JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry

TURKISH CHEMICAL SOCIETY

Amino Acid Content of the Seeds of Some Species from *Trigonella* L. Genus Collected from Turkey

Ş. Selma URAS GÜNGÖR[1*]

[1]Mersin University, Pharmacy Faculty, Pharmacognosy Department, Mersin, 33169, Turkey

Abstract: A study was undertaken to evaluate the amino acid composition of the seeds of *Trigonella cylindracea* Desv., *Trigonella mesopotamica* Hub.-Mor. and *Trigonella smyrnea* Boiss. which are growing wild in Turkey. The seeds of three species contain both essential (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, arginine, and tryptophan) and non-essential (alanine, aspartic acid, glycine, glutamic acid, proline, serine, and tyrosine) amino acids. The major amino acid was glutamic acid with a value of 4819 mg/100 g (*T. cylindracea*), 5888 mg/100 g (*T. mesopotamica*) and 4146 mg/100 g (*T. smyrnea*). Among the essential amino acids, lysine is found at the highest rate in all three species. The highest amount of lysine was found in the seeds of *T. mesopotamica*, followed by the seeds of *T. cylindracea* and *T. smyrnea* (3352±0.02 mg/100 g, 3059±0.03 mg/100 g and 2947±0.04 mg/100 g, respectively). The literature review showed that the amino acid composition of the studied seeds were evaluated for the first time in this study, therefore; our study provides important preliminary data to the literature in terms of evaluating the chemical compositions of three *Trigonella* species.

Keywords: *Trigonella cylindracea, Trigonella mesopotamica, Trigonella smyrnea,* Amino acid, Chemical composition.

Submitted: September 19, 2022. **Accepted:** March 14, 2023.

Cite this: Uras Güngör ŞS. Amino Acid Content of the Seeds of Some Species from Trigonella L. Genus Collected from Turkey. JOTCSA. 2023;10(2):381–4.

DOI: https://doi.org/10.18596/jotcsa.1177340.

***Corresponding author. E-mail:** urasselma@mersin.edu.tr.

1. INTRODUCTION

Traditional medicines based on medicinal plants which have been used to treat illness or to prevent progression of chronic disease have a long history dates back to approximately 2500 years (1). In recent years, plant foods play an important role in traditional medicine in health care. Some plant foods have rich bioactive and nutritive contents, and these have been used in traditional medicine to prevent or inhibit various human diseases (2).

Amino acids are found in plants and are the basic building blocks of proteins. Amino acids are essential for protein synthesis and have various functions in the body. It is necessary to take them into the diet, since their deficiency will cause severe decreases in protein biosynthesis, therefore, the amount of total protein in the organism may also decrease. For these reasons, it is concluded that amino acids play important roles in the human body (3).

The genus *Trigonella* L. which is widely distributed throughout the Mediterranean region, Europe, South and North Africa, West Asia, and South Australia is a genus of traditional medicinal plants belonging to the Fabaceae family and includes approximately 135 species (4). The most well-known Trigonella species is *Trigonella foenum-graecum*, known as fenugreek. Fenugreek possesses an important position throughout the world to add flavor and taste in various foodstuffs which has been long known as a potent herb in traditional medicine. Its seeds contain proteins with a nutritive amino acid profile, as well as lipids, saponin, flavonoid, polyphenol, alkaloid, mucilage and other functional elements (5, 6) and have many medicinal properties such as antidiabetic, antioxidant, anticancer, gastroprotective and hepatoprotective effects (7).

The genus *Trigonella* comprises about 34 taxa which are represented by 10 sections in Turkey (4). *T. cylindracea* Desv., *T. mesopotamica* Hub.-Mor.

and *T. smyrnea* Boiss. species belongs to the section Cylindricae Boiss. and are known as Boruboyotu, Dicleboyotu and Efeboyotu, respectively, in Turkey. The species *T. foenum-graecum* is widely studied worldwide however there is few studies on other species of this genus.

We have previously conducted studies on the chemical compositions of some *Trigonella* species (*T. kotschyi*, *T. filipes*, *T. cilicica*, *T. strangulata* and *T. rhytidocarpa*) and their amino acid compositions have also been investigated (8-10). In this study, it was aimed to investigate, for the first time, the amino acid profile of three different *Trigonella* species (*T. cylindracea* Desv., *T. mesopotamica* Hub.-Mor. and *T. smyrnea* Boiss.) collected from their natural habitats.

2. MATERIALS AND METHODS

2.1. Chemicals

In this study, most of the analytes were obtained from Sigma-Aldrich (St. Louis, MO, USA) except for acetonitrile, ammonium acetate, sodium hydroxide, ethanol, acetic acid, and hydrochloric acid were purchased from Merck (Darmstadt, Germany). All aqueous solutions were prepared with doubly distilled water obtained from Milli-Q System (Millipore, Bedford, MA, USA). During hydrolysis and sample preparation extra-pure nitrogen was used.

2.2. Plant Materials

T. cylindracea, *T. mesopotamica* and *T. smyrnea* were collected from different regions of Turkey (Table 1). Voucher specimens were identified by Prof. Dr. Ahmet İLÇİM (Department of Biology, Faculty of Sciences and Arts, Mustafa Kemal University, Antakya, Hatay, TURKEY) and Assist. Prof. Dr. Ş. Selma URAS GÜNGÖR (Department of Pharmacognosy, Faculty of Pharmacy, Mersin University, Mersin, TURKEY) and stored in the Herbarium of the Faculty of Sciences and Arts, Mustafa Kemal University.

Table 1: Localities and voucher numbers of the studied *Trigonella* species.

Species	Localities	Voucher number
T. cylindracea Desv.	C4 Içel:Tömük, 0-20 m, Ş.S.Uras Güngör	MKU1755
T. mesopotamica Hub.-Mor.	C6 Kahramanmaraş:Çağlayancerit, 1300-1500 m, Ş.S.Uras Güngör, A. İlçim	MKU1754
T. smyrnea Boiss.	C2 Antalya:Gömbe, 1150 m, Ş.S.Uras Güngör	MKU1782

2.3. Amino Acid Analysis

Acid hydrolysis and derivatization of proteins for the determination of 16 amino acids, including aspartic acid, serine, glutamic acid, glycine, histidine, arginine, alanine, threonine, lysine, leucine, proline, tyrosine, isoleucine, valine, phenylalanine, and methionine from seeds of *Trigonella* species were performed with a minor modification to the method reported by Eroglu et al., (2016) (11). The powdered seeds (0.1-1 g) were dissolved in HCl (6 M, 20 mL) in hydrolysis tubes and hydrolyzed for 24 hours at 110 °C in an oven under nitrogen atmosphere. The mixture was then allowed to cool to room temperature. Immediately after protein hydrolysis, precolumn derivatization with phenylisothiocyanate was used. Dry samples were dissolved in 20 µL of ethanol:water:triethylamine (2:2:1) and then dried under vacuum. Finally, derivatization was performed using 20 µL of derivatization reagent [ethanol: water: triethylamine: phenylisothiocyanate (7:1:1:1)] for 20 minutes at room temperature, then the reagent was separated under vacuum at 45 °C. Derivatized samples were dissolved in 0.1 mL of 0.14 M sodium acetate and adjusted to pH 6.4 with dilute acetic acid.

Alkaline hydrolysis to determine tryptophan was performed according to the method reported by Cevikkalp et al., (2016) (12). A standard stock solution of tryptophan (100 µg/mL) was prepared with water (pH 6.3) and stored in the dark at 4 °C for up to one month. The powdered seeds (0.1-1 g) were dissolved with 20 mL of 5 N NaOH under nitrogen atmosphere. After the mixture was hydrolyzed in an oven at 120 °C for 12 hours, the hydrolysates were cooled to room temperature and adjusted to pH 6.3 using diluted HCl. The Prominence ultra-fast liquid chromatography system (Shimadzu, Tokyo, Japan) equipped with a binary pump and UV/Vis detectors were used for analysis. A reversed phase analytical column [Shim-pact XR-ODS (75 mm x 3.0 mm i.d.)] with a fluorescence detector was used for separation and detection. The analysis conditions were as follows; mobile phase A: 10 mmol/L (potassium phosphate buffer (pH: 7.0), mobile phase B: acetonitrile [5% (0 to 0.3 minutes), 5% to 40% (0.3 to 3.4 minutes) min)], 40 °C column temperature, 1.2 mL/min flow rate, 1 µL injection volume. The amount of amino acids was presented as mg of amino acid/100 g of dry sample.

3. RESULTS AND DISCUSSION

The amino acid compositions of the studied species (*T. cylindracea*, *T. mesopotamica*, *T. smyrnea*) are presented in Table 2. The presence of various essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine,

threonine, valine, arginine, and tryptophan) and non-essential amino acids (alanine, aspartic acid, glycine, glutamic acid, proline, serine and tyrosine) has been demonstrated in the studied seeds.

It was determined that the amounts of lysine, isoleucine, arginine, aspartic acid, and glutamic acid in the seeds of all three species were higher than the other amino acids. Among the essential amino acids, lysine was found at the highest rate in all three species. The highest amount of lysine was found in the seeds of *Trigonella mesopotamica*, followed by the seeds of *Trigonella cylindracea* and *Trigonella smyrnea* (3352±0.02 mg/100 g, 3059±0.03 mg/100 g and 2947±0.04 mg/100 g, respectively). Arginine and isoleucine content of *Trigonella cylindracea*, *Trigonella mesopotamica,* and *Trigonella smyrnea* seeds were 2599±0.02 mg/100 g, 3264±0.03 mg/100 g, 2203±0.05 mg/100 g, and 2283±0.02 mg/100 g, 2679±0.05 mg/100 g, and 2401±0.03 mg/100 g, respectively. Among the non-essential amino acids, glutamic acid was determined at the highest rate in all three species. The highest amount of glutamic acid was found in the seeds of *Trigonella mesopotamica*, followed by the seeds of *Trigonella cylindracea* and *Trigonella smyrnea* (5888±0.15 mg/100 g, 4819±0.12 mg/100 g, and 4146±0.11 mg/100 g, respectively). This was followed by the aspartic acid and proline (Table 2).

Table 2: Amino acid composition of *T. cylindracea*, *T. mesopotamica* and *T. smyrnea* seeds.

Amino acid	Symbol	*T. cylindracea* mg/100 g	*T. mesopotamica* mg/100 g	*T. smyrnea* mg/100 g
Essential amino acids				
Histidine	HIS	981±0.04	1384±0.02	1192±0.05
Isoleucine	ILE	2283±0.02	2679±0.05	2401±0.03
Leucine	LEU	966±0.03	1145±0.04	1093±0.02
Lysine	LYS	3059±0.03	3352±0.02	2947±0.04
Methionine	MET	195±0.02	328±0.02	372±0.01
Phenylalanine	PHE	1431±0.04	1640±0.03	1451±0.02
Threonine	THR	789±0.02	960±0.01	968±0.01
Valine	VAL	1136±0.03	1350±0.04	1339±0.03
Arginine	ARG	2599±0.02	3264±0.03	2203±0.05
Tryptophan	TRP	314±0.02	334±0.03	312±0.01
Non-essential amino acids				
Alanine	ALA	1181±0.01	1409±0.02	1316±0.04
Aspartic acid	ASP	2305±0.09	2802±0.08	1343±0.05
Glycine	GLY	1588±0.02	1905±0.06	1679±0.02
Glutamic acid	GLU	4819±0.12	5888±0.15	4146±0.11
Proline	PRO	1778±0.02	2279±0.06	1958±0.05
Serine	SER	1197±0.03	1383±0.04	1271±0.03
Tyrosine	TYR	846±0.01	980±0.01	1018±0.04

Data presented as mean±SD (n=3).

Feyzi et al. (2015) studied fenugreek protein isolate. They found that high quantities of glutamic acid (199.80 g/kg), aspartic acid (116.80 g/kg), leucine (93.70 g/kg), threonine (80.01 g/kg), and arginine (75.70 g/kg) were observed in fenugreek protein isolate (13). Aljuhaimi et al. (2018) investigated amino acid compositions of *T. foenum-graecum* seeds from India, Saudi Arabia, Yemen, and Turkey. Those results showed that major amino acids were determined as glutamic acid (3.79-4.82 % w/w), aspartic acid (2.65-3.31 % w/w), arginine (2.45-3.26 % w/w), leucine (1.67-2.09 % w/w), and lysine (1.63-1.95 % w/w) (14). In another study conducted by our group, the amino acid compositions of *T. kotschyi*, *T. filipes* and *T. cilicica* seeds were studied, and it was determined that the amounts of leucine, lysine, aspartic acid and glutamic acid in the seeds of all three species were higher than the other amino acids (8). As a result of amino acid analysis of *T. strangulata* seeds, it was found that the amounts of lysine (2482 mg/100 g), arginine (2244 mg/100 g), and leucine (2053 mg/100 g) were higher than other amino acids (9). In the amino acid composition study conducted on *T. rhytidocarpa* seeds, we found that the amounts of glutamic acid, lysine and arginine were 5116 mg/100 g, 4278 mg/100 g, and 4001 mg/100 g, respectively (10). Yasothai (2021) studied amino acid composition of fenugreek seeds from Indian samples and found that the major amino acids were

determined as histidine (2.08 g/100 g), leucine (1.53 g/100 g), glycine (1.21 g/100 g) and lysine (1.13 g/100 g) (15). Our results for the studied seeds were quite similar to the report by Gungor et al. (10) with some quantitative differences.

4. CONCLUSION

Glutamic acid was the major amino acid in the seeds of *T. cylindracea*, *T. mesopotamica* and *T. smyrnea*. It is an acidic type of an amino acid that helps in the synthesis of glutathione. Glutamic acid, a multifunctional amino acid, is involved in excitatory neurotransmission and taste perception. It also plays an important role in the gastric phase of digestion by increasing gastric exocrine secretion when consumed with food (3, 16). As a result, the amino acid compositions of the studied species were determined for the first time in this study. It is thought that further research should be done for these species to be considered as nutritional food and for their possible biological activities.

5. ACKNOWLEDGMENTS

The authors gratefully acknowledge to TÜBİTAK Marmara Research Center. The author also thanks Prof. Dr. Ahmet İLÇİM (Department of Biology, Faculty of Sciences and Arts, Mustafa Kemal University, Antakya, Hatay, TURKEY) for the determination of the plant.

6. REFERENCES

1. Salma S, Suraby K, Ramakrishnan L. Antioxidant Analysis and Phytochemical Detection of the Some Selected Spices (Ajwain, Coriander, Cumin, Fennel, Fenugreek). Research journal of pharmaceutical biological and chemical sciences. 2017;8(6):78–84.

2. Özkan G, Göktürk RS, Kıralan M, Ramadan MF. Fatty acids and tocopherols of Turkish Salvia fruticose, Salvia tomentosa, Stachys aleurites and Stachys cretica subsp. anatolica seed oils. La rivista Italiana delle sostanze grasse. 2018;XCV(Ge/Ma).

3. Akram M, Asif H, Uzair M, Akhtar N, Madni A, Shah SA, et al. Amino acids: A review article. Journal of medicinal plants research. 2011;5(17):3997–4000.

4. Akan H, EkïCï M, Aytaç Z. The synopsis of the genus Trigonella L. (Fabaceae) in Turkey. Turk Journal of Botany. 2020 Nov 30;44(6):670–93. Available from: <URL>.

5. Żuk-Gołaszewska K, Wierzbowska J, Żuk-Gołaszewska K. Fenugreek: productivity, nutritional value and uses. Journal of Elementology [Internet]. 2017 Jun 6 [cited 2023 Mar 20];(3/2017). Available from: <URL>.

6. Wani SA, Kumar P. Fenugreek: A review on its nutraceutical properties and utilization in various food products. Journal of the Saudi Society of Agricultural Sciences. 2018 Apr;17(2):97–106. Available from: <URL>.

7. Srinivasan K. Fenugreek (*Trigonella foenum-graecum*): A Review of Health Beneficial Physiological Effects. Food Reviews International. 2006 Jul;22(2):203–24. Available from: <URL>.

8. Uras G, Kokdil G. Phytochemical screening and antioxidant activity of Trigonella cariensis seeds. Botanica Serbica. 2022;46(2):209–16. Available from: <URL>.

9. Selma UGS, Gamze K. Tocopherol, sterol and amino acid compositions of Trigonella strangulata Boiss. seeds. International Research Journal of Pharmacy and Medical Sciences (IRJPMS). 2018;2(1):36–9.

10. Gungor SSU, Guzel S, Ulger M, Kokdil G. Chemical Composition and Antioxidant and Antimicrobialactivities of Turkish Endemic Trigonella rhytidocarpa Seeds. Chemistry of Natural Compounds. 2021 Jan;57(1):136–40. Available from: <URL>.

11. Eroglu N, Akkus S, Yaman M, Asci B, Silici S. Amino Acid and Vitamin Content of Propolis Collected by Native Caucasican Honeybees. Journal of Apicultural Science. 2016 Dec 1;60(2):101–10. Available from: <URL>.

12. Çevikkalp SA, Löker GB, Yaman M, Amoutzopoulos B. A simplified HPLC method for determination of tryptophan in some cereals and legumes. Food Chemistry. 2016 Feb;193:26–9. Available from: <URL>.

13. Feyzi S, Varidi M, Zare F, Varidi MJ. Fenugreek (*Trigonella foenum graecum*) seed protein isolate: extraction optimization, amino acid composition, thermo and functional properties: Extraction optimization of fenugreek seed protein. Journal of the Science of Food and Agriculture. 2015 Dec;95(15):3165–76. Available from: <URL>.

14. Aljuhaimi F, Şimşek Ş, Özcan MM, Ghafoor K, Babiker EE. Effect of location on chemical properties, amino acid and fatty acid compositions of fenugreek (*Trigonella foenum-graecum* L.) seed and oils. Journal of Food Processing and Preservation. 2018 Apr;42(4):e13569. Available from: <URL>.

15. Yasothai R. Fatty acid composition of fenugreek (Trigonella foenum-graecum L.) seed and Galactomannan depleted fenugreek residue. The Pharma Innovation Journal. 2021;10(7):1509–11.

16. Zareian M, Ebrahimpour A, Bakar FA, Mohamed AKS, Forghani B, Ab-Kadir MSB, et al. A Glutamic Acid-Producing Lactic Acid Bacteria Isolated from Malaysian Fermented Foods. International Journal of Molecular Sciences. 2012 May 7;13(5):5482–97. Available from: <URL>.