Research Article

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Determination of polyphenols in Chinese jujube using ultra-performance liquid chromatography–mass spectrometry

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Abstract: The polyphenolic composition of the same variety of winter jujube was determined using ultra-performance liquid chromatography–mass spectrometry (UPLC–MS/MS). A metabolomic approach was employed to determine polyphenols at different developmental stages (S1, S2, and S3). The total phenolic content of Chinese jujube was quantified, and the metabolites were statistically analyzed using orthogonal partial least squares discriminant analysis (OPLS-DA) for differential metabolite screening and clustering analysis of key components. The findings revealed that 128 polyphenolic components of Chinese jujube had been identified. Different developmental periods could not be clearly distinguished in principal component analysis, and there was a crossover between S2 and S3 stages. In contrast, the OPLS-DA score plot could effectively distinguish between samples of different developmental periods, and its differential metabolites could be visualized by a volcano plot based on OPLS-DA. Ten polyphenolic differential metabolites in different developmental periods were clustered and analyzed, among which N'-p-coumaroylguanidinium, N-p-coumaroylbutylamine, caffeoyl guanidinium, N-feruloyl guanidinium, pinoresinol, isorhamnetin 5-O-hexoside, isorhamnetin O-acetyl-hexoside, quercetin, and lignan O-hexosyl-O-pentoside were higher in the S1 period and chrysoeriol 6-C-hexoside was higher in the S2 and S3 periods. In this study, the differences in jujube polyphenols were elucidated, which provided scientific guidance for the application of jujube polyphenols.

Keywords: polyphenols in Chinese jujube, ultra-performance liquid chromatography–mass spectrometry, principal component analysis, cluster analysis

1 Introduction

The jujube tree, a member of the deciduous shrubs of the Ziziphus jujuba Mill of the Rhamnaceae family [1], has been cultivated since early times for its economic value in over 30 countries across Asia, Europe, America, Africa, and Oceania [2]. China is abundant in jujube, and statistics show that jujube trees account for a significant proportion of fruit trees and Chinese jujube production is high. Winter jujube is a type of spineless jujube tree that is widely distributed worldwide and popularized and consumed in Asian countries [3]. Due to its thin skin, crunchy texture, tasty flesh, and high nutritional value, winter jujube has gained considerable popularity among consumers as a freshly consumed fruit [4–6]. Jujubes are high in vitamins, sugars, organic acids, fatty acids, oleic acid, linoleic acid, amino acids, carbohydrates, fiber, and phenols [7–9]. These are basic chemicals involved in disease treatment and provide health benefits such as allergies, urinary problems, constipation, chronic bronchitis, liver diseases, depression, and insomnia [10–12].

Many fruits contain phenolic compounds, such as berries, pear, kiwifruit, passion fruit, peach, orange, apple, bananas, and jujube [13], which are molecules that can act as antioxidants to prevent heart disease [14], reduce inflammation [15], reduce the incidence of cancers [16], and diabetes [16]. When compared to other edible fruits, the jujube fruit has been dubbed as the “fruit of life,” and it is widely used as medicine in Asian
countries, particularly Taiwan and China, for the treatment of allergies, constipation, urinary problems, depression, chronic bronchitis, insomnia, and liver diseases [17]. Jujubes contain a variety of phenolic compounds, including hydroxycinnamate, flavonols, flavan-3-ols, and especially procyanidins, as well as saponins [18,19]. The current focus of research on Chinese jujube phenolic substances is on extraction, isolation, and antioxidant activities [20]. A comprehensive phenolic analysis of winter jujube is rarely reported.

Metabolomics techniques were used to investigate inter-group differences in a large number of samples, allowing the original complex data information of the samples to be processed through a series of dimensionality reductions to obtain components with simple dimensions that reflect the main information of the complex data. Nonobjective multivariate analysis tools, such as principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and Hierarchical Cluster Analysis (HCA), were frequently used to help reveal differences between samples [21]. Ultra-performance liquid chromatography–mass spectrometry (UPLC–MS/MS) has been widely used in recent years to detect phenolic compounds [22,23] as a tool for high-throughput screening and quantitative analysis of substances. In this study, Chinese jujubes from the same variety of jujube trees were harvested at different times (S1, S2, and S3) and used for quantitative analysis of Chinese jujube polyphenolic compounds at different developmental stages using UPLC–MS/MS. After that, the OPLS-DA technique was used to discriminate the phenolic substances of Chinese jujube in different harvesting periods. The differential metabolites of Chinese jujube phenolic substances were screened, allowing the key differential phenolic substances in different harvesting periods to be clustered and analyzed. The study examined the distribution and content of phenolic components at various developmental stages, which can provide theoretical foundations for the use of winter jujube.

2 Materials and methods

2.1 Experimental samples

The tested Chinese jujube came from the Jujube Experimental Station at Luoyang Normal University in Luoyang, Henan, China. Samples from three different growth stages, named S1, S2, and S3, were collected on days 35, 80, and 120 following anthesis (Figure 1).

2.2 Reagents and apparatus

Merck (Darmstadt, Germany) provided the methanol, acetonitrile, and ethanol, all of which were high performance liquid chromatography (HPLC)-grade. A UPLC–MS/MS system (UPLC supplied by SHIMADZU Nexera X2, https://www.shimadzu.com.cn/; MS supplied by Applied Biosystems 6500 Q TRAP, https://www.thermofisher.cn/cn/zh/home/brands/applied-biosystems.html.cn/) was used.

2.3 Pretreatment of samples

For each sample, three biological replicates were independently analyzed (nine samples in total), which were cleaned, frozen, dried under vacuum, and ground into powder (30 Hz, 1.5 min) using a grinder. About 1.0 mL of the extraction solution (70% methanol) was added to

Figure 1: Chinese jujube in three different stages of development.
100 mg of powder, which was then dissolved and labeled before being stored in the refrigerator overnight at 4°C to prevent denaturation and decomposition of bioactive compounds. The Chinese jujube was vortexed three times during this period to extract the polyphenols. The extraction rate of polyphenols in Chinese jujube was increased. Finally, the samples were centrifuged at 10,000 rpm for 10 min in a freezing centrifuge to collect the supernatant. The supernatant was filtered through a 0.22 µm micro-porous filter membrane and stored in the injection vial for UPLC–MS/MS analysis.

2.4 The conditions of liquid chromatography–mass spectrometry

The liquid chromatographic conditions: the column used was Waters ACQUITY UPLC HSS T3 C18 1.8 µm, 2.1 mm*100 mm; the mobile phase was ultrapure water containing 0.04% acetic acid; the organic phase was acetonitrile containing 0.04% acetic acid; the elution gradient was 0–11.0 min for water/acetoneitrile (95:5 V/V), 11.0–12.0 min for 5:95 V/V, and 12.0–15.0 min for 95:5 V/V; flow rate, 0.4 mL/min; column temperature, 40°C; and injection volume, 2 µL. The mass spectrometry conditions included the use of an electrospray ionization source with spray temperatures and voltage set to 500°C and 500 V, curtain gas set to 25 psi, and collision-activated dissociation set to high. Each ion pair was scanned for detection in the quadruple quadrupole using the optimized declustering potential and collision energy.

2.5 Qualitative and quantitative analysis of polyphenolic compounds in Chinese jujube

The polyphenols in Chinese jujube were qualitatively analyzed in both public and self-built metabolite databases using secondary spectral information, with isotopes and duplicate signals removed from the analysis.

Triple quadrupole mass spectrometry with multiple reaction monitoring (MRM) was used to quantify polyphenols in Chinese jujube. In MRM mode, precursor ions were screened. Many fragmented ions were formed by collision chamber-induced ionization breakage, followed by filtering to select a characteristic fragmented ion for more accurate quantification. The peaks of the mass spectra of polyphenolic compounds at different developmental stages were integrated, and the peaks of the same metabolite in different samples were corrected to quantify the polyphenolic substances.

2.6 Total phenolic compound analysis

The spectrophotometric method was used to determine the total phenolic content [24]. Briefly, 1 mL of sample (1 mg/mL) was mixed for 5 min with 1 mL of Folin–Ciocalteu reagent, and then 10 mL of a 7% Na2CO3 solution was added to the mixture, followed by 13 mL of deionized distilled water and thoroughly mixed. The mixture was kept in the dark for 90 min at 23°C before the absorbance at 750 nm was measured. The total phenolic content was calculated by extrapolating the calibration curve created by preparing the gallic acid solution. The phenolic compound estimation was performed in triplicate. The total phenolic content was given in milligrams of gallic acid equivalents per 100 g of dried sample.

2.7 Data analysis

The data from the experiments were standardized using Microsoft Office Excel 2016 and SPSS before being analyzed. The data from the HPLC–MS/MS were subjected to a series of processes, such as reading, peak alignment, and peak matching. The data from the preprocessing were subjected to multivariate statistical analysis using SIMCA-14.1. PCA was used to obtain correlations between samples, followed by OPLS-DA screening for phenolic differential metabolites and cluster analysis of key components [25].

3 Results and discussion

3.1 Quantitative analysis of the total phenolic content of Chinese jujube throughout its growth

The total phenolic compound content of winter jujube varied across developmental stages. Figure 2 shows the specifics. Total phenols in Chinese jujube ranged from 315.68 (mg/100 g) in the S1 stage to 156.47 (mg/100 g) in
the S2 stage to 109.76 (mg/100 g) in the S3 stage. The polyphenol content of Chinese jujube was highest in the S1 stage. The total phenolic content of Chinese jujube decreased continuously with the continuous growth of Chinese jujube, with the least polyphenol content in the S3 stage, which is similar to the result obtained by Shi et al. [26], who discovered that the levels of total phenolic content were lowest in the maturity stage in “Tailihong” and “Junzao.” Phenols have a variety of biological effects, including antioxidant [24], antimicrobial [27], and inhibitory [28] properties. Therefore, winter jujube in the S1 stage can be used medicinally, while winter jujube in the S3 stage (mature) can provide good flavor and multi-nutrition, including polyphenols [29].

3.2 Discriminant analysis of phenolic compounds in Chinese jujube at different growth stages

PCA is an objective-free multivariate analysis tool that reduces a multidimensional dataset to a two-dimensional plane, where the differences between samples are represented by scatter plots [30]. In PCA, samples with greater similarity are more aggregated, while those with less similarity are dispersed with poor stability [31]. The results of the PCA (Figure 3a) of polyphenolic substances in Chinese jujube at different developmental periods show that polyphenolic compounds could not be clearly distinguished into three groups for Chinese jujube at different developmental periods. The jujube varieties were clearly distinguished at the S1 and S2 periods, and the S1 and S3 periods were distinctly identified, whereas the S2 and S3 periods failed to distinguish clearly with some overlapping peaks [32]. The reason is that PCA is an unsupervised identification model based on group non-classified samples [33]. Further, the dimensionality reduction analysis without considering the grouping cannot clearly distinguish the information of Chinese jujube polyphenolic substances in three different developmental periods, which are further distinguished by the OPLS-DA method.

OPLS-DA was applied to samples from various developmental stages, including S1 and S2, S1 and S3, and S2 and S3. The S1 and S2 OPLS-DA models are illustrated in Figure 3b. The models obtained from the values of $R^2_Y$ and $Q^2$ had a good fitness ($R^2_X = 0.869, R^2_Y = 0.999, Q^2 = 0.881$); the fitting and predictive ability of OPLS-DA for S1 and S2 phases can be achieved [34]; and S1 samples were in the X-negative half-axis and S2 samples in the X-positive half-axis. Furthermore, on the score plot, the coordinate points of different samples in the S1 phase were larger than those in the S2 phase, and the results revealed significant differences in phenolic compound composition between the S1 and S2 phases, with the differences between different samples in the S1 phase being larger than those in the S2 phase. The OPLS-DA of the S1 and S3 periods is shown in Figure 3c, where $R^2_X = 0.812$, $R^2_Y = 0.963$, and the predictive ability value $Q^2 = 0.831$, and the model fit and predictive ability could meet the requirements. All samples of the S1 period were located in the negative half-axis of X, and all samples of the S3 period were located in the positive half-axis of X. The different samples of the S1 and S3 periods were far apart in the figure. The findings revealed significant differences in phenolic compound composition between S1 and S3 stages and between S1 and S3 stages between samples harvested at the same time [35]. Figure 3d shows the S2 and S3 periods of OPLS-DA with good fit and predictive power ($R^2_X = 0.69, R^2_Y = 0.977$, and $Q^2 = -0.451$). All S2 phase samples were in the negative half-axis of $X$, while all S3 phase samples were in the positive half-axis of $X$. Furthermore, the different samples in the S3 phase were far apart on the graph. The results revealed significant differences in phenolic composition between the S2 and S3 phases, with differences between samples in the S3 phase being greater than in the S2 phase [36]. Overall, the S1, S2, and S3 samples were well differentiated through the construction of the OPLS-DA model between different groups, with more significant differences between S1 and S2 and significant differences between S2 and S3. The findings were consistent with those of Xie et al., who discovered significant differences in phenolic content in different developmental stages of jujube fruit [37].

![Figure 2: Total phenolic content during the developmental stages (n = 3; error bar, a standard deviation of the sample).](Image)
3.3 Screening of phenolic differential metabolites of Chinese jujube at different developmental stages

The variable importance in the project (VIP) for Chinese jujube at different developmental stages was obtained using the OPLS-DA model. The initial screening of differential metabolites was conducted based on the magnitude of the VIP value. The differential multiplier values were combined to further screen the differential metabolites of Chinese jujube at different developmental stages, which were represented via volcano plots. Metabolites with fold change (FC) ≥2 and ≤0.5 and VIP ≥1 were chosen for screening of phenolic differential metabolites, and metabolites with VIP ≥1 denoted significant differences [38]. Figure 4a and b depicts the phenolic differential metabolites of Chinese jujube used in this experiment at different developmental stages. The dots in the figure represent the metabolites, the blue and red dots indicate the downregulated and upregulated differential metabolites, respectively, and Table 1 details the specific differential metabolites. In the S1 and S2 phases, eight differential metabolites were detected, all of which were upregulated differential metabolites, including four phenolamines (N’-p-coumaroylguanidinium, N-p-coumaroylbutylamine, caffeoyl guanidinium, and N-feruloyl guanidinium), three flavonols (squaroside, isorhamnetin 5-O-hexoside, and isorhamnetin O-acetylhexoside), and one flavonoid (lignan O-hexosyl-O-pentoside). In the S2 and S3 stages of Chinese jujube, two differential metabolites were detected. Both were downregulated differential metabolites, including chrysoeriol 6-C-hexoside in the flavonoid carbon glycoside class and quercetin in the flavonol class. Different metabolites were found in different developmental stages of Chinese jujube, with phenolic compounds being more variable between the S1 and S2 stages than between the S2 and S3 stages.

3.4 Cluster analysis of key components of Chinese jujube at different developmental stages

The phenolic compounds are differential metabolites between groups of Chinese jujube at different developmental stages.
and were screened in this experiment using the OPLS-DA model and the t-test of VIP > 1 and p < 0.05 [38]. The experimental results can be visualized using volcano plots, showing significant differences in phenolic metabolites between the S1 and S2 stages and the S2 and S3 stages of Chinese jujube. Further HCA was used to analyze these ten key differential metabolites in different developmental stages of Chinese jujube to make the distribution of these ten key differential metabolites in different developmental stages of Chinese jujube more intuitive, as shown in Figure 5. The graph shows a gradual increase in phenolic compound content from green to red [39], and these ten key differential metabolites had a certain regularity in the S1, S2, and S3 phases. In contrast, N′-p-coumaroylguanidine butylamine, N-p-coumaroylbutyramidine, caffeylguanidinobutyramidine, N-feruloylguanidinobutyramidine, pinoamine, isorhamnetin 5-O-hexoside, isorhamnetin plumbagin O-hexoside, lignan O-hexosyl-O-pentoside, and quercetin had the highest content in the S1 period, which was significantly higher than in the S2 and S3 periods [40]. N′-p-coumaroylguanidine butylamine, N-p-coumaroylbutyramidine, caffeylguanidine butylamine, and N-feruloylguanidine butylamine belong to the phenolic amine group. Lignan, isorhamnetin 5-O-hexoside, isorhamnetin O-acetyl-hexoxide, and quercetin belong to the flavonoid group, and lignan O-hexosyl-O-pentoside belongs to the flavonoid group. For S2 and S3 periods, chrysanthenin 6-C-hexosides belonging to flavonoid carbon glycosides were highly abundant, while metabolites of N′-p-coumaramide, N-p-coumaramide, caffeyl agmatine, N-feruloyl agmatine, inosine, isorhamnetin 5-O-hexosides, isorhamnetin O-hexosides, luteolin O-hexosyl-O-pentoside, and quercetin were low in content. Because of the different effects of light and temperature on Z. jujuba Mill., the chrysin 6-C-hexosides in the S2 period and quercetin in the S3 period showed greater differences among different samples picked at the same time. The chrysin 6-C-hexoside content in S2 was highest in S1–1 and S1–2 and lowest in S1–3. Quercetin levels in the S3 period were low in S3–1 and S3–3 but high in S3–2. The contents of four metabolites, including N′-p-coumaroyl agmatine, N-p-coumaroyl agmatine, N-feruloyl agmatine, and luteolin O-hexosyl-O-pentoside, were lower in the S3 phase than in the S2. Five metabolites, including caffeyl agmatine, polyglucoside, isorhamnetin 5-O-hexosides, isorhamnetin O-hexosides, and quercetin, were slightly higher in the S3 phase than in the S2 phase. The composition and content of Chinese jujube phenolic compounds were higher in the S1 period than in the S2 and S3 periods. Among them, phenolic amines (N′-p-coumaroyl agmatine, N-p-coumaroyl guanidine butylamine) and quercetin had the highest content in the S1 period, which was significantly higher than in the S2 and S3 periods [40].

![Volcano map of Chinese jujube at S1 and S2 stages (a) and S2 and S3 stages (b).](image)

Table 1: Different phenolic metabolites of Chinese jujube during developmental stages

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Log 2 FC</th>
<th>VIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential metabolites in S1 and S2 stages</td>
<td>N-p-Coumaroylguanidine butylamine</td>
<td>1.76977</td>
</tr>
<tr>
<td></td>
<td>N-p-Coumaroylbutyramidine</td>
<td>1.67709</td>
</tr>
<tr>
<td></td>
<td>Caffeoylguanidine butylamine</td>
<td>2.21522</td>
</tr>
<tr>
<td></td>
<td>N-Feruloylguanidine butylamine</td>
<td>1.37062</td>
</tr>
<tr>
<td></td>
<td>Avicularin</td>
<td>1.49037</td>
</tr>
<tr>
<td></td>
<td>Isorhamnetin 5-O-hexoside</td>
<td>1.61606</td>
</tr>
<tr>
<td></td>
<td>Isorhamnetin O-acetyl-hexoside</td>
<td>1.52887</td>
</tr>
<tr>
<td></td>
<td>Luteolin O-hexosyl-O-pentoside</td>
<td>1.26665</td>
</tr>
<tr>
<td>Differential metabolites in S2 and S3 stages</td>
<td>Chrysoeriol 6-C-hexoside</td>
<td>-1.01124</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>-1.31075</td>
</tr>
</tbody>
</table>
agmatine, caffeoyl agmatine, and \(N\)-feruloyl agmatine), flavonols (polyglucoside, isorhamnetin 5-\(O\)-hexoside, isorhamnetin \(O\)-hexoside, and quercetin), and flavonoids (luteolin \(O\)-hexosyl-\(O\)-pentoside) were high in the S1 period and low in the S2 and S3 periods. Flavonoids (chrysogenin 6-\(C\)-hexoside) were abundant in S2 but scarce in S1.

4 Conclusion

This study investigated the phenolic composition of Chinese jujube at various stages of development. UPLC–MS/MS was used to identify 128 phenolic compounds in Chinese jujube at various developmental stages (S1, S2, and S3). Using PCA and OPLS-DA, different developmental periods of Chinese jujube could not be distinguished effectively. There were 20 differential phenolic compounds with VIP values greater than 1 in the S1 and S2 phases, while volcano plots based on \(FC \geq 2\) and \(FC \leq 0.5\) were obtained to visualize the data, and eight upregulated differential metabolites were screened, as well as 19 differential phenolic compounds with VIP values greater than 1 in the S2 and S3 phases. The data were visualized using \(FC \geq 2\) and \(FC \leq 0.5\), and two downregulated differential metabolites were identified, as well as ten compounds with indigenous differences at three different developmental stages. Further cluster analysis revealed that in the S1 period, for phenolic compounds of Chinese jujube, phenolamines (\(N'\)-\(p\)-coumaroylguanidinium, \(N\)-\(p\)-coumaroylbutylamine, caffeoyl guanidinium, and \(N\)-feruloyl guanidinium), flavonoids (lentil glycosides, isorhamnetin 5-o-hexoside, isorhamnetin O-acetyl-hexoside, and quercetin),

Figure 5: Heatmap of the main components during the developmental stages.
and flavonoids (lignan O-hex glycosyl-O-pentoside) were higher than those in the S2 and S3 periods. The key differential metabolites were more homogenous in the S2 and S3 periods, with only the flavonoid carbon glycosides (chrystoeriol 6-C-hexoside). These were abundant in the S2 and S3 periods and quite low in the S1 period. In this study, more consideration was given to the developmental periods to obtain the key differential substances.

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**Conflict of interest:** All authors in the study have declared that they have no conflicts of interest for the words or data published in this article.

**Ethical approval:** The conducted research is not related to either human or animal use.

**Data availability statement:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**References**


