  $\beta$ -carotene or  $\beta$ -cryptoxanthin possess provitamin A activity [16]. Additionally, carotenoids have demonstrated their positive effects on human health against cardiovascular disease and cancers [37–39]. In the present study, we found that carotenoids significantly differ among cultivars of both kale and collard, indicating that cultivar selection can greatly affect potential health benefits derived from carotenoids of kale and collard.

**3.3. Vitamins E and K.** Vitamin E consists of 4 tocopherols and 4 tocotrienols and  $\alpha$ - and  $\gamma$ -tocopherols are the predominant vitamin E in plant materials [17, 18]. We found the total tocopherol content ranging 44.1–174.9 and 64.8–93.4  $\mu\text{g/g DW}$  for kale and collard, respectively (Figure 2), with  $\alpha$ -tocopherol representing 85.5–97.9 and 93.9–97.7% of the total tocopherol content in kale and collard, respectively. There was no significant difference in individual and total tocopherol content among collard cultivars. In contrast, “Scarlet” was

the highest in  $\alpha$ - and total tocopherol and  $\gamma$ -tocopherol was highest in “Beira.” Although a few studies have reported tocopherol content in various vegetables [17, 34], information on tocopherol content in kale and collard is limited and to our knowledge, analysis of tocopherol in different collard cultivars has not been reported. Kurilich et al. [34] reported 1.03–2.80 and 0.15–0.31 mg/100 g FW of  $\alpha$ - and  $\gamma$ -tocopherols, respectively, in kale, which is in agreement with our data of  $\alpha$ -tocopherol being the predominant form. Similar to Kurilich et al. [34], the USDA nutrient database [19] reported the  $\alpha$ -tocopherol content in kale at 1.54 mg/100 g FW. In contrast,  $\alpha$ -tocopherol content in collards was higher than kale, 2.26 mg/100 g FW [19]. Considering 84% of water content in kale [19], this is equivalent to 96.3  $\mu\text{g/g DW}$  which is in the range of our result. Similarly,  $\alpha$ -tocopherol content in collard reported by USDA [19] is equivalent to 226  $\mu\text{g/g DW}$  (calculated based on 90% of water content reported by USDA [19]). The  $\alpha$ - and  $\gamma$ -tocopherols have shown potential benefits against cardiovascular disease, inflammation, diabetes, prostate cancer, and Alzheimer's disease [40]. Considering that the recommended intake of vitamin E is 15 mg/g for adults, 100 g of fresh kale and collard cultivars used in this study can provide 2.9–11.7 and 6.5–9.3% of RDA, respectively (calculated based on the water content reported by USDA nutrient database [19]). Therefore, cultivar selection, especially for kale, may have a significant impact on tocopherol consumption.

Phylloquinone (vitamin K<sub>1</sub>) is the major form of vitamin K in plants although there are a few different types of vitamin K (menaquinones) depending on the source. Among different forms, phylloquinone is thought to be the predominant form of dietary vitamin K (>90%) [41]. We detected phylloquinone from all kale and collard cultivars investigated, ranging from 37.9 to 71.3 and from 42.7 to 77.5  $\mu\text{g/g DW}$  for kale and collard, respectively (Figure 3). There was no

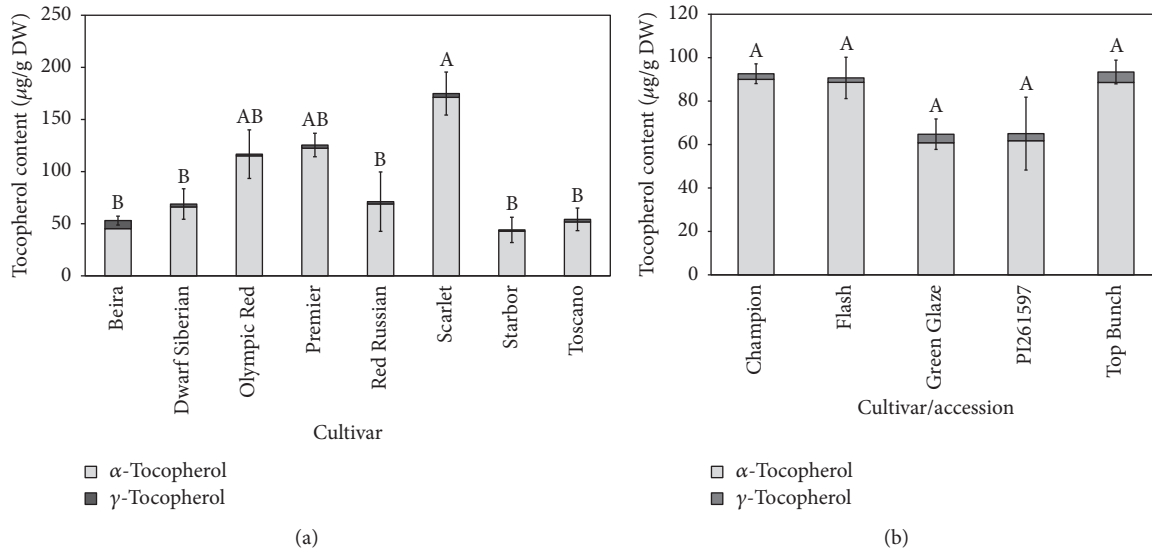


FIGURE 2: Tocopherol (vitamin E) content in kale (a) and collard (b). Light grey bars,  $\alpha$ -tocopherol; dark grey bars,  $\gamma$ -tocopherol. Data are shown as mean  $\pm$  standard error for the total vitamin E content ( $n = 3$ ). Total vitamin E content in collard was not different among cultivars of kale. Different letters for kale mean significant difference in the total vitamin E content among cultivars by Tukey's HSD at  $p \leq 0.05$ .

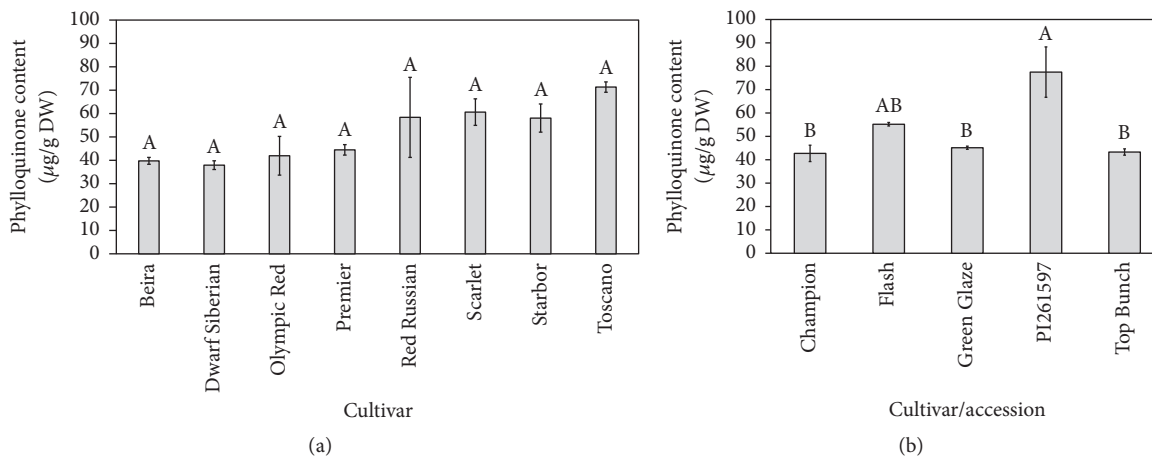


FIGURE 3: Phylloquinone (vitamin K<sub>1</sub>) content in kale (a) and collard (b). Data are shown as mean  $\pm$  standard error ( $n = 3$ ). Phylloquinone content in kale was not different among cultivars of kale. Different letters for collard mean significant difference among cultivars by Tukey's HSD at  $p \leq 0.05$ .

significant difference in phylloquinone content in kale but "Toscano" was numerically highest in phylloquinone. For collard, PI 261597 was the highest in phylloquinone. Information on phylloquinone content in vegetables is limited, and there are only few reports on collard phylloquinone content [4]. Broccoli, a *Brassica* vegetable, was reported to contain 102  $\mu\text{g}/100\text{ g FW}$  [42] or 110  $\mu\text{g}/100\text{ g FW}$  of phylloquinone [43]. The USDA nutrient database [19] reported phylloquinone content in kale and collard at 704.8 and 437.1  $\mu\text{g}/100\text{ g FW}$ , respectively. The difference between our result and USDA's report can be due to different cultivars and growth conditions as well as analytical protocols employed. Farnham et al. [4] reported the phylloquinone content in "Top Bunch," "Flash," and "Champion" collards at 498.6, 379.3, and 387.5  $\mu\text{g}/100\text{ g FW}$  in upper leaves, respectively.

Considering the moisture content of these cultivars reported by the authors, those values are equivalent to 57.34, 51.58, and 59.29  $\mu\text{g}/\text{g DW}$ , respectively, similar to our result. They also reported variation in phylloquinone content in 15 collard genotypes, consistent with our observation. Vitamin K is generally considered being involved in blood coagulation, bone health, and reducing the risk of vascular calcification and cardiovascular disease [22]. In the US, the adequate intake level of vitamin K for adult women and men is 90 and 120  $\mu\text{g}/\text{day}$ , respectively, but the guideline in the UK is slightly lower, at 60 and 75  $\mu\text{g}/\text{day}$ , respectively [44]. The RDA for vitamin K is relatively low compared to other vitamins and, according to our result, 100 g of fresh kale and collard can provide > 300% of RDA for the US adults (calculated based on the water content reported by USDA [19]).

Tocopherols have been analyzed in various foods including leafy vegetables and it has been reported that tocopherols from plant sources can be extracted and analyzed along with carotenoids when using liquid chromatography. In contrast, vitamin K has often been analyzed using fluorescent detector with postcolumn procedures and only a few studies have used a PDA detector for vitamin K analysis. Moreover, chlorophylls usually coelute with vitamin K, making detection and quantification of vitamin K difficult. Using a solid-phase extraction method, we obtained a relatively clean chromatogram from kale and collard samples. To our knowledge, this is the first report on simultaneous extraction of vitamin E and vitamin K for leafy vegetables using PDA detector.

#### 4. Conclusions

In the present study, we analyzed 8 kale and 5 collard cultivars/accession grown under uniform environmental conditions for content of phytonutrients including glucosinolates, carotenoids, and fat-soluble vitamins E (as  $\alpha$ - and  $\gamma$ -tocopherols) and K (phyloquinone). Although glucosinolate and carotenoid composition in kale is relatively well understood, reports on levels in collard are few. We found that phytonutrient composition differed among cultivars of each crop with “Beira” and “Olympic Red” being the highest in total glucosinolates for kales. “Toscano” kale was significantly higher in carotenoids than in the other cultivars. “Scarlet” kale was highest in tocopherols but there was no cultivar difference for phyloquinone in kale. Among collard cultivars, “Top Bunch” was the highest in the total glucosinolate while carotenoids were the highest in “Green Glaze”. An accession PI261597 was the highest in phyloquinone whereas there was no significant difference in tocopherol content among collard cultivars. Our results suggest that phytonutrient content significantly differs among cultivars of kale and collard, agreeing with previous studies. Additionally, cultivar selection may play a critical role in consuming kale and/or collard with greater health benefits. The result of this study will benefit breeders in selecting a cultivar with better nutritional value for a new cultivar development as well as growers and consumers for their cultivar selection.

#### Conflicts of Interest

The authors declare that there are no conflicts of interest.

#### Acknowledgments

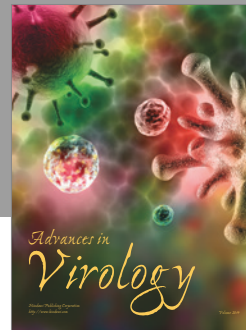
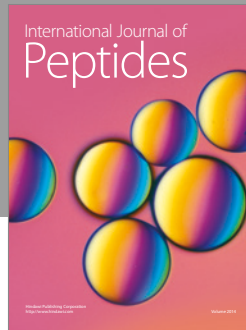
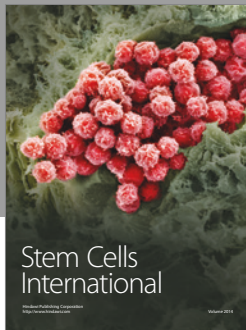
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