Research Article

Evaluation of Bioactive Compounds and Mineral Composition in Thai-Variety Amaranths

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Abstract
Amaranth is one of the high nutrient vegetables with high proteins, vitamins, and mineral contents. However, the accumulated pigments and nutrients may change by plant growth stages. Therefore, this study explored the phytonutrients and mineral composition in three amaranth varieties with five harvesting stages. The results revealed that plant pigments were highest accumulated at the reproductive stage with anthocyanin and β-carotene contents of 0.82 and 141.21 mg/g fresh weight (FW), respectively. Contrastingly, vitamin C contents were highest at the marketing stage (1,904 mg/g FW). Accumulations of Ca and K increased over cultivation time, whereas Zn contents were higher at the younger stages. However, Fe contents were not different by growth stages or varieties. From all measurements, only β-carotene and vitamin C accumulations were different among plant varieties. Compared to the WHO food recommendations, amaranths are excellent sources of β-carotene, antioxidants, calcium, potassium, and iron. Additionally, the associations among traits were evaluated and found that most traits were positively correlated with the growth stage, whereas zinc contents were strongly negatively correlated. These results provide a clear picture of amaranth's nutrition for consumers and suggest the appropriate harvesting time for specific nutritional benefits as well as promoting the consumption of these vegetables.

Keywords: *Amaranthus* spp., Harvesting times, Plant pigments, Antioxidant activity, Mineral profile

1 Introduction

Amaranthus spp. (family Amaranthaceae) or commonly called Amaranth, are the indigenous vegetables consumed in Thailand and many developing countries on several continents including Southeast Asia, South Asia, South, and Central America, and Africa. This genus consists of about 70 species including 400 varieties with a few domesticated varieties throughout the world. Out of 70 species, 17 species are consumed as leafy vegetables, 3 are used as edible seeds and the rest are weeds or ornamental plants [1]. However, there have been some reports that for some varieties of *Amaranthus*, both leaf and grain can be consumed as as food [2]. These plants have many valuable features as they grow very rapidly under full sunlight, high temperature, and dry soil due to their C4 carbon-fixation pathway [3]. Moreover, it grows easily and is well-adapted to harsh environmental conditions [4]. The species used for vegetables usually contain short plant types with wider

and smoother leaves, smaller auxiliary inflorescences, and thicker stems, compared to grainy species. Amaranth leaves are highly nutritious in protein, calcium (Ca), potassium (K), iron (Fe), vitamins A, C, K, B_2, B_6, and B_{12}, dietary fiber, plant pigments, such as β-carotene, β-cyanin and anthocyanin, and other antioxidants [5]–[8]. While, grain amaranths compose of high protein contents (13–21% depending on species), especially in lysine, high unsaturated fatty acids, and high fibers [9]. These phytonutrients, such as vitamin A and calcium are necessary for new normal lifestyles because many countries including Thailand where are become an aging society. Aging people at the age of 65 and up require 20% of vitamin A and 30% of calcium higher than adult people [10]. In addition, aging people lose their hormone production, resulting in the increase in cell aging, incidences of some cancers, and risk of cardiovascular diseases. The study showed that dietary antioxidants help to protect women with menopause against oxidative stress and reduce the risk of cancer and cardiovascular diseases [11]. An increase in these essential nutrients in their daily consumption improves the health of the elderly. Additionally, amaranth can be used as gluten-free flour for celiac disease patients [12].

Many leafy vegetables can be consumed in several growth stages: sprouts, microgreens, juvenile vegetative, marketable, and reproductive stages. Sprouts and microgreens become new vegetable products that gain popularity from their high nutritional values as well as the easiness to grow [13]. Plants in each growth stage synthesize and accumulate different levels of each phytonutrient [14]–[16]. Several studies are showing each phytonutrient accumulation varied among different types and growth stages. For example, dill (*Anethum graveolens* L.) decreased vitamin C content but tended to increase the content of carotenoids with growth [17]. In addition, kale at different harvesting stages contained the amounts of vitamin C, protein, and minerals [18]. Amaranths can be consumed in many growth stages from sprouts to their grains. There have been several studies in their nutrition with different varieties [1], [5], [14], [15], [19], [20]. Ebert *et al.* [19] studied four varieties of leafy amaranths (two varieties of *A. tricolor* and other two commercial varieties from China) in three growth stages sprouts, microgreens (a seedling stage with 2–4 true leaves), and the usual consumption stage. The results have shown that amaranths had the highest accumulations of calcium, β-carotene, and vitamin C at the oldest stages (the fully grown stage) as 406.83, 2.51, and 59.12 mg/100 g FW, respectively. Furthermore, phytonutrient compositions in *A. cruentus* L. were investigated at different harvesting stages [19]. The results were shown that calcium concentration peaked at three weeks after emergence, whereas potassium content reached the peak at six weeks after emergence.

As mentioned earlier, amaranths are the high nutritional crops that have potential as a new functional food in Thailand. Nevertheless, these crops are not popular in the Thai vegetable market, compared to their benefits. Moreover, amaranths can be used as sources of ingredients for food and supplement industries in every growth stage, from sprouts to grain. Thus, the study of phytonutrients in amaranths varieties consumed in Thailand could be the information for the consumers selection. However, the previous studies have shown that different varieties of *Amaranthus* accumulated different nutrition at different growth stages [5]–[8]. Unfortunately, none of them investigated all growth stages from sprout to reproductive stages with Thai commercial varieties that are consumed in Thailand. In this study, the nutritional profiles of the three amaranth varieties (*A. viridis*, *A. tricolor*, and *A. tricolor* var. *tricolor*) at five different growth stages were investigated to evaluate the levels of phytonutrients as well as some mineral accumulation for optimal consumption in terms of alternative raw material for a novel food industry in the future.

2 Materials and Methods

2.1 *Plant materials*

All commercial varieties of Thai amaranth seeds were bought from the local market, including three varieties of leafy amaranths (*A. viridis*, *A. tricolor*, and *A. tricolor* var. *tricolor*), representing green, bicolor, and red amaranth (Figure 1). To grow these plants, seeds of all varieties were soaked in distilled water overnight and set to germinate on wet peat moss in a plastic box. All plants were cultivated in a greenhouse with an average temperature and relative humidity of 30.4 °C and 73.1%, respectively. While, sprouts were collected 7 days after sowing (DAS), microgreens were harvested at 14 DAS. Seeds for juvenile vegetative, marketable

(a) (b) (c)

Figure 1: Plant varieties used in the experiment: (a) green amaranth (*A. viridis*) (two left columns), (b) bicolor amaranth (*A. tricolor*) (two middle columns), and (c) red amaranth (*A. tricolor* var. *tricolor*) (two right columns) growing in the plastic pots at the marketable stage.

and reproductive stages were sown in the tray with peat moss and then transplanted after 14 days to six-inch plastic pots with mixed soil media and a teaspoon of slow-release fertilizer (13-13-13) per pot. The plants werewatered daily. Amaranth at juvenile, marketable and reproductive stages were harvested at 21, 28, and 42 DAS, respectively (Figure 2).

2.2 *Bioactive compound assays*

2.2.1 Anthocyanin assay

One gram of each fresh sample was ground and extracted in 1% HCl in methanol as described by Mizukami *et al.* [21], then kept in the dark condition at room temperature for one day. After that, samples were centrifuged and filtered off for supernatants, which were measured in Microplate Reader (SPECTROstar Nano, BMG LabTech, Ortenberg, Germany) at 530 nm (A530) and calculated for anthocyanin content using Equation (1). The results were shown in mg/g FW.

$$\text{Total anthocyanin (mg/g FW)} = ((27.208 \times A_{530} + 0.059) \times \text{solvent (mL)}) \times ((10/1000) \times \text{FW (g)}) \quad (1)$$

2.2.2 β-carotene assay

β-carotene content was evaluated by using the modified method from Nagata and Yamashita [22] and results were presented as mg/g FW. One gram of each fresh sample was ground in 4:6 acetone-hexane solvent (v/v) and kept at room temperature for 1 h, then samples were centrifuged for 10 min at 3,000 rpm and were collected supernatants which were measured in Microplate Reader (SPECTROstar Nano, BMG LabTech, Ortenberg, Germany) at 435, 505, 645, and 663 nm (A_{435}, A_{505}, A_{645}, and A_{663}, respectively) to determine the absorption coefficient of plant pigments including chlorophyll a, chlorophyll b, lycopene, and β-carotene. Then, the amount of β-carotene was calculated from Equation (2).

$$\text{β-carotene (mg/g FW)} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A453 \quad (2)$$

2.2.3 Vitamin C measurement

Vitamin C contents from amaranth samples were measured by the modified method by Pantelidis *et al.*

(a) (b) (c) (d) (e)

Figure 2: Five different growth stages using in the experiment: (a) sprout (7 DAS), (b) microgreens (14 DAS), (c) juvenile vegetative (21 DAS), (d) marketable (28 DAS), and (e) reproductive (42 DAS).

[23]. Fresh samples (5 g) were ground with mortar and pestle, homogenized in 10 mL reverse osmosis (RO) water, and filtered for liquid samples. The samples were spotted on an ascorbic acid testing strip and measured ascorbic content in a reflectometer (RQFlex (Reflectoquant®), Merck, Darmstadt, Germany) by inserting the sampled strip into the reflectometer and the quantity of vitamin C content was shown on the machine. The results were shown in μg/g FW.

2.2.4 DPPH radical scavenging activity test for antioxidant activity assay

Samples for antioxidant activity assay were extracted by the method by Sensoy *et al.* [24]. Two grams of each fresh sample were collected at each growth stage, extracted in 4 mL absolute ethanol, and then incubated at room temperature for 24 h. The incubated samples were centrifuged at 8,000 rpm and supernatants were collected. The supernatants were analyzed with a stable radical, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (Sigma-Aldrich, St. Louis, Missouri, USA) using the methods by Ingkasupat *et al.* [25] with some modifications. The samples were kept in a 96-well microplate. 22 μL of each extraction were added to 200 μL of 0.1 mM DPPH radical solution in 95% aqueous ethanol and incubated for 30 min in the dark condition at room temperature. The mixed solution was measured with Microplate Reader (SPECTROstar Nano, BMG LabTech, Ortenberg, Germany) at 517 nm, by using 95% aqueous ethanol as a control. The % of DPPH radical scavenging activity was calculated using Equation (3).

$$\% \text{ DPPH scavenging activity} = [(A_{blank} - A_{sample}) \div A\text{blank}] \times 100 \quad (3)$$

Where A_{blank} and A_{sample} were the absorbance of blank and sample, respectively, at 517 nm.

2.3 *Mineral composition*

Amaranths from each stage were collected and dried for 72 h at 60 °C. The mineral compositions of all samples were measured using methods by Ong *et al.* [26]. The 0.5 g of dried samples were digested in 10 mL concentrated nitric acid (HNO_3) (AnalaR grade, BDH 69%) at 40 °C for 1 h, then were incubated at 140 °C for at least 3 h. After cooling down, samples were topped up with double de-ionized water to 40 mL and then filtered through filter paper (Whatman No. 1). Samples were stored in acid-washed pillboxes and determined by using an air-acetylene flame atomic absorption spectrophotometer (Agilent 240 FS, Agilent Technologies, California, USA). The lights with different wavelengths were used for different metal determination, 422.7 nm for Ca, 766.5 nm for K, 248.3 nm for Fe, and 213.9 nm for Zn [27]. Standard solutions for Ca, K, Fe, and Zn were prepared from 1,000 ppm stock solution (Merck Titrisol) and the results were shown in mg/g DW.

2.4 *Statistical analysis*

The experimental design was 3 × 5 factorial in a completely randomized design with three replications. The first factor was the plant variety and another factor was the harvesting stage. The results are presented as the mean of all replications along with standard deviations. Differences in plant nutrient compounds across varieties were evaluated using the analysis of variance (ANOVA) followed by the multiple comparisons using the Duncan's Multiple Range Test (DMRT) at $p < 0.05$. Correlations among traits were calculated by the Pearson correlation method at $p < 0.05$ [28]. All statistical analyzes were performed using R software [29].

3 Results and Discussion

3.1 *Phytonutrients and antioxidant activity*

Results from the ANOVA analysis shown in Table 1 represented two-factor ANOVA (plant variety of amaranth and growth stage) and the interaction between these factors. From Table 1, contents of anthocyanin, Ca, and Zn were only significant differences in their growth stages at $p < 0.001$, while contents of β-carotene and vitamin C were statistically different in plant varieties, plant growth stages, and their interactions between both factors, all of them at $p < 0.001$. Antioxidant activity and K were statistical different in their growth stages and interaction between the factors, at $p < 0.001$ and $p < 0.01$, respectively. However, there was no significant difference in any factor nor interaction in Fe.

Table 1: Summary of the ANOVA results from the factorial in CRD experiment for all traits

Trait	Variation Source	*p*-value	*p*-value Summary
Anthocyanin	Stage	<0.001	***
	Variety	0.06	NS
	Interaction	0.12	NS
β-carotene	Stage	<0.001	***
	Variety	<0.001	***
	Interaction	<0.001	***
Vitamin C	Stage	<0.001	***
	Variety	<0.001	***
	Interaction	<0.001	***
Antioxidant activity	Stage	<0.001	***
	Variety	0.24	NS
	Interaction	<0.001	***
Ca	Stage	<0.001	***
	Variety	0.82	NS
	Interaction	0.052	NS
K	Stage	0.007	**
	Variety	0.62	NS
	Interaction	0.007	**
Fe	Stage	0.69	NS
	Variety	0.35	NS
	Interaction	0.91	NS
Zn	Stage	<0.001	***
	Variety	0.41	NS
	Interaction	0.48	NS

Data shows significant difference at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.0001$. NS = no statistically significant difference.

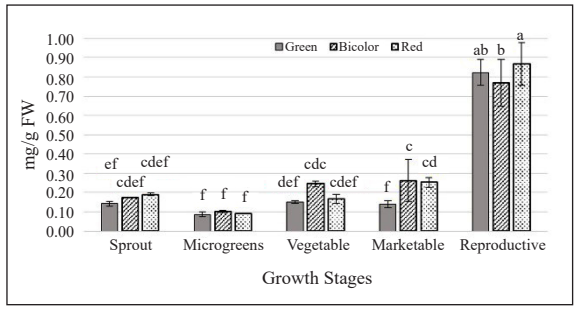

Figure 3: Anthocyanin accumulation from three amaranth varieties at five growth stages. Means within a column with different upper case letters are significantly different at $p < 0.05$.

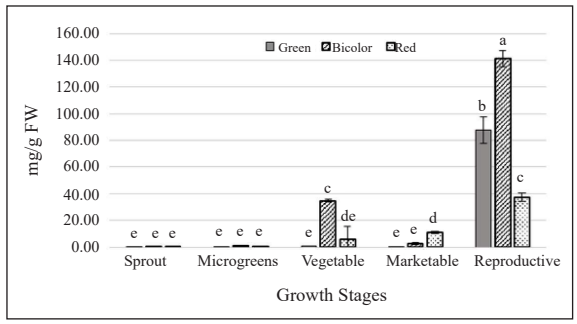

Figure 4: β-carotene accumulation from three amaranth varieties at five growth stages. Means within a column with different upper case letters are significantly different at $p < 0.05$.

Based on Table 1 and Figure 3, anthocyanin was significantly different among harvesting stages without any differences among varieties and no interactions between the two factors. However, the accumulations of anthocyanin were not significant differences during the vegetative stages except at the microgreen stage. This is due to the ages of leafy vegetables could affect the pigment accumulation from the changes in nutritional value [8]. Anthocyanin accumulation reached the highest point, at 0.87 mg/g FW during the reproductive stage of the red variety (Supplementary Table 1), which was 4 times higher than other stages. These results were similar to those of Khandaker *et al.* [8] that showed the increases of red pigment (β-cyanin) in 30-day-old amaranth leaves in every varieties. The increase of anthocyanin and other red pigments including β-carotene (Figure 4) and β-cyanin [8] during plant maturity were due to the loss of chlorophyll at plant matured stages caused by the suppression of NADPH: protochlorophyllide oxidoreductase (POR) gene in plant leaves [30]. Anthocyanins are the red-to-blue, water-soluble compounds, which are categorized asa subclass of dietary flavonoids, and they are accumulated in plant organs, such as leaves, flowers, and fruit [31]. These phytochemicals have been reported to promote a wide range of human health properties, such as antioxidant, anticarcinogenic and anti-inflammatory properties. Moreover, anthocyanin intake correlated with the reduction in the risk of diabetes and cognitive function symptoms. Although there have been several studies on the links between these phytonutrients and the health benefits, the amount of anthocyanin for daily intake was reported with a wide range from a few to hundreds of milligrams depending on food types and area-based of the population in the studies [32].

Contents of β-carotene and vitamin C were significant differences in both two factors and the

interactions at $p < 0.001$ (Supplementary Table 1). Vitamin A is an essential nutrient for the human to normalize many systems, such as the visual and immune systems [10]. Provitamin A carotenoids, such as β-carotene are the major sources of vitamin A, which becomes a major public health problem [33].

In our study, β-carotene contents were also highest accumulated at the reproductive stage with all varieties at 87.73, 141.21, and 37.52 mg/g FW for green, bicolor and red amaranths, respectively (Figure 4, Supplementary Table 1). Moreover, β-carotene content from each variety was also significantly different. The results showed higher accumulations of β-carotene in bicolor amaranth at vegetative and reproductive stages than other varieties. Compared to the previous studies that measured β-carotene content at the marketable stage, β-carotene contents from this experiment ranged from 3.00 to 1,111.67 mg/100 g FW, while the previous studies found the accumulation of β-carotene ranging from 21.43 to 31.01 mg/100 g [34] and from 1.16 to 4.45 mg/100 g FW [16]. These differences might result from the different varieties and the environment of each experiment and how to extract the compound. World Health Organization (WHO) estimates the safe intake of vitamin A in adults and the elderly at 9.3 μg retinol (RE)/kg, which is approximately 3,000 μg of β-carotene intake [10]. Therefore, the consumption of bicolor amaranth at the reproductive about 25 g FW should be sufficient for vitamin A daily needs.

Vitamin C (as known as ascorbic acid or ascorbate) is an essential nutrient involved in DNA utilizing enzymes, collagen formation, and maintenance of immune system function [10]. Vitamin C deficiency causes scurvy, instability of collagen performance, and incorrectly operating enzymes [35]. Vitamin C is one of the most important quality characteristics of amaranth [36]. This study discovered that vitamin C accumulation varied significantly by both growth stages and varieties (Figure 5). The vitamin C tended to increase the accumulation when they grew up. Vitamin C contents were highest in red amaranth at marketable and reproductive stages around 1,904 and 1,861 μg/g FW, respectively (Supplementary Table 1). This result was similar to the study by Nyonje *et al.* [37], which the amounts of ascorbic acids from all amaranth varieties at the post-flowering stage were at approximately 2 times higher than the vegetative stage.

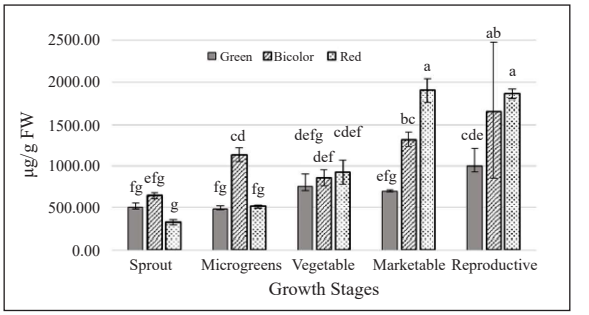

Figure 5: Vitamin C contents from three amaranth varieties at five growth stages. Means within a column with different upper case letters are significantly different at $p < 0.05$.

These results were consistent among different species of amaranths [38], [39]. The study by Franceschi *et al.* [40] demonstrated that mature leaves had a greater capacity for ascorbic biosynthesis and a lower rate of ascorbic acid loss compared to other tissue. Reference to the WHO, adults should consume vitamin C at approximately 1,500 mg/day. Therefore, the intake of red amaranths at the marketable or reproductive stage of 80 g/day would be sufficient enough for vitamin C recommendation [10]. In addition, plant genotypes were significantly different in vitamin C accumulations (Figure 5). These results were correlated to the previous studies with different varieties of leafy amaranths [20], [37], [38], [41], [42]. In addition, the interactions between amaranth varieties and their growth stages on vitamin C accumulation were observed. Green amaranth tended to increase less vitamin C accumulation via plant growth than the varieties with red pigment (Figure 5). This observation was similar to the study that was able to detect ascorbate in the red area of lettuce leaves rather than the green area [43].

Reactive oxygen species (ROS) are super active chemicals with free electrons that can be chemical signal or cause irreversible cell damage. ROS are generated in mitochondria, peroxisomes, chloroplast (in plant) and cytochrome P450 [44]. The meta-analysis reported the correlation between the reduction of ROS and the increase in fruit and vegetable consumption [45]. Dietary antioxidants in fruit and vegetable played an important role in decreasing the adverse effects of ROS [10]. Provitamin A, vitamin C, vitamin E, and polyphenols are the nutrients that possess radical-quenching properties [46]. The experiment

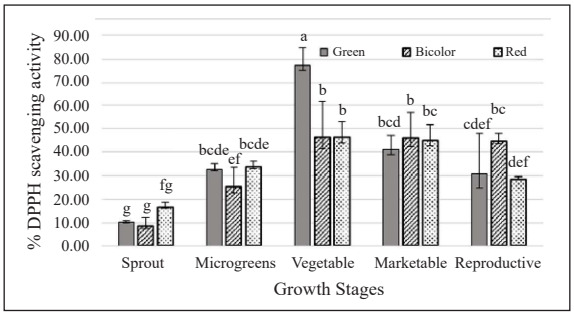

Figure 6: % DPPH scavenging activity of three amaranth varieties at five growth stages. Means within a column with different upper case letters are significantly different at $p < 0.05$.

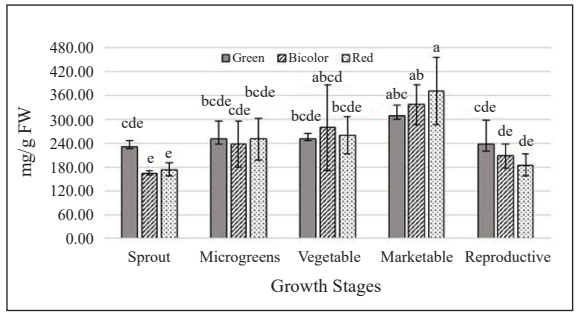

Figure 7: Ca contents from three amaranth varieties at five growth stages. Means within a column with different upper case letters are significantly different at $p < 0.05$.

stimulated the scavenging activity of DPPH radicals to observe the antioxidant activity in these vegetables [47], [48]. Considering the antioxidant activity, the results illustrated the significant differences from growth stages and the interaction at $p < 0.001$ (Table 1). % Inhibitions increased from sprout to vegetative stages, then slightly reduced when the plants were getting older (Figure 6). The highest % inhibition was observed in green amaranth at the vegetative stage, accounting for 77.60%, whereas the lowest % inhibition was found in bicolor amaranth at the sprout stage, which inhibited antioxidant activity at 8.53% (Table 1). These results were corresponding to the study from [36] that showed significant differences in antioxidant activity from pre-and post-flowering stages but similar results among varieties. These were probably a result of higher active plant metabolism, corresponding to the rapid growth of leafy vegetables in the younger period, but then the plant metabolism reduced when the plant matured and senesced [49]. However, these two experiments were dissimilar to the outcomes from Sarker *et al.* [42] which detected the significant differences in total antioxidant capacity from both DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods from different plant varieties. In addition, these results were also unlike the previous study that the antioxidant activity was relatively high during the sprout stage, then reduced at the microgreen stage, and peaked at the full growth stage [16]. These might be caused by the differences in plant varieties and environment that were used in each experiment. In the WHO recommendation for vitamin and mineral requirements, there are no exact amounts of dietary antioxidants for daily intake. However, the amount of vegetable and fruit consumption is recommended as five portions a day, which is at least 400 g/day [10].

3.2 *Mineral composition*

From Table 1, calcium and zinc were statistically different in the growth stage at $p < 0.001$. Ca accumulation tended to increase when the vegetables grew up and peaked at the marketable stage, on average among varieties at 339.36 mg/g DW but dropped rapidly during the reproductive stage to 210.54 mg/g DW (Figure 7, and Table 1). Similar to the previous studies, Ca accumulations in amaranths were detected during their growths [16], [37], and [47]. Ca accumulated contents are increased during plant growth because it is a part of cell wall and cell membrane [50]. Not only Ca is stored in the cell wall, but it is also found in free Ca in the cytosol and crystallized forms, such as calcium oxalate [51]. Unfortunately, there were no experiments exploring the reproductive stage of vegetable amaranths. However, there was an experiment discovering the decrease of calcium during megasporogenesis and megaspore degeneration from programmed cell death in lettuce [52]. Considering the WHO suggestion, adults require Ca at 1,000 mg/day but adolescents (10–18 years old) and aging people need an addition of 300 mg Ca/day [10]. Thus, all varieties of amaranth at the marketable stage, about 3–4 g DW, would be a good source of calcium for vegetarians or dairy allergy people.

Potassium (K) is an essential element that

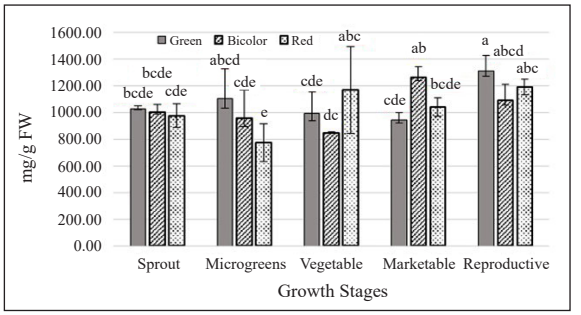

Figure 8: K contents from three amaranth varieties at five growth stages. Means within a column with different upper case letters are significantly different at $p < 0.05$.

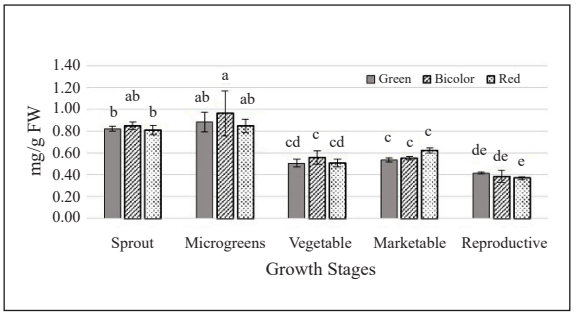

Figure 9: Zn contents from three amaranth varieties at five growth stages. Means within a column with different upper case letters are significantly different at $p < 0.05$.

helps blood pressure reduce, decreases the risk of cardiovascular diseases, and increases bone mineral density. WHO strongly recommends a potassium intake of at least 3,500 mg/day [53]. Nevertheless, a high level of potassium can cause an irregular heartbeat or heart attack, especially when the patients have alcoholism, diabetes and kidney diseases [54]. In this study, K contents were significant differences in the different growth stages and also the interaction between growth stage and variety at $p < 0.01$ (Table 1). The highest K accumulations was detected in the green variety at the reproductive stage (1,313.61 mg/g DW), followed by the bicolor variety at the marketable stage (1,268.17 mg/g DW) and the red variety at the reproductive stage (1,193.44 mg/g DW) (Table 1). K accumulations had similar patterns in every variety by having relatively high K content at the sprout stage, then reducing the accumulation while they grew up, finally increasing the accumulations again in the marketable or reproductive stage depending on the variety. The interaction showed the higher K contents in the green variety at the earlier stages, while the red variety tended to contain more K contents at the later stages (Figure 8), which was similar to Makobo *et al.* [19]. According to these outcomes, the consumption of amaranth in a healthy adult only requires approximately 3–4 g DW to get enough K intake. Although K contents in amaranth microgreens were relatively low compared to other harvesting stages, we did not recommend the patients with specific conditions that were listed above to consume amaranth at any growth stage.

Zinc is a microelement that becomes a component of more than 300 enzymes participating in the synthesis and degradation of proteins, lipids, carbohydrates, and nucleic acids. In addition, Zn involves in the metabolism of other microelements and stabilizes the component of cell structure. Men require Zn intake a little bit more than women [10]. This research found that Zn contents were highest at microgreen and sprout stages, corresponding to 0.90 and 0.83 mg/g DW, respectively (Figure 9, Table 1). These consequences were complementary to the previous studies that zinc accumulated in the younger stages rather than the older ones [19], [47], [48]. The reduction of Zn contents might have deviated during plant development [55] or might be gradually diluted as the plant matures [56]. However, there were no significant differences in plant variety and interaction between treatments. These results were different from some previous studies that discovered the differences in Ca and Zn contents among varieties [40], [41]. However, amaranth is considered to be low Zn availability food compared to meat and dairy product. Vegetarians might consider taking additional Zn supplements [10].

Iron is another crucial element in the body due to its function as an oxygen carrier to tissues by hemoglobin as well as a cofactor for several enzymes, such as cytochrome P450. Normally, an adult woman requires more Fe than a man because of menstruation, at 1.68 mg/day for a woman and 1.05 mg/day for a man. Many vegetables, grains and nuts are Fe-rich. However, the bioavailability of Fe from these foods is low since there are some phytochemicals, such as phytates that inhibit the absorption of this element, similarly to Zn [10]. In this experiment, Fe accumulations were not significant differences in any variety or

growth stage, on average at 2.10 mg/g DW, which was different from other studies that found the reduction of Fe contents at the later growth stages [47], [49]. This might result from the outlier Fe values of the bicolor variety at the marketable stage, making the large variation in data (Table 1). Although there were no significant differences in our results, amaranths are still good sources of Fe, requiring a few grams of dry weight to reach the daily intake recommendation.

3.3 *Correlations among traits*

From all the data, correlations among traits were calculated to determine the relationships between each couple of traits. Figure 10 represented the Pearson correlations among traits of interest. The correlation coefficient was shown in Table 2. From Figure 8, we observed the positive correlation among growth stage, anthocyanin, β-carotene, vitamin C, antioxidant activity, and K content. This result was similar to the previous studies that reported the positive associations between antioxidant activity and plant pigments [7], [40]. The results indicated that amaranths accumulated plant pigments (anthocyanin and β-carotene) over time. These increasing bioactive compounds including ascorbic acid act as the antioxidant compounds, resulting in a higher % inhibition of antioxidant activity. Moreover, we detected a positive correlation between antioxidant activity and calcium content. It is possible that accumulation of Ca plays an essential role in the stress signaling pathway and the bioavailability of Ca increases the activities of many antioxidant enzymes including superoxide dismutase (SOD), and ascorbate peroxidase (APOX), and catalase (CAT) [57]. K accumulation was also positively associated with anthocyanin and β-carotene contents. This is in agreement with the results from a previous study showing that potassium silicate could stimulate the production and accumulation of carotenoid and anthocyanin [58]. In contrast, zinc content was highly negatively correlated to the plant growth stage (Figure 10, Table 2), which was explained in the earlier result.

4 Conclusions

This study was objected to present amaranths with the

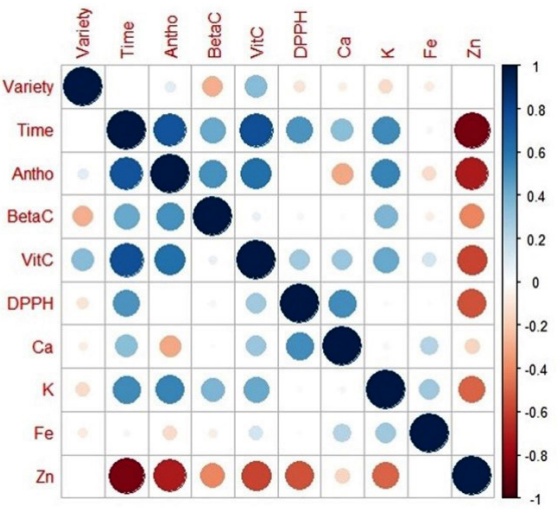

Figure 10: Correlation matrix calculating from Pearson correlation among plant variety, growth stage, bioactive compounds, and mineral compositions from three amaranth varieties at five growth stages. Circles show the correlations between two traits including Variety, Time (growth stage), Antho (anthocyanin content), BetaC (β-carotene content), VitC (vitamin C content), DPPH (antioxidant activity), Ca (calcium), K (potassium), Fe (iron), and Zn (zinc). Blue circles show positive correlations between traits whereas red circles represent negative correlations between traits.

varieties that were commercialized and consumed in Thailand. However, these high nutritional vegetables have not received enough attention comparing to other leafy vegetables. Thus, this research targeted to demonstrate their nutritional profiles in every growth stage. These nutritious plants were not only used as functional food, they were also able to be as ingredients for supplement industries. From the study, we observed that many nutrients, such as vitamin C and potassium were accumulated at the later growth stages, whereas zinc content peaked at the early growth stage. Additionally, we compared our results to the WHO recommendations for bioactive compounds and essential minerals. The investigation results showed that amaranths were good sources of β-carotene, antioxidants, calcium, potassium, and iron. Thus, this research provides a guideline for amaranth harvesting, and consumption to optimize the nutritional benefits.

Table 2: Correlation matrix among plant variety, growth stage, bioactive compounds and some minerals in three varieties of Thai amaranths at five different growth stages using Pearson correlation for calculation. Data shows significant difference at $*p < 0.05$, $**p < 0.01$, and $***p < 0.0001$, while no star symbol data are not significant differences. Traits that table represented are Variety, Time (growth stage), Antho (anthocyanin content), BetaC (β-carotene content), VitC (vitamin C content), DPPH (antioxidant activity), Ca (calcium), K (potassium), Fe (iron), and Zn (zinc). Minus symbols show negative correlations among traits whereas no negative symbols represent positive correlations among traits

	Variety	Time	Antho	BetaC	VitC	DPPH	Ca	K	Fe	Zn
Variety	1.00	0.00	0.08	–0.28	0.35	–0.11	–0.06	–0.13	–0.07	–0.01
Time	0.00	1.00	0.74**	0.43*	0.76***	0.50*	0.34	0.53*	0.03	–0.88***
Antho			1.00	0.51*	0.62**	0.00	–0.31	0.54**	–0.14	–0.71**
BetaC				1.00	0.06	0.03	0.02	0.37*	–0.06	–0.40*
VitC					1.00	0.27	0.29	0.43*	0.14	–0.58**
DPPH						1.00	0.52*	0.02	–0.01	–0.54**
Ca							1.00	0.03	0.23	–0.16
K								1.00	0.28	–0.49*
Fe									1.00	–0.01
Zn										1.00

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Author Contributions

J.C.: conceptualization, research design, investigation, methodology, data analysis, writing an original draft, reviewing and editing; B.M.: conceptualization, methodology, reviewing and editing; K.S.: investigation and data analysis; P.W.: methodology, reviewing and editing. All authors have read and agreed to the published this version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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