

# Mineral Content Differs among Microgreen, Baby Leaf, and Adult Stages in Three Cultivars of Kale

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**Abstract.** Kale (*Brassica oleracea* L. and other species) is considered a rich source of important minerals. Kale at the early stage of leaf development is assumed to contain higher levels of minerals than at maturity. However, literature supporting this assumption is scarce. In this study, the concentrations of macronutrients [potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P)] and micronutrients [sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu)] either essential to plant growth and development, or important to human health, were determined. Three kale cultivars (green leaf ‘Dwarf Blue Curled’ and red leaf ‘Scarlet’ in *B. oleracea*, and green leaf with purple midvein ‘Red Russian’ in *Brassica napus*) were evaluated at five different leaf developmental stages; cotyledon [microgreen 1 (MG1)], two true leaf [microgreen 2 (MG2)], four true leaf [baby leaf 1 (BL1)], six true leaf [baby leaf 2 (BL2)], and adult. As kale matured, total mineral (ash) decreased from 14.6–19.1% at the microgreen stages to 3.9–6.4% at the adult stage, on a dry weight (DW) basis. Microgreen kale contained higher concentrations of most minerals than adult kale, on a DW basis, in all cultivars. On a fresh weight (FW) (as consumed) basis, the highest level of total mineral concentration was detected at baby leaf stage 1 (1.3–1.7%) and there was no difference between microgreen and adult stages. Fresh microgreens generally contained lower K, Ca, Mg, Fe, and Zn than fresh baby leaves, and lower concentrations of Ca and Mg and higher Na compared with fresh adult kale. Overall, water content decreased from 95.1% at MG1 stage to 80.0% at adult stage. The variation in water content and mineral accumulation during leaf development might contribute to the discrepancy. In addition, fresh leaves of ‘Scarlet’ contained higher concentration of total minerals than that of ‘Dwarf Blue Curled’ or ‘Red Russian’. Although ‘Dwarf Blue Curled’ and ‘Red Russian’ are different species, their mineral content profile during leaf development was similar. Together, cultivar and leaf developmental stage influenced mineral content in kale.

Kale is a leaf vegetable and includes cultivars mainly belong to *B. oleracea* and some cultivars of *B. napus*. Traditionally,

kale has been used as a garnish, but is gaining popularity as a primary ingredient due to public perception that kale is one of the healthiest foods (Migliozzi et al., 2015). Kale placed 15th for nutrient content in a study conducted by the Centers for Disease Control ranking 47 “powerhouse” fruits and vegetables (Di Noia, 2014). However, the only dietary mineral included in the assessment of nutrient density was Fe.

Minerals play vital roles in various biological processes during different stages of growth and development in plants. Plants provide up to 17 essential minerals for normal plant growth and development

as well as for human nutrition (Ohkama-Ohtsu and Wasaki, 2010; Singh et al., 2013). These essential minerals for plant growth and development can be classified into two groups depending on the concentration required for plant growth and development: macronutrients and micronutrients. Macronutrients include carbon, hydrogen, oxygen, nitrogen, P, K, sulfur, Ca, and Mg, whereas Mn, Fe, Zn, Cu, molybdenum, boron, nickel, and chlorine belong to the micronutrient group (Singh et al., 2013). In addition, silicon and Na can be included among the macronutrients and micronutrients, respectively (Marschner, 2012; Santos et al., 2014; Taiz et al., 2015).

Potassium is present in abundance in most plant cells/tissues (Bindraban et al., 2015; Britto and Kronzucker, 2008; Maathuis, 2009). The concentration of K tends to be high in young tissues, because of K’s active involvement in photosynthesis, respiration, and water homeostasis (Kopsell et al., 2013; Singh et al., 2013), as well as its relatively high mobility within the plant (Maathuis, 2009). Similar to K, Ca is abundant in tissues (Maathuis, 2009). Calcium plays an important role in plant growth and development, coordinating cell responses to various internal and external stimuli, and to environmental stresses (Kopsell et al., 2013; McAinsh and Pittman, 2009; Maathuis, 2009; White and Broadley, 2003). Calcium tends to be present at low abundance within plant cells (Bindraban et al., 2015; Maathuis and Diatloff, 2013) and concentrations of cytosolic Ca<sup>2+</sup> are submicromolar, likely due to the difficulty in Ca transport to plant tissues (Karley and White, 2009). However, significant amounts of Ca are found in mature and senescing organs (White and Broadley, 2003). Another divalent cation, Mg, is associated with chlorophyll and protein synthesis, and plays an important role as an enzyme cofactor (Maathuis, 2009; McAinsh and Pittman, 2009; Singh et al., 2013).

One of the nutrients important in energy storage is P. Phosphorous is required for the synthesis of nucleic acids, phospholipids, and adenosine triphosphate (Bindraban et al., 2015; Singh et al., 2013). More than 90% of P in the soil cannot be used by plants (Maathuis and Diatloff, 2013). A large amount of P is usually stored in seeds and later used for embryo development, germination, and seedling growth (Marschner, 2012). Among the less required nutrients; Fe, Mn, and Cu are involved in photosynthesis, chlorophyll synthesis, and redox reactions, whereas Zn is associated with the formation of chlorophyll and N metabolism, and plays a role as a metal cofactor in various transcription factors and activates a large number of enzymes in plants (Maathuis and Diatloff, 2013; Singh et al., 2013; Taiz et al., 2015). Another micronutrient, Na, can substitute K in some metabolic functions (Taiz et al., 2015).

Essential nutrients are not only important for plant growth and development, but are also required in the human diet (Santos et al., 2014). Mineral deficiency is a global issue,

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with over 60% of the world's population being Fe deficient and over 30% being Zn deficient (White and Broadly, 2009), whereas Ca, Mg, and Cu deficiencies are prevalent in both developed and developing countries (White and Broadly, 2009). Iron, Zn, Mn, and Cu are important cofactors for endogenous antioxidant vitamins, which protect the human body against diseases due to free radical damage (Matés et al., 1999). Although Mn deficiency is rare in humans, Mn plays an important role as a cofactor for enzymes involved in antioxidant functions, bone development, and neurotransmitter production (Pope et al., 2016). Calcium deficiency results in bone loss, which increases risk of osteoporosis. Adequate P intake is also important for bone health. In human health, high Na and low K consumption increases the risk of hypertension (Binia et al., 2015). Plant foods are low in Na, whereas K is present in abundance in most plant cells (Bindraban et al., 2015; Britto and Kronzucker, 2008; Maathuis, 2009).

Studies evaluating the nutrient composition of kale have been limited to either old varieties (Migliozzi et al., 2015) or the adult stage tissues (Ayaz et al., 2006). Kawashima and Soares (2003) analyzed the mineral profile of eight leafy vegetables popularly consumed in Brazil and reported that kale offered the highest concentrations of K and Ca. Currently, popular kale market classes include curly ('Dwarf Blue Curled' and 'Scarlet'), lacinato, Siberian ('Red Russian'), and ornamental (Migliozzi et al., 2015). These cultivars exhibit differences in leaf pigmentation. 'Dwarf Blue Curled' has green leaves, 'Red Russian' has green leaf blades with purple midveins, and 'Scarlet' has red leaves. In some plant cultivars, pigmentation has been related to mineral content. Previously, we demonstrated that romaine and crisphead lettuces grown under greenhouse conditions showed significantly higher K and P concentrations in red leaf cultivars than green leaf cultivars (Kim et al., 2016).

Besides choice of cultivar, kale is also marketed by stage of maturity (Migliozzi et al., 2015). Kale leaves lose palatability with maturation due to increased toughness, causing microgreen (young seedlings with cotyledons with or without a couple of true leaves) to be preferred due to the tender texture. However, growth stage may also impact nutrient content. Generally, higher nutritional value was reported in younger leaves than mature lettuce leaves (Pinto et al., 2014). A recent comprehensive review of microgreens by Mir et al. (2016) reported that consumption of mustard, cabbage, radish, buckwheat, lettuce, and spinach microgreens has increased due to suggestions of higher concentrations of bioactive phytochemicals important for human health, such as dietary minerals, compared with mature greens. However, to our knowledge, there has been no published comparative study investigating mineral content among microgreen, baby green, and adult kale.

Our hypothesis was that genetic differences (cultivars with differing leaf pigmentation)

and developmental stages (from cotyledon to adult stage) influence the mineral content of kale. Therefore, the objective of this study was to determine whether harvesting kale at different growth stages affected mineral composition in three kale cultivars with differing leaf color.

## Materials and Methods

### Plant materials and growing conditions.

Three cultivars of kale, with green leaf 'Dwarf Blue Curled' ('DBC', *B. oleracea* var. *acephala*), red leaf 'Scarlet' (*B. oleracea* var. *acephala*), and green leaf with purple veins 'Red Russian' ('RR', *B. napus*) were grown to determine whether different developmental stages and pigmentation influenced mineral content. Seeds were purchased from Johnny's Selected Seeds (Winslow, ME). Kale was grown from seed in the soilless media (Sunshine® Mix #1; Sun Gro Horticulture, Agawam, MA). For microgreens, about 2800 seeds per tray ( $54.3 \times 27.9$  cm or 1.8 seedlings/cm<sup>2</sup>) were sown and harvested from the same tray, whereas 48 plants in a 1204 cell pack ( $53.3 \times 27.9$  cm; one plant per cell) were grown for baby leaf kales. Uniform kales with six true leaves from a 1204 cell pack were transplanted to 15.2-cm pots and grown until harvest (one plant per pot) for adult plants. They were grown in the greenhouse (Morgantown, WV) under natural irradiance from 6 June to 8 Sept. 2015 with supplemental lighting. High-pressure sodium lamps (600 W HS200 deep reflector; Hortilux, Pijnacker, the Netherlands) were used for supplemental lighting when natural radiance fell below 50 W·m<sup>-2</sup>. The average photosynthetic photon flux density was 334  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (mean daily light integral = 16.5  $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ). Mean greenhouse temperatures were 24.4/19.7  $\pm$  3.7/2.2 °C day/night (mean  $\pm$  sd) with daytime relative humidity of 72.0%  $\pm$  15.0%. A quarter strength Hoagland modified basal salt mixture (PhytoTechnology Laboratory, Shawnee Mission, KS) was carefully applied daily into the growing media without spilling the solution onto the aerial part of the kale until harvest.

Kale was grown to five defined developmental stages, fully expanded cotyledon (microgreen 1 or MG1), seedlings with two true leaves (microgreen 2 or MG2), seedlings with four true leaves (baby green 1 or BL1), seedlings with six true leaves (baby green 2 or BL2), and mature plants with more than eight true leaves (adult). The term for each developmental stage in our study was used to distinguish different stages of leaf development. 'DBC' and 'RR' were harvested 8, 13, 18, and 37 d after sowing for MG1, MG2, BL1, and BL2 stages, respectively, whereas 'Scarlet' took slightly longer 9, 16, 29, and 44 d to reach MG1, MG2, BL1, and BL2, respectively. For adult stage samples, all kale cultivars were harvested 89 d after seeds were sown. For microgreen and baby leaf stages, all plant tissues aboveground were harvested, whereas only leaves were harvested for adult stages. On harvest, FW was measured. The

harvested samples were immediately frozen and stored at -80 °C until freeze-dried (VirTis Freezemobile 12SL; SP Scientific, Warminster, PA) and ground into a fine powder for mineral analysis.

**Water content, total minerals, and essential minerals.** Water content was determined for each replication by comparing DW to FW at each developmental stage in the three kale cultivars. To analyze mineral composition, freeze-dried kale powder (0.3 g) was placed in a crucible, then ashed in a muffle furnace (Isotemp® Muffle Furnace 550-126; Fisher Scientific, Waltham, MA) at 550 °C for 16 h. The ashed samples were dissolved in 4 mL of 70% nitric acid (HNO<sub>3</sub>) for 4 h, filtered through Fisher-Scientific Grade Q2 filter paper (Fisher Scientific), and adjusted to a total volume of 20 mL with deionized distilled water. Mineral concentrations (K, Ca, Mg, P, Na, Fe, Mn, Zn, and Cu) in samples were determined by inductively coupled plasma spectrometry (Optima 2100DV; Perkin Elmer Corp., Waltham, MA). Standards Control Mate 8 and Multi-Element Solution 2 were prepared in 5% HNO<sub>3</sub> (AccuStandard, Inc., New Haven, CT). Analysis of a standard reference material (SRM) (tomato leaves, NIST 1573a) was performed to confirm the accuracy of the method used for determination of the minerals of interest. The SRM reference standard was prepared and analyzed in a manner identical to kale samples. The difference between the expected values on the certificate and the measured values for SRM for all minerals was less than 5%. Total mineral

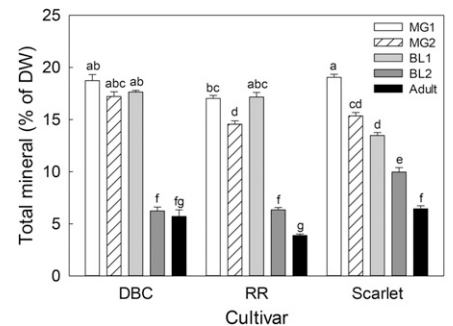


Fig. 1. The total mineral content of three kale cultivars at five different stages of leaf development as a fraction of the dry weight (DW). Microgreen 1 (MG1), kale with two fully expanded cotyledons; microgreen 2 (MG2), kale with two fully expanded true leaves; baby leaf 1 (BL1), kale with four fully expanded true leaves; baby leaf 2 (BL2), kale with six fully expanded true leaves; and Adult, kale with more than eight fully expanded true leaves. DBC, green leaf kale cultivar Dwarf Blue Curled; RR, green leaf with purple vein kale cultivar Red Russian; and Scarlet, red leaf kale cultivar Scarlet. Vertical bars are the standard errors of the means with four replications. *P* values for cultivar (C), developmental stage (S), and their interaction (C  $\times$  S) were all *P*  $\leq$  0.0001. Different letters indicate a significant difference among the cultivar by developmental stage (C  $\times$  S) treatments by Tukey's significance test (*P*  $\leq$  0.05).

was determined as percentage ash on both DW and FW bases for each developmental stage. Essential mineral concentrations in kale were expressed as  $\mu\text{g}\cdot\text{g}^{-1}$  or  $\text{mg}\cdot\text{g}^{-1}$  DW and  $\mu\text{g}/100\text{ g}$  or  $\text{mg}/100\text{ g}$  FW.

**Statistical analysis.** The experimental design was a randomized complete block with four replications. Analysis of variance was performed by SAS using PROC GLM version 9.3 (SAS Institute, Inc., Cary, NC) with cultivar, developmental stage, and their interaction as sources of variance. Kale cultivars were blocked by replication based on plant position in the greenhouse. Differences among the treatment means were assessed by Tukey's significance test at  $P \leq 0.05$ .

## Results and Discussion

Previous studies have investigated mineral concentrations in kale harvested at the adult stage and expressed values on a DW basis (Ayaz et al., 2006; Kopsell et al., 2013; Rosa and Heaney, 1996). The present study investigated mineral concentrations of three different kale cultivars harvested at five different developmental stages on both DW and FW bases. On a DW basis, kale contained

more minerals at early stages of leaf development (MG1 through BL1) than at the later stages (BL2 to adult stage) in all three cultivars (Fig. 1). There was a significant decrease in total mineral content between BL1 (17.6% and 17.2%) and BL2 (6.2% and 6.3%) stages in 'DBC' and 'RR', respectively, whereas a gradual decline in total mineral concentration was observed in 'Scarlet'. Potassium was the most abundant mineral, followed by Ca, Mg, and P, in all three cultivars (Table 1). Phosphorus concentration in adult kale ( $1.7\text{--}2.4\text{ mg}\cdot\text{g}^{-1}$  DW) was lower than reported by Ayaz et al. (2006) for black cabbage (*B. oleraceae* var. *acephala* DC.) grown in the field ( $5.7 \pm 0.9\text{ mg}\cdot\text{g}^{-1}$  DW), possibly due to foliar fertilization of P fertilizer. However, when compared with microgreen kale (MG1 and MG2) in our study, K, Mg, and P, were higher than those in adult kale grown in the field (Ayaz et al., 2006) (Table 1). Adult portuguese kale 'Galega' grown in the field also contained lower concentrations of K ( $17.0\text{--}24.3\text{ mg}\cdot\text{g}^{-1}$  DW), Mg ( $1.4\text{--}3.2\text{ mg}\cdot\text{g}^{-1}$  DW), and P ( $3.6\text{--}5.8\text{ mg}\cdot\text{g}^{-1}$  DW), but a higher concentration of Ca ( $23.8\text{--}39.7\text{ mg}\cdot\text{g}^{-1}$  DW) (Rosa and Heaney, 1996), than the microgreens in our study (Table 1).

Sodium, Fe, Mn, Zn, and Cu concentrations in kale were also measured due to their importance in the plants as well as for human development, nutrition, and health. In the present study, the concentrations of Fe at microgreen ( $48.1\text{--}67.2\text{ }\mu\text{g}\cdot\text{g}^{-1}$  DW) or adult ( $23.0\text{--}26.9\text{ mg}\cdot\text{g}^{-1}$  DW) stage in three cultivars were lower than reported for adult kale ( $72.6 \pm 1.3\text{ }\mu\text{g}\cdot\text{g}^{-1}$  DW) by Ayaz et al. (2006) (Table 2). The Fe concentration of adult kale in our study was lower than that of Portuguese kale grown in the field ( $55\text{--}137\text{ }\mu\text{g}\cdot\text{g}^{-1}$  DW) (Rosa and Heaney, 1996). This discrepancy may be due to differences in cultivars, analytical method, and growing conditions, including various irrigation schedules and light conditions. Manganese and Zn concentrations in adult kale was similar to values reported by Ayaz et al. (2006). However, the concentrations of Mn, Zn, or Cu detected in microgreens in our study were higher than those in adult kale reported by Ayaz et al. (2006) and Rosa and Heaney (1996). Higher concentrations of Na, as well as Fe, Mn, and Zn, were detected in kale microgreens compared with BL2 and adult stages (Table 2). In this study, the concentrations of Cu on a DW basis was generally higher in microgreens

Table 1. The concentrations of macronutrients in three cultivars of kale (*Brassica oleraceae* 'Dwarf Blue Curled', *Brassica napus* 'Red Russian', and *Brassica oleraceae* 'Scarlet'), at five different leaf developmental stages, on a dry weight (DW) basis.<sup>z</sup>

Stage <sup>y</sup>	K			Ca			Mg			P		
	Cultivar <sup>x</sup>			Cultivar			Cultivar			Cultivar		
	DBC	RR	Scarlet	DBC	RR	Scarlet	DBC	RR	Scarlet	DBC	RR	Scarlet
	----- $\text{mg}\cdot\text{g}^{-1}$ DW -----											
MG1	43.0 bc	43.0 bc	47.5 ab	18.5 bc	12.9 def	18.1 bc	11.7 a	9.5 bc	11.0 ab	9.7 a	10.0 a	10.7 a
MG2	39.9 cd	35.4 def	32.7 ef	20.1 ab	13.3 def	18.1 bc	9.5 bc	7.5 def	8.3 cd	6.5 b	6.2 b	5.1 bcd
BL1	36.2 de	49.6 a	29.4 f	22.7 a	14.5 de	18.4 bc	11.4 a	8.1 cde	9.1 cd	6.0 b	5.8 bc	4.3 cde
BL2	12.2 h	14.4 h	21.7 g	11.2 fg	9.9 g	15.5 cd	6.3 efg	6.2 fg	9.0 cd	2.1 fg	2.7 efg	3.5 def
Adult	13.2 h	8.3 h	13.9 h	12.0 efg	9.5 g	12.3 efg	5.5 g	4.5 g	5.8 fg	2.0 fg	1.7 g	2.4 fg
Cultivar (C)	0.2476			$\leq 0.0001$			$\leq 0.0001$			0.8934		
Stage (S)	$\leq 0.0001$			$\leq 0.0001$			$\leq 0.0001$			$\leq 0.0001$		
C × S	$\leq 0.0001$			$\leq 0.0001$			$\leq 0.0001$			0.0002		

K = potassium; Ca = calcium; Mg = magnesium; P = phosphorus.

For a given macronutrient, mean values followed by the same letter are not significantly different by Tukey's significance test ( $P \leq 0.05$ ).

<sup>z</sup>Values are the means of four replications.

<sup>y</sup>Microgreen 1 (MG1), kale with two fully expanded cotyledons; microgreen 2 (MG2), kale with two fully expanded true leaves; baby leaf 1 (BL1), kale with four fully expanded true leaves; BL2, kale with six fully expanded true leaves; and Adult, kale with more than eight fully expanded true leaves.

<sup>x</sup>DBC, green leaf kale cultivar Dwarf Blue Curled; RR, green leaf with purple vein kale cultivar Red Russian; and Scarlet, red leaf kale cultivar Scarlet.

Table 2. The concentrations of micronutrients in three cultivars of kale (*Brassica oleraceae* 'Dwarf Blue Curled', *Brassica napus* 'Red Russian', and *Brassica oleraceae* 'Scarlet'), at five different leaf developmental stages, on a dry weight (DW) basis.<sup>z</sup>

Stage <sup>y</sup>	Na			Fe			Mn			Zn			Cu		
	Cultivar <sup>x</sup>			Cultivar			Cultivar			Cultivar			Cultivar		
	DBC	RR	Scarlet	DBC	RR	Scarlet	DBC	RR	Scarlet	DBC	RR	Scarlet	DBC	RR	Scarlet
	----- $\mu\text{g}\cdot\text{g}^{-1}$ DW -----														
MG1	6.1 bc	8.8 a	3.6 de	55.4 ab	53.0 abc	67.2 a	138 bcd	102 defg	178 a	134 bc	118 cd	197 a	7.0 ab	4.4 ab	8.6 a
MG2	5.0 c	6.6 b	3.3 e	55.6 ab	48.1 bcd	50.3 bc	130 bcde	84.4 gi	163 ab	152 b	94.7 def	139 bc	6.1 ab	8.0 a	5.8 ab
BL1	3.3 e	4.8 cd	1.5 f	62.4 ab	49.0 bc	39.0 cde	159 abc	123 cdef	116 defg	147 b	115 cde	89.1 ef	4.9 ab	6.3 ab	6.0 ab
BL2	0.9 f	1.5 f	1.5 f	23.9 ef	25.7 ef	32.7 def	85.9 fgi	74.0 i	99.4 efg	57.9 g	48.2 gh	84.3 f	5.3 ab	6.5 ab	6.7 ab
Adult	1.0 f	0.6 f	0.7 f	23.9 ef	23.0 f	26.9 ef	73.8 i	48.1 i	74.8 i	31.0 hi	22.3 i	35.8 ghi	3.3 ab	2.0 b	3.1 ab
Cultivar (C)	$\leq 0.0001$			0.0661			$\leq 0.0001$			$\leq 0.0001$			0.5565		
Stage (S)	$\leq 0.0001$			$\leq 0.0001$			$\leq 0.0001$			$\leq 0.0001$			0.0003		
C × S	$\leq 0.0001$			$\leq 0.0001$			$\leq 0.0001$			$\leq 0.0001$			0.2249		

Na = sodium; Fe = iron; Mn = manganese; Zn = zinc; Cu = copper.

For a given micronutrient, mean values followed by the same letter are not significantly different by Tukey's significance test ( $P \leq 0.05$ ).

<sup>z</sup>Values are the means of four replications.

<sup>y</sup>Microgreen 1 (MG1), kale with two fully expanded cotyledons; microgreen 2 (MG2), kale with two fully expanded true leaves; baby leaf 1 (BL1), kale with four fully expanded true leaves; baby leaf 2 (BL2), kale with six fully expanded true leaves; and Adult, kale with more than eight fully expanded true leaves.

<sup>x</sup>DBC, green leaf kale cultivar Dwarf Blue Curled; RR, green leaf with purple vein kale cultivar Red Russian; and Scarlet, red leaf kale cultivar Scarlet.

(4.4–8.6  $\mu\text{g}\cdot\text{g}^{-1}$  DW), but lower in adult kale (2.0–3.3  $\mu\text{g}\cdot\text{g}^{-1}$  DW), compared with values for adult kale (5.1  $\pm$  0.1  $\mu\text{g}\cdot\text{g}^{-1}$  DW) reported by Ayaz et al. (2006). Supported by previously discussed observations, higher nutritional value was generally observed at the early development stages (MG1 through BL1) than at BL2 or adult stage (Fig. 1). This was further supported by the total mineral content of kale, which decreased from 17.0–19.0% at MG1 to 3.9–6.4% at adult stage in all cultivars (Fig. 1).

Although scientific papers typically express plant nutrient concentrations on a DW basis, it is important to include FW mineral concentrations since leafy greens are typically consumed fresh. On a DW basis, a higher concentration of total mineral was detected in younger kale leaves (MG1–BL1) than in BL2 or adult leaves (Fig. 1). Overall, on a FW basis, all three kale cultivars exhibited the highest total mineral content (1.3% to 1.7%) at the BL1 stage, but there were few significant differences in FW total mineral concentrations among the cultivars by developmental stage treatment at the microgreens and adult kale (Fig. 2).

As previously described, a steady decrease in mineral contents was observed in ‘Scarlet’, whereas ‘DBC’ and ‘RR’ kale exhibited large decreases between BL1 and BL2 on a DW basis. This was accompanied by a similar pattern of decreased water content between BL1 and BL2 stages (Fig. 3), suggesting that discrepancies in DW and FW values were likely due to the difference in water content and mineral accumulation at different developmental stages.

The higher concentration of K was detected at BL1 or BL2 and there was no difference between microgreens and adult stages in the three kales (Table 3). Generally, K was more abundant in ‘Scarlet’ than ‘DBC’ or ‘RR’. Calcium, as well as P and Mg, are important for bone health. The concentration of Ca in our study was 36.8% to 69.1% lower than the values reported by Kawashima and Soares (2003), whereas the Mg concentration was 53.5% to 92.1% higher in all three adult kales evaluated. Overall, fresh kale had higher concentrations of Ca and Mg at baby leaf (BL1 and BL2) and adult stages than at the microgreen stages (MG1 and MG2). However, fresh microgreen (MG1) provided more P than fresh adult kale, with the highest concentration of P observed in ‘Scarlet’ microgreen (Table 3), possibly due to the higher demand for P for active growth of young seedlings. Previously, we reported higher Ca, Mg, and P in red compared with green cultivars of lettuce (Kim et al., 2016). In the case of kale, red leaf ‘Scarlet’ generally contained more Ca, Mg, and P than the other green cultivars. This may be due to genetic variation associated with uptake, distribution and/or storage of Ca, Mg, and P in plant tissue. The effect of pigmentation on mineral metabolism merits future investigation.

The concentration of Na in kale was low (12–42.8 mg/100 g FW) and decreased as

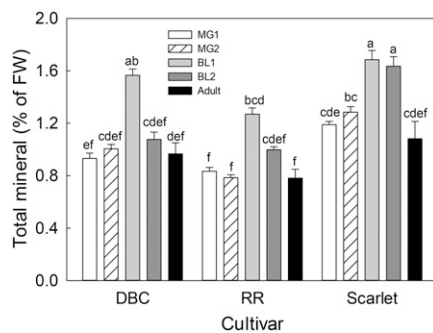


Fig. 2. The total mineral content of three kale cultivars at five different stages of leaf development as a fraction of the fresh weight (FW). Microgreen 1 (MG1), kale with two fully expanded cotyledons; microgreen 2 (MG2), kale with two fully expanded true leaves; baby leaf 1 (BL1), kale with four fully expanded true leaves; baby leaf 2 (BL2), kale with six fully expanded true leaves; and Adult, kale with more than eight fully expanded true leaves. DBC, green leaf kale cultivar Dwarf Blue Curled; RR, green leaf with purple vein kale cultivar Red Russian; and Scarlet, red leaf kale cultivar Scarlet. Vertical bars are standard errors of the means with four replications. *P* values for cultivar (C), developmental stage (S), and their interaction (C × S) were  $P \leq 0.0001$ ,  $P \leq 0.0001$ , and 0.0253, respectively. Different letters indicate a significant difference among the cultivar by developmental stage (C × S) treatments by Tukey’s significance test ( $P \leq 0.05$ ).

kale matured (Table 4). The concentration of Na was generally lower in ‘Scarlet’ and there was no difference at adult stage among the three cultivars. The concentrations of Fe, Mn, Zn, and Cu in adult kale were similar to values reported for adult fresh kale by Kawashima and Soares (2003) (Table 4). The concentration of Mn (0.96–1.27 mg/100 g FW) was higher, whereas the Zn concentration (0.45–0.61 mg/100 g FW) detected in adult fresh leaves was lower than the values reported in the U.S. Department of Agriculture (USDA) nutrition database (2016) of 0.56 and 0.7 mg/100 g FW, respectively. The concentration of Cu in adult ‘Scarlet’ leaves (52  $\mu\text{g}/100$  g FW) was considerably lower than reported by the USDA database (1500  $\mu\text{g}/100$  g FW) (USDA, 2016), but similar to Kawashima and Soares (2003) (40  $\mu\text{g}/100$  g FW). Cultivation condition, cultivar, and analysis method differences could have contributed to these discrepancies. Generally, the concentrations of Fe, Mn, and Zn at BL1 or BL2 were higher than at microgreen stages in ‘DBC’ and ‘RR’ (Table 4). Similar concentrations of Fe, Mn, and Cu were detected in baby leaf and adult leaves, whereas overall Na and Zn concentrations in adult kale were lower than at other stages (Table 4). Adult kale contained a higher concentration of Mn in ‘DBC’ and ‘RR’, and a lower concentration of Na in all three kale cultivars, as compared with the MG1 stage (Table 4). Thus, mineral content of kale was greatly influenced by leaf developmental stage and cultivar difference.

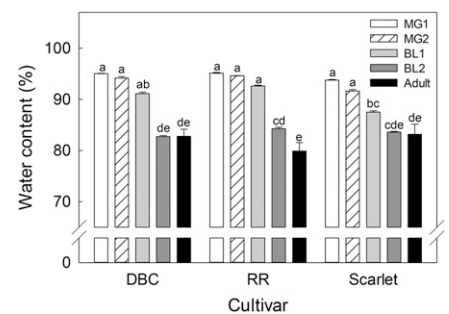


Fig. 3. The water content of three kale cultivars at five different stages of leaf development. Microgreen 1 (MG1), kale with two fully expanded cotyledons; microgreen 2 (MG2), kale with two fully expanded true leaves; baby leaf 1 (BL1), kale with four fully expanded true leaves; baby leaf 2 (BL2), kale with six fully expanded true leaves; and Adult, kale with more than eight fully expanded true leaves. DBC, green leaf kale cultivar Dwarf Blue Curled; RR, green leaf with purple vein kale cultivar Red Russian; and Scarlet, red leaf kale cultivar Scarlet. Vertical bars are standard errors of the means with four replications. *P* values for cultivar (C), developmental stage (S), and their interaction (C × S) were  $P = 0.0168$ ,  $P \leq 0.0001$ , and  $P \leq 0.0006$ , respectively. Different letters indicate a significant difference among the cultivar by developmental stage (C × S) treatments by Tukey’s significance test ( $P \leq 0.05$ ).

In addition to developmental stages, there were cultivar differences in total mineral content. Iron, Mn, and Zn were generally more abundant in fresh baby leaf stages than microgreen stages in ‘DBC’ and ‘RR’ kale. In contrast, micronutrient content in baby leaf ‘Scarlet’ was similar to that in microgreens. Based on 100 g of fresh kale leaf consumption per day, kale can contribute to 5% of the recommended daily allowance (RDA) for Fe and Cu, as well as for P and K, and more than 10% of the recommended daily intake for Zn, Ca, and Mg. Nearly 90% of the RDA for Mn can be met by consuming 100 g of fresh kale. Among the different cultivars in the present study, ‘Scarlet’ kale contributed the most Ca, Mg, Mn, and Zn, up to 24.8%, 45.1%, 88.0%, and 17.4% of RDA, respectively.

## Conclusion

Consumption of baby leaf salads has gained popularity as a culinary trend and consumer demand due to a belief that young plants have higher nutritional value. To address this, we compared different cultivars and developmental stages of kale for their content of minerals of particular health significance, on both DW and FW bases. On a DW basis, dietary mineral concentrations were higher at the early stages of leaf development. In contrast, on a FW basis, baby leaf (BL1) contained more minerals and there was no difference between microgreens and adult kales. Discrepancies in mineral concentrations between DW and FW bases may be due to the differences in DW, total minerals, and

Table 3. The concentrations of macronutrients in three cultivars of kale (*Brassica oleraceae* ‘Dwarf Blue Curled’, *Brassica napus* ‘Red Russian’, and *Brassica oleraceae* ‘Scarlet’), at five different leaf developmental stages, on a fresh weight (FW) basis.<sup>z</sup>

Stage <sup>y</sup>	K			Ca			Mg			P		
	Cultivar <sup>x</sup>			Cultivar			Cultivar			Cultivar		
	DBC	RR	Scarlet	DBC	RR	Scarlet	DBC	RR	Scarlet	DBC	RR	Scarlet
	-----mg/100 g FW-----											
MG1	214 def	210 def	296 bcd	91.8 ghi	63.3 i	117 f	58.1 efg	46.3 fg	68.5 def	48.3 bcd	49.0 bcd	66.5 a
MG2	234 cdef	191 ef	274 cde	118 gf	71.8 hi	152 ef	55.7 efg	40.5 g	69.8 de	38.2 def	33.4 ef	43.1 bcde
BL1	322 bc	367 ab	368 ab	202 bcd	107 gh	230 ab	101 bc	59.9 efg	114 b	53.2 abc	42.7 cde	53.1 abc
BL2	190 ef	226 def	413 a	175 cde	156 e	255 a	97.9 bc	97.7 bc	147 a	33.3 ef	41.8 cdef	57.1 ab
Adult	194 ef	165 f	237 cdef	169 de	189 cde	209 bc	78.3 cde	89.6 cd	98.0 bc	28.6 f	33.7 ef	40.6 cdef
Cultivar (C)	≤0.0001			≤0.0001			≤0.0001			≤0.0001		
Stage (S)	≤0.0001			≤0.0001			≤0.0001			≤0.0001		
C × S	≤0.0001			≤0.0001			≤0.0001			0.0022		

K = potassium; Ca = calcium; Mg = magnesium; P = phosphorus.

For a given macronutrient, mean values followed by the same letter are not significantly different by Tukey’s significance test ( $P \leq 0.05$ ).

<sup>z</sup>Values are the means of four replications.

<sup>y</sup>Microgreen 1 (MG1), kale with two fully expanded cotyledons; microgreen 2 (MG2), kale with two fully expanded true leaves; baby leaf 1 (BL1), kale with four fully expanded true leaves; baby leaf 2 (BL2), kale with six fully expanded true leaves; and Adult, kale with more than eight fully expanded true leaves.

<sup>x</sup>DBC, green leaf kale cultivar Dwarf Blue Curled; RR, green leaf with purple vein kale cultivar Red Russian; and Scarlet, red leaf kale cultivar Scarlet.

Table 4. The concentrations of micronutrients in three cultivars of fresh kale (*Brassica oleraceae* ‘Dwarf Blue Curled’, *Brassica napus* ‘Red Russian’, and *Brassica oleraceae* ‘Scarlet’), at five different leaf developmental stages, on a fresh weight (FW) basis.<sup>z</sup>

Stage <sup>y</sup>	Na			Mn			Zn			Fe			Cu		
	Cultivar <sup>x</sup>			Cultivar			Cultivar			Cultivar			Cultivar		
	DBC	RR	Scarlet	DBC	RR	Scarlet	DBC	RR	Scarlet	DBC	RR	Scarlet	DBC	RR	Scarlet
	-----mg/100 g FW-----												-----µg/100 g FW-----		
MG1	30.2 bc	42.8 a	22.7 cdef	0.69 gh	0.50 h	1.11 bcdef	0.67 efg	0.57 fgh	1.23 ab	276 ef	259 f	419 bcd	34.7 b	21.4 b	53.3 b
MG2	29.5 bc	35.6 ab	27.4 bcd	0.77 fgh	0.46 h	1.37 abc	0.89 d	0.51 gh	1.24 ab	326 def	259 f	421 bcd	36.0 b	43.0 b	48.5 b
BL1	29.2 bc	35.6 ab	18.6 defg	1.41 ab	0.91 efg	1.44 ab	1.30 ab	0.85 de	1.11 bc	554 a	362 cdef	487 ab	43.4 b	46.2 b	74.4 b
BL2	14.8 efg	22.8 cde	25.1 cd	1.34 abc	1.17 bcde	1.63 a	0.90 cd	0.76 def	1.38 a	373 cde	405 bcd	537 a	82.8 a	103 a	110 a
Adult	14.1 efg	12.8 fg	12.0 g	1.04 cdef	0.96 defg	1.27 bcd	0.48 gh	0.45 h	0.61 fgh	338 def	459 abc	456 abc	46.1 b	39.1 b	52.0 b
Cultivar (C)	≤0.0001			≤0.0001			≤0.0001			≤0.0001			0.0768		
Stage (S)	≤0.0001			≤0.0001			≤0.0001			≤0.0001			≤0.0001		
C × S	≤0.0001			0.0010			≤0.0001			≤0.0001			0.9447		

Na = sodium; Mn = manganese; Zn = zinc; Fe = iron; Cu = copper.

For a given micronutrient, mean values followed by the same letter are not significantly different by Tukey’s significance test ( $P \leq 0.05$ ).

<sup>z</sup>Values are the means of four replications.

<sup>y</sup>Microgreen 1 (MG1), kale with two fully expanded cotyledons; microgreen 2 (MG2), kale with two fully expanded true leaves; baby leaf 1 (BL1), kale with four fully expanded true leaves; baby leaf 2 (BL2), kale with six fully expanded true leaves; and Adult, kale with more than eight fully expanded true leaves.

<sup>x</sup>DBC, green leaf kale cultivar Dwarf Blue Curled; RR, green leaf with purple vein kale cultivar Red Russian; and Scarlet, red leaf kale cultivar Scarlet.

water contents. Water content is an important factor to take into consideration since leafy greens are typically consumed fresh. Among kale cultivars, fresh red leaf ‘Scarlet’ contained the lowest Na, but was generally higher in other minerals compared with green leaf kale cultivars (‘DBC’ and ‘RR’) at every developmental stage. Based on our results, red leaf kale ‘Scarlet’ provided the highest concentrations of minerals important to human health. Our results could assist growers in the timing of kale harvest and selection of cultivars to provide increased mineral contents for consumers.

#### Literature Cited

Ayaz, F.A., R.H. Glew, M. Millson, H.S. Huang, L.T. Chuang, C. Sanz, and S. Hayirlioglu-Ayaz. 2006. Nutrient contents of kale (*Brassica oleraceae* L. var. *acephala* DC.). Food Chem. 96:572–579.

Bindraban, P.S., C. Dimkpa, L. Nagarajan, A. Roy, and R. Rabbings. 2015. Revisiting fertilizers and fertilization strategies for improved nutrient uptake by plants. Biol. Fertil. Soils 51:897–911.

Binia, A., J. Jaeger, Y. Hu, A. Singh, and D. Zimmermann. 2015. Daily potassium intake and sodium-to-potassium ratio in the reduction

of blood pressure: A meta-analysis of randomized controlled trials. J. Hypertens. 33 (8):1509–1520.

Britto, D.T. and H.J. Kronzucker. 2008. Cellular mechanisms of potassium transport in plants. Physiol. Plant. 133:637–650.

Di Noia, J. 2014. Defining powerhouse fruits and vegetables: A nutrient density approach. Prev. Chronic Dis. 11:130390.

Karley, A.J. and P.J. White. 2009. Moving cationic minerals to edible tissues: Potassium, magnesium, calcium. Curr. Opin. Plant Biol. 12:291–298.

Kawashima, L.M. and L.M.V. Soares. 2003. Mineral profile of raw and cooked leafy vegetables consumed in southern Brazil. J. Food Compos. Anal. 16:605–611.

Kim, M.J., Y. Moon, D.A. Kopsell, S. Park, J.C. Tou, and N.L. Waterland. 2016. Nutritional value of crisphead ‘Iceberg’ and romaine lettuces (*Lactuca sativa* L.). J. Agr. Sci. 8(11):19–34.

Kopsell, D.E., D.A. Kopsell, C.E. Sams, and T.C. Barickman. 2013. Ratio of calcium to magnesium influences biomass, elemental accumulations, and pigment concentrations in kale. J. Plant Nutr. 36:2154–2165.

Maathuis, F.J.M. 2009. Physiological functions of mineral macronutrients. Curr. Opin. Plant Biol. 12:250–258.

Maathuis, F.J.M. and E. Diatloff. 2013. Roles and functions of plant mineral nutrients, p. 1–21. In: F.J.M. Maathuis (ed.). Methods in molecular

biology. Springer Science Business Media, LLC, New York, NY.

Marschner, H. 2012. Mineral nutrition of higher plants. 3rd ed. Academic, London, UK.

Matés, J.M., C. Pérez-Gómez, and I. Núñez, de Castro. 1999. Antioxidant enzymes and human diseases. Clin. Biochem. 32(8):595–603.

McAinsh, M.R. and J.K. Pittman. 2009. Shaping the calcium signature. New Phytol. 161:275–294.

Migliozzi, M., D. Thavarajah, P. Thavarajah, and P. Smith. 2015. Lentil and kale: Complementary nutrient-rich whole food sources to combat micronutrient and calorie malnutrition. Nutrients 7(11):9285–9298.

Mir, S.A., M.A. Shah, and M.M. Mir. 2016. Microgreens: Production, shelf life and bioactive components. Crit. Rev. Food Sci. Nutr. 2016 Feb. 8:0 [Epub ahead of print].

Ohkama-Ohtsu, N. and J. Wasaki. 2010. Recent progress in plant nutrition research: Cross-talk between nutrients, plant physiology and soil microorganisms. Plant Cell Physiol. 51(8):1255–1264.

Pinto, E., A.A. Almeida, A.A. Aguiar, and I.M. Ferreira. 2014. Changes in macrominerals, trace elements and pigments content during lettuce (*Lactuca sativa* L.) growth: Influence of soil composition. Food Chem. 152:603–611.

Pope, J., S. Nizielski, and A. McCook. 2016. Ch. 14. Trace minerals, p. 310–330. In: Nutrition for a changing world. McMillan, New York, NY.

- Rosa, E. and R. Heaney. 1996. Seasonal variation in protein, mineral and glucosinolate composition of Portuguese cabbages and kale. *Anim. Feed Sci. Technol.* 57:111–127.
- Santos, J., M.T. Oliva-Teles, C. Delerue-Matos, and M.B. Liverira. 2014. Muto-elemental analysis of ready-to-eat “baby leaf” vegetables using microwave digestion and high-resolution continuum source atomic absorption spectrometry. *Food Chem.* 151:311–316.
- Singh, U.M., P. Sareen, R.S. Sengar, and A. Kumar. 2013. Plant ionomics: A newer approach to study mineral transport and its regulation. *Acta Physiol. Plant.* 35:2641–2653.
- Taiz, L., E. Zeiger, I.M. Möller, and A. Murphy. 2015. Mineral nutrition, p. 119–142. In: A.D. Sinauer (ed.). *Plant physiology and development*. Sinauer Associates, Inc., Sunderland, MA.
- USDA. 2016. National Nutrient Database for Standard Reference Release 28. USDA, Washington, DC.
- White, P.J. and M.R. Broadley. 2003. Calcium in plants. *Ann. Bot. (Lond.)* 92:487–511.
- White, P.J. and R. Broadly. 2009. Biofortification of crops with seven mineral elements often lacking in human diets-iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Physiol.* 182:49–84.