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ORIGINAL ARTICLE

Hydrophilic interaction ultra-performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry for highly rapid and sensitive analysis of underivatized amino acids in functional foods

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Abstract This work presented a new analytical methodology based on hydrophilic interaction ultra-performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry in multiple-reaction monitoring mode for analysis of 24 underivatized free amino acids (FAAs) in functional foods. The proposed method was first reported and validated by assessing the matrix effects, linearity, limit of detections and limit of quantifications, precision, repeatability, stability and recovery of all target compounds, and it was used to determine the nutritional substances of FAAs in ginkgo seeds and further elucidate the nutritional value of this functional food. The result showed that ginkgo seed turned out to be a good source of FAAs with high levels of several essential FAAs and to have a good nutritional value. Furthermore, the principal component analysis was performed to classify the ginkgo seed samples on the basis of 24 FAAs. As a result, the samples could be mainly clustered into three groups, which were similar to areas classification. Overall, the presented method would be useful for the investigation of amino acids in edible plants and agricultural products.

Keywords Underivatized amino acids analysis · HILIC-UPLC–TQ-MS/MS · PCA · Functional foods · Ginkgo seed

Introduction

As an archaic living fossil, *Ginkgo biloba* has existed on the earth for 200 million years (Major 1967) and has great anti-adversity ability. It is widely distributed in extratropical, warm and subtropical zones especially in eastern China (Jiangsu, Shandong, Zhejiang, Anhui, and Guangxi provinces). The seeds of *G. biloba* have been used as traditional medicine and foodstuff in China as early as 5,000 years ago. Recently, the medicinal and health protection effects of ginkgo seeds have attracted considerable attention, and they have been widely used in commercial medical products and health foods in many countries (Singh et al. 2008). With the development of modern food technology, ginkgo seeds have been processed into canned products, paste, comfits, powder and other dietary supplements (Deng et al. 2011). As foods, there are many patents about ginkgo seed products such as desserts, glazed fruit, beverages and tipple.

Ginkgo seeds are complex matrixes that contain crude protein, carbohydrates, lipids, vitamins and microelements (Van Beek 2002). They were also reported to contain the limited number of free amino acids (FAAs) such as aspartic and glutamic acid (Wang et al. 2000). But up to now, there is no further report about the detailed information of FAAs

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in ginkgo seeds. From a nutritional and functional point of view, the consumption of FAAs is recommended as an important source of nutrition and function (Friedman and Levin 2012) in ginkgo seeds. It is well known that amino acids involve in many biological activities such as promoting relaxation, reducing blood pressure, and enhancing antitumor activity. Moreover, it was reported to have physiological activities including neuroprotection and anti-obesity (Wang et al. 2010). With these much benefits for human health, they have been attached great importance in the scientific field (Song et al. 2012), and many of them have been selected as quality control markers for several functional foods such as royal jelly (Wu et al. 2009) and Chinese teas (Chen et al. 2009). In addition, the principal nutritional value of proteins food is determined by their amino acid content, and amino acid analysis has been successfully used for the profiles of their nutritional value (Vioque et al. 2012). Hence, the characterization of the FAAs in ginkgo seeds would be very helpful for their potential value improvements as food and the better quality control.

The simultaneous analysis of amino acids was difficult due to their structure diversity, high polarity and the absence of specific chromophores. In the literature, several different techniques were used for analyzing amino acids in food or biological samples, and there were mainly three techniques used most frequently as following: separation of volatile amino acid derivatives by gas chromatography (GC) and detection by flame ionization detection (FID) and/or MS (Kvitvang et al. 2011); separation of derivatization amino acid by capillary electrophoresis (CE) and detection by ultraviolet (UV) (Sarazin et al. 2011), mass spectrometry (MS) (Sanchez-Hernandez et al. 2012) or capacitively coupled contactless conductivity (C^4) (Strieglerová et al. 2011); and separation of amino acid derivatives by liquid chromatography (LC) and detection by fluorescence, UV, nuclear magnetic resonance (NMR) (Callejón et al. 2010), evaporative light scattering detection (ELSD) (Yan et al. 2007) or MS (Langrock et al. 2006; Li et al. 2011; Cerrada-Gimenez et al. 2012). Although GC methods had shown high sensitivity and an excellent resolving power, the main disadvantage of GC seemed to be the procedure of derivatization, due to the complexity of reactions and types of reagents used (Callejón et al. 2010). CE methods had advantages of short analysis time, high sensitivity and less consumption of solvents, but the number of separated amino acids was usually much smaller than those provided by the LC methods (Jia et al. 2011). Conventional reverse phase LC was not appropriate for separations of most high polar amino acids due to its poor retention capability. Hence, pre- or post-column derivatization combined with reverse phase C18 (RP-C18) column separation had been widely accepted in recent years (Leggio et al. 2012; Qu et al. 2002a, b; Rebane and Herodes 2012). Most of the present methods required derivatization (in most cases, either the derivatization procedures were time consuming or the derivatives were not stable) and laborious sample preparation procedures when assaying food or biological samples.

Quantization of amino acids from food matrix without derivatization was advantageous: it not only eliminated laborious sample preparation procedures, but also reduced the errors introduced by some problems such as derivative instability, side reactions and reagent interferences. More and more researchers tried to establish methods for assaying amino acids without derivatization. In this study, we presented a new approach based on hydrophilic interaction ultra-performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (HILIC-UPLC–TQ-MS/MS) for identification and quantification of 24 underivatized FAAs in ginkgo seeds. The applications of HILIC for FAAs analysis have been steadily increasing. HILIC is a type of liquid chromatography that allows high-resolution separation of highly polar compounds (Jandera 2011). It employs a polar stationary phase and a MS-compatible mobile phase (Xing et al. 2012). Recent HILIC use mainly focused on general HPLC equipment with columns packed with particles of size 3–5 μm. With the separate technique development, HILIC columns packed with particles of size 1.7 μm for UPLC separations could be acquired from Waters Corporation (Cai et al. 2009). These columns gave more efficient separation of polar compounds because reduction of particle diameter of the packing material increased separation power, permitting faster analysis (Kaiser et al. 2012). For analysis of FAAs, these ultra-performance HILIC columns could provide more qualitative information compared to the columns packed with larger particles. In addition, since the eluent of HILIC was mainly the aqueous organic solution which enhanced the analytes ionization with ESI process, the HILIC operation mode was compatible for MS analysis with ESI source. Especially, the HILIC was coupled to triple-quadrupole mass spectrometry (TQ-MS) that had very high sensitivity and provided structural information (Wang et al. 2012) of amino acid structure. The combined HILIC-UPLC–TQ-MS/MS could provide better resolution, narrower peaks and shorter retention time. The quantification of amino acids from food matrix without derivatization is advantageous because it reduces the errors such as derivative stability, side reactions and reagent interferences. Hence, the technology of HILIC-UPLC–TQ-MS/MS was considered for the determination of underivatized FAAs. The objectives of the present study were (1) to develop an analytical method for a simple, rapid, accurate and repeatable determination of underivatized FAAs using a combined HILIC-UPLC–TQ-MS/MS method, (2) to apply the developed method to investigate the 24 underivatized FAAs in ginkgo seeds, and (3) to increase the interest in ginkgo seeds from a nutritional point of view by determination of so many FAAs.

Experimental

Sources of plant materials

Forty-eight batches of ginkgo seed samples, consisting of 15 samples of five different parts (episperm, mesosperm, endopleura, endosperm, and plumule), as shown in the Supporting information Fig. S1, 11 samples of different tree ages (8, 10, 15, 20, 25, 30, 40, 50, 100, 300, and 600 years of age), and 22 samples of different regions in China were analyzed. Specifications of ginkgo seed samples (cultivation regions, analytical parts, and age of ginkgo seeds) were listed in Table 1. The botanical origins of the samples were identified as the seeds of *Ginkgo biloba* L. by Dr. Hui Yan (Department of Pharmacognosy, Nanjing University of Chinese Medicine, China), and the voucher specimens were deposited at the Herbarium in Jiangsu Key Laboratory for TCM Formulae Research, Nanjing University of Chinese Medicine, China. After collection, the samples were dried by air.

Reagents and chemicals

The acetonitrile and formic acid were all of HPLC grade and purchased from Merck (Darmstadt, Germany). HPLC-grade ammonium formate and ammonium acetate were purchased from Shanghai Chemical Reagent Factory (Shanghai, China). Pure water for UPLC analysis was purified using Milli-Q water purification system (Millipore, Bedford, MA, USA) and was used for all solutions and dilutions. Other reagents and chemicals were of analytical grade.

Twenty-four amino acid standards including tryptophan, phenylalanine, leucine, iso-leucine, γ-aminobutyric acid, methionine, valine, proline, tyrosine, cysteine, alanine, hydroxyproline, threonine, glycine, glutamic acid, serine, glutamine, asparagine, citrulline, aspartic acid, arginine, histidine, lysine and ornithine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Some informations of these compounds were shown in the Supporting information Table S1 and Fig. S2.

Individual standards (1.0 mg) were prepared by dissolving in 5 mL distilled water. A working standard mixture of 100 μg/mL was prepared by diluting the intermediate stock standard solution, from which the calibration standards within the range 10–100,000 ng/mL were prepared by serial dilution with water. All the standard solutions were filtered through a 0.22-μm cellulose membrane filter before injection into UPLC-MS/MS system for analysis.

HILIC-UPLC–TQ-MS/MS analysis conditions

UPLC was performed using a Waters ACQUITY UPLC system (Waters, Milford, MA, USA), equipped with a binary solvent delivery system, and an auto-sampler. Hydrophilic interaction chromatographic separation was performed on an ACQUITY UPLC BEH amide column (2.1 mm × 100 mm, 1.7 μm) equipped with an ACQUITY UPLC BEH Amide 1.7 μm VanGuard Pre-column. Chromatographic analyses were carried out with a gradient mobile phase consisting of solution A (water, 5 mmol/L ammonium formate, 5 mmol/L ammonium acetate and 0.15 % formic acid) and consisting of solution B (acetonitrile, 1 mmol/L ammonium formate, 1 mmol/L ammonium acetate and 0.15 % formic acid) at a flow rate of 0.4 mL/min. The linear gradient conditions were: 0–6 min, 15–20 % A; 6–10 min, 20–30 % A; 10–12 min, 30–46 % A. The column temperature was maintained at 35 °C and the injection volume was 1 μL. Two cycles of weak and strong solvent washing of the injecting system were carried out in between injections. The column eluent was directed to the mass spectrometer.

Mass spectrometry analysis was carried out using a Waters Xevo TQ tandem quadrupole mass spectrometer (Micromass MS Technologies, Manchester, UK). All amino acids were detected using electrospray ionization (ESI) in positive 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; desolvation gas flow 1,000 L/h. Data were collected in MRM mode by screening parent and daughter ions simultaneously. All ESI and MS parameters were optimized individually for each target compound and listed in Table S1. Dwell time was automatically set by the software. Data acquisition was performed using MassLynx^{TM} XS software with TargetLynx program (Waters).

Sample preparation

The dried samples were ground using a mortar and pestle, and then sieved through a 40 mesh stainless-steel sieve before extraction. 1.0 g of each sample powder was accurately weighed into 100 mL conical flask, and 80 mL of distilled water was added to each conical flask, except for those of episperm, mesosperm and endopleura samples (S3, S4, S5, S8, S9, S10, S13, S14 and S15), into each of which 20 mL of water was added. All of the mixtures were precisely weighed and placed into an ultrasonic bath (40 kHz) for 60 min at room temperature, and then the same solvent was added to compensate for the weight lost during the

Table 1 Cultivation regions, analytical parts and age of 48 ginkgo seed samples

Sample number	Cultivation region	Part	Age (years)	Sample number	Cultivation region	Part	Age (years)
S.1	Lingchuan, Guangxi	Plumule	20	S.25	Tancheng, Shandong	Plumule and embryoid	300
S.2	Lingchuan, Guangxi	Embryoid	20	S.26	Tancheng, Shandong	Plumule and embryoid	600
S.3	Lingchuan, Guangxi	Endopleura	20	S.27	Changting, Fujian	Plumule and embryoid	20
S.4	Lingchuan, Guangxi	Mesosperm	20	S.28	Taixin, Jiangsu	Plumule and embryoid	20
S.5	Lingchuan, Guangxi	Episperm	20	S.29	Jangyan, Jiangsu	Plumule and embryoid	20
S.6	Xuzhou, Jiangsu	Plumule	20	S.30	Xuzhou, Jiangsu	Plumule and embryoid	20
S.7	Xuzhou, Jiangsu	Embryoid	20	S.31	Xuzhou, Jiangsu	Plumule and embryoid	20
S.8	Xuzhou, Jiangsu	Endopleura	20	S.32	Dandong, Liaoning	Plumule and embryoid	20
S.9	Xuzhou, Jiangsu	Mesosperm	20	S.33	Shijiazhuang, Hebei	Plumule and embryoid	20
S.10	Xuzhou, Jiangsu	Episperm	20	S.34	Changting, Fujian	Plumule and embryoid	20
S.11	Tancheng, Shandong	Plumule	20	S.35	Taian, Shandong	Plumule and embryoid	20
S.12	Tancheng, Shandong	Embryoid	20	S.36	Nanjing, Jiangsu	Plumule and embryoid	20
S.13	Tancheng, Shandong	Endopleura	20	S.37	Nantong, Jiangsu	Plumule and embryoid	20
S.14	Tancheng, Shandong	Mesosperm	20	S.38	Suzhou, Jiangsu	Plumule and embryoid	20
S.15	Tancheng, Shandong	Episperm	20	S.39	Lingchuan, Guangxi	Plumule and embryoid	20
S.16	Tancheng, Shandong	Plumule and embryoid	8	S.40	Yangzhou, Jiangsu	Plumule and embryoid	20
S.17	Tancheng, Shandong	Plumule and embryoid	10	S.41	Ningguo, Anhui	Plumule and embryoid	20
S.18	Tancheng, Shandong	Plumule and embryoid	15	S.42	Nanchong, Sichuan	Plumule and embryoid	20
S.19	Tancheng, Shandong	Plumule and embryoid	20	S.43	Chengdu, Sichuan	Plumule and embryoid	20
S.20	Tancheng, Shandong	Plumule and embryoid	25	S.44	Yongzhou, Hunan	Plumule and embryoid	20
S.21	Tancheng, Shandong	Plumule and embryoid	30	S.45	Zunyi, Guizhou	Plumule and embryoid	20
S.22	Tancheng, Shandong	Plumule and embryoid	40	S.46	Songxian, Henan	Plumule and embryoid	20
S.23	Tancheng, Shandong	Plumule and embryoid	50	S.47	Tancheng, Shandong	Plumule and embryoid	20
S.24	Tancheng, Shandong	Plumule and embryoid	100	S.48	Anji, Zhejiang	Plumule and embryoid	20

extraction. After centrifugation (13,000 rpm, 10 min), the supernatants were stored at 4 °C and filtered through 0.22-μm cellulose membrane filters and transferred to glass vials for the UPLC-MS/MS system injection.

Validation of the method

The working standard mixture of 100 μg/mL containing 24 amino acids was prepared and diluted to appropriate concentrations for the establishment of calibration curves. At least 6 concentrations of the 24 analytes solution were analyzed in triplicate, and then the calibration curves were constructed by plotting the peak areas versus the concentrations of each analyte. The limit of detection (LOD) and quantification (LOQ) for each amino acid was determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively. Intra- and inter-day variations were chosen to determine the precision of the method. For intra-day variability test, the mixed standards solution was analyzed for six replicates ($n = 6$) within 1 day; while for inter-day variability test, the solution was examined in duplicates for consecutive 3 days ($n = 6$). Variations were expressed by the relative standard deviation (RSD) for intra and inter-day. To examine the repeatability, six different working solutions prepared from the same sample (sample S1) were analyzed. The RSD was taken as a measure of repeatability. Stability of sample (sample S1) solution was tested. The sample solution was analyzed in every 4 h within 24 h. Variation was expressed as RSD. A recovery test was used to evaluate the accuracy of the above method. Known amount of the 24 analytes was added to an accurate 0.5 g of ginkgo seeds (sample S1), and then extracted and analyzed as described above. Each sample was analyzed in triplicate. The average percentage recoveries were calculated using the following formula: recovery (%) = 100 × (amount found − original amount)/amount spiked.

Matrix effects

The matrix effect was defined as the ion suppression or enhancement in the process of analyte ionization (Matuszewski et al. 2003). Due to the complexity of food samples investigated, the matrix effect was always relevant. The latent interfering effect from coeluting matrix constituents on ESI response was investigated in this paper by comparing the slopes of linear calibration curves from matrix-matched experiments with that obtained from pure solvent standards. The slope ratios (slope matrix/slope solvent) of 1 indicate that matrix does not significantly suppress or enhance the response of the MS, otherwise denotes ionization suppression (<1) or enhancement (>1) (Chen et al. 2012). The functional food sample extracts, which were spiked with appropriate amounts of standards as done for the apparent recovery measurement based on the above-described recovery parameter, 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 neat standards at the same concentration levels (Jia et al. 2011).

Data statistics and analysis

TargetLynx was employed to the quantification of FAAs. The principal component analysis (PCA) was a well-known, unsupervised pattern recognition method of data analysis, and it was done by SPSS 16.0 software (SPSS, Chicago, IL, USA).

Results and discussion

Optimization of the extraction procedure

In this study, extraction method (refluxing, stirring and ultrasonication), extraction solvent (water, 25 % aqueous methanol, 50 % aqueous methanol, 25 % aqueous ethanol, 50 % aqueous ethanol, v/v), extraction time (30, 60, 90, 120, and 180 min), and solvent volume (10, 20, 40, 80 and 100 mL) were investigated on 1.0 g, 40 mesh of different kinds of samples (S1-plumule, S2-embryoid, S3-endopleura, S4-mesosperm and S5-episperm) to obtain optimal extraction conditions. As a result, ultrasonication was the most effective extraction method compared with refluxing and stirring extraction. It was observed that water was the best solvent which could obtain the highest extraction efficiency for the 24 FAAs analyzed among these investigated samples. The solvent volume was examined to gain high extraction efficiency of the markers from different samples; the volume of solvent was chosen as 80 mL for samples of plumule (S1) and embryoid (S2), and 20 mL for those of endopleura (S3), mesosperm (S4) and episperm (S5). The extraction time was also optimized, which revealed that 60 min was the best choice.

Finally, suitable extraction conditions were optimized as follows: samples of plumule and embryoid were extracted by ultrasonication with 80 mL of water for 60 min; and samples of endopleura, mesosperm, and episperm were extracted by ultrasonication with 20 mL of water for 60 min. All of the samples were extracted at room temperature.

Optimization of HILIC-UPLC conditions

To compare the separation of highly polar FAAs on the C18 columns and HILIC columns, 10 μg/mL working standard mixture was injected into an Acquity UPLC BEH C18 (2.1 × 100 mm, 1.7 μm) and an Acquity HSS T3 (2.1 × 100 mm, 1.7 μm) column and analyzed under the same condition. Most of the highly polar target compounds could not be retained on the C18 columns, and were eluted in dead column (about 1 min). Fortunately, the retain of the highly polar amino acids on the HILIC column could gain amelioration. However, different HILIC columns have different performances on the separation of FAAs. Two kinds of HILIC columns, an Acquity UPLC BEH HILIC (2.1 × 100 mm, 1.7 μm) and an Acquity UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 μm) were investigated. Peaks shapes were sharper with less peak broadening, less tailing, and reduced variability of absolute retention times for the Acquity UPLC BEH Amide column. Therefore, this column was selected for further analysis.

An optimization strategy for UPLC conditions to develop a HILIC profile was performed initially with standards (above), and then with sample 1, to determine the best chromatographic separation within the constraints of a practical run time and the suitability of solvents for MS. Two types of mobile phase, acetonitrile–water and methanol–water, were compared. The results in Fig. S3 showed that the former had more excellent peak shape as well as better resolution than the latter on the same HILIC column. Solvent gradients were also explored, in hope of obtaining the moderately improved separation of weaker solvents but at reduced run time. As the organic solvent content decreased, the prominence of the various interactions involved in HILIC may vary (Johnsen et al. 2011). Thus, higher acetonitrile amounts were used in a few minutes after the beginning of chromatography, the concentration being decreased later to shorten the separation time and to improve the peak shape.

In the literature, buffer type and salt concentration usually affect the HILIC separation (Cai et al. 2009). Hence, several ammonium salts were investigated to determine the target compounds in our preliminary test, such as acetate, formate, bicarbonate, and trifluoroacetate. Ammonium acetate and/or ammonium formate were the salt of choice because they provided the best results in selectivity and reproducibility, presented excellent solubility even at high concentrations of organic solvents, and were highly volatile, making them suitable for eventual further mass spectrometry analysis (Fig. S4). Retention and peak shape were influenced by the presence of ammonium acetate and/or ammonium formate at different concentrations. Effects of ammonium acetate and/or ammonium formate concentration from 0 to 20 mM in solution A and from 0 to 3 mM in solution B on retention time and peak shape variation were studied. Further increase in the salt concentration was not possible due to solubility limitation in the mobile phase and gave longer retention time. When the concentration was lower than 10 Mm in solution A and 2 mM in solution B, the reproducibility of the retention time and peak area could not be guaranteed owing to an insufficient buffering effect of the mobile phase. Thus, 10 mM ammonium acetate or ammonium formate in solution A and 2 mM ammonium acetate or ammonium formate in solution B were selected, providing an optimum analysis separation. The mobile phase composition was further optimized by investigating the effects of formic acid, ethanoic acid, ammonium acetate, ammonium formate, and combinations of these added to the aqueous mobile phase, and acetonitrile, with and without formic acid, ethanoic acid, ammonium acetate and ammonium formate added as the organic mobile phase, on chromatographic retention, resolution and peak shape. The results in Fig. S5 showed, ammonium formate combined with ammonium acetate as a salt additive of aqueous and organic mobile phase could provide excellent peak shape and resolution for FAAs compared to single ammonium acetate or ammonium formate. For the majority of these compounds, the eluent with acidic pH could obtain better peak shapes and provide narrower peaks than those with neutral or basic pH. Considering all the above factors, the aqueous mobile phase selected contained 5.0 mmol/L ammonium formate, 5.0 mmol/L ammonium acetate ammonium and 0.15 % formic acid and the organic mobile phase of choice was acetonitrile containing 1.0 mmol/L ammonium formate, 1.0 mmol/L ammonium acetate ammonium and 0.15 % formic acid.

Column temperature and flow rate were also important parameters that affected the retention of polar compounds in HILIC. The effect of column temperature on retention was investigated in the temperature range of 15–40 °C. As shown in Fig. 1, the temperature increase caused a decrease in retention time for all the compounds, and poor resolution among compounds, especially for leucine and iso-leucine. Although the lower column temperature gave good resolution, the peak shape and resolution time were still not satisfactory for many compounds (aspartic acid, arginine, histidine, lysine and ornithine) when the column temperature was lower than 30 °C. Therefore, 35 °C was used for the column.

The flow rate range of 0.1–0.5 mL/min was also investigated in this study. The result showed that a high flow rate (0.5 mL/min) was applied for shorter retention time with better peak shape. However, the fast mobile phase flow could cause insufficient or unstable ionization of the ESI source. Lower flow rate (0.1–0.4 mL/min) was evaluated to effectively decrease the matrix effect. Accordingly, a flow rate of 0.4 mL/min was applied to solve the problems of retention time, peak shape and matrix effect in consideration of varied factors.

Optimization of TQ-MS/MS conditions

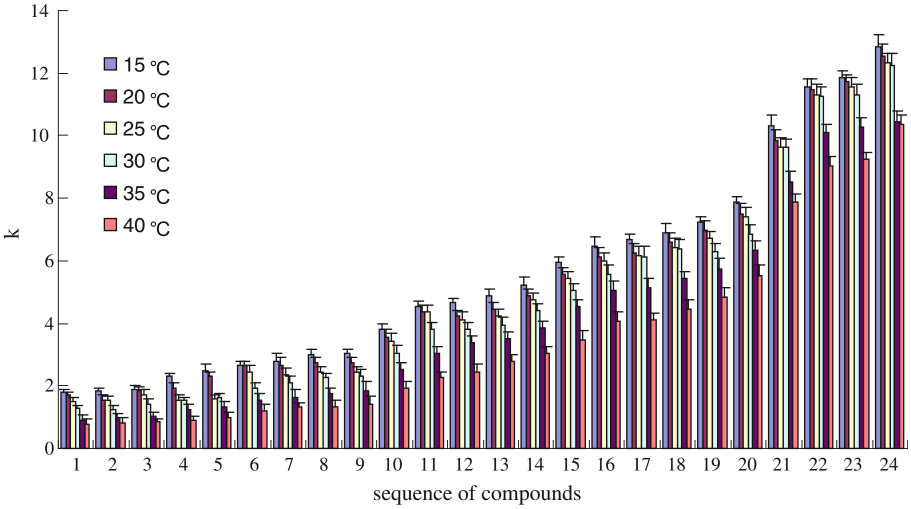
The analytes tested in this study were first detected by direct full scan mass spectrometry method in both positive and negative ionization modes, because not only the amino or imino groups can easily form positive ions but also the carboxyl groups can form a stable negative ion existing in the amino acid molecules. The results revealed that higher responses were obtained in positive ion mode for target compounds than in negative ion mode. Hence, ESI in positive mode was selected for the whole experiment. 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 one pair of precursor/product ions for each analyte was presented in this study. And 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 was determined, cone voltage and collision energy were optimized using the IntelliStart software. Supporting information Table S1 showed the MS/MS transitions as well as the cone voltages and collision energies optimized for each amino acid.

It can be observed that most amino acids involved the carboxyl and the α-amino group showed an abundant product ion at $[M+H-46]^+$, which was in correspondence to the neutral loss of formic acid by a rearrangement, whereas non-α-amino acid such as GABA had a common neutral loss of *m/z* 17 due to the loss of NH_3.

For the amino acids containing the amide group, such as Gln, the fragment ions of $[M+H-17]^+$ (*m/z* 130), $[M+H-46]^+$ (*m/z* 101) and $[M+H-46-17]^+$ (*m/z* 84) could be simultaneously provided, and $[M+H-46-17]^+$ (*m/z* 84) was the most abundant among them, so *m/z* 84 was selected as the product ion for MRM of Gln. For Asn, another amino acid containing amide group, it could not give the fragment ion of $[M+H-46-17]^+$, while giving the ion at *m/z* 74, and the intensity of this ion was higher than those of fragment ions of $[M+H-17]^+$ and $[M+H-46]^+$. Thus, the ion at *m/z* 74 was chosen as the product ion for MRM.

For most basic amino acids, the products of $[M+H-46]^+$ were very weak or hardly detected, probably due to the fact that these amino acids possess extra amino groups and their basicity is stronger than that of the α-amino. As a result, the common transition of $[M+H]^+$ to [M+H

Fig. 1 Influence of the temperature on the capacity factor (*k*) of 24 standard compounds on the ACQUITY UPLC BEH amide column. The data are presented as the average of three replicates; *error bars* represent the standard deviations



– 46]$^+$ hardly occurred. Therefore, 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.

Complete resolution for all the amino acids was not achieved, but the use of MS/MS in MRM mode allowed the analysis without chromatographic resolution between compounds. Furthermore, the application of the chromatographic technique also allowed the discrimination between isobaric compounds. For instance, one pair of the compounds Gln and Lys and another pair of Ile and Leu had the same precursor ion and fragmentation pattern, respectively (see Supporting information Table S1). However, they had different retention times. Therefore, they could be determined separately. On the other hand, Ile or Leu and Hpro had the same molecular (*m/z* 132) mass. However, they had different product ions and retention times (see Supporting information Table S1) and reliable determination of these compounds could be carried out. Representative MRM chromatograms for 10 μg/mL mixture standard analytes and sample 1 are shown in Fig. 2a and b, respectively.

The literatures reported that retention time deviations were also a problem in HILIC analysis (Neville et al. 2012). The intra-day retention time deviation was assessed by injecting 24 standard compounds six times during the day, while the inter-day retention time deviation was assessed by injecting samples for three consecutive days. Table S2 shows the data for both inter- and intra-day experiments ($n = 6$). There was a small change, 0.0080 ± 0.0038 min, in the average retention times of 24 FAAs, and there was also a small change (0.0027 ± 0.0014 min) in the time difference between the inter- and intra-day experiments. The inter- and intra-day experiments in this study were conducted by referring to the related literature (Neville et al. 2012), which could provide evidence in support of the robustness of retention times with the development HILIC method.

Method validation

The described method was required for compliance with the International Conference on Harmonization (ICH) regulations in this study. Several performance parameters should be studied, including matrix effect, linearity, LOD, LOQ, precision, repeatability, stability and recovery (Rambla-Alegre et al. 2012).

First, matrix effects were studied to ensure bias-free analytical results. Because the samples were not standard reference materials and no blank ginkgo sample samples were available, ginkgo seed samples were spiked, before extraction, with the amino acids at different concentrations (from 0.5 to 2 mg/kg), and the slopes of the calibration plots were compared with results obtained when the whole process was applied to standard solutions of the amino acids. The slope ratio of matrix curve to neat solution curve was calculated; the ratio value of 1.0 indicated no matrix effect. When ginkgo seeds were tested, most of the ratio values were close to 1.0 (Supporting information Table S3), implying that there were no significant matrix effects in relatively complex functional food matrices.

The calibration functions obtained by plotting the peak area versus the concentration of the compound were linear, with the determination coefficient higher than 0.997 for all compounds. The sensitivity was investigated as the values of LOD and LOQ. The lowest sensitivity was found for His (LOQ = 78.66 ng/mL and LOD = 270.12 ng/mL), while the best sensitivity was for GABA (LOQ = 0.15 ng/mL and LOD = 0.56 ng/mL). The intra- and inter-day precision variations, expressed as RSD, were from 0.54 to 3.35 % and from 1.70 to 6.79 %, respectively.

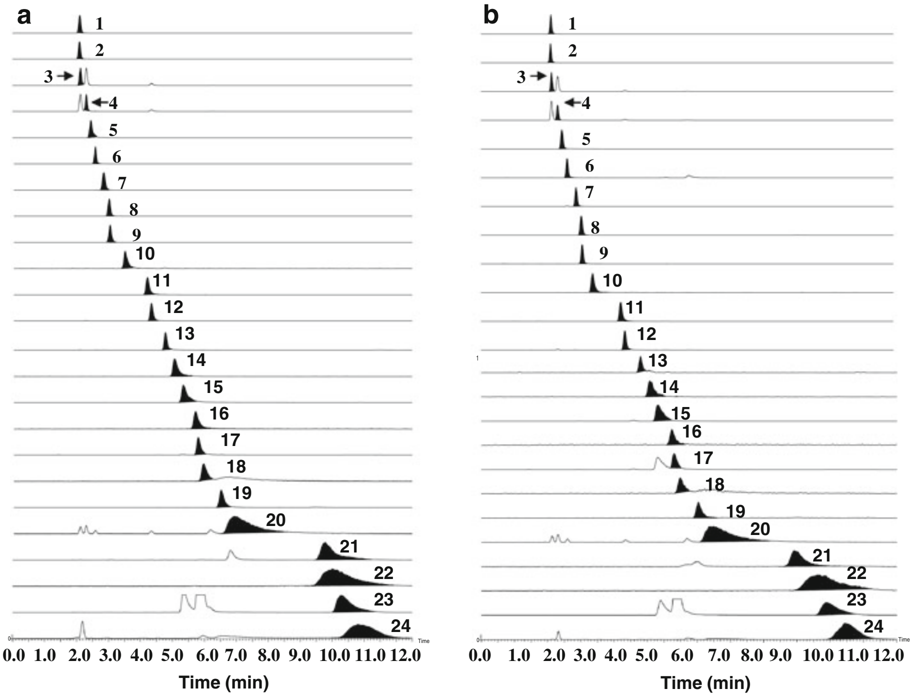


Fig. 2 Representative MRM chromatogram of 24 FAAs in 10.0 μg/mL mixture standards (**a**) and ginkgo seed sample 1 (**b**). Peaks: *1* Trp, *2* Phe, *3* Leu, *4* Ile, *5* GABA, *6* Met, *7* Val, *8* Pro, *9* Tyr, *10* Cys, *11* Ala, *12* Hpro, *13* Thr, *14* Gly, *15* Glu, *16* Ser, *17* Gln, *18* Asn, *19* Cit, *20* Asp, *21* Arg, *22* His, *23* Lys and *24* Orn

The repeatability and stability presented as RSD were in the range from 1.54 % to 6.99 % and from 0.95 % to 6.15 %. Trueness was estimated through recovery studies. Before extraction, different aliquots of ginkgo seeds ($n = 6$) were spiked at two levels, 0.1 and 1.0 mg/g, with the target compounds and were extracted with the developed method. Supporting information Table S3 shows the obtained results, and it can be observed that recoveries ranged from 93.7 % (Orn) to 106.3 % (Gly) for the selected compounds at 0.1 mg/g and from 91.5 % (Lys) to 105.9 % (Met) at 1.0 mg/g. These parameters were sufficient for quantification of the compounds in real samples. The results are all summarized in the Supporting information Table S3.

Method application

The validated HILIC-UPLC–TQ-MS/MS method was applied for the analysis of ginkgo seed five parts (episperm, mesosperm, endopleura, embryoid and plumule) collected from three major production areas of *G. biloba* in China (Jiangsu, Shandong and Guangxi provinces). The total content of all the target compounds ranged from 341.2 to 13,136.4 μg/g and the contents varied greatly among different parts. Among the five parts, plumule, episperm and embryoid had higher FAAs levels, whereas mesosperm and endopleura had lower content. The data demonstrated that the distribution and determination of FAAs in ginkgo seeds five parts were arranged in a decreasing order as plumule > episperm > embryoid > endopleura > mesosperm (Fig. 3a). It can be observed from Table 2 that the major FAAs were GABA, Pro, Gln and Glu for the plumule, Gln and Glu for the episperm, endopleura and mesosperm, and Glu and GABA for the embryoid. In addition, the episperms were much different from the other four parts. For example, the total contents of essential amino acids and four non-protein amino acids (GABA, Hpro, Cit and Orn) were 2,380.49 and 11,729.95 μg/g in the episperm, respectively, and occupied approximately 7.7 and 38.2 % of total amino acids, whereas they occupied approximately 20.0–26.6 % and 10.7–26.9 % in the other four parts.

To further demonstrate the regularity of FAAs in ginkgo seeds, the proposed method was also applied to analyze the edible parts of 11 ginkgo seeds materials (samples 16–26) of different plant-ages obtained from the largest *G biloba* region in China (Tancheng, Shandong province). The content of 24 FAAs ranged between 4,330.4 and 8,813.7 μg/g. The highest content was of Glu followed by GABA, Arg, Pro and Gln. It is noticeable that both the acidic amino acid (Glu) and the basic amino acid (Arg) were among the dominant contributors. The non-protein amino acid GABA presented in almost quadruple amount than the other three non-protein amino acids. The conclusion can be drawn from Table 2 that the content of FAAs in these investigated ginkgo seeds (samples 16–26) revealed remarkable results, among which the age-related changes were especially striking. Our data showed that the content of the FAAs in ginkgo seed edible parts changed with age. A steady increase is evident in the content of FAAs from

8 years until about 40 years of age. The significant age-related changes plotted in Fig. 3b were derived from such total analyses on the series of ginkgo seed specimens aged 8–600 years. Besides, the results plotted in the Supporting information Fig. S6 also showed that the content of each FAA increases in the ginkgo seeds with different age, reaching a maximum in the sample 22 (40 years).

The investigated compounds in samples 27–48 of ginkgo seed edible parts collected from different places in China were also simultaneously evaluated by the proposed HILIC-UPLC–TQ-MS/MS. The results showed that all of these ginkgo seed samples were rich in the FAAs, especially for essential amino acids such as Trp, Phe, Leu, Ile, Met, Val, Thr and Lys. Our study also revealed that FAAs exhibited a different concentration level in different localities, suggesting that the distribution of FAAs had geographical differentiation. Obviously, the contents of total and essential FAAs in samples 28–31 (the famed *G. biloba* growing area of Xuzhou and Taizhou, Jiangsu) and sample 47 (the largest *G. biloba* growing area of Tancheng, Shandong) were approximately 1.5 and 3.5 times higher than those in the other locality samples, respectively. As for the individual compounds determined in the experiments, GABA and Glu contributed the largest fractions to total FAAs. In particular, the non-protein amino acid GABA functions as an important neurotransmitter signal in humans, has blood pressure lowering activity, and has been used as a component of pharmaceuticals and functional foods (Syu et al. 2008). Except for GABA, the contents of three other non-protein amino acids in ginkgo seeds were at a trace level. Furthermore, the content of the rest 12 free Pro, Tyr, Cys, Ala, Gly, Gln, Ser, Asn, Asp, His, Arg and Lys amino acids in different sampling localities showed consistency and no variation at relate lower level (approximately 100 μg/g), indicating substantial and stable genetic diversity of these amino acid compositions in ginkgo seeds. From a nutritional and functional point of view, this research results indicated that ginkgo seeds were healthy and nutritional food for the supplement of FAAs, especially the essential amino acids and GABA. In addition, one obvious conclusion could be given that ginkgo seeds cultivated in major production regions (Jiangsu and Shandong province) have a high content of these nutritional and functional substances than other areas.

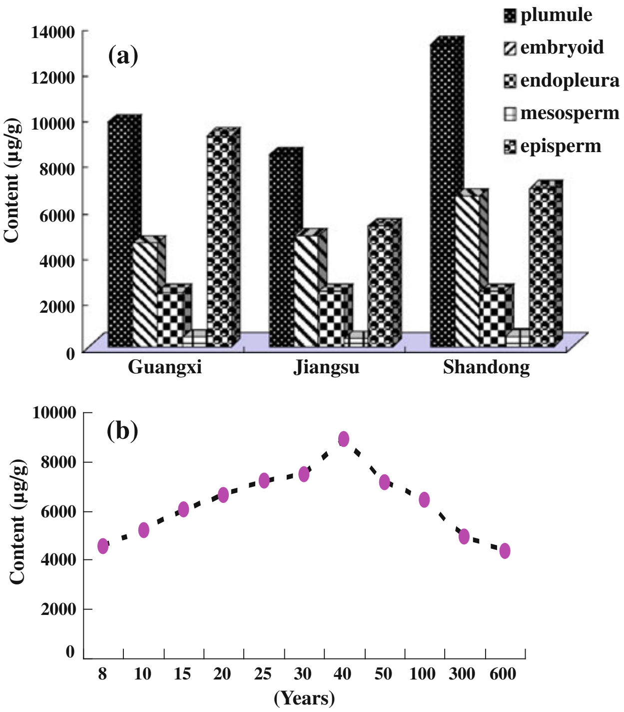


Fig. 3 Histogram showing the total content of FAAs in five parts of ginkgo seeds from three major production areas of China (**a**); polygram exhibiting the total content change tends of FAAs in 11 different aged ginkgo seeds (**b**). The different ages (8, 10, 15, 20, 25, 30, 40, 50, 100, 300, and 600 years) were not arithmetic progression points on the abscissa, and therefore the *dotted lines* in the polygram was regarded as the hypothesis content change trendline

In general, ginkgo seeds were rich in FAAs, which could be used as nutritional substance to provide and/or complement the need of life activity, and even to prevent and/or cure several diseases. Therefore, further studies are needed to investigate the relationship between FAAs and the pharmacological effects in ginkgo seeds.

PCA of the samples

To evaluate the variation of ginkgo seeds, PCA was used to further investigate the relationship between different region samples based on the contents of 24 determined compounds. The three principal components (PC 1, PC 2 and PC 3) represented >82 % of the whole variance. PC 1 accounted for 65.71 %, PC 2 and PC 3 accounted for 11.26 and 5.89 %, respectively. The remaining principal components with less effect on the model were discarded. According to the components loading matrix, PC 1 had good correlation with compounds **1**–**12**, **14**, **16**, **17** and **19**–**24**, PC 2 was related with compounds **13** and **18**, whereas PC 3 was related with compounds **15**. As shown in Fig. 4, PCA analysis showed three separate groups, and the main contributors to the three groups were representing as follow: samples 26–41, 48 were in group A, samples 42–46 were in group B, and samples 47 were in group C. Obviously, it can be observed that samples with similar chemical profiles were commonly divided into the same group. As for sample 47 (Tancheng, Shandong province), the content of each FAA was relatively higher than that in the other samples. This result also supported the fact that Tancheng was a famous and special growing area for

Table 2 Contents of 24 free amino acids in the tested samples of ginkgo seed

Sample number	Contents of analytes (μg/g, $n = 3$)																								Total
	Trp	Phe	Leu	Ile	GABA	Met	Val	Pro	Tyr	Cys	Ala	Hpro	Thr	Gly	Glu	Ser	Gln	Asn	Cit	Asp	Arg	Hit	Lys	Orn	
1	196.9	330.7	466.4	308.6	1030.6	7.6	454.7	1134.0	132.4	72.6	168.1	38.3	474.0	288.6	1334.4	179.1	1658.8	171.8	22.5	281.4	601.7	77.8	244.6	64.0	9739.7
2	104.3	160.1	150.5	95.8	539.8	28.8	152.0	240.9	168.5	60.8	90.3	16.4	449.5	181.5	906.9	176.0	203.0	254.8	8.8	138.5	207.0	26.1	105.9	30.7	4496.8
3	8.4	26.7	143.5	20.0	215.9	+[a]	38.7	98.1	28.2	20.8	65.3	8.2	98.2	22.8	348.1	85.3	849.4	135.7	12.7	15.4	88.9	7.8	14.7	3.8	2358.3
4	3.7	5.6	46.4	3.7	46.3	nd[b]	10.4	10.4	5.1	15.9	11.7	5.0	23.6	16.3	34.6	15.2	107.8	15.7	+	12.3	7.2	7.3	12.4	nd	417.6
5	7.6	35.8	374.6	38.9	463.5	nd	83.1	282.1	116.0	21.8	81.5	45.7	178.8	45.5	3274.1	406.8	2315.6	753.3	112.0	51.6	268.0	139.3	58.7	13.0	9167.5
6	115.4	368.9	555.3	356.1	1026.1	59.7	563.6	1033.6	181.1	80.9	139.8	24.3	401.7	267.0	886.8	254.4	215.6	335.5	6.5	203.1	778.3	70.3	332.1	77.2	8333.5
7	113.1	106.3	119.7	142.5	659.6	40.5	135.9	91.8	180.3	61.2	95.6	35.0	411.2	168.4	1129.9	145.6	145.6	238.6	26.3	157.5	212.6	35.6	257.6	83.6	4793.8
8	11.1	58.2	77.0	58.1	237.0	nd	80.0	355.8	35.4	24.0	38.5	6.7	184.3	19.2	370.9	110.0	405.9	142.2	1.8	21.0	114.1	14.6	13.8	2.0	2381.4
9	2.2	7.5	11.5	6.6	39.4	+	12.7	29.7	6.1	13.2	13.6	4.9	20.1	13.2	49.3	12.4	37.2	21.2	+	13.7	3.0	7.1	12.4	+	341.2
10	2.2	21.4	61.4	35.2	377.1	nd	61.5	644.7	65.3	35.2	97.7	14.0	239.5	24.3	1224.7	316.2	1250.3	527.9	+	71.4	114.0	13.6	16.3	3.3	5218.6
11	278.2	491.0	668.5	461.6	2714.9	41.8	708.7	1549.0	368.1	94.3	96.2	31.4	665.2	791.9	936.2	204.1	473.4	496.6	18.1	396.0	1318.2	44.9	228.9	59.3	13136.4
12	120.6	222.5	317.6	183.8	948.5	32.6	336.0	589.5	123.6	72.4	89.9	22.5	430.8	223.6	1253.0	97.9	321.8	192.8	66.6	143.2	495.7	33.5	165.9	30.6	6514.7
13	14.2	35.6	46.3	48.5	223.6	+	70.2	301.0	33.0	18.8	80.8	6.5	187.3	11.7	208.8	127.5	346.5	473.3	1.5	14.2	107.0	7.2	13.6	5.5	2384.1
14	2.2	3.7	5.1	6.3	20.8	nd	9.3	27.7	6.4	13.8	25.4	7.0	19.7	91.4	21.4	16.0	22.1	96.7	+	14.1	1.8	7.5	13.1	+	433.5
15	28.4	75.5	96.6	90.6	220.6	2.4	112.9	369.4	91.2	19.4	92.1	11.7	327.2	30.4	930.9	208.1	1602.2	1687.6	48.9	73.2	625.6	38.6	81.2	12.7	6877.3
16	95.3	188.5	214.9	156.5	683.1	31.3	236.0	483.7	93.4	73.2	51.2	23.1	254.1	81.6	1193.1	83.5	229.1	41.8	30.1	68.5	70.8	30.9	125.1	11.3	4550.3
17	113.5	214.7	261.5	187.5	889.4	33.0	275.9	488.0	108.8	73.0	66.0	22.0	297.9	182.2	1115.2	85.5	261.1	147.6	30.5	69.7	76.9	32.5	148.3	13.0	5193.7
18	132.8	218.6	315.7	198.1	956.9	33.9	333.8	556.1	134.3	75.7	65.0	22.7	419.2	191.5	1116.9	95.5	274.7	163.1	27.8	103.9	467.7	28.5	149.0	12.8	6094.0
19	129.3	224.4	332.9	200.0	957.6	34.4	349.0	586.2	130.5	80.0	65.0	23.8	436.7	233.6	1241.6	107.2	349.7	195.7	64.6	151.8	496.5	34.9	169.7	31.4	6626.8
20	130.3	217.4	343.8	209.9	1029.0	32.2	371.8	594.5	145.3	85.0	69.7	24.1	451.3	213.0	1302.6	121.9	498.6	198.6	132.6	152.5	599.2	32.0	181.3	54.4	7191.0
21	146.4	223.9	357.4	220.8	1016.6	35.1	350.3	598.1	152.0	88.4	71.0	25.0	453.0	224.4	1338.2	146.7	559.5	209.2	152.2	176.6	639.8	35.4	229.3	53.4	7502.8
22	173.0	311.0	380.7	239.5	1263.2	44.7	388.1	547.9	168.1	91.9	81.7	27.2	544.8	281.5	1596.9	167.0	682.3	218.5	309.5	196.5	728.2	35.7	256.1	79.9	8813.7
23	136.8	276.3	292.8	213.2	1001.0	33.2	361.4	559.9	131.0	78.3	64.1	26.5	487.6	255.3	1395.2	130.9	330.2	137.4	298.2	165.3	436.5	32.9	234.1	49.9	7128.0
24	111.4	226.5	284.3	189.3	980.9	32.3	300.9	482.8	118.7	78.3	66.5	24.7	450.9	238.8	1285.3	155.2	305.5	130.0	131.6	154.3	323.9	32.9	220.2	43.2	6368.6
25	92.0	219.6	245.6	185.3	548.3	31.7	306.3	444.7	117.0	68.7	64.8	21.6	381.8	226.4	919.5	115.8	259.1	71.4	71.3	144.7	105.9	30.1	181.1	31.5	4883.9
26	71.4	162.3	236.7	139.6	524.1	31.5	212.6	444.7	107.2	66.4	64.3	21.6	301.3	162.4	828.5	116.7	254.8	66.0	52.7	142.6	104.7	29.8	167.0	21.5	4330.4
27	98.4	129.9	153.7	177.8	569.9	18.6	188.4	178.4	156.7	78.9	86.7	23.3	396.6	222.9	1197.3	102.7	150.6	218.7	20.9	140.7	339.4	58.6	128.1	13.0	4850.1
28	256.4	463.1	389.2	351.2	920.5	38.6	452.4	740.0	265.7	174.7	116.2	76.1	183.3	316.7	392.2	224.6	114.0	95.9	80.2	290.7	536.2	65.0	223.5	87.5	6853.7
29	246.2	430.0	366.6	394.6	979.0	96.1	432.4	617.0	251.2	194.1	104.7	80.8	204.1	236.3	365.1	282.4	100.4	79.6	79.8	291.9	565.5	70.7	237.8	97.6	6803.8
30	196.7	457.6	370.0	339.1	1011.1	64.1	454.4	586.1	259.0	85.3	120.5	99.2	205.5	268.1	361.7	230.6	117.4	68.0	77.2	182.7	555.8	58.9	222.5	87.0	6478.4
31	106.6	157.2	166.8	103.9	753.4	43.4	182.9	251.6	177.8	63.0	114.5	43.2	400.8	198.1	912.6	153.8	205.9	218.1	13.2	175.0	234.6	39.4	116.5	97.1	4929.3
32	116.1	114.6	127.8	159.3	668.9	41.4	141.6	194.0	182.6	64.8	100.3	34.2	407.2	172.8	1111.3	158.5	149.9	241.9	24.9	146.2	215.0	37.4	249.8	82.1	4942.2
33	113.9	103.4	109.6	152.0	681.3	36.0	175.3	152.0	178.3	66.3	102.9	43.6	397.1	185.9	1257.4	151.7	169.3	213.8	14.3	158.5	271.5	32.3	107.3	51.4	4925.0
34	92.6	119.0	119.4	198.0	429.9	23.5	117.2	254.5	133.3	70.9	101.6	22.7	385.1	190.5	1219.9	164.9	217.0	268.8	14.4	144.4	313.3	30.6	151.0	20.4	4802.9
35	110.9	122.9	193.2	133.0	589.3	34.0	190.5	276.4	111.6	76.9	82.2	27.5	512.2	201.6	1048.0	143.6	176.8	204.5	28.7	190.0	324.2	30.2	183.1	21.8	5013.1
36	138.6	129.3	115.7	131.5	592.4	52.7	160.8	218.3	151.6	76.3	115.6	29.1	502.4	173.8	952.7	176.1	185.4	219.6	47.2	227.9	309.4	30.5	193.4	25.7	4956.0
37	126.8	116.6	123.2	127.1	643.7	39.7	144.9	226.9	162.5	67.5	112.0	27.2	464.4	204.5	1023.2	128.7	206.6	270.7	23.0	199.5	268.4	31.2	108.5	17.6	4864.2
38	104.5	126.0	171.4	133.3	557.4	48.6	201.6	204.9	122.5	72.3	118.8	21.9	511.8	206.2	919.7	129.1	247.0	280.7	10.5	161.8	277.2	28.9	158.7	29.7	4844.5
39	104.9	168.0	167.5	105.8	588.0	21.9	171.5	267.1	164.9	68.5	93.2	21.4	489.8	191.2	912.6	178.9	211.9	246.2	8.8	153.7	243.7	30.7	111.1	37.4	4758.6

Table 2 continued

Sample number	Contents of analytes (μg/g, $n = 3$)																								Total
	Trp	Phe	Leu	Ile	GABA	Met	Val	Pro	Tyr	Cys	Ala	Hpro	Thr	Gly	Glu	Ser	Gln	Asn	Cit	Asp	Arg	Hit	Lys	Orn	
40	114.0	100.3	120.6	154.1	746.7	38.8	158.5	255.2	156.7	71.3	84.3	22.0	404.9	127.4	1242.3	122.1	158.1	268.5	21.4	165.4	251.2	32.5	118.5	20.3	4955.1
41	116.5	115.3	149.6	157.8	686.3	46.3	152.7	208.5	137.2	65.3	108.7	28.7	486.3	176.6	1147.7	143.2	173.7	242.0	16.0	109.4	291.2	35.3	63.2	58.2	4915.7
42	44.7	145.3	114.0	157.9	193.4	42.2	144.7	200.9	132.2	65.6	58.7	34.1	376.9	174.8	1142.7	138.6	73.2	232.3	15.0	106.3	281.4	36.7	67.1	64.9	4043.2
43	34.7	148.5	117.9	152.2	198.3	44.0	138.6	242.7	174.8	73.2	67.1	32.3	400.9	144.7	1645.6	176.9	108.7	234.1	22.2	114.9	286.3	36.7	81.4	55.0	4731.4
44	35.5	130.8	118.2	112.6	177.7	25.8	129.9	152.8	155.4	72.0	79.5	23.2	387.3	95.3	1218.3	171.1	76.1	168.4	34.7	140.3	288.9	30.8	53.6	23.9	3902.0
45	23.0	141.7	87.1	115.4	184.3	20.1	156.4	120.1	153.7	41.0	58.1	57.5	385.6	144.8	1351.7	135.4	85.1	277.0	36.9	131.7	169.9	30.8	59.1	32.7	3999.0
46	73.0	147.5	95.4	120.1	184.3	47.1	165.4	251.6	144.8	65.1	49.1	27.0	320.1	156.4	941.0	185.6	128.1	257.5	13.7	142.6	231.7	29.8	69.9	36.9	3883.8
47	129.3	224.4	332.9	200.0	957.6	34.4	349.0	586.2	130.5	80.0	65.0	23.8	436.7	233.6	1241.6	107.2	349.7	195.7	64.6	151.8	496.5	34.9	169.7	31.4	6626.8
48	106.0	150.8	107.4	153.3	724.6	31.7	191.8	251.2	145.3	70.1	96.3	22.2	457.7	180.9	1011.2	139.3	177.5	239.7	7.8	111.7	279.1	31.0	170.2	28.9	4885.7

[a] Below the limit of quantitation

[b] Undetectable

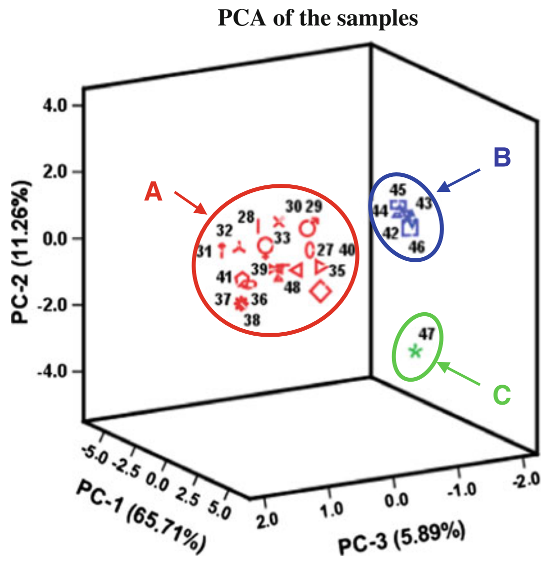


Fig. 4 The *scatter plot* obtained by PCA of the 22 samples of ginkgo seeds from different cultivation regions

G. biloba with largest number in production of China. On the contrast, for samples 42 (Nanchong, Sichuan), 43 (Chengdu, Sichuan), 44 (Yongzhou, Hunan), 45 (Zunyi, Guizhou) and 46 (Songxian, Henan), the content of the target compounds relatively lower than those in the other samples. These results indicated the factor of areas of ginkgo seed might play an important role in samples classification, which meant that chemical profiles of FAAs of ginkgo seed were mainly affected by production regions.

Conclusion

In summary, HILIC-UPLC–TQ-MS/MS has been successfully developed and employed to determine 24 underivatized FAAs in ginkgo seed samples. This method was simple, rapid, accurate and repeatable, and could be also used to simultaneously analyze FAAs in medicinal or edible plant materials and agricultural products such as cucumbers, tomatoes, cabbages, spinach, etc. The research results showed that ginkgo seeds were rich in both content and types of FAAs. Thus, ginkgo seeds as a main ingredient of some desserts, glazed fruit, beverages and dietary supplements, were good source of FAAs for good nutritional and functional products.

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