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Amino Acids Content in Different Korean Cultivars of Rapeseed (*Brassica napus*)

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This study assessed the variation in amino acid contents of five different Korean cultivars of rapeseed (*Brassica napus*). Twenty-two amino acids were detected in the cultivars of rapeseed and the amino acid content varied greatly among the cultivars. The Tamra cultivar contains the highest total quantity of amino acids, with values 43.29, 24.12, 6.46 and 3.43 % higher than the total amino acid contents of the Hanlla, Youngsan, Naehan and Tammi cultivars, respectively. The Tamra cultivar also contained the highest levels of eight different amino acids. The γ-amino butyric acid content was very high in the cultivars and was higher than the levels of half of the other amino acids. The levels of alanine and arginine were also higher than those of the other amino acids irrespective of cultivar. The Tammi cultivar contains the highest levels of alanine and arginine, which were 2.03 and 5.59 times higher, respectively, than those in the Hanlla cultivar, which had the lowest concentrations of these amino acids. Moderate levels of the amino acids serine, glutamine, threonine, cysteine, valine, norvaline, phenylalanine and isoleucine were found in each cultivar, at quantities more than 10 mg/100 g dry weight. The levels of serine, cysteine, valine and phenylalanine in the Tamra cultivar were 1.65, 1.78, 2.52 and 3.25 times higher, respectively, than those in the Hanlla cultivar, which had the lowest concentrations of these amino acids. The levels of glutamate, vitamin U, histidine, tyrosine, methionine, tryptophan and leucine were the lowest of the amino acids measured irrespective of cultivar, with Tamra containing the highest levels for most of these amino acids. In conclusion, the present results demonstrate that Korean rapeseed cultivars contain variable quantities of amino acids, with the Tamra cultivar showing the greatest potential to serve as a commercial source of amino acids.

Keywords: Amino acids, Cultivars, Oil content crop, Rapeseed.

INTRODUCTION

The family Brassicaceae includes plant species that represent major vegetable crops and play important roles in maintaining a well-balanced diet [1]. Their seeds are pressed to make mustard oil and the edible leaves can be eaten as salads or mixed with other salad greens [2]. An inverse correlation between the consumption of Brassicaceae and risk of cancer has been reported [3]. Globally, mustard (*Brassica* spp.) crops are considered to be the third most important source of vegetable oil after soybean and groundnut [4]. The mustard *Brassica juncea* (L.) is an important oilseed crop. Like other Brassicaceae species, *Brassica* spp. contain various bioactive compounds such as glucosinolates and phenolics, which contribute to their nutritional value [5-7]. Rapeseed and mustard are the third most important edible oilseed crops of the world after soybean and oil palm. Rapeseed is grown as a leafy vegetable (China and Japan), oilseed (India) and turnip-rape (Europe).

Most essential amino acids are obtained from the diet because human has a limited ability to synthesize adequate levels of essential amino acids. The production and distribution of free amino acids in plants are affected by environmental factors [8-10] or artificial treatments [11,12]. More than 60 % of the protein required by human comes from plant sources [13]. The most important function of amino acids is their role as the building blocks of proteins. Amino acids also have antioxidant effects [14-17] 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 [18].

Although many amino acids exist in nature, approximately 24 are reported to be essential to human nutrition [19,20]. Several previous studies have addressed the different nutritive properties of rapeseed and other crops. However, to our best of knowledge, no study has reported the amino acid contents of the different cultivars of rapeseed grown in Korea. The objective of the present study was to determine the profile and quantity of amino acids present in different cultivars of Korean rapeseed.

EXPERIMENTAL

Five Korean cultivars (Naehan, Youngsan, Tamra, Tammi and Hanlla) of rapeseed (*Brassica napus*) 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, after which they were ground with a mortar and pestle into a fine powder for amino acid analysis.

Trichloro acetic acid (TCA, 99.0 %) 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 (ACN) and methanol (MeOH) 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, followed by the addition of 1.2 mL 5 % (v/v) trichloro acetic acid solution. After being 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 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 milligrams per 100 grams dry weight (DW). All samples were run in triplicate.

RESULTS AND DISCUSSION

A total of 22 different amino acids were identified (Table-1) from the analysis of the different Korean cultivars of rape seed (*Brassica napus*). Among the five rape seed cultivars, the cultivar Naehan contained all 22 amino acids. Vitamin U was not detected in the Youngsan, Tamra, and Tammi cultivars. Methionine was not found in the Youngsan and Hanlla cultivars. The Tamra cultivar contained the highest total quantity of amino acids (918.69 mg/100 g dry wt.), which

TABLE-1
AMINO ACIDSCONTENT IN DIFFERENT CULTIVARS OF RAPESEED (mg/100 g dry wt.)

Amino acids	Cultivars				
	Naehan	Youngsan	Tamra	Tammi	Hanlla
Aspartate	22.98 ± 0.26	19.47 ± 0.05	43.38 ± 0.56	45.44 ± 0.73	19.76 ± 0.42
Glutamate	6.50 ± 0.33	4.07 ± 0.09	5.19 ± 0.03	8.98 ± 0.14	3.31 ± 0.04
Asparagine	55.17 ± 0.10	47.01 ± 0.39	54.13 ± 0.17	58.47 ± 0.36	39.39 ± 0.22
Serine	10.56 ± 0.22	9.70 ± 0.04	14.34 ± 0.20	11.08 ± 0.17	8.71 ± 0.11
Vitamin U	5.21 ± 0.00	0.00	0.00	0.00	1.60 ± 0.01
Glutamine	6.47 ± 0.56	7.00 ± 0.76	8.87 ± 0.91	12.25 ± 0.97	7.49 ± 0.63
Histidine	4.59 ± 0.10	1.80 ± 0.02	4.25 ± 0.04	3.84 ± 0.03	2.04 ± 0.04
Glycine	34.92 ± 0.00	14.03 ± 0.19	18.75 ± 0.09	13.02 ± 0.10	23.02 ± 0.10
Threonine	11.26 ± 0.08	5.72 ± 0.36	11.00 ± 0.02	14.60 ± 3.22	8.15 ± 0.09
Arginine	109.11 ± 1.70	22.70 ± 1.26	46.94 ± 0.10	44.08 ± 0.46	19.51 ± 0.51
Alanine	104.30 ± 1.00	87.01 ± 0.11	79.87 ± 0.65	129.69 ± 2.07	63.90 ± 1.34
γ-Amino butyric acid	366.98 ± 0.43	449.89 ± 1.82	509.76 ± 6.30	452.87 ± 1.78	366.89 ± 1.35
Tyrosine	5.00 ± 0.01	3.30 ± 0.57	6.46 ± 2.28	4.58 ± 0.10	3.47 ± 0.13
Cystine	12.18 ± 1.60	10.10 ± 0.00	18.01 ± 0.33	10.89 ± 0.11	11.08 ± 0.07
Valine	12.28 ± 0.57	7.77 ± 0.98	14.21 ± 1.65	13.42 ± 0.20	5.64 ± 0.33
Methionine	1.42 ± 0.04	0.00	4.12 ± 0.12	2.38 ± 0.78	0.00
Norvaline	15.59 ± 2.13	15.61 ± 0.98	18.24 ± 0.25	17.09 ± 0.13	16.17 ± 1.18
Tryptophan	6.92 ± 1.83	5.66 ± 3.50	4.29 ± 0.14	4.05 ± 1.06	1.69 ± 0.20
Phenylalanine	9.97 ± 0.86	5.91 ± 0.11	13.57 ± 0.04	6.23 ± 1.09	4.18 ± 0.36
Isoleucine	8.68 ± 0.03	3.94 ± 0.20	9.75 ± 0.35	7.57 ± 0.07	12.98 ± 0.85
Leucine	6.67 ± 0.31	4.66 ± 0.08	6.10 ± 0.36	5.50 ± 0.03	4.47 ± 0.22
Lysine	46.19 ± 0.33	14.85 ± 0.17	27.47 ± 0.07	22.16 ± 0.20	17.66 ± 0.26
Total	862.95 ± 4.15	740.19 ± 8.15	918.69 ± 11.47	888.19 ± 2.71	641.12 ± 5.17

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

was 1.43, 1.24, 1.06 and 1.03 times higher than the total amino acid content of the Hanlla, Youngsan, Naehan, and Tammi cultivars, respectively. The Tamra cultivar also contained the highest levels of eight different amino acids:serine, GABA, tyrosine, cysteine, valine, methionine, norvaline, and phenylalanine. The Tammi cultivar had the second highest total amino acid content and contained the highest levels of six different amino acids:aspartate, glutamate, asparagine, glutamine, threonine, and alanine. The Naehan cultivar had the third highest total amino acid content and contained the highest levels of seven different amino acids:vitamin U, histidine, glycine, arginine, tryptophan, leucine, and lysine. The Hanlla cultivar had lowest total amino acid content and contained the highest level of isoleucine.

The GABA content was very high and was almost similar to the total value of the other 21 amino acids. The level of GABA ranged from 366.89 to 509.76 mg/100 g dry weight among the cultivars,and Tamra had the highest level, which was38.94, 38.91, 13.31 and 12.56 % higher than that of Hanlla, Naehan, Youngsan, and Tammi, respectively. Afterthe level of GABA, the levels of alanine and arginine were also higher than the levels of the other amino acids irrespective of cultivar. The level of alanine ranged from 63.90 to 129.69 mg/100 g dry weight among the cultivars,and Tammi had the highest level, which was2.03, 1.62, 1.49, and 1.24 times higher than that of Hanlla, Tamra, Youngsan, and Naehan, respectively. The amount of arginine among the cultivars ranged from 19.51 to 109.11 mg/100 g dry weight. The cultivar Naehan contained the highest level of arginine, which was5.59, 4.81, 2.48 and 2.32 times higher than that of Hanlla, Youngsan, Tammi, and Tamra, respectively.

Aside from GABA, alanine, and arginine, the levels of aspartate, asparagine, glycine, and lysine were higher irrespective of cultivar. The amount of aspartate ranged from 19.47 to 45.44 mg/100 g dry weight among the cultivars and Tammi contained the highest level, which was 2.33, 2.30, 1.98 and 1.05 times higher than that of Youngsan, Hanlla, Naehan and Tamra, respectively.

The level of asparagine ranged from 39.39 to 58.47 mg/100 g dry weight among the cultivars. The variation of asparagine content was smaller among the cultivars. The Tammi cultivar contained the highest level of asparagine,which was 48.44%, 24.38%, 8.02%, and 5.98% times higher than that of Hanlla, Youngsan, Tamra, and Naehan, respectively. The amount of lysine among the cultivars ranged from 14.85 to 46.19 mg/100 g dry weight. The lysine content was 3.11, 2.62, 2.08, and 1.68 times higher in the Naehan cultivar than in Youngsan, Hanlla, Tammi, and Tamra, respectively. Moderate levels of the amino acids serine, glutamine, threonine, cysteine, valine, norvaline, phenylalanine, and isoleucine were found in each cultivar, atqu antitiesmore than 10 mg/100 g dry weight. The levels of serine, cysteine, valine, and phenylalanine in the Tamra cultivar were 1.65, 1.78, 2.52, and 3.25 times higher, respectively, than in the Hanlla cultivar, which had the lowest concentrations of these amino acids. The levels of glutamate, vitaminU, histidine, tyrosine, methionine, tryptophan, and leucine were the lowest irrespective of cultivar, with Tammi containing the highest quantity of most of these amino acids.

The variation of amino acid contents among cultivars, species and different parts of same species was previously reported by several researchers. The amino acid concentration in the phloem sap of leaves and the protein content in seeds, two *Brassica napus* genotypes and one *B. carinata* genotype with low, medium and high seed protein contents were analyzed where amino acid content varied between two genotypes [22]. Free amino acidcontent varied among the tea cultivars [23]. Significant differences were found between cultivars, where cultivar Ma'afala contained significantly higher total essential amino acidcontent than other varieties and higher-quality protein than staples such as corn, wheat, rice, soybean, potato and pea [24]. Variation of amino acid also varied depends on the type of canopy of fruits trees. It was observed from a study of fruits from three cultivars of apple (Malus × domestica Borkh.) 'McIntosh', 'Gala' and 'Mutsu' when harvested from the exterior and interior of the tree canopy where except for histidine, lysine, threonine and glycine, most amino acids accumulated at higher concentrations in the inner-canopy fruit[25]. Variation of free amino acid were observed in three jujube fruit pulp extracts from Boeun-deachu, Mechu, and Sanzoin cultivars and two seed extracts (Mechu and Sanzoin) from plants grown in Korea [26]. Quinoa (*Chenopodium quinoa* Willd.), which is considered a pseudocereal or pseudograin, has remarkable nutritional properties; not only from its protein content (15 %) but also from its great amino acid balance which varied among the cultivars [27]. Amino acids such as lysine and methionine are also low, and some research reports indicate the absence of methionine in cassava edible roots, where their content varied among the cultivars [28].

The chestnut cultivars were Longal, Judia and Martaínha. All three cultivars had high moisture contents but were low in ash, crude fat, and crude protein contents, with high starch and low fiber contents. The free amino acid contents, including various essential amino acids, varied depending on the cultivar [29]. Free amino acids showed little change at the storage temperatures tested and varied within certain ranges characteristic of each cultivar of potatoes [30]. Accurate and detailed amino acid determinations were carried out on nine northern adapted soybean cultivars to ascertain whether their amino acid profiles could be used as potentially useful indices for assessing their protein quality. The total amino acids content was also variable among these cultivars [31]. The changes in amino acid composition that occur with maturity of the Noble cultivar of the *Vitis rotundifolia* Michx. (muscadine) grape were determined by HPLC where 18 amino acids were identified. Histidine was the most prominent amino acid followed by alanine [32].

Conclusion

Varieties showed a very significant variation for the accumulation of amino acids in different Korean cultivars of rapeseed. Among the cultivars, Tamra has been considered the best for the highest total accumulation of amino acids and also for the containing of the highest levels of 8 different amino acids. It is clearly shown that the cultivars of different rapeseed exhibit variable amino acid concentrations which might suggest that these cultivar-specific amino acid profiles belongs a potentially significant source of amino acids.

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