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Abstract: Jujube is the homology of medicine and food, and polyphenols are key compounds that determined the functional effects of jujubes. In this study, characteristic polyphenols in 15 varieties of Chinese jujubes were investigated based on untargeted metabolomics. Result showed a total of 79 characteristic polyphenols were identified in 15 varieties of Chinese jujube, and 55 characteristic polyphenols such as syringetin, spinosin and kaempferol were reported for the first time. Scopoletin (63.94% in LZYZ), pectolinarin (22.63% in HZ) and taxifolin (19.69% in HZ) contributed great and presented significant (p<0.05) differences in 15 varieties of Chinese jujubes. HZ can be characterized by pectolinarin, erianin and wogonoside. While, XSHZ, NYDZ and RQHZ with similar polyphenol profile were characterized by (+)-catechin, combretastatin A4 and tectorigenin. JSBZ, HMDZ, TZ, JCJZ and HPZ had similar polyphenol profile of galangin, isoferulic acid and hydroxysafflor yellow A. In conclusion, metabolomics is critical to grasp the fully nutritional components of jujubes, and the differences of polyphenol profiles and characteristic individual polyphenol of 15 varieties of Chinese jujubes can be well analyzed by principal component analysis (PCA).

Keywords: jujube; polyphenol; variety; metabolomics; PCA

Introduction

Jujube (*Ziziphus jujuba* Mill.), also called red jujube or Chinese date belongs to Rhamnaceous family and is widely cultivated and consumed worldwide [1]. Jujube originates in China with 7000 years history and more than 400 cultivars are available [2,3]. In Chinese traditional culture and the latest announcement by National Health Commission and State Administration for Market Regulation of China (2024), jujube is a homologous herb of food and medicine. Research has shown that jujube is rich in carbohydrate, proteins, amino acids, minerals and various of bioactive compounds such as polyphenols, flavonoids, saponins, and polysaccharides [4,5]. Therefore, the bioactive activities such as antioxidant activities, anti-cancer and anti-insomnia of jujubes are excellent [6]. Jujubes are classified as table, dried, candied, multipurpose and ornamental group, and dried one accounts for over 80% [7,8]. In daily life, jujubes are eaten as fresh and dried forms directly, or consumed as jujube wine, compotes, jujube juice, bread, cake and yogurt [1,3].

As a kind of secondary metabolites, polyphenols widely exist in plant and plant-based foods, and they can be classified into flavonoids, non-flavonoids and tannins based on their characteristic chemical structure of aromatic rings with one or more hydroxyl moieties [9,10]. Polyphenols present varieties of functional effects such as antioxidative, antiglycative activities, antimicrobial, antiproliferative and anti-inflammatory properties and closely relate to the water-holding capacity, texture and color of the foods [11,12]. Wang, et al (2022) [13] found that in Chinese jujubes the content of total phenols was 1.33 ～ 10.92 mg/g and the total flavonoids was 0.10 ～ 7.66 mg/g. According to the present statistics, over 60 polyphenols have been reported in jujubes, and the common polyphenols are caffeic, rutin, protocatechuic, ferulic, gallic acid, p-coumaric acid, quercetin, coumarin and (-)-epicatechin [14-17]. The characteristic phenolic compounds in jujubes are

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significantly affected by maturity stages [15,16], processing conditions [18], and varieties [13,19]. Noteworthy, a total of 59 individual phenolic compounds have been reported in jujubes by metabolomics [14], however only 7 phenolics are detected in 26 varieties of jujubes by LC/MS [13], it indicates that metabolomics is superior in the phenolics analysis of jujubes.

Metabolome has the advantage to identify the whole metabolites (< 1000 Da) in plants, foods, and humans [20,21]. In practical applications, gas chromatography-mass spectrometry (GC-MS), liquid chromatography- mass spectrometry (LC-MS), or nuclear magnetic resonance (NMR) is applied for metabolome [22,23]. Unlike GC-MS adapted for the detection of low molecular weight metabolites [24,25], metabolome based on LC-MS could achieve the best metabolites coverage, covering from very polar to non-polar food molecules [26]. Recently, metabolome based on LC-MS has been successfully applied for the characteristic polyphenols analysis of strawberry [27], green teas [28], and barley [29]. Hence, in order to reveal the characteristic polyphenol compositions in commercial Chinese jujubes, metabolomics based on LC-MS was applied in this study. Moreover, the differences of 15 commercial Chinese jujubes were distinguished based on their polyphenols as well.

Materials and Methods

2.Plant Material

A total of 15 commercial Chinese jujubes were collected from the markets in different regions of China. The information of these jujubes was JSXZ (Jin Si Xiao Zao, originate in Cangzhou Country, Hebei Province, China), NHDZ (Nei Huang Da Zao, originate in Anyang, Country, Henan Province, China), SZ (Suan Zao, originate in Kashi County, Xinjiang Province, China), HMDZ (Ha Mi Da Zao, originate in Hami Country, Xinjiang Province, China), HZ (Hui Zao, originate in Akesu County, Shanxi Province, China), LZYZ (Lin Ze Yu Zao, originate in Zhangye Country, Gansu Province, China), JSBZ (Ji Shan Ban Zao, originate in Jishan County, Shanxi Province, China), ZHDZ (Zan Huang Da Zao, originate in Zanhuang Country, Hebei Province, China), TZ (Tan Zao, originate in Jinzhong Country, Shanxin Province, China), XSHZ (Xi Sha Hong Zao, originate in Zhongning Country, Ningxia Province, China), RQHZ (Ruo Qiang Hui Zao, originate in Ruoqiang Country, Xinjiang Province, China), JCJZ (Jiao Cheng Jun Zao, originate in Jiaocheng Country, Shanxi Province, China), HTDZ (He Tian Da Zao, originate in Hetian Country, Xinjiang Province, China), HPZ (Hu Ping Zao, originate in Jinzhong Country, Shanxi Province, China) and NYDZ (Ning Yang Da Zao, originate in Ningyang Country, Shandong Province, China). About 2.0 kg of each variety of commercial Chinese jujubes were obtained and stored at 4 °C with package. The picture of 15 variety of commercial Chinese jujubes was shown in Figure 1.

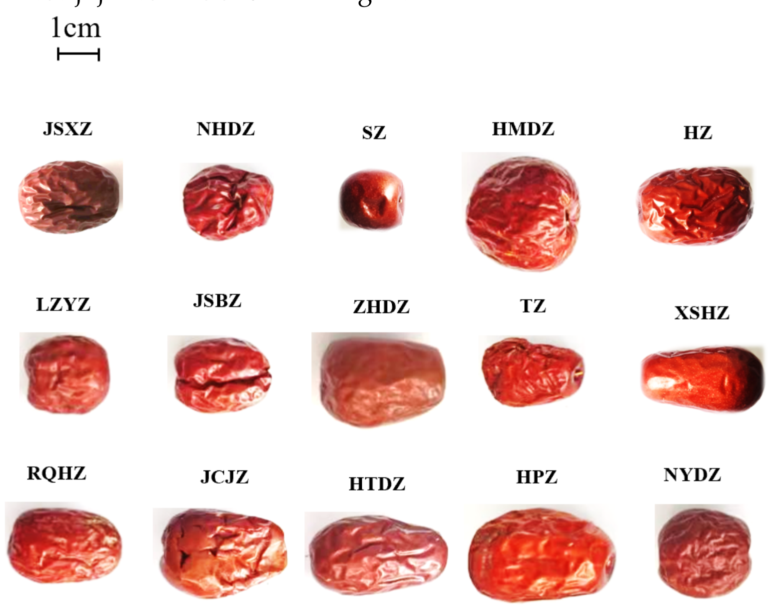

Figure 1. Pictures of 15 varieties of Chinese jujubes.

2.Metabolite Extraction

The metabolite extraction of 15 varieties of commercial Chinese jujubes was according to the method of Song, & Tang (2023) [22]. Briefly, accurate 100.00 mg of jujube samples (ground with liquid nitrogen) was respectively put in PE tube. Then 500 μL 80% methanol aqueous solution containing 0.1% formic acid (LC-MS grade) was added and treated with vortex concussion. Subsequently, the sample was kept in ice bath (5 min) and then centrifugated at 15000 g at 4℃ (10 min). The certain supernatant was diluted to 53% methanol content. Eventually, the supernatant was collected for injection and analysis.

2.LC-MS/MS Analysis

A Vanquish UHPLC (Thermo Fisher, Germany) coupled with an Q ExactiveTM HF mass spectrometer (Thermo Fisher, Germany) was performed for UHPLC-MS/MS analysis, and a C^{18} column (Hypesil Gold, 100 × 2.1 mm, 1.9 μm, Thermo Fisher, USA) was equipped. LC-MS grade of formic acid (0.1%) and methanol was respectively used as mobile phase A and B for positive mode analysis, ammonium acetate (5 mM, pH 9.0) and methanol was used as mobile phase A and B, respectively for negative mode analysis. The column temperature and the flow rate was 40℃ and 0.2 mL/min, respectively. The gradient elution was performed with 0～1.5 min of 98% A and 2% B, 12～14 min of 0% A and 100% B, 14.1～17.0 min of 98% A and 2% B. The Q ExactiveTM HF mass spectrometer of positive/negative polarity mode (3.2 kV spray voltage, 320°C capillary, 40 arb sheath gas and 10 arb aux gas) was operated.

2.Metabolite Identification

Peak alignment, peak picking, and quantitation of each metabolite was treated by the Compound Discoverer 3.1 (CD3.1, Thermo Fisher) of UHPLC-MS/MS. The tolerance of retention time, actual mass and signal intensity was 0.2 minutes, 5ppm, and 30%, respectively. While, the signal/noise ratio and minimum intensity was 3 and 100, 000, respectively. All the peak intensities were normalized to the total spectral intensity. Based on the additive ions, molecular ion peaks and fragment ions, the normalized molecular formulas were predicted. Then the identified polyphenols which at least presented full match with the mzCloud (https://www.mzcloud.org/), mzVault or MassList database were analyzed [30]. R (R version R-3.4.3), Python (Python 2.7.6 version) and CentOS (CentOS release 6.6) were performed for the statistical treatment. All determinations were analyzed in Novogene Co., Ltd (Beijing, China).

2.Data Preprocessing

Area normalization method was used for the content analysis of each identified polyphenol [31]. SPSS of 20.0 version (Inc., Chicago, IL) was used for verify differences analysis by Duncan's multiple tests at $p<0.05$ level. All determinations were three repetitions and the results were presented as mean ± SD (standard deviation). Principal component analysis (PCA) was carried out by EZinfo 3.0 software.

Results and Discussion

3.Identification of Characteristic Polyphenols in 15 Chinese Jujubes

Characteristic polyphenols in 15 Chinese jujubes were identified based on untargeted metabolomics, and they were listed according to their retention time (RT) of ascending order. The information of compound, m/z, CAS number, molecular weight, molecular formula and molecular structure of individual polyphenol was presented as well (Table S1), and the identified compositions which at least showed full match with the database of mzCloud, mzVault or massList of UHPLC-MS/MS were analyzed.

Table S1 showed a total of 79 characteristic polyphenols were identified in 15 varieties of Chinese jujube. Among these polyphenols, rutin, ferulic acid, (+)-catechin, *p*-coumaric acid, (-)-epicatechin

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and quercetin were common in jujubes [13,15,17,32,33]. However, only 7 polyphenols were reported in 26 varieties of Chinese jujubes [13], 10 polyphenols were determined in 'Junzao' jujubes [32], 12 polyphenols were found in 7 cultivars of Chinese jujube [17], 10 polyphenols were analyzed in 7 varieties of Chinese jujubes [15], 8 polyphenols were presented in Xinjiang jujubes [33], respectively by high-performance liquid chromatography (HPLC). It indicated metabolomics was superior in the polyphenols analysis of jujube, which was agreed with the result of Zhang, et al (2023) [14] that 59 phenolics were detected. Moreover, phloretin, resveratrol, trilobatin, taxifolin, nobiletin, scopoletin, eriodictyol, myricetin, isorhamnetin, luteolin and naringenin presented in this study were also analyzed previously [14]. Meaningfully, a total of 55 characteristic polyphenols such as syringetin, spinosin, kaempferol and apigenin were found in jujube for the first time [1,13-15, 17, 32, 33], which is critical to grasp the fully nutritional components and then deeply explore the functional effects of jujubes.

3.Content of Characteristic Polyphenols in 15 Chinese Jujubes

In this study, area normalization method was applied for the content analysis of the characteristic polyphenols in 15 Chinese jujubes and the individual phenolic with the content of > 1% were shown in Table With significant high content, scopoletin (63.94% in LZYZ), pectolinarin (22.63% in HZ), taxifolin (19.69% in HZ), camelliaside A (18.82% in NYDZ), combretastatin A4 (16.25% in XSHZ) and *p*-coumaric acid (10.67% in JSXZ) contributed great to the total polyphenol content of different variety of jujube (Table 1). Scopoletin, act as a coumarin derivative compound and widely presented in noni is closely relate to antioxidative, immunomodulatory, anti-inflammatory and hepatoprotective properties that determining the function of jujube [34]. Taxifolin is a kind of dihydroflavonol and presents strong bioactivities of antiviral, anti-aging, anticancer, anti-inflammatory, antiangiogenic effects, immunity regulation and liver protection [35]. p-Coumaric acid shows functional activities of antioxidant, anti-inflammatory, anti-apoptotic, neuroprotective and memory ameliorating effects, as well as can promote hippocampal neurogenesis and stimulate hippocampal synaptic plasticity, and protect scopolamine-induced hippocampal LTP degradation and cognitive impairment [36]. However, significant different content of scopoletin (from 0.03% to 63.94%), pectolinarin (from 0.46% to 22.63%), taxifolin (from 0,15% to 19.69%), camelliaside A (from 0.02% to 18.82%), combretastatin A4 (from 0.06% to 16.25%) and p-coumaric acid (from 0.31% to 10.67% in JSXZ) was respectively presented in 15 varieties of Chinese jujubes, it indicated different bioactive abilities of 15 varieties of Chinese jujubes.

Ferulic acid, (+)-catechin, epicatechin and coumarin were common in jujubes, and they presented significant (p<0.05) differences in 15 varieties of Chinese jujubes with the content of 0.18% (in HZ)-4.31% (in JSXZ), 0.02% (in SZ, LZYZ and JSBZ)-1.08% (in XSHZ), 0.12% (in SZ)-5.49% (in RQHZ), and 0.08% (in HZ)-3.00% (in HTDZ), respectively (Table 1). Similar result was also reported in the phenolic compounds analysis of 26 varieties of jujubes [13]. Ferulic acid has the ability to reduce body weight, modulate the gut microbiota composition in HFD-induced mice and improve glucose and lipid metabolisms by activating the insulin receptor/PI3K/AKT pathway [37]. (+)-Catechin is a potent antioxidant with therapeutic benefits of cancers, coronary heart disease, and inflammatory disorders [38]. Epicatechin is excellent in anti-oxidation, anti-inflammatory, increasing the activity or expression of antioxidant enzymes [39]. Other polyphenols such as syringetin, erianin, wogonoside and galangin found for the first time also contributed the characteristic polyphenol group of jujubes (Table 1). It indicated that the biological activities of different varieties of jujubes was significant, for their different characteristic phenolics content. Furthermore, metabolomics provides the technical support to discover the whole nutrients and explore the functional effects of jujubes.

Table 1. Content of individual polyphenol (>1%) in 15 varieties of Chinese jujubes.

Compounds	Relative content (%)														
	JSXZ	NHDZ	SZ	HMDZ	HZ	LZYZ	JSBZ	ZHDZ	TZ	XSHZ	RQHZ	JCJZ	HTDZ	HPZ	NYDZ
Phloretin	1.08±0.02h	0.50±0.01c	0.56±0.01d	0.85±0.03fg	0.27±0.01b	0.31±0.00c	0.10±0.00a	0.79±0.01f	0.70±0.02e	0.89±0.01g	0.25±0.01b	1.10±0.03h	0.61±0.02d	1.05±0.01h	0.52±0.00c
Ellagic acid	1.28±0.04j	0.12±0.00b	0.21±0.01cd	0.29±0.01f	0.10±0.00a	0.23±0.00de	0.10±0.00a	0.18±0.01c	0.13±0.01b	0.25±0.01e	0.30±0.00f	0.65±0.02h	1.24±0.04j	0.83±0.04i	0.35±0.01g
p-Coumaric acid	10.67±0.35j	3.68±0.11d	4.14±0.15e	5.29±0.09f	0.31±0.02a	3.83±0.12de	5.35±0.20f	6.95±0.19h	10.40±0.33j	3.26±0.14c	7.32±0.17h	6.06±0.08g	8.12±0.26i	10.30±0.41j	2.77±0.05b
Pectolinarin *	1.62±0.02c	0.46±0.01a	0.48±0.00a	1.63±0.03c	22.63±0.72i	0.85±0.03b	2.88±0.10d	1.56±0.02c	0.89±0.03b	9.27±0.38h	4.71±0.21f	3.79±0.05e	7.22±0.09g	3.80±0.04e	4.03±0.12e
Baohuoside II *	3.05±0.07h	2.11±0.03d	0.62±0.01a	2.37±0.02ef	3.00±0.04h	0.98±0.01b	2.31±0.00e	2.97±0.05h	1.26±0.04c	4.59±0.15i	5.81±0.19j	2.50±0.03fg	2.69±0.05g	2.16±0.01d	2.57±0.04g
Syringetin *	0.31±0.00j	0.02±0.00a	0.08±0.01f	0.11±0.00h	7.04±0.18k	0.02±0.00a	0.03±0.00b	0.08±0.00f	0.04±0.00c	0.09±0.00g	0.10±0.00h	0.07±0.00e	0.12±0.00i	0.06±0.00d	0.07±0.00e
Coumarin	0.45±0.01f	0.20±0.00d	0.16±0.01bc	0.15±0.00b	0.08±0.00a	0.49±0.02g	0.29±0.00e	0.40±0.01e	0.15±0.01a	0.17±0.00c	0.17±0.01c	0.85±0.02h	3.00±0.12j	1.35±0.03i	0.44±0.01ef
Morin Hydrate *	1.64±0.04h	0.35±0.01a	0.32±0.01a	1.39±0.03g	1.38±0.02g	0.41±0.01b	0.65±0.02d	1.01±0.01e	0.51±0.01c	1.64±0.05h	1.43±0.02g	1.78±0.03h	1.34±0.01g	1.09±0.02ef	1.10±0.00f
Hydroxysafflor yellow A *	0.12±0.00i	0.02±0.00a	0.03±0.00b	1.10±0.02j	0.05±0.00d	0.04±0.00c	0.02±0.00a	0.10±0.00g	0.05±0.00d	0.08±0.00f	0.11±0.00h	0.07±0.00e	0.08±0.00f	0.07±0.00e	0.11±0.00h
Taxifolin	15.86±0.85f	4.75±0.32a	4.64±0.21a	11.78±0.47de	19.69±0.73g	6.67±0.59b	10.38±0.48cd	13.34±0.62e	9.60±0.52c	15.72±0.83f	17.96±0.91fg	10.92±0.35cd	13.32±0.27e	11.16±0.44d	0.15±0.00e
Combretastatin A4 *	4.10±0.12cd	2.39±0.09b	0.90±0.03a	3.76±0.17c	6.10±0.25g	2.42±0.16b	5.62±0.28fg	7.81±0.19h	2.40±0.05b	16.25±0.63i	4.73±0.11e	4.31±0.06d	5.30±0.03f	5.74±0.14fg	0.06±0.00c

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5-O-Caffeoylshikimic acid *	3.42±0.12m	0.08±0.00a	1.42±0.03k	0.26±0.00g	0.17±0.01c	0.98±0.01j	0.13±0.00b	0.88±0.02i	0.13±0.00b	0.33±0.01h	3.03±0.04l	0.21±0.00e	0.18±0.01cd	0.20±0.00de	0.29±0.00h
Rosmarinic acid	0.78±0.01f	0.15±0.00b	0.60±0.01e	0.60±0.01e	0.13±0.00a	0.36±0.01d	0.29±0.01c	5.09±0.12m	1.35±0.03j	2.04±0.02l	1.66±0.02k	0.98±0.03h	1.13±0.01i	0.90±0.00g	0.05±0.00d
Nobiletin	1.09±0.02jk	0.43±0.01ef	0.16±0.00b	1.11±0.05k	0.27±0.01d	0.25±0.01d	0.05±0.01a	0.22±0.01c	1.04±0.03j	0.45±0.01f	0.58±0.01h	0.68±0.02i	0.40±0.01e	0.45±0.01f	0.03±0.00c
Isomucronulatol *	1.02±0.02h	0.22±0.00b	0.22±0.00b	0.66±0.01f	0.20±0.00a	0.25±0.01c	0.20±0.00a	0.83±0.02g	0.52±0.02d	1.14±0.03i	0.64±0.01f	0.58±0.01e	0.49±0.01d	0.83±0.02g	0.03±0.00c
Erianin *	0.20±0.00g	0.12±0.00g	0.03±0.00a	0.07±0.00d	1.08±0.02h	0.03±0.00a	0.10±0.00f	0.07±0.00d	0.06±0.00c	0.15±0.00e	0.09±0.00e	0.22±0.01g	0.12±0.00g	0.17±0.01f	0.06±0.00e
Wogonoside *	0.04±0.00a	0.18±0.00h	0.05±0.00b	0.14±0.00g	7.26±0.28n	0.08±0.00c	0.12±0.00f	0.15±0.01g	0.11±0.00e	1.42±0.05m	0.09±0.00d	0.28±0.01k	0.34±0.01l	0.20±0.00i	0.03±0.00c
Scopoletin	26.26±0.73d	72.60±1.04k	76.96±0.98k	47.48±1.16h	5.88±0.17a	63.94±0.55j	53.50±0.70i	34.90±1.01f	52.02±1.37hi	15.68±0.52b	20.44±0.77c	43.46±1.12gh	30.00±0.27e	42.73±0.85g	0.03±0.00c
Camelliaside A *	0.08±0.00e	0.08±0.00e	0.02±0.00a	0.03±0.00b	3.28±0.12h	0.02±0.00a	0.04±0.00c	0.03±0.00b	0.05±0.00d	0.15±0.00g	0.11±0.00f	0.08±0.00e	0.05±0.00d	0.05±0.00d	18.82±0.62g
Isoliquiritin *	0.72±0.01g	0.74±0.02g	0.25±0.01c	0.23±0.01c	0.01±0.00a	0.73±0.01g	0.50±0.02e	0.82±0.02h	0.32±0.01d	0.11±0.01b	0.96±0.02i	1.79±0.04j	5.13±0.34l	2.29±0.18k	4.55±0.09de
Galangin *	0.74±0.02h	0.26±0.01d	0.14±0.00a	0.14±0.00a	0.26±0.00d	2.71±0.05k	0.14±0.00a	0.37±0.01f	1.22±0.03i	0.18±0.00c	0.33±0.00e	0.56±0.02g	2.31±0.05j	0.41±0.01f	0.24±0.00f
Ferulic acid	4.31±0.07j	0.73±0.02c	0.59±0.01b	2.95±0.08i	0.18±0.00a	1.31±0.04e	1.60±0.03f	1.41±0.02e	0.92±0.02d	2.89±0.07i	2.60±0.05h	2.38±0.04h	1.61±0.05	1.87±0.02g	1.41±0.02j
Isoferulic acid *	0.74±0.01j	0.27±0.01e	0.14±0.00a	0.15±0.01ab	0.27±0.01e	2.71±0.06m	0.21±0.01d	0.37±0.00g	1.22±0.03k	0.18±0.00c	0.34±0.01f	0.56±0.01i	2.31±0.05l	0.40±0.01h	0.49±0.00g
Demethylnobiletin *	0.47±0.01d	0.96±0.02h	0.89±0.01g	0.96±0.01h	0.02±0.00a	1.11±0.03i	0.29±0.00c	1.21±0.01j	0.65±0.02e	1.10±0.02i	0.13±0.00b	0.81±0.01f	0.43±0.01d	0.80±0.02f	0.66±0.02j

Kaempferol *	0.90±0.01h	0.34±0.01c	0.18±0.00a	0.44±0.02d	2.10±0.05k	0.33±0.01c	0.70±0.01f	0.84±0.02h	0.44±0.01d	1.03±0.02i	1.21±0.02j	0.52±0.01e	0.54±0.02e	0.29±0.01b	0.56±0.01h
(+)-Catechin	0.06±0.00d	0.05±0.00c	0.02±0.00a	0.05±0.00c	0.05±0.00c	0.02±0.00a	0.02±0.00a	0.36±0.01f	0.05±0.00c	1.08±0.02g	0.07±0.00d	0.37±0.00f	0.04±0.00b	0.05±0.00c	0.78±0.01g
Tectorigenin *	3.37±0.05g	1.74±0.02cd	0.75±0.02a	1.97±0.05e	2.68±0.07f	1.34±0.01b	5.40±0.04j	2.72±0.10f	1.38±0.06b	5.04±0.06i	4.60±0.03h	1.87±0.02de	1.82±0.02d	1.61±0.03c	0.05±0.00b
Rhapontigenin *	1.07±0.03k	0.52±0.01h	0.17±0.00b	0.58±0.01i	0.01±0.00a	0.24±0.00c	0.35±0.01e	0.31±0.00d	0.39±0.01f	0.46±0.01g	0.82±0.02j	1.47±0.03l	0.31±0.00d	0.50±0.01gh	0.14±0.00f
Epicatechin	2.05±0.06j	0.31±0.00b	0.12±0.00a	2.21±0.04j	0.43±0.01d	0.50±0.01e	3.34±0.07k	0.55±0.01f	1.13±0.03h	1.21±0.02h	5.49±0.11l	0.83±0.02g	0.40±0.01d	0.35±0.00c	0.62±0.01f
Schisanhenol *	1.28±0.03j	0.70±0.00f	0.77±0.01gh	0.99±0.02i	0.04±0.00a	0.81±0.02h	0.29±0.00c	0.62±0.01e	0.23±0.00b	0.50±0.02d	0.74±0.01fg	0.60±0.01e	0.63±0.02e	0.52±0.01d	0.58±0.02j
Schizandrol A *	0.04±0.00b	0.05±0.00c	0.03±0.00a	0.04±0.00b	6.96±0.11j	0.05±0.00c	0.08±0.00e	0.08±0.00e	0.07±0.00d	0.15±0.00i	0.04±0.00b	0.10±0.00g	0.09±0.00f	0.07±0.00d	0.67±0.02l
Procyanidin B2	1.76±0.02d	0.27±0.00b	1.15±0.02c	2.63±0.05g	0.15±0.00a	2.29±0.04f	0.27±0.00b	7.06±0.16j	5.62±0.08i	1.98±0.04e	4.64±0.11h	1.91±0.06e	2.39±0.07fg	2.00±0.03e	0.21±0.01fg
Cyanidin 3-rutinoside *	1.70±0.03j	0.07±0.00a	0.22±0.00g	0.16±0.00f	0.13±0.00d	0.24±0.00h	0.12±0.00c	0.13±0.00d	0.08±0.00b	0.14±0.00e	0.24±0.00h	0.26±0.00i	0.27±0.01i	0.27±0.00i	0.10±0.00f

JSXZ, Jin Si Xiao Zao; NHDZ, Nei Huang Da Zao; SZ, Suan Zao; HMDZ, Ha Mi Da Zao; HZ, Hui Zao; LZYZ, Lin Ze Yu Zao; JSBZ, Ji Shan Ban Zao; ZHDZ, Zan Huang Da Zao; TZ, Tan Zao; XSHZ, Xi Sha Hong Zao; RQHZ, Ruo Qiang Hui Zao; JCJZ, Jiao Cheng Jun Zao; HTDZ, He Tian Da Zao; HPZ, Hu Ping Zao; NYDZ, Ning Yang Da Zao. Mean values with different lower-case letters in the same column correspond to significant differences at $p < 0$.Data are represented as the mean ± SD (standard deviation). Compounds marked with "*" were reported in jujubes for the first time

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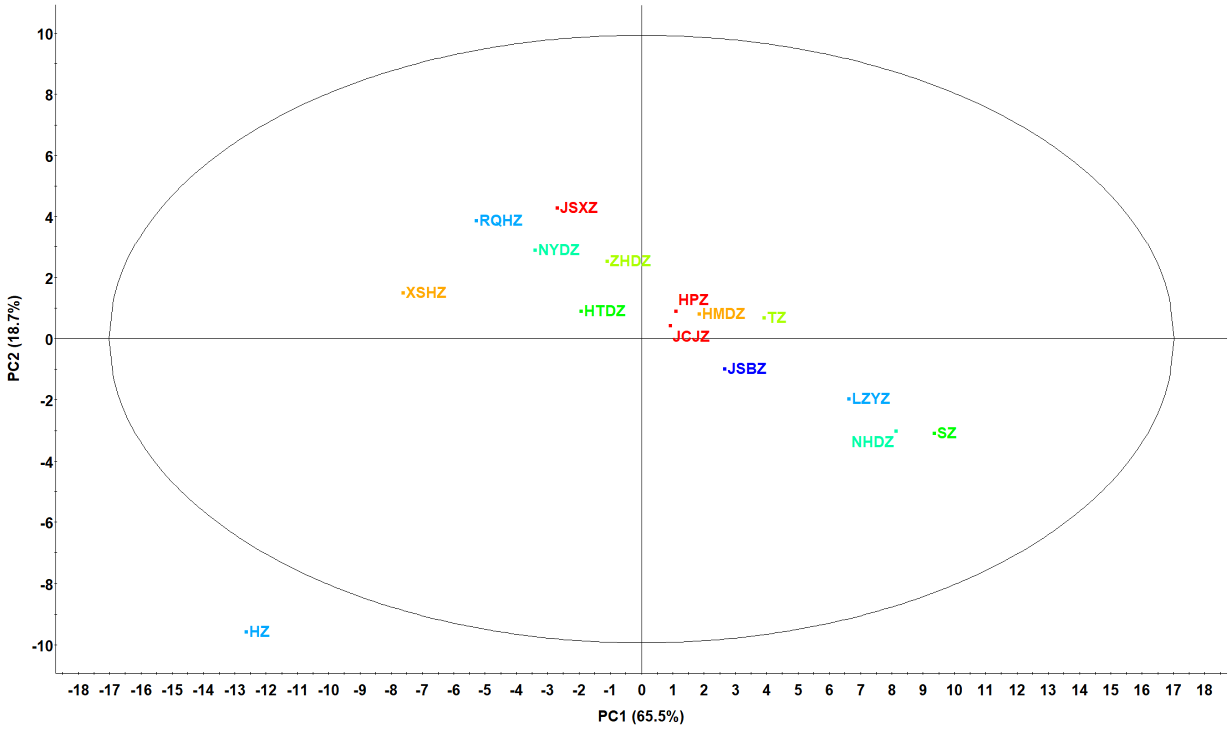
3.PCA of 15 Commercial Chinese jujubes

As a multivariate statistical method, principal component analysis (PCA) has the advantages of analyzing complex datasets by identifying two or more principal component factors, and examining the correlation between multiple variables [4,40]. To well distinguished the differences of polyphenol profiles and found the characteristic individual polyphenol of different commercial Chinese jujubes, PCA was applied according to the content of the identified polyphenols (Table S1 and Table 1). As shown in Figure 2, a 2D-PCA plot was established with the whole polyphenol compounds (Table S1), and the total contribution of PC1 (65.5%) and PC2 (18.7%) was 84.2% which was enough to explain the dataset [24]. As shown in the PCA plot (Figure 2), the observations of 15 commercial Chinese jujubes were well separated, it indicated the differences of polyphenol profiles of them were significant. HPZ, HMDZ, JCJZ, TZ, JSBZ and HTDZ showed close distance to each other, indicating they have similar polyphenol profile. Moreover, HZ presented far distance from other jujube samples, ae well as SZ (NHDZ, LZYZ) was far away from XSHZ (RQHZ, JSXZ) indicating they have different polyphenol profile, respectively [41]. However, the characteristic individual polyphenol of each jujube sample was hard to see clearly and distinguish when the whole identified polyphenols (Table S1) was presented in one PCA plot.



SXZ, Jin Si Xiao Zao; NHDZ, Nei Huang Da Zao; SZ, Suan Zao; HMDZ, Ha Mi Da Zao; HZ, Hui Zao; LZYZ, Lin Ze Yu Zao; JSBZ, Ji Shan Ban Zao; ZHDZ, Zan Huang Da Zao; TZ, Tan Zao; XSHZ, Xi Sha Hong Zao; RQHZ, Ruo Qiang Hui Zao; JCJZ, Jiao Cheng Jun Zao; HTDZ, He Tian Da Zao; HPZ, Hu Ping Zao; NYDZ, Ning Yang Da Zao.

Figure 2. PCA of 15 commercial Chinese jujubes based on their whole polyphenols.

To made clear the critical and characteristic individual polyphenol of different varieties of jujubes, the characteristic polyphenols in 15 varieties Chinese jujubes with the content > 1% (Table 1) were selected for analysis. As shown in Figure 3, the total contribution of PC1 (68.3%) and PC2 (20.2%) was 88.5% and the result was well agreed with that of Figure. It indicated the polyphenols with the content > 1% (Table 1) can well represented the whole identified polyphenols (Table S1) in 15 varieties of Chinese jujubes. Figure 3 showed HZ can be characterized by pectolinarin, erianin, wogonoside, schizandrol A, syringetin and camelliaside A for their highest content (p<0.05), respectively (Table 1). While, XSHZ, NYDZ and RQHZ with similar polyphenol profile were

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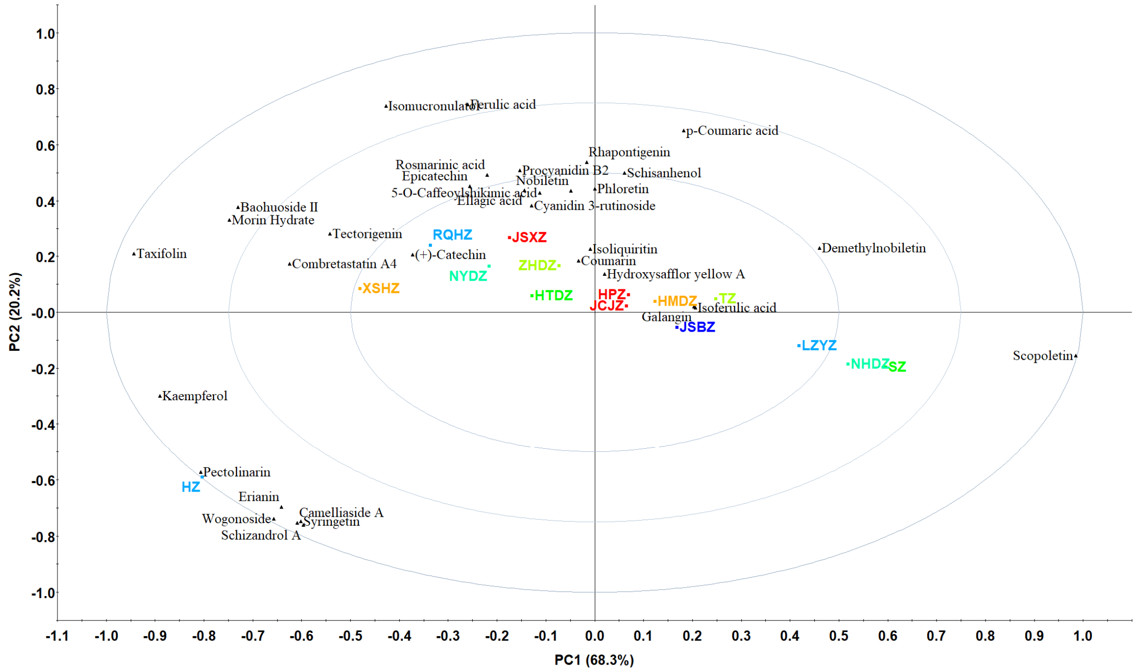
characterized by (+)-catechin, combretastatin A4 and tectorigenin. JSBZ, HMDZ, TZ, JCJZ and HPZ was closed to each other for their similar polyphenol profile which can be characterized by galangin, isoferulic acid and hydroxysafflor yellow A (Figure 3, Table 1). Clearly, polyphenol profiles of 15 varieties of Chinese jujubes were significant and characteristic individual polyphenol of each jujube sample can be well analyzed by PCA.



SXZ, Jin Si Xiao Zao; NHDZ, Nei Huang Da Zao; SZ, Suan Zao; HMDZ, Ha Mi Da Zao; HZ, Hui Zao; LZYZ, Lin Ze Yu Zao; JSBZ, Ji Shan Ban Zao; ZHDZ, Zan Huang Da Zao; TZ, Tan Zao; XSHZ, Xi Sha Hong Zao; RQHZ, Ruo Qiang Hui Zao; JCJZ, Jiao Cheng Jun Zao; HTDZ, He Tian Da Zao; HPZ, Hu Ping Zao; NYDZ, Ning Yang Da Zao.

Figure 3. PCA of 15 commercial Chinese jujubes based on the content of polyphenols > 1%.

Conclusion

A total of 79 characteristic polyphenols were identified in 15 varieties of Chinese jujube, and 55 characteristic polyphenols such as syringetin, spinosin, kaempferol and apigenin were reported for the first time. Scopoletin (63.94% in LZYZ), pectolinarin (22.63% in HZ), taxifolin (19.69% in HZ), camelliaside A (18.82% in NYDZ), combretastatin A4 (16.25% in XSHZ) and *p*-coumaric acid (10.67% in JSXZ) contributed great to the total polyphenol and presented significant different in 15 varieties of Chinese jujubes. Among these jujubes, HZ can be characterized by pectolinarin, erianin, wogonoside, schizandrol A, syringetin and camelliaside. While, XSHZ, NYDZ and RQHZ with similar polyphenol profile were characterized by (+)-catechin, combretastatin A4 and tectorigenin. JSBZ, HMDZ, TZ, JCJZ and HPZ had similar polyphenol profile of galangin, isoferulic acid and hydroxysafflor yellow A. Moreover, the differences of polyphenol profiles and characteristic individual polyphenol of 15 varieties of Chinese jujubes can be well analyzed by PCA.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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