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Amino Acid, Amino Acid Metabolite, and GABA Content of Three Domestic Tomato Varieties

Jun-Bae Ahn¶
¶Dept. of Food Service & Culinary Arts, Seowon University

ABSTRACT: To determine the nutritional value of domestic tomatoes, the levels of amino acids, amino acid metabolites, and the bioactive compound γ-aminobutyric-acid (GABA) were analyzed in three domestic tomato varieties (Rafito, Momotaro, and Medison). Eighteen free amino acids were found, and total free amino acid content was 3,810.21～4,594.56 mg/100 g (dry weight). L-glutamic acid (L-Glu) was the most abundant amino acid, ranging from 1,866.60 mg/100 g for Momotaro to 2,417.45 mg/100 g for Medison. The next most abundant amino acids were L-glutamine (L-Gln) and L-aspartic acid (L-Asp). The three tomato varieties had a good balance of all the essential amino acids except tryptophan. Total essential amino acid content was 274.26～472.71 mg/100 g (dry weight). The following amino acid metabolites were found: L-carnitine (L-Car), hydroxylysine (Hyl), o-phosphoethanolamine (o-Pea), phosphoserine (p-Ser), β-alanine (β-Ala), N-methyl-histidine (Me-His), ethanolamine (EtNH2),and L-citrulline(L-Cit). Large quantities of GABA were found in all three varieties: 666.95-868.48 mg/100g (dry weight). These results support the use of these tomato varieties as nutritious food materials.

Keywords: tomato variety, amino acid, metabolite, GABA, analysis, health food

INTRODUCTION

Tomatoes are a good source of various nutrients and bioactive substances and have been considered to be one of the most important vegetables consumed throughout the world[1,2]. They contain several vitamins (A, B, C, E and K) and minerals, β-carotene, and lycopene[3]. In addition, tomatoes have a high content of organic acids, sugar, unique flavor, and pigments[1]. They have therefore been widely used as an ingredient in various processed foods, such as beverages, purees, and ketchup.

Various studies on the bioactive components and functionality of tomatoes have been conducted, many of which indicate that the lycopene and β-carotene in tomatoes have inhibitory effects on prostate cancer[4,5], and low-density lipoprotein (LDL) oxidation[6], as well as antioxidant effects[7].

In Korea, several varieties have been bred and distributed to farms[8]. However, there have been no comprehensive systematic studies on common nutrients and bioactive compounds within these varieties. A few reports have included the levels of common nutrients[3], the physicochemical changes during growth[9], changes in hardness and inorganic components during ripening[10], and the levels of bioactive compounds in cherry tomato[11]. To elucidate the nutritional value of domestic tomatoes, studies on the common and bioactive components are needed. In the present study, free amino acids and amino acid metabolites were analyzed from three varieties of domestic tomato via ion chromatography. In addition, the level of γ-aminobutyric acid (GABA), a neurotransmitter and biomolecule increasing brain function[12-14] was measured. The results of this study are expected to be useful in elu-

¶ Corresponding Author: Jun-Bae Ahn, Dept. of Food Service & Culinary Arts, Seowon University, 377-3, Musimseoro, Seowon-gu, Cheongju-si, Chungchungbuk-do 28674, South Korea. Tel. +82-43-299-8461, Fax. +82-43-299-8460, E-mail: given@seowon.ac.kr

cidating the nutritional value of domestic tomatoes.

MATERIALS AND METHODS

Tomato Varieties

The Korean commercial tomato cultivars Rafito, Momotaro, and Medison, were obtained from Buyeo Tomato Experiment Station (Chungchengnamdo, Korea).

Preparation of Tomato Samples

To prepare each sample, 10 uniformly sized tomatoes from each variety were randomly selected. After the stem end had been removed, the fruits were sliced 4~5 mm thick. The slices were soaked in liquid nitrogen to ensure they were completely frozen, and then lyophilized with a freeze dryer (model PVTFD 10R, Ilsinbiobase, Korea).

The dried fruits were powdered by a Wiley mill (Model 4, Thomas Scientific, Swedesboro, USA) and passed through a 20-mesh screen. Due to the fruits' high hygroscopicity, the samples were stored in dark amber bottles sealed hermetically at −25℃.

Extraction of Amino Acids, Amino Acid Metabolites, and GABA

A 50 mg sample of each batch of prepared tomato was placed in a 25 mL volumetric flask, and 80% (v/v) methanol in water was added, up to 25 mL. To extract amino acids, amino acid metabolites, and GABA, the sample and solvent mixture was placed in an ultrasonic bath for 60 min at 30℃.

After extraction, the mixture was filtered under vacuum through Watman No. 2 filter paper and centrifuged for 10 min at 18,000 ×g. To prepare the extract for analysis, the supernatant was passed through a 0.45 μm syringe filter (Millipore, Bedford, MA, USA). The filtered extract was divided into 1 mL aliquots and preserved at −25℃.

Determination of Moisture and Crude Protein Content

The moisture content of each sample was determined by comparing the weight of the sample before and after freeze-drying. The crude protein content was determined by the Micro-Kjeldahl method, using an auto analyzer (model Kjeltec 2300, FOSS analytical, Hilleroed, Denmark). Crude protein analysis was conducted in accordance with the manufacturer's manual.

Analysis of Amino Acids, Amino Acid Metabolites, and GABA

The amino acids, amino acid metabolites, and GABA were analyzed by means of ion exchange chromatography. A 10 μL aliquot of the filtered extract was injected into an amino acid analyzer (model L-8800, Hitachi, Tokyo, Japan). The column was packed with ion exchange resin 2622 (Hitachi, 4.6 × 60 mm, particle size=5 mm). For the post-column reaction, lithium citrate buffer and ninhydrin were used at flow rates of 0.35 and 0.30 mL/min, respectively.

Statistical Analysis

Statistical differences were analyzed using one-way ANOVA followed by Duncan's *post hoc* test with a confidence level of 95% ($p<0.05$).

RESULTS AND DISCUSSION

The Shapes of Tomato Fruits

The shapes of tomatoes used in this study were described in Table 1.

All tomatoes were harvested at full maturity and were red in color. The weights ranged from 144.64~

Table 1. Colors, dimensions, and weights of three varieties of domestic tomato fruits (mean±SD)

Variety	Color	Length (mm)	Width (mm)	Weight (g)
Rafito	Red	64.57±1.79	85.26±3.25	267.44±17.49
Momotaro	Red	52.23±3.31	68.93±0.80	144.64±17.88
Medison	Red	57.37±1.09	69.23±3.50	164.01±16.97

Table 2. Moisture and crude protein content of three varieties of domestic tomato (mean±SD)

Variety	Moisture (%, wet weight)	Protein (%, wet weight)
Rafito	93.09±0.12[ab]	0.63±0.02[b]
Momotaro	93.01±0.18[b]	0.71±0.03[a]
Medison	93.52±0.26[a]	0.72±0.02[a]

[a,b] Values in the same column with different superscript letters are statistically different at $p<0.05$.

267.44 g. Rafito, the largest variety, was on average 1.8 times heavier than Momotaro.

Moisture and crude Protein Content

The moisture content of the tomato varieties was 93.01~93.52 g/100 g (wet weight), and Medison had the highest water content (Table 2).

The crude protein contents of Medison and Momotaro were 0.72 and 0.71% (wet weight) and were not statistically different at $p<0.05$. Rafito had the lowest crude protein content(0.63%).

Free Amino Acid Content

The amino acid content was analyzed via ion exchange chromatography. Free amino acids react with the other components of a foodstuff more rapidly than complete proteins do. They can also cause a browning reaction with sugars, resulting in changes in color and flavor during cooking or food processing. These changes could either improve or worsen the quality of the food[15].

Nutritionally, free amino acids can serve as a source of nitrogen and essential amino acids. Assessing the free amino acid content of tomatoes is thus an important part of determining their value as a food material.

The content of total free amino acids was 3,810.21~4,594.56 mg/100 g (dry weight; Table 3). Overall, Medison tomatoes were the richest in free amino acids, and the total free amino acid content was significantly different among all three varieties ($p<0.05$).

Eighteen free amino acids were found in the tomatoes. The levels of L-glutamic acid (L-Glu) were consistently the highest in all three varieties: 2,417.45, 2,347.65, and 1,866.60 mg/100 g (dry weight) in Medison, Rafito, and Momotaro, respectively (Table 3). These quantities were significantly different ($p<0.05$).

Lee et al[3] reported that L-Glu is the primary free amino acid in fresh domestic tomatoes, and observed a wet weight content of 13.67 mg/100 g. Choi et al[16] found that L-Glu increases gradually with tomato maturation, until it is the most abundant amino acid in the fully ripe tomato. The results of the present study are consistent with previous findings.

L-Glutamine (L-Gln) was the second-most abundant free amino acid. The concentrations ranged from 629.29 mg/100 g (dry weight) in Rafito to 912.97 mg/100 g in Medison (Table 3). The next most abundant was L-aspartic acid (L-Asp), which ranged from 401.39 mg/100 g in Rafito to 490.98 mg/100 g in Momotaro. There were significant differences in the content of both of these between all three varieties. L-Glu, L-Gln, and L-Asp were the primary amino acids in the sampled tomatoes, comprising 82.19~84.14% of total free amino acids.

Fifteen other amino acids were observed, such as L-proline (L-Pro), L-phenylalanine (L-Phe), L-alanine (L-Ala), and L-serine (L-Ser). However, L-cysteine (L-Cys) and L-tryptophan (L-Trp) were not found in three varieties.

The essential amino acid content ranged from 274.26 mg/100 g (dry weight) in Momotaro to 472.71 mg in Medison (10.29% of total free amino acids). There were significant differences between the essential amino acid content of all three varieties. The quality of the protein in a foodstuff can be evaluated by measuring the levels of the nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). All three varieties of domestic tomatoes had a good balance of all the essential amino acids except tryptophan. Domestic tomatoes can be therefore considered a nutritionally valuable foodstuff.

Table 3. Free amino acid content of three varieties of domestic tomato (mean±SD)

Amino acids	Medison		Rafito		Momotaro	
	mg/100 g (dry weight)	% of total amino acids	mg/100 g (dry weight)	% of total amino acids	mg/100 g (dry weight)	% of total amino acids
L-Glu	2,417.45±7.84[a]	52.62	2,347.65±9.38[ab]	57.42	1,866.60±29.73[b]	48.99
L-Gln	912.97±2.44[a]	19.87	629.29±2.55[c]	15.39	848.03±10.25[b]	22.26
L-Asp	445.59±0.28[b]	9.70	401.39±0.40[c]	9.82	490.98±16.74[a]	12.89
L-Ser	160.37±0.33[b]	3.49	181.06±1.15[a]	4.43	150.74±4.09[c]	3.96
L-Phe (EAA[1])	131.06±0.42[a]	2.85	100.95±2.10[b]	2.47	69.77±4.33[c]	1.83
L-Thr (EAA)	99.63±0.87[a]	2.17	69.17±0.60[b]	1.69	64.09±1.58[c]	1.68
L-Pro	45.19±3.08[a]	0.98	48.23±0.25[a]	1.18	41.05±8.07[b]	1.08
L-Val (EAA)	55.74±0.44[a]	1.21	41.28±0.22[b]	1.01	32.94±0.39[c]	0.86
L-Lys (EAA)	48.06±0.17[a]	1.05	38.96±0.79[b]	0.95	31.93±1.19[a]	0.84
L-Ala	25.40±1.23[b]	0.55	38.89±3.16[a]	0.95	40.97±1.07[a]	1.08
L-His (EAA)	46.27±0.96[a]	1.01	36.03±0.97[b]	0.88	31.24±1.77[c]	0.82
L-Leu (EAA)	41.12±0.71[a]	0.89	31.88±0.17[b]	0.78	21.80±1.02[c]	0.57
L-Arg	51.84±0.48[a]	1.13	29.16±1.54[b]	0.71	27.82±2.04[b]	0.73
L-Ile (EAA)	37.95±0.47[a]	0.83	29.04±0.13[b]	0.71	22.49±1.06[c]	0.59
L-Asn	26.31±0.04[b]	0.57	24.89±0.11[b]	0.61	34.37±0.89[a]	0.90
L-Gly	19.68±0.19[a]	0.43	18.65±1.23[ab]	0.46	17.50±0.05[b]	0.46
L-Tyr	17.05±0.54[a]	0.37	12.53±0.31[b]	0.31	17.89±1.54[a]	0.47
L-Met (EAA)	12.88±0.59	0.28	9.68±0.45	0.24	nd[3]	-
Total essential amino acids[2]	472.71±3.11[a]	10.29	356.99±4.16[b]	8.73	274.26±4.11[c]	7.20
Total amino acids	4,594.56±10.20[a]		4,088.74±17.91[b]		3,810.21±34.84[c]	

Amino acid abbreviations follow the International Union of Pure and Applied Chemistry (IUPAC) standard.
[1] EAA: Essential amino acid.
[2] The total essential amino acid content includes Thr, Val, Met, Ile, Leu, Phe, Lys and His.
[3] The content was less than 0.01 mg/100 g (dry weight).
[a~c] Values in the same row with different superscript letters are significantly different at $p<0.05$.

Amino Acid Metabolite Content

In addition to amino acids coded by genes (Table 3), the following amino acid derivatives were found in all three tomato varieties, except where indicated (Table 4): L-carnitine (L-Car), hydroxylysine (Hyl), o-phosphoethanolamine (o-Pea), phosphoserine (p-Ser), β-alanine (β-Ala, found in Rafito and Momotaro only), N-methyl-histidine (Me-His), ethanolamine ($EtNH_2$), and L-citrulline (L-Cit, found in Medison only).

L-Car was the most abundant of these in all three varieties. L-Car transports long-chain fatty acids into the mitochondrial matrix, resulting in the generation of energy via β-oxidation of fatty acids[17]. Although there is no evidence that tomato can be helpful in energy generation for humans, it is clear that

Table 4. Amino acid derivative content of three varieties of domestic tomato

Amino acid derivatives	Content (mg/100 g dry weight)		
	Rafito	Momotaro	Medison
L-Car	78.51±1.42	64.85±11.53	67.34±5.26
Hyl	30.21±0.71	29.73±0.54	28.92±0.65
o-Pea	28.25±0.52	28.68±4.28	31.95±1.04
p-Ser	24.14±0.22	26.02±0.47	20.18±0.91
β-Ala	20.79±0.59	22.56±1.04	nd
Me-His	13.47±0.38	10.75±0.27	11.25±1.15
$EtNH_2$	9.43±0.13	9.81±0.35	10.32±0.19
L-Cit	nd[1]	nd	5.48±0.31

[1] The content was less than 0.01 mg/100 g (dry weight).

tomato is a good source of L-Car.

GABA Content

GABA (Gamma-aminobutyric acid), synthesized from L-Glu, occurs in several plants[18]. GABA is a component of foods that has been recognized as an important inhibitory neurotransmitter[19]. It also has antihypersensitivity, natriuretic, and other positive effects on human health[18,20].

It is thus useful to identify or develop tomato varieties that have a high GABA content. To screen these varieties, the metabolism and functional significance of GABA in plants must be understood. However, the roles of GABA are not yet well understood. It may be related to stress responses, because it accumulates in plant tissues after exposure to various stress conditions, including high acidity, mechanical damage, high salt, heat, and cold[21]. Deewatthanawong et al[22] showed that the GABA content of tomatoes increases during storage in modified atmospheres containing 10% CO_2. GABA content can thus be controlled during growth and post-harvest storage.

In Korea and Japan, special attention has been given to the functional effects of GABA on human health. As a result, many health foods containing GABA have been released in the Korean and Japanese markets.

A large quantity of GABA was found in all three varieties of domestic tomatoes (Figure 1). The GABA

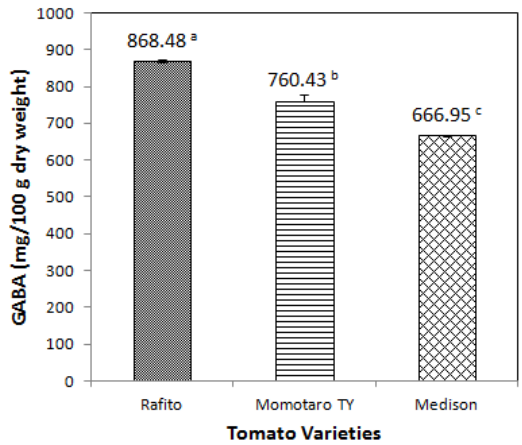

Figure 1. GABA content of three domestic tomato varieties. Bars with different superscript letters are significantly different at $p<0.05$.

content was 666.95~868.48 mg/100 g (dry weight). Rafito contained the most GABA (868.48 mg/100 g), and Medison the least (666.95 mg/100 g). There was a significant difference in GABA content among the varieties ($p<0.05$). Overall, these three varieties of tomato have high levels of GABA and can be used as effective health foods.

SUMMARY

Tomatoes, which contain many nutrients and bioactive compounds, are considered to be one of the

most nutritionally important vegetables. In Korea, several varieties have been bred and distributed to farms. However, systematic studies on the common nutrients and bioactive compounds of domestic tomato varieties are lacking.

In the present study, to elucidate the value of domestic tomato varieties as food materials, free amino acids and amino acid metabolites of three kinds of domestic tomato were analyzed via ion chromatography. I also analyzed their content of GABA, a neurotransmitter and biomolecule that increases brain function.

The tomatoes used in this study were harvested at full maturity, and weights ranged from 144.64～267.44 g. The moisture content of them was 93.01～93.52 g/100 g (wet weight). The crude protein content of the fresh tomatoes was 0.63～0.72%. The total free amino acid content was 3,810.21～4,594.56 mg/ 100 g (dry weight).

To examine the nutritional value of them, it is necessary to determine the amino acid pattern. Eighteen free amino acids were found in all three tomato varieties. The L-Glu content was the highest in all varieties: 2,417.45 mg/100 g, 2,347.65 mg/100 g, and 1,866.60 mg/100 g (dry weight) for Medison, Rafito, and Momotaro, respectively. Of the total free amino acids in the Rafito tomatoes, L-Glu comprised 57.42%. L-Gln was the second-most abundant free amino acid in all varieties. The next most abundant was L-Asp. These three were the principal free amino acids, comprising 82.19～84.14% of total free amino acids in the tomato varieties tested in this study.

Protein quality can be evaluated by examining the content of the nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). The tomatoes had a good balance of all the essential amino acids except tryptophan. The total essential amino acid content was approximately 472 mg/100 g (dry weight) for Medison, 357 mg/100 g for Rafito, and 274 mg/100 g for Momotaro.

The following amino acid derivatives were also found in the three domestic tomato varieties: L-Car, Hyl, o-Pea, p-Ser, β-Ala, Me-His, $EtNH_2$ and L-Cit. L-Car was particularly abundant (64.85～78.51 mg/100 g dry weight) in all three varieties. L-Car transports long-chain fatty acids into the mitochondrial matrix, resulting in the generation of energy via β-oxidation of fatty acids. Although there is no evidence that tomato can be helpful in energy generation for humans, it is clear that tomato is a good source of L-Car.

The tomatoes also had a high GABA content: 666.95～868.48 mg/100 g dry weight. GABA has been recognized as a nutritious food component that has antihypersensitivity, natriuretic, and other positive effects on human health. There was a significant difference in GABA content among the varieties ($p<$ 0.05). Rafito contained the most GABA (868.48 mg/100 g), and Medison the least (666.95 mg/100 g).

In this study, the nutritional properties of three domestic tomato varieties were investigated by analyzing their free amino acid, amino acid derivatives, and GABA content. Our results support the use of domestic tomato varieties as effective health foods. However, to fully elucidate the nutritional properties of domestic tomatoes, more of the bioactive compounds they contain should be analyzed in future studies.

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