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Bioactive, nutritional composition, heavy metal and pesticide residue of four Chinese jujube cultivars

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Abstract This study aims to conduct a detailed investigation on four cultivars grown in northwest China, concentrating on the analysis of the bioactive contents, nutrients, heavy metal concentrations, and pesticide residue contents. Those Chinese jujubes consist of 51.99–71.75% edible part, 82.35–89.63% carbohydrates, 4.43–6.01% protein, 0.48–0.63% lipid, 2.80–4.80% polysaccharide, 45.64–88.97 mg/100 g ascorbic acid, 132.16–196.58 mg/100 g phenolics and 101.17–132.04 mg/100 g flavonoids in dry matter. In those four Chinese jujube cultivars, sulfur amino acids are the first limiting amino acids for adults, and aromatic amino acids are for children. The amount of heavy metal and pesticide residue concentrations in those jujubes was way below the limit. All four cultivars were found to have different nutritional values except for the carbohydrates; they had higher rates of carbohydrates and polysaccharide than those previously reported ones from Eastern China; and they are a better source for carbohydrates, vitamin C and functional amino acids.

Keywords Chinese jujube · Nutritional composition · Bioactive content · Heavy metals · Pesticide residue

Abbreviations

HPZ	*Ziziphus jujube cv.* Hupingzao
HZ	*Ziziphus jujube cv.* Huizao
XZ	*Ziziphus jujube cv.* Xiaozao
JZ	*Ziziphus jujube cv.* Junzao
GAE	Garlic acid equivalent
TAA	Total amino acids
EAA	Essential amino acids
FAA	Functional amino acids
CNS	Chinese national standard
HCB	Hexachlorobenzene
HCHs	Hexachlorocyclohexane isomers
o,p′-DDT	1,1,1-(trichloro)-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)ethane
p,p′-DDE	2,2-bis(4-chlorophenyl)-1,1-dichloroethylene
p,p′-DDD	2,2-bis(4-chlorophenyl)-1,1-dichloroethane
p,p′-DDT	1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane

Introduction

Jujube (*Ziziphus jujuba* Mill.) belongs to Rhamnaceae family, and widely cultivated in tropical and subtropical areas of the world [1]. People have used jujube for food and medicinal purposes for thousands of years. In China, jujube pulp and seed are listed as functional food [2]. Jujube is also used in traditional medicine to treat inflammation, sedation, ulcer, hypotensive, nephritic, cardio-tonic, antioxidant, immune-stimulant, fungal, bacterial diseases, also used as healing material [1, 3–5]. Clinical trials also found jujube fruit could reduce chronic constipation and neonatal jaundice [6, 7]. Previous studies on jujube mostly

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focused on its shape, classification, preservation, and methods of processing [8–16] due to its importance as a food source both in dried and fresh forms. Jujube is a widespread plant, and it's found that more than 700 cultivars are being grown all around China, mainly in the areas with warm continental climate such as Shandong, Hebei, Henan, and Shanxi etc. However, north-west China with cold desert climate, mainly Xinjiang and Gansu, have become new novel planting areas for Chinese jujube in recent decades, but most of the information available for us so far is about the jujube cultivars in eastern regions with warm continental climate, and there are no adequate or reliable scientific reports about functional and nutritional compositions of the jujube cultivars from north-western cold desert climate regions. Moreover, there is very limited information about the potential health risks of heavy metal and pesticide residue contents in Chinese jujube. In addition to that, amino acid profile of Chinese jujube was absent. Therefore, the objective of this study is to determine the functional and nutritional value, amino acid profile, heavy metal concentration, and pesticide residue of four Chinese jujube cultivars that widely planted in north-west China, and as well as their suitability for being source of daily nutrients.

Materials and methods

Materials

Four cultivars of jujube, botanically identified as *Ziziphus jujube cv*. Hupingzao, *Ziziphus jujube cv*. Huizao, *Ziziphus jujube cv*. Xiaozao, *Ziziphus jujube cv*. Junzao (Figs. 1, 2) (2014 and 2015 harvest seasons with different harvest time) were supplied by Minqin county Gansu province, China. The jujubes had been stored at − 20 °C until all analysis being completed, and seeds have been removed from all samples before performing any analysis. All of the data obtained represent averages of triple duplicate determinations.

Basic composition analysis

Water content was determined by measuring weight differences after drying samples based on CNS GB 5009.3-2010. Protein content was calculated from the nitrogen content (N × 6.25) analyzed by Kjeldahl method according to CNS GB 5009.5-2010. Lipid content was determined by Soxhlet method according to CNS GB/T 5009.6-2003. Carbohydrate content was analyzed using phenol–sulfuric acid colorimetric method [17].

Polysaccharide content

Polysaccharide content was determined by using phenol–sulfuric acid colorimetric method based on CNS GB/T 18672-2014. 0.4 g Sample has been filtered after being refluxed with 80% ethanol. The filtrate has been measured following the standard procedure (GB/T 18672-2014).

Total phenolic

Total phenolic content was determined by using the Folin–Ciocalteu assay [18, 19]. 1 g sample was mixed by using high speed dispersator in 25 mL of ethanol, and extracted twice at 45 °C in ultrasonic cleaner for 30 min. Sample was centrifuged at 6000 rpm for 10 min, and sediments have been extracted once again. The phenolic content has been measured after combining the two supernatants. Sample (100 µL) was introduced into test tubes followed by 500 µL of Folin–Ciocalteau's reagent (10-times dilution) and 400 µL of sodium carbonate (7.5%, w/v). The mixture was mixed by vortex and incubated in the dark condition at room temperature for 60 min. The absorbance was measured by spectrophotometer (SpectraMax M2e, USA) at 765 nm. The result was expressed as garlic acid equivalent (mg GAE/100 g).

Total flavonoids

The total flavonoid contents were calculated by subtraction of the total phenolic content with non-flavonoid content. The phenolic content was extracted following the method mentioned above. The total non-flavonoid content was measured according to Deetae et al. [18]. 500 µL extraction was mixed with HCl (500 µL, 20% v/v) followed by 37% formaldehyde solution (250 µL). The mixture was mixed by vortex and left for 24 h at room temperature in the dark condition. The sample was then centrifuged at 12,000 rpm for 10 min. The supernatant was measured according to the Folin–Ciocalteu method mentioned above. The results were expressed as mg GAE/100 g.

Ascorbic acid analysis

Ascorbic acid content was determined by using colorimetric method based on CNS GB/T 5009.86-2003.

Amino acids analysis

Amino acids composition has been analyzed using Hitachi L-8900 amino acid analyzer according to CNS GB/T 5009.124-2003. Essential amino acid scores were calculated according to reference amino acid requirements of adults (FAO/WHO/UNU, 2007) and children (FAO/WHO/

Fig. 1 Four different cultivars of Chinese jujube. (**A**) *Ziziphus jujube* cv. Hupingzao (HPZ); (**B**) *Ziziphus jujube* cv. Huizao (HZ); (**C**) *Ziziphus jujube* cv. Junzao (JZ) (**D**) *Ziziphus jujube* cv. Xiaozao (XZ)

UNU, 1985). The score was calculated using the formula: amino acid score = amount of amino acid per test protein (mg g^{-1})/amount of amino acid per protein in the reference pattern (mg g^{-1}) × 100.

Heavy metal analysis

Heavy metal contents including Pb, Cd, As, Hg, and Cr have been analyzed by using graphite furnace method according to CNS GB 2009.12-2010, CNS GB/T 2009.15-2003, CNS GB/T 2009.11-2003, CNS GB/T 2009.17-2003, and CNS GB/T 2009.123-2003, respectively.

Pesticide residue analysis

Pesticide residues including hexachlorobenzene (HCB), hexachlorocyclohexane isomers (HCHs), organochlorine pesticides (DDTs), dimethoate, and deltamethyrin have been analyzed using Agilent 7980A gas chromatograph (Agilent, USA) with an Agilent electron capture detector (Agilent, USA) according to Chinese Standard NY/T 761-2008. 25.0 g sample was extracted with 50.0 mL of acetonitrile for 2 min in high speed dispersator. Then the filtrate was collected into 100 mL mixing cylinder with stopper (7 g NaCl included). The mixture was vigorously shaken for 1 min. After 30 min at room temperature, 10.00 mL of acetonitrile layer was transferred and concentrated to near dry by nitrogen in 80 °C water bath. The

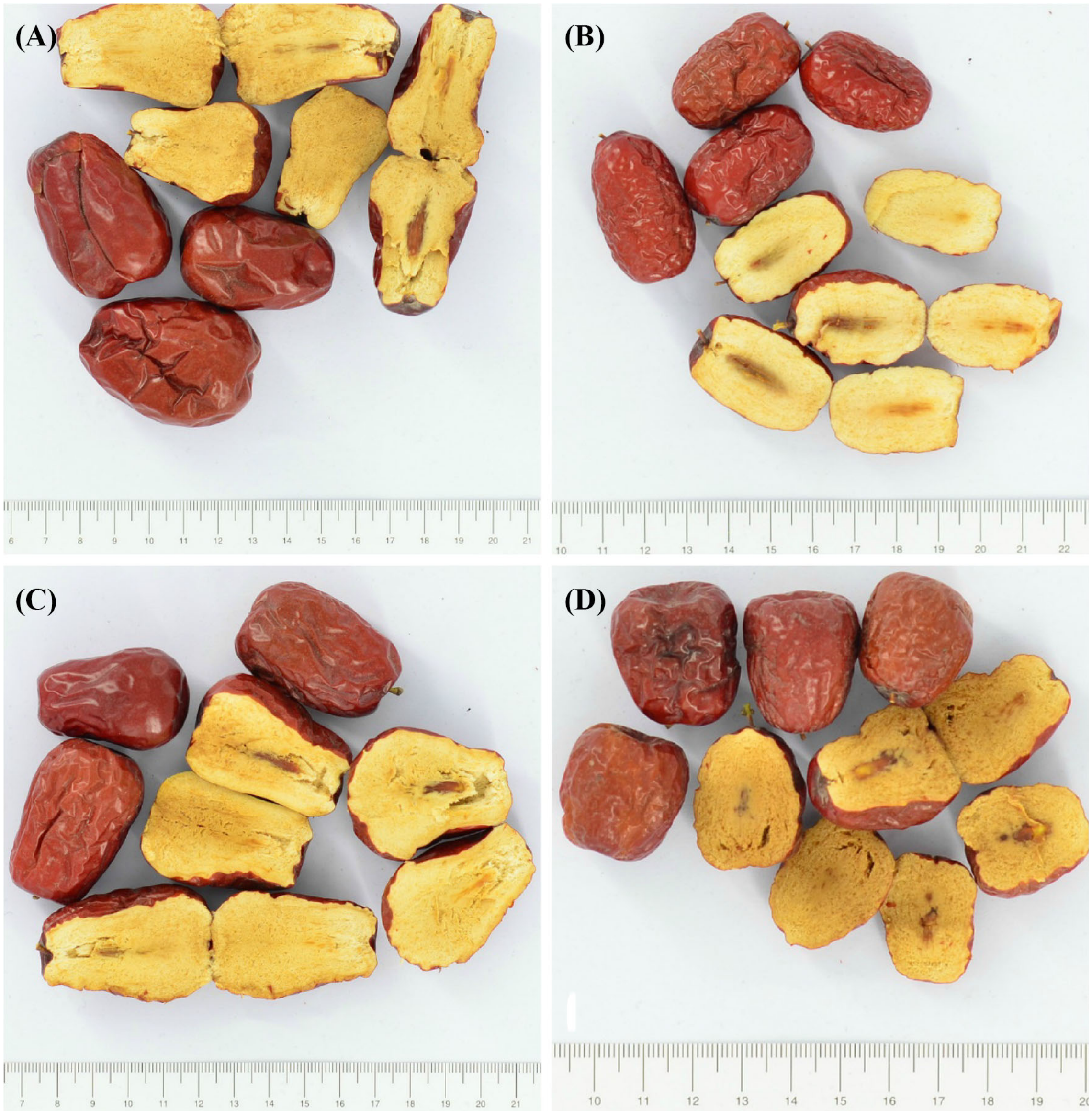

Fig. 2 Sliced Chinese jujube of four cultivars. (**A**) *Ziziphus jujube* cv. Hupingzao (HPZ); (**B**) *Ziziphus jujube* cv. Huizao (HZ); (**C**) *Ziziphus jujube* cv. Junzao (JZ) (**D**) *Ziziphus jujube* cv. Xiaozao (XZ)

final extract was dissolved in 5.0 mL acetone, and filtered through 0.22 μm filter. An Agilent DB-5 MS capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, USA) was used for separation, and the standard substance to assess recovery of target analytes. Recoveries of pesticide standard substance were found to be between 92.5 and 107.5%, while the limit of detection was 0.001 mg/kg ww for all target pesticides.

Statistical analysis

All sample analysis was performed in triplicate. Statistical analysis was done by SPSS V20.0 (SPSS Inc., Chicago, USA). The results have been presented as means of three determinations ± SD. The results obtained were analyzed using one-way analysis of variance (ANOVA) for mean differences among four cultivars.

Results and discussions

Basic composition analysis

The result of chemical composition analysis of jujubes is shown in Table 1. Moisture contents in all four jujube cultivars are significantly different from each other. HZ have been found to have the highest moisture content of 44.75%, while XZ has 23.71%. XZ has the highest content

Table 1 Basic composition analysis of four jujube cultivars (dry weight of basis)

Component	Unit	HPZ	HZ	XZ	JZ
Water	%	35.32 ± 0.30c	44.65 ± 0.20a	23.71 ± 0.26d	42.47 ± 0.41b
Edible part	%	62.00 ± 1.50b	51.99 ± 2.40c	71.75 ± 4.30a	55.06 ± 3.10d
Weight	g	8.22 ± 0.61b	3.68 ± 0.40c	4.06 ± 0.52c	9.39 ± 1.50a
Carbohydrates	g/100 g	89.63 ± 7.96a	88.23 ± 6.31a	89.73 ± 5.43a	82.35 ± 4.50a
Total lipid(fat)	g/100 g	0.480 ± 0.04b	0.499 ± 0.06b	0.450 ± 0.04b	0.630 ± 0.01a
Protein	g/100 g	4.432 ± 0.66b	4.922 ± 0.05ab	5.512 ± 0.16ab	6.013 ± 0.58a

Each value is expressed as mean ± SD (n = 3). Means with different letters within a row is significantly different ($p < 0.05$) by Tukey *t* test

with 71.75% and HZ has the lowest 51.99% for edible parts (pulp weight/whole fruit weight). JZ has the highest individual weight (9.39 g) regardless of its position as number three in the amount of edible parts, and HZ has the lowest individual weight (3.68 g) consistent with its amount of edible parts. Among four Chinese jujube cultivars, different basic compositions have significant differences ($p < 0.05$) except for carbohydrate. Even though there is no significant difference have been found in the amount of carbohydrates of the four different jujube cultivars, the carbohydrate contents of north-west cultivars are mostly higher than the cultivars reported from eastern China (85.63 g/100 g) [20].

All four jujube cultivars have lower lipid contents with a range of 0.48–0.63% (Table 1). JZ has higher lipid content, the three cultivars had no significant difference. Even though the lipid contents are in the range of east part cultivars (0.37–1.02%), JZ has lower lipid content than located in east part of China for the same cultivar [20, 21].

Protein contents of jujubes have significant differences ranging from 4.43 to 6.01%, and they show similarity to the protein contents of some other cereal crops [20, 22].

Bioactive content analysis

Bioactive compounds of jujubes have a wide range of health benefits such as improving cardiovascular system, enhancing immunity, and protecting liver and gastrointestinal etc. [1]. Jujube cultivars contain bioactive compounds like polyphenols, flavonoids, ascorbic acid, and polysaccharides (see Table 2), and these may vary depending on geographical environment, cultivar and processing, and storage conditions [1, 23].

Recent studies have shown that polysaccharides from jujube have anti-inflammatory, immunomodulating, hepatoprotective, and antioxidant effects [24–28]. Although there are no significant differences in polysaccharides among different jujube cultivars except for HPZ and XZ. However, all four jujube cultivars in question have shown higher values of polysaccharide contents (HPZ 4.80 g/100 g, JZ 4.12 g/100 g, HZ 3.63 g/100 g, XZ 2.80 g/100 g as shown in Table 2) than the ones reported from eastern China (2.1 g/100 g) [29].

Even though ascorbic acid content decreased during ripping stage, and due to the length of storage time, but jujube fruits can still be served as a good source of ascorbic acid [20, 30]. The ascorbic acid contents of four jujube cultivars are JZ 88.97 mg/100 g, HPZ 77.13 mg/100 g, HZ 71.50 mg/100 g, and XZ 45.64 mg/100 g respectively (Table 2), and three of them almost meet the recommended daily allowance of ascorbic acid by Chinese Nutrition Society which is 100 mg per day [31]. The ascorbic acid contents between JZ (88.97 mg/100 g) and XZ (45.64 mg/100 g), and between HPZ (77.13 mg/100 g) and XZ (71.50 mg/100 g) respectively have shown significant differences, and the amount of ascorbic acid contents in those four jujube cultivars have been proven to be lower than the jujube cultivars reported from eastern China (167 mg/100 g) [29], but higher than common fruits such as tomatoes (1.00 mg/100 g), apples (0.33 mg/100 g), and mangos (1.54 mg/100 g) [32]. The difference in the amount of ascorbic acid contents of jujubes from two different parts

Table 2 Bioactive composition of four jujube cultivars (dry weight basis)

Component	Unit	HPZ	HZ	XZ	JZ
Polysaccharide	g/100 g	4.798 ± 0.89a	3.627 ± 0.18ab	2.799 ± 0.18b	4.121 ± 0.53ab
Ascorbic acid	mg/100 g	77.13 ± 5.27a	71.50 ± 8.21ab	45.64 ± 1.28b	88.97 ± 6.68a
Phenolics	mg/100 g	132.18 ± 7.80b	184.65 ± 4.53a	196.58 ± 5.57a	193.61 ± 10.89a
Flavinoids	mg/100 g	101.17 ± 4.11b	119.85 ± 10.96ab	132.04 ± 7.36a	104.00 ± 4.93b

Each value is expressed as mean ± SD (n = 3). Means with different letters within a row is significantly different ($p < 0.05$) by Tukey *t* test. Total phenolic and flavonoid contents are expressed as mg GAE/100 g

Table 3 Amino acid composition of four jujube cultivars (g/100 g of dry weight of basis)

Component		HPZ	HZ	XZ	JZ
Aspartic acid[b]	ASP	0.39	0.60	0.84	1.214
Threonine[a]	THR	0.06	0.08	0.10	0.13
Serine	SER	0.08	0.09	0.11	0.13
Glutamic acid[b]	GLU	0.17	0.15	0.26	0.22
Glycine[b]	GLY	0.06	0.07	0.08	0.10
Alanine	ALA	0.06	0.07	0.08	0.11
Valine[a]	VAL	0.07	0.10	0.11	0.14
Methionine[a,b]	MET	0.01	0.01	0.01	0.01
Isoleucine[a]	ILE	0.04	0.07	0.06	0.08
Leucine[a,b]	LEU	0.08	0.11	0.11	0.15
Tyrosine[a,b]	TYR	0.01	0.00	0.01	0.02
Phenylalanine[a]	PHE	0.05	0.07	0.09	0.10
Lysine[a]	LYS	0.06	0.09	0.08	0.12
Histidine[a]	HIS	0.03	0.04	0.05	0.07
Arginine[b]	ARG	0.05	0.12	0.19	0.18
Proline[b]	PRO	0.68	1.242	1.458	1.762
Tryptophan[a,b]	TRP	0.01	0.02	0.02	0.02
Cysteine[b]	CYS	0.02	0.01	0.02	0.02
TAA		1.918	2.956	3.656	4.564
FAA/TAA(%)		76.5	79.4	81.6	80.8
EAA/TAA (%)		22.3	20.2	17.3	18.3

Each value is expressed as means; amino acid acronyms and abbreviations follow IUPAC standard; FAA stands for functional amino acids, which participate in and regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of organisms; EAA stands for total essential amino acids; TAA stands for total amino acids

[a]Essential amino acids

[b]Functional amino acids

of China may also due to the differences in the analytical method and sampling process [30].

The amount of total phenolic and flavonoid contents of those four jujubes did not show significant differences from the jujubes reported by Gao et al., and the numbers range from 196.58 (XZ) to 132 (HPZ) mg GAE/100 g for phenolic contents and from 132.04 (XZ) to 101.17 (HPZ) mg GAE/100 g for flavonoids contents respectively, which are similar to the numbers reported by Gao et al. [23, 29], but among those four, HPZ showed the lowest amount of phenolics (132.18 mg GAE/100 g) while XZ showed the highest amount of flavonoids, significantly differing from the other three (see Table 2).

Amino acid compositions

Amino acids, both of free and bonded forms, in plants are source of nitrogen and essential amino acids such as lysine (Lys), methionine (Met) and threonine (Thr) [33]. The amino acid profile of four jujube cultivars (presented in Table 3) shows that the most abundant ones are proline, aspartic acid and glutamic acid, and these constitutes the 64.5–70.0% of the total amino acids (TAA) in those four jujube cultivars. These amino acids, as functional amino acids, play anti-oxidative and anti-inflammatory roles, also demonstrate metabolic regulation and wound healing functionality [34].

The essential amino acid (EAA) score (presented in Table 4) shows that all EAAs belong to limiting amino acid for adults and children. The scores also explain that threonine, tryptophan and histidine have the highest scores in those four jujube cultivars in comparison to the reference amino acid pattern for adults (Table 4). Sulfur amino acids (Met + Cys) and aromatic amino acids (Phe + Tyr) are found to be the first limiting amino acids for adults and for

Table 4 Essential amino acids scores of Jujube cultivars

Amino acid	Reference[a]	Score (for adults)				Reference[b]	Score (for children)			
		HPZ	HZ	XZ	JZ		HPZ	HZ	XZ	JZ
His	15	48	58	58	79	19	38	46	46	62
Ile	30	31	45	38	43	28	33	48	41	46
Leu	59	31	40	34	41	66	28	35	30	37
Lys	45	30	39	33	45	58	23	30	25	35
Met + Cys	22	25	21	21	25	25	22	18	18	22
Phe + Tyr	38	36	40	47	52	63	21	24	28	31
Thr	23	62	73	76	94	34	42	49	51	64
Try	6	52	68	55	53	11	29	37	30	29
Val	39	39	51	50	58	35	44	57	56	65

Abbreviations of amino acid are in Table 3

[a]Reference amino acid requirements of adults (FAO/WHO/UNU, 2007)

[b]Reference amino acid requirements of child (FAO/WHO/UNU, 1985)

Table 5 Concentrations of heavy metals in four Chinese jujube cultivars (in mg/kg fresh weight)

	Pb	Cd	As	Hg	Cr
HPZ	ND	0.0139	0.0103	0.00245	0.162
HZ	ND	ND	0.0261	0.00244	0.0307
XZ	0.0566	ND	0.100	0.00510	ND
JZ	0.0990	0.0127	0.0492	0.00251	0.122

ND is not detected

Table 6 Concentration of pesticide residue in Chinese jujube (in mg/kg fresh weight)

	HCB	HCHs	*o,p′*-DDT	*p,p′*-DDE	*p,p′*-DDD	*p,p′*-DDT	Dimethoate	Deltamethyrin
HPZ	ND	ND	ND	ND	ND	ND	ND	ND
HZ	ND	ND	ND	ND	ND	ND	ND	ND
XZ	ND	ND	ND	ND	ND	ND	ND	ND
JZ	ND	ND	ND	ND	ND	ND	ND	ND

ND is not detected

children respectively, while valine, threonine and histidine have the highest scores for pre-school children. Different cultivars also demonstrate different scores for adults and children, the JZ gets the highest score for all EAAs than the other three. However, in regard of the new concept of functional amino acids (FAA) which hold great promise in prevention and treatment of metabolic diseases [34], the ratio of FAA to TAA in those jujubes ranges from 76.5 to 80.8%. The rich FAA in jujube protein may be one of the main reasons for jujubes to be widely utilized as a traditional remedy in some Asian countries including China, South and North Korea.

Heavy metal and pesticide residue analysis

Contaminants such as heavy metals and pesticides have become a matter of serious concern due to their toxicity and tendency to accumulate in food chains [35]. There is no pesticide residues have been detected from the four Chinese jujube cultivars (see Table 6), and even though large variations of heavy metal concentrations have been observed in all four Chinese jujube cultivars (see Table 5), but the amount of them found to be safe for consuming. The level of lead (Pb) concentration in HPZ and HZ, cadmium (Cd) concentration in HZ and XZ, chromium (Cr) concentration in XZ, and pesticide residues including hexachlorobenzene, hexachlorocyclohexane isomer, organochlorine, dimethoate, and deltamethyrin have been found to be below the instrumental limit of detection. The reported dietary intake limits for Pb, Cd and Cr for adults are 0.21 mg, 0.06 mg, and 2.0 mg respectively [35]. The results shown in Tables 5 and 6 reveals that the heavy metal concentrations and pesticide residues of those examined jujube samples are way below the limits, indicating that there is no potential health risk for consuming those jujubes.

This study investigated nutritional and bioactive compositions, amino acid profiles, and heavy metal and pesticide residue concentrations of the four Chinese jujube cultivars (HPZ, HZ, XZ and JZ) grown in the cold desert climate area of north-west China in comparison to the same species grown in warm continental climate area of Eastern China. The data we obtain through this investigation indicates that the contents of bioactive and nutritional compounds in jujubes vary among cultivars. JZ have higher contents of pulp weight, protein, fat, essential and non-essential amino acids in dry mass condition comparing to other cultivars, while XZ has the highest amount of total sugar, polyphenol and flavonoids both in fresh and dry mass conditions, indicating that cultivars of JZ and XZ have nutritional superiority in their family. Therefore, JZ can be recommended for direct consumption, and XZ maybe recommended as an ingredient for jujube related food and drinks. Those four cultivars have mostly higher carbohydrates and polysaccharides, and lower lipid contents comparing to cultivars from eastern China, but the amount of phenolic and flavonoid contents sit in the range where Eastern China cultivars do. Even though the dried jujubes have lower ascorbic acid contents, but they still can meet with basic human needs, and considered to be a good source of ascorbic acid. Although the amount of FAA showed different numbers among cultivars, those jujubes can still be a reliable source for FAA supplements. Analysis on the heavy metal and pesticide residue concentrations also proved that direct consuming those jujubes will not cause any potential health risks.

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Compliance with ethical standards

Conflict of interest There is not any conflict of interest with all authors.

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