Emirates Journal of Food and Agriculture. 2019. 31(3): 164-171
doi: 10.9755/ejfa.2019.v31.i3.1928
http://www.ejfa.me/

RESEARCH ARTICLE

Application of capillary electrophoresis for assessing amino acids composition of Jujube from different geographical origins

Jianyu Qiao[1,2], Ruibin Gao[3], Zhongyang Wang[1,2], Yaming Sun[1,2], Shuqing Dong[1]*, Liang Zhao[1]*

[1]Key laboratory of Chemistry of Northwestern Plant Resources and Key laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, The Chinese Academy of Sciences, Lanzhou, China, [2]University of Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing, China, [3]Third Provincial People's Hospital of Gansu, Department of Pharmacy, Lanzhou, China

ABSTRACT

A simple capillary electrophoresis (CE) method that using β-cyclodextrin (β-CD) as buffer modifier has been developed for the detection of ten amino acids of jujube from five different geographical origins. The CE methodology was optimized through the variation of type, pH and concentration of the buffer solution, concentration of the additive β-CD, applied voltage, injection time and wavelength of the UV detection. It was found that the best separation of ten kinds of amino acids was achieved within less than 6 min under the optimum separation conditions: 50 mmol L^{-1} pH 9.5 borate-phosphate buffer with 5.0 mmol L^{-1} β-CD, 15 kV applied voltage, 25°C column temperature, 210 nm detection wavelength, and 5 s injection time. This method showed good repeatability with RSD values of 1.4-3.4% for peak area, and 1.4-4.7% for migration time, when β-CD was used as buffer modifier. Under the optimum conditions, the method has been successfully applied to the detection of actual jujube samples, which also verifies the effectiveness and practicability of the method. Recovery of real samples was in ranging of 90%-105%, which proved the feasibility of the method. It also proved that the method was successfully applied to the quantitative analysis of amino acids of interest in plant samples.

Keywords: Capillary electrophoresis; Amino acids; Derivatization; Jujube

INTRODUCTION

Jujube (Ziziphus jujuba Mill.) belongs to Rhamnaceae, which is a plant resource originating in China and widely distributed in North China (Li et al. 2005; Li, 2003). Jujube is natural, healthy, and rich in sugars, vitamins and iron, with a good function of blood tonic used as food, and possesses multiple medicinal properties such as antimicrobial, antioxidant (Cheng et al. 2014), immune stimulating (Benammar et al. 2010), hypoglycemic (Glombitza et al. 1994) and haemagglutination activities (Ahmad et al. 2011). Based on the nutritional value of jujube, its active ingredients have received a great deal of interest. The most studied active ingredients in jujube are polysaccharide (Li et al. 2007), cyclic adenosine monophosphate (Shi et al. 2006), saponins (Zhao et al. 2006), and flavonoids (Jiang et al. 2007), but quantitative analysis of amino acids in jujube have been described seldom (Choi et al. 2011; Lin et al. 2013).

As an important component of protein molecules, amino acids widely exist in body fluids, tissues, foods and seeds, are a source of energy, and closely related to biological activities. Analyzing the types and contents of amino acids in food or some medicines can provide important food nutrition information or the medicines quality control information (Fang et al. 2014). As important chemical constituents of jujube, amino acids remarkably influence the nutritional quality of jujube. Thus, the determination of amino acids is important in evaluating the overall composition and the nutritional quality of jujube (Guo et al. 2013).

HPLC is a traditional method for detecting amino acids, but this method usually requires longer analysis time,

***Corresponding authors:**
Shuqing Dong, Key laboratory of Chemistry of Northwestern Plant Resources and Key laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, The Chinese Academy of Sciences, Lanzhou, China. **E-mail:** sqdong@licp.cas.cn
Liang Zhao, Key laboratory of Chemistry of Northwestern Plant Resources and Key laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, The Chinese Academy of Sciences, Lanzhou, China. **E-mail:** zhaol@licp.cas.cn

Received: 04 December 2018; **Accepted:** 11 February 2019

larger samples consumption (Ming et al. 2014; Zhao et al. 2012; Meussen et al. 2013). Compared with HPLC, CE is a powerful separation technique which could provide shorter analysis time, lower sample amount, fewer buffer consumption, and better separation efficiency (Frost et al. 2010; Geiger et al. 2012). These advantages make CE an alternative and complementary technique, and widely employ in amino acids analysis (Souza Crespo et al. 2015; Jeong et al. 2013; Faserl et al. 2015).

It is well known that amino acids are very polar compounds and most of them lack strong ultraviolet (UV) absorbance, so their detection and accurate quantitation is a challenge by UV detection (Guo et al. 2013). In order to obtain accurate quantification and good detection sensitivity, amino acids must be derivatized before CE separation under UV detection conditions. At present, the commonly used derivatization reagents are fluorescein isothiocyanate (FITC) (Liu et al. 2014), dansyl chloride (Erbas et al. 2005), phenylisothiocyanate (PITC) (Viadel et al. 2000), *o*-phthalaldehyde (OPA) (Filik and Çetintaş, 2012; Zhao et al. 2012). Due to that OPA could instantly react with amino acids at room temperature, and the derivatization reaction condition is mild, quick and easy. So, the OPA was usually used to label amino acids to make them have ultraviolet absorption.

In order to develop a sensitive, simple and practical method, it was used CE coupled with UV detector to determine amino acids in jujube that grows in five main and different origins. The β-CD was used as additive for the separation of ten kinds of amino acids and the OPA was chosen as derivatization agent to derivatize amino acids. The content of ten amino acids containing of L-arginine (L-Arg), L-tyrosine (L-Tyr), L-tryptophan (L-Trp), L-threonine (L-Thr), L-glutamine (L-Gln), L-asparaginate (L-Asn), L-valine (L-Val), L-glutamic acid (L-Glu), L-cysteine (L-Cys) and L-aspartic acid (L-Asp) in the jujube from different regions is significantly different.

MATERIALS AND METHOD

Apparatus

All the experiments were performed on a CE system equipped with a diode-array detector (Beckman P/ACE MDQ, USA). Fused silica capillary (68.5 cm×75 μm i.d, 60.0 cm effective length) was bought from Yongnian Photoconductive Fiber Factory (Hebei, China). The Beckman 3.2 karat software (version 7.0) was used to control the CE system.

Chemicals and reagents

All chemicals and reagents used were analytical grade. Ten standard amino acids, *o*-phthalaldehyde and β-cyclodextrin were purchased from J&K Scientific Ltd. (Beijing, China). Methanol, ethanol and all other chemicals were bought from Shanghai First Reagent Factory (Shanghai, China). Distilled water was used throughout the experiment. The sample of jujubes were purchased from local supermarkets in Shandong (Golden silk jujube), Shanxi (Jun jujube), Shaanxi (Linze Jujube), Gansu provinces (Jingyuan shimen jujube) and Xinjiang autonomous region (Grey jujube) (Picking date close).

Derivatization of amino acids

OPA was accurately weighed and dissolved in absolute ethanol to a concentration of 1.0 mg L^{-1} stock solution. Amino acids were dissolved in distill water to a concentration of 1.0 mg L^{-1} stock solution. The above two solutions were stored in the dark at 4°C. OPA and standard amino acids were added to buffer solutions at a volume ratio of 1:1. The mixed solution was derivatived at 35°C for 5 min. Then, all solutions were filtered through 0.45 polypropylene acrodisc syringe filter and degassed by agitation in an ultrasonic bath for 5 min to remove bubbles.

Preparation of sample

The samples of jujube was dried in the vacuum drying oven at 60°C, pulverized into powder after removing the nucleus. A 50.0 g sample of five kinds of jujube date powder was accurately weighed and soaked in 500 mL distilled water for 12 h in the dark, refluxed at 75°C for 2 h, and refluxed three times. The combined extract solution was filtered by qualitative filter paper and then concentrated through rotary evaporator. When the extract solution was concentrated to 30 mL, 200 mL of ethanol (90%) was added to the concentrate to precipitate it, and then the precipitate was removed by filtration through a filter paper. The remaining filtrate was concentrated to 10 mL by using a rotary evaporator. If necessary, the final solutions were diluted 5-10 times with distilled water before the analysis. The derivatization procedures were same as the standard amino acids. Prior to the CE analysis, 500 μL sample solution was diluted to 1.0 ml with a buffer solution and filtered through a 0.45 μm filter.

Capillary electrophoresis procedures

Before the CE separation, the capillary was rinsed sequentially with 0.1 M HCl, doubly distilled water, 0.1 M NaOH. After that, the capillary was treated with corresponding running buffer until the current in the capillary remained stable to obtain a repeatable electroosmotic flow (EOF).

CE separations were carried out using 15 kV voltage, 0.05 mol L^{-1} pH 9.5 borate-phosphate mixture solutions with 5 mmol L^{-1} β-CD as buffer additive. All the samples were prepared with buffer solution and injected at 0.5 psi for 5 s. The above process were performed at room temperature.

RESULTS AND DISCUSSION

Optimization of CE separation conditions

Effect of buffer type, pH and concentration

In order to improve the resolution and sensitivity for the ten amino acids, borate, phosphate, and borate-phosphate mixture were investigated as running buffer in the CE separation. It was found that the separation effect of borate-phosphate mixture buffer was the best.

Changing pH has an impact on the separation efficiency and selectivity. Therefore, the optimization of pH of the borate-phosphate mixture buffer could further improve the separation efficiency of analytes. Herein, the influence of buffer pH on the separation of OPA-amino acids was evaluated from 6.0 to 10.0. The results of different buffer pH conditions were illustrated in Fig. 1. As shown, when pH of the running buffer was 9.5 without β-CD, most amino acids could achieve baseline separation, except for L-glutamine and L-asparaginate. Therefore, the pH of the running buffer was set to 9.5, considering the separation efficiency of the further experiment.

In CE, the concentration of buffer can significantly affect the separation of analytes. When the pH of the buffer solution was 9.5, the effect of the concentration of the buffer on the separation was examined in the range of 25-80 mmol L^{-1}. In all cases, an applied voltage of 15 kV was used. According to the experiments results, the migration order of each amino acid slightly changed, but the resolution did not improve as the concentration of the buffer increased. Moreover, the migration time became longer. The experimental results showed that the concentration of borate-phosphate mixture buffer of 50 mmol L^{-1} was used as the optimum condition.

Effect of the additive β-CD concentration and separation mechanism

It is known that the concentration of modifier has a great influence on CE separation. In this work, β-CD was added to the buffer solution, the influence of β-CD concentration on the migration time of the amino acids was evaluated from 2.0 to 8.0 mmol L^{-1}. As can be seen from Fig. 2, the ten amino acids could achieve baseline separation with the addition of β-CD at a concentration 5.0 mmol L^{-1}. However, the separation effect is unsatisfactory when the concentration of β-CD was below or above 5 mmol L^{-1}.

The reason was that the different formation constants between the amino acids and β-CD resulted in different degrees of complexation (Borisov et al. 2016; Roy et al. 2016), and thus yielded different electrophoretic mobility,

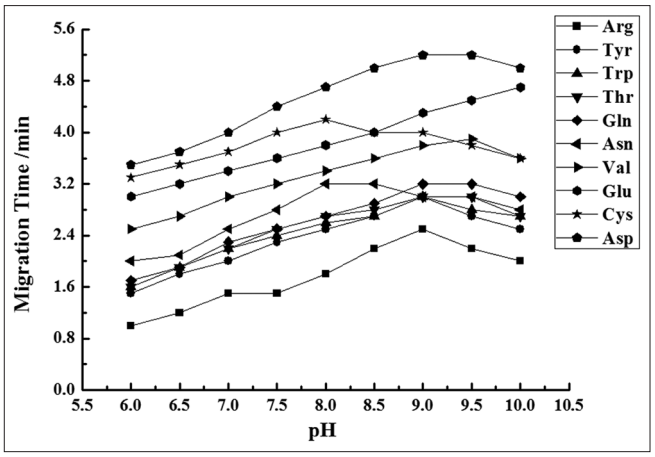

Fig 1. The effect of the running buffer pH values on migration time. Other experimental conditions and labels are the same as in Fig 4.

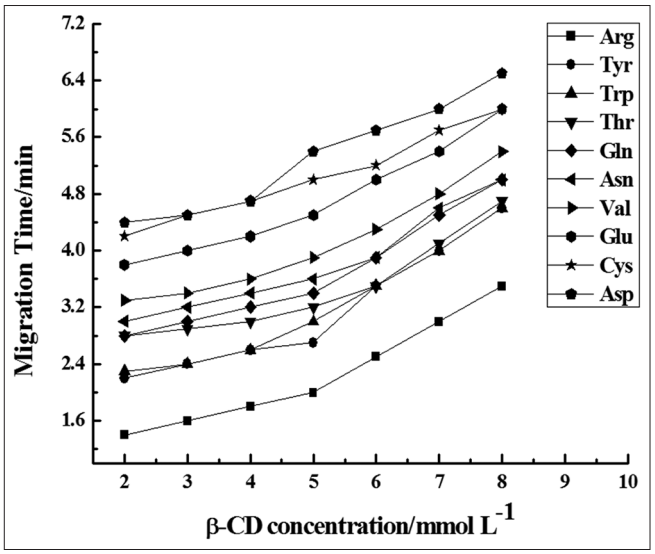

Fig 2. The effect of the β-CD concentration on migration time. Other experimental conditions and labels are the same as in Fig 4.

which may result in good separation in CE. Besides, the addition of β-CD to the running buffer could change the polarity and viscosity of the electrolyte, thereby affecting the EOF. As a result, the optimum β-CD concentration was fixed at 5.0 mmol L^{-1} considering of the higher resolution and shorter migration time.

Effect of applied voltage and injection time

The effect of the applied voltage on the separation of amino acids was evaluated by changing the applied voltage from 15 to 30 kV. As the applied voltage increased, the migration time of amino acids was obviously shortened, and their electrophoresis peaks were also sharpened. However, if the applied voltage was too large, the higher the internal current of the capillary, the more Joule heat was generated, which lead to the reduction in separation efficiency. Hence, 15 kV was chosen as the optimum applied voltage for all the separation experiments.

Under the pressure of 0.5 psi, different injection time (3, 4, 5, 6, 8 and 10 s) was selected to evaluate the impact of injection time on separation effect. The effect of injection time on migration time was shown in Fig. 3. According to the results, the peak current increased with the injection time. However, when the injection time was greater than 8 s, the current peaks of the analyte significantly widened and overlapped, so 5 s was selected as the injection time to obtain satisfactory results.

Effects of the UV detection wavelength

The on-line UV spectrum of the ten amino acids was obtained on PAD detector. The detection wave length was selected according to the adsorption wave lengths of ten OPA-amino acids at 210, 230 and 250 nm. According to the experiments, we found that the most OPA-amino acids have the maximum adsorption at 210-220 nm. Based on the above considerations, 210 nm was chosen as the optimal detection wavelength for simultaneous detection of the ten amino acids.

Analysis performance

The optimized separation conditions were as follows: 50 mmol L^{-1} pH 9.5 borate-phosphate buffer with 5.0 mmol L^{-1} β-CD, 15 kV applied separation voltage, 25°C column temperature, 210 nm UV detection wavelength, and 5 s electrokinetic injection time at a pressure of 0.5 psi. The electrophoregram of the ten OPA-amino acids (5.0 μg mL^{-1}) under the optimal experimental conditions was shown in Fig 4. As shown in the figure, we clearly saw that 10 amino acids achieved baseline separation within 6 minutes.

Method validation

Linearity and detection limits

A series of mixed standard solutions of the amino acids were detected to determine the linearity for ten OPA-amino acids. The linear ranges, regression equations, correlation coefficients and detection limits of these ten amino acids were listed in Table 1. The linear range of each peak area corresponding to the concentration for amino acid was

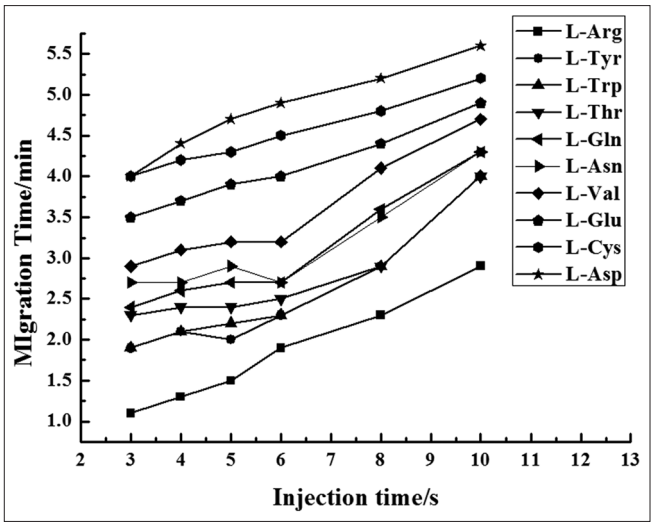

Fig 3. The effect of the injection time on migration time. Other experimental conditions and labels are the same as in Fig 4.

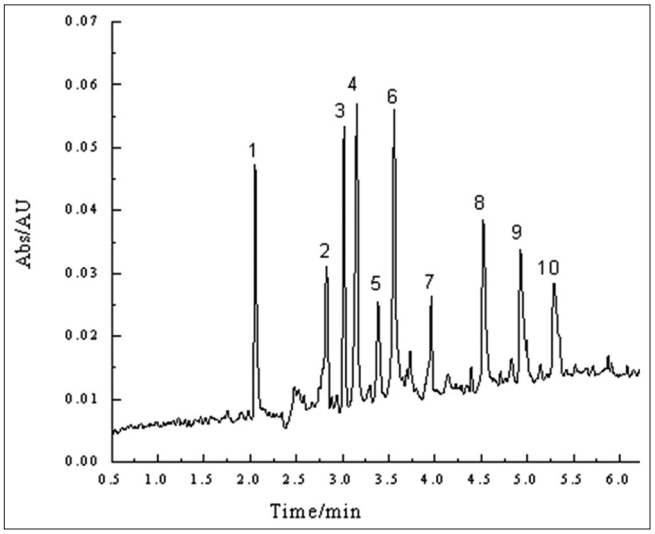

Fig 4. Electrophoregram of solution containing ten amino acids (5.0 μg mL^{-1}). Capillary: 65.5 cm×50 μm I.D.; Running buffer: 0.05 mol L^{-1} borate-phosphate (pH 9.5); separation voltage: 15 kV; column temperature: 25°C; UV detection: 210 nm. (1) L-arginine, (2) L-tyrosine, (3) L-tryptophan, (4) L-threonine, (5) L-glutamine, (6) L-asparagine, (7) L-valine, (8) L-glutamic acid, (9) L-cysteine and (10) L-aspartic acid.

Table 1: Results of regression analysis on calibration and the detection limits

Analyte	RSDs,% inter-day, intra-day	Regression equation[a]	Correlation coefficient	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
Arg	3.26 3.15	y=142.8309x-52.9897	0.9998	3.5-55.0	0.15	0.59
Tyr	1.78 1.65	y=319.7722x-51.0454	0.9991	1.5-25.0	0.11	0.34
Trp	3.53 3.64	y=34.3465x-36.4917	0.9996	4.5-135.0	0.21	0.61
Thr	2.84 2.76	y=16.6427x-33.7297	0.9995	1.5-225.0	0.37	1.01
Gln	2.95 2.87	y=84.4245x-146.8750	0.9998	4.5-135.0	0.14	0.47
Asn	2.12 2.35	y=151.6720x-33.6943	0.9997	5.0-75.0	0.13	0.45
Val	3.78 3.56	y=38.5794x-8.5129	0.9990	3.5-55.0	0.20	0.61
Glu	1.95 2.13	y=36.1361x-48.7875	0.9996	5.0-200.0	0.21	0.65
Cys	2.63 2.66	y=63.8763x-111.5010	0.9982	5.0-115.0	0.19	0.55
Asp	2.37 2.54	y=33.9804x+0.2209	0.9983	4.5-150.0	0.22	0.68

[a] x, concentration of L-amino acids (μg mL^{-1}) and y, peak area (Au.min).

between 1.5 and 225 μg mL^{-1}. The calibration curves exhibited good linear behavior over the concentration range with correlation coefficients (r) ranging between 0.9982 and 0.9998. The detection limits (LOD) were evaluated using a signal-to-noise ratio (S/N) of 3. In this experimengt, the detection limits for the ten analytes ranged from 0.11 to 0.37 μg mL^{-1}.

Stability

The stability of the method was evaluated using the relative standard deviation (RSDs) of peak area and migration time by taking the same standard mixture solution for determination at 0, 6, 12, 24, 48 h, respectively. The RSDs of peak area and migration time values were 2.5% and 4.8% for L-Arg, 3.4% and 4.5% L-Tyr, 3.2% and 4.7% for L-Trp, 2.4% and 3.7% for L-Thr, 3.1% and 3.7% for L-Gln, 2.1% and 3.8% for L-Asn, 2.9% and 4.4% for L-Val, 2.6% and 3.9% for L-Glu, 3.5% and 3.3% for L-Cys, 2.4% and 4.4% for L-Asp. From the results, the stability RSDs values of the ten amino acids were all below 5.0%, which indicated the good stability of the method.

Reproducibility

Under optimal conditions, the standard mixture solution was repeatedly injected 5 times to evaluate the reproducibility of the peak areas and migration time. The RSDs of peak area and migration time were 2.9% and 3.8% for L-Arg, 1.4% and 2.5% L-Tyr, 3.2% and 4.7% for L-Trp, 3.4% and 1.7% for L-Thr, 3.2% and 2.7% for L-Gln, 3.1% and 1.7% for L-Asn, 2.9% and 3.8% for L-Val, 2.2% and 1.9% for L-Glu, 3.2% and 2.3% for L-Cys, 3.4% and 1.4% for L-Asp. The high reproducibility indicated that the system was stable, and this method was dependable.

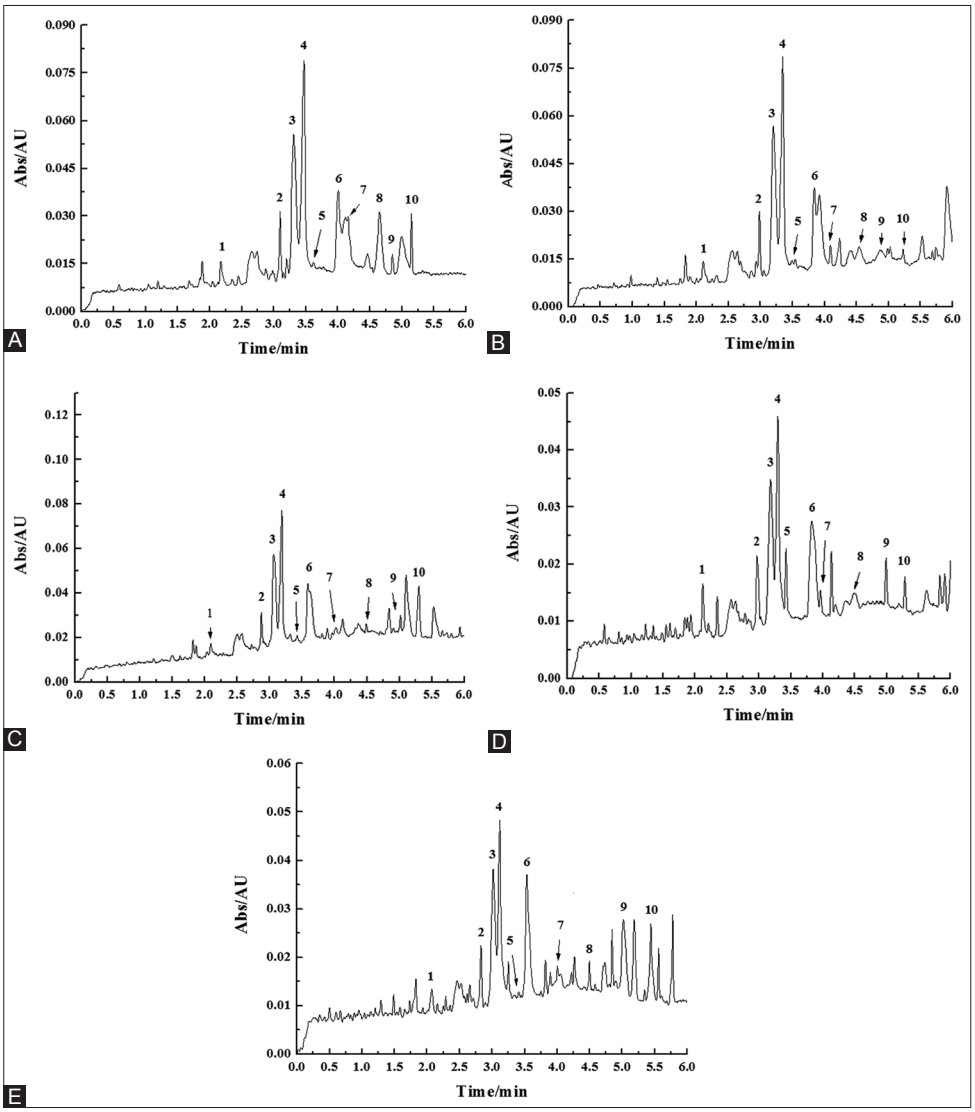

Fig 5. Electrophoregrams of 5.0 mg L^{-1} Jujube in (A) Shandong, (B) Shanxi, (C) Shaanxi, (D) Gansu, (E) Xingjiang. The conditions were the same as those in Fig. 3. (1) L-arginine, (2) L-tyrosine, (3) L-tryptophan, (4) L-threonine, (5) L-glutamine, (6) L-asparagine, (7) L-valine, (8) L-glutamic acid, (9) L-cysteine and (10) L-aspartic acid.

Analysis of amino acids in jujube of different origins and recoveries

In order to verify the actual effect of the method, the amino acids in jujube samples from five different sources were analyzed by the method under optimal conditions. Comparing the electropherograms of the five actual jujube samples and the electropherogram of the standard amino acid mixture solution and standard addition approach were

Table 2:Determination results of the recovery, accuracy and precision for this method in different origins jujube samples (n=5)

Jujube origin	Analyte	Measured (μg/mL)	Added (μg/mL)	Sample (μg/mL)	Mean recovery (%)[a]	R.S.D (%)[b]	Accuracy (%)	Precision (CV%)
Shandong	Arg	5.46	5.3	10.62	93.49	3.45	8.12	6.38
	Tyr	4.30	4.1	8.38	99.20	3.08	11.39	9.46
	Trp	6.01	5.9	11.71	96.30	7.29	13.29	3.01
	Thr	11.35	10.4	20.76	90.29	3.70	11.25	3.79
	Gln	9.88	8.9	19.14	103.28	7.49	3.04	5.25
	Asn	6.90	7.1	13.85	97.41	5.20	9.29	7.29
	Val	7.04	6.8	13.39	92.59	8.30	4.20	2.49
	Glu	14.16	13.2	26.51	93.49	9.19	5.73	5.02
	Cys	12.70	11.5	24.86	104.83	5.19	9.24	6.10
	Asp	6.79	6.5	12.25	96.29	3.15	4,20	7.10
Shanxi	Arg	7.57	7.4	14.94	92.20	4.73	5.32	7.30
	Tyr	4.44	4.3	8.79	90.39	5.14	8.51	8.17
	Trp	6.43	6.4	12.79	94.89	8.20	10.55	5.12
	Thr	6.12	6.1	12.23	102.92	6.68	4,04	7.71
	Gln	5.34	5.3	10.69	95.43	9.74	4.72	3.04
	Asn	4.17	4.2	8.33	97.91	3.91	11.63	9.46
	Val	7.49	7.4	14.93	93.76	7.73	8.39	8.14
	Glu	4.36	3.9	7.01	94.85	9.25	1.80	6.17
	Cys	5.96	5.8	10.86	102.23	6.11	3.33	7.10
	Asp	7.12	7.0	14.25	95.10	11.10	5.14	9.04
Shaanxi	Arg	4.80	4.7	8.51	97.19	3.82	4.89	8.20
	Tyr	6.28	6.2	12.50	95.20	10.24	4.29	11.23
	Trp	5.75	5.6	10.22	90.98	9.14	7.98	4.22
	Thr	4.45	4.3	8.65	92.92	5.23	3.78	6.44
	Gln	5.56	5.5	10.16	101.29	4.04	9.07	8.72
	Asn	6.28	6.2	12.51	93.84	9.22	10.32	7.25
	Val	5.96	5.8	10.84	102.48	3.40	6.22	4.71
	Glu	8.73	8.5	16.15	93.41	3.49	10.32	3.66
	Cys	7.42	7.4	14.82	98.10	5.71	9.31	5.99
	Asp	6.66	6.4	12.10	90.17	8.92	11.04	8.05
Gansu	Arg	7.26	7.1	14.23	93.25	4.95	4.49	3.24
	Tyr	5.94	5.8	10.73	93.10	5.19	7.29	4.23
	Trp	6.32	6.2	12.61	101.35	10.60	8.22	6.10
	Thr	7.44	7.3	14.87	105.32	5.29	4.77	5.29
	Gln	8.52	8.5	16.00	95.33	4.11	9.53	11.49
	Asn	8.11	8.1	16.20	90.33	9.71	11.95	6.77
	Val	9.32	9.2	18.60	99.13	3.14	2.55	3.19
	Glu	5.66	5.5	10.22	104.04	11.59	11.45	6.40
	Cys	6.28	6.3	12.58	94.22	7.15	8.19	8.48
	Asp	8.30	8.2	16.56	91.26	5.14	8.13	4.16
Xinjiang	Arg	8.01	8.2	15.06	94.74	6.39	10.48	7.45
	Tyr	5.92	5.8	10.78	103.24	5.39	6.20	4.29
	Trp	6.27	6.1	12.19	91.32	3.82	5.32	9.32
	Thr	5.36	5.0	10.21	92.12	5.37	10.33	6.34
	Gln	8.53	8.6	16.24	102.48	2.48	9.24	10.45
	Asn	6.54	6.3	12.74	94.55	10.25	15.30	8.20
	Val	7.65	7.4	14.96	91.37	4.22	6.92	6.55
	Glu	9.22	9.0	18.29	103.46	3.21	4.33	4.44
	Cys	5.57	5.1	10.63	97.98	8.56	5.30	3.49
	Asp	6.11	6.9	12.99	94.58	8.26	7.13	7.20

used for further demonstration. Fig. 4 and Fig. 5 showed the electropherograms of standard amino acids and actual jujube samples, respectively. From the experiments results, it was found that the different active ingredients were included in all the samples and it is clear that the contents of these ten amino acids in jujube from different sources are different. The total amount of amino acids is highest in Shandong jujube and lowest in Shanxi which is similar with Shaanxi Gansu is similar with Xinjiang. This result demonstrates that the amount of amino acids maybe depend on the soil and climate conditions, and it is subject to geographical distribution of the five provinces. Located in the eastern coast of China, Shandong Province has the most suitable climate and soil conditions for plant growth. Shanxi Province is located in the inland of central China, which is adjacent to Shaanxi Province. So their climate and soil are similar. Gansu Province is located in the inland of western China, adjacent to Xinjiang. Furthermore, the results of the experiment may show that the ten amino acids can be as supplement of quality control standards for jujube. Compared with other reports, this developed method is simple and fast for amino acids determination, especially for Gln, Tyr, Asn analysis and the limit of detection is lower than related literature (Lin et al. 2013; Souza Crespo et al. 2015).

The ten OPA-amino acids were accurately added to the actual samples for recovery experiments. Table 2 listed the average recoveries, RSDs, accuracy and precision for the ten analytes in different samples. From the experimental data, it is obvious that the method has high recovery and good precision for the analysis of 10 amino acids in the actual sample.

CONCLUSION

In summary, a simple CE method using β-cyclodextrin (β-CD) as additive for the determination of ten kinds of amino acids in five different habitats jujube was developed in our work. Excellent separation of ten kinds of amino acids was achieved within less than 6 min by optimizing separation conditions such as pH, electrolyte concentration, β-CD, applied voltage, injection time and the UV detection wavelength. The method exhibited good repeatability (RSD<3.4%; n=5) and intermediate precision (RSD<4.7%; n=5). For the determination of real samples of five origins in China by this method, the results demonstrate that the total contents of amino acids are distinguishing to different origins, which may be subject to geographical distribution of the five provinces, especially, climate and soil conditions. The study can serve as a reference for the quality control of jujube and design of novel jujube products, and provide further supplement for the quantity control of jujube.

ACKNOWLEDGEMENT

This work is supported by the National Natural Science Foundation of China (Nos. 21675163, 21405161), and the top priority program of "One-Three-Five" Strategic Planning of Lanzhou Institute of Chemical Physics, CAS.

REFERENCES

Ahmad, B., I. Khan, S. Bashir, S. Azam and F. Hussain. 2011. Screening of zizyphus jujuba for antibacterial, phytotoxic and haemagglutination activities. Afr. J. Biotechnol. 10: 2514-2519.

Benammar, C., A. Hichami, A. Yessoufou, A. M. Simonin, M. Belarbi, H. Allali and N. A. Khan. 2010. *Zizyphus lotus* L. (Desf.) modulates antioxidant activity and human T-cell proliferation. BMC Complement. Altern. Med. 10: 54-63.

Borisov, Y. A. and S. S. Kiselev. 2016. Structure and energy of formation of beta and gamma cyclodextrin complexes with amino acid enantiomers. Russ. J. Phys. Chem. A 90: 1822-1827.

Cheng, N., B. Du, Y. Wang, H. Gao, W. Cao, J. Zheng and F. Feng. 2014. Antioxidant properties of jujube honey and its protective effects against chronic alcohol-induced liver damage in mice. Food Funct. 5: 900-908.

Choi, S. H., J. B. Ahn, N. Kozukue, C. E. Levin and M. Friedman. 2011. Distribution of free amino acids, flavonoids, total phenolics, and antioxidative activities of Jujube (*Ziziphus jujuba*) fruits and seeds harvested from plants grown in Korea. J. Agric. Food Chem. 59: 6594-6604.

Erbas, M., M. F. Ertugay, M. O. Erbas and M. Certel. 2005. The effect of fermentation and storage on free amino acids of tarhana. Int. J. Food Sci. Nutr. 56: 349-358.

Fang, Z., O. U. Jun, Y. F. Huang, Q. Li, G. Z. Xu, Z. Z. Liu and S. H. Yang. 2014. Determination of 21 free amino acids in fruit juices by HPLC using a modification of the 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) method. Food Anal. Methods. 8: 428-437.

Faserl, K., L. Kremser, M. Muller, D. Teis and H. H. Lindner. 2015. Quantitative proteomics using ultralow flow capillary electrophoresis-mass spectrometry. Anal. Chem. 87: 4633-4640.

Filik, H. and G. Çetintaş. 2012. Determination of sulfite in water and dried fruit samples by dispersive liquid-liquid microextraction combined with uv-vis fiber optic linear array spectrophotometry. Food Anal. Methods. 5: 1362-1367.

Frost, N. W., M. Jing and M. T. Bowser. 2010. Capillary electrophoresis. Anal. Chem. 82: 4682-4698.

Geiger, M., A. L. Hogerton and M. T. Bowser. 2012. Capillary electrophoresis. Anal. Chem. 84: 577-596.

Glombitza, K. W., G. H. Mahran, Y. W. Mirhom, K. G. Michel and T. K. Motawi. 1994. Hypoglycemic and antihyperglycemic effects of *Ziziphus spina-christi* in rats. Planta Med. 60: 244-247.

Guo, S., J. A. Duan, D. Qian, Y. P. Tang, Y. F. Qian, D. W. Wu, S. L. Su and E. X. Shang. 2013. Rapid determination of amino acids in fruits of *Ziziphus jujuba* by hydrophilic interaction ultra-high-performance liquid chromatography coupled with triple-quadrupole mass spectrometry. J. Agric. Food Chem. 61: 2709-2719.

Jeong, J. S., S. K. Kim and S. R. Park. 2013. Amino acid analysis of dried blood spots for diagnosis of phenylketonuria using capillary electrophoresis-mass spectrometry equipped with a sheathless electrospray ionization interface. Anal. Bioanal.

Chem. 405: 8063-8072.

Jiang, J. G., X. J. Huang, J. Chen and Q. S. Lin. 2007. Comparison of the sedative and hypnotic effects of flavonoids, saponins, and polysaccharides extracted from Semen *Ziziphus jujube*. Nat. Prod. Res. 21: 310-320.

Li, J. W., S. D. Ding and L. Ding. 2007. Optimization of the ultrasonically assisted extraction of polysaccharides from *Zizyphus jujuba* Cv. Jinsixiaozao. J. Food Eng. 80: 176-183.

Li, X. G. 2003. Research on status of jujube region in north Shanxi. J. Northwest For. Univ. 18: 80-83.

Li, X. G., J. Huang and W. H. Gao. 2005. A study on high-quality production regions of dry Chinese jujube in China. J. Fruit Sci. 22: 620-625.

Lin, Q. B., L. L. Che, J. Guo and R. Z. Wang. 2013. Use of 4-chloro-3, 5-dinitrobenzotrifluoride (CNBF) Derivatization and ultrahigh-performance liquid chromatography tandem mass spectrometry for the determination of 20 free amino acids in Chinese jujube date. Food Anal. Methods. 7: 571-579.

Liu, C. C., X. Feng, H. L. Qian, G. Z. Fang and S. Wang. 2014. Determination of norfloxacin in food by capillary electrophoresis immunoassay with laser-induced fluorescence detector. Food Anal. Methods. 8: 596-603.

Meussen, B. J., A. N. T. van Zeeland, M. E. Bruins and J. P. M. Sanders. 2013. A fast and accurate UPLC method for analysis of proteinogenic amino acids. Food Anal. Methods. 7: 1047-1055.

Ming, T. Y., J. J. Li, P. Huo, Y. L. Wei and X. H. Chen. 2014. Analysis of free amino acids in *Russula griseocarnosa* harvested at different stages of maturity using iTRAQ®-LC-MS/MS. Food Anal. Methods. 7: 1816-1823.

Roy, M. N., A. Roy and S. Saha. 2016. Probing inclusion complexes of cyclodextrins with amino acids by physicochemical approach. Carbohydrate Polym. 151: 458-466.

Shi, H. M., Z. R. Yin and W. Q. Jiang. 2006. Determination of cyclic adenosine-3,5-monophosphate in golden silk jujube by high performance chromatography. Food Sci. 27: 216-218.

Souza, C. I. C., M. T. Resende, A. D. P. Netto and F. F. C. Marques. 2015. Capillary zone electrophoresis method for the direct determination of amino acids in recombinant human erythropoietin preparations used for the treatment of anemia. Electrophoresis. 36: 1179-1185.

Viadel, B., A. Alegria, R. Farre, P. Abellan and F. Romero. 2000. Amino acid profile of milk-based infant formulas. Int. J. Food Sci. Nutr. 51: 367-372.

Zhao, J., S. P. Li, F. Q. Yang, P. Li and Y. T. Wang. 2006. Simultaneous determination of saponins and fatty acids in *Ziziphus jujuba* (Suanzaoren) by high performance liquid chromatography-evaporative light scattering detection and pressurized liquid extraction. J. Chromatogr. A. 1108: 188-194.

Zhao, M., Y. Ma, L. L. Dai, D. L. Zhang, J. H. Li, W. X. Yuan, Y. L. Li and H. J. Zhou. 2012. A high-performance liquid chromatographic method for simultaneous determination of 21 free amino acids in tea. Food Anal. Methods. 6: 69-75.