ARTICLE TYPE: Original research article

TITLE: Minerals, vitamin C, phenolics, flavonoids and antioxidant activity of *Amaranthus* leafy vegetables

AUTHOR NAMES AND AFFILIATIONS: Dulce M. Jiménez-Aguilar, Michael A. Grusak*

USDA-ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, 1100 Bates Avenue, Houston, TX 77030-2600, USA

***Corresponding author.** Tel.: 713-798-7044. Fax: 713-798-7078. E-mail: mike.grusak@ars.usda.gov

Acknowledgments

This research has been supported by CONACYT Postdoctoral Fellowship Grant No 234764 to DMJA and by the USDA Agricultural Research Service through Cooperative Agreement No. 58-6250-0-008 to MAG. We are grateful to Ashley Hudson, David Dworak and Chee-Ming Li for providing excellent technical support. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products or organizations imply endorsement by the U.S. government.

Abstract

The objective of this study was to evaluate mineral, vitamin C, phenolic and flavonoid concentrations and antioxidant activity levels in 15 leafy *Amaranthus* species. Across species, the concentration ranges of Ca, K, Mg, P and phenolics, and activity ranges of antioxidants in amaranth leaves were 1.5-3.5mg/g, 5.5-8.8 mg/g, 1.8-4.5 mg/g, 0.5-0.9 mg/g, 3.2-5.5 mg gallic acid equivalents/g, and 38-90 μ mol Trolox equivalents/g (all values on a fresh weight [FW] basis), respectively. *Amaranthus acanthochiton* had the highest concentrations of Ca, Mg, Ni, Zn, and *A. deflexus* and *A. viridis* had the highest concentrations of Fe. A serving of any of the *Amaranthus* leaves (1 cup; 30 g FW) would contribute from 13 to 34% of the daily value (DV) of Mg (DV = 400 mg; as established by the US Food and Drug Administration), and up to 68% of the DV of vitamin C (DV = 60 mg). In addition, *A. acanthochiton* would be considered a good source of Ca, Mn and Mo (10%-19% of the DV), and an excellent source of Mg and vitamin C (20% or more of the DV). Amaranth leafy vegetables should be promoted as a dietary source of essential nutrients and health-beneficial compounds.

Keywords

Amaranthus leaves; Leafy vegetables; Minerals; Vitamin C; Phenolics; Flavonoids; Antioxidant activity; Daily value.

Abbreviations

L-ascorbic acid (AA), 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH), catechin equivalents (CE), daily value (DV), US Food and Drug Administration (U.S. FDA), fresh weight (FW), gallic acid equivalents (GAE), inductively coupled plasma optical emission spectroscopy (ICP-OES), trichloroacetic acid (TCA), trolox equivalents (TE).

1. Introduction

Worldwide there are about 795 million undernourished people who suffer chronic calorie deficiency due to issues related to food insecurity (FAO et al., 2015). Additionally, there are more than two billion people suffering hidden hunger, which is defined as a chronic deficiency of vitamins or minerals (Muthayya et al., 2013; von Grebmer et al., 2014). It is worrying that more than double the 795 million undernourished people suffer from hidden hunger. Most of the countries with alarming global hunger index scores are in

Africa, south of the Sahara (von Grebmer et al., 2014). Staple crops (cereals and root crops) are a source of energy, but they are deficient in micronutrients, mainly provitamin A carotenoids, vitamin C and E, and iron, zinc and iodine (Afari-Sefa et al., 2011). Therefore, a diet based mostly on staple crops frequently results in hidden hunger (von Grebmer et al., 2014). Fruit and vegetables are sources of vitamins and minerals and their consumption, in conjunction with staple food crops, can provide a balanced and healthy diet. Additionally, the consumption of fruit and vegetables is associated with protective roles in human health and with a reduction in the risk of cancer, cardiovascular, and other chronic diseases. Phytochemical compounds such as vitamin C, phenolics and flavonoids are thought to contribute to those health benefits (Grosso et al., 2013; Isabelle et al. 2010; Randhawa et al., 2015).

Amaranthus spp. are a group of versatile food crops that consist of 60 species, of which three species are grown for edible grains and 17 species are grown for edible leaves (Achigan-Dako et al., 2014). *Amaranthus* leaves are used as food in many countries from Africa, where they are consumed as infusions, salads, soups, sauces, or mixed with other vegetables or legumes (Achigan-Dako et al., 2014). *Amaranthus* leaves are considered the most commonly harvested and consumed African leafy vegetable throughout the continent (Odhav et al., 2007; van Jaarsveld et al., 2014). They are considered an inexpensive and rich source of minerals and vitamin C (Achigan-Dako et al., 2014; Alegbejo, 2013; Barminas et al., 1998; Odhav et al., 2007; Orech et al. 2007; Singh et al., 2001). In addition, both phenolic and flavonoid compounds and antioxidant activity have been reported in a few species of amaranth (Li et al., 2015).

According to the 2015 Dietary Guidelines for Americans, it is recommended that five servings of vegetables be consumed per day, based on a 2000 calorie intake. Additionally, one of the five subgroups of vegetables recommended is the group of green leafy vegetables (HHS & USDA, 2015). Leafy vegetables constitute a major part of any balanced diet and are sources of minerals and antioxidants (Isabelle et al., 2010; Jiménez-Aguilar & Grusak, 2015; Khanam & Oba, 2013; Olsen et al., 2012; Schönfeldt & Pretorius, 2011). The consumption of amaranth leafy vegetables could contribute important nutrients to populations at risk for hidden hunger. Hence, the goal of this research was to evaluate the

mineral, vitamin C, phenolic and flavonoid concentrations and the antioxidant activity in leaves of diverse *Amaranthus* species. These values were used to calculate the mineral and vitamin C daily value contributions per serving and to make recommendations for how these *Amaranthus* species could contribute to human nutritional needs.

2. Material and methods

2.1. Reagents

Trichloroacetic acid, N-ethylmaleimide, α - α -bipyridyl, L-ascorbic acid, catechin, gallic acid, Folin–Ciocalteu phenol reagent (2N), fluorescein, trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), and 2,2'-Azobis(2-methylpropionamidine) dihydrochloride were obtained from Sigma–Aldrich Corp. (St. Louis, MO, USA). 99% DL-dithiothreitol was obtained from Bio-Rad Laboratories (Richmond, CA, USA). Certified tomato leaf reference material (SRM 1573a) was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA) and certified mineral standards were obtained from Inorganic Ventures (Christiansburg, VA, USA).

2.2. Plant material

Amaranthus seeds were obtained from the USDA-ARS National Plant Germplasm System with exception of *A. thunbergii* seeds, which were obtained from AVRDC, The World Vegetable Center (see **Table 1** for accession numbers). Seeds were imbibed in deionized water in seed germination pouches (Cyg pouches, Mega International, Minneapolis, MN, USA) for a minimum of three days at 32 °C (dark conditions). Germinated seeds were planted and grown in pots filled with a 2:1 (v:v) mixture of synthetic soil (Metro-Mix 366, Sun Gro Horticulture Co., Seba Beach, Alberta, Canada) and vermiculite (Premium grade Vermiculite, Sun Gro Horticulture Co., Bellevue, WA, USA). Plants of each accession were grown in quadruplicate in an environmental growth chamber (Conviron Model PGW36; Winnipeg, Manitoba, Canada) using a 15 hr, 25 °C / 9 hr, 15 °C day/night regime, 50% relative humidity, and photon flux density during the day of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation, supplied by a mixture of halogen bulbs and fluorescent lamps.

Leaves were harvested at 5 weeks after planting (7-12 g of fresh leaves were collected per pot). For the mineral analysis, the leaves were dried in an oven at 70 °C; and for vitamin C, phenolic, flavonoid and antioxidant activity analyses, the leaves were frozen at -80 °C and lyophilized at -55 °C. Percent moisture of the fresh leaves was determined by weight loss after drying at 70 °C for 3 days. All samples were ground before analysis.

2.3. Minerals

Minerals were evaluated according to the method described by Jiménez-Aguilar & Grusak (2015). Samples (250 mg) were digested with concentrated HNO₃ over night (room temperature) and then for 2.5 h at 125 °C, followed with 30% H₂O₂ for 2 h at 125 °C. The temperature was then increased to 200 °C, and the samples were heated until they were completely dry. After cooling, the samples were re-suspended in 15 mL 2% HNO₃. Concentrations were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Ciros ICP- FCE12, Kleve, Germany) using the following wavelengths (nm): Ca (219.77), K (404.721), Mg (294.20), P (253.565), S (182.034), B (249.773), Cu (327.39), Fe (262.82), Mn (257.6), Mo (202.03), Na (589.592), Ni (231.60), and (Zn 206.19). A tomato leaf standard (SRM 1573a; National Institute of Standards and Technology) was used daily as a reference in the digestion and quantification of minerals. Additionally, the ICP-OES was calibrated daily with certified mineral standards. Results are expressed in mg or µg per gram of sample fresh weight (FW).

2.4. Vitamin C

Vitamin C was extracted and quantified according to the method described by Gillespie & Ainsworth (2007) and adapted by Jiménez-Aguilar & Grusak (2015). Vitamin C extraction was performed in 40 mg of sample with 6% TCA followed by centrifugation (13,000g and 4 °C) for 5 min. The supernatant (50 µL) was recovered and mixed with 75 mM potassium phosphate buffer (25 µL). Total vitamin C was evaluated by mixing 75 µL of buffered sample and 25 µL 10 mM DL-dithiothreitol. After 10 min, 25 µL of 0.5% N-ethylmaleimide was added. Reduced vitamin C (ascorbic acid) was quantified by mixing 75 µL of buffered sample and 50 µL of water. For all analyses (total and reduced vitamin C), 375 µL of a mixture with 3% FeCl (50 µL), 4% α-α' bipyridyl (100 µL), 10% TCA (125 µL) and 43% H₃PO₄ (100

μL) were added. The samples were incubated for 1 h at 37 °C, and then 200 μL were transferred to a 96 well flat bottom microplate. The absorbance was measured at 525 nm in a microplate reader (Synergy HT, Biotek Instrument, Inc., Winooski, VT, USA). Results are expressed in mg L-ascorbic acid (AA) per gram of sample FW.

2.5. Phenolics

Phenolic compounds were extracted according to Agbor et al. (2010) and modified by Jiménez-Aguilar & Grusak (2015). Extraction of total phenolics was carried out using 25 mg of sample in 2.5 mL of 1.2 M HCl in 90% methanol in a water bath (90 °C) for 2 h. The samples were centrifuged at 7500 rpm for 20 min after the volume was readjusted (2.5 mL). Extraction of free phenolics was performed in 25 mg of sample with 2.5 mL of 90% methanol and then following the same procedure as with total phenolics, the supernatants were recovered.

Total or free phenolic extracts (100 μL) were mixed with 2 N Folin-Ciocalteu reagent (50 μL). After 5 min, 2 N Na₂CO₃ (400 μL) and water (1 mL) were added. Samples were incubated at 37 °C for 90 min and were then transferred to a 96 well flat bottom microplate. The absorbance was measured at 740 nm in a microplate reader. Results are expressed in mg of gallic acid equivalents (GAE) per gram of sample FW. Note that results could be slightly overestimated because these samples contained ascorbic acid (Prior et al., 2005).

2.6. Flavonoids

Total flavonoids were extracted and quantified according to Lamien-Meda et al. (2010), with some adjustments as described by Jiménez-Aguilar & Grusak (2015). Samples (100 mg) were mixed with 5 mL of 50% methanol in water and treated by ultrasound for 1 h. They were then centrifuged for 10 min at 13,000 g (4 °C), and the supernatants were recovered. Flavonoid extracts (400 μL) were homogenized with water (500 μL), 5% NaNO₂ (60 μL), 10% AlCl₃ (140 μL). After 10 min, 1 mM NaOH (400 μL) was added. Samples were incubated for 10 min at room temperature and were then transferred to a 96 well flat bottom microplate. The absorbance was measured at 500 nm in a microplate reader. Results are expressed in mg of catechin equivalents (CE) per gram of sample FW.

2.7. Antioxidant activity

Extracts used for this measurement were prepared with the same process as total flavonoid quantification (as described above), and the antioxidant activity was evaluated according to Huang et al. (2002). Diluted extracts (25 μ L) were transferred to a 96 well black round bottom microplate and 150 μ L of 1 μ M fluorescein were added to each well. The microplate was incubated at 37 °C for 30 min (dark) and then 25 μ L of AAPH were added to each well. Fluorescence readings were carried out at 485 nm (excitation) and 520 nm (emission) every 2 min during 90 min. Results are expressed in μ mol trolox equivalents (TE) per g of sample FW.

2.8. Quality control of analytical techniques

Linearity, linear range, limit of detection and accuracy of measurements made for minerals, vitamin C, phenolics, flavonoids and antioxidant activity are shown in Appendix 1 (**Table A. 1**).

2.9. Statistical analysis

Mineral analysis was evaluated in four independent samples per accession (each sample was measured in duplicate), antioxidant activity in three independent samples per accession (each sample was measured in duplicate), and vitamin C, phenolic and flavonoid analyses in three independent samples per accession. Results were expressed as mean value $\pm$ standard deviation per species. Every mean represents the average of all measurements for all accessions of the same species (**Table 1, 2 and 3**). Results per accession were provided in **Appendix 1 (Tables A. 2, A. 3 and A. 4)**. ANOVA and Tukey tests were performed using JMP (13.0.1, SAS Institute Inc, Cary, NC, USA) with a significance level of $\alpha = 0.05$.

3. Results

3.1. Minerals

Mineral concentrations of amaranth leaves are presented in **Tables 1 and 2**. *Amaranthus acanthochiton* had the highest concentrations of Ca, Mg, Ni and Zn, and *A. viridis* had the highest concentrations of P, S, B, Cu and Fe. *A. blitum*, *A. dubius*, *A. palmeri* and *A. retroflexus* were found to have similar concentration profiles across the measured macroelements. For each individual microelement (except Na), *A. caudatus*, *A. spinosus*, *A. tricolor* and *A. thunbergii* had similar concentrations. In comparison to other leafy

vegetables, *Amaranthus* species have higher macroelement concentrations (Ca, K and Mg) than spinach (*Spinacia oleracea*), kale (*Brassica oleracea*) and black nightshade (*Solanum scabrum*) (**Table 4**), and several *Amaranthus* species had similar microelement concentrations (Fe, Mn, Mo and Zn) to spider flower (*Gynandropsis gynandra*) (**Table 4**).

3.2. Vitamin C

Vitamin C can be found reduced (ascorbic acid) or oxidized (dehydroascorbic acid); the sum of these is total vitamin C. The predominant form of vitamin C in *Amaranthus* leaves was the reduced form, which was found at levels between 55 and 99% of the total vitamin C (**Table 3**). These results are consistent with data published for other leafy vegetables by Jiménez-Aguilar & Grusak (2015) and Noctor (2006), who reported higher concentrations of the reduced form than the oxidized form in healthy leaves. *Amaranthus blitum*, *A. dubius*, *A. tricolor*, *A. viridis* and *A. thunbergii* had the highest total vitamin C concentrations (1.3-1.4 mg AA/g FW), being over twice the value of *A. spinosus* (0.63 mg AA/g FW) (**Table 3**). All *Amaranthus* species had higher concentrations of total vitamin C than spinach and kale (**Table 4**).

3.3. Phenolics

Phenolic compounds can be found free or bound, with their sum representing total phenolics. In *Amaranthus* leafy vegetables, phenolics were found mainly in the free form, at levels from 51 to 73% of the total phenolic compounds (**Table 3**). *A. acanthochiton* and *A. blitum* were the species with the highest concentrations of total phenolics (5.5 and 4.9 mg GAE/g FW, respectively). These concentrations were higher than spinach (1.1-1.2 mg GAE/g FW) (**Table 4**) (Isabelle et al., 2010; Mavhungu, 2011). *Amaranthus* species (with the exception of *A. acanthochiton* and *A. blitum*) have statistically similar concentrations of total phenolics ($p<0.05$) (3.4-4.5 mg GAE/g FW), and these values were similar to spider flower (**Table 4**).

3.4. Flavonoids

Total flavonoid concentrations in *Amaranthus* leaves ranged from 0.98 to 4.1 mg CE/g (**Table 3**). *A. acanthochiton* was the species with the highest flavonoid concentration, being roughly twice the values

found in *A. deflexus*, *A. graecizans* and *A. dubius*. In comparison to other leafy vegetables, *A. retroflexus* and *A. tricolor* had similar flavonoid concentrations (**Table 3**) to black nightshade (**Table 4**). Flavonoid concentrations across the leafy *Amaranthus* species were all higher than reported for spinach (0.70 mg CE/g FW) and spider flower (0.51-0.82 mg CE/g FW) (Jiménez-Aguilar & Grusak, 2015; Mavhungu, 2011) (**Table 4**).

3.5. Antioxidant activity

Amaranth leaves had antioxidant activity levels between 38 and 90 $\mu\text{mol TE/g FW}$ (**Table 3**). The *A. hybr.* species had the highest level of antioxidant activity, and the *A. viridis* species were found to have the lowest levels of antioxidants. On the other hand, *A. acanthochiton*, *A. blitum*, *A. caudatus* or *A. hybridus* had around 40% more antioxidant activity than *A. deflexus*, *A. dubius*, *A. palmeri* or *A. retroflexus*. All *Amaranthus* species had higher antioxidant activity than spider flower, black nightshade and spinach (**Table 3** and **4**).

4. Discussion

4.1. Minerals

In Africa, the consumption of indigenous leafy vegetables such as *Amaranthus* species is highly dependent on factors such as poverty status or degree of urbanization. Poor families tend to consume these types of leafy vegetables more than wealthy families (Jansen van Rensburg et al., 2007). Actually, amaranth is considered as the poor man's vegetable because it is one of the less expensive leafy vegetables in markets (Achigan-Dako et al., 2014). It has been reported that the consumption of indigenous leafy vegetables has decreased over the years, possibly because they are perceived to be inferior in taste and lower in nutritional value than more conventional leafy vegetables (Uusiku et al., 2010). However, several *Amaranthus* species have higher mineral concentrations than commonly consumed leafy vegetables, such as spinach and kale (**Table 4**).

The U.S. Department of Agriculture's National Nutrient Database for Standard Reference (USDA, 2015) lists a serving size of spinach as 30 g FW (1 cup). Accordingly, in this study, a serving size of *Amaranthus* leaves was considered with the same weight. Furthermore, the US Food and Drug Administration (U.S.

FDA, 2013) has established daily value (DV) recommendations for key nutrients, relative to a 2000 calorie diet (see **Tables 1** and **2**). Based on these guidelines, DV percentages were calculated for a serving size of amaranth leaves. A serving of *A. acantochitan*, which was the species with the highest concentration of several minerals, contains 11, 6, 34, 2, 2, 3, 12, 13 and 3% of the DV for Ca, K, Mg, P, Cu, Fe, Mn, Mo and Zn, respectively. *A. caudatus*, *A. cruentus*, *A. dubius*, *A. hybr.*, *A. hybridus*, *A. retroflexus* and *A. tricolor* are considered good sources of magnesium because they contribute between 10% and 19% of the Mg DV per serving. The rest of the species are considered excellent sources of Mg because they contain 20% or more of the DV per serving (U.S. FDA, 2013). A serving of all *Amaranthus* species, with the exception of *A. deflexus*, would be considered a good source of manganese. *A. acantochitan*, *A. blitum*, *A. graecizans*, *A. palmeri*, *A. spinosus*, *A. tricolor* and *A. viridis* are good sources of molybdenum. In addition, *Amaranthus* leaves contribute from 2% to 3% of the DV of P and Zn, and from 1 to 4 % of Fe per serving.

4.2. Vitamin C

Vitamin C is essential for the biosynthesis of collagen and carnitine, and is an antioxidant and anticarcinogenic compound. A lack of sufficient vitamin C causes scurvy, a condition characterized by bleeding, arthralgias, and impaired wound healing (Grosso et al., 2013). According to the U.S. Food and Drug Administration (U.S. FDA, 2013), the DV for vitamin C is 60 mg. A serving size of *Amaranthus* leaves has from 21 to 41 mg AA, which contributes between 32 and 68% of the DV. A serving size of *A. blitum*, *A. dubius*, *A. palmeri*, *A. tricolor*, *A. viridis* or *A. thunbergii* provides from 63 to 68% of the DV for vitamin C. In addition, a serving size of *A. hybr* or *A. spinosus* contributes between 32 and 35% of the DV for vitamin C. Thus, a serving of any species of *Amaranthus* is considered an excellent source of vitamin C (U.S. FDA, 2013).

4.3. Phenolics, flavonoids and antioxidant activity

Vitamin C, phenolics and flavonoids are phytochemical compounds responsible for much of the antioxidant activity in fruit and vegetables. Phenolics and flavonoids reduce the risk of cardiovascular, chronic and neurodegenerative diseases and certain types of cancer (Das et al., 2012; Randhawa et al.,

2015; Velderrain-Rodriguez et al., 2014). Antioxidant activity is associated with several health benefits, and has been reported to provide anticarcinogenic, anti-inflammatory, hypoglycemic and antiatherogenic effects (Das et al., 2012; Grosso et al., 2013; Gülçin 2012). Although, the consumption of phenolics, flavonoids and antioxidant compounds is highly recommended, there currently are no specific recommendations for daily intake amounts for these compounds. Thus, it is not possible to calculate DV percentages for these phytochemicals in a serving of these leafy amaranths.

The presence of vitamin C, phenolics, and flavonoids in amaranth leaves might be directly related to some of the medicinal properties of these vegetables. Amaranth leaves, besides being used as food, are also used to treat pain, inflammation, diarrhea, gastroenteritis and arthritis (Achigan-Dako et al., 2014). Additionally, *Amaranthus hybridus*, *A. spinosus* and *A. caudatus* have antimicrobial activity (Maiyo et al., 2010), and *Amaranthus viridis* has potential antihyperglycemic and hypolipidemic activities (Krishnamurthy et al., 2011).

With respect to measured antioxidant levels, there were no correlations with individual or combined levels of vitamin C, total phenolics, or total flavonoids (data not shown). This could be due to the antioxidant contribution of other phytochemicals, such as chlorophylls, carotenoids, betacyanins and/or betaxanthins, which have been found in amaranth leaves (Khanam & Oba 2013; Li et al., 2015; Schönfeldt & Pretorius, 2011), but were not measured in this study. Similarly, the lack of correlation could have been due to differences in the underlying phenolic and flavonoid molecules found in leaves across each of the *Amaranthus* species. Different phenolic and flavonoid molecules promote different levels of antioxidant activity (Rice-Evans et al., 1995). Phenolic compounds that have been detected in *A. hypochondriacus* and *A. tricolor* leaves include several hydroxybenzoic acids (gallic, vanilic, syringic, *p*-hydroxybenzoic, salicylic and ellagic acids) and hydroxycinnamic acids (caffeic, chlorogenic, *p*-coumaric, *m*-coumaric, ferulic, sinapic and *trans*-cinnamic acids) (Khanam & Oba, 2013). Flavonoids that have been detected in *A. hypochondriacus* and *A. tricolor* leaves include quercetin 3-glucoside, quercetin 3-galactoside and quercetin 3-rutinoside (Khanam & Oba 2013). Many or all of these compounds could

be potentially present in the amaranth leaves studied in this research, but they may have been present in different percentages across the species.

5. Conclusions

The current study has demonstrated that leafy *Amaranthus* species are potential vegetable sources of micronutrients and phytochemicals that could contribute to human nutrition and health. They are good sources of manganese (with the exception of *A. deflexus*), good or excellent sources of magnesium, and excellent sources of vitamin C. More specifically, *Amaranthus acanthochiton* had the highest concentrations of Ca, Mg, Ni, Zn, and *A. deflexus* and *A. viridis* had the highest concentrations of Fe. Because these species are adapted to a wide range of environments (Achigan-Dako et al., 2014), their cultivation as a food source should be encouraged, especially in rural areas where micronutrient deficiencies are prevalent. While a serving of any of the leafy amaranths would contribute only minimally to iron or zinc requirements directly (1-4% or 2-3% of DV, respectively), it should be kept in mind that the vitamin C, phenolics, and/or flavonoids in amaranth leaves could act as reducing agents or chelators for these metals (Andjelković et al., 2006; Etcheverry et al., 2012; Wei and Guo, 2014). These compounds could enhance the bioavailability of iron or zinc from other components of the diet. Thus, the combination of leafy amaranths with staple foods (e.g., rice, bean, cassava, banana) could offer another strategy to reduce the incidence of hidden hunger across the globe.

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Table 1. Mineral composition (macroelements) in *Amaranthus* leaves

Species	Accession numbers	Moisture	Ca	K	Mg	P	S
<i>Amaranthus acanthochiton</i>	PI 632238	80±0.82	3.5±0.10 ^a	6.5±1.1 ^{bc}	4.5±0.092 ^a	0.73±0.13 ^{abcde}	1.2±0.34 ^{abc}
<i>Amaranthus blitum</i>	PI 606751, PI 652433, PI 490298, PI 606282, PI 612860, PI 632245	80±1.8	2.0±0.40 ^c	5.8±1.0 ^c	3.1±0.40 ^b	0.66±0.11 ^{cde}	1.1±0.18 ^{ab}
<i>Amaranthus caudatus</i>	PI 553073, PI 654432	81±1.9	2.2±0.33 ^{bc}	8.8±1.5 ^a	2.6±0.35 ^{bcd}	0.88±0.15 ^a	0.99±0.092 ^{abc}
<i>Amaranthus cruentus</i>	Ames 15188, Ames 15196, PI 477913, PI 566897	81±1.5	1.5±0.13 ^d	6.7±1.2 ^{bc}	1.8±0.31 ^e	0.53±0.084 ^e	1.1±0.26 ^{abc}
<i>Amaranthus deflexus</i>	PI 633576, PI 649303	82±0.96	1.9±0.45 ^{cd}	6.8±1.2 ^{bc}	2.9±0.48 ^{bc}	0.80±0.21 ^{abc}	0.95±0.074 ^{bc}
<i>Amaranthus dubius</i>	PI 605352	79±0.84	2.1±0.061 ^{bcd}	5.6±0.67 ^{bc}	2.4±0.081 ^{bcde}	0.53±0.022 ^{de}	0.86±0.10 ^{bc}
<i>Amaranthus graecizans</i>	PI 608661, PI 173837, PI 288277 PI 658732	82±0.87	2.6±0.50 ^b	5.5±0.93 ^c	2.8±0.42 ^{bc}	0.73±0.10 ^{abcd}	0.86±0.092 ^c
<i>Amaranthus hybr.</i>	PI 604567	80±0.92	1.9±0.033 ^{bcd}	6.8±0.39 ^{abc}	2.6±0.053 ^{bcd}	0.68±0.033 ^{abcde}	0.76±0.021 ^c
<i>Amaranthus hybridus</i>	PI 500249, PI 605351, PI 632247 PI 641052	81±1.1	2.0±0.15 ^c	6.7±1.1 ^{bc}	2.3±0.43 ^{de}	0.63±0.17 ^{cde}	0.91±0.22 ^c
<i>Amaranthus palmeri</i>	PI 549158, PI 632235, PI 633586	80±0.83	2.1±0.25 ^c	6.4±1.1 ^{bc}	2.7±0.55 ^{bcd}	0.66±0.15 ^{bcde}	1.0±0.21 ^{abc}
<i>Amaranthus retroflexus</i>	PI 572263, PI 649310	81±1.2	2.5±0.42 ^{bc}	5.8±0.37 ^{bc}	2.6±0.50 ^{bcd}	0.65±0.15 ^{bcde}	0.92±0.11 ^{bc}
<i>Amaranthus spinosus</i>	PI 482057, PI 612848	82±0.93	2.6±0.51 ^b	6.2±0.32 ^{bc}	2.8±0.14 ^{bcd}	0.61±0.061 ^{cde}	1.1±0.18 ^{abc}
<i>Amaranthus tricolor</i>	Ames 5166, PI 349553, PI 419057 PI 604669	82±1.7	2.1±0.44 ^c	7.0±1.2 ^b	2.5±0.39 ^{cd}	0.81±0.14 ^{ab}	0.86±0.13 ^c
<i>Amaranthus virindis</i>	PI 536439	80±1.3	2.4±0.19 ^{bc}	6.2±0.19 ^{bc}	3.3±0.30 ^{bc}	0.93±0.073 ^a	1.3±0.034 ^a
<i>Amaranthus thunbergii</i>	VI050456, VI050467, VI050468	82±0.71	2.3±0.49 ^{bc}	5.8±1.2 ^{bc}	2.7±0.44 ^{bcd}	0.67±0.082 ^{bcde}	0.85±0.15 ^c
*Daily value (mg)			1000	3500	400	1000	NA
mg per serving (1 cup: 30 g FW)			46-106	164-265	53-135	16-28	33-40
Percent DV			5-11	5-8	13-34	2-3	NA

Results expressed as mg/g fresh weight (FW). Not available (NA).

*Daily value (DV) based on a caloric intake of 2,000 calories as established by the U.S. FDA (2013).

Each species represents the average and standard deviation of the accessions.

Different letters in each column represent significant differences ($p<0.05$).

Table 2. Mineral composition (microelements, µg/g FW) in *Amaranthus* leaves

Species	B	Cu	Fe	Mn	Mo	Na	Ni	Zn
<i>Amaranthus acanthochiton</i>	7.2±0.58 ^{abc}	1.5±0.27 ^{bcd}	15±3.7 ^{bcdg}	7.7±2.6 ^{ab}	0.33±0.19 ^{abc}	15±5.7 ^{def}	0.14±0.046 ^a	16±1.9 ^a
<i>Amaranthus blitum</i>	6.9±2.8 ^b	1.6±0.20 ^b	17±2.8 ^b	9.7±5.2 ^a	0.39±0.13 ^{ab}	154±52 ^a	0.064±0.033 ^{bc}	11±5.1 ^{ab}
<i>Amaranthus caudatus</i>	5.3±1.3 ^{bcd}	1.0±0.17 ^{de}	11±2.3 ^{cefg}	9.0±1.7 ^{ab}	0.13±0.062 ^c	11±5.5 ^{def}	0.017±0.093 ^c	14±5.5 ^{ab}
<i>Amaranthus cruentus</i>	4.6±1.0 ^{cd}	1.0±0.35 ^d	8.4±1.8 ^f	7.8±2.2 ^{ab}	0.19±0.10 ^c	5.4±2.3 ^f	0.075±0.018 ^{abc}	13±1.8 ^{ab}
<i>Amaranthus deflexus</i>	5.7±1.8 ^{bcd}	1.1±0.36 ^d	22±4.2 ^a	3.8±1.9 ^b	0.17±0.064 ^c	128±22 ^{ab}	0.11±0.026 ^{ab}	12±3.5 ^{ab}
<i>Amaranthus dubius</i>	5.9±0.43 ^{bcd}	0.56±0.10 ^e	14±1.1 ^{bcddeg}	11±3.9 ^{ab}	0.08±0.061 ^c	31±9.4 ^{cdef}	0.044±0.011 ^{abc}	8.1±1.2 ^b
<i>Amaranthus graecizans</i>	5.2±0.65 ^{cd}	1.1±0.16 ^d	15±1.7 ^b	9.3±5.1 ^a	0.47±0.13 ^a	86±45 ^{bc}	0.072±0.014 ^{abc}	11±1.9 ^{ab}
<i>Amaranthus hybr.</i>	5.9±0.10 ^{bcd}	1.1±0.083 ^{cde}	9.5±1.1 ^f	6.4±1.4 ^{ab}	0.10±0.024 ^c	6.1±0.60 ^{def}	0.035±0.011 ^{bc}	9.4±0.50 ^{ab}
<i>Amaranthus hybridus</i>	6.2±1.1 ^{bc}	1.1±0.12 ^d	9.9±1.3 ^{efg}	7.6±1.3 ^{ab}	0.19±0.073 ^c	4.7±1.4 ^f	0.057±0.014 ^{bc}	10±2.0 ^b
<i>Amaranthus palmeri</i>	4.2±0.55 ^d	1.5±0.34 ^{bc}	15±2.9 ^{bcd}	8.5±3.0 ^{ab}	0.31±0.34 ^{abc}	49±49 ^{cde}	0.077±0.061 ^{abc}	10±2.2 ^{ab}
<i>Amaranthus retroflexus</i>	4.5±1.8 ^{cd}	1.1±0.17 ^d	12±4 ^{cdefg}	11±4.4 ^a	0.18±0.15 ^c	6.2±1.1 ^{ef}	0.092±0.026 ^{ab}	11±0.93 ^{ab}
<i>Amaranthus spinosus</i>	5.2±0.76 ^{bcd}	1.1±0.11 ^d	15±1.2 ^{bcd}	9.6±1.1 ^{ab}	0.31±0.12 ^{abc}	23±9.2 ^{def}	0.049±0.026 ^{bc}	11±2.2 ^{ab}
<i>Amaranthus tricolor</i>	5.7±1.4 ^{bcd}	1.2±0.17 ^d	15±1.9 ^{bcd}	11±3.4 ^a	0.25±0.17 ^{bc}	79±27 ^c	0.073±0.025 ^{abc}	12±1.5 ^{ab}
<i>Amaranthus virindis</i>	10±0.71 ^a	2.4±0.091 ^a	22±1.7 ^a	8.5±1.9 ^{ab}	0.31±0.023 ^{abc}	29±5.0 ^{cdef}	0.073±0.0099 ^{abc}	13±0.62 ^{ab}
<i>Amaranthus thunbergii</i>	6.0±0.64 ^{bcd}	1.2±0.27 ^{cd}	12±2.5 ^{ceg}	7.7±4.0 ^{ab}	0.23±0.07 ^{bc}	56±13 ^{cd}	0.071±0.018 ^{abc}	13±2.6 ^{ab}
*Daily value (µg)	NA	2000	18000	2000	75	2400000	NA	15000
mg per serving (1 cup: 30 g FW)	124-300	17-72	252-672	115-344	3.0-14	142-4626	0.62-4.2	243-479
Percent DV	NA	1-4	1-4	6-17	4-19	0.01-19.0	NA	2-3

Results expressed as mg/g fresh weight (FW). Not available (NA).

*Daily value (DV) based on a caloric intake of 2,000 calories as established by the U.S. FDA (2013).

Each species represents the average and standard deviation of the accessions listed in Table 1.

Different letters in each column represent significant differences ($p < 0.05$)

Table 3. Vitamin C, phenolics, total flavonoids and antioxidant activity in *Amaranthus* leaves

Species	Vitamin C (mg AA/g FW)		Phenolics (mg GAE/g FW)		Flavonoids (mg CE/g FW)	Antioxidant activity (μ mol TE/g FW)
	Reduced	Total	Free	Total		
<i>Amaranthus acanthochiton</i>	0.62 $\pm$ 0.05 ^{2abcd}	1.1 $\pm$ 0.08 ^{2abcde}	3.7 $\pm$ 0.05 ^{8a}	5.5 $\pm$ 0.35 ^a	4.1 $\pm$ 0.14 ^a	82 $\pm$ 7.9 ^{abcd}
<i>Amaranthus blitum</i>	1.2 $\pm$ 0.28 ^a	1.3 $\pm$ 0.32 ^a	3.3 $\pm$ 0.62 ^{ab}	4.9 $\pm$ 1.1 ^a	2.6 $\pm$ 0.53 ^b	81 $\pm$ 30 ^{abcd}
<i>Amaranthus caudatus</i>	0.93 $\pm$ 0.13 ^{abc}	1.1 $\pm$ 0.33 ^{abcde}	2.9 $\pm$ 0.84 ^{abcd}	4.1 $\pm$ 1.1 ^{abc}	1.7 $\pm$ 0.33 ^{cd}	84 $\pm$ 28 ^{abcd}
<i>Amaranthus cruentus</i>	0.64 $\pm$ 0.20 ^{cd}	0.80 $\pm$ 0.22 ^{de}	2.2 $\pm$ 0.29 ^{def}	3.2 $\pm$ 0.26 ^c	1.0 $\pm$ 0.11 ^e	89 $\pm$ 19 ^a
<i>Amaranthus deflexus</i>	0.99 $\pm$ 0.17 ^{abc}	1.0 $\pm$ 0.13 ^{abcde}	2.7 $\pm$ 0.19 ^{bcdef}	4.4 $\pm$ 0.21 ^{ab}	2.1 $\pm$ 0.52 ^{bc}	54 $\pm$ 10 ^{abcd}
<i>Amaranthus dubius</i>	1.2 $\pm$ 0.031 ^{abc}	1.3 $\pm$ 0.11 ^{abcde}	2.6 $\pm$ 0.10 ^{bcdef}	4.5 $\pm$ 0.29 ^{abc}	2.2 $\pm$ 0.11 ^{bc}	55 $\pm$ 1.8 ^{abcd}
<i>Amaranthus graecizans</i>	0.91 $\pm$ 0.20 ^{abc}	1.1 $\pm$ 0.26 ^{abcd}	2.1 $\pm$ 0.23 ^{ef}	4.0 $\pm$ 0.35 ^{bc}	2.0 $\pm$ 0.35 ^c	49 $\pm$ 13 ^c
<i>Amaranthus hybr.</i>	0.64 $\pm$ 0.054 ^{abcd}	0.70 $\pm$ 0.093 ^{cde}	2.3 $\pm$ 0.24 ^{cdef}	4.2 $\pm$ 0.24 ^{abc}	0.98 $\pm$ 0.093 ^{de}	90 $\pm$ 7.3 ^{abcd}
<i>Amaranthus hybridus</i>	0.80 $\pm$ 0.19 ^{bcd}	0.94 $\pm$ 0.17 ^{bcde}	2.3 $\pm$ 0.22 ^{def}	3.7 $\pm$ 0.23 ^{bc}	1.2 $\pm$ 0.35 ^{de}	78 $\pm$ 31 ^{abcd}
<i>Amaranthus palmeri</i>	1.2 $\pm$ 0.49 ^{ab}	1.3 $\pm$ 0.44 ^{abc}	2.9 $\pm$ 0.34 ^{abc}	4.0 $\pm$ 0.64 ^{bc}	1.7 $\pm$ 0.59 ^{cd}	51 $\pm$ 9.1 ^{bc}
<i>Amaranthus retroflexus</i>	0.66 $\pm$ 0.051 ^{cd}	0.82 $\pm$ 0.043 ^{cde}	2.1 $\pm$ 0.34 ^{def}	3.4 $\pm$ 0.31 ^{bc}	1.5 $\pm$ 0.24 ^{cde}	56 $\pm$ 20 ^{abcd}
<i>Amaranthus spinosus</i>	0.41 $\pm$ 0.13 ^d	0.63 $\pm$ 0.082 ^e	2.1 $\pm$ 0.29 ^{def}	3.5 $\pm$ 0.48 ^{bc}	1.7 $\pm$ 0.56 ^{cd}	47 $\pm$ 22 ^{bc}
<i>Amaranthus tricolor</i>	1.2 $\pm$ 0.28 ^a	1.4 $\pm$ 0.25 ^a	2.2 $\pm$ 0.21 ^{ef}	3.9 $\pm$ 0.36 ^{bc}	1.5 $\pm$ 0.30 ^{cde}	61 $\pm$ 16 ^{abcd}
<i>Amaranthus virindis</i>	1.0 $\pm$ 0.024 ^{abc}	1.3 $\pm$ 0.057 ^{abcde}	2.4 $\pm$ 0.13 ^{cdef}	4.2 $\pm$ 0.10 ^{abc}	1.7 $\pm$ 0.058 ^{cde}	38 $\pm$ 8.1 ^{bcd}
<i>Amaranthus thunbergii</i>	1.1 $\pm$ 0.34 ^{ab}	1.3 $\pm$ 0.37 ^{ab}	2.0 $\pm$ 0.28 ^f	3.8 $\pm$ 0.37 ^{bc}	1.8 $\pm$ 0.22 ^{cd}	61 $\pm$ 7.8 ^{abcd}

Results expressed on fresh weight (FW) as ascorbic acid (AA), gallic acid equivalents (GAE), catechin equivalents (CE) and trolox equivalents (TE). Each species represents the average and standard deviation of the accessions listed in Table 1.

Different letters in each column represent significant differences ($p < 0.05$).

Table 4. Macroelements, microelements and *Amaranthus* leaves

Species	Macroelements (mg/g FW)			
	Ca	K	Mg	P
<i>Amaranthus</i> spp.	1.4-3.5	4.6-9.2	1.5-4.5	0.43-1.0
Spider flower (<i>Gynandropsis gynandra</i>)	1.1-2.3 ^{Φζ}	3.4-7.3 ^{Φζ}	0.76-1.5 ^{Φζ}	0.97-1.4 ^{Φζ}
Black nightshade (<i>Solanum scabrum</i>)	1.4 ^Φ	4.3-4.6 ^Φ	1.0-1.1 ^Φ	0.57-0.58 ^Φ
Pumpkin leaves (<i>Cucurbita maxima</i>)	3.8 ^Θ	---	1.4 ^Θ	1.2 ^Θ
Spinach (<i>Spinacia oleracea</i>)	0.99 ^Ψ	5.6 ^Ψ	0.79 ^Ψ	0.49 ^Ψ
Kale (<i>Brassica oleracea</i>)	1.5 ^Ψ	4.9 ^Ψ	0.47 ^Ψ	0.92 ^Ψ

	Microelements (μg/g FW)				
	Cu	Fe	Mn	Mo	Zn
<i>Amaranthus</i> spp.	0.56-2.4	6.6-26	2.2-17	0.062-0.78	6.7-20
Spider flower (<i>Gynandropsis gynandra</i>)	1.3-2.5 ^{Φζ}	12-25 ^{Φζ}	3.9-6.3 ^{Φζ}	0.091-0.58 ^Φ	9.3-16 ^{ΦΘζ}
Black nightshade (<i>Solanum scabrum</i>)	1.3 ^Φ	11-12 ^Φ	8.1-8.6 ^Φ	0.19-0.23 ^Φ	4.5-4.9 ^Φ
Spinach (<i>Spinacia oleracea</i>)	1.1 ^ε	27 ^Ψ	5.9 ^ε	---	3.5-5.3 ^{Ψε}
Kale (<i>Brassica oleracea</i>)	0.40 ^Σ	4.0-15 ^{ΨΣ}	3.0 ^Σ	---	2.9-5.6 ^{ΨΣ}

	Phytochemical compounds and antioxidant activity			
	Vitamin C (mg AA/g FW)	Phenolics (mg GAE/g FW)	Flavonoids (mg CE/g FW)	Antioxidant activity (μmol TE/g FW)
<i>Amaranthus</i> spp.	0.56-2.0	2.9-6.9	0.91-4.1	28-138
Spider flower (<i>Gynandropsis gynandra</i>)	1.1-1.4 ^Φ	2.3-4.4 ^Φ	0.51-0.82 ^Φ	15-33 ^Φ
Black nightshade (<i>Solanum scabrum</i>)	0.94-1.3 ^Φ	3.2-3.4 ^Φ	1.4 ^Φ	35-36 ^Φ
Spinach (<i>Spinacia oleracea</i>)	0.073-0.63 ^{Qμε}	1.1-1.2 ^{Qκ}	0.70 ^κ	16 ^{Qκ}
Kale (<i>Brassica oleracea</i>)	0.52-0.67 ^{Qδ}	1.5-5.6 ^{Qδ}	---	33 ^Q

Ranges from *Amaranthus* spp. were obtained from the **Appendix 1**. Results expressed on fresh weight (FW) as ascorbic acid (AA), gallic acid equivalents (GAE), catechin equivalents (CE) and trolox equivalents (TE).

^QIsabelle et al. (2010), ^ΦJiménez-Aguilar & Grusak (2015), ^ΣKawashima & Valente Soares (2003), ^ΘKoh et al. (2012), ^κMavhungu (2011), ^εOlsen et al. (2012), ^ΘSchönfeldt & Pretorius (2011), ^εSingh et al. (2001), ^ζvan Jaarsveld et al. (2014), ^ΨUSDA (2015).