

Amino Acids in Callus Derived from Leaves, Leaf-Derived Callus and Fruits of Muzao Jujubes (*Ziziphus jujuba* Mill var *muzao*)

CHEN Zong-li^{1,2,3}, HE Xiao-long^{1,2,3}, ZHANG Xiang-qian^{1,2,3}, ZHAO Man-xing^{1,2,3}, XUE Bi-rong¹

(1. Shaanxi Key Laboratory of Chinese Jujube (Yan'an University), Yan'an 716000, China;

2. Shaanxi Engineering and Technological Research Center for Conservation and Utilization of Regional Biological Resources, Yan'an 716000, China; 3. College of Life Science, Yan'an University, Yan'an 716000, China)

Abstract: This study was performed to determine the content and constituents of amino acids in tree leaves, leaves of *in vitro* plantlets, fruits, and leaf-derived callus from Muzao jujube (*Ziziphus jujuba* Mill var *muzao*). Amino acids were extracted by the hydrochloric acid hydrolysis method and were determined on an automatic amino acid analyzer. From each type of tissues, 16 amino acids were identified, consisting of 7 essential amino acids for human nutrients, 2 essential amino acids for children, 9 medicinal amino acids, 6 aromatic amino acids, 4 umami tasty amino acids, 4 sweetness amino acids and 3 branched amino acids. The highest content of total amino acid content was leaves from *in vitro* plantlets (77.14 mg/100 g), next was in the callus (11.52 mg/100 g) and in the tree leaves (11.10 mg/100 g), and the lowest content of total amino acid content was in fruits (4.59 mg/100 g). Leaves from *in vitro* plantlets had the highest mass fraction (in total amino acid) of essential amino acids (43.21%), that in turn was the tree leaves (30.45%), the calli (25.26%), and the fruits (17.21%). The order of percent of amino acids of medicinal functions was: the calli (72.05%) > tree leaves (71.89%) > leaves from *in vitro* plantlets (65.35%) > the fruits (32.03%). Leaves from *in vitro* plantlets were the richest in aromatic amino acids (43.60%) followed by calli (33.33%) and tree leaves (30.54%) and jujube fruits (13.73%). and fruits had the highest the ratio of BCAA/AAA (55.56%), the lowest ratio of BCAA/AAA was found in leaves from *in vitro* plantlets (36.72%), tree leaves (43.59%) and leaves from *in vitro* plantlets (52.04%) also had the higher ratios. This study has confirmed that each of the four types of tissues contained the complete set of 16 amino acid species. In addition to being rich in amino acids, the contents of medicinal amino acids and tasty amino acids are higher than those of common vegetables and fruits. Additionally, different total amino acid contents were found among the different tissue types. Tissue-cultured leaves, leaves of cultured plants and leaf-derived calli presented increases in total amino acid content by 116.81-, 2.42-, and 2.51-fold and in essential amino acid content by 42.19-, 4.28- and 3.68-fold as compared to jujube fruits. This study demonstrates that jujube fruits and leaves as well as leaf-derived calli all have promising development prospects.

Key words: Muzao jujube; leaves of jujube trees; callus; amino acids

木枣叶片、叶片愈伤组织及果实的氨基酸分析

陈宗礼^{1,2,3}, 贺晓龙^{1,2,3}, 张向前^{1,2,3}, 赵满兴^{1,2,3}, 薛碧荣¹

(1.陕西省红枣重点实验室(延安大学), 陕西 延安 716000; 2.陕西省区域生物资源保育与利用工程技术研究中心, 陕西 延安 716000; 3.延安大学生命科学学院, 陕西 延安 716000)

摘 要: 测定枣叶、枣果实和愈伤组织中的氨基酸组成及其含量, 为深入研究和开发利用中国特有枣树资源提供科学依据。以中国陕北特产的栽培木枣和组培木枣的叶片、枣果及叶片培养诱导的愈伤组织为材料, 采用盐酸水解法, 利用氨基酸分析仪对其所含氨基酸进行定性、定量分析。分析结果显示, 各试验材料中均含有至少16种氨基酸。其中, 有7种人体必需氨基酸、2种儿童必需氨基酸、9种药效氨基酸、6种芳香族氨基酸、4种鲜味氨基酸、4种甜味氨基酸和3种支链氨基酸。它们中的总氨基酸含量以组培枣叶中最高(77.14mg/100g), 其次为愈伤组

收稿日期: 2012-03-13

基金项目: 陕西省科技统筹创新工程计划项目(2011HBGC-21); 陕西省科技发展计划项目(2009K01-09);

陕西省高水平大学建设专项(2012SXTS06); 延安市科技计划项目(2009KN-25); 延安大学重点科研项目(YDKY2010003)

作者简介: 陈宗礼(1954—), 男, 教授, 本科, 主要从事红枣遗传育种与组织培养研究。E-mail: zongli_chen@yahoo.com.cn

织(11.52mg/100g),栽培枣叶中为11.10mg/100g,枣果中含量最低(4.59mg/100g);支链氨基酸的质量分数大小次序为:组培枣叶>栽培枣叶>愈伤组织>枣果;药效氨基酸占的比例以愈伤组织最高(72.05%),依次为栽培枣叶(71.89%)、组培枣叶(65.35%)、枣果(32.03%);芳香族氨基酸占的比例以组培枣叶最高(43.60%),依次为愈伤组织(33.33%)、栽培枣叶(30.54%)、枣果(13.73%);支/芳比值以枣果中最高(55.56%),组培枣叶中最低(36.72%),栽培枣叶(43.59%)和愈伤组织(52.04%)也含有较高的比例。木枣叶片、枣果及愈伤组织中均含有相同组分的16种氨基酸,它们含量丰富,尤以药用氨基酸和味觉氨基酸的含量较高。但它们的总氨基酸含量差别较大,其中,组培枣叶、栽培枣叶和叶片愈伤组织中的氨基酸总量分别是枣果的116.81、2.42和2.51倍,必需氨基酸总量分别是枣果的42.19、4.28倍和3.68倍。说明,不仅枣果,枣叶片和组培获得的愈伤组织亦有很好的开发利用价值。

关键词: 木枣; 叶片; 枣叶片愈伤组织; 氨基酸含量

中图分类号: S665.1

文献标志码: A

文章编号: 1002-6630(2013)10-0197-07

doi:10.7506/spkx1002-6630-201310043

Chinese date, also known as jujube, (*Zizyphus jujuba* Mill) belongs to genus *Zizyphus* Mill in the family Rhamnace. Originated in the middle to lower reaches of Yellow River, the species has been cultivated for more than 7000 years^[1] in China. According to the historical records, the most popular and unique variety ‘Big Chinese Date’ in Northern China was on the list of “Five Nuts”, which are peaches, apricot, plum, chestnuts and Chinese date.

Each part of a jujube tree can be utilized for human benefit. Since ancient times many different ways have been attempted and researched on how to wisely use those resources. The sweet and nutritious fruits have medicinal values including nourishing yin and strengthening the kidney, regulating the liver, relaxing blood vein, promoting appetite, invigorating the spleen, thus promoting health, etc. The medicinal and health-care functions were recorded as early as in the 3rd century B.C in the book *Ming Yi Bie Lu*. Later in another book titled *Shennung Ben Ts’ao King* from the 6th century B.C, it was highly recommended as a medicinal fruit. Books on “herbs” through the history all have an entry of Chinese date^[2].

Recent studies are mostly on germplasm, propagation and breeding, cultivation and management, and processing and utilization. Those researches occurred mainly in China, few in other countries. Contents of proteins, minerals, lipids, sugars, vitamins, flavonoids, saponin, cyclic nucleotides, terpenoid, and steroid, phenolic compound^[2-4] were measured. The objective of this study was to determine amino acid content and composition in tree leaves, fruits and callus tissues, in order to propose the potential application in nutrition and health care of Chinese date and products.

1 Materials and Methods

1.1 Materials

Fresh leaves from six-year old trees contained 46% of water. Leaf tissues were collected from the Chinese Date Tree Repository in the Red Date Key Laboratory of Yan’an University, Shaanxi Province in August, 2010. Water content of leaves from *in vitro* plantlets was 89%, and 91% in calli. These samples were propagated as described previously^[5]. Fresh fruit water content was 31.2%.

1.2 Instrumentation

Instruments used in this study included: a L-8900 automatic amino acid analyzer (Hitachi, Japan), a R170 ultralow freezer (Thermo, Germany), a LT-105 freeze-drier (Christ, Germany), a high-speed all-purpose micro-grinder (Tianjin City Taisite Instrument Co. Ltd), a JY96-II ultrasound cell grinder (Ningbo Scientz Biotechnology Co. Ltd.), 80 sieve standard meshes (Dailheng analytical instrument manufactory, Shagyu, Zhejiang); a RE-52B rotary evaporator (Shanghai Yarong Biochemical Instrument Factory); a XH-1050 refrigerated circulator (Beijing Boyikang Laboratory Apparatus Co. Ltd.); a Yuha water circulation vacuum pump (Gongyi Yuha Instrument Co. Ltd), and a 101-Electric heating air-blowing oven (Beijing KeweiYongxing instrument limited company).

1.3 Reagents

Reagents used for the analysis were amino acid standard H at a concentration 2 nmol/L and super-pure grade ninhydrin reagent reaction solution (Wako Pure Chemical Industries, Ltd. Wako), and L-8500 PH-KIT buffer (Mitsubishi Chemical Corporation); Grade A pure hydrochloric acid (Tianjin Kermel Chemical Reagent Co. Ltd.).

1.4 Pretreatment of samples

Fruits, leaves from trees and *in vitro* plantlets, and calli

were washed in double distilled water, air-dried to remove water on the surface, and cut into small, thin pieces. After prechilled in a $-80\text{ }^{\circ}\text{C}$ freezer for 5h, samples were dried to constant weight in a freezer drier, and then ground into a fine power. Materials passing through an 80 sieve mesh were stored in amber -bottles, labeled and stored at $4\text{ }^{\circ}\text{C}$.

1.5 Preparation of samples for amino acid analysis

Dry sample powder was weighed with an accuracy specified in Table 1, and mixed into HCl (6 mol/L) in a beaker. After sonication at 100 W and 50 kHz for 30 min, samples were transferred into volumetric flasks. The flask was filled with nitrogen gas, sealed with the flask cap, and then placed into a drier to proceed to the hydrolysis reaction at $(110\pm 1)\text{ }^{\circ}\text{C}$ for 24 h.

After completion of hydrolysis, samples were evaporated to complete dryness using a rotary evaporator. Then, diluted HCl (0.02 mol/L) was added into each flask in the amount as described in Table 1. After being mixed thoroughly, the hydrolysate was filtered through a $0.45\text{ }\mu\text{m}$ filter.

The filtrates were diluted with ultra-pure deionized water to a final concentration as recorded in Table 1. After removal of color from samples with C_{18} column, the elutes were filtered through a $0.22\text{ }\mu\text{m}$ filter into sample vials. The filtrate was further diluted, and the dilution factor of each sample was determined based on the content of cellulosic materials and pectin^[6].

Table 1 Preparation of hydrolyzed protein solution from tissues of Chinese Date

Type of samples	Quantity added /mg	HCl for hydrolysis reaction (6 mol/L HCl) /mL	Diluted HCl (0.02 mol/L) to solubilize hydrolysate sample/mL	Ultrapure deionized water for filtrate dilution/mL
Fruit powder	500	40	80	320
Tree leaves	500	40	80	320
<i>In vitro</i> plantlet leaves	500	50	100	400
Leaf-derived calli	2000	100	200	800

1.6 Sample injection and analysis

The hydrolysate sample from each tissue and the amino acid standard solution were injected into the instrument each in a volume of $20\text{ }\mu\text{L}$. The instrument was installed with a Hitachi 2622SC-PH ion exchange chromatography column of $4.6\text{ mm}\times 60\text{ mm}$, packed with the Hitachi $3\text{ }\mu\text{m}$ sulfonic acid ion exchange resin. The conditions employed for amino acid analysis were: column temperature: $57\text{ }^{\circ}\text{C}$, reaction unit temperature: $135\text{ }^{\circ}\text{C}$; pump I (buffer) flow rate: 0.4 mL/min , pump pressure: $0.3\sim 25\text{ MPa}$; pump II (reaction solution) flow rate: 0.35 mL/min , pump pressure: $0\sim 0.2\text{ MPa}$; the detection wavelengths: channel one at 570 nm and channel two at 440 nm ; sample inlet volume: $20\text{ }\mu\text{L/min}$. The time

length of analysis: channel one for 32 min, and channel two for 10min. Data was analyzed using the EZChrom EliteTM chromatography processing system analysis software^[6]. The coefficient of variance (C.V.) of technical replicates of the analytical method was below 0.5%.

1.7 Calculation of amino acid content in each tissue sample

Using the software installed in the instrument, each amino acid in the tissue sample was identified by comparing with the spectrum in the standard. The ESTD method in the software was used to determine the curve integral, and the external standard concentration (ESC, nmol/L) which is the amino acid content in the sample relative to the standard, was used to estimate amino acid content in the tissue sample using the following equation:

$$\text{AAC} = \frac{\text{ESC}}{20} \times n \times 10^{-9} \times 10^3 \times M \times 100$$

Where AAC is amino acid content in the tissue sample/(mg/100 g); ESC is the external standard concentration (nmol/L); n is the dilution factor of the tissue sample; 10^{-9} is to convert nanomolar to molar ($1\text{ nmol}=10^{-9}\text{ mol}$) and 10^3 to convert gram into milligram; M is the molar mass of the amino acid in the tissue sample; 20 is the sample injection volume ($20\text{ }\mu\text{L}$).

2 Results and Analysis

2.1 Content and constituents of amino acids of Chinese Date

Amino acid mapping of standard samples is illustrated in Fig. 1, and the determination composition and contents of the amino acids in Table 2. The results of Table 2 showed that 16 kinds amino acids were all detected in the jujube leaves, tissue cultured jujube leaves, induced callus by ujube leaves culture of the Mu-Zao. Amino acids were eluted in the following order: Asp, Thr, Ser, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, Lys, His, Arg, Pro. The seven essential amino acids for human beings (Thr, Val, Phe, Met, Ile, Leu, and Lys) were identified in all the four types of tissues.

The tissues were rich in total amino acids, but different in the constituents of amino acid species. The content of total amino acids was ranked in descending order as leaf of *in vitro* plantlets>calli>tree leaves>fruits. The total amino acid content in *in vitro* plantlet leaves, calli, and tree leaves was 16.81-, 2.51- and 2.42-fold higher than fruits. Amino acid content in the leaves of *in vitro* plantlets seedlings was 6.95-fold higher than the tree leaves.

When compared at individual amino acid level, leaves of *in vitro* plantlets were high in Leu, Asp and Glu (> 8 mg/100 g), but low in His and Met. Tree leaves contained higher level of Asp (2.69 mg/100 g, with composition mass fraction of 24.23% in total amino acids) followed by Glu, and the last one was Met (Table 2).

Table 2 Composition and content of amino acids in different parts of Chinese date

Amino acids	Mean mass fraction/(mg/100 g)				Composition mass fraction/%			
	Fruits	Leaves of mature trees	Leaves of <i>in vitro</i> plantlets	Calli derived from leaf	Fruits	Leaves of mature trees	Leaves of <i>in vitro</i> plantlets	Calli derived from leaf
Asp ^{cf}	0.70	2.69	8.30	3.30	15.25	24.23	10.76	28.65
Thr ^a	0.08	0.37	3.38	0.37	1.74	3.33	4.38	3.21
Ser ^d	0.09	0.43	2.98	0.49	1.96	3.87	3.86	4.25
Glu ^{cf}	0.16	1.87	8.15	1.14	3.49	16.85	10.57	9.90
Gly ^{cf,g}	0.07	0.45	3.88	0.43	1.53	4.05	5.03	3.73
Ala ^g	0.11	0.47	6.15	0.49	2.40	4.23	7.97	4.25
Val ^{ade}	0.17	0.51	5.11	0.46	3.70	4.59	6.62	3.99
Met ^c	0.08	0.09	1.56	0.05	1.74	0.81	2.02	0.43
Ile ^{ad}	0.06	0.31	3.95	0.32	1.31	2.79	5.12	2.78
Leu ^{ade}	0.12	0.67	8.44	0.63	2.61	6.04	10.94	5.47
Tyr ^{ce}	0.01	0.19	3.72	0.18	0.22	1.71	4.82	1.56
Phe ^{ace}	0.18	0.86	5.15	0.40	3.92	7.75	6.68	3.47
Lys ^{ace}	0.10	0.57	5.74	0.68	2.18	5.14	7.44	5.90
His ^b	0.01	0.2	0.87	0.27	0.22	1.80	1.13	2.34
Arg ^{bce}	0.05	0.59	5.47	1.49	1.09	5.32	7.09	12.93
Pro ^f	2.60	0.83	4.29	0.82	56.64	7.48	5.56	7.12
T	4.59	11.10	77.14	11.52	100.00	100.00	100.00	100.00
Σa	0.79	3.38	33.33	2.91				
Σb	0.06	0.79	6.34	1.76				
Σc	1.47	7.98	50.41	8.30				
Σd	0.35	1.49	17.50	1.41				
Σe	0.63	3.39	33.63	3.84				
Σf	1.04	5.48	26.48	5.36				
Σg	2.87	2.18	17.30	2.23				
Σa/T/%	17.21	30.45	43.21	25.26				
Σb/T/%	1.31	7.12	8.22	15.28				
Σc/T/%	32.03	71.89	65.35	72.05				
Σd/T/%	7.63	13.42	22.69	12.24				
Σe/T/%	13.73	30.54	43.60	33.33				
Σf/T/%	22.66	49.37	34.33	46.53				
Σg/T/%	62.53	19.64	22.43	19.36				
Σd/Σc/%	55.56	43.95	52.04	36.72				

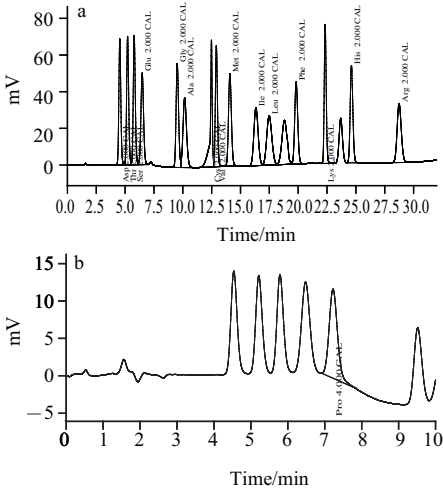
Note: T. the content of total amino acids; a-essential amino acids for human nutrition; b. essential amino acids for children; c. medicinal amino acids; d. branched-chain amino acids; e. aromatic amino acids; f. umami tasty amino acids; g. sweet amino acids; Σa. total essential amino acids; Σb. total essential amino acid for children; Σc. total medicinal amino acids; Σd. total branched-chain amino acids; Σe. total aromatic amino acids; Σf. total umami tasty amino acids; Σg. total sweetness amino acids.

In the fruits, Pro content was the highest at 2.60 mg/100 g, accounting for 56.64% of total amino acids, followed by Asp, and then Phe, Val, Glu. The content of His and Arg was at the lower end.

In the leaf-derived calli, the most abundant amino acid was Asp at 3.30 mg/100 g, which is equal to 28.65% of total amino acid content. Content of Arg and Glu was at the

moderate level, and Met and Tyr was at the lowest level.

In this study, amino acids were extracted using the hydrochloric acid hydrolysis method. During the extraction process, Trp could be broken down and Gln and Asn be converted into Glu and Asp after losing NH₃⁺. This could lead to underestimation of Trp, Gln and Asn content^[7].



a. First channel 570 nm, 32 min; b. Second channel 440 nm, 10 min.

Fig.1 Amino acid mapping of standard samples

2.2 Amino acid constituents in different type of tissues of Chinese Date

2.2.1 Content and proportion of essential amino acids

In different types of tissues, the proportion of amino acids, including the seven essential amino acids for human nutrition (Thr, Val, Met, Ile, Leu, Phe and Lys) and two for children (His and Arg) was analyzed (Table 2). Trp was excluded because of possible hydrolysis during the extraction process. The content of the seven essential amino acids for human health was listed in descending order as the *in vitro* plantlet leaves (33.33 mg/100 g)>tree leaves>leaf calli>fruits. The content of this group of amino acids in the *in vitro* plantlet leaves, tree leaves and calli was 34.36-, 4.28- and 3.68-fold higher than fruits, and in addition leaves of *in vitro* plantlets contained 9.86-fold and 11.45-fold higher in these amino acids compared to tree leaves and calli. The composition mass fraction of essential amino acids in total amino acids was 43.21% in the leaves from *in vitro* plantlets, 30.45% in mature tree leaves, 25.26% in calli, and 17.21% in fruits. Content of essential amino acids for children was also the highest in leaves of *in vitro* plantlets (6.34 mg/100 g), followed by calli, and then the tree leaves, and the lowest in fruits. The content of these amino acids in leaves from *in vitro* plantlets, calli, and tree leaves was 105.67-, 29.33-, and 13.17-fold higher than fruits. The composition mass fraction was calli (15.28%)>*in vitro* plantlet leaves (8.22%)>tree leaves (7.12%)>fruits (1.31%).

2.2.2 Evaluation of the amino acid nutritional value using model spectra for human health

2.2.2.1 Evaluation of amino acid nutrients using the FAO/WHO models

The percentage of Thr, Val, Leu, Ile, Lys, Met + Cys and Phe + Tyr in the four tissue types was compared to the FAO/WHO modified essential amino acids model spectra for human health, version 1973^[8] (Table 3). For leaves of *in vitro* plantlets, the content of Met + Cys was below the standard, while the other essential amino acids content was in excess of human nutrient need. In the tree leaves, the Phe + Tyr content exceeded the standard, but Met + Cys content was far below, Ile was slightly lower, and content of the rest of the amino acids was adequate. The leaf calli contained an adequate level of Lys while the other amino acids were slightly below the standard level (1~0.7 point), and the Met + Cys content was serious deficient. In the fruits, content of all amino acids was below standards in the models. These results indicate that leaves and callus tissues are more valuable resources to be developed into food supplement and animal feed products.

Table 3 The ratio of essential amino acid for human in different tissues of Chinese date and comparison to the model standards

Amino acid/ FAO/WHO model standard	Item	Materials			
		Fruits	Leaves of mature trees	Leaves of <i>in vitro</i> plantlets	Calli derived from leaves
Thr/4.00	MF	1.74	3.33	4.38	3.21
	AAS	0.44	0.83	1.10	0.80
	RC	0.55	1.05	1.38	1.01
Val/5.00	MF	3.70	4.59	6.62	3.99
	AAS	0.74	0.92	1.32	0.80
	RC	0.78	0.97	1.40	0.84
Leu/7.00	MF	2.61	6.04	10.94	5.47
	AAS	0.37	0.86	1.56	0.78
	RC	0.42	0.96	1.75	0.87
Ile/4.00	MF	1.31	2.79	5.12	2.78
	AAS	0.33	0.70	1.28	0.70
	RC	0.44	0.93	1.71	0.93
Lys/5.50	MF	2.18	5.14	7.44	5.90
	AAS	0.40	0.93	1.35	1.07
	RC	0.42	1.00	1.44	1.14
Phe+Tyr/6.00	MF	4.14	9.46	11.50	5.03
	AAS	0.69	1.58	1.92	0.84
	RC	0.55	1.26	1.53	0.67
Met+Cys*/3.50	MF	1.74	0.81	2.02	0.43
	AAS	0.50	0.23	0.58	0.12
	RC	1.39	0.65	1.62	0.34

Note: MF. Mass fraction of amino acid; AAS. Amino acid score; RC. Ratio coefficient of amino acid. RC = Ratio of amino acid (RAA)/Mean ratio coefficient of amino acid; RAA = Content of amino acid in the sample / Content of amino acid of FAO model standard. *. Cys was not detected, only Met content was listed.

2.2.2.2 The relative ratio coefficient (RC) of essential amino acids compared to the model standards

A protein's quality for human consumption is determined by its amino acid composition. The closer the essential amino acid constituents to human nutrient needs, the better the quality. The relative ratio coefficient of essential amino acids (RC) was calculated using the ratio of essential amino acids contained in a sample (the RAA)^[9-10]. Results in Table 3 shows that all the amino acid species in the leaves of *in vitro* plantlets were at excessive levels. In the tree leaves, Thr and Lys met the standard, Phe + Tyr was in excess, Met + Cys were the first limiting amino acids, and the rest of the amino acids basically was at the adequate levels. In the fruits, Met + Cys content was too high, whereas the others were too low, and Leu was the first limiting amino acid. In calli, Thr met the standard level, Ile was within the range, Lys was slightly too high, and Val, Leu was slightly too low, Phe + Tyr was moderately low, and Met + Cys were the limiting amino acids.

2.3 The medicinal amino acids and their specific functions

The amino acids with putative medicinal functions include Asp, Glu, Gly, Met, Leu, Tyr, Phe, Lys and Arg nine species^[11]. In general, they have been recognized for some health-care functions for human or animals. From Table 2, it can be seen that Chinese date tree leaves, leaves from *in vitro* plantlets, fruits, and calli each contained 7.98, 50.41, 1.47, 30 mg/100 g of medicinal amino acids, which is 71.89%, 65.35%, 32.03% and 72.05% of the total mass from the 16 amino acids in the respective tissues. The ratio for medicinal amino acids is quite high, especially the tree leaves and callus tissues^[12-16].

Additionally, Pro has been found to have a unique function in human body: it is effective in treating scald, mal-nutrient status and acute gastrointestinal diseases. It is included in the protein supplement for patients during surgery aftercare^[17].

In tree leaves, leaves from *in vitro* plantlet, fruits and calli, the proline content was 0.83, 4.29, 2.60 mg/100 g and 0.82 mg/100 g, respectively, which equals to the composition mass fraction of 7.48%, 5.56%, 56.64% and 7.12% of the total 16 amino acids in the respective tissues. Especially in the fruit, composition mass fraction of proline was 56.64% of the total amino acids, which is rarely seen in any other fruit species.

In summary, every part of Chinese date contains very high level of functional amino acids, therefore these resources are recommended to be developed into some types of health-care products.

2.4 The ratio of branched-chain amino acids and aromatic amino acids

The three branched-chain amino acids (BCAA) are Val, Leu and Ile, and the three aromatic amino acids (AAA) are Tyr, Phe and Tryptophan^[18]. From Table 2, it can be seen in tree leaves, leaves from *in vitro* plantlets, fruits and calli, the BCAA content was 1.49, 17.50, 0.35 mg/100 g and 1.41 mg/100 g compared to 3.39, 33.63, 0.63 mg/100 g and 3.84 mg/100 g of AAA, the ratio of BCAA/AAA ratio in the respective tissue was 43.95%, 52.04%, 55.56% and 36.72%. The high BCAA/AAA ratio (>50%) in fruits and leaves from *in vitro* plantlets indicate that these materials have better medicinal value in this field.

2.5 Tasty amino acid content

Tasty amino acids include Asp, Glu, Ala, Gly four species, they bring into the food freshness and crispy taste. Content of these amino acids affects food flavor^[19-20]. Results in Table 2 show that tree leaves, leaves from *in vitro* plantlets, fruits and calli each contained 49.37%, 34.33%, 22.66% and 46.53% (in total amino acids) of the tasty amino acids. The high percentage, especially in the tree leaves and calli, suggests that these two types of tissues should be investigated to produce condiments that add freshness to foods.

Four amino acids, Ala, Gly, Pro, Ser, give a sweetness taste. When added into the food, they would enhance the sweetness taste^[21]. The Chinese date tree leaves, leaves from *in vitro* plantlets, fruits and calli contained 19.64%, 22.43%, 62.53% and 19.36% of those amino acids. The high concentration in fruits suggests that they should be very effective in enhancing the sweat taste when added into foods.

The six aromatic amino acids, Val, Leu, Tyr, Phe, Lys, and Arg six species can enhance aroma, and these amino acids affect the smell of foods^[11]. The aromatic amino acid content in tree leaves, leaves from *in vitro* plantlets, fruits and calli, was 30.54%, 43.50%, 13.73% and 33.33%, respectively. The mass fraction of these amino acids are very high in these tissues. Furthermore leaves and calli contained much higher amount of aromatic amino acids compared to fruits, therefore, they should be considered as the priority in selection of materials for flavor enhancement.

Conclusively, each part of the Chinese date contains different amino acids that give off different tastes and flavors. Products made from these materials should have properties of enhancing appetite as well as nutritious values. They can be used as food additives, freshness preservatives, condiments, and nutrient supplements, where a comprehensive plan should be made for utilizing these resources.

3 Conclusion

Results from this study indicate that in Chinese Date, tree leaves, leaves from *in vitro* plantlets, fruits, and calli each contains at least 16 amino acids that have nutritional value for humans^[5]. Among the 16 amino acid species, seven are essential for human health, two are required for children development, nine have medicinal values, six are aromatic amino acids, and four are responsible for the freshness-taste and four for the sweetness taste, and 3 branched-chain amino acids. The highest content of total amino acid content was leaves from *in vitro* plantlets (77.14 mg/100 g), next were in the calli (11.52 mg/100 g) and in the tree leaves (11.10 mg/100 g), and the lowest content of total amino acid content was in the fruits (4.59 mg/100 g). Leaves from *in vitro* plantlets had the highest the mass fraction (in total amino acid) of essential amino acids (43.21%), that in turn was the tree leaves(30.45%), the calli (25.26%) , and the fruits(17.21%). The order of percent of amino acids of medicinal functions was: the calli (72.05%)>tree leaves (71.89%)>leaves from *in vitro* plantlets (65.35%)>the fruits (32.03%). The highest percent of aromatic amino acids was leaves from *in vitro* plantlets (43.60%), next were the calli (33.33%) and tree leaves (30.54%), and the lowest was fruits (13.73%). Fruits had the highest the ratio of BCAA/AAA (55.56%), the lowest ratio of BCAA/AAA was found in leaves from *in vitro* plantlets (36.72%), tree leaves (43.59%) and leaves from *in vitro* plantlets (52.04%) also had the higher ratios. Due to the high content of these amino acids, Chinese date should be used as high-value materials in food and medicinal industries.

Based on the composition mass fraction of each amino acid, fruits contained more species rendering the sweatiness taste, the ratio for the medicinal amino acids and the mass ratio of BCAA and the ratio of BCAA/AAA were also higher, but the content of tasty amino acids and essential amino acid was not high. Because of such amino acid spectra, fruits are sweet and aromatic, also nourishing and health-promoting, and are superior materials to be developed to functional health care food supplement.

Very few species of foods and medicinal products are prepared using raw materials from this species. Furthermore, no information can be found on the extraction of the effective components, the utilization of leaf and calli has not even started. Therefore, more researches are needed to develop Chinese date into a unique value-added marketable product.

References:

- [1] LIU Mengjun, ZHAO Zhihui. Germplasm resources and production of jujube in China[J]. J Acta Horticulturae, 2009, 36(9): 25-31.
- [2] GUO Yuxin, SHAN Gonghua. Chinese jujuba[M]. Shanghai: Shanghai Scientific and Technical Publishers, 2010: 3-7.
- [3] WANG Bini, CAO Wei, HUI Gao, et al. Simultaneous determination of six phenolic compounds in jujube by LC-ECD[J]. Chromatographia, 2010, 71(7/8): 703-707.
- [4] ZHANG Xiangqian, CHEN Zongli, YANG Xuanwen, et al. Analysis of phenolic compounds in north Shaanxi red jujube by UV spectrophotometry[J]. Chinese Agricultural Science Bulletin, 2010, 26(24): 83-88.
- [5] CHEN Zongli, WANG Xiaojian, LIU Shipeng, et al. Development of somatic embryo and adventitious buds from the differentiating cultured calli of Chinese jujube mu-zao[J]. Acta Horticulturae, 2009, 840(9): 273-282.
- [6] Hitachi high-technologies corporation. User manual for L-8900 high speed amino acid analyzer (Mainunit)[M]. Tokyo, Japan: Hitachi Ltd: 2005.
- [7] ZOU Yuanfeng, CHEN Xingfu, YANG Wenyu, et al. Analysis of amino acids in different ages of radix codonopsis and their nutritional evaluation[J]. Food and Fermentation Industries, 2010, 36(6): 146-150.
- [8] FAO/WHO. Energy and protein requirements[J]. FAO Nutrition Meeting Report Series, 1985, 724: 1-206.
- [9] ZHU Sengtao, WU Kun. Nutritional evaluation of protein: ratio coefficient of amino acid[J]. Acta Nutrimenta Sinica, 1988, 10(2): 187-190.
- [10] QIAN Aiping, LIN Qiu, YU Yabai, et al. The content of amino acid in the flesh of oranges produced in Fujian province and its nutritive evaluation[J]. Chinese Agricultural Science Bulletin, 2008, 24(6): 86-90.
- [11] XU Chongyuan, CHEN Zhende, CHEN Zhiliang, et al. Content determination of the amino acid ridge of *Cibotium barometz* (L.) J. Sm[J]. The Journal of Pharmaceutical Practice, 2000, 18(5): 299-300.
- [12] PAN Xuejun, ZHANG Wener, LIU Wei, et al. Fatty acids and amino acids content of walnut kernels in Guizhou[J]. Southwest China Journal of Agricultural Sciences, 2010, 23(2): 497-501.
- [13] JIANG Yugang, XU Qishou. Advances of study on effect and mechanism of conditionally essential amino acid on wound healing[J]. Amino Acids & Biotic Resources, 2002, 24(3): 59-62.
- [14] SUMITA B. Arginine metabolism and airflow obstruction in asthma[J]. Amino Acids, 2009, 37(Suppl 1): S73.
- [15] HURSON M, REGAN M C, KIRK D, et al. Metabolic effects of arginine in a healthy elderly population[J]. JPEN, 1995, 19(3): 227-232.
- [16] ZHANG Jiasheng, FANG Linxiang. Evaluation of the amino acid content and nutrition of Si-Hua rice[J]. Agriculture Technology, 2001, 21(2): 44-45.
- [17] MIAO Zhengxing, ZHANG Zhongming, LI Baozhong, et al. Production and application of the *L*-proline[J]. Shanghai Medical Intelligence Research, 2004, 33(2): 1-2.
- [18] PENG Youshun, WANG Shuyuan, JIA Dandan, et al. Determination and analysis of amino acids in different parts of *Asparagus officinalis* L. stem[J]. Journal of Anhui Agri Sci, 2011, 39(1): 129-130.
- [19] GUO Yaowu, FULLER W, ROBBERT C, et al. Spencer amino acid nutrition and fetal growth[J]. Amino Acids, 2009, 37(Suppl 1): 6.
- [20] NING Liaozen, LI Meijuan. Biological function of branched-chain amino acids in sports nutrition supplies and its application in athletic practice[J]. Journal of Gansu Lianhe University: Natural Sciences, 2009, 23(7): 117-119.
- [21] WANG Bin, CAI Yongqiang, ZHENG Wei. Analysis on the amino acid content and the composition in the pitaya fruit[J]. Chinese Agricultural Science Bulletin, 2009, 25(8): 210-214.