Hindawi
Journal of Food Quality
Volume 2018, Article ID 7673652, 15 pages
https://doi.org/10.1155/2018/7673652

Research Article

Determination of Free Amino Acids in Three Species of Duckweed (Lemnaceae)

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Received 6 July 2018; Revised 22 October 2018; Accepted 18 November 2018; Published 5 December 2018

Academic Editor: Antimo Di Maro

In this study, a fast, simple, precise, and sensitive hydrophilic interaction liquid chromatography (HILIC) method was established for simultaneous determination of free amino acids in three different varieties of duckweed including *Spirodela polyrhiza* (L.) Schleid., *Landoltia punctata* (G. Mey.) Les & D. J. Crawford, and *Lemna aequinoctialis* Welwitsch by ultrahigh performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). Method validation was processed in terms of linearity, precision, stability, repeatability, and accuracy as well as limits of detection and quantification. The developed method was applied for quantification of 59 batches of samples. Then chemometric analysis was used to evaluate different duckweeds by principle component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA). The results demonstrated that there was no significant difference in FAAs' profile among three varieties of duckweed.

1. Introduction

Amino acids are kinds of nitrogenous components that play vital and diverse roles in metabolism and have attracted significant attention in food, feedstuff, and alimentary supplements. Amino acids are basic units and important ingredients of proteins and involved in the progress of biosynthesis for glycoprotein, porphyrins, neurotransmitters, polyamines, and nitric oxide [1–5]. Nutritional studies show that amino acids can modulate gene expression and enhance the growth of skeletal muscle and small intestine [6].

The family of Lemnaceae colloquially known as duckweed has been consumed as human food since long [7]. Duckweeds have the striking capacity of explosive reproduction [8]. They can grow almost everywhere with appropriate temperature and nutrition in water. But their greatest potential is to produce large quantities of protein-rich biomass that is a promising food resource for humans [9] and is suitable for feeding a wide range of animals including fish, poultry, and cattle [10–12]. The contents of free amino acids (FAAs) are very important for the evaluation of the protein-rich food. Fresh duckweed contains a large amount of water with moisture content of 86%–94%. But for dried samples, their protein concentration ranges from 25% to 40% with much lower fibrous material than that of land forage [13]. The habitat influences the content of proteins and fibers dramatically. Content of amino acids and protein always depend on their species pattern and growth environment, such as sunshine, nutrition media, and their growth pattern: gregarious or solitary. Under high nutritional conditions, more proteins and fewer fibers are accumulated in duckweed and vice versa. The amino acid type of duckweed is very close to that of animals and thus could be used by animals efficiently [7, 14]. The first limited amino acid (lysine) is similar to soybean and is more superior to that in other food such as sorghum and maize.

There are several methods reported in literature for analysis of free amino acids. In general, the FAAs are extracted with solvents water, formic acid, hydrochloric acid, or ethanol, followed by filtration or centrifugation before analysis [15–17]. Several techniques have been described for detection of free amino acids including ion-exchange chromatography [18], precolumn derivatization followed by reversed high-performance liquid chromatography coupled with diode array or fluorescence detector [19, 20], gas chromatography, mass spectrometry, and capillary electrophoresis [21]. In recent years, determination of FAAs using ultrahigh performance liquid chromatography hyphenated to mass spectrometry has gained great notice because of the merit of selective separation for the polar underivatized analytes under HILIC condition with acetonitrile-dominated mobile phase [22–25].

In present study, a feasible and reliable UPLC-QTRAP-MS/MS method was developed and validated for quantitative analysis of 24 underivatized FAAs simultaneously. Multivariate statistical analysis was employed to assess the differences in the profiles of FAAs among three species of duckweed. This simple and fast analytical method would be used for quality control of duckweeds.

Table 1: Information of duckweeds from different regions.

Sample	Species	Site
SP1-7	*Spirodela polyrhiza*	Yangzhou City of Jiangsu Province
SP8-9	*Spirodela polyrhiza*	Yancheng City of Jiangsu Province
SP10-11	*Spirodela polyrhiza*	Nanjing City of Jiangsu Province
SP12-13	*Spirodela polyrhiza*	Huaian City of Jiangsu Province
SP14-17	*Spirodela polyrhiza*	Lianyungang City of Jiangsu Province
SP18-19	*Spirodela polyrhiza*	Linyi City of Shandong Province
SP20-21	*Spirodela polyrhiza*	Hefei City of Anhui Province
SP22-23	*Spirodela polyrhiza*	Hangzhou City of Jiangsu Province
SP24-25	*Spirodela polyrhiza*	Baoding City of Hebei Province
LP1-11	*Landoltia punctata*	Yangzhou City of Jiangsu province
LP12-16	*Landoltia punctata*	Huaian City of Jiangsu province
LA1-3	*Lemna aequinoctialis*	Yangzhou City of Jiangsu Province
LA4-8	*Lemna aequinoctialis*	Yancheng City of Jiangsu Province
LA9-14	*Lemna aequinoctialis*	Nanjing City of Jiangsu Province
LA15-16	*Lemna aequinoctialis*	Lianyungang City of Jiangsu Province
LA17-18	*Lemna aequinoctialis*	Linyi City of Shandong Province

2. Materials and Methods

2.1. Reagents and Materials. 59 batches of duckweed were collected from different regions in China as shown in Table 1. The samples were identified and classified by PCR [26–28], and the number of populations was as follows: 25 populations from species *Spirodela polyrhiza*, 16 populations from species *Landoltia punctata* (its another previously invalid name is *Spirodela oligorrhiza* (Kurz) Hegelm.), and 18 populations from *Lemna aequinoctialis*. Fresh materials were washed by tap water, dried in sunshine, and pulverized, and the powder was screened through 60-mesh sieve. Reference standards of tyrosine (Tyr), alanine (Ala), glutamic acid (Glu), phenylalanine (Phe), histidine (His), isoleucine (Ile), aspartic acid (Asp), lysine (Lys), glycine (Gly), valine (Val), citrulline (Cit), cystine (Cys2), proline (Pro), tryptophan (Trp), leucine (Leu), arginine (Arg), cysteine (Cys), hydroxylproline (Hpro), methionine (Met), threonine (Thr), asparagine (Asn), and serine (Ser) were purchased from National Institutes for Food and Drug Control (Beijing, China) with the purity more than 98%. Reference standards of γ-aminobutyric (GABA) and glutamine (Gln) with the purity more than 98% were obtained from Aladdin Industrial Corporation (Shanghai, China). Acetonitrile (HPLC grade) was purchased from Merk (Darmstadt, Germany). Formic acid and ammonium formate (HPLC grade) were provided by Mreda Technology Incorporation (USA). Deionized water used for analysis procedure was produced by a Milli-Q Academic ultrapure water system (Millipore, Bedford, MA, USA).

2.2. Instrumentation and Chromatographic and Mass Conditions. Chromatographic analysis was performed on a Shimadzu LC-30 ultrahigh performance liquid chromatography system (Shimadzu, Japan), which consisted of a communication bus module (CMB-20A), a vacuum degasser, binary gradient pumps (LC-30AD), an autosampler (SIL-30A), and a column oven (CTO-30A) coupled with an AB Sciex QTRAP 5500 (AB SCIEX, USA). A Waters XBridge Amide column (2.1 mm × 150 mm, 2.5 μm, Waters, USA) was used for chromatographic separation. The binary mobile phase was composed of acetonitrile (A) and 10 mM ammonium formate with 0.2% (v/v) formic acid (B) at a flow rate of 0.4 mL/min. The linear gradient elution was carried out as follows: 0–5 min, 90%–85.5% A; 5–11 min, 85.5%–54% A; 11-12 min, 54%–10% A; 12–14 min, 10% A; 14-15 min, 10%–90% A; 15–20 min, and 90% A. The reequilibration time was 4 min with a total running time of 20 min. The column compartment was kept at 20°C, while the autosampler trial was maintained at 15°C, and injection volume was 2 μl. The needle was washed with mixtures of acetonitrile and water.

The mass spectrometry assay was performed on a triple quadruple mass spectrometer equipped with an electrospray ionization source. The spectra were recorded under positive-ion type with the multiple reaction monitoring (MRM) mode. The capillary voltage was 5500 V, and desolvation gas temperature was 550°C. The curtain gas was 35 psi with both nebulizer and drying gas of 55 psi. Nitrogen was used as source gas with purity more than 95% and collision gas with purity over 99.999%. The 24 components of amino acids were optimized in the tuning mode with mass only to obtain the mass of the precursor and product ion, the best declustering potential (DP), and collision energy (CE), respectively. The details are shown in Table 2. The entrance potential (EP) and collision cell exit potential (CXP) were set as default value. The typical LC-QTRAP-MS/MS chromatograms are shown in Figure 1.

Table 2: The precursor ions, product ions, declustering potential, and collision energy of amino acids.

Number	Compound	Precursor ion	Product ion	DP (V)	CE (V)
1	Tyr	182.0	91.1	60	37
2	Ala	90.0	44.1	50	14
3	Glu	148.1	84.0	60	21
4	Phe	166.1	120.1	60	19
5	His	156.1	110.3	80	20
6	Ile	132.1	86.1	50	14
7	Asp	134.1	74.0	50	19
8	Lys	147.0	84.1	60	23
9	Gly	76.1	29.9	40	16
10	Val	118.1	71.9	50	15
11	Cit	176.2	70.0	60	31
12	GABA	104.1	86.8	50	14
13	Cys2	241.1	73.9	80	40
14	Pro	116.0	70.1	60	22
15	Trp	205.1	146.0	50	24
16	Leu	132.1	86.1	50	14
17	Arg	175.2	70.2	90	30
18	Cys	122.1	59.0	40	32
19	Hpro	132.1	86.1	60	20
20	Met	150.1	103.9	60	14
21	Thr	120.1	74.1	40	14
22	Asn	133.1	74.0	60	21
23	Gln	147.0	84.1	60	23
24	Ser	106.0	60.1	60	15

2.3. Preparation of Reference Compound Solution. Stock solutions of individual standards were made by dissolving accurately weighed components in 0.5% formic acid at a concentration of 0.2 mg/mL approximately and stored at 4°C before use. The mixed working solutions of all the standards were diluted with 0.5% formic acid solution to a series of appropriate concentrations immediately before analysis and used to attain the calibration curves.

2.4. Preparation of Sample Solution. Each sample powder was weighed 0.2 g accurately and transferred into a 25 mL stainless tube with 6 stainless beads with a diameter of 0.6 mm. Then, the sample was extracted in a high-throughput tissue-grinding apparatus with vibrating frequency of 70 kHz for 120 s with 15 mL 0.5% formic acid. The sample solution was centrifuged at 15000 rpm for 10 min, and then the supernatant was screened through a 0.22 μm polytetrafluoroethylene membrane filter and stored in a glass bottle at 4°C for later LC-MS/MS analysis.

2.5. Method Validation. For each reference substance, the calibration curve was confirmed by linear regression of the peak area versus concentration. The limits of detection (LOD) and limits of quantification (LOQ) were determined by diluting the standard solution till the signal-to-noise ratios were about 3 and 10, respectively. Precision was determined by intraday and interday variability. The intraday variability was conducted by determining the same standard solution in six replicates on the same day. The interday variability was conducted by determining the same solution for three consecutive days. The relative standard deviation (RSD) values were calculated to denote the precision. Stability of the sample solution was analysed by peak areas of analytes at 0, 2, 4, 8, 12, and 24 h at room temperature. To evaluate the repeatability of the developed method, the same sample was analysed in six replicates and variations were expressed as RSD. The recovery test was performed to assess the accuracy of the method. For the test, a known amount of the 24 standard components was spiked into a certain amount (0.1 g) of sample. Then, the spiked sample was extracted and analysed as described. The recovery rate was calculated by using the following formula: recovery (%) = (measured amount − original amount)/spiked amount $\times$ 100%. Six replicates were performed for the recovery test.

2.6. Data Analysis. The quantitative analyses of acquired data were processed by MultiQuant 3.0.2 (AB SCIEX, USA). Multivariate statistical analysis including PCA and OPLS-DA was used to classify the sample. SIMCA-P 14.1 (Umetrics AB, Sweden) was used to perform statistical analysis for the data of 59 batches of duckweed.

2.7. Analysis of Minerals and Total Proteins. Twenty-four minerals of each environment water where duckweed lived were quantified by ICP-MS. Total nitrogen and phosphorus of the water body were measured according to the alkaline potassium persulfate digestion method GB11894-89 and the ammonium molybdate spectrophotometric method GB11893-89 published by the State Bureau of Technological Supervision of China. Total proteins of each duckweed were determined by the Kjeldahl method. Each sample of duckweeds was hydrolyzed in acidic conditions, and then total amino acids were quantified by the HPLC-MSMS method mentioned above.

3. Results and Discussion

3.1. Optimization of Extraction Procedure. Free amino acids can dissolve in methanol, ethanol, water, diluted acid, and so on. In order to acquire an efficient extraction strategy of amino acids from duckweed, variables of the extraction process were investigated. The solvents (methanol, ethanol, water, 0.1% formic acid, and 0.5% formic acid), methods (heating reflux, ultrasonication, and tissue-grinding), and time (20, 30, and 40 min for ultrasonication; 60, 90, and 120 s for tissue-grinding) were studied. The results showed that the amount of amino acids had no significant difference between the ultrasonication time of 30 min in 0.5% formic acid and tissue-grinding time of 120 s in 0.5% formic acid. The amounts of extraction by other methods were not more than that by these two modalities. From the viewpoint of working efficiency, the tissue-grinding method was much more time-saving than the ultrasonication method by the extracting time of 120 s versus 30 min.

3.2. Optimization of UPLC Condition. Chromatographic conditions were optimized to attain a satisfactory separation for amino acid especially for isomeric molecules. Columns

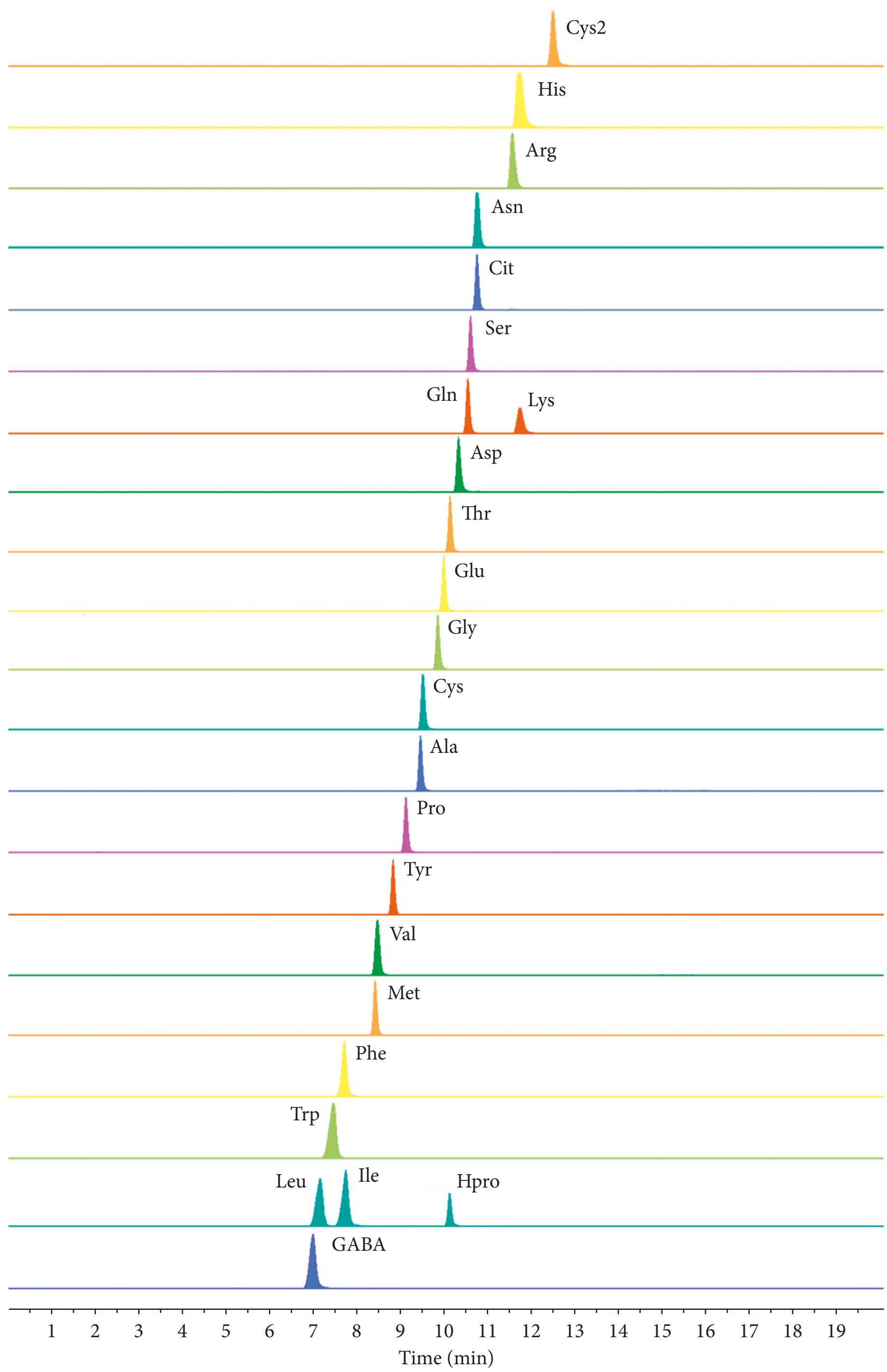

Figure 1: Typical MRM chromatograms of the 24 amino acids.

(Waters XBridge Amide, 2.5 μm, 2.1 × 150 mm and Agilent Poroshell 120 HILIC, 2.7 μm, 2.1 × 150 mm), column temperature (20, 25, 30, 35, and 40°C), mobile phase (acetonitrile-0.1% formic acid, acetonitrile-0.2% formic acid, acetonitrile with 0.2% formic acid-0.2% formic acid, and acetonitrile-10 mM ammonium formate with 0.2% formic acid), and different kinds of gradient elution were investigated.

The results demonstrated that the peak shape of amino acids became good when the concentration of formic acid rose, but retention time would be shortened significantly especially if some formic acid was complemented into the acetonitrile. The buffers such as ammonium formate increased the resolution degrees of amino acids. Better retention times and separations were observed by conducting analysis on Waters Amide column than on Agilent Poroshell 120 HILIC column, and the best chromatograms were gained with temperature controlled at 20°C. Especially for the isomers of leucine and isoleucine, baseline separation was

Table 3: Calibration curves, LODs, and LOQs for the 24 compounds.

Number	Compound	Calibration curve	R^2	Linear range (ng/mL)	LOQ (ng/mL)	LOD (ng/mL)
1	Tyr	$y = 6534.1x + 6063.4$	0.9984	1.16–5780	0.92	0.46
2	Ala	$y = 7168.9x + 4932.8$	0.9969	5.30–5300	3.71	1.24
3	Glu	$y = 12796x + 47334$	0.9960	6.41–2564	4.49	1.50
4	Phe	$y = 59157\ x + 42917$	0.9965	0.99–992.0	0.79	0.40
5	His	$y = 20263x + 247917$	0.9962	5.57–5570	3.90	1.30
6	Ile	$y = 70857x + 46587$	0.9957	1.07–1074	0.86	0.43
7	Asp	$y = 7084.7x + 86152$	0.9963	5.43–5430	3.80	1.27
8	Lys	$y = 4305.1x + 17648$	0.9980	5.53–5530	3.87	1.94
9	Gly	$y = 1063.0x + 3407.3$	0.9990	0.94–4680	0.75	0.37
10	Val	$y = 31972x + 35728$	0.9962	1.11–1112	0.89	0.44
11	Cit	$y = 8520.8x + 9811.0$	0.9980	1.01–5070	0.81	0.41
12	GABA	$y = 18495x + 14623$	0.9981	1.10–5490	0.88	0.44
13	Cys2	$y = 2389.7x + 3146.6$	0.9978	1.09–5440	0.87	0.44
14	Pro	$y = 51204x + 58539$	0.9981	1.02–510.0	0.82	0.41
15	Trp	$y = 19030x + 4465.3$	0.9977	1.24–2476	0.99	0.50
16	Leu	$y = 56715x + 40047$	0.9974	1.01–1012	0.81	0.40
17	Arg	$y = 11505x + 84828$	0.9974	5.96–5960	4.17	1.39
18	Cys	$y = 2496.6x + 1017.5$	0.9988	1.20–6012	0.96	0.48
19	Hpro	$y = 15783x + 3968.1$	0.9977	0.95–1892	0.76	0.38
20	Met	$y = 8626.4x + 1545.9$	0.9984	1.05–5250	0.84	0.42
21	Thr	$y = 8807.8x + 19073$	0.9957	4.46–4460	3.12	1.56
22	Asn	$y = 4939.9x + 22367$	0.9967	1.20–5980	0.96	0.48
23	Gln	$y = 7733.9x + 8338.4$	0.9961	0.93–4650	0.74	0.37
24	Ser	$y = 7731.3x + 54859$	0.9977	5.09–5090	3.56	1.19

Table 4: Precision, stability, repeatability, and accuracy of the investigated analytes ($n = 6$).

Number	Compound	Precision		Stability RSD (%)	Repeatability RSD (%)	Accuracy	
		Intraday RSD (%)	Interday RSD (%)			Recovery (%)	RSD (%)
1	Tyr	1.36	1.85	0.94	3.05	98.56	1.33
2	Ala	2.60	3.31	3.47	3.09	102.49	2.18
3	Glu	2.07	2.56	2.10	2.11	106.94	3.76
4	Phe	0.66	1.03	2.60	1.66	97.23	4.03
5	His	1.07	3.30	1.58	1.17	98.32	2.20
6	Ile	0.97	3.21	3.82	0.71	99.09	3.57
7	Asp	1.96	3.98	2.42	1.84	97.86	2.36
8	Lys	2.70	3.51	1.44	0.99	102.30	3.02
9	Gly	1.66	2.65	3.43	3.74	104.48	3.07
10	Val	2.42	2.89	3.30	0.83	107.51	1.52
11	Cit	1.47	1.88	3.12	3.86	99.14	2.21
12	GABA	3.14	2.32	2.27	0.82	102.54	3.41
13	Cys2	2.53	4.27	3.24	1.49	96.60	1.27
14	Pro	1.21	3.39	1.09	2.09	101.94	3.20
15	Trp	0.37	3.93	1.36	1.39	99.11	3.46
16	Leu	1.50	3.90	3.40	1.50	95.27	3.61
17	Arg	2.72	4.15	3.72	1.45	102.37	1.79
18	Cys	2.82	3.63	1.16	0.53	103.65	3.82
19	Hpro	3.05	2.23	0.87	2.38	97.55	3.91
20	Met	1.57	3.02	1.26	0.52	104.89	2.14
21	Thr	2.11	2.13	1.77	3.01	95.97	3.00
22	Asn	2.93	2.85	1.48	3.68	105.35	2.95
23	Gln	0.97	1.87	1.22	2.72	102.33	2.16
24	Ser	2.71	2.86	0.98	1.48	97.44	1.88

successfully achieved when using the mobile phase consisting of acetonitrile-10 mM ammonium formate with 0.2% formic acid. Under other abovementioned conditions, these two isomeric amino acids were not well separated reciprocally.

3.3. Optimization of MS/MS Conditions. Primary experiments were conducted to acquire the best mass spectrometry conditions of amino acids individually. For this purpose, each reference component of amino acids with the concentration of approximate 100 ng/mL was delivered into the

Table 5: Contents of 24 free amino acids, total amino acids, total proteins (μg/g), and ratio of free to total amino acids (%) in three species of duckweed.

Sample	Tyr			Ala			Glu			Phe			His			Ile			Asp			Lys			Gly		
	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio
SP1	3.16	6259.48	0.0505	6.95	10680.18	0.0651	25.27	16774.31	0.1506	0.44	9597.56	0.0046	1.52	4255.26	0.0357	0.38	8532.07	0.0045	9.31	11169.49	0.0834	11.95	7164.09	0.1668	3.33	8764.89	0.0380
SP2	6.73	6161.25	0.1092	65.17	10089.59	0.6459	54.61	25366.04	0.2153	0.95	10729.73	0.0089	4.43	3888.26	0.1139	1.35	10094.12	0.0134	56.68	15862.58	0.3573	21.28	10090.45	0.2109	8.38	8695.46	0.0964
SP3	39.72	6115.87	0.6495	173.11	9881.22	1.7519	96.43	15463.41	0.6236	7.97	8667.06	0.0920	22.26	3824.22	0.5821	11.90	7226.86	0.1647	69.12	12387.34	0.5580	112.90	8153.11	1.3847	128.27	6293.65	2.0381
SP4	29.21	6251.45	0.4673	157.69	12424.62	1.2692	49.52	22986.51	0.2154	5.01	10467.29	0.0479	32.80	3569.79	0.9188	11.14	8392.28	0.1327	170.28	17175.49	0.9914	137.48	10225.20	1.3445	78.17	9626.14	0.8121
SP5	5.58	6257.00	0.0892	29.04	13866.45	0.2094	34.93	23518.46	0.1485	0.86	11427.08	0.0075	6.41	4312.95	0.1486	0.96	9753.24	0.0098	16.39	18654.70	0.0879	21.25	11094.76	0.1915	5.53	10174.01	0.0544
SP6	27.42	6245.68	0.4390	273.35	12005.84	2.2768	49.09	18787.83	0.2613	2.55	10284.72	0.0248	45.46	3424.75	1.3274	13.55	8465.53	0.1601	132.84	15752.94	0.8433	139.27	8608.52	1.6178	77.36	7010.22	1.1035
SP7	39.61	5884.11	0.6732	229.20	12123.87	1.8905	71.86	18146.81	0.3960	4.90	8585.01	0.0571	46.17	4088.80	1.1292	11.22	7231.96	0.1551	94.84	14833.85	0.6393	230.52	7636.34	3.0187	65.35	5417.04	1.2064
SP8	5.71	6124.81	0.0932	38.00	9734.57	0.3904	53.14	17640.33	0.3012	1.17	8527.84	0.0137	4.56	3751.52	0.1216	1.40	7266.84	0.0193	42.82	14315.75	0.2991	22.77	7392.06	0.3080	5.63	7832.50	0.0719
SP9	13.90	6054.66	0.2296	120.58	9953.18	1.2115	70.75	16620.79	0.4257	3.68	8384.54	0.0439	18.01	3496.99	0.5150	5.25	6745.54	0.0778	94.10	11606.55	0.8107	73.99	6323.41	1.1701	21.63	7899.86	0.2738
SP10	16.70	6097.17	0.2739	118.61	13272.16	0.8937	74.72	25451.33	0.2936	3.72	11056.99	0.0336	28.19	4110.01	0.6859	4.41	9671.14	0.0456	36.11	17757.11	0.2034	70.81	8546.75	0.8285	25.55	7505.72	0.3404
SP11	23.10	6004.10	0.3847	205.08	11155.38	1.8384	67.31	26088.53	0.2580	10.26	9883.88	0.1038	61.35	3238.60	1.8943	14.23	8891.25	0.1600	56.14	15601.46	0.3598	133.66	8386.70	1.5937	87.43	9088.41	0.9620
SP12	9.38	6127.68	0.1531	93.06	9760.92	0.9534	51.58	26516.14	0.1945	7.60	7997.37	0.0950	31.87	3300.60	0.9656	4.09	9260.26	0.0442	225.20	12413.46	1.8142	74.86	5684.24	1.3170	24.76	6908.86	0.3584
SP13	93.61	6130.06	1.5271	195.69	8956.55	2.1849	52.85	16377.44	0.3227	23.33	7668.17	0.3042	46.78	2889.26	1.6191	14.85	7650.07	0.1941	4.76	10136.24	0.0470	135.91	6635.82	2.0481	73.19	5663.18	1.2924
SP14	19.98	6120.49	0.3264	161.86	13337.15	1.2136	96.09	15114.77	0.6357	5.94	10678.41	0.0556	26.03	4189.64	0.6213	6.56	9933.71	0.0660	29.20	13225.32	0.2208	102.55	11141.92	0.9204	34.50	7778.37	0.4435
SP15	1.13	6057.44	0.0187	19.99	13617.28	0.1468	17.01	21045.25	0.0808	0.62	11056.19	0.0056	9.77	4213.32	0.2319	0.30	8452.34	0.0035	0.95	12630.34	0.0075	17.70	8018.34	0.2207	6.64	6872.17	0.0966
SP16	61.29	5884.22	1.0416	280.92	12551.36	2.2382	46.82	20775.77	0.2254	19.27	10311.88	0.1869	54.60	3881.89	1.4065	20.18	9290.92	0.2172	112.81	13791.37	0.8180	158.24	7293.79	2.1695	52.41	8307.13	0.6309
SP17	0.89	5938.26	0.0150	23.96	10170.30	0.2356	46.90	15656.17	0.2996	0.42	10562.87	0.0040	2.49	5358.17	0.0465	1.11	10201.54	0.0109	2.95	12263.36	0.0241	13.08	8069.24	0.1621	4.44	5669.36	0.0783
SP18	12.28	5985.32	0.2052	157.75	13673.70	1.1537	81.27	15272.53	0.5321	15.58	10449.06	0.1491	28.93	4949.42	0.5845	10.04	9948.89	0.1009	85.38	20641.51	0.4136	79.39	11463.15	0.6926	29.19	6848.95	0.4262
SP19	45.66	5897.55	0.7742	182.98	12217.80	1.4977	87.43	20888.93	0.4185	28.84	10128.19	0.2847	48.02	4231.77	1.1347	20.85	9161.44	0.2276	10.22	14574.99	0.0701	83.12	9060.52	0.9174	34.17	6664.53	0.5127
SP20	28.03	5860.01	0.4783	241.91	9302.34	2.6005	46.17	14837.19	0.3112	23.18	7603.97	0.3048	64.10	3206.91	1.9988	14.80	6774.94	0.2185	109.03	11544.71	0.9444	164.87	6737.28	2.4471	73.15	6715.81	1.0892
SP21	2.75	6264.80	0.0439	55.55	10989.25	0.5055	91.79	22493.65	0.4081	0.84	9615.10	0.0087	23.80	4257.07	0.5591	1.00	8364.75	0.0120	—	11442.81	—	31.30	6932.76	0.4515	9.46	6910.06	0.1369
SP22	7.95	5839.15	0.1361	29.20	10343.17	0.2823	22.86	16692.99	0.1369	1.07	8957.29	0.0119	5.17	3585.28	0.1442	1.06	8011.79	0.0132	0.91	13590.39	0.0067	15.15	6361.56	0.2381	11.21	7059.65	0.1588
SP23	6.86	6265.35	0.1095	68.62	11510.74	0.5961	100.28	20064.52	0.4998	2.53	9584.25	0.0264	32.22	3573.51	0.9016	1.84	7753.50	0.0237	33.31	15126.56	0.2202	42.69	7622.22	0.5601	11.21	8763.05	0.1279
SP24	2.86	6318.22	0.0453	121.06	11508.52	1.0519	133.69	16946.16	0.7889	1.99	9477.13	0.0210	25.81	4474.44	0.5768	2.27	7926.91	0.0286	47.91	15776.45	0.3037	30.02	7939.55	0.3781	10.04	8867.42	0.1132
SP25	43.24	5967.19	0.7246	261.74	11024.75	2.3741	53.23	21609.69	0.2463	16.89	9293.80	0.1817	64.22	4222.87	1.5208	20.92	9560.51	0.2188	162.59	18624.39	0.8730	209.44	7299.84	2.8691	63.50	7917.96	0.8020
LP1	15.93	5971.26	0.2668	66.50	12151.90	0.5472	30.75	24958.79	0.1232	11.80	10826.10	0.1090	35.08	4061.19	0.8638	4.88	9319.60	0.0524	108.30	14896.35	0.7270	47.16	8613.79	0.5475	14.76	9583.97	0.1540
LP2	38.97	5921.55	0.6581	140.81	11584.07	1.2155	63.83	21969.13	0.2905	15.15	9814.43	0.1544	30.41	4647.49	0.6543	2.61	8750.97	0.0298	59.96	16728.80	0.3584	134.72	7842.84	1.7177	30.64	6109.14	0.5015
LP3	10.25	6287.19	0.1630	65.63	11013.29	0.5959	55.16	18366.66	0.3003	2.58	10992.07	0.0235	16.14	3302.54	0.4887	0.65	8567.75	0.0076	28.45	14101.74	0.2017	40.15	6593.31	0.6090	7.10	4632.59	0.1533
LP4	47.76	6004.07	0.7955	180.48	11148.05	1.6189	83.06	18093.89	0.4590	7.65	9312.27	0.0821	48.49	3515.42	1.3794	9.18	8187.52	0.1121	103.28	15513.40	0.6657	94.68	8007.43	1.1824	74.60	8640.35	0.8634
LP5	74.45	6011.02	1.2386	318.14	9785.11	3.2513	50.05	16830.48	0.2974	17.43	8490.88	0.2053	38.14	3571.09	1.0680	11.66	7900.11	0.1476	218.70	13106.01	1.6687	129.60	8308.91	1.5598	44.93	5574.15	0.8060
LP6	22.44	6404.35	0.3504	187.93	9320.51	2.0163	51.11	19093.97	0.2677	10.76	10746.47	0.1001	17.66	4145.39	0.4260	4.29	8189.38	0.0524	137.49	13879.01	0.9906	57.61	6486.39	0.8882	13.94	6180.53	0.2255
LP7	17.40	6413.54	0.2713	167.19	10950.61	1.5268	73.42	20616.00	0.3561	10.75	9606.73	0.1119	20.94	3464.43	0.6044	2.23	8717.93	0.0256	207.21	15567.27	1.3311	51.72	8761.56	0.5903	14.60	6213.59	0.2350
LP8	35.02	6285.44	0.5572	139.27	11569.10	1.2038	72.38	18381.09	0.3938	18.51	10138.80	0.1826	28.90	4434.85	0.6517	7.46	9558.72	0.0780	73.30	15357.08	0.4773	78.65	7886.32	0.9973	24.80	8792.99	0.2820
LP9	41.17	5951.60	0.6917	149.55	11075.37	1.3503	48.55	22021.01	0.2205	9.10	11619.61	0.0783	52.62	3058.17	1.7206	12.36	8587.29	0.1439	182.44	15444.00	1.1813	202.10	10583.78	1.9095	176.76	6207.16	2.8477
LP10	23.74	6012.91	0.3948	187.15	10077.16	1.8572	57.20	22847.31	0.2504	3.20	10674.83	0.0300	11.62	3881.87	0.2993	6.47	10514.94	0.0615	48.04	19845.70	0.2421	111.09	8283.89	1.3410	107.73	7557.95	1.4254
LP11	14.86	6110.90	0.2432	94.14	9256.17	1.0171	16.70	26967.52	0.0619	5.60	7780.40	0.0720	7.52	3541.02	0.2124	3.81	7300.09	0.0522	6.45	13678.56	0.0472	75.66	7596.11	0.9960	46.74	6276.56	0.7447
LP12	8.06	6259.52	0.1288	25.80	10729.35	0.2405	11.61	28848.73	0.0402	1.45	9609.21	0.0151	4.31	4095.51	0.1052	0.93	8635.84	0.0108	2.44	11847.35	0.0206	29.23	8736.23	0.3346	7.00	7434.59	0.0942
LP13	33.36	6270.49	0.5320	274.21	10059.65	2.7258	85.36	26195.21	0.3259	5.22	11114.42	0.0470	44.10	3500.71	1.2597	8.74	9771.06	0.0894	78.86	17064.92	0.4621	148.06	8026.46	1.8446	92.39	7807.71	1.1833
LP14	29.35	6070.40	0.4835	160.58	13818.32	1.1621	57.00	27399.97	0.2080	5.18	12682.81	0.0408	38.41	5535.27	0.6939	9.59	11826.29	0.0811	139.52	14718.19	0.9479	158.90	7779.67	2.0425	85.79	7101.48	1.2081
LP15	14.08	6310.67	0.2231	108.54	12226.01	0.8878	11.43	20415.24	0.0560	4.29	10235.21	0.0419	6.83	4786.65	0.1427	3.49	10163.51	0.0343	4.42	16813.25	0.0263	70.82	8454.76	0.8376	57.87	8826.59	0.6556
LP16	38.10	6308.99	0.6039	224.42	10920.13	2.0551	58.19	18915.27	0.3076	12.98	9252.57	0.1403	25.58	3501.54	0.7305	6.06	8361.97	0.0725	187.80	14243.86	1.3185	79.64	8381.28	0.9502	24.49	7349.44	0.3332
LA1	39.53	5844.15	0.6764	153.40	11080.16	1.3845	53.94	17900.19	0.3013	5.26	8940.22	0.0588	38.98	3436.65	1.1342	10.44	7739.17	0.1349	92.27	11396.06	0.8097	116.20	8514.88	1.3647	16.63	8366.72	0.1988
LA2	7.06	6163.58	0.1145	110.58	11359.90	0.9734	38.25	21707.93	0.1762	8.65	9105.55	0.0950	45.78	3419.43	1.3388	10.95	9140.90	0.1198	84.67	16077.78	0.5266	87.09	8669.05	1.0046	14.22	7375.81	0.1928
LA3	49.51	6127.32	0.8080	189.37	10480.08	1.8070	47.31	23969.23	0.1974	23.46	8251.42	0.2843	73.17	3213.54	2.2769	30.13	8053.11	0.3741	190.71	18067.61	1.0555	206.67	10157.90	2.0346	118.69	11012.41	1.0778
LA4	9.45	6231.43	0.1517	101.15	10764.28	0.9397	43.93	18445.78	0.2382	6.83	9106.47	0.0750	5.48	3968.13	0.1381	2.07	7183.48	0.0288	14.98	11095.78	0.1350	44.31	8400.33	0.5275	18.02	5093.33	0.3538
LA5	8.21	6468.09	0.1269	101.70	10782.93	0.9432	49.41	17857.46	0.2767	4.67	9378.50	0.0498	27.88	4320.50	0.6453	7.67	8732.91	0.0878	245.22	11348.94	2.1607	101.42	9642.82	1.0518	59.10	7589.10	0.7787
LA6	95.03	6441.94	1.4752	53.56	10969.89	0.4882	45.63	19113.36	0.2387	34.05	8826.04	0.3858	22.67	3432.16	0.6605	15.73	7873.91	0.1998	194.49	13395.53	1.4519	203.37	9453.82	2.1512	165.17	5220.68	3.1638
LA7	43.57	6390.61	0.6818	136.48	9697.57	1.4074	44.59	17227.16	0.2588	26.85	10617.54	0.2529	53.25	3342.84	1.5930	8.20	8078.65	0.1015	99.41	13308.10	0.7470	156.91	8444.81	1.8581	124.79	6172.25	2.0218
LA8	25.51	5969.99	0.4273	159.29	9551.72	1.6677	21.69	22493.72	0.0964	7.29	12500.69	0.0583	28.21	5068.31	0.5566	12.52	10500.36	0.1192	2.23	19936.15	0.0112	66.00	11282.00	0.5850	75.19	11732.39	0.6409
LA9	3.26	6188.23	0.0527	19.10	14974.86	0.1275	71.65	23929.16	0.2994	0.84	12303.21	0.0068	3.65	4847.99	0.0753	0.36	10895.91	0.0033	2.47	21371.14	0.0116	18.43	10548.00	0.1747	7.33	7698.82	0.0952
LA10	29.15	5884.38	0.4954	95.43	11229.43	0.8498	89.66	20489.06	0.4376	16.96	9051.65	0.1874	21.61	3412.09	0.6333	16.06	8721.17	0.1841	2.82	17303.92	0.0163	74.14	8057.43	0.9201	27.50	5058.68	0.5436
LA11	10.88	6259.53	0.1738	40.56	13131.47	0.3089	121.99	22020.10	0.5540	2.48	10555.63	0.0235	5.92	4847.60	0.1221	0.48	9784.56	0.0049	7.09	16593.97	0.0427	26.86	7655.07	0.3509	7.73	7284.80	0.1061

Table 5: Continued.

Sample	Tyr			Ala			Glu			Phe			His			Ile			Asp			Lys			Gly		
	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio
LA12	48.02	6403.75	0.7499	149.63	9735.88	1.5369	62.74	14671.39	0.4276	20.20	8380.51	0.2410	56.05	3384.94	1.6559	5.41	6896.77	0.0784	91.38	11321.11	0.8072	109.18	5802.35	1.8817	46.13	7209.85	0.6398
LA13	22.08	5983.25	0.3690	77.34	12359.55	0.6258	49.89	24426.48	0.2042	13.64	10650.97	0.1281	31.43	4286.46	0.7332	3.68	8877.22	0.0415	184.36	14885.17	1.2385	77.19	10677.05	0.7230	23.24	10012.96	0.2321
LA14	29.06	6197.60	0.4689	219.41	12097.18	1.8137	68.78	22967.64	0.2995	7.41	10651.10	0.0696	33.00	4079.19	0.8090	10.50	8376.22	0.1254	21.77	16404.09	0.1327	122.70	8502.69	1.4431	92.02	9152.03	1.0055
LA15	28.90	6409.17	0.4509	11.61	12035.67	0.0965	43.15	25371.86	0.1701	5.48	11118.01	0.0493	35.81	3597.27	0.9955	6.72	6464.97	0.1039	159.51	12763.92	1.2497	120.12	7008.78	1.7139	55.67	5676.13	0.9808
LA16	20.91	6243.38	0.3349	95.37	11719.51	0.8138	41.47	21759.15	0.1906	2.48	11669.90	0.0213	27.93	5249.47	0.5321	5.48	10752.39	0.0510	113.55	14828.06	0.7658	123.52	9586.25	1.2885	34.37	10915.62	0.3149
LA17	33.89	6029.51	0.5621	12.57	12627.27	0.0995	65.10	23746.45	0.2741	13.69	10538.29	0.1299	34.97	4468.75	0.7825	7.61	9975.36	0.0763	117.69	13313.53	0.8840	122.84	9248.08	1.3283	36.44	8620.93	0.4227
LA18	29.56	6046.27	0.4889	137.19	10245.05	1.3391	54.86	18325.38	0.2994	10.74	8928.25	0.1203	48.27	3846.06	1.2551	6.44	7467.00	0.0862	91.91	12882.36	0.7135	125.43	7366.73	1.7027	36.87	4820.33	0.7649

Sample	Val			Cit			GABA			Cys2			Pro			Trp			Leu			Arg			Cys		
	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio
SP1	4.42	6012.65	0.0735	2.49	4673.88	0.0533	3.46	3353.11	0.1032	—	11525.08	—	4.15	6607.74	0.0628	0.24	3637.74	0.0066	1.37	12211.14	0.0112	34.88	9489.73	0.3676	—	5538.05	—
SP2	15.20	10648.71	0.1427	6.07	3955.21	0.1535	21.27	4028.12	0.5280	—	13123.45	—	12.90	6341.28	0.2034	0.51	6868.79	0.0074	4.08	11230.44	0.0363	87.38	8979.88	0.9731	—	8675.28	—
SP3	15.36	6327.29	0.2428	24.59	4218.39	0.5829	83.54	2906.04	2.8747	0.67	9520.68	0.0070	27.55	7911.24	0.3482	2.67	6256.52	0.0427	33.86	9531.17	0.3553	68.39	8667.37	0.7891	—	3752.42	—
SP4	16.09	10136.97	0.1587	9.45	4525.17	0.2088	112.07	1860.38	6.0240	0.71	8713.26	0.0081	22.64	9816.87	0.2306	3.14	6886.81	0.0456	25.37	11900.95	0.2132	41.42	7830.13	0.5290	—	5020.00	—
SP5	12.81	12270.45	0.1044	3.68	5960.38	0.0617	9.31	2163.00	0.4304	—	11194.54	—	5.92	8973.82	0.0660	0.39	6741.47	0.0058	3.03	17710.97	0.0171	75.96	11624.78	0.6534	—	8152.58	—
SP6	18.63	6921.90	0.2691	54.08	6475.87	0.8351	110.08	2010.87	5.4742	0.26	9803.15	0.0027	28.19	7126.12	0.3956	4.73	3570.11	0.1325	26.75	15586.82	0.1716	43.86	5689.69	0.7709	—	3789.97	—
SP7	17.07	9259.39	0.1844	29.75	4140.35	0.7185	103.95	2825.91	3.6785	2.41	9778.44	0.0246	24.62	8226.72	0.2993	3.95	4856.66	0.0813	27.66	10861.08	0.2547	34.40	4988.56	0.6896	—	3471.13	—
SP8	15.86	7296.00	0.2174	3.13	4702.29	0.0666	20.48	1682.03	1.2176	—	6139.57	—	10.52	6680.26	0.1575	0.56	4883.67	0.0115	4.38	9640.58	0.0454	64.28	4886.46	1.3155	—	3120.50	—
SP9	37.20	6514.46	0.5710	10.18	5254.55	0.1937	57.02	2501.97	2.2790	0.14	10056.41	0.0014	15.77	5340.61	0.2953	1.20	4253.18	0.0282	14.42	11713.99	0.1231	124.64	5090.33	2.4486	—	3897.56	—
SP10	38.29	9908.44	0.3864	13.50	7105.23	0.1900	57.85	1953.05	2.9620	0.26	10687.68	0.0024	19.03	7381.05	0.2578	1.17	7036.56	0.0166	13.77	14235.69	0.0967	108.51	4268.65	2.5420	—	8653.16	—
SP11	21.90	10089.65	0.2171	8.30	5747.51	0.1444	108.20	3155.71	3.4287	0.79	9550.72	0.0083	24.66	5804.81	0.4248	1.94	7077.68	0.0274	26.89	14118.06	0.1905	67.72	5222.67	1.2967	—	6165.20	—
SP12	36.91	5104.20	0.7231	31.74	3267.50	0.9714	56.19	3092.80	1.8168	0.58	8821.94	0.0066	11.01	6511.44	0.1691	2.35	4185.92	0.0561	10.46	8633.77	0.1212	99.42	6433.17	1.5454	—	2940.84	—
SP13	27.76	5423.78	0.5118	27.14	3816.49	0.7111	82.12	2484.15	3.3058	0.99	6828.14	0.0145	22.06	7524.55	0.2932	11.42	3149.92	0.3625	31.25	8945.92	0.3493	73.82	5010.51	1.4733	—	6503.52	—
SP14	44.12	10896.32	0.4049	11.57	6328.11	0.1828	70.23	4220.23	1.6641	0.28	9165.62	0.0031	21.63	8005.88	0.2702	2.44	4732.57	0.0516	18.29	13035.53	0.1403	65.62	6826.41	0.9613	—	8677.59	—
SP15	6.17	7444.35	0.0829	5.45	3756.96	0.1451	4.23	3324.82	0.1272	—	10198.26	—	14.42	5416.47	0.2662	—	5143.26	—	1.80	14814.40	0.0122	30.19	5059.02	0.5968	—	6694.08	—
SP16	26.04	6396.60	0.4071	36.82	3762.26	0.9787	134.46	3870.97	3.4735	0.72	8704.16	0.0083	27.10	7353.81	0.3685	15.39	7545.48	0.2040	30.79	12588.03	0.2446	43.32	6483.46	0.6682	—	4369.57	—
SP17	9.24	8969.61	0.1030	4.12	3703.39	0.1112	2.34	3148.92	0.0743	—	8001.88	—	14.78	8403.61	0.1759	—	4014.56	—	5.35	10274.36	0.0521	9.96	4876.50	0.2042	—	6360.07	—
SP18	26.19	12386.71	0.2114	26.35	5209.87	0.5058	98.88	3534.87	2.7973	0.12	12402.18	0.0010	17.21	9211.57	0.1868	2.08	7798.78	0.0267	22.77	17831.20	0.1277	108.35	4662.91	2.3237	—	7665.04	—
SP19	25.16	8312.52	0.3027	8.51	4849.89	0.1755	73.42	1868.93	3.9285	0.28	8968.53	0.0031	17.64	8754.89	0.2015	3.68	5290.70	0.0696	16.70	11607.35	0.1439	80.47	7406.94	1.0864	—	7063.71	—
SP20	25.85	8395.03	0.3079	17.22	4586.93	0.3754	131.28	2873.62	4.5685	0.40	8894.24	0.0045	18.50	4472.47	0.4136	9.86	3888.62	0.2536	24.40	8829.37	0.2764	42.54	4662.51	0.9124	—	4657.46	—
SP21	11.27	5982.04	0.1884	24.90	4366.50	0.5703	10.82	3551.90	0.3046	0.24	8933.72	0.0027	28.86	6002.68	0.4808	0.15	6460.55	0.0023	3.89	12300.36	0.0316	55.98	5963.67	0.9387	—	5955.22	—
SP22	10.06	9469.47	0.1062	5.09	5545.62	0.0918	1.90	1902.37	0.0999	—	10023.81	—	18.22	5611.46	0.3247	0.14	3261.78	0.0043	9.76	12591.58	0.0775	18.52	8217.10	0.2254	—	6884.34	—
SP23	19.80	6941.68	0.2852	35.46	4560.09	0.7776	10.96	2519.87	0.4349	0.31	11078.52	0.0028	25.31	7474.12	0.3386	0.55	4776.94	0.0115	5.34	9323.61	0.0573	75.70	8379.33	0.9034	—	4296.33	—
SP24	24.68	7991.34	0.3088	29.19	6319.28	0.4619	4.94	3668.10	0.1347	0.09	10178.72	0.0009	23.29	5293.61	0.4400	0.60	4551.39	0.0132	3.83	14205.93	0.0270	58.04	6170.55	0.9406	—	5644.77	—
SP25	28.45	7459.65	0.3814	46.15	5088.78	0.9069	163.48	3897.18	4.1948	1.16	11215.85	0.0103	24.67	7584.57	0.3253	7.63	7080.31	0.1078	35.40	14162.74	0.2500	74.34	9656.72	0.7698	—	6166.09	—
LP1	21.36	7244.74	0.2948	5.16	4405.39	0.1171	58.37	2407.83	2.4242	0.13	13065.90	0.0010	11.76	8651.28	0.1359	3.62	5123.83	0.0707	13.93	10764.87	0.1294	30.24	8884.37	0.3404	—	4324.94	—
LP2	14.46	7373.76	0.1961	16.36	4874.65	0.3356	40.10	2138.03	1.8756	0.77	7988.91	0.0096	14.86	8851.40	0.1679	14.60	3817.53	0.3824	15.17	13357.73	0.1136	26.71	9701.11	0.2753	—	7977.29	—
LP3	9.83	8734.76	0.1125	60.38	3744.80	1.6124	14.14	2099.63	0.6735	—	9725.45	—	18.43	4540.45	0.4059	0.45	4728.65	0.0095	3.52	9822.04	0.0358	74.16	5810.06	1.2764	—	3530.69	—
LP4	35.80	9919.45	0.3609	3.79	3526.80	0.1075	122.04	2438.49	5.0047	0.37	9387.49	0.0039	18.34	8296.58	0.2211	1.14	5349.26	0.0213	22.21	9157.07	0.2425	48.84	8460.67	0.5773	—	6861.56	—
LP5	35.99	8871.65	0.4057	11.90	4587.62	0.2594	133.05	3159.85	4.2106	4.21	7298.47	0.0577	25.23	5061.04	0.4985	4.34	5510.02	0.0788	31.89	11224.14	0.2841	45.88	6939.12	0.6612	—	3854.06	—
LP6	24.78	7215.09	0.3434	9.73	4416.05	0.2203	91.48	2092.89	4.3710	2.32	7641.78	0.0304	14.71	6655.83	0.2210	5.76	3078.31	0.1871	13.10	10225.91	0.1281	77.03	6842.51	1.1258	—	3272.62	—
LP7	18.41	6396.21	0.2878	10.84	4275.51	0.2535	64.21	2197.40	2.9221	4.25	11232.05	0.0378	9.15	8097.10	0.1130	4.71	3289.73	0.1432	11.93	9991.64	0.1194	30.66	7921.66	0.3870	0.14	6607.63	0.0021
LP8	23.88	7401.70	0.3226	16.38	4758.26	0.3442	69.10	3565.33	1.9381	0.55	8739.92	0.0063	14.73	8982.82	0.1640	2.82	4541.13	0.0621	24.29	16149.82	0.1504	31.42	6643.33	0.4730	—	5673.14	—
LP9	22.74	8090.74	0.2811	32.17	6784.16	0.4742	95.67	3874.88	2.4690	0.34	11756.40	0.0029	25.48	9895.35	0.2575	3.59	4755.18	0.0755	30.55	11213.15	0.2724	118.53	7876.17	1.5049	0.22	4743.85	0.0046
LP10	44.70	10435.87	0.4283	15.47	3862.27	0.4005	82.77	2085.51	3.9688	0.95	13573.29	0.0070	21.56	8888.77	0.2426	0.77	7378.31	0.0104	16.31	14411.77	0.1132	63.57	9539.07	0.6664	0.14	8866.38	0.0016
LP11	30.18	7118.23	0.4240	10.12	3840.01	0.2635	38.62	2188.18	1.7649	0.39	9579.85	0.0041	14.31	6477.73	0.2209	0.56	4389.22	0.0128	9.34	10905.75	0.0856	74.49	5696.30	1.3077	—	5334.87	—
LP12	10.67	9073.21	0.1176	9.45	4795.94	0.1970	6.74	2798.07	0.2409	—	9754.10	—	10.14	5687.18	0.1783	0.22	6220.07	0.0035	2.99	11120.12	0.0269	89.48	9822.46	0.9110	—	7787.85	—
LP13	24.43	7986.97	0.3059	10.20	5208.39	0.1958	105.99	3587.82	2.9542	1.19	10899.43	0.0109	25.32	9578.40	0.2643	2.01	6255.14	0.0321	24.85	10572.72	0.2350	76.46	7230.87	1.0574	—	8350.79	—
LP14	21.85	12895.61	0.1694	10.61	6247.35	0.1698	114.88	3385.86	3.3929	0.65	12941.50	0.0050	24.21	10165.28	0.2382	0.42	5801.66	0.0072	24.35	12767.46	0.1907	38.76	9267.96	0.4182	0.10	4749.52	0.0021
LP15	30.04	10600.31	0.2834	8.02	6112.10	0.1312	39.61	2063.22	1.9198	0.26	10874.29	0.0024	14.42	6109.32	0.2360	0.63	7137.55	0.0088	7.68	12394.41	0.0620	71.29	6514.00	1.0944	—	4351.01	—
LP16	26.39	5728.98	0.4606	10.83	4203.58	0.2576	96.25	3176.63	3.0299	3.59	6782.15	0.0529	16.36	8733.17	0.1873	4.94	6147.21	0.0804	18.97	9376.15	0.2023	51.19	8498.90	0.6023	0.14	5213.83	0.0027
LA1	45.00	5702.67	0.7891	13.87	3437.80	0.4035	97.36	3259.71	2.9868	0.34	9934.14	0.0034	16.88	5375.18	0.3140	5.39	5649.12	0.0954	25.80	11813.05	0.2184	46.47	7148.37	0.6501	—	6609.48	—
LA2	19.62	10334.80	0.1898	28.58	3747.09	0.7627	81.64	2390.45	3.4153	0.86	7845.80	0.0110	12.38	8095.98	0.1529	2.57	5265.52	0.0488	23.74	15177.45	0.1564	33.03	7150.11	0.4620	—	5064.86	—

Table 5: Continued.

Sample	Val			Cit			GABA			Cys2			Pro			Trp			Leu			Arg			Cys		
	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio
LA3	39.08	11531.59	0.3389	11.17	7658.73	0.1458	96.78	3420.33	2.8296	0.34	9648.03	0.0035	25.22	6740.71	0.3741	14.39	6990.99	0.2058	28.29	16947.54	0.1669	35.87	11157.91	0.3215	0.12	5317.49	0.0023
LA4	30.49	9094.55	0.3353	5.75	3337.54	0.1723	59.10	1960.88	3.0140	—	7030.26	—	21.76	7834.00	0.2778	1.38	6225.95	0.0222	12.30	8279.16	0.1486	67.99	6933.19	0.9806	—	5458.32	—
LA5	41.41	10636.04	0.3893	53.62	5018.49	1.0684	47.11	3686.56	1.2779	1.53	7468.47	0.0205	28.74	6068.81	0.4736	1.29	4893.15	0.0264	22.91	11517.82	0.1989	104.70	5679.74	1.8434	—	6326.99	—
LA6	26.86	10152.01	0.2646	10.02	5879.06	0.1704	109.90	3477.43	3.1604	0.66	10000.06	0.0066	23.06	5135.21	0.4491	7.12	3736.03	0.1906	33.26	15229.07	0.2184	38.54	6481.81	0.5946	0.24	5289.57	0.0045
LA7	37.39	5299.21	0.7056	7.21	4582.47	0.1573	137.64	3164.85	4.3490	—	6820.33	—	25.78	4508.55	0.5718	2.81	5062.03	0.0555	30.16	14035.50	0.2149	37.11	8557.89	0.4336	—	6719.24	—
LA8	33.07	10470.63	0.3158	14.37	6157.76	0.2334	26.46	2514.35	1.0524	—	12359.55	—	27.27	8147.42	0.3347	3.27	7846.61	0.0417	18.76	12842.68	0.1461	88.93	8060.89	1.1032	—	4516.77	—
LA9	3.40	12345.60	0.0275	9.98	5675.92	0.1758	2.04	4742.21	0.0430	—	15127.20	—	6.76	10585.00	0.0639	0.29	8961.94	0.0032	1.34	12900.12	0.0104	13.42	10716.38	0.1252	—	5820.67	—
LA10	32.37	8506.23	0.3805	15.12	4869.46	0.3105	40.05	3515.47	1.1393	0.11	11225.08	0.0010	15.86	4962.73	0.3196	1.40	6261.69	0.0224	8.88	9143.75	0.0971	86.86	5400.65	1.6083	—	7815.34	—
LA11	5.06	11187.35	0.0452	16.69	6130.94	0.2722	2.35	2533.92	0.0927	0.36	13012.18	0.0028	5.43	10083.36	0.0539	0.97	5607.42	0.0173	1.87	15543.78	0.0120	7.40	7751.63	0.0955	—	4413.68	—
LA12	38.36	6567.71	0.5841	37.15	4331.21	0.8577	73.93	1638.83	4.5111	0.13	10001.78	0.0013	19.64	5219.17	0.3763	1.98	3334.61	0.0594	16.66	13924.60	0.1196	43.90	4477.46	0.9805	—	3708.31	—
LA13	33.49	9334.49	0.3588	21.25	5427.96	0.3915	57.89	3900.56	1.4841	0.15	11927.09	0.0013	18.94	7570.98	0.2502	1.52	3832.52	0.0397	14.66	14801.24	0.0990	130.27	9104.06	1.4309	—	4437.40	—
LA14	48.22	9862.78	0.4889	10.25	5078.48	0.2018	68.84	1820.89	3.7806	0.18	12167.89	0.0015	20.49	9331.57	0.2196	3.12	6487.16	0.0481	27.88	15343.15	0.1817	73.66	5754.34	1.2801	—	4845.05	—
LA15	33.62	4870.39	0.6903	38.52	4318.57	0.8920	46.08	2538.52	1.8152	—	8893.47	—	18.06	5895.62	0.3063	0.58	3366.22	0.0172	18.67	7744.33	0.2411	97.99	6807.78	1.4394	0.19	6361.27	0.0030
LA16	28.73	10676.60	0.2691	17.16	5956.75	0.2881	30.34	4234.41	0.7165	0.10	10936.85	0.0009	20.90	7579.89	0.2757	1.06	6621.76	0.0160	17.03	15351.32	0.1109	39.65	7199.84	0.5507	0.16	4666.63	0.0034
LA17	25.33	11534.16	0.2196	8.43	5093.69	0.1655	57.74	3692.35	1.5638	0.41	10297.60	0.0040	12.50	7491.18	0.1669	4.43	3719.53	0.1191	16.02	17696.48	0.0905	32.56	9782.67	0.3328	—	8946.44	—
LA18	24.44	9269.41	0.2637	7.67	3801.25	0.2018	56.82	3179.11	1.7873	0.60	9490.75	0.0063	15.74	6342.64	0.2482	2.92	3811.54	0.0766	15.43	11215.99	0.1376	37.22	8358.76	0.4453	—	3946.18	—

Sample	Hpro			Met			Thr			Asn			Gln			Ser			Total free amino acids	Total proteins (%)
	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio		
SP1	0.34	12275.50	0.0028	—	2102.26	—	12.78	7963.72	0.1605	39.82	3834.80	1.0384	1.04	1971.45	0.0528	4.76	6466.09	0.0736	172.07	16.19
SP2	0.84	11585.53	0.0073	—	1927.32	—	38.01	7239.98	0.5250	215.37	4986.79	4.3188	3.20	2096.01	0.1527	22.43	5929.40	0.3783	646.85	18.88
SP3	10.37	9975.53	0.1040	4.20	1384.33	0.3034	157.92	4945.98	3.1929	133.70	3711.29	3.6025	68.98	1951.23	3.5352	144.02	5954.58	2.4186	1437.51	15.19
SP4	3.50	12741.33	0.0275	—	1958.62	—	168.24	8709.13	1.9318	139.13	4253.91	3.2706	143.70	2036.62	7.0558	163.42	5609.49	2.9133	1520.16	17.82
SP5	0.48	15829.31	0.0030	—	2151.19	—	34.91	8355.57	0.4178	258.84	4912.45	5.2691	3.06	2256.83	0.1356	16.69	10234.90	0.1631	546.01	20.46
SP6	6.53	12371.35	0.0528	—	1608.64	—	185.93	5726.31	3.2469	131.14	4602.59	2.8493	53.20	2164.53	2.4578	144.62	8041.81	1.7984	1568.88	17.51
SP7	3.37	12423.61	0.0271	0.10	1470.47	0.0068	168.48	4848.42	3.4749	137.82	3978.83	3.4638	115.64	2145.57	5.3897	153.92	8601.82	1.7894	1616.80	15.59
SP8	0.83	9760.19	0.0085	—	1320.98	—	36.00	5251.98	0.6855	243.18	3721.89	6.5338	4.33	2074.31	0.2087	23.02	7285.14	0.3160	601.76	14.41
SP9	2.33	10641.34	0.0219	0.10	1927.64	0.0052	101.08	6224.18	1.6240	287.73	3643.59	7.8969	22.11	2123.14	1.0414	80.72	7300.64	1.1057	1176.53	14.43
SP10	5.44	15070.02	0.0361	0.26	2233.47	0.0116	111.26	9030.91	1.2320	295.67	4158.01	7.1109	19.66	2820.23	0.6971	98.55	9877.31	0.9977	1162.05	19.14
SP11	11.23	13641.99	0.0823	0.28	1744.92	0.0160	176.59	7985.90	2.2113	140.18	4655.28	3.0112	144.55	2112.77	6.8417	179.12	6088.37	2.9420	1570.89	17.01
SP12	12.01	11162.90	0.1076	0.24	1881.32	0.0128	125.79	4403.32	2.8567	137.25	3369.96	4.0727	41.76	1600.68	2.6089	57.70	6605.01	0.8736	1145.82	14.13
SP13	2.39	10615.66	0.0225	3.51	1382.26	0.2539	228.15	6391.62	3.5695	165.25	4095.51	4.0349	269.51	1696.44	15.8868	168.80	6708.25	2.5163	1755.14	13.75
SP14	1.44	13205.06	0.0109	—	2393.23	—	132.66	8976.95	1.4778	149.56	4798.05	3.1171	125.79	2465.90	5.1012	129.14	7298.41	1.7694	1255.50	19.06
SP15	6.10	10366.51	0.0588	—	1814.22	—	15.94	7685.12	0.2074	22.02	4676.65	0.4708	6.45	2382.45	0.2707	7.22	5548.49	0.1301	194.11	15.85
SP16	7.67	12903.86	0.0594	0.23	2509.21	0.0092	85.06	5550.87	1.5324	142.06	4329.68	3.2811	176.62	3056.34	5.7788	145.74	5866.53	2.4843	1678.57	18.29
SP17	2.03	10626.57	0.0191	—	1794.68	—	10.28	5329.55	0.1929	3.24	3731.24	0.0868	2.10	2828.19	0.0743	6.37	4657.92	0.1368	166.05	15.26
SP18	3.88	12874.45	0.0301	0.25	2623.84	0.0095	124.83	7649.77	1.6318	148.44	4745.86	3.1278	123.11	3038.81	4.0513	102.24	8464.54	1.2079	1304.51	19.91
SP19	6.64	13923.42	0.0477	0.39	1611.21	0.0242	116.76	6748.38	1.7302	49.92	4539.22	1.0997	45.19	3232.29	1.3981	77.95	5525.06	1.4108	1063.99	17.50
SP20	1.26	8623.89	0.0146	0.17	1655.87	0.0103	184.54	4751.82	3.8836	152.52	3938.78	3.8723	120.92	2150.51	5.6229	176.26	7450.83	2.3656	1670.95	13.61
SP21	10.27	10862.57	0.0945	—	1789.85	—	19.55	7093.47	0.2756	38.03	4126.30	0.9216	7.72	2908.79	0.2654	11.94	6041.00	0.1976	460.11	16.55
SP22	3.58	11476.96	0.0312	0.36	1441.11	0.0250	11.15	5896.68	0.1891	3.63	3801.35	0.0955	8.88	2075.29	0.4279	6.66	7009.63	0.0950	192.55	15.30
SP23	6.80	11931.55	0.0570	—	1557.75	—	48.22	5634.77	0.8558	90.90	3869.18	2.3493	14.04	1927.16	0.7285	18.66	8290.65	0.2251	651.61	16.73
SP24	6.27	10593.16	0.0592	0.12	2127.69	0.0056	64.57	5101.21	1.2658	104.24	3837.96	2.7160	14.64	2823.06	0.5186	24.13	9012.82	0.2677	734.27	16.87
SP25	1.86	13824.44	0.0135	0.44	2716.54	0.0162	186.66	4842.06	3.8550	146.65	4187.28	3.5023	97.55	2771.87	3.5193	189.30	8595.86	2.2022	1899.50	19.76
LP1	0.47	14631.38	0.0032	1.18	1948.75	0.0606	65.83	6231.55	1.0564	95.95	4013.49	2.3907	86.11	3138.12	2.7440	85.73	5753.40	1.4901	814.99	18.47
LP2	0.64	12206.18	0.0052	1.85	2297.38	0.0805	78.50	5838.37	1.3446	145.43	4899.76	2.9681	52.18	2104.26	2.4797	73.92	6212.65	1.1898	1012.65	17.73
LP3	4.52	8885.71	0.0509	0.13	1858.23	0.0070	41.09	5789.60	0.7097	124.04	2882.55	4.3031	10.24	2456.95	0.4168	10.18	6286.97	0.1619	597.23	13.95
LP4	5.45	10112.04	0.0539	—	1630.58	—	163.52	7525.00	2.1730	143.69	3758.46	3.8231	51.80	1875.09	2.7625	160.80	7731.94	2.0797	1426.96	16.05
LP5	1.71	9028.04	0.0189	—	1621.37	—	152.81	4615.56	3.3108	134.30	3260.74	4.1187	112.30	1704.64	6.5879	122.16	4651.12	2.6265	1718.87	15.03
LP6	0.71	11053.96	0.0064	—	1379.86	—	85.21	6409.94	1.3293	270.66	3875.46	6.9839	136.75	1528.04	8.9494	72.78	6453.61	1.1277	1308.25	13.94
LP7	0.41	10437.44	0.0039	0.99	1616.03	0.0613	48.37	6713.60	0.7205	199.42	3616.48	5.5142	71.18	2426.57	2.9334	73.61	5273.31	1.3959	1113.74	16.11
LP8	0.60	12028.82	0.0050	2.77	2130.37	0.1300	64.52	8264.66	0.7807	93.71	4102.87	2.2840	128.62	3030.50	4.2442	77.21	8764.09	0.8810	1028.91	17.52

Table 5: Continued.

Sample	Hpro			Met			Thr			Asn			Gln			Ser			Total free amino acids	Total proteins (%)
	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio	Free	Total	Ratio		
LP9	2.85	15395.17	0.0185	1.09	2611.93	0.0417	95.31	6079.19	1.5678	135.68	5800.10	2.3393	21.02	2847.50	0.7382	160.76	6714.80	2.3941	1620.64	20.09
LP10	3.15	12510.19	0.0252	0.19	2294.16	0.0083	139.99	5986.95	2.3383	316.13	4149.54	7.6184	20.34	2755.14	0.7383	126.41	9249.73	1.3666	1408.67	18.88
LP11	2.02	11304.83	0.0179	0.44	1616.02	0.0272	92.06	7087.95	1.2988	100.62	3134.85	3.2097	9.67	2255.64	0.4287	81.26	7479.28	1.0865	735.57	14.11
LP12	1.26	11500.95	0.0110	0.34	1721.63	0.0197	24.77	6419.44	0.3859	25.52	4463.29	0.5718	3.93	2645.29	0.1486	15.06	7598.99	0.1982	291.40	16.46
LP13	6.41	13163.18	0.0487	0.30	2716.29	0.0110	172.44	9432.07	1.8282	143.41	4225.29	3.3941	134.72	2932.22	4.5945	169.75	8497.59	1.9976	1667.79	19.79
LP14	3.64	16225.57	0.0224	0.17	2793.92	0.0061	175.47	6457.65	2.7172	140.65	5052.79	2.7836	118.28	3874.56	3.0527	171.21	9352.55	1.8306	1529.56	21.15
LP15	1.70	11614.17	0.0146	0.09	2501.01	0.0036	89.92	6615.18	1.3593	269.56	3725.94	7.2347	3.34	2877.91	0.1161	82.11	8616.92	0.9529	900.44	18.46
LP16	0.94	11631.20	0.0081	0.99	1651.61	0.0599	95.46	5189.08	1.8396	201.46	3964.71	5.0813	106.74	2083.97	5.1220	89.52	5649.83	1.5845	1381.04	15.81
LA1	0.75	10525.37	0.0071	0.33	1722.41	0.0192	119.80	5747.08	2.0845	146.08	4354.39	3.3548	117.79	2828.86	4.1639	99.93	8354.17	1.1962	1262.44	15.27
LA2	0.71	11050.46	0.0064	2.14	2075.76	0.1031	88.95	5755.14	1.5456	114.22	4714.20	2.4229	113.30	2590.48	4.3737	128.49	8446.54	1.5212	1057.50	16.42
LA3	1.58	14292.88	0.0111	—	2387.79	—	85.96	9803.09	0.8769	129.12	5651.48	2.2847	172.44	2107.17	8.1835	147.16	11408.14	1.2900	1716.54	20.72
LA4	0.78	11457.33	0.0068	—	2109.40	—	75.39	7598.12	0.9922	177.20	4225.71	4.1934	34.18	2341.13	1.4600	45.77	7073.90	0.6470	778.32	15.58
LA5	6.18	11098.92	0.0557	0.60	1527.50	0.0393	127.09	6426.35	1.9776	196.17	4798.09	4.0885	29.66	2500.29	1.1863	66.00	7750.15	0.8516	1332.28	16.58
LA6	1.69	11256.40	0.0150	0.14	1485.02	0.0094	92.57	6651.83	1.3916	129.99	4328.83	3.0029	86.79	1722.57	5.0384	157.13	8406.98	1.8690	1547.66	16.04
LA7	2.07	11307.16	0.0183	—	1577.93	—	92.43	4656.66	1.9849	145.34	4362.67	3.3314	135.71	1877.25	7.2292	170.21	8033.47	2.1188	1517.92	14.71
LA8	10.79	15918.39	0.0678	0.19	2259.56	0.0084	117.78	7883.83	1.4939	154.15	5792.38	2.6613	216.67	2621.75	8.2643	61.37	7941.33	0.7728	1171.00	21.30
LA9	0.76	14178.74	0.0054	0.14	2618.77	0.0053	6.51	6538.81	0.0996	7.87	4624.51	0.1702	2.68	2321.32	0.1155	6.76	10561.97	0.0640	189.02	21.46
LA10	1.44	11088.93	0.0130	0.26	1806.72	0.0144	62.25	6942.43	0.8967	53.97	4802.60	1.1238	47.46	2041.76	2.3245	36.17	5403.37	0.6694	775.54	16.41
LA11	0.84	15220.71	0.0055	1.65	2528.91	0.0652	8.60	7038.75	0.1222	1.31	5236.37	0.0250	4.91	2429.40	0.2021	7.80	6092.24	0.1280	289.21	19.05
LA12	2.55	9164.65	0.0278	0.18	1894.44	0.0095	174.89	4404.00	3.9712	138.27	3675.47	3.7620	72.48	1770.28	4.0943	85.28	5128.63	1.6628	1294.14	14.54
LA13	2.16	13906.13	0.0155	—	2514.70	—	116.52	7070.17	1.6481	294.15	3964.62	7.4194	42.77	2727.93	1.5679	65.75	7050.95	0.9325	1282.38	18.31
LA14	9.08	13760.74	0.0660	0.60	2297.56	0.0261	164.90	9014.10	1.8294	131.85	5045.39	2.6133	63.90	2704.59	2.3627	144.27	5601.90	2.5754	1371.90	17.81
LA15	3.69	8467.71	0.0436	0.30	1826.09	0.0164	135.18	5450.03	2.4804	62.94	3497.92	1.7994	46.02	1747.54	2.6334	51.56	4656.58	1.1073	1020.37	13.89
LA16	2.78	16217.53	0.0171	0.29	2603.75	0.0111	97.73	8455.20	1.1559	22.61	5055.17	0.4473	18.70	2206.25	0.8476	48.71	9026.29	0.5396	811.03	20.42
LA17	0.68	12202.21	0.0056	2.23	2011.22	0.1109	81.41	5966.49	1.3645	207.86	3770.32	5.5131	55.84	3327.23	1.6783	76.89	7660.33	1.0037	1027.14	18.44
LA18	0.55	9875.86	0.0056	2.42	1754.15	0.1380	76.82	5468.75	1.4047	143.75	3704.63	3.8803	52.67	1760.52	2.9917	90.61	6192.83	1.4631	1068.96	14.93

—, analytes were not detected.

Table 6: Contents of 26 minerals in living water of three species of duckweed (μg/L).

Sample	Ti	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Sr	Mo	Cd	Sn	Sb	Ba	Tl	Pb
SP1	22.831	2.900	2.178	47.015	0.181	1.860	1.061	90.405	1.530	1.578	261.614	2.840	0.007	0.087	0.440	96.840	0.003	0.058
SP2	27.277	1.017	2.922	36.990	0.177	1.648	0.806	80.395	2.091	1.288	199.006	3.275	0.006	0.007	0.516	60.613	0.003	0.001
SP3	35.621	1.915	1.838	41.295	0.162	2.899	1.500	56.437	1.127	1.983	172.960	3.017	0.016	0.168	0.386	70.196	0.002	0.024
SP4	22.183	3.099	1.687	55.683	0.214	1.767	1.690	63.717	3.164	1.935	178.985	0.407	0.014	0.168	0.487	77.659	0.001	0.041
SP5	45.839	3.162	1.413	24.431	0.189	2.829	0.615	71.741	0.920	1.861	275.418	2.211	0.011	0.098	0.542	63.126	0.004	0.074
SP6	41.446	1.783	2.371	2.110	0.240	2.157	2.805	87.665	2.146	1.464	193.120	0.577	0.002	0.121	0.536	39.704	0.004	0.046
SP7	37.099	2.423	2.068	13.993	0.219	2.583	1.424	88.936	2.601	1.747	165.967	1.562	0.010	0.095	0.316	69.916	0.003	0.050
SP8	28.377	2.251	2.276	54.028	0.197	1.709	2.941	46.581	2.262	2.139	249.180	2.345	0.015	0.125	0.359	110.809	0.004	0.047
SP9	17.084	1.069	2.452	40.559	0.192	3.140	1.247	29.974	2.770	1.664	163.694	2.659	0.015	0.139	0.637	60.895	0.004	0.018
SP10	37.166	3.316	1.705	35.254	0.184	1.793	2.040	78.451	1.247	1.407	174.872	0.632	0.002	0.137	0.720	86.360	—	0.003
SP11	24.163	1.033	1.368	60.738	0.190	3.261	2.894	66.981	1.135	2.291	207.084	1.840	0.003	0.160	0.488	117.603	0.004	0.046
SP12	38.630	2.597	1.146	53.768	0.252	2.550	1.505	76.263	1.772	2.126	180.679	0.626	0.003	0.124	0.628	142.280	0.001	0.062
SP13	39.728	2.347	1.577	26.733	0.173	3.173	2.882	75.098	1.628	1.112	225.261	0.323	0.012	0.106	0.550	95.230	0.001	0.001
SP14	39.483	2.488	2.539	3.742	0.249	2.137	1.427	55.271	1.670	1.581	154.375	3.214	0.019	0.135	0.338	119.138	0.003	0.043
SP15	19.629	2.734	2.326	22.405	0.198	3.153	1.427	55.631	1.175	2.065	247.855	2.928	0.011	0.011	0.633	136.536	0.004	0.030
SP16	34.619	1.068	2.064	52.250	0.163	2.169	3.017	54.488	2.321	1.985	253.553	2.155	0.002	0.120	0.377	68.487	0.001	0.022
SP17	28.672	3.244	2.192	31.414	0.159	1.880	2.871	91.946	1.444	1.287	175.165	1.721	0.018	0.138	0.669	91.883	0.004	0.030
SP18	25.018	1.789	2.378	45.984	0.183	2.570	2.365	24.786	1.877	2.146	194.164	3.450	0.007	0.049	0.318	95.469	0.002	0.057
SP19	22.802	2.136	2.199	53.284	0.212	2.704	1.054	85.613	0.913	2.073	269.684	3.174	0.014	0.043	0.423	111.149	0.003	0.004
SP20	40.638	1.682	1.746	14.621	0.180	1.611	2.705	73.740	3.221	2.055	225.646	3.023	0.013	0.159	0.450	137.841	0.004	0.019
SP21	23.513	1.413	2.811	12.908	0.236	1.959	0.607	22.616	2.711	1.170	227.681	3.187	0.005	0.037	0.205	62.615	0.003	0.016
SP22	32.010	1.755	2.461	56.750	0.202	2.515	1.114	80.116	1.210	2.322	223.754	1.075	0.015	0.155	0.573	96.469	0.002	0.012
SP23	17.817	2.259	2.391	43.452	0.219	1.903	0.700	88.007	3.277	1.855	155.450	2.793	0.014	0.164	0.311	82.020	0.002	0.010
SP24	24.306	2.384	1.841	32.935	0.253	1.896	3.027	63.243	1.975	1.109	214.325	2.979	0.008	0.044	0.642	47.431	0.003	0.061
SP25	30.842	2.878	1.693	29.377	0.254	2.786	1.811	69.276	1.082	1.349	218.438	0.896	0.011	0.125	0.474	126.036	0.001	0.032
LP1	37.974	1.441	2.756	42.898	0.251	1.828	2.325	46.010	2.655	2.264	162.610	0.298	0.004	0.049	0.368	106.287	0.002	0.055
LP2	25.601	3.144	1.914	10.035	0.173	2.425	2.422	53.173	1.031	1.740	211.860	0.256	0.017	0.153	0.596	73.995	0.002	0.001
LP3	40.576	2.424	1.579	36.158	0.203	2.635	1.373	32.781	1.839	1.363	234.793	1.450	0.001	0.145	0.697	107.956	0.001	0.032
LP4	29.919	1.451	2.468	31.060	0.239	3.221	2.253	43.918	2.873	1.754	193.086	2.154	0.013	0.027	0.249	36.637	0.001	0.015
LP5	43.240	2.466	2.331	46.991	0.209	3.218	1.577	26.153	1.994	2.211	234.265	1.895	0.010	0.017	0.224	84.449	0.004	0.006
LP6	29.925	2.796	2.933	11.764	0.248	2.974	2.174	43.896	2.394	1.784	247.178	3.487	0.012	0.017	0.336	125.785	0.003	0.016
LP7	28.783	1.090	2.067	41.129	0.244	2.388	1.514	76.640	1.923	1.886	157.760	2.486	0.003	0.031	0.241	87.240	0.004	0.053
LP8	45.076	3.030	1.857	7.724	0.247	2.328	2.152	87.415	2.455	2.044	225.691	2.976	0.011	0.009	0.607	111.718	0.003	0.023
LP9	20.651	2.339	2.731	42.403	0.257	1.681	1.012	89.960	1.066	1.855	210.521	2.237	0.007	0.052	0.424	32.520	—	0.033
LP10	23.769	1.646	1.288	52.927	0.243	2.319	2.950	93.752	2.929	1.882	217.655	2.496	0.017	0.165	0.196	135.624	0.003	0.051
LP11	32.364	2.560	3.084	33.335	0.208	3.219	2.180	82.812	1.451	1.918	269.769	3.250	0.019	0.008	0.406	66.278	0.003	0.051
LP12	24.918	1.470	2.999	37.041	0.243	1.893	1.928	95.305	2.415	1.698	224.103	1.906	0.008	0.041	0.570	64.033	0.004	0.008
LP13	33.468	1.704	2.451	45.575	0.176	2.507	1.882	38.837	2.524	1.580	187.087	1.245	0.003	0.133	0.285	44.075	0.003	0.030
LP14	28.614	2.426	1.879	39.614	0.209	3.272	1.963	74.622	2.875	1.962	261.572	2.514	0.013	0.136	0.287	100.138	0.002	0.048
LP15	35.617	2.088	2.116	42.214	0.162	2.284	1.641	39.037	1.412	1.185	244.588	0.489	0.007	0.158	0.512	110.953	0.003	0.032
LP16	32.522	2.845	1.545	41.087	0.186	2.100	1.860	94.105	3.060	1.921	165.053	1.398	0.015	0.008	0.451	69.796	0.002	0.066
LA1	36.451	2.711	1.271	38.156	0.233	2.893	0.909	65.183	3.041	1.926	194.455	3.342	0.005	0.055	0.262	51.502	0.002	0.023
LA2	43.394	2.188	2.067	17.427	0.165	2.206	1.564	58.425	3.159	1.247	254.469	1.789	0.006	0.146	0.280	33.628	0.003	0.052
LA3	24.311	1.120	2.917	35.712	0.247	2.359	2.087	59.228	3.345	2.092	247.432	1.778	0.010	0.108	0.362	105.945	—	0.069

Table 6: Continued.

Sample	Ti	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Sr	Mo	Cd	Sn	Sb	Ba	Tl	Pb
LA4	26.794	1.110	1.710	13.192	0.232	1.909	1.963	25.002	3.351	1.618	198.614	1.801	—	0.011	0.452	33.107	0.003	0.024
LA5	39.248	2.722	1.585	24.608	0.203	2.755	2.186	68.836	1.896	1.634	279.990	3.413	0.016	0.037	0.213	37.247	0.004	0.077
LA6	20.981	2.024	1.392	25.368	0.248	2.878	2.542	45.526	1.863	1.095	215.126	3.019	0.009	0.148	0.651	117.017	0.003	0.010
LA7	30.279	1.148	2.330	62.335	0.231	2.772	2.791	44.135	2.816	1.556	187.777	3.290	0.012	0.151	0.436	36.940	0.003	0.041
LA8	39.053	0.971	3.084	15.630	0.243	3.097	1.738	42.571	2.567	1.411	231.825	2.281	0.010	0.013	0.499	33.670	0.003	0.053
LA9	41.198	2.925	2.254	52.257	0.230	2.490	2.132	95.196	2.872	2.220	170.239	1.302	0.002	0.132	0.395	80.576	0.003	0.015
LA10	16.833	1.384	2.832	34.567	0.188	2.044	1.951	62.570	0.922	1.571	169.715	0.952	0.001	0.110	0.496	81.418	0.002	0.044
LA11	38.564	2.900	3.171	30.344	0.257	2.860	2.083	37.280	1.155	1.073	172.207	1.959	0.008	0.117	0.433	108.524	0.002	0.014
LA12	43.364	1.975	2.442	7.660	0.253	1.986	0.775	85.359	2.247	1.143	237.191	0.673	0.009	0.155	0.201	41.025	—	0.002
LA13	30.181	3.229	1.146	53.644	0.230	2.341	2.091	77.183	0.842	2.028	177.614	2.395	0.009	0.105	0.689	97.023	0.001	0.022
LA14	31.940	3.492	1.508	64.537	0.181	2.947	2.883	24.336	3.049	2.137	243.782	1.989	0.017	0.117	0.272	125.192	0.003	0.021
LA15	22.170	2.327	3.077	51.812	0.175	3.050	0.998	60.176	2.860	1.716	206.597	0.281	0.006	0.058	0.397	108.215	—	0.025
LA16	42.638	1.570	1.978	49.228	0.258	1.959	0.924	88.563	2.498	1.683	201.422	3.242	0.014	0.029	0.295	80.373	0.003	0.075
LA17	39.670	1.249	2.650	27.755	0.259	2.112	2.250	55.219	2.830	1.217	154.053	1.978	0.006	0.099	0.561	116.452	—	0.047
LA18	23.423	2.528	2.932	10.179	0.199	2.743	2.953	66.269	3.199	1.527	209.326	3.075	0.016	0.116	0.306	79.441	0.001	0.040

Sample	Na	Mg	Al	K	Ca	Fe	N	P	Total
SP1	410579.062	209771.718	18661.431	178472.536	591631.101	334974.476	4.303	0.914	1744628.970
SP2	546214.809	232797.639	9209.284	416708.514	386703.829	349989.801	2.389	0.675	1942044.979
SP3	384732.341	302374.639	16864.219	351597.651	645593.344	330485.336	18.804	5.589	2032063.470
SP4	763978.060	217006.139	24174.116	515345.319	525036.011	165789.077	19.687	4.182	2211765.493
SP5	498927.575	304758.187	29743.190	617594.946	663524.773	200323.584	25.750	23.839	2315416.327
SP6	568688.625	219822.814	31062.346	305039.982	486491.766	202085.631	25.562	22.681	1813617.704
SP7	815621.400	203217.299	32660.784	559562.408	566642.730	171755.702	2.565	1.507	2349855.405
SP8	704283.730	188285.004	23602.584	315772.349	624642.806	439955.497	23.372	6.689	2297077.677
SP9	876213.262	151574.474	27629.879	516294.381	409003.501	35506.241	18.458	4.258	2016572.669
SP10	873643.979	294027.085	21164.307	737471.818	383002.078	37324.576	33.550	19.033	2347111.719
SP11	491391.823	256934.016	10147.423	610308.309	636084.667	367347.644	31.694	25.205	2372762.062
SP12	796497.255	269227.175	17999.811	414658.818	512138.780	171064.616	16.646	2.703	2182110.814
SP13	366808.463	156147.673	11576.232	471660.374	756208.109	347515.518	11.647	4.202	2110408.155
SP14	512150.165	243500.636	31493.169	641312.090	499270.353	208734.109	11.077	1.602	2136861.051
SP15	533557.790	280721.134	16614.646	267728.355	378226.451	306162.902	13.593	4.564	1783528.184
SP16	644403.624	276057.049	21110.856	521532.157	583524.045	59176.189	11.942	25.264	2106319.987
SP17	751229.025	173163.933	10495.020	625004.125	472688.229	345116.023	8.458	5.204	2378144.753
SP18	522140.520	176639.957	29371.612	557932.109	690911.336	157124.877	29.906	10.221	2134563.150
SP19	579415.759	238453.094	30677.565	439717.245	686006.551	382672.972	10.823	3.662	2357515.153
SP20	476281.317	182915.790	9070.049	325811.742	373586.252	432553.750	34.616	21.891	1800784.763
SP21	760676.992	188574.381	12126.295	629919.272	484913.883	230880.412	26.927	9.833	2307491.687
SP22	709408.940	239417.173	10783.138	619462.993	550226.213	88795.927	20.170	9.877	2218626.941
SP23	513303.754	213899.504	26441.948	351694.267	732144.084	326876.462	34.159	26.699	2164823.520
SP24	824465.276	238490.190	31283.397	717257.458	679154.325	297001.594	14.031	10.796	2788075.528
SP25	815267.536	215764.623	30718.721	536395.881	508251.297	371174.761	24.650	2.494	2478087.325
LP1	897950.747	158686.979	27871.364	590366.194	696960.175	327232.239	17.263	7.080	2699502.116
LP2	531566.916	294929.433	14640.021	570417.870	648189.841	273059.464	15.536	1.239	2333208.859

Table 6: Continued.

Sample	Na	Mg	Al	K	Ca	Fe	N	P	Total
LP3	858626.936	266231.149	32272.481	727712.714	480257.660	348345.090	12.319	1.894	2713926.251
LP4	431098.253	221743.162	34467.794	311381.975	785570.359	339960.811	26.584	17.225	2124617.501
LP5	818404.736	237360.411	25820.309	683695.992	566015.946	406722.563	20.219	7.521	2738498.958
LP6	614726.783	203772.151	23626.598	170198.351	715325.262	132978.487	8.402	1.447	1861115.202
LP7	498622.492	200069.361	34249.218	182114.487	450498.128	213156.897	26.337	12.664	1579155.065
LP8	407734.217	308322.609	12414.630	462642.984	726996.054	273473.025	23.952	9.428	2192112.264
LP9	591923.425	196758.141	20795.603	169080.807	379447.395	244832.850	24.638	15.719	1603288.325
LP10	506188.280	278745.249	26183.977	548614.526	619327.368	71487.282	27.250	10.475	2051124.319
LP11	484360.416	252740.828	27267.497	394747.762	420528.173	411590.345	15.427	3.462	1991756.825
LP12	755545.368	290158.455	18713.693	269669.218	653034.467	303936.794	26.120	0.889	2291545.587
LP13	644431.270	171083.444	14922.153	701260.007	799614.153	241821.499	27.383	10.915	2573534.387
LP14	855511.785	226105.300	11812.507	382201.190	657443.790	421778.147	4.108	0.257	2555378.850
LP15	840333.425	261183.216	19461.126	468066.062	568653.040	96502.984	17.469	12.422	2254714.244
LP16	662138.232	247763.574	23217.103	653430.420	481555.835	52632.672	16.299	0.242	2121172.397
LA1	542867.038	291631.774	16101.327	208393.119	534546.718	287585.245	20.487	11.217	1881559.346
LA2	768502.457	174767.313	34293.763	538598.282	767009.154	130620.652	24.093	8.098	2414246.025
LA3	836100.485	232547.859	8383.317	197659.044	475008.913	70420.274	26.668	18.771	1820654.452
LA4	554969.006	231606.445	11451.728	365538.304	505163.079	269818.459	11.932	3.908	1938873.753
LA5	794677.014	293809.893	30832.050	293530.490	510493.154	262663.280	12.498	6.307	2186491.358
LA6	704360.729	287183.610	31019.483	189142.179	384371.287	327789.933	12.239	4.852	1924324.212
LA7	894339.623	204701.453	27985.214	281212.437	595254.900	371594.312	24.934	18.350	2375510.268
LA8	427283.433	184599.086	23700.009	211637.098	596714.072	325251.967	22.097	1.616	1769588.097
LA9	879491.821	180076.383	12682.543	611024.725	416252.729	426535.290	22.887	10.851	2526553.669
LA10	712373.467	297199.560	9966.649	218385.532	678770.388	129164.596	32.940	4.216	2046274.948
LA11	625486.302	275066.946	25810.603	496586.040	470812.328	438093.869	14.927	1.233	2332275.197
LA12	796469.226	199565.531	26532.869	616737.846	538184.180	286481.065	25.413	16.087	2464438.679
LA13	587462.535	234893.095	16533.152	573600.610	441381.833	215218.753	5.375	3.154	2069549.281
LA14	598797.362	200101.308	32285.037	179408.885	737688.278	321911.868	10.937	7.808	2070719.888
LA15	775176.535	182419.544	9875.652	375172.731	686178.073	146549.529	20.767	18.862	2175875.636
LA16	486462.514	269482.486	19514.461	382028.835	385846.710	419515.606	25.566	4.690	1963357.619
LA17	397975.248	220647.104	20225.722	404790.872	406870.509	69049.196	25.782	16.448	1520009.288
LA18	852308.317	145491.528	11612.476	499143.838	570244.949	84828.689	23.929	16.569	2164078.568

—, analytes were not detected.

mass spectrometer directly via a syringe pump with the velocity of 7 μL/min. While tuning the standard dissolved in water, most response of amino acids was very low when the sodium-adducted ion, $[M+Na]^+$, was relatively high. On the contrary, when dissolving the standard in 0.1% or higher concentration of formic acid solution, the response of protonated molecule, $[M+H]^+$, was dramatically higher and the metal-adducted ion was barely observed. Consequently, precursor ion (Q1), product ion (Q3), declustering potential (DP), and collision energy (CE) of each amino acid were acquired and optimized.

3.4. Method Validation. As shown in Table 3, the calibration curves of twenty-four amino acids showed good linear regression (all correlation coefficients > 0.995) within a wide range of concentration. The LOD and LOQ of each analyte with the signal-to-noise ratio (S/N) of 3 and 10 were in the range of 0.37–1.94 ng/mL and 0.74–4.49 ng/mL, respectively. The RSD values of intraday and interday precision are summarized in Table 4. From the results, the present method was found to be precise with intraday variability less than 3.14% and interday variability less than 4.27%. The sample solutions were stable within 24 h with stability RSD < 3.82%. The RSD values of repeatability were 0.52%–3.86%. The recoveries of 24 amino acids ranged from 95.27%–107.51% with RSD < 4.03%.

3.5. Quantitative Analysis for Samples. The developed UPLC-MS/MS method was applied for simultaneous determination of 24 free amino acids in 59 batches of three species of duckweed. All the contents are displayed in Table 5. The results showed that the total amount of amino acids varied dramatically among each sample from different regions and species. Total content of AAs in SP25 from Baoding City of Hebei Province reached as high as 1899.50 μg/g, which was much more than that in SP17 of 166.05 μg/g from Lianyungang City of Jiangsu Province. For each amino acid, the content also varied significantly even in the same sample. Coincidentally, the contents of free amino acids that include sulfur in their molecules were much less than others, including cysteine, cystine, and methionine with the concentration of 0.24, 4.25, and 4.20 μg/g in maximum, respectively. Especially for cysteine, it was not found in all of *Spirodela polyrhiza* and only detected in several samples of other two species. The putative reason was that the thiol in cysteine was not stable and easily oxidized. But in general, there was no significant difference observed in the total content of all FAAs among different species.

Twenty-six minerals, total proteins, and the content of amino acids after hydrolysis are shown in Tables 5 and 6. From the tables, it could be found that the total protein contents in 59 samples ranged from 13.61% to 21.46%. The relative contents of amino acids such as Lys, Ala, Ser, Thr, GABA, Gln, and Asn were all a little higher than other components based on the average value of each species among three different varieties of duckweed. The contents of Cys, Cys2, and Met were higher after hydrolysis. Obviously, it could be seen that the contents of free amino acids in duckweed were much lower than that of protein-bound amino acids. Moreover, the data showed that the contents of free amino acids in duckweed were negatively correlated with binding amino acids.

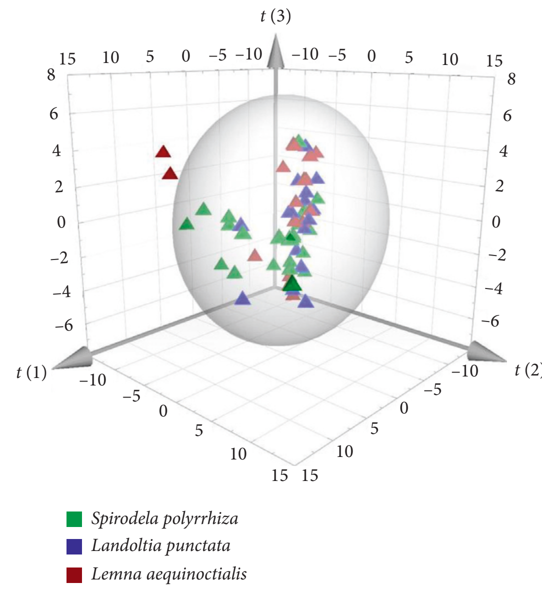

Figure 2: Three-dimensional score plot of principle component analysis (PCA) based on the content of free amino acids.

The elements with higher content in the samples were sodium, calcium, potassium, iron, magnesium, and aluminum. Their concentration ranges were 366808.463–897950.747, 373586.252–799614.153, 169080.807–737471.818, 35506.241–439955.497, 145491.528–308322.609, and 8383.317–34467.794 μg/L, respectively. The concentration ranges of strontium, barium, zinc, manganese and titanium were 154.053–279.990, 32.520–142.280, 22.616–95.305, 2.110–64.537, 16.833–45.839 μg/L. The concentrations of vanadium, chromium, cobalt, nickel, copper, arsenic, selenium, molybdenum, cadmium, tin, antimony, thallium, and lead are very low. It ranged from 0.0 to 3.492 μg/L. Evidently, the contents of macroelements in the samples were higher while they contained much lower trace elements.

3.6. Statistical Analysis. As one of the most important multivariate analysis techniques, PCA was employed to evaluate the variation among three species of duckweed. PCA is an unsupervised pattern recognition method without prior information of the data set and retained maximum variance of multidimensional data while reducing its dimensionality into two- or three-dimension [29–31]. Furthermore, all of the data information about amino acids was subjected to a supervised method, OPLS-DA [32], to get more information about different duckweeds. All the raw data were processed by normalization and Pareto scaling (Par) before modeling. Three dimensions were established in both the PCA and OPLS-DA models based on the content of free amino acids and the ratio of free to total amino acids with R2X (cum) value of 0.628, 0.596 and 0.758, 0.676, respectively. As shown in Figures 2–5, both PCA and OPLS-DA methods demonstrated that *Spirodela polyrhiza*,

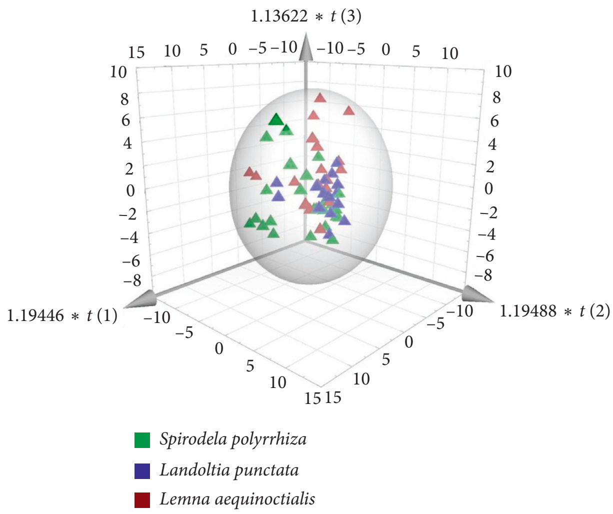

Figure 3: Three-dimensional score plot of orthogonal partial least square-discriminant analysis (OPLS-DA) based on the content of free amino acids.

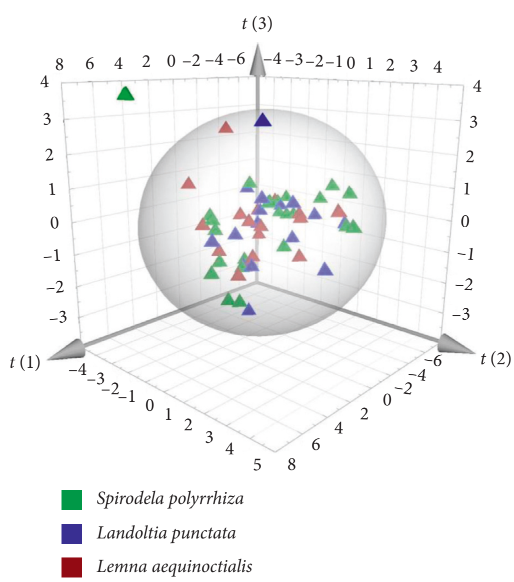

Figure 4: Three-dimensional score plot of principle component analysis (PCA) based on the ratio of free to total amino acids.

Landoltia punctata, and *Lemna aequinoctialis* were clustered together with each other in the score plot. There was no remarkable difference in different duckweeds.

4. Conclusions

In this study, a simple, fast, and convenient method with UPLC-QTRAP-MS/MS was established for the identification and quantification control of duckweeds by HILIC separation without derivatization. The results of method validation suggested that the developed method was sensitive, accurate, and precise for determination of 24 free amino acids. 59 batches of sample in three species were quantified, and then the data were statistically analysed by using PCA and OPLS-DA. The multivariate analysis

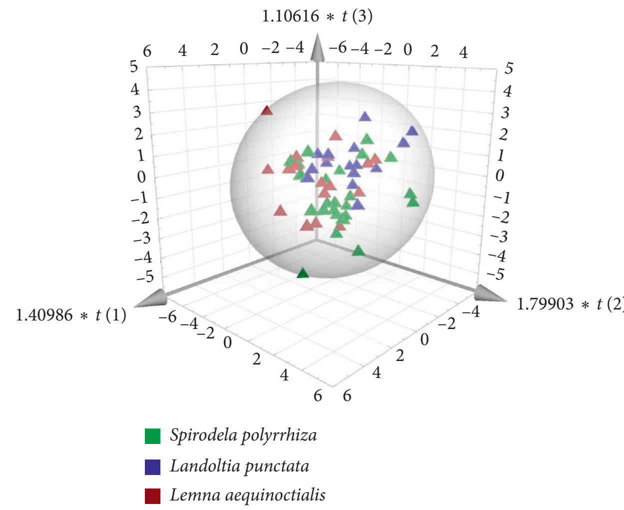

Figure 5: Three-dimensional score plot of orthogonal partial least squares-discriminant analysis (OPLS-DA) based on the ratio of free to total amino acids.

illustrated that the content of free amino acids was not significantly different among *Spirodela polyrhiza*, *Landoltia punctata*, and *Lemna aequinoctialis* in pattern recognition. But as a food, more amino acids mean more nutrition it has and more valuable it is. In other words, all of the three species of duckweed could be used as food equally if the absolute amount of AAs was more or less the same. In conclusion, a reliable and feasible method was developed for the nutritional evaluation of duckweeds.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This study was supported by the Chinese National Natural Science Foundation (81073002), the Program of Collaborative Innovation Center of Chinese Medicinal Material Resources Industrialization of Jiangsu Province (2016), and the National "Twentieth Five-Year" Technology Support Program (2011BAI04B06).

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