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Construction of a free-form amino acid database for vegetables and mushrooms

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Abstract

Free-form amino acids were quantitatively measured in over 30 species of vegetables and mushrooms. Significant amounts of free-form amino acids were found in each species analyzed. Additionally, there appear to be no rules or patterns of free-form amino acid distribution among species. Highly functional free-form amino acids, like γ-aminobutyric acid, are found in significant levels and suggest that amino acid analysis could play an informative role in nutrition. Our analysis should provide valuable data for the establishment of nutritional databases that include free-form amino acids and more accurately represent the entire amino acid profile of foods.

Introduction

Amino acids play significant biological roles in human. The amino acids required for human metabolism arise from the digestion and utilization of amino acids from food, and from free-form amino acids within food components (For a list of amino acid abbreviations used in this study: Table 1). Free-form amino acids may be utilized as nutrients and/or act as messengers that target specific receptors.

Free-form amino acids exist in foods derived from plants and animals. β-alanine (β-Ala), citrulline (Cit), γ-aminobutyric acid (GABA), and ornithine (Orn) usually exist as free-form amino acids and perform various biological functions including coenzyme components, control of osmoregulation, ammonia detoxification, and as neurotransmitters [1,2]. In human, tryptophan (Trp), a precursor of serotonin and melatonin, acts to regulate circadian rhythm [3]. Some free-form amino acids act as neurotransmitters: in the central nervous system GABA and glycine (Gly) function as inhibitory signals while glutamic acid (Glu) is an excitatory signal [4-8] and may have similar functions in non-neuronal cells [5]. The roles of free-form amino acids in dietary supplements have been extensively explored and dietary branched chain amino acids (BCAA; including valine (Val), leucine (Leu), and isoleucine (Ile)), improve exercise performance, endurance performance, and upper body power in human [9]. Moreover, GABA can reduce high blood pressure [10], and in Japan is authorized as "Tokuho", which is a "food for specified health uses", and other health foods containing GABA are widely available.

Glu receptors have been identified on taste buds and the gastrointestinal tract, including stomach cells [11], which suggests that free-form Glu may be involved in enhancing digestion, nutrient absorption, and/or utilization of nutrients [12]. The study was extended to examine the use of daily dietary Glu supplements in the meals of elderly people in hospital [12]. The results of this analysis showed that Glu supplementation improved the nutritional status of elderly and nutritionally deficient patients through improved food intake [12-14]. Therefore, free-form Glu is a highly effective supplement with the potential to improve quality of life (QOL) [12,15,16]. It is likely that free-form amino acids exert useful effects on physiologic functions, particularly during food digestion and nutrient absorption.

Some free-form amino acids act as palatable compounds and are key determinants in the taste of food. Free-form Glu, also known as monosodium glutamate or MSG, is an umami compound isolated from kelp, and is also rich in tomatoes and cheese [16]. Other free-form amino acids that confer taste include Gly, alanine (Ala), and proline (Pro) which provide sweetness, and Leu, Ile, and Val which provide a bitter taste [17,18]. L-serine (Ser) gives mainly sweetness and minor umami taste, and D-Ser, enantiomer of L-Ser, has sweetness. As for taste of D-amino acids, it is known that D-Ala and D-Leu are sweeter than their L-enantiomers [19]. Amino acids elicit not only single basic tastes but also complex taste when they are combined.

Information about the "amino acid" content of various agricultural products is available in databases, typically called "Standard Tables of Food Composition". However, amino acid databases usually list amino acid composition measured in acid hydrolyzed samples, so most of these databases do not accurately reflect free-form amino acid contents [20]. The Stein-Moore laboratory, at Rockefeller University, has shown that acid hydrolysis destroys glutamine (Gln), asparagine (Asn), Trp, and some other amino acids such as GABA, Orn, Cit [21,22]. Thus, little is known about the kinds and amounts of free-form amino acids present in foods.

Studies of nutrition, particularly concerning the quality of proteins, have progressed extensively since information about the amino acid composition of foods has become readily available. However,

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Table 1. List of amino acids and their abbreviations

Amino acid	3 Letter abbreviation
Alanine	Ala
β-Alanine	β-Ala
Arginine	Arg
Asparagine	Asn
Aspartic acid	Asp
Citrulline	Cit
Cysteine	Cys
Cystine	Cys-Cys
γ-Amino butyric acid	GABA
Glutamic acid	Glu
Glutamine	Gln
Glycine	Gly
Histidine	His
Hydroxyproline	HYP
Isoleucine	Ile
Leucine	Leu
Lysine	Lys
Methionine	Met
Ornithine	Orn
Phenyl alanine	Phe
Proline	Pro
Serine	Ser
Threonine	Thr
Tryptophan	Trp
Tyrosine	Tyr
Valine	Val

information about the amino acid composition of both hydrolyzed samples and free-form amino acids in the original food samples are necessary for analyses of palatability and the beneficial functions of amino acids. The Japan Society of Nutrition and Food Science website lists some information about free-form amino acids in food, but the data presented was collated from literature published about 30 years ago [23-26]. Therefore, the amino acid contents listed in some of these databases are likely to be misleading or incomplete.

Amino acid analysis has advanced over the last few decades, and the time has come to renew these data using modern techniques that provide increased sensitivity and reliability. Currently, only a few databases specializing in free-form amino acids are available. To understand the amount of free-form amino acids in our daily foods, free-form amino acids must be "extracted" and then measured. Previously, we reported initial attempts to establish a free-form amino acid database for approximately 140 species of fruits and vegetables [27]. We showed that distribution of free-form amino acids is characteristic for each sample and significantly different from the amino acid distribution recorded in acid hydrolyzed databases. Here, we have extended our analysis of free-form amino acid contents to include additional vegetables and some mushroom species.

Materials and methods

Extraction of free amino acids from foods

We examined 38 species of vegetables and mushrooms (Table 2). Some samples were purchased from local supermarkets in Shiga Prefecture and others were supplied by local farmers. The food samples came from various geographic regions. All food samples were washed in water and the edible and inedible parts, including peels, seeds, and skins, were separated. Samples were stored at -25°C until use.

Sample preparation

Samples (5-10 g) were ground to a fine powder with a mortar and pestle. The powdered samples were added to 100 mM HEPES-Na buffer, pH 7.0, and homogenized on ice with a Tissue-Tearor (Biospec). The homogenate was centrifuged at 10,000×g for 10 min and the supernatant was collected. Suitable supernatant aliquots were treated with 60% perchloric acid to remove proteins. The protein content of samples was estimated by Bradford protein assays (BioRad). Ultrafiltration was performed using CENTRICON®10 (Millipore) when further removal of protein contamination was necessary. After ultrafiltration, the protein content was measured by Bradford protein assay.

Amino acid standards

GABA, hydroxyproline (HYP), L-Gln, L-Asn, β-Ala, L-cysteine (Cys), 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 each amino acid (100 μmol/L in 0.1 N HCl).

Amino acid analysis

Free-form amino acids extracted from food samples were analyzed on a Hitachi Ultra High Speed Liquid Chromatography system

Table 2. List of foods analyzed for free-form amino acids

	Scientific Name	Food
Vegetables	*Abelmoschus esculentus*	Okra "Yashiro-shio okura"
	Allium cepa	Onion
	Allium cepa	Red onion
	Capsicum annuum	Paprika
	Chrysanthemum morifolium	Edible chrysanthemum
	Chrysanthemum morifolium	Edible purple chrysanthemum "Enmeiraku"
	Cucurbita maxima	Winter squash
	Cucurbita moschata	Japanese squash
	Daucus carota	young carrot (Leaf)
	Daucus carota	Moso bamboo
	Phyllostachys heterocycla	Pea
	Pisum sativum	Peas (Tutankhamen)
	Pisum sativum	Podded pea
	Raphanus sativus	Radish sprout
	Rheum rhabarbarum	Garden rhubarb
	Solanum mlycopersicum	Cherry tomato
	Solanum melongena	Egg plant "Beinasu"
	Solanum melongena	Egg plant "Kamonasu"
	Solanum melongena	Egg plant "Kyokonasu"
	Solanum melongena	Egg plant "Marunasu"
	Solanum melongena	Egg plant "Zebranasu"
	Spinacia olerace	Spinach "Okame"
	Vicia faba	Broad bean
	Vigna unguiculata	Cowpea
	Vigna unguiculata	Cowpea beans
		Pumpkin "Korinnki"
Mushrooms	*Agaricus bisporus*	Button mushroom (brown)
	Agaricus bisporus	Button mushroom (white)
	Auricularia auricula-judae	Jew's ear
	Boletus edulis	Porcini mushrooms (Dried)
	Flammulina velutipes	Winter mushroom
	Grifola frondosa	Mushroom "Maitake"
	Hypsizygus marmoreus	Beech mushroom
	Hypsizygus marmoreus	Beech mushroom(Tanba)
	Letinula edodes	Mushroom "Shiitake"
	Lyophyllum shimeji	Mushroom "Honshimeji"
	Pleurotus eryngii	King oyster mushroom

equipped with UV-Vis detector. Samples were reacted with 4-fluoro-7-nitrobenzofurazan (NBD-F) and derivatized NBD-amino acids were separated on a Zorbax SB-C18 column (3.0 × 50 mm, 2.7 μm; Agilent Technologies) and elution was monitored 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 B solution: equilibration with 15%, 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, where solution A mainly consists of ammonium formate and solution B of acetonitrile. The flow rate was maintained at 0.550 mL/min throughout the analysis.

Linearity of the dose response was evaluated using an amino acid standard solution within the range of 10 to 60 pmol. Minimum and maximum correlation coefficients (r^2) for Asn, as an example, were 0.974 and 0.997, respectively. Ten consecutive standard solution injections were analyzed to evaluate reproducibility (Table 3). The relative standard deviation of peak area for Tyr and His was in the range of 0.3% and 5.1%, respectively. The mean limit of detection was 0.8 pmol.

Results

Chromatograms for the amino acid mixture standard and eggplant "kamonasu" (*Solanum melongena*) were obtained and analyzed (Figures 1 and 2). In our system, the typical time to baseline NBD-amino acid separation, with the exception of Pro and β-Ala, was about 8 min.

The free-form amino acid contents of the vegetables and mushrooms analyzed varied considerably (Tables 4 and 5). The total free-form amino acids found in the extracts ranged from 59.91 (Garden rhubarb) to 4,093 and 424.6 (Jew's ear) to 12,773 μmol per 100 g of vegetables and mushrooms, respectively. Of the vegetables and mushrooms analyzed, the podded pea (4,093 μmol per 100 g) and dried porcini mushrooms (12,773 μmol per 100 g) had the highest total free-form amino acids of vegetables and mushrooms, respectively. The most abundant free-form amino acids found in the vegetables examined were Asn, Gln, Glu, and GABA, all of which are important metabolites in human. In mushrooms, arginine (Arg), Glu, Gln, and Ala were the most abundant free-form amino acids. Orn was found in mushrooms, but rarely in vegetables and HYP, Cys, and methionine (Met) were found at low levels in both vegetables and mushrooms.

Table 3. Means of peak area and retention time (min) 10 replicates analysis

	Peak Area		Elution Time (min)	
	Mean ± SE	**%RSD**	**Mean ± SE**	**%RSD**
His	107839 ± 1724	5.05	0.784 ± 0.001	0.290
Arg	100894 ± 1427	4.47	0.909 ± 0.004	1.250
HYP	113930 ± 589	1.64	1.715 ± 0.004	0.740
Asn	34137 ± 497	4.61	2.003 ± 0.001	0.216
Gln	50739 ± 470	2.93	2.158 ± 0.002	0.224
Cit	79837 ± 342	1.36	2.250 ± 0.001	0.166
Ser	53349 ± 478	2.83	2.331 ± 0.002	0.301
Asp	48268 ± 530	3.47	2.760 ± 0.001	0.156
Glu	51824 ± 418	2.55	2.927 ± 0.001	0.101
Thr	107560 ± 300	0.88	3.073 ± 0.001	0.094
Pro+β-Ala	106552 ± 302	0.90	3.156 ± 0.001	0.132
Gly	90638 ± 637	2.22	3.254 ± 0.002	0.166
GABA	62614 ± 510	2.57	3.551 ± 0.002	0.141
Ala	53683 ± 593	3.49	3.817 ± 0.001	0.124
Val	78636 ± 237	0.95	5.465 ± 0.002	0.139
Met	61485 ± 95	0.49	5.612 ± 0.001	0.080
Leu	68875 ± 316	1.45	6.363 ± 0.002	0.099
Ile	84374 ± 233	0.87	6.519 ± 0.003	0.140
Cys-Cys	56979 ± 197	1.09	6.689 ± 0.002	0.118
Trp	69741 ± 295	1.34	6.820 ± 0.002	0.078
Orn	74663 ± 277	1.17	6.864 ± 0.002	0.089
Phe	80193 ± 134	0.53	6.947 ± 0.002	0.069
Lys	79550 ± 210	0.83	7.026 ± 0.001	0.063
Cys	82425 ± 178	0.68	7.226 ± 0.001	0.029
Tyr	61109 ± 62	0.32	7.450 ± 0.001	0.031

Amino acids are listed as abbreviated according to Table 1.

The free-form amino acid compositions of five different kinds of eggplant were analyzed. Slight differences were observed in the free-form amino acid contents of the five species, but Asn, Gln, and GABA were abundant in all.

BCAAs, common ingredients in drinks designed for athletes, were not detected in most foods analyzed. However, moso bamboo and some peas, including the podded pea, contained considerable amounts of Val, Leu, and Ile BCAAs.

Discussion

Here, we identified and measured significant levels of free-form amino acids in both vegetables and mushrooms. These free-form amino acids should contribute to the taste and palatability of foods. Indeed, Glu provides umami taste and has been shown to improve appetite [12,14,15], both of which contribute to QOL. Glu is particularly important in the foods of elderly and sick people, as it improves QOL by increasing nutrient intake. It is often discussed that excess intake of amino acids is toxic for human. For example, it has been warned that Glu has a potential risk of neurotoxicity like headache. However, this warning was almost eliminated after the joint report of FAO, WHO, and the Expert Committee on Food Additives (JECFA) in 2000 [28]. Thus, Glu is considered relatively safe and could be consumed without any concerns.

GABA is known for health-promoting functions, including an anti-hypotensive effect. Some studies suggest that supplementation with 10~20 mg (equivalent to 97~194 μmol) GABA reduces blood pressure in human [29,30]. Commercially available GABA enriched "Tokuho" foods are typically supplemented with 20 mg of GABA in Japan. Our present study suggests that two pieces of eggplant "kamonasu", equivalent to 90 g, per day provides enough GABA to maintain blood pressure at a normal level.

Free-form amino acids are significantly beneficial to human health. The presence of these amino acids in vegetables and mushrooms suggests that they also provide a benefit to those species. In plants, free-form amino acids are likely absorbed directly from soil via the roots [31]. Some of these free-form amino acids are metabolized or stored, and some are utilized as signaling molecules to regulate cellular functions including enzyme activity, gene expression, and redox-homeostasis [32]. Pro, Arg, Met, Glu, and GABA are involved in the regulation of plant responses to various stress conditions [33-35]. Asn, the most abundant free-form amino acid in the foods analyzed in our study, is accumulated under stress conditions or mineral deficiencies [36]. In plants, free-form Asn also plays a central role in nitrogen storage and transport [36,37]. The roles of free-form amino acids in mushrooms are not well understood and are still being determined.

Here, we have demonstrated the presence of free-form amino acids in plants. However, there are many factors that influence

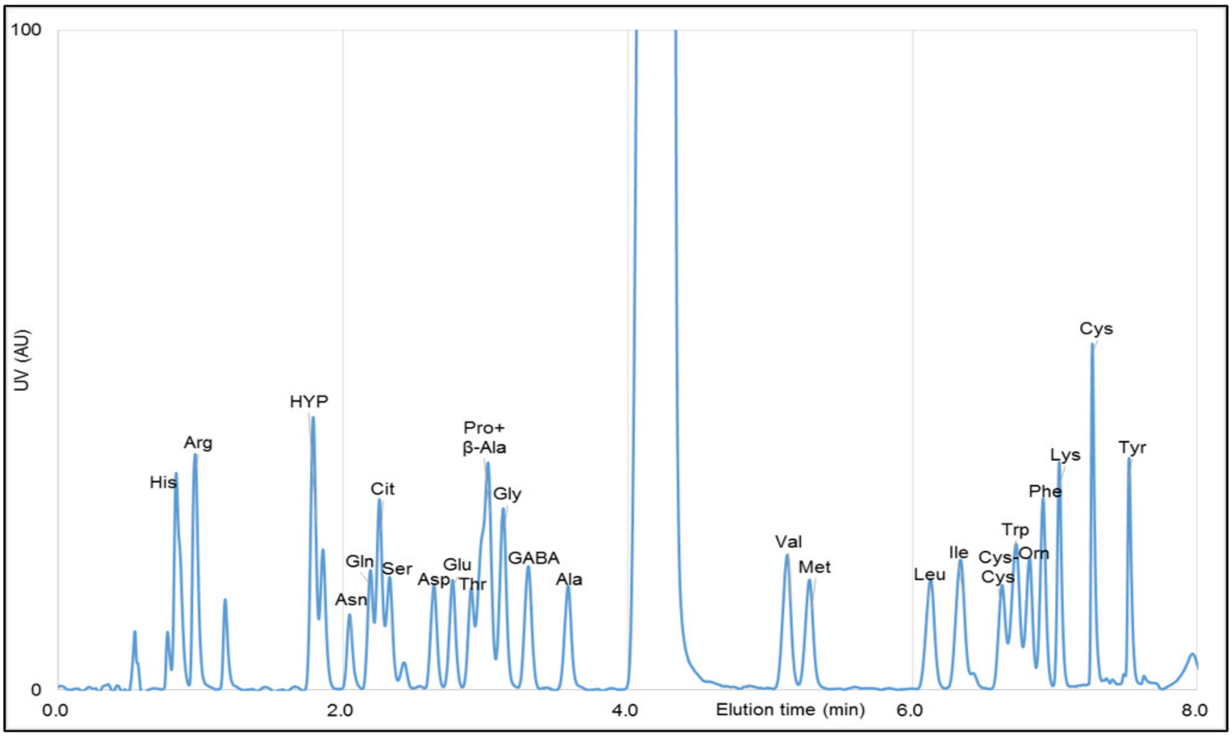

Figure 1. HPLC chromatogram of the amino acids standard mixture derivatized with NBD-F and UV-VIS detection

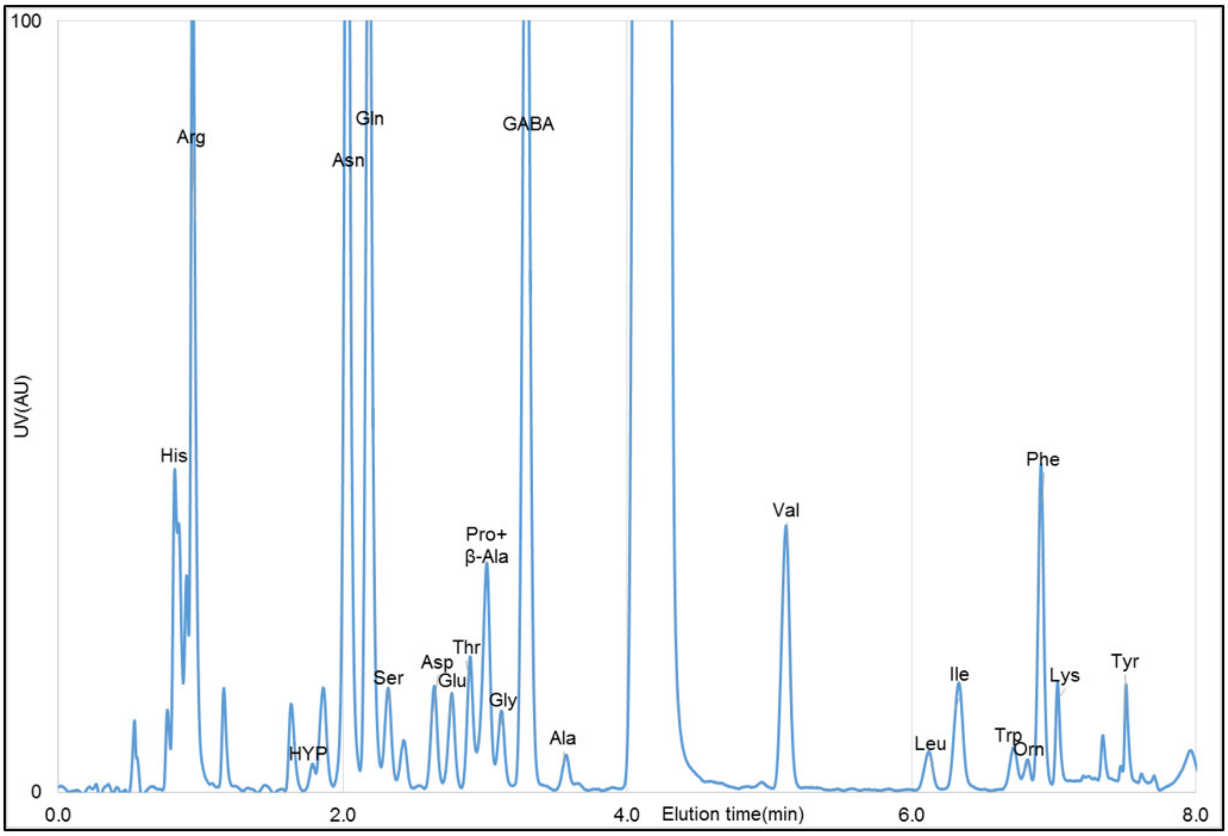

Figure 2. HPLC chromatogram of the "kamonasu" derivatized with NBD-F and UV-VIS detection

Table 4. Contents of free-form amino acids assayed in vegetables (µmol/100 g sample on a wet weight basis)

	Okra"Yashiro-shio okura"	Onion	Red onion	Paprika	Edible chrysanthemum "Kinkaramatsu"	Edible chrysanthemum "Enmeiraku"
His	39.5 ± 0.977	16.06 ± 4.234	8.394 ± 0.674	10.68 ± 1.84	13.44 ± 3.137	9.415 ± 1.225
Arg	86.12 ± 2.473	83.6 ± 5.363	53.49 ± 4.528	44.01 ± 2.39	32.75 ± 7.504	17.23 ± 3.145
HYP	nd	nd	nd	0.32 ± 0.261	nd	nd
Asn	1480 ± 40.59	79.14 ± 8.421	87.73 ± 4.638	473.1 ± 45.43	408.1 ± 116	128.1 ± 7.235
Gln	908 ± 2.846	157.3 ± 10.06	286.2 ± 19.35	255.8 ± 23.08	469.3 ± 111.9	596.7 ± 51.47
Cit	nd	nd	nd	nd	nd	nd
Ser	129.1 ± 3.901	15.97 ± 1.216	24.37 ± 1.356	99.13 ± 12.57	53.32 ± 14.24	61.87 ± 4.289
Asp	46.51 ± 0	15.3 ± 1.811	16.42 ± 0.921	83.82 ± 15.04	76.64 ± 14.9	115.3 ± 11.64
Glu	105.3 ± 5.084	29.89 ± 1.902	29.09 ± 1.11	36.91 ± 4.401	98.7 ± 19.51	143.7 ± 9.575
Thr	42.67 ± 1.133	135.9 ± 9.568	95.39 ± 5.964	36.7 ± 7.325	58.42 ± 15.05	55.44 ± 3.696
Pro+β-Ala	18.15 ± 0.806	4.231 ± 0.167	6.233 ± 0.567	19.2 ± 3.532	103.1 ± 17.66	24.09 ± 1.438
Gly	22.59 ± 0.228	4.777 ± 0.204	3.875 ± 0.332	17.28 ± 5.297	6.604 ± 3.816	7.871 ± 0.686
GABA	79.61 ± 11.49	4.982 ± 0.523	2.74 ± 0.237	36.21 ± 4.637	0.831 ± 0.831	nd
Ala	53.62 ± 3.453	14.66 ± 1.369	22.93 ± 1.376	21.45 ± 2.529	17.69 ± 5.758	33.81 ± 3.291
Val	35.36 ± 2.323	19.74 ± 1.286	19.77 ± 0.994	40.05 ± 7.5	26.74 ± 6.647	23.73 ± 2.163
Met	nd	nd	nd	4.971 ± 0.58	nd	nd
Leu	21.44 ± 0	28.89 ± 1.684	31.6 ± 1.652	15.5 ± 1.354	5.079 ± 2.957	nd
Ile	13.34 ± 0.802	15.14 ± 0.701	13.66 ± 1.315	9.44 ± 0.669	14.58 ± 5.23	6.451 ± 0.718
Cys-Cys	nd	nd	nd	nd	nd	nd
Trp	16.61 ± 0.042	9.72 ± 1.496	6.809 ± 0.757	nd	3.274 ± 1.375	nd
Orn	nd	nd	nd	nd	nd	nd
Phe	44.37 ± 0.51	21.26 ± 1.237	20.46 ± 1.604	9.36 ± 0.673	21.3 ± 5.56	4.035 ± 0.349
Lys	4.88 ± 0.641	25.19 ± 3.425	27.54 ± 2.864	4.736 ± 2.777	0.456 ± 0.456	4.164 ± 0.705
Cys	0.442 ± 0.312	nd	nd	25.03 ± 5.24	0.647 ± 0.373	nd
Tyr	23.56 ± 0.98	31.61 ± 1.768	20.01 ± 0.273	11.63 ± 0.219	4.63 ± 1.658	nd
	Winter squash	**Japanese squash**	**Corinky squash**	**Young carrot (Root)**	**Young carrot (Leaf)**	**Moso bamboo**
His	74.06 ± 1.997	57.78 ± 1.118	77.19 ± 4.327	0.665 ± 0.47	7.221 ± 0.163	36.11 ± 2.543
Arg	37.9 ± 10.38	15.37 ± 1.592	26.6 ± 3.459	1.141 ± 0.807	0.485 ± 0.343	52.1 ± 5.874
HYP	1.134 ± 0	nd	nd	nd	nd	nd
Asn	82.25 ± 2.595	1025 ± 37.8	162.1 ± 16.04	2.948 ± 0.458	17.41 ± 1.325	995.7 ± 151.2
Gln	90.75 ± 6.974	367.3 ± 14.21	281.9 ± 35.3	16.13 ± 5.61	55.48 ± 6.236	368.6 ± 68.51
Cit	nd	nd	nd	nd	nd	nd
Ser	63.67 ± 3.586	231.2 ± 15.89	90.86 ± 9.239	11.83 ± 0.405	24.09 ± 1.003	232.7 ± 42.35
Asp	192.5 ± 15.57	118.9 ± 5.924	179.7 ± 17.7	18.45 ± 4.924	51.98 ± 3.635	101.7 ± 10.36
Glu	97.7 ± 4.456	95.33 ± 14.87	21.5 ± 2.688	14.09 ± 0.914	59.27 ± 5.786	63.96 ± 6.861
Thr	23.66 ± 2.029	46.92 ± 2.309	44.8 ± 5.556	7.677 ± 0.336	24.43 ± 2.651	103.1 ± 13.78
Pro+β-Ala	14.37 ± 1.468	59.87 ± 1.693	15.03 ± 1.776	1.222 ± 0.096	8.544 ± 1.058	132.6 ± 26.76
Gly	8.521 ± 0.167	34.47 ± 1.265	9.774 ± 1.11	0.286 ± 0.202	2.966 ± 0.239	32.78 ± 4.574
GABA	112.2 ± 10.17	82.02 ± 3.727	70.72 ± 6.325	40.13 ± 6.319	40.4 ± 1.376	75.83 ± 12.81
Ala	43.4 ± 1.337	181.8 ± 9.326	50.15 ± 4.89	9.717 ± 2.299	49.69 ± 4.37	128.9 ± 31.91
Val	14.99 ± 1.734	55.74 ± 2.064	56.93 ± 5.973	5.45 ± 0.333	21.05 ± 2.243	140.8 ± 26.69
Met	7.215 ± 1.251	26.21 ± 1.322	20.58 ± 2.241	nd	nd	44.02 ± 5.688
Leu	40.01 ± 1.073	42.37 ± 5.847	54.94 ± 6	1.852 ± 0.413	5.699 ± 0.683	110.6 ± 11.53
Ile	15.85 ± 2.566	28.14 ± 0.981	35.2 ± 3.677	2.252 ± 0.407	10.44 ± 1.489	88.01 ± 12.15
Cys-Cys	nd	nd	nd	nd	nd	nd
Trp	4.827 ± 0.255	20.78 ± 1.077	5.034 ± 0.615	nd	nd	39.6 ± 2.042
Orn	nd	nd	nd	nd	nd	nd
Phe	29.65 ± 1.843	30.89 ± 1.656	41.53 ± 4.733	2.879 ± 0.04	5.631 ± 0.047	55.34 ± 5.782
Lys	2.929 ± 0.313	1.216 ± 0	14.8 ± 2.154	1.134 ± 0.802	4.007 ± 0.486	23.13 ± 2.955
Cys	0.476 ± 0	nd	nd	nd	nd	1.128 ± 0.142
Tyr	34.79 ± 4.246	54.89 ± 3.071	44.66 ± 5.257	2.195 ± 0.367	4.417 ± 0.317	361.9 ± 32.95

	Pea	Peas “Tutankhamen”	Podded pea	Radish sprout	Garden rhubarb	
His	49.61 ± 9.239	28.71 ± 1.118	54.13 ± 4.181	70.19 ± 12.28	0.598 ± 0.226	
Arg	1304 ± 77.94	703.6 ± 38.32	70.3 ± 12.99	33.05 ± 7.199	1.391 ± 0.452	
HYP	nd	nd	nd	1.762 ± 1.205	nd	
Asn	291 ± 60.56	171.5 ± 12.55	1257 ± 61.92	60.09 ± 13.23	7.21 ± 1.421	
Gln	688.1 ± 50.39	546 ± 60.41	890.1 ± 7.863	438.7 ± 77.95	16.42 ± 1.526	
Cit	nd	nd	nd	nd	nd	
Ser	153.8 ± 11.01	118.1 ± 9.087	444.1 ± 24.78	60.98 ± 12.41	3.174 ± 0.397	
Asp	161.4 ± 10.26	53.84 ± 7.253	114.5 ± 0.625	5.169 ± 1.378	4.756 ± 0.497	
Glu	257 ± 18.73	233.9 ± 4.07	84.87 ± 6.466	27.8 ± 3.028	10.9 ± 1.067	
Thr	407 ± 30.14	239.9 ± 11.59	355.7 ± 2.423	58.36 ± 11.07	1.149 ± 0.366	
Pro+β-Ala	14.21 ± 0.639	28.73 ± 5.658	42.94 ± 8.26	17.98 ± 3.882	0.704 ± 0.231	
Gly	35.94 ± 2.387	26.26 ± 0.778	34.17 ± 2.539	40.21 ± 7.198	nd	
GABA	105.8 ± 7.17	40.19 ± 1.837	84.76 ± 3.756	9.175 ± 1.978	7.235 ± 0.31	
Ala	253.4 ± 27.27	351.5 ± 5.481	387.7 ± 10.44	39.58 ± 6.501	2.866 ± 0.252	
Val	159.4 ± 9.839	155.4 ± 3.191	140.8 ± 4.633	50.55 ± 10.05	nd	
Met	12.49 ± 0.862	4.772 ± 0.17	8.843 ± 0.528	0.461 ± 0.461	nd	
Leu	37.07 ± 2.496	32.55 ± 2.118	25.58 ± 1.322	15.18 ± 2.881	0.16 ± 0.16	
Ile	45.71 ± 2.607	50.4 ± 0.83	57.76 ± 6.047	29.32 ± 5.613	nd	
Cys-Cys	nd	nd	nd	nd	nd	
Trp	3.819 ± 0.777	4.433 ± 0.697	nd	11.58 ± 1.086	nd	
Orn	2.935 ± 0.371	2.347 ± 0.009	nd	3.12 ± 0.643	nd	
Phe	26.63 ± 2.33	9.983 ± 0.101	15.8 ± 0.034	15.76 ± 2.987	nd	
Lys	28.73 ± 3.061	17.24 ± 0.54	13.65 ± 2.164	9.518 ± 1.765	nd	
Cys	1.588 ± 0.085	1.596 ± 0.055	1.26 ± 0.069	2.105 ± 0.142	nd	
Tyr	49.61 ± 9.239	12.5 ± 0.283	9.671 ± 0.02	16.45 ± 3.433	3.353 ± 0.684	
	Cherry tomato	**Eggplant "Beinasu"**	**Eggplant "Kamonasu"**	**Eggplant "Kyokonasu"**	**Eggplant "Marunasu"**	**Eggplant "Zebranasu"**
His	13.39 ± 5.62	31.46 ± 8.565	39.41 ± 3.139	88.56 ± 4.134	15.98 ± 2.025	30.67 ± 1.806
Arg	19.27 ± 5.129	26.41 ± 1.75	80.53 ± 8.906	76.24 ± 2.669	16.53 ± 4.35	90.2 ± 8.215
HYP	nd	nd	2.386 ± 0.125	nd	nd	nd
Asn	166.6 ± 71.7	355.4 ± 28.42	472.1 ± 63.92	992.8 ± 29.96	98.11 ± 19.69	152 ± 10.4
Gln	472.9 ± 214.5	206 ± 22.98	269.1 ± 36.24	130.6 ± 7.851	89.43 ± 13.8	204.1 ± 10.87
Cit	nd	nd	nd	nd	nd	nd
Ser	48 ± 16.45	10.26 ± 0.677	25.77 ± 2.991	45.42 ± 2.354	10.78 ± 1.583	18.25 ± 0.891
Asp	81.52 ± 41.2	29.51 ± 2.832	21.19 ± 0.597	60.67 ± 3.534	21.88 ± 2.575	25.66 ± 4.704
Glu	687.4 ± 290.4	20.54 ± 1.197	20.41 ± 0.961	32.95 ± 2.064	17.63 ± 2.032	16.21 ± 1.467
Thr	24.85 ± 7.489	23.64 ± 1.108	33.09 ± 3.141	62.48 ± 0.968	18.51 ± 2.113	20.87 ± 1.14
Pro+β-Ala	22.29 ± 1.618	12.98 ± 0.407	17.22 ± 1.132	30.29 ± 1.987	9.24 ± 1.061	18.5 ± 2.429
Gly	7.274 ± 2.767	4.403 ± 0.265	9.574 ± 0.55	7.413 ± 0.306	4.307 ± 0.463	7.901 ± 0.476
GABA	93.59 ± 55.17	86.84 ± 7.584	221.5 ± 18.1	74.7 ± 3.888	184.2 ± 15.42	83.94 ± 6.821
Ala	37.04 ± 16.65	3.619 ± 0.614	6.567 ± 0.482	9.47 ± 0.887	5.975 ± 0.901	8.224 ± 1.059
Val	8.005 ± 3.296	21.67 ± 0.835	45.94 ± 2.538	61.48 ± 0.896	20.98 ± 2.597	35.03 ± 1.74
Met	3.382 ± 1.15	nd	nd	nd	nd	nd
Leu	19.01 ± 6.342	4.738 ± 0.545	6.691 ± 0.134	10.81 ± 0.493	3.824 ± 0.9	16.46 ± 1.966
Ile	7.223 ± 2.122	12.46 ± 0.639	20.22 ± 1.233	27.8 ± 0.593	11.12 ± 1.615	23.61 ± 1.435
Cys-Cys	nd	nd	nd	nd	nd	nd
Trp	1.438 ± 4.39	4.056 ± 0.645	7.87 ± 1.805	14.45 ± 1.306	2.713 ± 0.182	4.411 ± 0.285
Orn	nd	1.772 ± 0.13	7.206 ± 2.17	5.766 ± 0.577	1.46 ± 0.072	9.496 ± 1.016
Phe	27.81 ± 6.499	17.34 ± 0.356	37.74 ± 1.647	97.05 ± 2.003	12.98 ± 1.54	18.1 ± 1.283
Lys	8.565 ± 4.092	12.77 ± 1.369	10.92 ± 0.899	30.36 ± 1.895	8.691 ± 1.686	9.977 ± 1.494
Cys	7.715 ± 0.878	nd	nd	1.626 ± 0	nd	nd
Tyr	11.72 ± 2.1	8.541 ± 0.919	11.25 ± 1.104	31.58 ± 0.765	7.867 ± 1.1	9.702 ± 0.373

	Spinach "Okame"	Broad bean	Cowpea	Cowpea beans
His	nd	126.6 ± 23.9	38.08 ± 6.123	163.6 ± 4.632
Arg	0.372 ± 0.372	654.7 ± 54.74	38.2 ± 7.142	162.4 ± 3.91
HYP	2.259 ± 1.311	nd	nd	nd
Asn	26.24 ± 1.131	945 ± 66.41	613 ± 30.48	392.6 ± 25.88
Gln	35.31 ± 9.173	154.1 ± 4.755	551.4 ± 28.61	248.4 ± 8.96
Cit	nd	2.675 ± 1.693	nd	nd
Ser	24.18 ± 1.985	142.1 ± 9.879	73.27 ± 2.145	72.32 ± 1.861
Asp	48.59 ± 3.46	146.2 ± 13.99	46.51 ± 0	46.51 ± 0
Glu	85.43 ± 7.502	181.6 ± 11.21	46.48 ± 1.804	265.7 ± 7.392
Thr	9.409 ± 1.653	143.3 ± 14.71	45.86 ± 0.463	90.39 ± 1.414
Pro+β-Ala	4.336 ± 0.561	36.5 ± 2.915	18.17 ± 0.047	67.29 ± 3.878
Gly	1.165 ± 0.681	31.17 ± 1.757	6.967 ± 0.301	16.37 ± 0.785
GABA	22.75 ± 1.147	226.6 ± 16.13	34.2 ± 1.668	15.39 ± 1.526
Ala	21.94 ± 2.911	654.5 ± 64.95	31.8 ± 4.071	125.9 ± 3.136
Val	4.754 ± 1.102	79.84 ± 2.213	41.82 ± 1.037	120 ± 4.047
Met	nd	1.7 ± 0.558	8.761 ± 0.393	25.52 ± 0.461
Leu	3.405 ± 0.362	27.39 ± 0.715	21.44 ± 0	21.44 ± 0
Ile	nd	25.34 ± 0.754	23.29 ± 0.467	74.98 ± 2.901
Cys-Cys	5.861 ± 3.487	nd	nd	nd
Trp	nd	32.18 ± 2.075	3.813 ± 0.47	18.24 ± 1.499
Orn	nd	nd	nd	3.388 ± 0.124
Phe	3.291 ± 0.61	11.6 ± 0.318	20.45 ± 0.577	58.47 ± 0.536
Lys	nd	60.26 ± 0.73	7.725 ± 0.576	17.8 ± 0.988
Cys	109.3 ± 0	1.153 ± 0.126	nd	2.286 ± 0.136
Tyr	12.14 ± 1.411	60.24 ± 3.935	18.83 ± 1.493	72.12 ± 2.174

Amino acids are listed as abbreviated according to Table 1.
Average of 2~5 analyses (Means ± SE), nd: Not detected.

Table 5. Contents of free-form amino acids assayed in mushrooms (μmol/100 g sample on a wet weight basis)

	Beech mushroom	Beech mushroom (Tanba)	Button mushroom (brown)	Button mushroom (white)	Jew's ear	King oyster mushroom
His	29.38 ± 7.477	99.7 ± 5.005	43.63 ± 2.66	133.7 ± 3.481	9.663 ± 1.963	80.38 ± 3.689
Arg	149.8 ± 8.816	311.2 ± 10.49	90.49 ± 2.751	99.8 ± 4.613	57.94 ± 13.16	302.6 ± 13.05
HYP	2.095 ± 1.236	nd	1.15 ± 0.734	1.847 ± 1.173	nd	13.52 ± 0.789
Asn	290.9 ± 31.47	113 ± 2.854	80.17 ± 3.27	178.1 ± 9.544	72.1 ± 12.47	106.5 ± 3.763
Gln	149.8 ± 10.74	283.2 ± 17.75	322.7 ± 12.09	802.7 ± 41.74	68.81 ± 14.11	382.9 ± 15.61
Cit	nd	nd	nd	nd	nd	nd
Ser	60.76 ± 4.634	184.2 ± 7.715	101.5 ± 3.719	182.6 ± 8.61	14.09 ± 2.211	151.8 ± 3.506
Asp	6.673 ± 0.615	7.738 ± 0.887	204.5 ± 8.97	210.3 ± 13.68	27.32 ± 6.626	37.37 ± 9.767
Glu	222.9 ± 21.88	62.48 ± 7.219	299.3 ± 13.01	372.5 ± 29.14	26.04 ± 3.239	190.1 ± 16.83
Thr	66.86 ± 4.54	121.2 ± 5.029	131.5 ± 5.477	206.8 ± 8.645	11.01 ± 1.4	145.7 ± 4.064
Pro+β-Ala	24.26 ± 1.547	84.83 ± 2.042	97.13 ± 3.355	127.2 ± 4.381	2.392 ± 0.317	64.84 ± 1.564
Gly	53.36 ± 3.844	141.4 ± 8.039	88.39 ± 3.428	163.2 ± 10.44	8.306 ± 1.602	129.2 ± 10.84
GABA	nd	98.47 ± 7.632	59.07 ± 2.68	140.9 ± 20.12	32.61 ± 8.053	35.12 ± 0.667
Ala	86.72 ± 8.508	123.5 ± 9.437	240.8 ± 9.665	598.3 ± 50.35	43.95 ± 11.21	507.6 ± 61.07
Val	33.9 ± 3.485	85.08 ± 5.763	94.7 ± 3.492	151.4 ± 8.963	4.24 ± 2.007	103.8 ± 6.616
Met	nd	15.67 ± 0.605	5.684 ± 0.26	8.567 ± 0.423		4.674 ± 0.199
Leu	39.65 ± 4.621	96.53 ± 6.645	93.28 ± 3.86	144.5 ± 10.15	8.475 ± 3.134	144 ± 21.41
Ile	18.91 ± 1.756	46.8 ± 2.982	46.45 ± 1.692	83.98 ± 5.49	3.667 ± 2.126	75.18 ± 6.733
Cys-Cys	nd	nd	nd	nd	nd	nd
Trp	nd	nd	nd	32.86 ± 1.011	nd	14.15 ± 0.772
Orn	156.3 ± 20.96	242.2 ± 25.8	110.1 ± 8.37	98.03 ± 15.28	23.93 ± 8.903	199.2 ± 29.78
Phe	38.54 ± 2.916	103.1 ± 3.677	100.2 ± 3.305	118.4 ± 4.251	nd	73.4 ± 1.9
Lys	12.8 ± 1.983	18.78 ± 3.628	25.74 ± 2.02	44.98 ± 7.77	6.433 ± 3.884	52.82 ± 9.439
Cys	nd	4.687 ± 0.72	nd		nd	1.773 ± 0.293
Tyr	24.48 ± 1.605	75.12 ± 4.388	65.53 ± 2.636	50.22 ± 2.619	3.627 ± 0.616	76.28 ± 2.503
	Mushroom "Honshimeji"	**Mushroom "Maitake"**	**Mushroom "Shiitake"**	**Porcini mushrooms (Dried)**	**Winter mushroom**	

His	178.5 ± 10.14	41.05 ± 2.678	123.7 ± 5.593	527.7 ± 54.46	118.2 ± 3.371	
Arg	410.6 ± 18.82	47.35 ± 2.468	226.4 ± 12.59	724.1 ± 35.73	114 ± 3.03	
HYP	nd	0.312 ± 0.312	nd	244.2 ± 43.95	19.8 ± 1.552	
Asn	44.37 ± 3.853	16.44 ± 1.439	87.64 ± 3.504	1277 ± 142.8	101.7 ± 2.697	
Gln	755.9 ± 55.49	119.2 ± 9.674	1237 ± 83.49	629.6 ± 72.69	414.9 ± 11.71	
Cit	nd	nd	nd	nd	nd	
Ser	93.53 ± 8.26	35.49 ± 3.123	97.06 ± 4.664	1499 ± 83.72	78.06 ± 2.904	
Asp	25.04 ± 0.899	11.71 ± 0.468	187.3 ± 15.24	325.5 ± 230.1	19.19 ± 3.483	
Glu	125.7 ± 4.428	75.59 ± 4.98	180.1 ± 13.53	462.6 ± 84.5	95.03 ± 3.556	
Thr	113.4 ± 8.527	42.18 ± 3.339	169.8 ± 10.55	694.9 ± 26.45	113.1 ± 4.342	
Pro+β-Ala	87.9 ± 6.65	17.79 ± 2.266	71.66 ± 1.074	396.4 ± 42.3	68.92 ± 3.138	
Gly	155.5 ± 6.678	21.9 ± 1.938	255.7 ± 15.22	1222 ± 144	123 ± 2.055	
GABA	46.12 ± 1.717	0.467 ± 0.467	80.73 ± 4.854	1378 ± 91.32	183.9 ± 5.589	
Ala	185.9 ± 6.48	92.2 ± 6.107	113.7 ± 6.708	2088 ± 148.1	252.6 ± 5.196	
Val	66.5 ± 3.585	21.51 ± 1.703	128.8 ± 7.157	262.2 ± 40.33	95.39 ± 1.62	
Met	10.53 ± 1.111	nd	3.345 ± 1.156	346.9 ± 99.25	5.32 ± 0.205	
Leu	63.38 ± 3.125	8.217 ± 0.811	80.65 ± 4.762	65.07 ± 0.218	115.6 ± 5.381	
Ile	31.56 ± 1.551	11.55 ± 0.373	51.86 ± 2.891	92.72 ± 23.11	60.88 ± 1.216	
Cys-Cys	nd	nd	nd	nd	nd	
Trp	nd	nd	nd	103.6 ± 18.91	20.72 ± 0.598	
Orn	274.4 ± 13.8	62.71 ± 1.961	156 ± 14.37	66.86 ± 11.24	85.33 ± 2.289	
Phe	70.26 ± 4.225	22.54 ± 0.784	58.04 ± 2.473	123.4 ± 23.3	217.3 ± 5.183	
Lys	22.34 ± 1.081	7.209 ± 0.438	49.39 ± 5.58	96.48 ± 7.537	27.24 ± 1.79	
Cys	nd	nd	0.566 ± 0.19	8.504 ± 6.013	nd	
Tyr	39.86 ± 1.959	24.42 ± 1.461	12.55 ± 2.442	136.9 ± 17.97	142.8 ± 6.058	
Amino acids are listed as abbreviated according to Table 1.						

the distribution of amino acids, including cultivation conditions, geographic production location, picking season, breed, and soil. In this study, we were unable to examine, or control, these variables. Nevertheless, we believe our results provide ample information with which to establish a free-form amino acid database to serve and inform nutritional studies.

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