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Analysis of the water-soluble vitamins B_2 and B_6 of crops in the Amaranthaceae family by HPLC-FLD

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Abstract

The levels of vitamins B_2 (riboflavin) and B_6 (pyridoxine) in the main edible parts of five crops in the Amaranthaceae family, namely *Amaranthus* spp. (amaranth grain), *Beta vulgaris* subsp. *vulgaris* var. *cicla* (Swiss chard leaf), *B. vulgaris* subsp. *vulgaris* var. *conditiva* (beet root), *Chenopodium quinoa* (quinoa grain), and *Spinacia oleracea* (spinach leaf) were analysed by high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). This analysis detected both vitamins in all of the samples. The highest content of vitamin B_2 was found in spinach leaf (0.439 ± 0.094 µg/g FW). Amaranth grain (0.431 ± 0.023 µg/g FW) and quinoa grain (0.419 ± 0.055 µg/g FW) showed similar vitamin B_2 content to that of spinach leaf. The highest content of vitamin B_6 was found in quinoa grain (0.321 ± 0.030 µg/g FW), followed by amaranth grain (0.184 ± 0.003 µg/g FW). Taken together, the main edible parts of crops in the Amaranthaceae family, especially quinoa grain, might be a good vegetable source for the consumption of both vitamins, and these results could serve as valuable preliminary data for estimating both vitamin contents of crops in the Amaranthaceae family.

Introduction

Vitamins B_2 (riboflavin) and B_6 (pyridoxine) are water-soluble members of the B vitamins group, and are essential for human health (Roje, 2007; Asensi-Fabado and Munné-Bosch, 2010). Generally, water-soluble vitamins are not stored in the human body, and thus, they need to be continuously supplied through a steady dietary intake. Additionally, the human body can excrete water-soluble vitamins when their levels exceed the required levels (Bellows and Moore, 2012).

Vitamin B_2 is the precursor of the cofactors flavin adenine dinucleotide and flavin mononucleotide, and plays a vital role in energy production by metabolising fats, proteins, and carbohydrates into glucose (Fischer and Bacher, 2006). Riboflavin deficiency causes cracks in the corners of the mouth, dermatitis on the nose and lips, high sensitivity to sunlight, cataracts, and glossitis (Bellows and Moore, 2012). Vitamin B_6 is a derivative of the cofactor pyridoxal 5'-phosphate (PLP), which is required for many enzyme reactions mainly in amino acid metabolism (Tambasco-Studart *et al.,* 2005). Human cells synthesise PLP from pyridoxine, pyridoxal, and pyridoxamine; thus, these compounds should be obtained from the diet (Tambasco-Studart *et al.,* 2005). Vitamin B_6 deficiency causes skin disorders, dermatitis, cracks in the corners of the mouth, anaemia, kidney stones, and nausea (Bellows and Moore, 2012).

Vitamins B_2 and/or B_6 are present in many common foods such as meat, eggs, milk, grains, and vegetables (IOM, 1998; Bellows and Moore, 2012). However, vitamins B_2 and B_6 derived from plant sources are of great interest because of their impact on human health (Asensi-Fabado and Munné-Bosch, 2010). Spinach, which is a member of the Amaranthaceae family, is an excellent vegetable source of vitamins B_2 and B_6 (Vicente *et al.,* 2009; Verma, 2018). Therefore, other vegetables or grains in the Amaranthaceae family, including amaranth, Swiss chard, beet, and quinoa are likely good sources of vitamins B_2 and B_6. Nevertheless, the vitamins B_2 and B_6 levels in some edible parts of vegetables or grains in the Amaranthaceae family have not yet been compared.

In the present work, we analysed vitamins B_2 and B_6 in the main edible parts of five crops in the Amaranthaceae family, including amaranth grain, Swiss chard leaf, beet root, quinoa grain, and spinach leaf using HPLC-FLD, and then compared the contents of the two vitamins in these foods.

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Materials and methods

Plant materials

In the present work, we used the main edible parts of five crops in the Amaranthaceae family, including amaranth grain (*Amaranthus* spp.), Swiss chard leaf (*Beta vulgaris* subsp. *vulgaris* var. *cicla*), beet root (*Beta vulgaris* subsp. *vulgaris* var. *conditiva*), quinoa grain (*Chenopodium quinoa*), and spinach leaf (*Spinacia oleracea*), as shown in Figure 1. All of the samples were purchased three times from the market located in Jeju City in 2018. Four samples, except for quinoa grain which was imported from Peru, were harvested at the right time in Korea. In the case of leaf or root samples, the whole was sliced into smaller pieces, and then stored at -80°C. The grain samples were stored at 4°C.

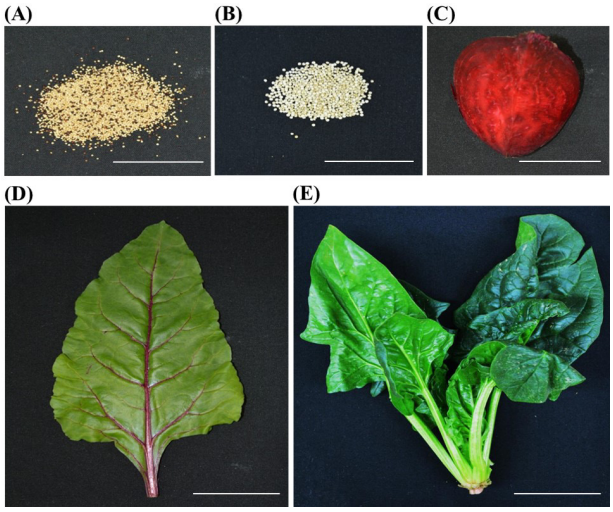

Figure 1. Representative pictures of the samples used in the present work. (A) amaranth grain (*Amaranthus* spp.), (B) quinoa grain (*Chenopodium quinoa*), (C) beet root (*Beta vulgaris* subsp. *vulgaris* var. *conditiva*), (D) Swiss chard leaf (*Beta vulgaris* subsp. *vulgaris* var. *cicla*), and (E) spinach leaf (*Spinacia oleracea*). Scale bar: 5 cm.

Sample preparation

Vitamins B_2 and B_6 were extracted according to a previously described method (Sami *et al.,* 2014). Samples were ground with liquid nitrogen using a mortar and pestle, and approximately 2 g of sample powder (fresh weight, FW) was mixed with 10 mL of 0.1 N sulphuric acid (Daejung, Siheung, Korea). The mixture was incubated at 121°C for 30 min using an autoclave. Later, the extract was placed at 4°C for 2 h, and then, the pH was adjusted to 4.5 with 2.5 M sodium acetate (Daejung, Siheung, Korea). After adjusting the pH, 50 mg of Taka-Diastase enzyme (Sigma, St. Louis, USA) was added, and the extract was incubated overnight at 37°C. The volume of the extract was adjusted to 15 mL with pure water and filtered through filter paper (No. 5C). The final solution was filtered again through a 0.22 µm syringe filter and used for HPLC analysis.

Standard preparation

Stock solutions of vitamins B_2 and B_6 were prepared by separately dissolving 10 mg of vitamin B_2 and B_6 standards (Supelco, Bellefonte, USA) in 100 mL of 0.05 M disodium phosphate at pH 6.5 (Yakuri, Osaka, Japan). Six different concentrations of working solution (0.01, 0.05, 0.1, 0.5, 1.0, and 2.0 µg/mL for vitamin B_2; and 0.005, 0.01, 0.05, 0.1, 0.5, and 1.0 µg/mL for vitamin B_6) were prepared by diluting the stock solution with the dissolving solution.

HPLC conditions

Based on a previous method (Antakli *et al.,* 2015), the extract was separated on a Shim-pack GISODS column (250 × 4.6 nm, 5 µm) at 40°C, under gradient conditions of mobile phase A and B; where A was 5.84 mM hexane-1-sulfonic acid sodium:acetonitrile (95:5) with 0.1% triethylamine (pH = 2.5, adjusted with 1 M phosphoric acid), and B was 5.84 mM hexane-1-sulfonic acid sodium:acetonitrile (50:50) with 0.1% triethylamine (pH = 2.5, adjusted with 1 M phosphoric acid). Gradient conditions started with a 100% mobile phase composition of A. Over 5 min, the gradient elution was performed until the mobile phase composition was 50% A and 50% B. After maintaining the conditions for 5 min, the mobile phase composition was returned to 100% A for 5 min. The flow rate was 1.6 mL/min, and the injection volume was 20 µL. Detection was performed with FLD, which was programmed at λex = 296 nm and λem = 390 nm for vitamin B_2 during the first 7 min, and then at λex = 450 nm and λem = 530 nm for vitamin B_6 from 7 min to 20 min (Antakli *et al.,* 2015).

Statistical analysis

All experiments were conducted in triplicate. The data are presented as mean ± SD. Analysis of variance (ANOVA) was performed using SPSS version 20 (IBM, New York, USA). Differences among sample means were generally evaluated using Duncan's multiple range test at a 95% confidence level ($p < 0.05$).

Results and discussion

Detection of vitamins B_2 and B_6 by HPLC-FLD

To quantify vitamins B_2 and B_6, six different concentrations of each standard solution were analysed by HPLC-FLD. The peaks corresponding to vitamins B_2 and B_6 were detected at 7.630

and 6.430 min, respectively (Figure 2). The calibration curves of vitamins B_2 and B_6 obtained by plotting the peak area versus concentration were linear with high correlation coefficients (both $R^2 >$ 0.999). The peaks corresponding to vitamins B_2 and B_6 were successfully detected in all sample extracts, showing retention times of 7.622 ± 0.004 and 6.446 ± 0.047 min, respectively (Figure 2). These results indicated that the HPLC-FLD conditions used in the present work were adequate to estimate the riboflavin and pyridoxine contents in our samples. The HPLC used in the present work was connected not only to FLD, but also to UVD (190 - 800 nm). However, two compounds present in our samples were well detected by FLD but not by UVD (270 nm), suggesting that FLD was more sensitive than UVD to analyse vitamins B_2 and B_6. Moreover, our HPLC-FLD system was efficient in analysing these two components simultaneously with different absorption and emission wavelengths.

Contents of vitamin B_2 in the Amaranthaceae family

All samples investigated in the present work contained vitamin B_2 which ranged from 0.074 ± 0.011 to 0.439 ± 0.094 µg/g FW (Figure 3). Relatively high contents of vitamin B_2 were found in spinach leaf (0.439 ± 0.094 µg/g FW), amaranth grain (0.431 ± 0.023 µg/g FW), and quinoa grain (0.419 ± 0.055 µg/g FW), which all showed insignificant difference, followed by Swiss chard leaf (0.212 ± 0.003 µg/g FW) and beet root (0.074 ± 0.011 µg/g FW).

In the USDA food composition database, vitamin B_2 contents of the edible portion of each cereal or vegetable used in the present work were recorded as follows: raw spinach, 1.89 µg/g; uncooked amaranth grain, 2.00 µg/g; uncooked quinoa, 3.18 µg/g; raw chard, 0.90 µg/g; and raw beet, 0.40 µg/g (USDA, 2018). These contents are much higher than those in our samples, and these differences might be caused by differences in experimental materials, cultivars, and cultivation conditions. Indeed, in a previous report, it was reported that some vegetables distributed in Korea such as spinach, bean sprout, cabbage, and carrot contained approximately 0.48, 0.40, 0.08, and 0.068 µg/g FW of vitamin B_2 , respectively (Chung *et al.,* 2016). The vitamin B_2 content in spinach in this report is similar to our result, which also showed that the highest content of vitamin B_2 was in spinach as compared to the other analysed samples. Other samples such as amaranth and quinoa grain analysed in the present work also contained a very good level of vitamin B_2, which was comparable with other vegetables such as spinach and bean sprout distributed in Korea. These results indicated that the spinach leaf, amaranth grain, and quinoa grain could be good vegetable sources for vitamin B_2 in the Amaranthaceae family.

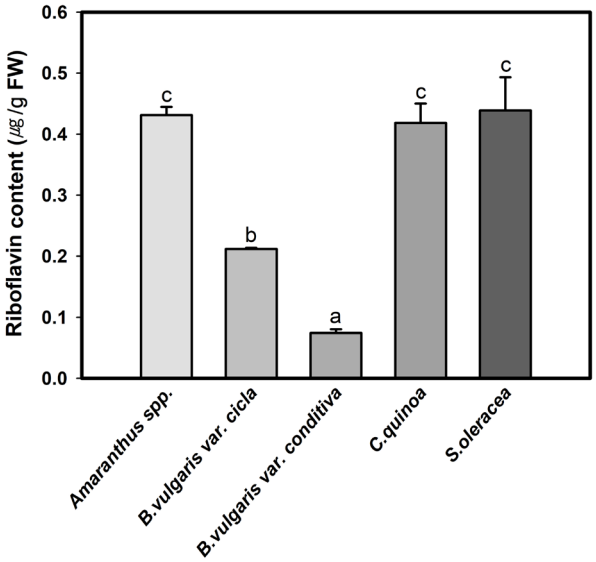

Figure 3. Vitamin B_2 (riboflavin) contents in the main edible part of crops in the Amaranthaceae family. Data are mean with error bar indicating SD. Different letters indicate statistically significant difference ($p < 0.05$).

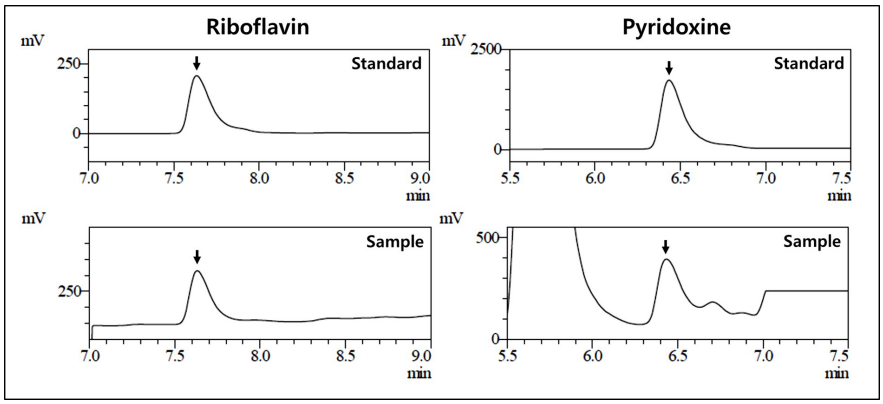

Figure 2. Representative HPLC-FLD chromatograms of vitamin B_2 (riboflavin) and vitamin B_6 (pyridoxine) in the authentic standard and sample.

Contents of vitamin B_6 in the Amaranthaceae family

Vitamin B_6 was also detected in all of the samples investigated in the present work which ranged from 0.070 ± 0.010 to 0.321 ± 0.030 μg/g FW (Figure 4). Quinoa grain contained the highest content of vitamin B_6 (0.321 ± 0.030 μg/g FW), followed by amaranth grain (0.184 ± 0.003 μg/g FW), beet root (0.145 ± 0.007 μg/g FW), spinach leaf (0.093 ± 0.009 μg/g FW), and Swiss chard leaf (0.070 ± 0.010 μg/g FW).

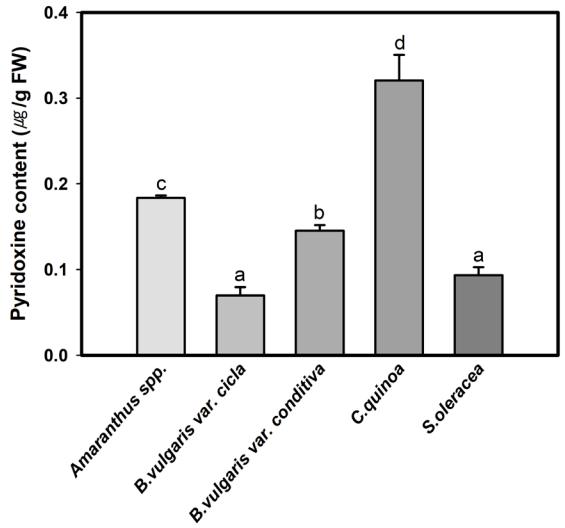

Figure 4. Vitamin B_6 (pyridoxine) contents in the main edible part of crops in the Amaranthaceae family. Data are mean with error bar indicating SD. Different letters indicate statistically significant difference ($p < 0.05$).

In a previous report (Choi *et al.,* 2017), the vitamin B_6 content in quinoa cultivated in Korea was estimated to be 0.03 μg/g FW, which is lower than that in our samples. On the other hand, the vitamin B_6 contents in red and yellow amaranth grains cultivated in Korea were determined to be 0.32 and 0.51 μg/g FW, respectively (Choi *et al.,* 2017), which are higher than that in our amaranth grain sample. Such differences in contents might be caused by differences in experimental materials, cultivars, and cultivation conditions. Choi *et al.* (2017) also reported the vitamin B_6 content in various agricultural products (62 species and 114 fresh products) cultivated in Korea, from which vitamin B_6 was detected in 105 fresh products with an average content of 0.303 μg/g FW. Based on this report, the quinoa grain distributed in Korea with the above average content could be a good vegetable source for vitamin B_6 among members of the Amaranthaceae family.

Conclusion

In the present work, vitamin B_2 (riboflavin) and vitamin B_6 (pyridoxine) contents in the main edible parts of five crops in the Amaranthaceae family, namely amaranth grain, Swiss chard leaf, beet root, quinoa grain, and spinach leaf were determined by HPLC-FLD analysis. Both vitamins were detected in all samples, and their contents ranged from 0.074 - 0.439 and 0.070 - 0.321 μg/g FW, respectively. The highest content of vitamin B_2 was found in spinach leaf (0.439 ± 0.094 μg/g FW), followed by amaranth grain (0.431 ± 0.023 μg/g FW), and quinoa grain (0.419 ± 0.055 μg/g FW), which both had similar vitamin B_2 contents to that in spinach leaf. The highest content of vitamin B_6 was found in quinoa grain (0.321 ± 0.030 μg/g FW), followed by amaranth grain (0.184 ± 0.003 μg/g FW). Among the five crops, quinoa grain contained the highest contents of both vitamins as compared to the other samples. Based on these results, we conclude that the main edible part of crops in the Amaranthaceae family, especially quinoa grain, might be a good vegetable source for the consumption of both vitamins B_2 and B_6. Particularly, as quinoa possesses unique nutritional value among crops of the Amaranthaceae family (Angeli *et al.,* 2020), our findings will further enhance the nutritional value of quinoa. Although the contents of both vitamins might be different depending on the cultivar, cultivation condition, and etc., the results obtained in the present work could still serve as valuable preliminary data for estimating their contents in crops of the Amaranthaceae family.

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