Rapid Determination of Amino Acids in Fruits of *Ziziphus jujuba* by Hydrophilic Interaction Ultra-High-Performance Liquid Chromatography Coupled with Triple-Quadrupole Mass Spectrometry

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Ⓢ *Supporting Information*

ABSTRACT: In this study, a sensitive and rapid method for the simultaneous determination of free amino acids without derivatization using hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry (HILIC−MS/MS) was developed. The method was performed on an ultra-high-performance liquid chromatography (UHPLC) separation system coupled with a triple-quadrupole mass spectrometry (TQ-MS) instrument. Sufficient separation of 23 underivatized amino acids was achieved on an Acquity BEH Amide column (2.1 mm × 100 mm, 1.7 μm) in a single run of 12 min. Then the method was applied for the analysis of the free amino acids in 46 batches of *Ziziphus jujuba* fruits which comprised 39 cultivars from 26 cultivation regions. Multivariate statistical analysis was also used to investigate the differences in free amino acid profiles among the samples. This study showed that HILIC−UHPLC−TQ-MS is an effective technique to analyze underivatized amino acids in the food samples.

KEYWORDS: amino acid, Ziziphus jujuba, hydrophilic interaction chromatography, ultra-high-performance liquid chromatography, triple-quadrupole mass spectrometry

■ INTRODUCTION

Amino acids are arguably the most important compounds from a biological point of view. They are not only the basic structural units of proteins, but also a source of energy and serve as precursors for the biosynthesis of neurotransmitters, porphyrins, polyamines, and nitric oxide. Furthermore, some amino acids play important roles as neurotransmitters.[1] Thus, the determination of these compounds is important in evaluating the nutritional quality of foods and in the quality control of some medicines[2,3] as well as in clinical diagnosis.[4,5]

Since amino acids are more polar compounds and most of them have little ultraviolet (UV) absorbance, their detection and accurate quantitation is a challenge with weak retention and poor separation as well as difficult detection using the conventional reversed-phase high-performance liquid chromatography (RP-HPLC)−UV method. To avoid these problems, many alternative approaches have been established, and they could be classified as two types: the indirect methods with derivatization of amino acids and the direct methods without derivatization. For the derivatization method, which was commonly used until now, the sensitive detection and good separation could be provided with RP-HPLC or capillary electrophoresis (CE) coupled with optical or MS detection. However, these methods must include some derivatization procedures using derivative molecules with strong chromophore groups, such as ninhydrin, *o*-phthalaldehyde, etc.[6−9] Besides, GC−MS with the precolumn derivatization method was also used for analysis of amino acids.[10,11] Unfortunately,

most of the derivatization methods are affected by some disadvantages, including derivative instability, insufficient reproducibility of derivative yield, interferences caused by the reagent, or time-consuming derivatization procedures.[1,12,13] Subsequently, analysis techniques free of the derivatization process have been established, such as ion-exchange HPLC coupled with electrochemical detection[2] and CE−MS methods.[14,15] However, most of these methods suffered from the drawback of either lack of analyte specificity or low throughput and comparatively poor reproducibility.[1] Recently, the use of perfluorinated acids as ion-pairing agents was reported to improve the separation of these polar compounds on C18 columns without the requirement for specialty columns or derivatization.[3,12,16,17] In this way, the underivatized amino acids could achieve good separation and could be detected using MS or evaporative light scattering detection (ELSD). However, retention times of amino acids were found to increase when a number of analyses were performed with the perfluorinated acids as ion-pairing agents,[12] and most of the ion-pairing agents could decrease the sensitivity of the MS detector.[18] All these problems limited the application of this method.

Alternatively, the highly polar compounds can obtain good retention and separation on hydrophilic interaction

chromatography (HILIC). In contrast with RP-HPLC, HILIC separation is based on the strong hydrophilic interaction of polar compounds with the hydrophilic polar stationary phase.[19] It is suitable for the separation of a broad spectrum of highly polar compounds, including peptides, amino acids, carbohydrates, nucleobases, and nucleosides as well as many other biologically important compounds.[13,20−22] Besides, since the eluent of HILIC is mainly the aqueous organic solution which enhances the analyte ionization with ESI process, the HILIC operation mode is friendly for MS analysis. Recently, an HILIC separation method with a 3 μm particle column, coupled with a high-resolution MS method, was developed for rapid determination of amino acids.[23] Unfortunately, the peaks of leucine and isoleucine were not well separated in the above method. Because these two amino acids have the same molecular weights and similar MS/MS characteristics, they could not be determined accurately even using high-resolution Orbitrap MS. It is well-known that the HILIC method coupled with the tandem mass spectrometry (MS/MS) method could provide higher sensitivity and selectivity, better resolution, narrower peaks, and shorter retention time when it was performed on the UHPLC column with sub 2 μm particles.[24] This opens the possibility that trace amounts of amino acids can be determined without derivatization.

Here we report the development, validation, and application of a fast and sensitive HILIC−UHPLC separation system coupled with triple-quadrupole mass spectrometry (TQ-MS) for underivatized amino acid detection and quantitation. The applicability of this method is demonstrated by the analysis of amino acids in the samples of *Ziziphus jujuba* fruit (ZJF), which is a medicine and food dual-purpose plant used in China[25] and has been found to contain many kinds of amino acids.[26] In addition, multivariate statistical analysis was used to investigate the differences in free amino acid profiles among the samples.

■ MATERIAL AND METHODS

Reagents and Materials. The acetonitrile and formic acid were all of HPLC grade and purchased from Merck (Darmstadt, Germany). Ammonium formate (analytical grade) was purchased from the Shanghai Chemical Reagent Factory (Shanghai, China). Pure water for UHPLC analysis was purified using a Milli-Q water purification system (Millipore, Billerica, MA) and was used for all solutions and dilutions. Other reagents and chemicals were of analytical grade.

Reference compounds tryptophan (Trp, **1**), phenylalanine (Phe, **2**), leucine (Leu, **3**), isoleucine (Ile, **4**), methionine (Met, **5**), γ-amino-butyric acid (GABA, **6**), valine (Val, **7**), proline (Pro, **8**), tyrosine (Tyr, **9**), cysteine (Cys, **10**), alanine (Ala, **11**), hydroxyproline (Hpro, **12**), threonine (Thr, **13**), glycine (Gly, **14**), glutamine (Gln, **15**), serine (Ser, **16**), glutamic acid (Glu, **17**), asparagine (Asn, **18**), citrulline (Cit, **19**), aspartic acid (Asp, **20**), histidine (Hit, **21**), arginine (Arg, **22**), and lysine (Lys, **23**) were purchased from Sigma-Aldrich (St. Louis, MO). The purity of each compound was more than 98%, determined by HPLC analysis.

Forty-six batches of ZJF (samples 1−46), consisting of 39 cultivars from 26 cultivation regions in China, were collected in September 2008, and the information on these samples is summarized in Table 1. Their botanical origins were identified as *Z. jujuba* Mill. by the corresponding author, and voucher specimens were deposited at the Herbarium at the Nanjing University of Chinese Medicine, China. After collection, the fruits were dried at 45 °C for six days.

Preparation of Standard Solutions. A mixed standard stock solution containing the reference compounds **1−23** was prepared by dissolving them in water, and the concentrations of these analytes were as follows: **1**, 20.4 μg mL^{-1}; **2**, 36.6 μg mL^{-1}; **3**, 38.2 μg mL^{-1}; **4**, 54.4 μg mL^{-1}; **5**, 21.2 μg mL^{-1}; **6**, 34.2 μg mL^{-1}; **7**, 29.8 μg mL^{-1}; **8**, 37.0 μg mL^{-1}; **9**, 23.6 μg mL^{-1}; **10**, 68.4 μg mL^{-1}; **11**, 47.2 μg mL^{-1}; **12**, 36.2 μg mL^{-1}; **13**, 29.4 μg mL^{-1}; **14**, 48.2 μg mL^{-1}; **15**, 29.2 μg mL^{-1}; **16**, 36.2 μg mL^{-1}; **17**, 31.0 μg mL^{-1}; **18**, 61.2 μg mL^{-1}; **19**, 38.2 μg mL^{-1}; **20**, 62.5 μg mL^{-1}; **21**, 63.4 μg mL^{-1}; **22**, 37.4 μg mL^{-1}; **23**, 60.0 μg mL^{-1}. This mixed standard stock solution was then diluted with water to different concentrations for construction of calibration curves. All the solutions were stored at 4 °C and brought to room temperature before use.

Preparation of Sample Solutions. After the cores had been removed, the dried fruits were pulverized to homogeneous powders

Table 1. Summary of the Tested Samples of *Z. jujuba*

sample no.	*Z. jujuba* cv.	cultivation region	sample no.	*Z. jujuba* cv.	cultivation region
1	Lizao	Hetian, Xinjiang	24	Yuanlingzao	Ningyang, Shandong
2	Junzao	Hetian, Xinjiang	25	Yuanlingzao	Shouguang, Shandong
3	Huizao	Hetian, Xinjiang	26	Yuanlingzao	Yantai, Shandong
4	Hetianyuzao	Hetian, Xinjiang	27	Dongzao	Zhanhua, Shandong
5	Hamidazao	Hami, Xinjiang	28	Hetaowen	Heze, Shandong
6	Ruoqiangdazao	Ruoqiang, Xinjiang	29	Mangguozao	Heze, Shandong
7	Ningxiayuanzao	Zhongwei, Ningxia	30	Lingzao	Wuhu, Anhui
8	Lingwuchangzao	Lingwu, Ningxia	31	Sihongdazao	Sihong, Jiangsu
9	Huizao	Lingwu, Ningxia	32	Fupingdazao	Fuping, Hebei
10	Gansuyuanzao	Pingliang, Gansu	33	Xingtangdazao	Xingtang, Hebei
11	Jinzao	Binxian, Shaanxi	34	Zanhuangdazao	Zanhuang, Hebei
12	Yazao	Liulin, Shanxi	35	Jinsixiaozao	Cangxian, Hebei
13	Muzao	Liulin, Shanxi	36	Xuanchengyuanzao	Cangxian, Hebei
14	Hupingzao	Taigu, Shanxi	37	Dalilongzao	Cangxian, Hebei
15	Junzao	Jiaocheng, Shanxi	38	Guantanzao	Cangxian, Hebei
16	Lizao	Taigu, Shanxi	39	Yiwuzao	Cangxian, Hebei
17	Lizao	Yuncheng, Shanxi	40	Jidanzao	Cangxian, Hebei
18	Banzao	Jishan, Shanxi	41	Longxuzao	Cangxian, Hebei
19	Lingbaoyuanzao	Lingbao, Henan	42	Damuzao	Cangxian, Hebei
20	Lingzao	Xinzheng, Henan	43	Xiangzao	Cangxian, Hebei
21	Jixinzao	Xinzheng, Henan	44	Mianzao	Cangxian, Hebei
22	Huizao	Xinzheng, Henan	45	Xiaozao	Cangxian, Hebei
23	Changhongzao	Ningyang, Shandong	46	Wuhezao	Cangxian, Hebei

(40 mesh). The dried powder (1.0 g) was weighed accurately into a 50 mL conical flask with a stopper, and 20 mL of water was added. After accurate weighing, ultrasonication (40 kHz) was performed at room temperature for 30 min, and then the same solvent was added to compensate for the weight lost during the extraction. The solution was adequately mixed and followed by centrifugation at 13 000 rpm for 10 min. For the quantification of Asn and Pro, the supernatants were diluted 10- or 100-fold with water, while for the others, the supernatants did not require dilution. All the solutions were stored at 4 °C and filtered through a 0.22 μm nylon membrane filter before injection into the UHPLC system for analysis.

Chromatography. UHPLC was performed by using a Waters ACQUITY UPLC system (Waters, Milford, MA), equipped with a binary solvent delivery system and an autosampler. HILIC separation was performed on an ACQUITY UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 μm, Waters) equipped with an ACQUITY UPLC BEH Amide 1.7 μm VanGuard precolumn. The mobile phase was composed of solvent A (water, 10 mM ammonium formate, and 0.15% formic acid, pH 3.0) and solvent B (acetonitrile, 2 mM ammonium formate, and 0.15% formic acid) with a gradient elution: 0−6 min, 15−20% A; 6−10 min, 20−30% A; 10−12 min, 30−40% A. Then the column was equilibrated for 6 min in the initial conditions. The flow rate of the mobile phase was 0.4 mL min^{-1}, and the column temperature was maintained at 35 °C. Two cycles of weak (80% acetonitrile) and strong (20% acetonitrile) solvent washing of the injecting system were carried out between injections. The injection volume was 2 μL. The column eluent was directed to the mass spectrometer. All the analyses were operated using MassLynx XS software.

Mass Spectrometry. Mass spectrometry was performed on a Waters Xevo TQ tandem quadrupole mass spectrometer (Micromass MS Technologies, Manchester, U.K.) using an ESI source operated in positive ion mode. The parameters in the source were set as follows: capillary voltage, 3.0 kV; source temperature, 150 °C; desolvation temperature, 550 °C; cone gas flow, 50 L h^{-1}; desolvation gas flow, 1000 L h^{-1}. The analyte detection was performed by using multiple reaction monitoring (MRM). The cone voltage and collision energy were optimized for each analyte, and selected values are given in Table 2. The dwell time was automatically set by the software.

Validation of the Method. The method was validated for linearity, limits of detection and quantification (LODs and LOQs), precision (interday and intraday precision), reproducibility, and stability following the International Conference on Harmonisation (ICH) guideline[27] and some reports on determination analysis.[28,29]

Calibration curves were constructed from peak areas of the reference standards versus their concentrations. Each calibration curve was performed with at least six appropriate concentrations in triplicate (Table S1, Supporting Information). Linearity evaluation of the calibration curve was accomplished by applying the lack-of-fit test using the software SPSS 16.0. The LOD and LOQ for each analyte were determined at signal-to-noise ratios (S/N) of about 3 and 10, respectively.

Intra- and interday variations were chosen to determine the precision of the developed method. For the intraday variability test, the mixed standard solutions were analyzed for six replicates within a day, while for the interday variability test, the solutions were examined in triplicate for three consecutive days. To confirm the repeatability, six sample solutions were independently prepared via the method described in the section "Preparation of Sample Solutions" from the same sample (sample 12) and were analyzed. One of the above sample solutions was stored at 20 °C and analyzed at 0, 2, 4, 8, 12, and 24 h to evaluate the solution's stability. All these variations were expressed by the relative standard deviation (RSD).

A recovery test was used to evaluate the accuracy of this method. It was performed by adding the corresponding marker compounds with high (150%), middle (100%), and low (50%) levels to 0.5 g of sample 12 which had previously been analyzed. The spiked samples were then extracted, processed, and quantified in accordance with the methods mentioned above, and triplicate experiments were performed at each level. The average recoveries were estimated by the formula recovery (%) = [(amount found − original amount)/amount added] × 100.

Table 2. Precursor/Product Ion Pairs and Parameters for MRM of Compounds Used in This Study

analyte	retention time (min)	[M + H]$^+$ (m/z)	MRM transition (precursor → product)	cone voltage (V)	collision energy (eV)
1, Trp	1.99	205.10	205.1 → 146.0^a	16.0	18.0
			205.1 → 118.0	16.0	24.0
2, Phe	1.99	166.09	166.1 → 120.1^a	18.0	14.0
			166.1 → 103.0	18.0	22.0
3, Leu	2.06	132.10	132.1 → 86.1^a	16.0	10.0
			132.1 → 69.1	16.0	18.0
4, Ile	2.34	132.10	132.1 → 86.1^a	16.0	10.0
			132.1 → 69.1	16.0	18.0
5, Met	2.46	150.06	150.1 → 104.0^a	14.0	10.0
			150.1 → 56.0	14.0	16.0
6, GABA	2.77	104.07	104.1 → 87.0^a	16.0	10.0
			104.1 → 68.9	16.0	14.0
7, Val	2.77	118.09	118.1 → 72.1^a	12.0	10.0
			118.1 → 55.1	12.0	18.0
8, Pro	2.87	116.07	116.1 → 70.0^a	20.0	10.0
			116.1 → 43.0	20.0	22.0
9, Tyr	2.87	182.08	182.1 → 136.0^a	16.0	16.0
			182.1 → 91.0	16.0	26.0
10, Cys	3.30	122.03	122.0 → 75.9^a	14.0	17.0
			122.0 → 58.9	14.0	20.0
11, Ala	4.01	90.06	90.1 → 44.0^a	16.0	10.0
			90.1 → 62.0	16.0	6.0
12, Hpro	4.02	132.07	132.1 → 67.9^a	18.0	16.0
			132.1 → 86.0	18.0	10.0
13, Thr	4.47	120.07	120.1 → 74.0^a	38.0	20.0
			120.1 → 93.0	38.0	14.0
14, Gly	4.70	76.04	76.0 → 30.0^a	12.0	6.0
			76.0 → 48.0	12.0	14.0
15, Gln	5.30	147.08	147.1 → 83.9^a	8.0	16.0
			147.1 → 56.0	8.0	24.0
16, Ser	5.49	106.05	106.1 → 60.0^a	14.0	8.0
			106.1 → 70.0	14.0	14.0
17, Glu	5.58	148.06	148.1 → 83.9^a	12.0	14.0
			148.1 → 56.0	12.0	22.0
18, Asn	5.68	133.06	133.1 → 73.9^a	12.0	14.0
			133.1 → 87.0	12.0	18.0
19, Cit	6.26	176.10	176.1 → 69.9^a	16.0	20.0
			176.1 → 106.0	16.0	10.0
20, Asp	7.31	134.05	134.1 → 88.0^a	14.0	10.0
			134.1 → 74.0	14.0	12.0
21, Hit	9.30	156.08	156.1 → 110.0^a	20.0	16.0
			156.1 → 82.9	20.0	22.0
22, Arg	9.30	175.12	175.1 → 70.0^a	22.0	18.0
			175.1 → 60.0	22.0	14.0
23, Lys	10.00	147.11	147.1 → 83.9^a	14.0	14.0
			147.1 → 56.1	14.0	24.0

^{a}Transition used for quantitation.

The matrix effect was defined as the ion suppression or enhancement of the ionization of analytes. Because it is very difficult to find blank matrix samples free of amino acids, the slope comparison method[7,30,31] was used to evaluate the matrix effect for this study. The sample extracts, which were spiked with appropriate amounts of standards as done for the apparent recovery measurement, were used to construct standard addition calibration curves. Then the slopes of the calibration curves from the standard addition experiments were compared with the slopes obtained from the pure aqueous standards at the same concentration levels. The slope ratio (slope matrix/slope solvent) of 1 indicates that the matrix does not significantly suppress
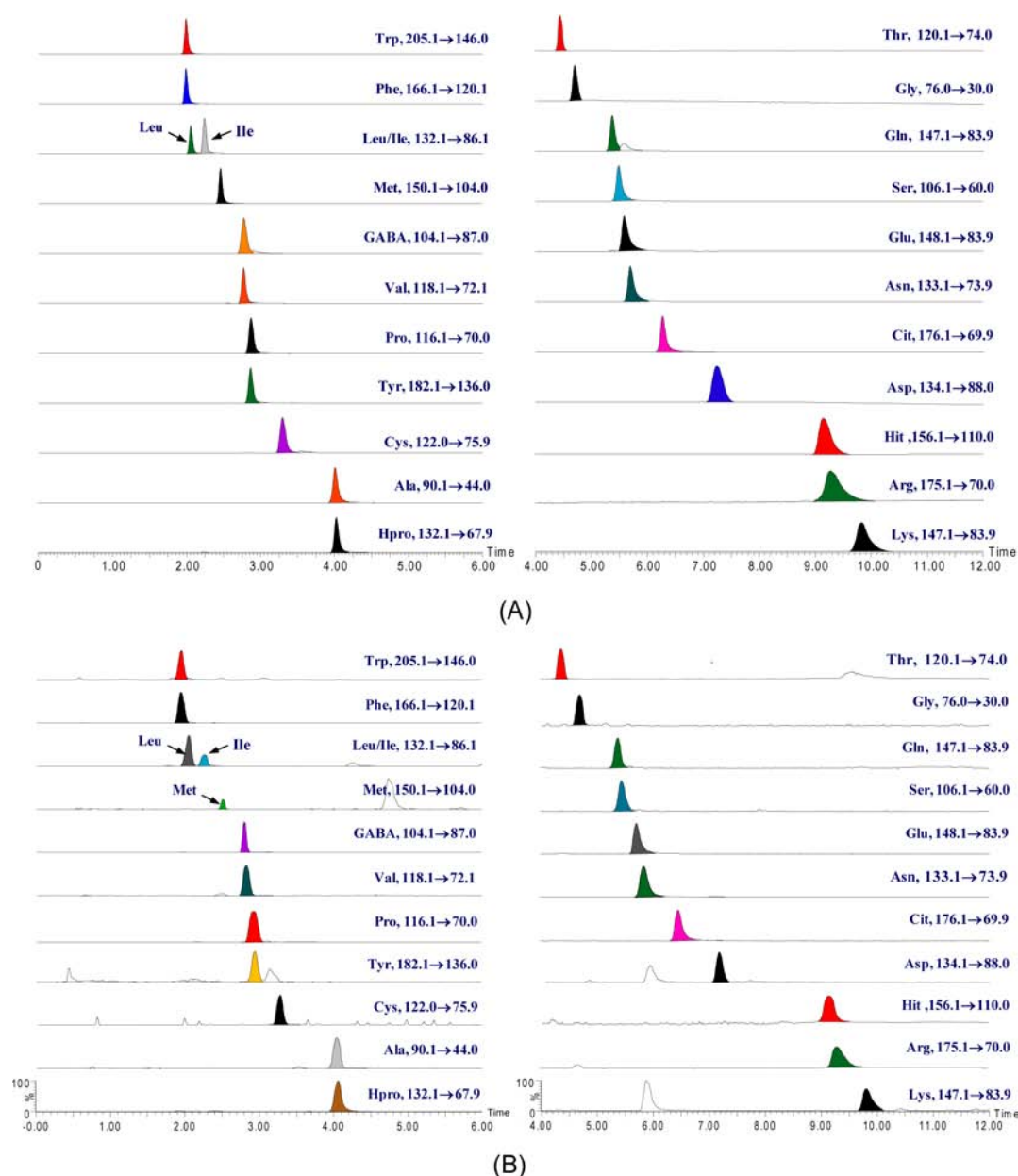
Figure 1. Typical UHPLC chromatograms of (A) mixed standards and (B) the fruits of *Z jujuba* with the MRM method.

or enhance the response of the mass spectrometer, otherwise denoting ionization suppression (<1) or enhancement (>1).[30]

Data Processing and Statistical Analysis. Data were processed using the TargetLynx application manager for the quantification of compounds. Principle component analysis (PCA) was performed using SPSS 16.0 software.

■ RESULTS AND DISCUSSION

Optimization of TQ-MS Conditions. Given the fact that not only the amino or imino groups, which can easily form positive ions, but also the carboxyl groups, which can form stable negative ions, simultaneously exist in the amino acid molecules, the analytes tested in this assay were first detected by the direct full scan mass spectrometry method using sample and standard solutions in both positive and negative ionization modes. The results showed that the sensitivity and intensity of analyte signals obtained from the positive ion mode were higher than those from the negative ion mode. Thus, the ESI+ mode

was selected in the following studies. To select a proper transition for the MS/MS detection of the analyte, all the compounds were examined separately in direct infusion mode, and at least two precursor/product ion pairs for each analyte were presented in this study. Then, according to the quantitative results, the highest sensitive and specific ion pairs were selected for the MRM determination. Once the most appropriate precursor/product ion pairs were determined, the values of the cone voltage and collision energy were optimized using the IntelliStart software (Figure S1, Supporting Information). For all amino acids analyzed in this study, the protonated molecule $[M + H]^+$ was detected with abundant intensities and selected as the precursor ion. Most amino acids have an abundant product ion at $[M + H − 46]^+$, which corresponds to the neutral loss of a formic acid by a rearrangement. This transition is specific to α-amino acid, because it involves both the carboxyl and the α-amino group.[4] Therefore, this transition for the detection of most amino acids was selected in this study. As for a

Table 3. Regression Equations, Correlation Coefficients, Linearity Ranges, and Limits of Detection (LODs) and Quantitation (LOQs) of the 23 Amino Acids

analyte	calibration curve	r^2	linear range ($\mu g\ mL^{-1}$)	LOD ($ng\ mL^{-1}$)	LOQ ($ng\ mL^{-1}$)
1, Trp	$y = 59672x + 192$	0.9994	0.01−10.20	1.02	4.08
2, Phe	$y = 198287x + 2854.2$	0.9993	0.01−9.15	0.61	2.26
3, Leu	$y = 173877x + 6802$	0.9989	0.01−9.55	1.91	5.73
4, Ile	$y = 171590x + 5775$	0.9991	0.01−13.60	2.72	8.16
5, Met	$y = 40626x − 2631.4$	0.9984	0.01−10.60	1.06	4.24
6, GABA	$y = 91680x + 8777.7$	0.9977	0.01−8.55	0.17	0.68
7, Val	$y = 112650x + 2178.3$	0.9996	0.04−7.45	7.45	28.80
8, Pro	$y = 182085x + 13533$	0.9951	0.05−9.25	0.31	1.16
9, Tyr	$y = 19740x + 1416.3$	0.9990	0.03−11.80	5.90	18.10
10, Cys	$y = 4064.2x − 6572.8$	0.9910	0.34−34.20	57.00	199.50
11, Ala	$y = 19318x + 489.3$	0.9995	0.12−23.60	23.60	94.40
12, Hpro	$y = 21925x − 788.48$	0.9982	0.02−9.05	4.53	15.82
13, Thr	$y = 177.89x − 66.65$	0.9992	0.74−29.40	73.50	294.00
14, Gly	$y = 3050.2x − 527.15$	0.9980	0.12−24.10	48.20	144.20
15, Gln	$y = 36183x − 4495.3$	0.9937	0.04−7.30	7.30	21.90
16, Ser	$y = 16558x − 847.31$	0.9993	0.18−18.10	36.20	108.60
17, Glu	$y = 23395x − 26645$	0.9943	0.31−31.00	77.75	272.13
18, Asn	$y = 19984x − 29699$	0.9989	0.31−30.60	61.20	244.80
19, Cit	$y = 33790x − 7274.1$	0.9953	0.02−19.10	5.05	17.75
20, Asp	$y = 7231.9x − 8844.1$	0.9937	0.63−62.50	62.50	218.75
21, Hit	$y = 76529x − 319331$	0.9935	0.63−63.40	79.25	277.38
22, Arg	$y = 73913x − 159557$	0.9956	0.94−37.40	62.34	187.00
23, Lys	$y = 39347x − 43692$	0.9999	0.60−60.00	50.00	200.00

Table 4. Precisions, Repeatabilities, Stabilities, Recoveries, and Matrix Effects of the 23 Amino Acids

analyte	precision (RSD, %)		repeatability (RSD, %, $n = 6$)	stability (RSD, %, $n = 6$)	recovery (%, $n = 3$)		matrix effect[a]
	intraday ($n = 6$)	interday ($n = 6$)			mean	RSD	
Trp	1.32	6.34	4.31	1.42	96.4	3.11	0.94
Phe	1.29	3.97	0.80	1.27	102.1	2.28	0.96
Leu	1.14	3.77	2.57	1.27	98.2	3.09	0.95
Ile	0.83	2.63	3.93	2.48	99.0	2.51	1.03
Met	0.81	3.28	5.78	5.92	97.5	4.87	0.99
GABA	0.98	4.79	5.24	3.58	103.4	5.13	0.96
Val	0.94	1.33	3.89	3.66	94.4	4.07	0.93
Pro	1.16	1.06	3.47	2.60	99.0	2.15	0.97
Tyr	2.01	5.00	4.86	3.57	94.9	3.99	0.95
Cys	3.09	6.01	5.15	4.16	95.7	5.06	0.91
Ala	1.56	2.68	2.03	1.79	97.4	2.91	0.98
Hpro	1.95	3.71	2.15	1.68	97.7	3.57	0.96
Thr	4.79	2.14	4.45	6.31	94.8	4.95	0.95
Gly	3.81	6.37	5.99	6.97	103.6	5.05	1.01
Gln	4.24	4.13	5.82	5.60	94.8	4.77	0.90
Ser	3.21	4.33	4.48	2.44	99.1	5.00	0.98
Glu	2.76	2.00	6.06	1.11	93.5	3.65	0.91
Asn	3.00	2.76	5.01	4.07	96.9	3.94	1.04
Cit	1.27	2.80	10.62	3.18	98.1	2.81	1.05
Asp	4.19	3.70	5.12	5.19	94.3	6.44	0.90
Hit	3.71	6.60	2.13	3.02	93.8	5.06	0.95
Arg	2.79	4.14	3.70	6.31	96.5	3.83	0.92
Lys	2.79	6.68	4.16	5.78	97.0	4.79	0.90

[a]Matrix effects are calculated by slope of matrix curve/slope of solvent curve.

non-α-amino acid, such as GABA, the product $[M + H − 17]^+$ ($−NH_3$) was more abundant than $[M + H − 46]^+$, so the former was used for the MRM of this compound. Amino acids containing the amide group, such as Gln, could simultaneously provide the fragment ions $[M + H − 17]^+$ (m/z 130), $[M + H − 46]^+$ (m/z 101), and $[M + H − 46 − 17]^+$ (m/z 84), and the

last was the most abundant among them, so this ion was selected as the product ion for MRM of Gln. Asn, another amino acid containing an amide group, could not give the fragment ion $[M + H − 46 − 17]^+$, while giving the ion at m/z 74, and the intensity of this ion is higher than those of fragment ions $[M + H − 17]^+$ and $[M + H − 46]^+$. Thus, the ion at m/z

Table 5. Contents ($\mu g\ g^{-1}$) of 23 Free Amino Acids in the Tested Samples of Z. jujuba Fruits

contents of analytes (mean ± SD, $n = 3$)

sample no.[a]	Trp	Phe	Leu	Ile	Met	GABA	Val	Pro	Tyr	Cys	Ala	Hpro
1	16.47 ± 0.63	14.13 ± 0.45	15.82 ± 0.43	7.91 ± 0.26	0.77 ± 0.02	4.83 ± 0.12	6.51 ± 0.17	2475.25 ± 50.28	3.92 ± 0.15	2.32 ± 0.13	20.33 ± 0.75	34.47 ± 0.87
2	7.83 ± 0.34	12.21 ± 0.53	11.51 ± 0.25	5.24 ± 0.16	0.71 ± 0.03	4.11 ± 0.16	8.78 ± 0.44	3458.41 ± 62.45	3.20 ± 0.12	nd[b]	17.90 ± 0.37	34.12 ± 0.66
3	2.26 ± 0.03	7.20 ± 0.25	5.50 ± 0.21	2.01 ± 0.10	0.22 ± 0.01	2.67 ± 0.10	4.39 ± 0.20	3021.90 ± 45.11	1.35 ± 0.05	nd	9.42 ± 0.15	23.98 ± 0.57
4	4.10 ± 0.09	11.57 ± 0.42	14.93 ± 0.44	5.13 ± 0.18	0.17 ± 0.01	2.42 ± 0.07	7.97 ± 0.27	3313.41 ± 75.06	3.92 ± 0.13	3.54 ± 0.14	25.07 ± 0.62	43.74 ± 0.90
5	1.68 ± 0.04	4.72 ± 0.11	3.72 ± 0.08	1.19 ± 0.05	0.11 ± 0.01	0.91 ± 0.03	3.21 ± 0.14	2347.33 ± 39.78	1.58 ± 0.06	2.32 ± 0.10	4.35 ± 0.11	21.26 ± 0.65
6	8.84 ± 0.33	14.85 ± 0.33	21.48 ± 0.60	9.29 ± 0.25	0.59 ± 0.02	15.20 ± 0.38	7.35 ± 0.33	3010.09 ± 58.05	3.42 ± 0.14	3.07 ± 0.16	47.27 ± 0.99	49.25 ± 1.15
7	2.61 ± 0.08	7.11 ± 0.23	6.26 ± 0.13	3.42 ± 0.10	0.53 ± 0.02	1.28 ± 0.03	2.90 ± 0.11	1058.35 ± 35.45	3.46 ± 0.11	0.90 ± 0.03	4.74 ± 0.13	12.05 ± 0.43
8	4.63 ± 0.20	8.32 ± 0.18	8.20 ± 0.21	3.44 ± 0.07	0.70 ± 0.03	3.28 ± 0.12	6.39 ± 0.25	2482.33 ± 55.13	2.70 ± 0.10	2.47 ± 0.08	8.91 ± 0.19	19.14 ± 0.46
9	10.94 ± 0.29	28.07 ± 0.78	38.11 ± 0.66	15.14 ± 0.36	0.96 ± 0.02	5.26 ± 0.12	24.01 ± 0.92	3492.11 ± 78.54	11.96 ± 0.35	3.78 ± 0.15	48.24 ± 1.14	29.10 ± 0.77
10	2.20 ± 0.06	5.79 ± 0.17	5.47 ± 0.20	1.75 ± 0.06	0.32 ± 0.01	2.94 ± 0.08	3.29 ± 0.10	1761.10 ± 45.23	3.12 ± 0.12	nd	5.19 ± 0.16	12.45 ± 0.35
11	31.52 ± 1.03	104.55 ± 1.63	121.36 ± 1.97	55.76 ± 1.07	11.77 ± 0.37	3.17 ± 0.07	49.32 ± 1.36	753.62 ± 28.56	85.58 ± 2.58	0.76 ± 0.03	142.10 ± 2.19	8.71 ± 0.15
12	1.78 ± 0.05	9.01 ± 0.31	9.02 ± 0.25	2.39 ± 0.05	0.24 ± 0.01	4.08 ± 0.10	4.77 ± 0.21	2980.63 ± 67.23	2.86 ± 0.11	3.03 ± 0.14	22.19 ± 0.45	44.38 ± 0.90
13	4.61 ± 0.16	13.39 ± 0.55	14.98 ± 0.34	5.03 ± 0.14	1.02 ± 0.04	3.20 ± 0.12	7.01 ± 0.18	3597.99 ± 90.44	3.63 ± 0.14	3.98 ± 0.18	28.37 ± 0.47	38.86 ± 0.92
14	1.99 ± 0.09	9.79 ± 0.12	11.09 ± 0.40	4.43 ± 0.16	0.27 ± 0.01	3.90 ± 0.14	6.32 ± 0.20	3041.64 ± 35.28	4.05 ± 0.10	3.07 ± 0.13	26.44 ± 0.61	33.88 ± 0.78
15	11.32 ± 0.53	21.54 ± 0.61	23.19 ± 0.25	7.63 ± 0.24	1.27 ± 0.03	5.26 ± 0.18	13.71 ± 0.38	3259.27 ± 55.89	4.81 ± 0.17	3.40 ± 0.10	42.27 ± 1.37	38.55 ± 0.93
16	4.65 ± 0.22	9.44 ± 0.39	7.74 ± 0.36	2.53 ± 0.08	0.59 ± 0.03	3.91 ± 0.11	4.77 ± 0.12	1219.12 ± 45.04	6.10 ± 0.21	nd	7.45 ± 0.35	10.65 ± 0.38
17	11.60 ± 0.45	23.40 ± 0.57	23.76 ± 0.58	9.33 ± 0.16	0.68 ± 0.02	10.50 ± 0.34	10.92 ± 0.31	2832.54 ± 78.99	10.20 ± 0.33	2.88 ± 0.07	78.51 ± 1.18	48.25 ± 1.17
18	8.95 ± 0.35	20.41 ± 0.64	18.56 ± 0.71	6.74 ± 0.25	3.20 ± 0.11	1.45 ± 0.05	6.67 ± 0.22	3359.37 ± 88.13	5.20 ± 0.29	nd	20.11 ± 0.33	28.80 ± 0.84
19	5.08 ± 0.23	13.10 ± 0.42	11.83 ± 0.55	4.22 ± 0.12	0.36 ± 0.01	1.11 ± 0.06	5.32 ± 0.15	3117.90 ± 76.67	4.38 ± 0.16	3.37 ± 0.11	23.00 ± 0.72	29.95 ± 0.71
20	2.92 ± 0.13	12.34 ± 0.46	10.38 ± 0.47	4.46 ± 0.19	0.77 ± 0.03	2.01 ± 0.06	3.97 ± 0.11	1121.15 ± 36.00	5.09 ± 0.15	0.99 ± 0.05	9.84 ± 0.30	10.99 ± 0.30
21	4.16 ± 0.19	16.86 ± 0.39	14.58 ± 0.67	5.44 ± 0.22	0.50 ± 0.01	3.55 ± 0.09	6.45 ± 0.32	2902.55 ± 76.53	5.67 ± 0.19	2.96 ± 0.12	90.07 ± 1.85	38.99 ± 1.11
22	5.93 ± 0.13	16.52 ± 0.65	14.30 ± 0.33	4.55 ± 0.15	0.67 ± 0.02	3.55 ± 0.12	7.53 ± 0.33	2779.79 ± 48.49	8.16 ± 0.30	2.75 ± 0.13	55.90 ± 0.87	31.68 ± 0.82
23	4.70 ± 0.09	15.20 ± 0.36	14.12 ± 0.51	4.09 ± 0.11	0.40 ± 0.02	2.52 ± 0.07	6.22 ± 0.21	2703.90 ± 87.87	5.69 ± 0.28	2.68 ± 0.09	33.59 ± 0.64	36.10 ± 0.96
24	3.89 ± 0.07	14.02 ± 0.45	12.83 ± 0.30	3.64 ± 0.07	0.61 ± 0.02	1.88 ± 0.04	5.43 ± 0.15	3091.85 ± 98.12	5.16 ± 0.26	3.21 ± 0.10	18.22 ± 0.49	29.47 ± 0.70
25	4.10 ± 0.11	17.56 ± 0.63	19.46 ± 0.29	7.38 ± 0.30	0.76 ± 0.03	2.98 ± 0.12	6.83 ± 0.12	2663.60 ± 86.04	6.20 ± 0.19	2.66 ± 0.05	36.84 ± 0.88	33.26 ± 0.69
26	14.28 ± 0.38	20.61 ± 0.45	17.01 ± 0.76	8.94 ± 0.26	0.22 ± 0.01	16.09 ± 0.75	14.59 ± 0.68	2680.92 ± 35.08	8.51 ± 0.25	2.81 ± 0.08	108.38 ± 2.40	59.93 ± 1.77
27	7.68 ± 0.32	24.66 ± 0.69	14.37 ± 0.32	7.77 ± 0.22	0.27 ± 0.01	5.94 ± 0.28	14.13 ± 0.55	1736.54 ± 57.33	14.12 ± 0.32	1.66 ± 0.07	102.53 ± 2.47	42.83 ± 1.23
28	28.44 ± 0.76	86.71 ± 1.87	119.60 ± 2.27	46.36 ± 0.97	6.02 ± 0.01	8.48 ± 0.32	38.16 ± 1.08	3172.28 ± 85.11	38.28 ± 0.81	3.54 ± 0.14	242.29 ± 4.15	54.53 ± 1.64
29	3.54 ± 0.11	16.18 ± 0.60	14.66 ± 0.45	5.55 ± 0.30	0.31 ± 0.01	2.20 ± 0.06	7.57 ± 0.28	2976.72 ± 61.39	6.82 ± 0.15	3.03 ± 0.08	49.92 ± 1.19	35.63 ± 0.79
30	37.67 ± 1.05	38.27 ± 0.81	35.80 ± 0.68	28.85 ± 0.98	2.25 ± 0.07	39.03 ± 0.89	22.80 ± 0.80	1804.12 ± 61.95	20.95 ± 0.48	1.81 ± 0.08	166.62 ± 4.58	105.14 ± 2.88
31	8.02 ± 0.18	13.45 ± 0.42	12.32 ± 0.34	4.73 ± 0.27	0.26 ± 0.01	6.13 ± 0.17	7.01 ± 0.25	2484.68 ± 58.64	6.86 ± 0.17	2.43 ± 0.05	74.77 ± 2.11	35.37 ± 0.80
32	34.03 ± 0.94	69.94 ± 1.08	82.31 ± 1.79	38.83 ± 1.02	15.57 ± 0.46	5.81 ± 0.18	23.52 ± 0.89	3001.69 ± 55.03	24.85 ± 0.70	3.08 ± 0.15	112.78 ± 1.89	56.72 ± 1.56
33	4.08 ± 0.12	14.73 ± 0.50	12.82 ± 0.25	3.89 ± 0.15	0.68 ± 0.03	2.51 ± 0.13	4.92 ± 0.18	2145.08 ± 67.28	5.27 ± 0.15	2.04 ± 0.11	21.46 ± 0.55	22.66 ± 0.71
34	3.85 ± 0.09	11.55 ± 0.33	12.10 ± 0.28	6.01 ± 0.28	0.46 ± 0.01	2.34 ± 0.06	5.23 ± 0.17	2731.11 ± 50.82	4.22 ± 0.18	2.53 ± 0.08	27.71 ± 0.67	24.55 ± 0.64
35	6.60 ± 0.15	7.18 ± 0.27	8.38 ± 0.29	3.23 ± 0.17	0.32 ± 0.01	6.40 ± 0.23	6.23 ± 0.13	3905.42 ± 79.77	2.35 ± 0.09	4.55 ± 0.15	25.89 ± 0.66	36.45 ± 0.90
36	6.63 ± 0.08	12.35 ± 0.41	14.03 ± 0.25	4.21 ± 0.19	1.43 ± 0.06	4.47 ± 0.22	5.59 ± 0.22	1490.68 ± 46.83	6.69 ± 0.25	1.35 ± 0.05	21.96 ± 0.46	25.84 ± 0.83
37	13.45 ± 0.45	24.16 ± 0.59	23.70 ± 0.25	8.45 ± 0.22	0.87 ± 0.04	9.07 ± 0.31	12.49 ± 0.54	2773.09 ± 59.37	10.36 ± 0.27	2.79 ± 0.06	69.54 ± 1.78	57.40 ± 1.67
38	15.98 ± 0.57	32.34 ± 0.68	34.25 ± 0.58	19.76 ± 0.40	0.82 ± 0.03	27.27 ± 0.81	12.60 ± 0.35	2269.69 ± 60.85	10.26 ± 0.19	2.28 ± 0.07	64.81 ± 2.01	33.04 ± 1.10
39	6.09 ± 0.13	18.71 ± 0.55	29.18 ± 0.74	13.59 ± 0.25	1.04 ± 0.03	20.74 ± 0.84	11.24 ± 0.22	3685.67 ± 50.28	6.54 ± 0.15	4.57 ± 0.12	68.29 ± 1.58	57.94 ± 1.58
40	5.20 ± 0.09	25.86 ± 0.62	28.61 ± 0.68	14.16 ± 0.29	0.68 ± 0.03	10.62 ± 0.35	10.03 ± 0.34	2909.09 ± 67.36	7.96 ± 0.22	2.79 ± 0.08	70.05 ± 2.15	40.04 ± 1.24
41	4.25 ± 0.13	8.08 ± 0.22	6.84 ± 0.22	2.32 ± 0.09	0.16 ± 0.01	2.81 ± 0.08	4.62 ± 0.20	2699.69 ± 75.85	2.75 ± 0.10	2.74 ± 0.06	10.22 ± 0.34	28.30 ± 0.81

Table 5. continued

contents of analytes (mean ± SD, n = 3)

sample no.[a]	Trp	Phe	Leu	Ile	Met	GABA	Val	Pro	Tyr	Cys	Ala	Hpro	total
42	5.87 ± 0.16	23.30 ± 0.51	24.72 ± 0.39	10.36 ± 0.46	0.53 ± 0.02	11.82 ± 0.42	8.31 ± 0.44	2505.18 ± 50.50	10.44 ± 0.35	2.30 ± 0.09	51.10 ± 1.33	43.60 ± 0.85	
43	12.54 ± 0.41	26.76 ± 0.77	32.58 ± 0.80	15.21 ± 0.42	2.28 ± 0.05	0.66 ± 0.03	9.74 ± 0.36	2673.44 ± 65.19	9.88 ± 0.20	2.66 ± 0.07	45.90 ± 1.57	38.61 ± 0.78	
44	0.87 ± 0.01	10.91 ± 0.60	11.40 ± 0.26	3.42 ± 0.18	0.52 ± 0.01	3.02 ± 0.09	4.23 ± 0.18	2521.38 ± 58.53	3.79 ± 0.12	2.42 ± 0.10	22.87 ± 0.67	44.61 ± 1.03	
45	4.67 ± 0.07	5.69 ± 0.28	7.26 ± 0.21	4.04 ± 0.14	0.23 ± 0.01	3.87 ± 0.09	3.84 ± 0.13	3277.70 ± 81.32	2.94 ± 0.08	3.23 ± 0.15	11.85 ± 0.24	21.19 ± 0.44	
46	4.68 ± 0.13	9.03 ± 0.34	10.52 ± 0.37	3.82 ± 0.09	1.12 ± 0.04	1.57 ± 0.06	4.12 ± 0.15	3594.73 ± 72.82	2.27 ± 0.10	3.66 ± 0.12	22.04 ± 0.61	29.85 ± 0.67	

contents of analytes (mean ± SD, n = 3)

sample no.[a]	Thr	Gly	Gln	Ser	Glu	Asn	Cit	Asp	Hit	Arg	Lys	total
1	nd	6.86 ± 0.23	2.33 ± 0.08	26.66 ± 0.72	41.40 ± 1.24	428.16 ± 12.45	1.31 ± 0.03	84.41 ± 2.36	9.66 ± 0.23	92.97 ± 3.51	5.72 ± 0.15	3302.23
2	nd	7.37 ± 0.30	4.09 ± 0.19	30.30 ± 0.69	23.92 ± 0.80	277.48 ± 7.18	1.41 ± 0.05	43.50 ± 1.58	11.15 ± 0.44	68.90 ± 1.68	5.29 ± 0.25	4037.42
3	nd	4.36 ± 0.12	2.44 ± 0.06	12.61 ± 0.28	15.58 ± 0.33	145.75 ± 4.24	0.38 ± 0.02	41.66 ± 1.22	6.12 ± 0.31	69.90 ± 2.07	2.26 ± 0.10	3381.97
4	nd	7.92 ± 0.20	2.45 ± 0.09	29.24 ± 0.66	36.54 ± 0.79	1116.17 ± 32.55	0.72 ± 0.03	124.97 ± 3.22	10.81 ± 0.39	113.16 ± 3.45	4.69 ± 0.18	4882.65
5	nd	1.69 ± 0.05	0.77 ± 0.04	7.21 ± 0.24	5.35 ± 0.14	150.74 ± 4.57	nd	105.52 ± 2.78	5.16 ± 0.24	60.44 ± 1.77	1.29 ± 0.05	2730.51
6	25.14 ± 0.56	15.61 ± 0.47	15.27 ± 0.55	72.95 ± 2.21	57.69 ± 1.28	1581.16 ± 43.06	2.76 ± 0.12	217.99 ± 6.11	7.39 ± 0.28	123.99 ± 3.51	12.37 ± 0.44	5323.03
7	nd	3.13 ± 0.08	3.13 ± 0.11	5.43 ± 0.23	9.12 ± 0.31	62.16 ± 1.88	nd	16.87 ± 0.54	4.77 ± 0.17	77.26 ± 1.89	1.84 ± 0.08	1287.33
8	nd	2.51 ± 0.07	2.23 ± 0.06	7.68 ± 0.31	12.05 ± 0.33	261.13 ± 6.11	0.86 ± 0.04	110.25 ± 4.02	17.03 ± 0.43	127.53 ± 4.07	2.07 ± 0.06	3093.84
9	31.35 ± 0.81	9.92 ± 0.25	30.04 ± 0.89	51.74 ± 1.56	63.08 ± 1.95	962.91 ± 25.10	2.33 ± 0.05	198.09 ± 5.64	44.49 ± 1.68	358.47 ± 8.45	14.24 ± 0.55	5474.33
10	2.47	nd	1.56 ± 0.07	8.02 ± 0.26	5.27 ± 0.13	128.66 ± 3.33	nd	17.42 ± 0.58	7.79 ± 0.26	94.08 ± 2.57	1.89 ± 0.06	2070.75
11	141.67 ± 2.99	53.02 ± 1.26	164.05 ± 3.79	122.70 ± 3.17	342.15 ± 9.98	121.11 ± 2.95	0.57 ± 0.02	131.17 ± 4.12	95.60 ± 2.33	283.43 ± 6.67	50.88 ± 1.25	2874.57
12	22.31 ± 0.66	4.39 ± 0.18	5.85 ± 0.21	22.46 ± 0.64	33.05 ± 0.97	633.10 ± 15.34	0.55 ± 0.02	49.23 ± 1.67	5.37 ± 0.20	110.72 ± 2.34	5.06 ± 0.30	3954.15
13	15.24 ± 0.45	6.01 ± 0.25	20.88 ± 0.88	20.99 ± 0.54	27.15 ± 0.88	337.73 ± 11.20	0.36 ± 0.02	92.23 ± 2.87	10.02 ± 0.45	83.46 ± 1.99	5.94 ± 0.21	4342.09
14	nd	5.35 ± 0.16	2.97 ± 0.10	14.73 ± 0.46	20.32 ± 0.54	677.32 ± 25.09	0.18 ± 0.01	46.17 ± 1.51	10.27 ± 0.27	97.89 ± 2.06	3.86 ± 0.09	4025.95
15	46.48 ± 1.28	7.52 ± 0.29	8.39 ± 0.35	54.52 ± 1.69	72.70 ± 2.01	1126.94 ± 31.45	1.13 ± 0.03	199.93 ± 4.99	23.97 ± 0.68	124.90 ± 3.66	8.95 ± 0.28	5107.63
16	nd	2.04 ± 0.07	3.67 ± 0.11	6.42 ± 0.18	11.01 ± 0.35	103.72 ± 2.99	0.36 ± 0.01	66.50 ± 1.42	8.88 ± 0.42	105.62 ± 3.60	2.64 ± 0.07	1587.81
17	34.32 ± 0.87	13.89 ± 0.42	11.73 ± 0.29	34.89 ± 1.02	65.64 ± 1.59	953.90 ± 20.47	0.96 ± 0.04	133.71 ± 3.57	14.20 ± 0.51	215.48 ± 6.33	15.08 ± 0.48	4556.39
18	nd	6.40 ± 0.23	9.75 ± 0.26	18.80 ± 0.61	23.65 ± 0.57	170.84 ± 5.78	nd	71.08 ± 2.03	17.25 ± 0.65	75.55 ± 1.89	7.40 ± 0.26	3880.18
19	nd	6.10 ± 0.16	3.84 ± 0.12	13.14 ± 0.33	15.77 ± 0.48	136.78 ± 4.26	nd	74.35 ± 1.56	7.23 ± 0.22	55.83 ± 1.24	3.51 ± 0.07	3536.18
20	17.93 ± 0.69	3.10 ± 0.12	3.73 ± 0.09	9.04 ± 0.40	9.45 ± 0.34	109.24 ± 2.85	nd	33.62 ± 0.99	9.04 ± 0.26	79.80 ± 1.90	3.50 ± 0.11	1463.34
21	8.99 ± 0.30	7.73 ± 0.26	5.97 ± 0.21	27.64 ± 0.64	9.67 ± 0.29	876.66 ± 19.06	0.52 ± 0.02	115.17 ± 3.59	4.19 ± 0.20	55.37 ± 2.10	7.72 ± 0.20	4211.41
22	nd	7.78 ± 0.15	4.24 ± 0.16	23.40 ± 0.82	7.86 ± 0.16	348.07 ± 10.66	0.32 ± 0.01	77.50 ± 1.87	9.62 ± 0.33	59.35 ± 1.37	4.82 ± 0.14	3474.29
23	nd	8.47 ± 0.20	9.00 ± 0.29	23.02 ± 0.57	14.75 ± 0.41	520.96 ± 15.53	0.51 ± 0.03	113.95 ± 3.45	11.19 ± 0.37	84.45 ± 2.96	5.05 ± 0.21	3620.54
24	nd	5.91 ± 0.22	6.65 ± 0.30	16.21 ± 0.49	12.24 ± 0.50	229.50 ± 4.37	nd	157.06 ± 4.66	10.53 ± 0.35	68.65 ± 1.44	5.08 ± 0.17	3702.04
25	nd	11.21 ± 0.31	15.16 ± 0.57	27.12 ± 0.80	30.75 ± 0.91	458.45 ± 14.17	nd	117.77 ± 4.03	12.15 ± 0.41	85.57 ± 1.78	8.26 ± 0.34	3568.05
26	29.87 ± 0.96	10.83 ± 0.24	51.41 ± 1.82	55.49 ± 1.46	47.77 ± 1.22	1903.53 ± 55.83	2.49 ± 0.08	440.23 ± 9.77	15.44 ± 0.39	182.94 ± 5.58	10.59 ± 0.28	5702.87
27	19.40 ± 0.57	9.39 ± 0.27	10.08 ± 0.29	36.45 ± 0.99	14.88 ± 0.39	1620.45 ± 45.39	1.06 ± 0.03	117.30 ± 3.69	23.20 ± 0.55	324.63 ± 7.77	11.36 ± 0.29	4160.69
28	97.12 ± 2.55	59.06 ± 1.34	85.08 ± 2.15	152.16 ± 4.11	497.60 ± 9.59	1351.81 ± 31.24	0.57 ± 0.02	65.89 ± 1.23	66.16 ± 1.79	300.46 ± 6.92	109.73 ± 2.85	6630.33
29	22.76 ± 0.69	5.76 ± 0.27	6.93 ± 0.24	33.24 ± 1.09	12.38 ± 0.47	540.22 ± 20.20	0.72 ± 0.03	139.17 ± 3.63	12.66 ± 0.48	153.75 ± 5.59	8.26 ± 0.26	4057.97
30	68.50 ± 1.67	35.69 ± 1.03	45.65 ± 1.23	113.41 ± 2.65	136.75 ± 4.00	4557.05 ± 97.87	5.65 ± 0.23	1057.19 ± 25.77	29.23 ± 0.72	125.79 ± 1.51	22.05 ± 0.74	8500.28
31	20.72 ± 0.60	7.70 ± 0.18	8.91 ± 0.20	22.06 ± 0.57	18.40 ± 0.37	904.74 ± 32.55	0.62 ± 0.03	65.56 ± 1.53	10.83 ± 0.30	75.56 ± 2.47	7.24 ± 0.31	3798.37
32	83.40 ± 2.31	41.02 ± 1.44	128.00 ± 2.94	90.33 ± 2.90	249.36 ± 6.94	746.87 ± 21.24	1.52 ± 0.05	84.27 ± 2.01	78.01 ± 2.14	257.25 ± 6.99	114.14 ± 4.12	5343.32
33	8.49 ± 0.27	6.56 ± 0.15	8.53 ± 0.33	11.25 ± 0.43	20.95 ± 0.41	158.38 ± 4.78	nd	49.13 ± 1.62	6.98 ± 0.26	61.69 ± 1.74	4.92 ± 0.20	2577.04

Table 5. continued

contents of analytes (mean ± SD, $n = 3$)

| sample no.[a]	Thr	Gly	Gln	Ser	Glu	Asn	Cit	Asp	Hit	Arg	Lys	total
34	nd	6.13 ± 0.11	3.86 ± 0.11	17.00 ± 0.62	10.47 ± 0.38	249.00 ± 7.34	0.25 ± 0.01	71.28 ± 2.03	5.56 ± 0.20	59.34 ± 1.88	4.24 ± 0.12	3258.81
35	nd	6.53 ± 0.20	8.70 ± 0.23	17.91 ± 0.44	48.77 ± 1.09	700.83 ± 27.33	0.69 ± 0.02	178.49 ± 4.14	11.59 ± 0.27	54.18 ± 1.26	4.29 ± 0.20	5045.02
36	nd	4.39 ± 0.16	8.51 ± 0.29	17.04 ± 0.58	20.65 ± 0.73	308.39 ± 10.25	0.37 ± 0.02	169.48 ± 5.09	11.04 ± 0.35	116.74 ± 3.71	4.89 ± 0.12	2256.73
37	nd	13.45 ± 0.33	9.25 ± 0.41	38.76 ± 0.96	56.65 ± 1.45	1191.94 ± 30.10	0.88 ± 0.04	56.49 ± 1.73	23.87 ± 0.62	144.41 ± 4.28	17.08 ± 0.43	4558.17
38	61.08 ± 1.55	21.68 ± 0.49	15.21 ± 0.44	64.46 ± 1.72	23.94 ± 0.92	1168.93 ± 26.91	0.60 ± 0.03	237.98 ± 5.42	9.18 ± 0.21	76.51 ± 1.67	23.82 ± 0.56	4226.48
39	nd	17.48 ± 0.42	18.78 ± 0.65	58.68 ± 1.09	67.50 ± 1.55	1176.11 ± 33.08	0.92 ± 0.04	343.26 ± 8.25	18.36 ± 0.44	142.06 ± 4.02	22.52 ± 0.54	5789.29
40	21.93 ± 0.69	10.89 ± 0.44	13.38 ± 0.57	51.65 ± 1.68	34.50 ± 0.57	709.55 ± 28.97	0.54 ± 0.02	354.60 ± 9.18	21.15 ± 0.38	94.26 ± 3.05	18.86 ± 0.36	4456.40
41	nd	3.50 ± 0.10	1.45 ± 0.08	10.04 ± 0.46	7.16 ± 0.25	216.37 ± 8.52	0.34 ± 0.01	35.35 ± 1.01	8.57 ± 0.33	48.27 ± 1.26	5.28 ± 0.11	3109.12
42	24.45 ± 0.72	13.29 ± 0.37	7.09 ± 0.20	23.76 ± 0.91	17.71 ± 0.40	782.39 ± 26.34	0.31 ± 0.01	206.13 ± 5.21	11.35 ± 0.27	71.59 ± 2.24	14.32 ± 0.40	3869.95
43	26.17 ± 0.58	13.64 ± 0.30	8.11 ± 0.19	29.78 ± 0.67	25.58 ± 0.77	610.11 ± 20.54	nd	196.74 ± 4.89	11.74 ± 0.29	79.87 ± 2.21	13.83 ± 0.33	3885.84
44	17.12 ± 0.41	5.88 ± 0.21	7.05 ± 0.21	13.89 ± 0.53	14.21 ± 0.51	520.70 ± 14.57	0.13 ± 0.00	74.50 ± 2.04	8.89 ± 0.26	59.40 ± 1.78	4.93 ± 0.18	3356.11
45	nd	3.01 ± 0.09	4.22 ± 0.14	9.50 ± 0.19	21.78 ± 0.58	423.11 ± 13.18	0.35 ± 0.01	108.41 ± 2.88	6.36 ± 0.20	57.35 ± 1.99	3.42 ± 0.09	3984.01
46	nd	5.80 ± 0.12	5.99 ± 0.23	12.43 ± 0.39	19.35 ± 0.68	233.85 ± 6.36	nd	63.20 ± 2.12	5.51 ± 0.16	59.37 ± 1.33	4.91 ± 0.25	4097.80

[a]The samples are the same as in Table 1. [b]Not detected.

74 was chosen as the product ion for MRM. As for most basic amino acids, the product $[M + H - 46]^+$ was very weak or hardly detected, probably due to the fact that these amino acids possess extra amino groups whose basicity is stronger than that of the α-amino, and these amino groups are easily ionized rather than the α-amino. As a result, the transition of $[M + H]^+$ to $[M + H - 46]^+$ hardly occurs.[12] Therefore, in this study, the product ions of these basic amino acids were automatically searched by the IntelliStart software, and the ions at m/z 70, 70, and 84 were chosen as the optimal products of Cit, Arg, and Lys, respectively. All the MRM transitions and parameters applied in the study are listed in Table 2.

Optimization of UHPLC Conditions. To obtain a desirable chromatographic profile with satisfactory retention and peak shape without excessive peak tailing, the UHPLC conditions were investigated under the optimized MS/MS conditions. First, two types of HILIC columns, a BEH Amide column (2.1 mm × 100 mm, 1.7 μm) and a BEH HILIC column (2.1 mm × 100 mm, 1.7 μm), were compared. The results showed that the former had a stronger retention ability as well as better resolution for these hydrophilic components than the latter with the same mobile phase. Second, different mobile phase additives, such as ammonium formate, ammonium acetate, formic acid, acetic acid, and ammonia−water, were compared. The results showed that ammonium formate used as a salt additive of the mobile phase could provide much improved peak shapes for these amino acids compared to ammonium acetate as well as other additives. Furthermore, the better peak shapes were observed when the buffer salt concentration of the eluents was increased. Considering the facts that the solubility of these salts in solvent B (acetonitrile) are low and a higher concentration of these salts could decrease the sensitivity of the mass spectrometer, the salt modifier concentrations of the eluents were determined for solvent A (water, 10 mM ammonium formate) and solvent B (acetonitrile, 2 mM ammonium formate). Adjustments of the buffer at different pH values (8.0, 7.0, 5.0, 3.0, and 2.5) with ammonia−water, acetic acid, and formic acid showed differences not only in the retention, but also in the peak shapes of these amino acids. For the majority of these compounds, except for some basic amino acids, such as histidine, arginine, and lysine, the eluent with acidic pH could obtain better peak shapes while providing lower retention than those with neutral and basic pH. Given the above facts, the mobile phase comprising solvents A (water, 10 mM ammonium formate, and 0.15% formic acid, pH 3.0) and B (acetonitrile, 2 mM ammonium formate, and 0.15% formic acid) was finally selected as a compromise, and under this condition, all the retentions and peak shapes were acceptable. Besides, some analytes with similar MS/MS characteristics should be separated well, because they could influence each other even using the specific MS/MS detection. Such problematic amino acids are Leu and Ile, as well as Glu, Gln, and Lys. For separation above analytes, the gradient elution procedure was optimized. The results showed that the optimized elution procedure mentioned above could fully separate these problematic amino acids as well as the others within 12 min. The typical chromatograms of the 23 amino acids in the MRM mode are presented in Figure 1.

Method Validation. The proposed HILIC−UHPLC−TQ-MS method for quantitation of amino acids was validated to determine the linearity, LOD, LOQ, intraday and interday precisions, stability, accuracy, and matrix effect. The results are summarized in Tables 3 and 4. The correlation coefficient
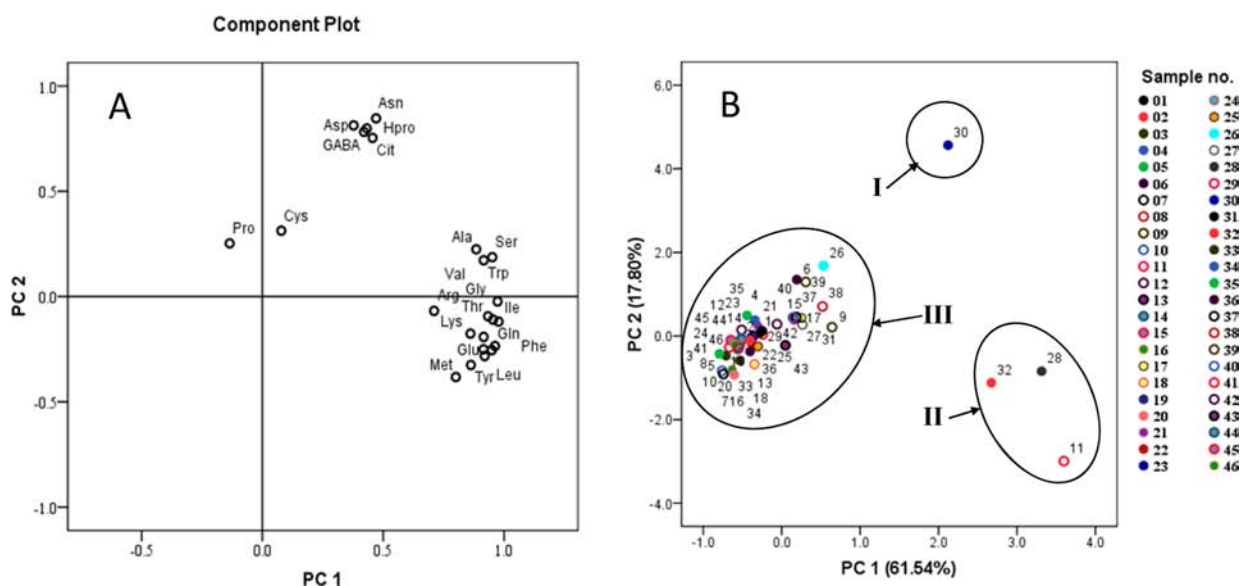
Figure 2. Loading plot (A) obtained by PCA of the 23 amino acids and the scatter plot (B) obtained by PCA of the 46 samples of *Z. jujuba*. The 46 samples are the same as in Table 1, and the 23 amino acids are the same as in Table 2.

values (r^2) were better than 0.9910 for all analytes. Linearity evaluation of the calibration curve was accomplished by applying the lack-of-fit test. As a result, significance values greater than 0.05 were obtained for all analytes at the 95% confidence level, indicating good correlations between the investigated compound concentrations and their peak area within the test ranges. The LODs and LOQs (Figure S2, Supporting Information) were in the ranges from 0.17 to 79.25 ng mL^{-1} and from 0.68 to 294.00 ng mL^{-1}, and the overall intra- and interday variations (RSDs) of the 23 analytes were in the ranges of 0.81−4.79% and 1.06−6.68%, respectively. The repeatability and stability presented as RSDs were in the ranges from 0.80% to 10.62% and from 1.11% to 6.97%. The overall recoveries lie between 93.5% and 103.6% with the RSD between 2.15% and 6.44%. The slope ratio values of the matrix curve to neat solution curve were between 0.90 and 1.05, indicating that the matrix effect on the ionization of analytes was not obvious under these conditions. These results indicated that the developed HILIC−UPLC−TQ-MS method was sensitive, repeatable, and accurate for the quantification analysis of these amino acids.

Application to ZJF Extracts. To show the utility of the method in profiling studies of amino acids, an application in real samples was performed. A total of 46 batches of ZJF, which comprised 39 cultivars from 26 cultivation regions, were analyzed with the established HILIC−UHPLC−TQ-MS method to evaluate the differences in the amino acid profile in the samples. Due to the fact that the contents of Pro and Asn are equal to or more than 2 orders of magnitude greater than those of the other amino acids in most samples, their quantitations were performed by diluting the sample solutions as described in the Materials and Methods before analysis.

All the contents are summarized in Table 5. The results showed that all of these ZJF samples were rich in the free amino acids, especially those essential amino acids such as Trp, Phe, Leu, Ile, Met, Val, Thr, and Lys. This result indicated that ZJF is a healthy food for the supplement of free amino acids. As shown in Table 5, the contents of these amino acids obviously varied in the different samples. The total content of these 23 amino acids in sample 30 (cv. Lingzao from Wuhu, Anhui)

reached as high as 8.50 mg g^{-1}, whereas it was only 1.29 mg g^{-1} in sample 7 (cv. Ningxiayuanzao from Zhongwei, Ningxia). As for the individual compounds determined in the experiments, Pro was found to be the most abundant free amino acid in all samples except for sample 30, and its average content in these investigated samples was 2.67 mg g^{-1}, which accounted for more than 60% of the total amino acids tested in this study. Next was Asn, whose average content in the tested samples was 0.70 mg g^{-1}. It is notable that there was a report that, during the thermal processing with glucose, Asn can contribute to formation of acrylamide, a browning product with potential toxicity.[26,32] Therefore, considering the fact that this amino acid was found in all samples with significant quantity, the processing method of this fruit should be controlled to avoid the formation of acrylamide. In addition to the protein amino acids, three nonprotein amino acids, including GABA, Hpro, and Cit, were found in the ZJF samples.

PCA of the Samples. To evaluate the variation of ZJF, PCA was performed on the basis of the contents of 23 tested compounds from UHPLC profiles. The first two principal components (PC 1 and PC 2) with >79% of the whole variance were extracted for analysis. Among them, PC 1 and PC 2 accounted for 61.54% and 17.80% of the total variance, respectively. The remaining principal components, which had a minor effect on the model, were discarded. The component loading matrix is shown in Figure 2A. According to their loadings, PC 1 had good correlation with all of the analytes except for **6**, **12**, and **18−20**, which exhibited their main relationship with PC 2. The results mentioned above suggested that all 23 compounds may contribute to the classification of the samples. Interestingly, all three nonprotein amino acids exhibited good correlation with PC 2; thus, PC 2 could be regarded as the nonprotein amino acid factor. The sample scatter plot is shown in Figure 2B, where each sample is represented as a marker. It was noticeable that the samples were clearly clustered into three domains, with sample 30 in domain I, samples 11, 28, and 32 in domain II, and the others in domain III. These results indicated that samples with similar chemical profiles were commonly divided into one domain. As for sample 30 (cv. Lingzao from Wuhu, Anhui), the contents of three nonprotein amino acids as well as Asp and Asn were the

highest in all the samples. Actually, the result was consistent with their natural properties in that although samples 30 and 20 were from the same cultivar of Lingzao, the ripe season of sample 30 (collected from Wuhu, Anhui) was the middle of August and was earlier by about one month than those of sample 20 (collected from Xingzheng, Henan) and the other samples. These properties may be due to the different climates in different cultivation regions. For samples 11 (cv. Jinzao from Binxian, Shaanxi), 28 (cv. Hetaowen from Heze, Shandong), and 32 (cv. Fupingdazao from Fuping, Hebei), the contents of the amino acids except for the three nonprotein amino acids as well as Asp and Asn were relatively higher than those in the other samples. In addition, the classified result of samples 11, 28, and 32 not in the same domain as the other samples was also in accordance with our previous report that these three cultivars were obviously different from the other cultivars of ZJF with their chemical profiles of nucleosides and nucleobases.[33]

In conclusion, a reliable, simple, and sensitive method capable of quantifying all protein amino acids as well as three nonprotein amino acids without derivatization was established by using an HILIC−UHPLC−TQ-MS method in this study. Then the proposed method was applied to analysis of 23 amino acids in 46 ZJS samples. Compared with the existing methodology for amino analysis, the sample solution was prepared right after a very simple extraction step, greatly reducing the laborious and time-consuming derivatization procedures required by most other methods. By combination of the HILIC and UHPLC methods, the more polar compounds were sufficiently separated within 12 min without utilization of the ion-pairing reagents, which was not suggested for an ESI source. The analysis results of ZJF samples showed that ZJF is a healthy food rich in free amino acids, and our results in the present study clearly suggest that the HILIC−UHPLC−TQ-MS method could be employed as a useful tool for quality assessment of ZJF using certain amino acids (such as Pro, Asp, Arg, and Hpro) as the markers. Furthermore, this validated method could be valuable for the routine quantitation of amino acids from food samples.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional tables and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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