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Amino Acids Content in Different Cultivars of Young Radish (*Raphanus sativus*)

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The variations in the amino acid contents of five different Korean cultivars of young radish were investigated in this study. Twenty-one amino acids were detected among the different cultivars and the presence and quantity of amino acids varied greatly among the cultivars. The cultivar Martjjang contained the highest total quantity of amino acids, with values 3.65, 2.26, 2.10 and 1.80 times higher than the total amino acid content in the Saebaram, Chuha, Chudong and Super Red cultivars, respectively. Martjjang also contains the highest levels of all amino acids except for a few. The levels of leucine, lysine, γ-amino butyric acid, asparagine and alanine were much higher in the Martjjang cultivar, which had 23.58, 12.01, 3.71, 3.16 and 2.83 times higher concentrations of these amino acids, respectively, than the Saebaram cultivar, which had the lowest content. In all cultivars, the amino acids aspartate, serine, glycine, threonine, cysteine, valine, methionine, phenylalanine and isoleucine were found in moderate quantities, with Martjjang containing the highest quantity of each. The levels of aspartate, serine, glycine, threonine, cysteine, valine, phenylalanine and isoleucine in Martjjang were 3.83, 5.39, 2.10, 5.22, 4.31, 6.58, 5.02 and 9.36 times higher, respectively, than those in the Saebaram cultivar, which had the lowest concentrations of these amino acids. The levels of vitamin U, glutamine and histidine were the lowest irrespective of cultivar, with Martjjang containing the highest quantity of each except histidine. The cultivar Super Red contains the second highest quantity of total amino acids (738.26 mg/100 g dry wt.), where only histidine and arginine were found in the highest quantities. In conclusion, the present results demonstrate that Korean young radish cultivars contain variable quantities of amino acids, while the Martjjang cultivar showing the greatest potential to serve as a commercial source of amino acids.

Keywords: Amino acids, Cultivars, Young radish, Vegetables, Korean side dish.

INTRODUCTION

Brassicaceae crops are among the most commonly grown vegetables worldwide and include radishes, cabbage, kale, brusselssprouts, cauliflower, broccoli and kohlrabi. These plants containing lucosinolates and their breakdown products [1]. Anthocyanins isolated from red-coloured radishes are widely used as natural food colouring agents [2]. These compounds can be differentiated from those that are isolated from other plants based on the structure of aglycon [3,4]. The young stages of Brassicaceae vegetables, including radish, have attracted attention as functional vegetables because of their beneficial nutritional components, which include amino acids, fiber, minerals, carbohydrates andproteins [5]. In fact, most of the protein required by humans comes from plant sources [6]. The most important function of amino acids is their role as the building blocks of proteins. Amino acids also have antioxidant effects [7-10] and in plants, free amino acids are needed in secondary metabolism and the biosynthesis of compounds, such as the glucosinolates and phenolics, that play important roles, either directly or indirectly, in plant-environment interactions and human health [11].

Young radish is a very popular vegetable in Korea, where it is known as yeolmu. Young radish water kimchi is one of the many types of kimchi, a popular banchan (Korean side dish). Although young radish is small and thin and does not have much use, its thick and abundant green leaves are frequently used throughout spring and summer to make yeolmu kimchi. Yeolmu kimchi is popular in the summer and is often eaten with cold noodles. Many authors have reported that kimchi has beneficial properties, which include antimutagenic [12], anticancer [13], antioxidant [14], antiaging [15], antiatherogenic [16] and antidiabetic [17] activities and fibrinolytic effects [18].

Several studies have addressed the different nutritive properties and various uses of young radish. However, to our best of knowledge, no study has reported the amino acid contents of the different cultivars of young radish grown in Korea [19,20]. The objective of the present study was to determine the profile and quantity of amino acids present in different cultivars of Korean young radish.

EXPERIMENTAL

Five Korean cultivars (Super Red, Chudong, Saebaram, Martjjang and Chuha) of young radish were maintained in a greenhouse at the Chungnam National University Experiment Farm, Daejeon, Korea. Twenty plants of each cultivar were grown in a greenhouse maintained at 25 °C, 70 % RH and a 14:10 light:dark cycle. Seeds of these five cultivars were harvested on July 30, 2015 and immediately freeze-dried at -80 °C for at least 72 h and then ground with a mortar and pestle into a fine powder for amino acid analysis.

Trichloroacetic acid (TCA, 99 %) was obtained from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). Sixteen amino acid standards and four amino acid supplements were obtained from Agilent Technologies (Waldbronn, Germany). Vitamin U (DL-methionine methylsulfonium chloride) standards and sodium phosphate monobasic monohydrate (NaH_2PO_4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). High performance liquid chromatography (HPLC)-grade acetonitrile and methanol were supplied by J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water with a resistivity of 18.2 MΩ/cm was produced using a PureLab Option system from ELGA LabWater (Model LA 621; Marlow, UK).

Extraction and HPLC of free amino acids: 100 mg of freeze-dried plant powder was measured and placed in a 2 mL Eppendorf tube, to which 1.2 mL 5 % (v/v) trichloro acetic acid solution was added. After it was vortexed, the mixture was allowed to stand for at least 1 h at room temperature and then centrifuged at 15,000 ×g for 15 min at 4 °C. The supernatant was filtered through a 0.45 μm hydrophilic polyvinylidene difluoride (PVDF) syringe filter (Ø 13 mm, Cat. no. 6779-1304; Whatman Int. Ltd., Maidstone, UK) into an HPLC vial.

HPLC analysis of the free amino acids was conducted according to Kim *et al*. [21]. Briefly, 22 different free amino acids were identified using an Agilent 1200 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with Zorbax Eclipse Amino Acid Analysis (AAA) columns (150 × 4.6 mm i.d., particle size 5 μm) and Zorbax Eclipse AAA Guard columns (12.5 × 4.6 mm i.d., particle size 5 μm, 4-pack). The HPLC conditions were set at a wavelength of 338 nm, 40f and a flow rate of 2.0 mL/min. The mobile phase consisted of 40 mM NaH_2PO_4 (pH 7.8, solvent A) and CH_3CN: MeOH:H_2O (45:45:10, v/v/v) (solvent B). The HPLC gradient protocol was as follows: a linear step from 0 to 57 % of solvent B from 1.9 to 21.1 min; from 57 to 100 % of solvent B from 21.1 to 21.6 min; isocratic conditions with 100 % solvent B from 21.6 to 25.0 min; followed by a rapid drop to 0 % solvent B at 25.1 min and then isocratic conditions with 0 % solvent B until completion (total 30 min). A solution (50 pmol/μL [0.05 mM]) of 20 amino acids was prepared as a standard. The quantification of free amino acids was based on HPLC peak areas calculated as equivalents of the standard compounds and all quantities are expressed as mg per 100 g dry weight (DW). All samples were run in triplicate.

RESULTS AND DISCUSSION

Twenty-two amino acids were compared to determine the identity and quantity of amino acids from five cultivars of young radish. The amino acid norvaline was not found in any of the young radish cultivars. Vitamin U, tyrosine and methionine were not detected in the Saebaram cultivar. In addition, no vitamin U was detected in the Chudong cultivar.

The Martjjang cultivar contained the highest total quantity of amino acids (1330.84 mg/100 g dry wt.), which was 3.65,

TABLE-1
AMINO ACID CONTENT IN DIFFERENT CULTIVARS OF YOUNG RADISH (mg/100 g dry wt.)

Amino acids	Cultivars				Chuha
	Super Red	Chudong	Saebaram	Martjjang	
Aspartate	28.44 ± 2.40	15.35 ± 0.07	14.24 ± 0.29	54.60 ± 1.47	17.82 ± 0.59
Glutamate	19.71 ± 0.23	23.51 ± 0.22	19.81 ± 0.65	27.37 ± 1.44	29.97 ± 2.34
Asparagine	61.20 ± 7.88	78.09 ± 10.87	48.88 ± 3.33	154.27 ± 12.71	126.10 ± 8.45
Serine	13.92 ± 0.89	11.89 ± 1.38	6.63 ± 0.17	35.73 ± 0.39	13.39 ± 0.42
Vitamin U	3.55 ± 0.86	0.00	0.00	5.11 ± 2.27	1.08 ± 1.53
Glutamine	4.94 ± 0.11	6.95 ± 1.49	4.74 ± 0.33	13.83 ± 2.68	9.87 ± 2.31
Histidine	11.05 ± 0.07	8.62 ± 0.25	5.93 ± 1.38	9.16 ± 2.05	5.09 ± 1.07
Glycine	31.87 ± 2.12	22.43 ± 1.76	18.93 ± 1.63	39.49 ± 5.56	22.21 ± 3.08
Threonine	15.46 ± 2.65	12.04 ± 3.00	6.65 ± 1.96	34.75 ± 3.83	10.31 ± 2.94
Arginine	87.90 ± 0.24	54.31 ± 9.71	54.80 ± 2.26	83.63 ± 11.33	55.99 ± 6.84
Alanine	79.43 ± 4.53	84.66 ± 7.14	49.54 ± 5.86	140.19 ± 7.28	99.43 ± 8.08
γ-Amino butyric acid	63.28 ± 3.63	43.71 ± 7.95	28.91 ± 4.33	107.16 ± 8.56	36.78 ± 8.40
Tyrosine	57.96 ± 15.65	1.86 ± 2.63	0.00	88.65 ± 46.53	1.51 ± 2.14
Cystine	37.89 ± 9.34	19.77 ± 1.83	10.70 ± 1.56	46.15 ± 8.31	11.54 ± 3.16
Valine	28.05 ± 4.90	14.33 ± 0.58	9.09 ± 2.29	59.78 ± 3.47	12.08 ± 1.20
Methionine	17.90 ± 6.93	3.40 ± 0.81	0.00	36.59 ± 4.59	2.94 ± 1.68
Norvaline	0.00	0.00	0.00	0.00	0.00
Tryptophan	56.57 ± 9.23	183.21 ± 89.66	56.35 ± 23.89	69.45 ± 7.97	92.88 ± 42.11
Phenylalanine	17.80 ± 3.28	14.16 ± 1.16	9.84 ± 0.20	49.36 ± 12.27	11.13 ± 1.24
Isoleucine	15.60 ± 2.17	7.80 ± 1.04	4.86 ± 0.51	45.51 ± 14.91	5.66 ± 1.17
Leucine	25.48 ± 2.70	8.34 ± 1.22	4.17 ± 0.37	98.32 ± 4.50	4.90 ± 0.08
Lysine	60.26 ± 9.91	17.81 ± 2.90	10.97 ± 0.12	131.78 ± 1.54	16.95 ± 0.40
Total	738.26 ± 22.39	632.26 ± 95.13	365.05 ± 5.87	1330.84 ± 132.24	587.64 ± 11.77

The values represent the mean ± standard deviation (n = 3).

2.26, 2.10 and 1.80 times higher than the total amino acid contents of the Saebaram, Chuha, Chudong and Super Red cultivars, respectively. Martjjang also contained the highest levels of all amino acids except for glutamate, histidine, arginine and tryptophan.The levels of asparagine, arginine, alanine, GABA, tyrosine, tryptophan, leucine and lysine were much higher irrespective of cultivar. The level of asparagine ranged from 48.88 to 154.27 mg/100 g dry weight among the cultivars andMartjjang contained the highest level, which was 3.16, 2.52, 1.97 and 1.22 times higher than that in Saebaram, Super Red, Chudong and Chuha, respectively. The amount of arginine among the cultivars ranged from 54.31 to 87.90 mg/100 g dry weight; thus, the variation of arginine content was lower among the cultivarsthan that of asparagine.The alanine content was 2.83, 1.76, 1.66 and 1.41 times higher in the Martjjang cultivar compared to that in Saebaram, Super Red, Chudong and Chuha, respectively.The variations of the leucine and lysine contentsin the cultivars were much higher than for any of the other studied amino acids. The leucine content ranged from 4.17 to 98.32 mg/100 g. The Martjjang cultivar contained the highest level of leucine, which was23.58, 20.10, 11.79 and 3.86 times higher than that of Saebaram, Chuha, Chudong and Super Red, respectively.Thelysine content among the cultivarsrangedfrom 10.97 to 131.78 mg/100 g. The Martjjang cultivar contained the highest level of lysine, which was12.01, 7.77, 7.40 and 2.19 times higher than that of Saebaram, Chuha, Chudong and Super Red, respectively. The tyrosine content among the cultivars was too much higher. No tyrosine was detected in the Saebaram cultivar. On the other hand,in both the Martjjang and Super Red cultivars, the tyrosine content was much higher than in Chudong and Chuha. The highest level of tyrosine was obtained in the Martjjang cultivar (88.65 mg/100 g dry wt), followed by the Super Red cultivar (57.96 mg/100 g dry wt). Both Chuha (1.51 mg/100 g dry wt) and Chudong (1.86) contained a small amount of tyrosine. The GABA content varied widely among the cultivars, ranging from 28.91 to 107.16 mg/100 g. The Martjjang cultivar contained the highest level of GABA, which was 3.71, 2.91, 2.45 and 1.69 times higher than that of Saebaram, Chuha, Chudong and Super Red, respectively.

The amino acids aspartate, serine, glycine, threonine, cysteine, valine, methionine, phenylalanine and isoleucine were found in moderate quantities in each cultivar, with Martjjang containing the highest quantity of each. Theseamino acids were found at levels above 30 mg/100 g dry weight in the Martjjang cultivar. The levels of aspartate, serine, glycine, threonine, cysteine, valine, phenylalanine and isoleucine in Martjjang were 3.83, 5.39, 2.10, 5.22, 4.31, 6.58, 5.02 and 9.36 times higher, respectively, than those in the Saebaram cultivar, which had the lowest concentrations of these amino acids. The level of methionine was 12.44 times higher in Martjjang than in the Chuha cultivar, which had the lowest concentration of this amino acid. The levels of vitamin U, glutamine and histidine were the lowest of all measured amino acids irrespective of cultivar, with Martjjang containing the highest quantity of each except histidine. The cultivar Super Red contained the second highest quantity of total amino acids (738.26 mg/100 g dry wt) and this cultivar had the highest contents of only histidine and arginine.

Although in most cases the cultivar Martjjang had the highest levels of amino acids among all cultivars, the cultivar Super Red contained the highest level of histidine and arginine. The levels of histidine in the Super Red cultivar were 2.17, 1.86, 1.28 and 1.21 times higher than in Chuha, Saebaram, Chudong and Martjjang, respectively. The Super Red cultivar also contained the highest level of arginine, which was slightly higher than that in the Martjjang cultivar and 1.62, 1.60 and 1.57 times higher than that in Chudong, Saebaram and Chuha, respectively. The Chuha cultivar contained the highest level of glutamate, which was1.52, 1.51, 1.27 and 1.10 times higher than that in Super Red, Saebaram, Chudong and Martjjang, respectively. The tryptophan content ranged from 56.35 to 183.21 mg/100 g and the Chudong cultivar contained the highest level of this amino acid, which was3.25, 3.24, 2.64 and 1.97 times higher than that in Saebaram, Super Red, Martjjang and Chuha, respectively.

Amino acids content varied among the cultivars, species and different parts of same species are reported previously from several studies. From a recent study it was reported that among the amino acid profiles of 3 different varieties of dry beans *i.e.*, black bean, dark red bean and cranberry bean revealed that the black bean contained the highest concentration of amino acids followed by dark red bean and cranberry samples [21]. Kim *et al*. [22,23], who found that of all the amino acids isolated from *Momordica charantia*, arginine was present in remarkably high quantities, whereas cysteine and methionine were present at the lowest concentrations. From our study, we found that the level of asparagine ranged from 48.88 to 154.27 mg/100 g dry weight among the cultivars where our study coincided with the findings of previously reported study where they found that Asparagine concentrations in the 150 genotypes ranged from 32 to 156 mg/100g dry matter in whole meal wheat flours [24]. Recent cultivar techniques on vineyards can have a marked influence on the final nitrogen content of grapes, specifically individual amino acid contents [25]. Both cultivar and culture conditions have significant effects on GABA production [26]. Previously, Li *et al*. [27] reported that the amino acid and GABA contents varied in cultivars of *Liriope platyphylla*, a finding supported by the results of the present study. Levels of cysteine and glutathione both in leaves and tubers were 1.5-fold higher in average than in control plants [28]. Variations in amino acid content have also been observed in different organs of *Scutellaria baicalensis* [29], green and red mustard [30] and different species of aloe [31]. Cultivar Nepalese high-protein soybeans having a desirable amino acid composition hold potential to increase the protein quality and diversity of North American cultivars [32].

Conclusion

The level of amino acids in different Korean cultivars of young radish varied widely. The cultivar Martjjang contained the highest total quantity of amino acids and the highest levels of most amino acids. In addition, Chodang was the only variety that contained all amino acids. The results of this study demonstrate that the different young radish cultivars exhibit variable amino acid concentrations. These cultivar-specific amino acid profiles suggest that young radishis a potentially significant source of amino acids, especially Martjjang, which contained the highest concentrations of all amino acids identified.

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