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Research Article

Comparative Study of Amino Acid Composition in the Seeds, Pulp and Rind from *Citurllus colocynthis* Fruits

Zead Helmi Mahmoud Abudayeh[1*], Haiane Rachykivna Lamazian[2]*, Petro Sereda[4], Ivan Chekman[2], Ihab Ibrahim Al Khalifa[1], Khaldun Mohammad Al Azzam[3], and Loay Khaled Mohammad Hassouneh[1]

[1]*Faculty of Pharmacy, Isra University, 11622 Amman, Jordan.*
[2]*O.O. Bogomolets National Medical University, T. Shevchenko Boulevard, 13, Kiev, Ukraine.*
[3]*Department of Pharmaceutical Chemistry, Pharmacy Program, Batterjee Medical College for Sciences and Technology (BMC), 21442 Jeddah, Kingdom of Saudi Arabia.*
[4]*The State Expert Center MoH Ukrain, Kiev, Ukraine.*

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ABSTRACT
Different parts of Citrullus colocynthis fruits were studied to determine the qualitative and quantitative composition of amino acids. For this purpose, ion-exchange chromatography technique using an Amino Acid Analyzer (T 339 M Mikrotecha Praha) was used. In all parts of the fruit studied, 17 amino acids were found, and 9 among them were essential. The most concentrated essential amino acid in all parts studied (mg/100 mg of sample) was arginine. The dominant amino acid contents present in each part: in seeds, glutamic acid; in rind, aspartic acid; and in pulp, arginine. On the other hand, the least concentrated amino acid contents in seeds, cystine; in pulp, methionine; and in rind, phenylalanine. The most abundant total amino acids were in seeds. We conclude that although the qualitative composition of amino acids in all parts was same, but the quantitative contents differ significantly from each other with a prevalence of amino acids in seeds. Citrullus colocyynthis fruit parts, especially seeds, are good protein sources of essential and non essential amino acids and can be used as food supplement which are the functional unit of protein required on a daily basis for good health of adults. This study is considered as the first approach to characterize *Citrullus colocynthis* based on its amino acids contents. Furthermore, the contents of amino acids found may provide researchers with useful information when used in combination with other parameters for determination of the quality of *Citrullus colocynthis*.

Keywords: *Citrullus colocynthis*, Egusi melon, amino acid, protein, supplement.

INTRODUCTION

Citrullus colocynthis or Egusi melon (family: Cucurbitaceae) also called egusi, is a bitter apple, bitter cucumber, or vine of Sodom as documented in the CRC World Dictionary of Plant Names[1]. It is a desert plant that grows in sandy arid soil, native to the Mediterranean Basin and Asia, and is widely distributed among the west coast of northern Africa, eastward through the Sahara, and Egypt until India. It also grows in southern European countries such as in Spain and on the islands of the Grecian archipelago[2,3]. Moreover, the plant has a large brown seed with thick black edges thickened towards the apex, about 16 x 9.5 mm and is common in the northern and western parts of Nigeria[4].

The fruit is smooth, spherical (5 – 10) cm in diameter with an extremely bitter taste. Yellow-green fruit which becomes marble (yellow stripes) at maturity, filled with a soft, dry, and spongy white pulp, in which the seeds are embedded. Each plant produces 15 to 30 fruits. The main chemical compounds of fruit pulp are colocynthin (the bitter principle up to 14%), colocynthein (resin), colocynthetin, cucurbitane type triterpen glycoside viz colocynthoside A and B, cucurbitacin E 2-O-beta-D-glcoside, aglycone Cucurbitacin E, pectin gum and glucopyranosyl cucurbitacin[5]. Additionally, egusi seeds are rich in fat and protein and thus are eaten as whole or even used as an oil seed[6].

The Cucurbitaceae, also called cucurbits, are a plant family known for its high proteins and oil content. Seeds of cucurbits are rich sources of oils and protein as prescribed by the work of Schafferman *et al*.[7]. The oil content of the seeds was found to be in the range of 17 – 19% (w/w)[7]. It has also been reported that its oil composition is similar to safflower one with a total of 80 – 85% of unsaturated fatty acids. The estimated oil yield value upon harvesting ranged between 250 – 400 L/ hectare[7] and composed of up to 35% protein, mainly, arginine, tryptophan, and sulfur-containing amino acids[8]. Therefore, due to the above mentioned reasons, they are cultivated and consumed the world over.

It has also been reported that the discovery of the nutritional and amino acid components of *Citrullus colocynthis* and *Citrullus vulgaris* seeds will enhance their potential use as food supplements and in food

**Author for Correspondence*

compounding to fortify the protein and fat contents of food substances for local consumption and industrial applications[8].

Almost all "melons" have nutritional and therapeutic values with the reported number of medicinal uses ranging from antiviral, antidiabetic, antiulcerogenic, antioxidant and hepatoprotective[9] to anticancer and cardio protective properties[10]. A wide range of pharmacological activity of *Citrullus colocynthis* is due to the different classes of chemical compounds present, in particular, saponins, glycosides, and aglycone of Cucurbitacin E[11].

Plant proteins are abundant and relatively inexpensive source of proteins that are widely recognized due to their nutritional values and excellent physicochemical properties[12]. Protein is the resource material used to build every cell in the body and play a vital role in most metabolic activities. Furthermore, it provides the structure for bones and muscles, which helps in preserving the skeletal structure of the body[13]. Amino acids (AA) are a class of organic compounds that play a major role in the less developed organisms and in human. It is considered as block components in protein synthesis[14]. Also, it serves as donors in the synthesis of non-protein and nitrogen-containing compounds, including nucleotides, heme, creatine, choline, and other substances[5]. Additionally, AA have been classified traditionally as nutritionally essential (indispensable) and non-essential (dispensable) for animals and humans[2].

Nutritionally essential amino acids (EAA) are those whose carbon skeletons are not synthesized by animal cells and therefore must be provided from the diet. In contrast, non-essential amino acids (NEAA) are those AA that are synthesized *de novo* in a species-dependent manner[15]. EAA are threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine and tryptophan. NEAA are aspartic acid, serine, glutamic acid, proline, glycine, alanine, cystine and tyrosine[16].

The aim of this work was to determine the qualitative and quantitative composition of AA in different parts of *Citrullus colocynthis* fruits using ion-exchange chromatography technique (Amino Acid Analyzer T 339 M, Mikrotecha Praha).

MATERIALS AND METHODS

Instrumentation

Amino acid analysis was done by the ion-exchange chromatography technique using an Amino Acid Analyzer (T 339 M Mikrotecha Praha).

Reagents and solutions

Hydrochloric acid, ninhydrin and the individual amino acid standards were purchased from Sigma-Aldrich (St Louis, MO, USA). All solutions were kept in the refrigerator. All other reagents used were of analytical grade. The ultrapure demonized water obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for preparing the solutions and for all dilutions.

Plant material and sample extracts

Citrullus colocynthis fruits were purchased from the local market, Cairo city, Egypt. They were dried in an oven at 60 °C. The seeds, pulp, and rind were ground separately with the aid of a food processor Power Plus 1300 (Braun, Germany). The obtained powders from each part of the fruit were used for the determination of AA composition which was carried out at the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine (Kiev).

Isolation of linked amino acids

Acid hydrolysis was carried out according to the following procedure. The weighed sample, to the nearest 0.01 g, was placed in the bottom of the glass tube and appropriate amount of 6 N hydrochloric acid was added to the dried sample. Then the tube was cooled in liquid nitrogen. After the content of the tube was frozen, the air was evacuated from it by vacuum pump to prevent the oxidation of AA by hydrolysis. The tube was scaled and placed in a thermostat for 24 h at a constant temperature (about 106 °C). After hydrolysis, the tube was cooled to room temperature and disclosed. The content was transferred to a glass weighing bottle and hydrochloric acid was evaporated with the aid of water bath. In the same way, the sample was prepared by dissolving in 0.30 N lithium citrate buffers (pH 2.2), (Lithium citrate buffers are used especially for the determination of the free AA from physiological samples), and applied to an ion exchange column of the Amino Acid Analyzer.

Amino acid detection method (post derivatization)

To detect AA in elutes, the ninhydrin detection method was used. In brief, ninhydrin reagent (derivatization agent) was added to the liquid that eluted from the column. Then the mixture was heated to 100 °C on reaction bath (the ninhydrin reacts with AA forming a dye complex). For calculation of the number of AA in the test sample, a mixture of standard of AA was added in the column of AA analyzer.

Qualitative determination of amino acids

According to Kozarenko T.D 1975, qualitative composition of AA mixture was determined by comparing the retention times in chromatograms of standard solutions and test sample mixtures of AA[17].

RESULTS

The values of AA contents in the seeds, pulp, and rind of *Citrullus colocynthis* fruits are shown in Table 1 and Table 2.

The study revealed that all parts of *Citrullus colocynthis* fruit contain 17 AA, including 9 EAA such as lysine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine, and phenylalanine (Table 1), and 8 NEAA namely; aspartic acid, serine, glutamic acid, proline, glycine, alanine, cysteine, and tyrosine (Table 2).

The total amino acids (TAA) for each part are shown clearly in Table 3. The contents of TAA in seeds, pulp, and rind were 6.74, 2.427, and 2.19 mg/100 mg of sample, respectively. The highest content was found in the seeds compared with the other two parts (pulp and rind). The total non-essential amino acids (TNEAA) in seeds, pulp, and rind were 4.059, 1.44 and 1.374 mg/100 mg of sample, respectively, while the total essential amino acids (TEAA)

Table 1: Essential amino acids (EAA) profile of *Citrullus colocynthis* in seeds, pulp, and rind (n = 3).

Amino Acid	General Formula	Molecular Weight (g/mol)	Amino Acids Content (mg/ 100 mg)		
			Seeds	Pulp	Rind
Lysine	$C_6H_{14}O_2N_2$	146.19	0.209	0.120	0.127
Histidine	$C_6H_9O_2N_3$	155.16	0.153	0.056	0.037
Arginine	$C_6H_{14}O_2N_4$	174.21	0.886	0.447	0.210
Threonine	$C_4H_9O_3N$	119.12	0.235	0.076	0.101
Valine	$C_5H_{11}O_2N$	117.15	0.182	0.051	0.099
Methionine	$C_5H_{11}O_2NS$	149.21	0.177	0.020	0.028
Isoleucine	$C_6H_{13}O_2N$	131.17	0.134	0.046	0.047
Leucine	$C_6H_{13}O_2N$	131.17	0.414	0.117	0.141
Phenylalanine	$C_9H_{11}O_2N$	165.19	0.291	0.054	0.026

Table 2: None-essential amino acids (NEAA) profile of *Citrullus colocynthis* in seeds, pulp, and rind (n = 3).

Amino acid	General Formula	Molecular Weight (g/mol)	Amino Acids Content (mg/ 100 mg)		
			Seeds	Pulp	Rind
Aspartic acid	$C_4H_7O_4N$	133.10	0.728	0.429	0.361
Serine	$C_3H_7O_3N$	105.09	0.404	0.162	0.170
Glutamic acid	$C_5H_9O_4N$	147.13	1.488	0.325	0.312
Proline	$C_5H_9O_2N$	115.13	0.296	0.139	0.117
Glycine	$C_2H_5O_2N$	75.07	0.523	0.141	0.150
Alanine	$C_3H_7O_2N$	89.09	0.434	0.145	0.170
Cystine	$C_6H_{12}O_4N_2S$	240.29	0.074	0.046	0.056
Tyrosine	$C_9H_{11}O_3N$	181.19	0.112	0.053	0.038

in seeds, pulp, and rind were 2.681, 0.987, 0.816 mg/100 mg of sample, respectively (Table 3).

The corresponding percentages of TNEAA in seeds, pulp, and rind were 60.22, 59.33, and 62.74%, respectively, while for TEAA 39.78, 40.67, and 37.26% for seeds, pulp, and rind, respectively (Table 3).

DISCUSSION

The study revealed that all parts of *Citrullus colocynthis* fruit contain 17 AA, including 9 EAA and 8 NEAA (Tables 1 and 2). Tryptophan was not determined either in seeds, pulp or in the rind, (it is separated from common AA by anion-exchange chromatography and directly detected by integrated pulsed amperometry), which correspond to the previous study reported by Ogundele *et al.*[18]. The obtained results of AA composition in seeds from *Citrullus colocynthis* fruit correspond to the one reported by Abbah *et al*., and Igwenyi *et al*.[19,20].

The most concentrated EAA found in all parts studied was arginine. The contents of arginine in seeds, pulp, and rind were 0.886, 0.447, and 0.210 mg/100 mg of sample, respectively. Therefore, this encourages the consumption of *Citrullus colocynthis* fruit due to the presence of arginine as it is EAA necessary for human life especially in children's growth[21].

The dominant AA found in the seeds was glutamic acid; in pulp, arginine; and in the rind, aspartic acid with 1.488, 0.447, and 0.361 mg/100 mg of sample, respectively (Table 1). On the other hand, the least concentrated AA in seeds, cystine (0.074 mg/100 mg of sample); in pulp, methionine (0.020 mg/100 mg of sample); and in rind, phenylalanine (0.026 mg/100 mg of sample) (Table 1), which are in agreement with the previous results reported by Ogundele *et al*.[18].

The TEAA contents in seeds, pulp, and rind were 2.681, 0.987, and 0.816 mg/100 mg of sample, respectively. On the other hand, the corresponding percentages were 39.78, 40.67, and 37.26% for the seeds, pulp and rinds, respectively, which are comparable with the results reported by Ogundele *et al*.[18]. Additionally, these results can compete favorably with those of Soya bean and *Adenopus breviflorus benth* seeds in being as rich plant protein sources[22].

Compared to TEAA, the percentages of TNEAA in all parts of *Citrullus colocynthis* fruit were higher than that for TEAA but the level of TEAA was valuable and highest, especially in the pulp (40.67%) (Table 3). In general, TAA and TNEAA contents of seeds were significantly higher than that for pulp and rind (Table 3) which are consistent with the results reported by Ogundele *et al*.[18]. Moreover, it showed higher protein and fat contents which are of biological values to be used as good sources in the daily AA requirements for adults[23].

Finally, although proteins from plant sources tend to have a relatively low biological value in comparison with proteins from eggs or milk, nevertheless, “complete” in that they contain at least trace amounts of all of the AA that are essential in human nutrition[24].

CONCLUSION

From the results of investigation mentioned above, we conclude that although the qualitative composition of AA in all parts were the same, but the quantitative contents in seeds, pulp and rind differ from each other. Moreover, *Citrullus colocyynthis* fruit parts, especially seeds, are

Table 3: Total amino acids composition of *Citrullus colocynthis* in seeds, pulp and rind.

Amino Acids	Seeds	Pulp	Rind
Total amino acids (TAA)	6.74	2.427	2.19
Total non-essential amino acids (TNEAA)	4.059	1.44	1.374
% TNEAA	60.22	59.33	62.74
Total essential amino acids (TEAA)	2.681	0.987	0.816
% TEAA	39.78	40.67	37.26

good protein source as they contain EAA and NEAA and thus could be used as a food supplement. Also, AA are considered as functional units of proteins used daily for good health of adults in calculated quantities.

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