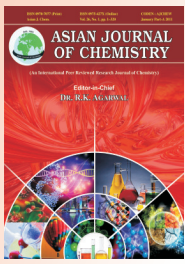
Asian Journal of Chemistry; Vol. 26, No. 7 (2014), 1910-1912

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.15568



Free Amino Acids in Different Organs of *Scutellaria baicalensis*

YEON BOK KIM[1], MD ROMIJ UDDIN[1], MIN-KI LEE[2], SUN-JU KIM[2], HAENG HOON KIM[3], JAI-HEON LEE[4,*] and SANG UN PARK[1,*]

[1]Department of Crop Science, College of Agriculture & Life Sciences, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, Republic of Korea
[2]Department of Bio-Environmental Chemistry, College of Agriculture & Life Sciences, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, Republic of Korea
[3]Department of Well-being Resources, Sunchon National University, 413 Jungangno, Suncheon, Jeollanam-do, 540-742, Republic of Korea
[4]Department of Genetic Engineering, Dong-A University, Busan 604-714, Republic of Korea

*Corresponding authors: E-mail: jhnlee@dau.ac.kr; supark@cnu.ac.kr

Received: 2 April 2013; *Accepted*: 25 June 2013; *Published online*: 22 March 2014; AJC-14927

We investigated the free amino acids content in different plant parts of golden root (*Scutellaria baicalensis*) and identified 21 different free amino acids, the levels of which varied widely between organs. The amount of free amino acids in the flower was much higher than that in the leaf (3.4-fold), stem (1.9-fold) and root (5.3-fold). Most of the free amino acids were present in higher amounts in the flower; in particular, a markedly higher amount of glutamine was observed in the flower, which contained 27, 11 and 2 times more glutamine than the root, leaf and stem. Norvaline was found only in the root, which also contained much higher amounts of leucine (37-, 28- and 9-fold higher than in the leaf, stem and flower). Our findings demonstrate that *S. baicalensis* contains varying amounts of free amino acids between plant tissues and the levels were the highest in the flower.

Keywords: Free amino acids, Golden root, Flower, Glutamine.

INTRODUCTION

Golden root (*Scutellaria baicalensis* Georgi) is a popular herb used to treat conditions such as inflammation, respiratory tract infections, diarrhea, liver disorders, hypertension, hemorrhage, and insomnia[1]. It also used as a mild relaxant that affects the neural and muscular-skeletal systems[2] and it contains a variety of flavones, phenylethanoids, amino acids, sterols and essential oils[3]. The dried root of *S. baicalensis* is rich in more than 30 different flavonoids. Baicalin, baicalein, and wogonin are the most commonly studied flavone constituents of *Scutellaria* species[4,5]. These flavonoids have many biological, pharmacological and antioxidant activities, and have been used for cancer prevention and the treatment and prevention of coronary heart disease[3,6].

Approximately 65 % of the human protein requirement comes from plant sources[7]. The importance of plant proteins varies from highly developed to poorly developed regions, depending on average local diets. Consumer awareness of food composition has increased and comprehensive information beyond that available in food composition tables is often demanded. Information on amino acid composition is limited in these tables and when it is included, it is primarily provided in terms of free and bound amino acids. The most important function of amino acids is their role as protein building blocks. Numerous researchers have reported the antioxidant effects of several amino acids[8-11]. Free amino acids are needed in secondary plant metabolism and in the biosynthesis of compounds such as glucosinolates and phenolics that play important roles, either directly or indirectly, in plant-environment interactions and human health[12].

Many amino acids exist in nature, although not all are necessary for human health. Approximately 24 different amino acids are essential to human nutrition[13,14]. Several studies have addressed the medicinal importance of *S. baicalensis* and most have been phytochemical investigations (mainly baicalin, baicalein and wogonin) from fresh or dried root. To date, no information regarding the free amino acid content in the different organs of *S. baicalensis* has been reported. We reported here the free amino acids in different organs of *Scutellaria baicalensis*.

EXPERIMENTAL

Plant material and growth conditions: *S. baicalensis* seedlings were grown under greenhouse conditions (25 °C and 50 % humidity) for 1 month and then transferred to pots filled

with perlite-mixed soil on May 30, 2012. Samples were grown under light (16 h) and dark (8 h) conditions in the greenhouse at Chungnam National University (Daejeon, Korea). Each organ (flowers, stems, leaves, and roots) was harvested 2 weeks after flowering. The organs were frozen in liquid nitrogen upon collection, stored at -80 °C and freeze-dried at -80 °C for 72 h. Dried samples were ground into a fine powder using a mortar and pestle before analysis by high-performance liquid chromatography (HPLC).

Trichloroacetic acid (TCA, 99.0 %) was obtained from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). Fifteen amino acid standards and 4 amino acid supplements were obtained from Agilent Technologies (Waldbronn, Germany). Vitamin U (DL-Methionine methylsulfonium chloride) standard and sodium phosphate monobasic monohydrate (NaH_2PO_4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile (ACN) and methanol (MeOH) were supplied by J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water with resistivity 18.2 MΩ/cm was produced by pure lab option from ELGA lab water (Model LA 621, Marlow, UK).

Extraction and HPLC analysis for free amino acids: Freeze-dried plant powder (100 mg) was weighed in a 2 mL-Eppendorf tube and 1.2 mL of 5 % (v/v) trichloroacetic acid (TCA) was added. After vortexing, the mixture was allowed to stand for at least 1 h at room temperature. After centrifugation (15,000 rpm, 4 °C, 15 min), the supernatant was filtered through a 0.45-µm hydrophilic PVDF Syringe Filter (Φ 13 mm, Whatman Int. Ltd.) into the HPLC vial. HPLC analysis of free amino acids was conducted according to the 'Rapid, accurate, sensitive and reproducible HPLC analysis of amino acids analysis' using Zorbax Eclipse-AAA Columns and the Agilent 1100 HPLC (http://www.chem.agilent.com/ Library/ chromatograms/59801193.pdf). Briefly, 21 kinds of free amino acids were assessed on the Agilent Technologies 1200 series HPLC system equipped with Zorbax Eclipse AAA analytical column (150 × 4.6 mm i.d.; particle size, 5 µm) and guard column Zorbax Eclipse AAA 4-Pack (12.5 × 4.6 mm i.d., 5 µm). The HPLC conditions were set at 338 nm wavelength, 40 °C oven temperature and 2.0 mL/min flow rate. The mobile phase consisted of 40 mM NaH_2PO_4 (pH 7.8, solvent A) and ACN:MeOH:water (45:45:10, v/v/v) (solvent B). The gradient programs were as follows: a linear step from 0 to 57 % solvent B from 1.9 to 21.1 min and from 57 to 100 % solvent B at 21.6 min, then isocratic conditions with 100 % solvent B at 25 min, followed by a rapid drop to 0 % solvent B at 25.1 min and then isocratic conditions with 0 % B at 30 min (total, 30 min). A 50 pmol/µL (0.05 mM) solution was prepared as a standard mixture of 21 amino acids. Quantification of free amino acids was based on HPLC peak areas and calculated as standard equivalents; all amounts were expressed as mg/100 g fresh weight (FW).

RESULTS AND DISCUSSION

Free amino acids in the root: A total of 21 different free amino acids were recorded in different plant parts of golden root (Table-1). The root contained all recorded free amino acids. The free amino acid norvaline was found only in the root (945.47 mg/100 g FW). The levels of vitamin U, valine, methionine, isoleucine and leucine were the highest in the root (Table-1). A markedly higher amount of leucine was observed in the root compared to other parts of the plant (Table-1). The level of leucine was approximately 37, 28 and 9 times higher in the root than in the leaf, stem and flower, respectively (Table-1). The amount of total free amino acids was much lower in the root.

Free amino acids in stem tissue: There were 19 free amino acids observed in the stem. No vitamin U or norvaline was detected in the stem (Table-1). The level of asparagine was higher in the stem, approximately 21-, 3- and 2-fold higher than in the root, leaf and flower, respectively. The glutamine

TABLE-1
FREE AMINO ACID CONTENTS (mg/100 g FRESH wt.) IN GOLDEN ROOT

No.	Free amino acids	Root	Stem	Leaf	Flower
1	Aspartate	19.89 ± 0.31	34.92 ± 0.64	60.63 ± 1.75	69.50 ± 0.16
2	Glutamate	15.43 ± 10.71	17.36 ± 0.89	25.02 ± 1.29	45.89 ± 0.29
3	Asparagine	9.48 ± 0.22	198.17 ± 3.65	70.40 ± 0.60	93.88 ± 2.02
4	Serine	6.88 ± 0.07	46.45 ± 0.86	70.76 ± 0.44	74.77 ± 1.41
5	Vitamin U	21.71 ± 0.59	ND[a]	11.81 ± 0.26	20.08 ± 1.57
6	Glutamine	109.11 ± 2.13	1513.02 ± 20.04	269.40 ± 1.82	2972.15 ± 35.54
7	Histidine	27.79 ± 0.35	29.94 ± 0.68	36.72 ± 0.20	74.40 ± 1.99
8	Glycine	3.10 ± 1.07	3.18 ± 0.12	10.48 ± 0.18	12.93 ± 0.32
9	Threonine	24.05 ± 0.63	50.43 ± 4.03	99.02 ± 0.27	201.78 ± 1.62
10	Arginine	43.50 ± 1.82	318.50 ± 4.70	390.47 ± 1.83	329.67 ± 2.77
11	Alanine	20.39 ± 0.86	17.45 ± 0.28	47.38 ± 0.45	51.21 ± 0.64
12	Tyrosine	16.17 ± 3.82	9.49 ± 0.42	51.76 ± 0.52	172.77 ± 1.36
13	Cystine	30.29 ± 1.06	29.18 ± 0.32	49.09 ± 0.60	40.68 ± 0.78
14	Valine	147.85 ± 30.02	15.29 ± 0.31	13.91 ± 0.16	53.13 ± 0.26
15	Methionine	22.93 ± 0.24	17.68 ± 1.10	12.74 ± 0.82	18.64 ± 0.37
16	Norvaline	45.47 ± 1.39	ND	ND	ND
17	Tryptophan	21.96 ± 0.11	17.77 ± 2.60	21.58 ± 1.32	17.29 ± 0.17
18	Phenylalanine	9.41 ± 0.39	12.34 ± 0.41	9.61 ± 0.12	34.68 ± 0.20
19	Isoleucine	50.50 ± 1.29	13.17 ± 0.40	15.18 ± 0.04	49.39 ± 0.31
20	Leucine	193.65 ± 6.69	6.84 ± 0.29	5.20 ± 0.36	21.08 ± 0.12
21	Lysine	7.29 ± 0.12	12.11 ± 0.56	7.24 ± 0.05	21.77 ± 0.07
Total		827.14 ± 23.36	2363.29 ± 35.35	1278.38 ± 10.95	4375.70 ± 51.78

content was also much higher in the stem than in the root and leaf. The stem contained 14- and 5.6-fold more glutamine than the root and leaf, respectively. The stem ranks second to the flower in terms of total free amino acid content. The stem contained 2.9- and 1.8-fold more total free amino acids than the root and leaf, respectively. The content of other free amino acids except asparagine and glutamine were the lowest in the stem.

Free amino acids in the leaf: Twenty free amino acids were recorded in the leaf (Table-1). The amino acid norvaline was not detected in the leaf, although this organ contained higher amounts of arginine and cystine than any other part of the golden root. The level of arginine was 9 times higher in the leaf than in the root and was slightly higher than that in the stem and flower. The leaf contained 1.54-fold greater total free amino acids than the root, but less than that in the flower and stem.

Free amino acids in the flower: The golden root flower contained higher levels of free amino acids than other organs (Table-1). Twenty different free amino acids were recorded in the flower. No norvaline was detected, although a markedly higher amount of glutamine was detected in the flower than in other parts of the plant. The flower contained 27, 11 and 2 times more glutamine than the root, leaf and stem, respectively. In addition to glutamine, the levels of aspartate, glutamate, serine, histidine, glycine, threonine, alanine, tyrosine, phenylalanine, and lysine were highest in the golden root flower. The flower contained 5.3, 3.4 and 1.9 times more total free amino acids than the root, leaf and stem, respectively.

Present results indicate that all the organs (leaf, stem, flower, and root) of *S. baicalensis* contained several free amino acids. The flower contained far more free amino acids than did other plant parts. The flower contained 5.3, 3.4 and 1.9 times more total free amino acids than the root, leaf, and stem, respectively. Some free amino acids were present in greater amounts in certain parts of *S. baicalensis*. For example, a markedly higher amount of glutamine was observed in the flower, which contains 27, 11, and 2 times more glutamine than the root, leaf and stem, respectively. Leucine content was approximately 37, 28 and 9 times higher in the root than in the leaf, stem and flower, respectively. Asparagine content was approximately 21-, 3- and 2-fold higher in the stem than in the root, leaf and flower, respectively. The level of arginine was 9 times higher in the leaf than in the root and slightly higher than in the stem and flower, respectively. Similar patterns may be found in other crops, such as higher concentrations of proline in the flower buds and flowers than in the vegetative organs of *Brassica napus*, consistent with our results[15]. An analysis of common buckwheat showed that the flowers have the highest levels of phenolic compounds compared to other plant organs[16].

Variation in amino acids among cultivars was reported previously by Kim *et al.*[17], who found that, among all the amino acids isolated from *Momordica charantia*, arginine was present in remarkably high quantities while cysteine and methionine were present at the lowest concentrations. Similar results regarding variation of amino acid content in different organs were observed in this study of *S. baicalensis*.

Conclusion

Amino acids content varied widely in the different parts of *S. baicalensis*. Flower parts contained the highest levels of total amino acids and in most cases, the highest levels of individual amino acids. Present results demonstrate that different parts of *S. baicalensis* contain varying amounts of amino acid accumulation, with the greatest accumulation in the flower. Plant part-specific amino acid profiles could prove a good resource in the development of food supplements.

ACKNOWLEDGEMENTS

This study was supported by research funds from Dong-A University, Korea, 2014.

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