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Amino Acid Contents in Different Korean Cultivars of *Zea mays*

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The present study investigated the variation in amino acids from different Korean cultivars of *Zea mays*. Twenty-one amino acids were detected in four different cultivars of *Z. mays*. The presence and quantity of amino acids varied greatly among cultivars. The cultivar Chodang contained all 21 amino acids and also had the highest total amount of amino acids, with values 3.68, 2.92 and 2.40 times higher than the total amino acid concentration in the cultivars Chalok, Jahuegchal and Huegma, respectively. Among amino acids, the level of asparagine and alanine were much higher irrespective of cultivar and were found at the highest level in Chodang. In all cultivars, glutamate, serine, γ-amino butyric acid and lysine were found in moderate quantities, but were highest in Chodang. The quantity of glutamine, threonine, tyrosine and valine were much higher in the Chodang cultivar, which had 8.0, 8.6, 2.5 and 5.8 times higher concentrations of these amino acids, respectively than the Chalok cultivar, which had the lowest content. Levels of histidine, isoleucine, leucine, methionine, norvaline, tryptophan and phenylalanine were low, irrespective of cultivar. However, Chodang contained the highest level of these amino acids. The cultivar Jahuegchal contained 1.8, 1.7 and 1.2 times higher levels of glycine than that of Chalok, Huegma and Chodang, respectively. Although Chalok contained the lowest total amount of amino acids and the levels of most amino acids were the lowest, this cultivar contained the highest level of aspartate, which was 1.4, 1.3 and 1.2 times higher than that in the Huegma, Chodang and Jahuegchal cultivars, respectively. In conclusion, our results demonstrate that Korean *Z. mays* cultivars contain variable quantities of amino acids, with the Chodang cultivar showing the greatest potential to serve as a marketable source of amino acids.

Keywords: Amino acids, Cultivars, *Zea mays*, Grain cereal.

INTRODUCTION

Maize (*Zea mays* L.) is the most widely grown crop in the Americas, with 70-100 million acres grown annually in the US alone, accounting for nearly 40 % of global maize production. This versatile crop can be grown in several different environments and on every continent except Antarctica. Maize also has a higher yield than many other grains, making it relatively inexpensive. Together with wheat (*Triticum*) and rice (*Oryza*), maize is one of the three most important food crops grown worldwide.

The maize kernel is composed of approximately 72 % starch, 10 % protein, 5 % oil, 2 % sugar and 1 % ash, with the remainder being water [1]. Maize oil is an important energy source in livestock feed and is one of the most common unsaturated fats consumed by humans [1]. Increasing the supply of nitrogen to maize generally results in increased yield and protein concentration [2-7]. Tsai *et al.* [8] suggested that the protein concentration of maize grains increases with nitrogen, because of the preferential deposition of zein compared to other endosperm proteins. As the protein concentration of corn grain increases, zein comprises an increasing proportion of the protein [6,9].

More than 60 % of the protein required by humans comes from plant sources [10]. The most important function of amino acids is their role as the building blocks of proteins. Amino acids have antioxidant effects [11-14] and free amino acids are needed in secondary plant 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 [15].

Although many amino acids exist in nature, approximately 24 are reported to be essential to human nutrition [16,17]. Several previous studies have addressed the different nutritive properties of maize and other crops. However, to our best of knowledge, no study has reported the amino acid content in the different cultivars of maize 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 maize.

EXPERIMENTAL

Four Korean cultivars (Chalok, Chodang, Jahuegchal and Huegma) of *Z. mays* were maintained in a greenhouse at the Chungnam National University Experiment Farm, Daejeon, Korea. The seed coat colour of Chalok, Chodang, Jahuegchal and Huegma is white, yellow, purple and black, respectively. 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 four 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, 99.0 %) 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) trichloroacetic 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.* [18]. Briefly, 20 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 mm, 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 ACN: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 were expressed as milligrams per 100 g fresh weight (FW). All samples were run in triplicate.

RESULTS AND DISCUSSION

Twenty-one amino acids were compared to determine the identity and quantity of amino acids from four cultivars of *Z. mays*. Among the four cultivars, Chodang contained all 21 amino acids. Methionine, norvaline, tryptophan, phenylalanine and leucine were not detected in the Chalok cultivar. In addition, methionine and norvaline were not detected in the cultivar Jahuegchal and no methionine, norvaline, or tryptophan was detected in the Huegma cultivar. Chodang contained the highest total quantity of amino acids (444.43 mg/100 g dry wt.), which was 3.68, 2.92 and 2.40 times higher than the total amino acid content of the Chalok, Jahuegchal and Huegma cultivars, respectively. Chodang also contained the highest levels of all amino acids except for aspartate and glycine (Table-1).

TABLE-1
AMINO ACID CONTENT IN
DIFFERENT CULTIVARS OF *Zea mays*

Amino acids	Cultivars (amino acid: mg/100 g dry wt.)			
	Chalok	Chodang	Jahuegchal	Huegma
Aspartate	25.92±0.30	19.34±0.34	22.50±0.00	18.22±0.05
Glutamate	4.87±3.64	30.21±0.40	10.26±0.70	12.11±0.05
Asparagine	22.40±0.49	112.12±1.36	28.62±0.52	35.80±0.14
Serine	4.28±0.12	28.55±0.13	7.44±0.00	10.28±0.02
Glutamine	1.49±0.11	11.92±1.45	2.40±0.39	2.29±0.07
Histidine	2.44±0.07	6.31±0.15	3.24±0.19	3.87±0.01
Glycine	6.33±0.34	9.62±0.51	11.09±0.23	6.60±0.26
Threonine	1.91±0.01	16.84±0.06	3.10±0.12	4.84±0.12
Arginine	6.26±1.93	14.17±0.10	6.04±0.04	8.90±1.04
Alanine	20.97±1.01	99.43±0.62	18.29±0.06	31.37±0.06
GABA	8.18±0.39	24.72±0.19	12.47±0.05	17.89±0.27
Tyrosine	4.18±0.01	10.35±0.09	4.85±0.10	4.95±0.03
Cystine	3.68±0.26	6.73±0.06	3.33±0.14	4.74±0.07
Valine	2.44±0.05	14.27±0.08	3.51±0.10	5.47±0.00
Methionine	0.00	3.53±0.01	0.00	0.00
Norvaline	0.00	1.74±0.06	0.00	0.00
Tryptophan	0.00	1.90±0.03	0.84±1.19	0.00
Phenylalanine	0.00	3.06±0.04	2.14±0.07	1.47±0.02
Isoleucine	1.63±0.17	4.02±0.09	1.81±0.09	2.05±0.04
Leucine	0.00	5.20±0.03	1.60±0.04	2.14±0.19
Lysine	3.66±0.06	20.41±0.07	8.79±0.03	12.44±0.06
Total	120.64±0.15	444.43±3.69	152.31±1.46	185.43±0.95

[a]ND = not detected; GABA = γ-amino butyric acid

Among all amino acids, the levels of asparagine and alanine were much higher irrespective of cultivar. The range of asparagine was 22.40 to 112.12 mg/100 g the dry weight of all cultivars. Chodang contained the highest level of asparagine, which was 5.0, 3.9 and 3.1 times higher than that of Chalok, Jahuegchal and Huegma, respectively. On the other hand, the level of alanine ranged from 18.29 to 99.43 mg/100 g the dry weight of all cultivars. Chodang also contained the highest level of alanine, which was 5.4, 4.7 and 3.2 times higher than that of Jahuegchal, Chalok and Huegma, respectively. The amino acids glutamate, serine, γ-amino butyric acid and lysine were found in moderate quantities in each cultivar, with Chodang containing the highest quantity of each. The quantity of these amino acids was more than 20 mg/100 g the dry weight of the Chodang cultivar. Levels of glutamate, serine, γ-amino butyric acid and lysine in Chodang were 6.2, 6.7, 3.0 and 5.6

times higher, respectively, than that of the Chalok cultivar, which had the lowest concentration of these amino acids. The quantities of glutamine, threonine, tyrosine and valine were much higher in Chodang and were 8.0, 8.6, 2.5 and 5.8 times higher, respectively, than those of the Chalok cultivar, which had the lowest concentration of these amino acids. Levels of histidine, isoleucine, leucine, methionine, norvaline, tryptophan and phenylalanine were the lowest irrespective of cultivar, with Chodang containing the highest quantity of each.

The cultivar Huegma contained the second highest quantity of amino acids (185.43 mg/100 g dry wt), but not the greatest quantity of any particular amino acid. The cultivar Jahuegchal contained the highest level of glycine, which was 1.8, 1.7 and 1.2 times higher than that of Chalok, Huegma and Chodang, respectively. Although Chalok contained the lowest total quantity of amino acids and most were found at their lowest concentrations, this cultivar contained the highest level of aspartate, which was 1.4, 1.3 and 1.2 times higher than that of Huegma, Chodang and Jahuegchal cultivars, respectively.

Variation in amino acids among cultivars was previously reported by Kim *et al*. [18], 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. Variation in amino acid content has also been observed in different organs of *Scutellaria baicalensis* [19] and green and red mustard [20] and in different species of aloe [21]. Li *et al*. [22] reported that the amino acid and γ-amino butyric acid content varied in cultivars of *Liriope platyphylla*, a finding supported by the results of the present study.

Conclusion

The level of amino acids in different Korean cultivars of *Z. mays*varied greatly. The cultivar Chodang contained the highest total quantity of amino acids and the highest levels of 19 different amino acids. In addition, Chodang was the only variety that contained all 21 amino acids. The results of this study demonstrate that the kernels of different *Z. mays*cultivars exhibit variable amino acid concentrations. These cultivar-specific amino acid profiles suggest that *Z. mays*is a potentially significant source of amino acids, especially Chodang, which contained the highest concentration of all the amino acids identified.

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