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Variation of Amino Acid Content in *Magnolia denudata* and *Magnolia liliiflora* Flowers

JAE KWANG KIM[1], SUN JU BONG[2] and SANG UN PARK[2,*]

[1]Division of Life Sciences and Bio-Resource and Environmental Center, Incheon National University, Incheon 406-772, Republic of Korea
[2]Department of Crop Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, Republic of Korea

*Corresponding author: Fax: +82 42 8222631; Tel: +82 42 8215730; E-mail: supark@cnu.ac.kr

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Amino acids are the base of all life functions, as they are absolutely essential for every metabolic process. Magnolia is used as an ornamental tree in gardens and has several pharmacological properties as well. The present study investigated the variation in amino acids in the flowers of *Magnolia denudata* and *Magnolia liliiflora*. We found that 21 different amino acids varied in concentration between *M. denudata* and *M. liliiflora* flowers. *M. denudata* had the highest levels of all amino acids with the exception of glutamate. The amino acids tyrosine and methionine were not detected in *M. denudata*. However, *M. liliiflora* contained some level of all 21 amino acids. The levels of asparagine, glutamine, alanine, cysteine and aspartate were much higher than those of the other amino acids irrespective of *Magnolia* species, although in *M. denudata* the levels of asparagine, glutamine, alanine, cysteine and aspartate were 1.50, 1.57, 1.23, 2.99 and 1.82 times higher, respectively, than those measured in *M. liliiflora*. The amino acids valine, serine, threonine, γ-aminobutyric acid, leucine, arginine, glutamate and tryptophan were found in moderate quantities in the *Magnolia* species, with *M. denudata* containing the highest quantity of each amino acid. Levels of glycine, norvaline, phenylalanine, isoleucine and lysine were the lowest irrespective of *Magnolia* species; however, *M. denudata* had higher quantities of each amino acid when compared to that in *M. liliiflora.* Our results show that flowers from different *Magnolia* species contain varying amounts of amino acids, suggesting that the flowers of *Magnolia* species have excellent potential as a source of amino acids.

Keywords: Amino acids, Variation, *Magnolia denudata*, *Magnolia liliiflora.*

INTRODUCTION

Magnolia denudata, known as Yulan Magnolia is native to central and eastern China. The 10-16 cm white flowers emerge in early to late spring and have a citrus-lemon fragrance, but are prone to browning if subjected to frost. This plant is used as an ornamental tree in gardens. Another species, *Magnolia liliiflora* is a small tree native to southwest China and have been cultivated for centuries in China and Japan. *Magnolia kobus* is a medicinal plant with a wide distribution throughout Korea and Japan and the flower buds of this plant have been used as a traditional herbal medicine for the treatment of headache, nasal obstruction during cold and toothache [1]. Phytochemical studies of *M. kobus* have reported the isolation of lignans [2-4] and sesquiterpenes [3]. *Magnolia sieboldii* K. Koch (Magnoliaceae) is an important plant used in traditional Chinese medicine and is available in various forms, such as Magnoliae Cortex and Magnoliae Flos. A number of biologically active substances such as magnolol and honokiol [5-7] are isolated from plants of the Magnoliaceae family. Previous investigations have reported that some constituents of syringin, a new phenylpropanoid glycoside and sinapyl alcohol were isolated from the stem bark of *M. sieboldii*, which exhibited nitric oxide (NO) synthase inhibition in endotoxin-activated murine macrophage cells [8].

Amino acids are at the foundation of all life processes, as they are essential for metabolic processes. Among their most important functions are the optimal transport and storage of nutrients (*i.e.*, water, fat, carbohydrates, proteins, minerals and vitamins). The majority of diseases such as obesity, high-cholesterol levels, diabetes, insomnia, erectile dysfunction and arthritis can essentially be traced back to metabolic disruptions. This also applies to hair loss and serious cases of wrinkle formation. This is confirmed by various studies that stress the importance amino acids have to keep the hair healthy and the skin firm [9-11]. Furthermore, the arginine leads to a considerable expansion of the blood vessels, allowing significant reduction in blood pressure [12]. Two studies from 2013 have shown similar results [13,14], as well as provide evidence that arginine is also useful in the treatment of diabetes-related foot diseases [15].

Most of the amino acids required by humans can be derived from plant sources [16]. The most important function of amino acids is their role as the building blocks of proteins.

Previous researches have characterized the antioxidant effects of several amino acids [17-20]. Free amino acids are required for plant secondary metabolism and the biosynthesis of different compounds play important roles in plant-environment interactions. These compounds also have an impact on human health [21].

Many amino acids exist in nature but not all are essential for human bodily function. Twenty-four different amino acids are reported to be essential to human nutrition [22,23]. Several studies have addressed management strategies, different uses and nutritional values of *Magnolia* species. To date, no study has clearly reported the amino acid content in *Magnolia* species. The objective of the present study was to identify and quantify the amino acids present in the flowers of *M. denudate* and *M. liliiflora*.

EXPERIMENTAL

M. denudate and *M. liliiflora* were maintained at the Chungnam National University Experiment Farm, Daejeon, Korea. Flowers of *M. denudate* and *M. liliiflora* were harvested on April 15, 2015 and immediately freeze-dried at -80 °C for at least 72 h and then ground using a mortar and pestle into a fine powder for amino acid analysis.

Trichloroacetic acid (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: An amount of 100 mg of freeze-dried plant powder was 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 (18-21 °C) and was 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*. [24]. 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 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 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; 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 the 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. All samples were analyzed in triplicate.

RESULTS AND DISCUSSION

A total of 21 different amino acids were found at varying concentrations in the flowers of both *M. denudata* and *M. liliiflora* cultivars (Table-1). *M. denudata* contained 1.55 times higher amounts of the total amino acids (4727.78 mg/100 g dry weight) than *M. liliiflora* (3057.04 mg/100 g dry weight), with the exception of glutamate. Tyrosine and methionine were not detected in *M. denudata*. However, *M. liliiflora* contained all the 21 amino acids measured. Among all the amino acids, the levels of asparagine, glutamine, alanine, cysteine and aspartate were measured at much higher levels irrespective of *Magnolia* species. The levels of asparagine, glutamine, alanine, cysteine and aspartate ranges between the species were 1233.46-1842.70, 616.14-964.27, 584.73-751.97, 111.22-331.50 and 158.83-289.10 mg/100 g dry weight, respectively. In *M. denudate* the levels of asparagine, glutamine, alanine, cysteine and aspartate were 1.50, 1.57, 1.23, 2.99 and 1.82 times higher, respectively, than those in *M. liliiflora*. The amino acids valine, serine, threonine, γ-aminobutyric acid (GABA), leucine, arginine, glutamate and tryptophan were found in moderate quantities in the *Magnolia* species, with *M. denudata* containing the highest quantity of each of these amino acids.

TABLE- 1
AMINO ACID CONTENT IN FLOWERS OF *Magnolia denudata* AND *M. liliiflora*

Amino acid	*Magnolia* flowers (amino acid: mg/100 g dry wt.)	
	M. denudata	*M. liliiflora*
Aspartate	289.10 ± 5.47	158.83 ± 3.71
Glutamate	30.04 ± 3.02	33.61 ± 2.62
Asparagine	1842.70 ± 7.56	1233.46 ± 4.25
Serine	85.72 ± 5.60	49.32 ± 1.71
Glutamine	964.27 ± 75.27	616.14 ± 45.72
Histidine	26.09 ± 0.11	8.82 ± 0.24
Glycine	10.87 ± 0.05	9.49 ± 0.31
Threonine	79.07 ± 0.30	44.45 ± 0.46
Arginine	32.73 ± 0.58	18.98 ± 0.12
Alanine	751.97 ± 200.18	584.73 ± 4.86
GABA	59.65 ± 22.34	45.18 ± 0.14
Tyrosine	0.00	43.40 ± 2.19
Cystine	331.50 ± 4.50	111.22 ± 2.19
Valine	116.13 ± 13.53	37.02 ± 1.79
Methionine	0.00	9.82 ± 3.43
Norvaline	4.01 ± 2.93	2.52 ± 1.17
Tryptophan	28.60 ± 0.68	19.32 ± 0.39
Phenylalanine	14.99 ± 0.29	12.50 ± 0.31
Isoleucine	12.11 ± 0.22	4.22 ± 0.02
Leucine	34.47 ± 9.57	8.32 ± 0.68
Lysine	13.75 ± 0.46	5.71 ± 0.03
Total	4727.78 ± 190.85	3057.04 ± 34.21

The values represent the mean ± SD

The quantity of these amino acids was more than 25 mg/100 g dry weight in *M. denudata*.

The ranges of valine, serine, threonine, γ-aminobutyric acid, leucine, arginine, tryptophan and histidine were 37.02-116.13, 49.32-85.72, 44.45-79.07, 45.18-59.65, 8.32-34.47, 18.98-32.73, 19.32-28.60 and 8.82-26.09 mg/100 g dry weight, respectively between the *Magnolia* species. In *M. denudata*, the amounts of valine, serine, threonine, γ-aminobutyric acid, leucine, arginine, tryptophan and histidine were 3.14, 1.74, 1.78, 1.32, 4.14, 1.72, 1.48 and 2.96 times higher, respectively, than that in *M. liliiflora*. The levels of glycine, norvaline, phenylalanine, isoleucine and lysine were lower irrespective of *Magnolia* species, although between the two species, the highest levels of this set of amino acids were observed in *M. denudata*.

Variations in the amino acid profiles among cultivars was previously reported by Kim *et al.* [25], who showed that among all the amino acids isolated from *Momordica charantia*, arginine was present in remarkably high quantities, whereas cysteine and methionine were present in low concentrations. Similar variation has also been observed in the different organs of *Scutellaria baicalensis* [26], green and red mustard [21] and in different species of aloe [27]. Similar results regarding variation in the amino acid content of different sword bean cultivars were observed in this study. Li *et al*. [28] reported that the amino acid and γ-aminobutyric acid content varied by cultivar, which is supported by the findings of the current study.

Conclusion

The levels of amino acids in the flowers of *M. denudata* and *M. liliiflora* varied greatly. *M. denudata* contained the highest total amount of amino acids and the highest levels of 18 different amino acids. Although the total amino acid content was lower in *M. liliiflora,* tyrosine and methionine were detected only in this species. The results of present study demonstrate that the different *Magnolia* species exhibit unique amino acid concentrations. The species-specific amino acid profiles generated by this study suggest that magnolia flowers could potentially be a significant source of amino acids for human consumption.

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