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RESEARCH ARTICLE

Free Amino Acid Compositions for Fruits

Hinako Ito[1], Hiroshi Ueno[2], Hiroe Kikuzaki[1]

[1]Department of Food and Nutritional Sciences, Graduate School of Humanities and Sciences, Nara Women's University, Nara 630-8506, Japan.
[2]Laboratory of Biochemistry and Microbiology, School of Agriculture, Ryukoku University, Seta, Shiga 520-2194, Japan.

Abstract

Twenty five free amino acids were quantitatively measured in 11 species of fruits. All species analyzed gave free amino acids, some like asparagine were at abundant and some like methionine and cysteine were at low levels, but each species showed characteristic profiles. γ-Amino butyric acid, known for its health benefit, was found at significant levels. It is important to evaluate nutritional values of free amino acids in fruits where amino acid analysis is still a powerful tool. Our analysis should provide valuable data for the establishment of nutritional databases and also contribute to nutritional surveys including health benefit of amino acids.

Keywords: Fruits, Free Amino Acids

Introduction

Amino acids play important roles in human health [1]. Although amino acids derived from protein sources have been studied extensively, our knowledges of free amino acids are still limited. Only recently, supplemental amounts of branched chain amino acids (BCAA), including leucine (Leu), isoleucine (Ile) and valine (Val), are shown to accelerate a recovery from muscle damages, soreness and fatigues after exercise [2,3]. γ-Aminobutyric acid (GABA) is known for its ability to lower high blood pressure [4] and called as a bioactive compound for human [4], where it is authorized as a "food for specified health uses (called Tokuho)" in Japan. Some free amino acids are key determinants in food taste. L-Glutamate (Glu) is recognized for Umami taste and is rich in cheese, tomato and kelp [5]. L-Glycine (Gly), L-alanine (Ala), and proline (Pro) provide sweetness, and BCAA provides bitter taste [6]. L-Serine (Ser) gives mainly sweetness and minor Umami taste, and D-Ser, an enantiomer of L-Ser, gives sweet taste [7]. It has been recognized that free amino acids are aroma precursors and utilized for the synthesis of aroma components during fruits maturation [8-10].

A wide range of fruits, typified strawberry and banana [11-13], provide various nutrients including vitamins and minerals. Fruits also contain various phytochemicals, which are defined as bioactive non-nutrient compounds and associated with reducing the risks of chronic diseases such as cardiovascular diseases and cancers [14,15]. The nutritional promotion strategies for moderate amount of intake of plant foods including fruits have been developed in many countries [16]. Likewise other bioactive compounds, free amino acids should be able to supply beneficial effects by eating fruits if fruits contain substantial amount of free amino acids. In this extent, the presence of GABA in fruits has been shown [17]. The databases of amino acid composition in foods have been published from WHO/FAO and some countries including Japan [18]. However, these database are specialized for amino acids after foods being acid hydrolyzed where acid sensitive amino acids, such as glutamine (Gln), asparagine (Asn) and tryptophan (Trp) are destroyed. Other minor amino acids, like GABA, ornithine (Orn) and citrulline (Cit), are often not shown. Some information of free amino acids in fruits are available; however most were published about 20 years ago and recent data is rare [19]. From these reasons, we are attempting to construct a new database for specializing free amino acids existing in various plant foods including vegetables, mushrooms, and fruits [20-21]. In this article, we report free amino acid compositions in some freshly harvested fruits which have not been reported until now.

Materials and Methods

Fruits Treatments

Eleven species of fruits as fresh or processed in dried or canned were either purchased from local supermarkets or obtained from local farmers in Shiga Prefecture (Table 1).

Food	Scientific Name
Apricot Goldcot	*Prunus armeniaca*
Apricot Harcot	*Prunus armeniaca*
Banana	*Musa spp*
Banana "Monkey"	*Musa spp*
Canned mandarin orane pulp	*Citrus unshiu*
Canned white peach pulp	*Prunus persica*
Dried white fig	*Ficus carica*
Dried goji berry	*Lycium chinense*
Loquat	*Eriobotrya japonica*
Oriental melon	*Cucumis melo*
Strawberry "Akihime"	*Fragaria × ananassa*

Table 1: List of foods analyzed for free-form amino acids.

Correspondence to: Hiroshi Ueno, Laboratory of Biochemistry and Microbiology, School of Agriculture, Ryukoku University, Seta, Shiga 520-2194, Japan. Tel: +81-77-599-5686 Email: ueno[AT]agr[DOT]ryukoku[DOT]ac[DOT]jp

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These fruits were selected because of their seasoning and easiness of obtaining from local supermarkets. These samples were produced not only locally but also transported from various geographic regions. Fresh fruit samples were washed with water and separated into the edible and inedible parts, including peels, seeds and skins. Samples were then stored at -25°C until the extraction of free amino acids.

Extraction of free amino acids from fruits

Frozen samples were cut into the size about 10 g, added to 100 mM HEPES-Na buffer, pH 7.0, and homogenized on ice with a Tissue-Tearor (Biospec).

Homogenate was centrifuged at 10,000 × g for 15 min and the supernatant was collected. Proteins in the supernatant were removed by adding 60% perchloric acid where precipitate was removed by centrifugation. Remaining protein contents in samples were estimated by Bradford protein assays (BioRad). If still some proteins were present, samples were applied on an ultrafiltration using CENTRICON®10 (Millipore). After the ultrafiltration, protein content was again measured by Bradford protein assay.

Amino acid standards

GABA, L-hydroxyproline (HYP), L-Gln, L-Asn, β-alanine (β-Ala), L-Cys, L-Orn, L-Cit and L-Trp were purchased from Wako Chemicals. Purchased amino acids were added to type H amino acid mixture standard solution (Wako Chemicals) to produce a working standard solution of 25 amino acids, where each amino acid concentrations were 100 µmol/L in 0.1 N HCl.

Amino acid analysis

Amino acid samples were reacted with 4-fluoro-7-nitrobenzofurazan (NBD-F), where derivatized NBD-amino acids were separated on a Zorbax SB-C18 column (3.0 × 50 mm, 2.7 µm; Agilent Technologies) attached to a Hitachi Ultra High Speed Liquid Chromatography system equipped with UV-Vis detector monitoring at 470 nm. Derivatization of amino acids was performed according to the Hitachi Ultra High Speed Liquid Chromatography manual. NBD-amino acids were eluted using a stepwise gradient of increasing elution solution B as follows: equilibration with 15%, 15-22% or 15-25% for 2.7 min, 25-35% for 3.5 min, 35-70% for 1.0 min, 70-85% for 0.1 min, and kept at 85% for 1.5 min, in sequence. Elution solutions A and B were purchased from Hitachi High-Technologies. The flow rate was maintained at 0.550 mL/min throughout the analysis.

Reproducibility was routinely examined by injecting standard solution 10 consecutive times (Table 2). Typically, relative standard deviation of peak area for cysteine (Cys) and Ala was obtained within the range of 0.46 and 6.82%, respectively. The mean limit of detection was 0.8 pmol.

Each food samples were analyzed three times per extraction and average values were used for analysis.

Results and Discussion

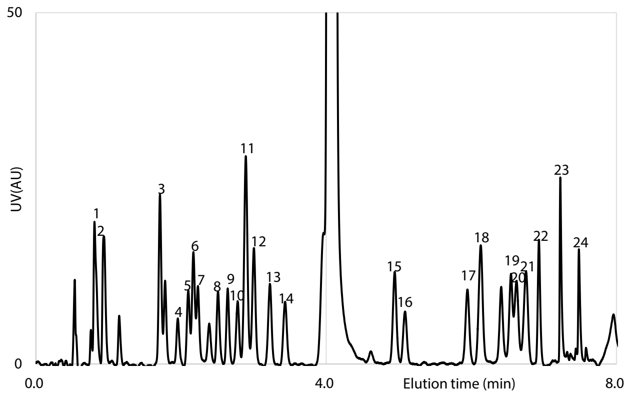
Typical chromatograms of amino acid separation are shown in Figures 1 and 2 for the amino acid mixture standard and apricot Goldcot. All of the NBD-amino acids gave baseline separation within 8 min analysis except for Pro and β-Ala, those are eluted together. Our analytical system is highly reproducible.

Free amino acid contents for 11 fruits were analyzed as summarized in Tables 3A and 3B, in which amino acid distributions varied considerably. Total free amino acids found in the extracts ranged from 48.90 µmol for loquat to 2,160 µmol for apricot Goldcot per 100 g.

Many fruits analyzed contain Asn abundantly. On the other

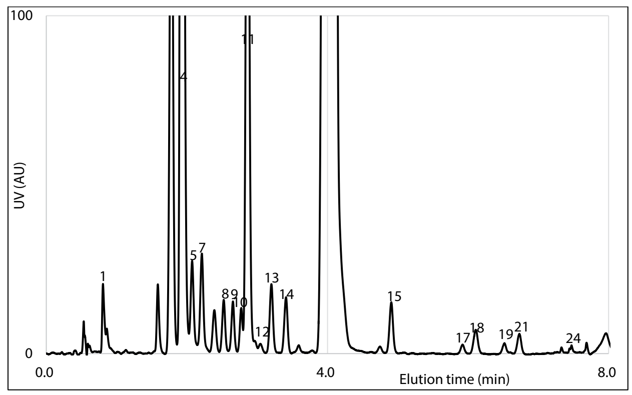
Amino Acid	Three letter code	Peak area				Elution time (min)			
		Means	±	SE	%RSD	Means	±	SE	%RSD
Histidine	His	60670	±	1237	6.45	0.804	±	0.001	0.26
Arginine	Arg	57213	±	1100	6.08	0.928	±	0.003	0.91
Hydroxyproline	HYP	61715	±	603	3.09	1.692	±	0.004	0.73
Asparagine	Asn	20439	±	379	5.86	1.940	±	0.003	0.47
Glutamine	Gln	28527	±	349	3.87	2.093	±	0.001	0.23
Citrulline	Cit	47212	±	336	2.25	2.161	±	0.002	0.33
Serine	Ser	31665	±	271	2.71	2.226	±	0.002	0.22
Aspartate	Asp	30304	±	447	4.67	2.505	±	0.001	0.10
Glutamate	Glu	30545	±	325	3.36	2.640	±	0.001	0.16
Threonine	Thr	27305	±	419	4.85	2.773	±	0.002	0.18
Proline+β-alanine	Pro+β-Ala	98012	±	326	1.05	2.886	±	0.002	0.17
Glycine	Gly	53070	±	614	3.66	2.999	±	0.001	0.13
GABA	GABA	37508	±	652	5.50	3.220	±	0.001	0.11
Alanine	Ala	30429	±	656	6.82	3.430	±	0.001	0.08
Valine	Val	43564	±	201	1.46	4.942	±	0.001	0.07
Methionine	Met	26324	±	180	2.16	5.085	±	0.001	0.04
Leucine	Leu	37194	±	398	3.39	5.941	±	0.002	0.10
Isoleucine	Ile	65445	±	184	0.89	6.127	±	0.001	0.06
Tryptophan	Trp	41894	±	201	1.51	6.547	±	0.002	0.09
Ornithine	Orn	45056	±	283	1.98	6.620	±	0.002	0.08
Phenylalanine	Phe	55173	±	152	0.87	6.748	±	0.002	0.09
Lysine	Lys	43219	±	141	1.03	6.928	±	0.001	0.05
Cysteine	Cys	43939	±	63	0.46	7.223	±	0.001	0.05
Tyrosine	Tyr	30535	±	69	0.71	7.482	±	0.001	0.05

Table 2: Means of peak area and retention time (min) 10 replicates analysis.



1: Histidine, 2: Arginine, 3: Hydroxyproline, 4: Asparagine, 5: Glutamine, 6: Citrulline, 7: Serine, 8: Aspartate, 9: Glutamate, 10: Threonine, 11: Proline + β-alanine, 12: Glycine, 13: g-amino butyric acid (GABA), 14: Alanine, 15: Valine, 16: Methionine, 17: Leucine, 18: Isoleucine, 19: Tryptophan, 20: Ornithine, 21: Phenylalanine, 22: Lysine, 23: Cysteine, 24: Tyrosine

Figure 1: HPLC chromatogram of the 50 μmol/l amino acids standard mixture derivatized with NBD-F and UV-VIS detection.



1: Histidine, 2: Arginine, 3: Hydroxyproline, 4: Asparagine, 5: Glutamine, 6: Citrulline, 7: Serine, 8: Aspartate, 9: Glutamate, 10: Threonine, 11: Proline + β-alanine, 12: Glycine, 13: g-amino butyric acid (GABA), 14: Alanine, 15: Valine, 16: Methionine, 17: Leucine, 18: Isoleucine, 19: Tryptophan, 20: Ornithine, 21: Phenylalanine, 22: Lysine, 23: Cysteine, 24: Tyrosine

Figure 2: HPLC chromatogram of the apricot Goldcot derivatized with NBD-F and UV-VIS detection.

Amino acids Three letter code[1]	Apricot Goldcot	Apricot Harcot	Banana	Banana "Monkey"	Canned mandarin orane pulp	Canned white peach pulp
His	8.614 ± 0.630	4.977 ± 0.851	228.2 ± 4.850	135.5 ± 27.75	12.97 ± 4.124	2.249 ± 0.140
Arg	nd	0.414 ± 0.273	58.14 ± 11.61	48.13 ± 9.717	33.71 ± 3.765	nd
HYP	nd	nd	nd	1.386 ± 0.000	nd	nd
Asn	1861 ± 138.8	1450 ± 234.6	29.52 ± 6.887	93.75 ± 16.52	84.55 ± 8.756	267.0 ± 22.20
Gln	35.29 ± 1.251	47.38 ± 11.27	7.174 ± 1.247	22.30 ± 3.550	nd	nd
Cit	nd	nd	nd	nd	nd	nd
Ser	36.89 ± 2.720	6.110 ± 13.73	65.38 ± 15.93	51.64 ± 11.94	24.89 ± 3.654	7.846 ± 0.759
Asp	26.00 ± 1.442	35.34 ± 8.244	3.763 ± 1.884	14.53 ± 4.949	5.971 ± 1.173	4.168 ± 1.312
Glu	19.89 ± 1.693	13.13 ± 2.484	2.292 ± 1.254	7.391 ± 1.923	5.992 ± 0.888	2.281 ± 0.276
Thr	18.13 ± 1.254	27.46 ± 5.767	29.84 ± 5.397	29.44 ± 6.369	5.154 ± 0.545	2.226 ± 0.190
Pro+β-Ala	74.17 ± 4.766	21.92 ± 6.363	31.62 ± 5.115	19.78 ± 3.769	89.76 ± 6.583	3.974 ± 0.629
Gly	1.765 ± 0.439	3.086 ± 1.018	27.86 ± 7.836	16.76 ± 3.864	4.857 ± 0.472	nd
GABA	20.20 ± 1.696	24.40 ± 5.566	18.40 ± 4.959	22.45 ± 5.392	23.30 ± 3.876	nd
Ala	22.96 ± 2.367	100.2 ± 34.12	9.843 ± 2.342	9.367 ± 2.475	16.33 ± 2.413	2.273 ± 0.266
Val	16.82 ± 1.140	18.62 ± 3.758	20.63 ± 5.116	21.37 ± 5.687	2.900 ± 0.374	1.298 ± 0.712
Met	nd	nd	1.255 ± 1.255	nd	nd	1.917 ± 0.000
Leu	2.555 ± 0.436	3.641 ± 1.926	59.80 ± 17.73	47.77 ± 12.23	1.553 ± 0.000	nd
Ile	7.349 ± 0.555	11.53 ± 2.226	9.788 ± 2.862	7.166 ± 1.756	1.876 ± 0.215	1.654 ± 0.117
Trp	0.487 ± 0.319	nd	12.14 ± 2.584	10.96 ± 2.578	nd	nd
Orn	nd	nd	nd	3.935 ± 0.522	2.719 ± 0.231	nd
Phe	5.807 ± 0.428	5.397 ± 0.856	18.21 ± 2.824	17.66 ± 3.621	4.249 ± 0.560	nd
Lys	nd	0.792 ± 0.398	11.36 ± 5.286	5.546 ± 0.776	1.419 ± 0.258	nd
Cys	nd	nd	0.354 ± 0.354	nd	nd	nd
Tyr	2.036 ± 0.071	5.447 ± 1.334	14.57 ± 2.723	13.97 ± 4.269	4.566 ± 0.544	6.685 ± 0.233
TFAA	2160	1779	660.2	600.8	326.8	303.6

Data presented as means ± SE (n = 3).

TFAA: Total free amino acids, nd: not detected

[1] Three letter code of amino acids are referred to the Table 1

Table 3A: Contents of free-form amino acids assayed in fruits (μmol/100 g sample on a wet weight basis).

Amino acids Three letter code[1)]	Dried white fig	Dried goji berry	Loquat	Oriental melon	Strawberry "akihime"
His	nd	11.65 ± 4.978	0.129 ± 0.129	5.496 ± 0.177	5.225 ± 0.653
Arg	5.041 ± 1.566	59.90 ± 15.15	nd	1.572 ± 0.117	1.629 ± 1.629
HYP	nd	nd	nd	nd	nd
Asn	42.27 ± 13.18	555.7 ± 119.4	11.88 ± 0.246	19.22 ± 0.596	384.0 ± 25.82
Gln	1.589 ± 1.122	249.5 ± 62.17	0.586 ± 0.586	68.46 ± 4.234	339.4 ± 59.27
Cit	nd	nd	nd	6.531 ± 0.263	nd
Ser	4.275 ± 1.441	173.0 ± 45.86	nd	38.56 ± 0.617	46.36 ± 12.11
Asp	nd	21.20 ± 7.668	7.167 ± 1.144	34.88 ± 2.332	14.81 ± 2.988
Glu	nd	15.40 ± 5.239	22.18 ± 1.973	18.86 ± 1.247	20.67 ± 5.427
Thr	49.86 ± 7.867	42.70 ± 1.419	nd	8.849 ± 0.353	21.97 ± 6.714
Pro+β-Ala	246.7 ± 62.83	1694 ± 324.0	1.323 ± 0.276	6.463 ± 0.260	2.586 ± 1.692
Gly	nd	nd	nd	26.98 ± 0.593	11.19 ± 3.437
GABA	2.208 ± 0.895	13.00 ± 5.215	0.941 ± 0.572	30.58 ± 1.728	12.82 ± 3.133
Ala	11.81 ± 3.873	190.5 ± 51.44	4.440 ± 0.580	127.0 ± 5.245	59.52 ± 15.98
Val	nd	10.72 ± 3.328	nd	4.724 ± 0.165	11.85 ± 3.569
Met	nd	nd	nd	2.524 ± 0.141	nd
Leu	nd	7.310 ± 2.968	nd	13.18 ± 0.176	15.34 ± 1.230
Ile	nd	2.210 ± 1.421	nd	nd	2.356 ± 1.764
Trp	nd	2.156 ± 1.371	nd	6.520 ± 0.116	6.961 ± 1.426
Orn	nd	6.756 ± 1.794	nd	nd	nd
Phe	nd	1.053 ± 0.673	nd	3.755 ± 0.167	4.544 ± 2.273
Lys	nd	2.950 ± 0.398	nd	nd	nd
Cys	nd	0.888 ± 0.561	nd	nd	2.967 ± 1.348
Tyr	2.964 ± 0.476	4.699 ± 0.346	0.252 ± 0.252	2.773 ± 0.424	12.32 ± 0.824
TFAA	368.2	3065	48.90	426.9	976.5

Data presented as means ± SE (n = 3).
Most fruits are presented on a wet weight basis except dried white fig and dried goji berry which are presented on a dry weight basis.
TFAA: Total free amino acids, nd: not detected [1)] Three letter code of amino acids are referred to the Table 1

Table 3B: Contents of free-form amino acids assayed in fruits (Continued) (μmol/100 g sample on a wet/ dry weight basis).

hand, HYP, Cys, Orn, Cit and methionine (Met) are the free amino acids existing at low levels in all fruits tested. Banana is unique in a sense containing abundant amount of histidine (His) that is not seen in other fruits.

Two Apricots, Goldcot and Harcot, belong to different cultivars, gave somehow different free amino acid compositions, for example, six times more Ser in Goldcot than in Harcot. Goldcot contains more total free amino acids than Harcot.

Most fruits we have tested contain GABA as a free amino acid at above 10.0 μmol per 100 g level, where oriental melon has the most. GABA is known of its health-promoting functions for human, such as an anti-hypotensive effect [4]. Some studies suggest that daily supplementation of GABA with 10~20 mg (equivalent to 97~194 μmol) is enough to lower blood pressure in human [22,23]. Hence, GABA is listed as "Tokuho" in Japan for its beneficial effects on human. Commercial teas, for example, are enriched with GABA at 20 mg. In this extent, eating one apricot a day, Harcot weighing about 40 g, provides approximately 10 μmol GABA, not sufficient to maintain blood pressure at a normal level. As we reported earlier [20,21], it is highly probable to supplement daily GABA for the purpose to lower blood pressure by eating a combination of fruits, mushroom, and plant supplies.

In plants including fruits, these free amino acids are metabolized or stored as nutritional source, and some are utilized as signaling molecules to regulate cellular functions including enzyme activity, gene expression, and redox-homeostasis [24]. Gln, which is abundant in the strawberry analyzed, has function as a signal molecule inducing the gene expression involved in metabolism, transport, signal transduction, and stress responses [25].

GABA and Pro are involved in the regulation of plants responding to various stress conditions like mineral deficiencies [26,27]. From these points, the different composition of the total free amino acids and individual amino acids in fruits were influenced by various factors including cultivation conditions, geographic production location, picking season, breed, and soil.

Our present results provide free-form amino acids in fruits, those typically available in the supermarket. Furthermore, our database of free amino acids would provide ample information to serve for the nutritional studies.

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