Drying enhances the antioxidant activity of *Allium mongolicum* Regel through the phenylpropane and AA-MA pathway as shown by metabolomics

Wangjing Liu [a], Aihuan Yu [a], Yaodi Xie [a], Haibo Yao [a], Chenxu Sun [a], Huixia Gao [a], Jianjian He [a], Changjin Ao [b], Defu Tang [a,*]

[a] *College of Animal Science and Technology, Gansu Agricultural University, No.1 Yingmen Village Anning, Lanzhou, Gansu 730070, People's Republic of China*
[b] *Inner Mongolia Key Laboratory of Animal Nutrition and Feed Science, College of Animal Science, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia 010018, People's Republic of China*

ARTICLE INFO

ABSTRACT

Fresh *Allium mongolicum* Regel (FA) and dried *A. mongolicum* Regel (DA) are significantly different in antioxidant activity. However, the relevant mechanisms have not yet been explored. We evaluated the antioxidant activities of two varieties of FA and DA and characterized their metabolites using targeted metabolomics. The effect of different metabolites on the antioxidant activity of *A. mongolicum* Regel was investigated by multivariate analysis. A total of 713 metabolites were detected in all samples. Pearson correlation analysis demonstrated that the key primary metabolites were directly and significantly correlated with the total phenolic content (TPC) and total flavonoid content (TFC), while the secondary metabolites were directly correlated with antioxidant activity. The higher antioxidant activity of DA may be mainly attributed to the higher TPC and TFC. This study revealed the potential mechanism by which drying enhances the antioxidant activity of *A. mongolicum* Regel.

1. Introduction

Allium mongolicum Regel is a perennial plant of the genus *Allium* in the *lily* family, mainly found in semiarid regions of China, Russia, Kazakhstan and southwestern Mongolia; a traditional Mongolian medicinal plant; and a nutritious, uniquely flavoured wild vegetable (Zhao et al., 2022). According to the Mongolian Pharmacopoeia, *A. mongolicum* Regel has many health benefits for people with high blood pressure, hyperlipidaemia and diabetes, as well as a variety of unique properties, such as anti-inflammatory, immune-enhancing and antioxidant abilities (Hossain, Lebelle, Birsan, & Rai, 2018). *A. mongolicum* Regel is rich in natural antioxidants, such as a variety of polyunsaturated fatty acids (PUFAs), flavonoids, phenolic acids and other primary and secondary metabolites (Liu, Tang, & Ao, 2022; Zhao et al., 2022). Further, it was reported that the main constituents of the aerial parts of *A. mongolicum* Regel were flavonoids and phenolic acids, with the flavonoid glycosides mainly quercetin, kaempferol and isorhamnetin; while coumaric acid, caffeic acid and ferulic acid were

phenolic acid glycosides (Dong et al., 2020). Wang et al. (2019) also confirmed that the high phenolic content [10.20 mg gallic acid equivalents (GAE) per gram dry weight)]and flavonoid content [4.02 mg quercetin equivalents (QE) per gram dry weight] in the aqueous extract of *A. mongolicum* Regel, with good antioxidant and inhibitory effects on lipase and angiotensin-converting enzyme, can be used as an antioxidant, and also has the potential to be used as a functional food or nutraceutical for the prevention and treatment of obesity and hypertension. Secondary metabolites play a decisive role in the antioxidant activity of *A. mongolicum* Regel and are derived from corresponding primary metabolites, and secondary metabolites play an important role in the adaptation of the plant to its environment or its resistance to pathogens, benefiting human nutrition and health (Kennedy & Wightman, 2011). Studies have shown that these antioxidants play a key role in protecting vegetables from pests and diseases, inhibiting the multiplication of pathogenic microorganisms and extending the shelf life of vegetables (Lakhani, Kamra, Lakhani, & Alhussien, 2019). Produced locally in Gansu, which gives them excellent quality and

---

popularity among consumers, may be a response of the flavonoids, polyphenols and other active ingredients in *A. mongolicum* Regel to the unique geographical and ecological conditions of Gansu, thus distinguishing it from other regions (Liu et al., 2023).

To date, the main product available on the market is fresh *A. mongolicum* Regel (FA), but after the drying process, the dried *A. mongolicum* Regel (DA) powder is easier to preserve and can be stored for a long time. DA can also be widely added to animal feed as a growth promoter (Ding, Liu, Erdene, Du, & Ao, 2021; Liu & Ao, 2021) and can be used in small quantities as a food seasoning (Hithamani & Srinivasan, 2016), and even to extend the shelf life of sausages (Zhao et al., 2022) and sheep meat (Liu et al., 2022) because of its antioxidant activity. Drying *A. mongolicum* Regel leaves reduces their moisture content to a safe level, overcoming the disadvantages of FA leaves, which are seasonal, difficult to store and cannot be consumed year-round (Zhang, Cao, Li, & Wang, 2022). Currently, the main methods of drying *A. mongolicum* Regel leaves are daylight, hot air, microwave, convection, vacuum and freeze drying methods, mainly depending on the amount of *A. mongolicum* Regel powder desired (Qu et al., 2015; Wang et al., 2019; Zhang et al., 2022). It has been found that drying may lead to changes in the flavour and bioactive composition of some vegetables or fruits, which in turn may affect their nutritional value and antioxidant activity (Zhou, Gao, Mitcham, & Wang, 2018). Unfortunately, in comparison with other food products, studies on the drying of *A. mongolicum* Regel are very limited. In addition, the correlation coefficients between the antioxidant activities of phenolics, flavonoids and their corresponding contents were 0.82–0.92 (Wu et al., 2021). The antioxidant activity of *A. mongolicum* Regel is usually significantly correlated with its total phenolic content (TPC) and total flavonoid content (TFC) and is usually evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging rate and the 2,2-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging rate (Dean, 2018; Fu, Qu, Yang, & Zhang, 2016).

Plant metabolomics can reflect the biochemical and physiological state of plant cells at specific developmental stages under specific environmental conditions and is therefore closely related to plant phenotypes (Abdelrahman, Burritt, & Tran, 2018). To date, there is relatively little research into the postmowing processing and storage of *A. mongolicum* Regel, and production and consumption are still largely based on experience or subjective evaluation, which directly affects the large-scale cultivation and promotion of *A. mongolicum* Regel. The key metabolites and metabolic pathways associated with the antioxidant components of *A. mongolicum* Regel remain to be explored, and the composition and metabolism of antioxidant substances in different varieties of DA and FA have not been systematically studied. In this study, metabolomics was used to characterize and compare the metabolite composition of both fresh and dried greenhouse *A. mongolicum* Regel (GAMR) leaves and wild *A. mongolicum* Regel (WAMR) leaves from Gansu and to investigate the mechanism of change in the antioxidant activity of *A. mongolicum* Regel before and after drying.

2. Materials and methods

2.1. Materials

The study materials included two different varieties of *A. mongolicum* Regel, including GAMR plants situated at 38°78′N, 101°08′E, 2919 m above sea level, and WAMR plants growing in the Gobi desert situated at 38°91′N, 101°02′E, 3015 m above sea level. All samples were collected from the same commercial planting area in Zhangye, Gansu Province, China. *A. mongolicum* Regel leaves were picked at the squaring stage in July 2022. All *A. mongolicum* Regel leaf samples were divided into two groups: FA leaves and DA leaves. In the FA group, the fresh samples were cleaned and wrapped in aluminium foil, flash-frozen in liquid N_2 and stored at −80 °C in an ultralow temperature freezer. In the DA group, the freshly washed *A. mongolicum* Regel leaves (each *A. mongolicum* Regel

leaf has an average weight of 1–2 g, a length of 20–30 cm, and a diameter of 0.5–1.5 mm) were freeze-dried by a vacuum freeze dryer (FD-1D-50, Shanghai Sun System Instrument Manufacturing Co., Ltd., Shanghai, China), in which the temperature was set at −65 °C, the time was set to 72 h, and vacuum pressure was adjusted to 0.00 Mpa. The vacuum was temporarily interrupted by removing the trays from the vacuum chamber every 30 mins during the experiment. Samples were weighed using an electronic balance (M20S, Sartorius, Berlin, German). All measurements were made within 1 min. The trays were again returned to the vacuum chamber to continue drying until the moisture content of the *A. mongolicum* Regel leaves was reduced to 10%. Drying until the moisture content of the sample is below 10% on a dry basis. The dried *A. mongolicum* Regel leaves were ground by a crusher (XL-30C, Guangzhou Xulang Machinery Equipment Co., Ltd., Guangzhou, China) and filtered through a 60-mesh sieve, and the prepared powder was stored at 4 °C in the dark until use. In order to make fresh and dried *A. mongolicum* Regel comparable, the moisture content of the two varieties of GAMR and WAMR was determined and used for dry matter content correction.

2.2. Chemicals and reagents

Anhydrous ether, anhydrous ethanol, sodium nitrite, aluminium nitrate, sodium hydroxide, sodium carbonate, copper sulfate, and potassium tartrate were obtained from Jining Shiye Reagent Company Limited (Tianjin, China). Gallic acid, rutin, and standard compounds were obtained from Sigma Aldrich (Milwaukee, WI, Germany) with a purity of >98%. DPPH, ABTS, and Folin–Ciocalteu phenol reagent were purchased from Sigma Aldrich (Milwaukee, WI, Germany). Other reagents were all of analytical grade.

2.3. Measurement of antioxidant activity

The measurement of total phenolic content (TPC) and total flavonoids content (TFC) was carried out as described by Han et al. (2019). Briefly, 0.1 g of *A. mongolicum* Regel leaf sample ground in liquid N_2 was added to 2 mL of (ethanol/water 3:5, *v/v*) ethanol solution, followed by vortexing for 2–3 min. The extract was placed in a constant temperature water bath (Precision GP 10, ThermoFisher Scientific, United States of America) at 60 °C for 30 min and centrifuged (Frontier™ 5000 Multi Pro, Guangzhou Giddy Instrument Co., China) at 4000 ×*g* for 10 min at 25 °C, and the supernatant was taken for the determination of TPC, TFC, and DPPH and ABTS scavenging activity.

2.3.1. The total phenolic content assay

The TPC of each sample was assessed in adherence to the colorimetric Folin–Ciocalteu method reported by Kortei et al. (2014). Briefly, 1 mL of sample or gallic acid solution dissolved in distilled water at different concentrations was mixed with 0.5 mL of 10% Folin–Ciocalteu reagent, and the mixture in assay tubes was incubated at 37 °C for 30 min. Finally, the mixture was transferred to a cuvette, and the absorbance was measured at 680 nm using an ultraviolet–visible spectrophotometer (UV5 Bio, Shanghai, China). The calibration curve was prepared using various concentrations of gallic acid (0–500 μg/mL) dissolved in 5% methanol/95% ethanol (*v/v*) solution. The results are expressed as mg GAE per gram of *A. mongolicum* Regel leaves on a dry weight basis (mg GAE/g).

2.3.2. The total flavonoids content assay

The TFC of each sample was assessed using the aluminium nitrate method as described by Liu et al. (2009). Briefly, 1 mL of sample or rutin solution dissolved in 95% ethanol (*v/v*) was mixed with 1 mL of 5% sodium nitrite, and then 1 mL of 10% aluminium nitrate and 10 mL of 4% sodium hydroxide were added in sequential order. Finally, the mixture was transferred to a cuvette, and the absorbance was measured at 510 nm using an ultraviolet–visible spectrophotometer (UV5 Bio,

Shanghai, China) after 15 min of standing. The calibration curve was prepared using various concentrations of rutin (0–250 μg/mL) dissolved in 5% methanol/95% ethanol (v/v) solution. The TFC results are shown as mg rutin equivalents (RTE) per gram of *A. mongolicum* Regel on a dry weight basis (mg RTE/g).

2.3.3. DPPH and ABTS assays

The antioxidant activity of *A. mongolicum* Regel leaves was measured in terms of DPPH radical scavenging and ABTS radical scavenging. DPPH radical scavenging (A015–2-1) and ABTS radical scavenging (A143–1-1) were assayed using chemical kits from Nanjing Jiancheng Bioengineering Institute. The results of TPC, TFC, and DPPH and ABTS scavenging activities were calculated on a dry weight basis of *A. mongolicum* Regel leaf samples.

2.4. Extraction and LC–MS/MS identification of primary and secondary metabolites

The method for the extraction and identification of primary and secondary metabolites in DE and FA leaves was performed according to the method described Want et al. (2013) with minor modifications.

2.4.1. Methods of sample pretreatment

Tissue samples (100 mg) were individually ground with liquid nitrogen, and the homogenate was resuspended with prechilled 80% methanol by vortexing (IKA Vortex, IKA India.

Private Limited, Staufen, Germany). The samples were incubated on ice for 5 min and then centrifuged (Micro-15R, KEWLAB, Australia) at 15,000 $\times g$ at 4 °C for 20 min. Some of the supernatant was diluted to a final concentration containing 53% methanol with LC–MS grade water. The samples were subsequently transferred to a fresh eppendorf tube and then centrifuged at 15,000 $\times g$ for 20 min at 4 °C. Finally, the supernatant was injected into the LC–MS/MS system for analysis. The data comes from six biological replicates in each group.

2.4.2. LC–MS/MS analysis

LC–MS/MS analyses were performed using an ExionLC™ AD system (SCIEX) coupled with a QTRAP® 6500$^+$ mass spectrometer (SCIEX) at Novogene Co., Ltd. (Beijing, China). Samples were injected onto an Xselect HSS T3 column (2.1 mm × 150 mm, 2.5 μm) using a 20-min linear gradient at a flow rate of 0.4 mL/min for positive/negative polarity modes. The mobile phases were eluent A (0.1% formic acid-water) and eluent B (0.1% formic acid-acetonitrile). The solvent gradient was set as follows: 2% B, 2.0 min; 2–100% B, 15.0 min; 100% B, 17.0 min; 100–2% B, 17.1 min; and 2% B, 20.0 min. The QTRAP® 6500+ mass spectrometer was operated in positive polarity mode with a curtain gas pressure of 35 psi, a collision gas setting of medium, an ion spray voltage of 5500 V, a temperature of 550 °C, an ion source gas 1 setting of 60, and an ion source gas 2 setting of 60. The QTRAP® 6500$^+$ mass spectrometer was operated in negative polarity mode with a curtain gas pressure of 35 psi, a collision gas setting of medium, an ion spray voltage of −4500 V, a temperature of 550 °C, an ion source gas 1 setting of 60, and an ion source gas 2 setting of 60.

2.4.3. Metabolite identification and quantification

The detection of the experimental samples using multiple reaction monitoring (MRM) was based on a Novogene in-house database. Q3 was used for metabolite quantification. Q1, Q3, retention time (RT), declustering potential (DP) and collision energy (CE) were used for metabolite identification. The data files generated by HPLC–MS/MS were processed using SCIEX OS Version 1.4 to integrate and correct the peaks. The main parameters were set as follows: minimum peak height, 500; signal/noise ratio, 5; and Gaussian smooth width, 1. The area of each peak represents the relative content of the corresponding substance.

2.5. Analysis of fatty acids

We used targeted metabolomics to verify the content of fatty acids in DA and FA leaves. A stock solution of individual fatty acids (ZZ Standards Co., LTD. Shanghai, China) was mixed and prepared in a fatty acid-free matrix to obtain a series of fatty acid calibrators at concentrations of 40,000, 20,000, 10,000, 4000, 2000, 1000, 400, 200, 100, 40, 20 and 10 ng/mL. Certain concentrations of decanoic acid-d_{19}, myristic acid-d_2, octadecanoic acid-d_{35}, eicosanoic acid-d_{39} and lignoceric acid-d_4 (Thermo-Fisher Scientific, FairLawn, NJ, USA) were mixed as internal standards (ISs). The stock solutions of all of these ISs and standards and working solutions were stored in a freezer at −20 °C. The samples (100 μL) were homogenized with 300 μL of isopropanol/acetonitrile (1:1), which contained mixed ISs, and centrifuged at 12,000 rpm for 10 min. Finally, the supernatant (2 μL) was injected into the LC–MS/MS system for analysis.

An ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) system (ExionLC™ AD UHPLC-QTRAP 6500$^+$, AB SCIEX Corp., Boston, MA, USA) was used to quantitate fatty acids by Novogene Co., Ltd. (Beijing, China). Separation was performed on a Waters ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm), which was maintained at 50 °C. The mobile phase, consisting of 0.05% formic acid in water (solvent A) and isopropanol/acetonitrile (1:1) (solvent B), was delivered at a flow rate of 0.30 mL/min. The solvent gradient was set as follows: 30% B, 1 min; 30–65% B, 2 min; 65–100% B, 11 min; 100% B, 13.5 min; 100–30% B, 14 min; and 30% B, 15 min. The mass spectrometer was operated in negative MRM mode. The parameters were as follows: ion spray voltage, −4500 V; curtain gas, 35 psi; ion source temperature, 550 °C; and ion source gas 1 and 2, 60 psi. Fatty acids of *A. mongolicum* Regel were quantified based on the IS content (ng/g) and fresh weight.

2.6. Statistical analyses

One-way ANOVA was used to test antioxidant activity between dried *A. mongolicum* Regel leaves and fresh *A. mongolicum* Regel leaves using SAS (v 9.4) (SAS Inst. Inc., Cary, NC, USA). Differences were considered significant at $P < 0.05$. Histograms of DPPH scavenging activity, ABTS scavenging activity, TPC and TFC comparisons between two varieties of FA and DA were visualized using GraphPad Prism 5 package (GraphPad Inc., San Diego, CA, USA). Principal component analysis (PCA) and partial least squares discriminant analysis (PLS—DA) were performed with metaX (Luo et al., 2015). We applied univariate analysis (*t*-test) to calculate the statistical significance (*P* value). Metabolites with VIP > 1 and *P* value<0.05 and fold change≥2 or FC ≤0.5 were considered to be differential metabolites. For clustering heatmaps, the data were normalized using z scores of the intensity areas of differential metabolites and were plotted by the Pheatmap package in *R* language. Significant correlations between differential metabolites were calculated by cor.mtest () in R language. A *P* value <0.05 was considered significant, and correlation plots were plotted by the corrplot package in *R* language. The functions of these metabolites and metabolic pathways were studied using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Metabolic pathway enrichment of differential metabolites was performed. When the ratio was satisfied by x/n > y/N, metabolic pathways were considered enriched. When the *P* value of metabolic pathways <0.05, metabolic pathways were considered significant enriched.

3. Results

3.1. Antioxidant activity, total phenolic content and total flavonoid content differences between dtied A. mongolicum Regel and fresh A. mongolicum Regel leaves

To determine the mechanisms by which drying impacts the

antioxidant activity of *A. mongolicum* Regel, we first determined the TPC, TFC and antioxidant activity differences. DPPH and ABTS assays as well as TPC and total TFC determination were used to assess the antioxidant activity differences between DA and FA. The results suggested that DA had better antioxidant performance than FA in all tested *A. mongolicum* Regel leaf samples (Fig. 1A-D). Specifically, compared with that of FA, the DPPH value of DA was increased 19.2% (GAMR) to 19.6% (WAMR); the ABTS value of DA was increased by 52.4% (WAMR) to 53.1% (GAMR) (Fig. 1A and B). For the TPC and TFC, the highest contents were 3.69 mg GAE/g (GAMR) and 3.19 mg RTE/g (WAMR) in FA and 20.78 mg GAE/g (GAMR) and 15.49 mg RTE/g (WAMR) in DA, respectively. Compared with those in FA, the TPC and TFC increased by 19.47% and 20.15% on average in DA, respectively (Fig. 1C and D). Interestingly, in FA, there were no significant differences in TPC between GAMR and WAMR, but the results of the DPPH and ABTS assays were significantly different. However, there were significant differences in antioxidant activity between GAMR and WAMR after drying, with DA leaves exhibiting significantly stronger antioxidant activity than FA leaves ($P < 0.05$). Moisture content is an important indicator to distinguish between DA and FA leaves. The moisture content ranged from 87.32% to 90.45% in FW, and ranged from 7.59 to 9.26% in DW (Fig. 1E).

3.2. LC–MS/MS-based class-targeted metabolomics analysis of dried *A. mongolicum* Regel and fresh *A. mongolicum* Regel leaves

The primary and secondary metabolites of GAMR and WAMR were identified by LC–MS/MS to better investigate the changes in the relevant metabolites in the fresh and dried leaves of *A. mongolicum* Regel. The Heatmap combined with PCA was used to.

investigate the differences in metabolites between GAMR and WAMR as well as before and after drying. Hierarchical clustering analysis of all differential metabolites revealed that the 24 samples were clearly divided into 2 groups (FA vs. DA) (Fig. 2A), and PCA also revealed a clear separation between FA (including FA-GAMR and FA-WAMR) and DA (including DA-GAMR and DA-WAMR) (Fig. 2B). This plan consists of the first two factors; PCA1 and PCA2, which explain 61.44% and 13.19% of the variability, respectively. The R2 value (0.0, 0.34) in the PLS-DA was greater than the Q2 value (0.0, −0.69), and the intercept between the Q2 regression line and the Y-axis was <0 (Fig. 2D). This result proves that the proposed model is not overfitted (Wang et al., 2014).

As shown in Fig. 2C, 713 (688 metabolites upregulated, 25 metabolites downregulated) metabolites were identified and could be categorized into at least 16 different classes, but the majority of metabolites were categorized into eight classes, including amino acids and derivatives (20.48%), flavonoids (14.17%), lipids (8.98%), nucleotides
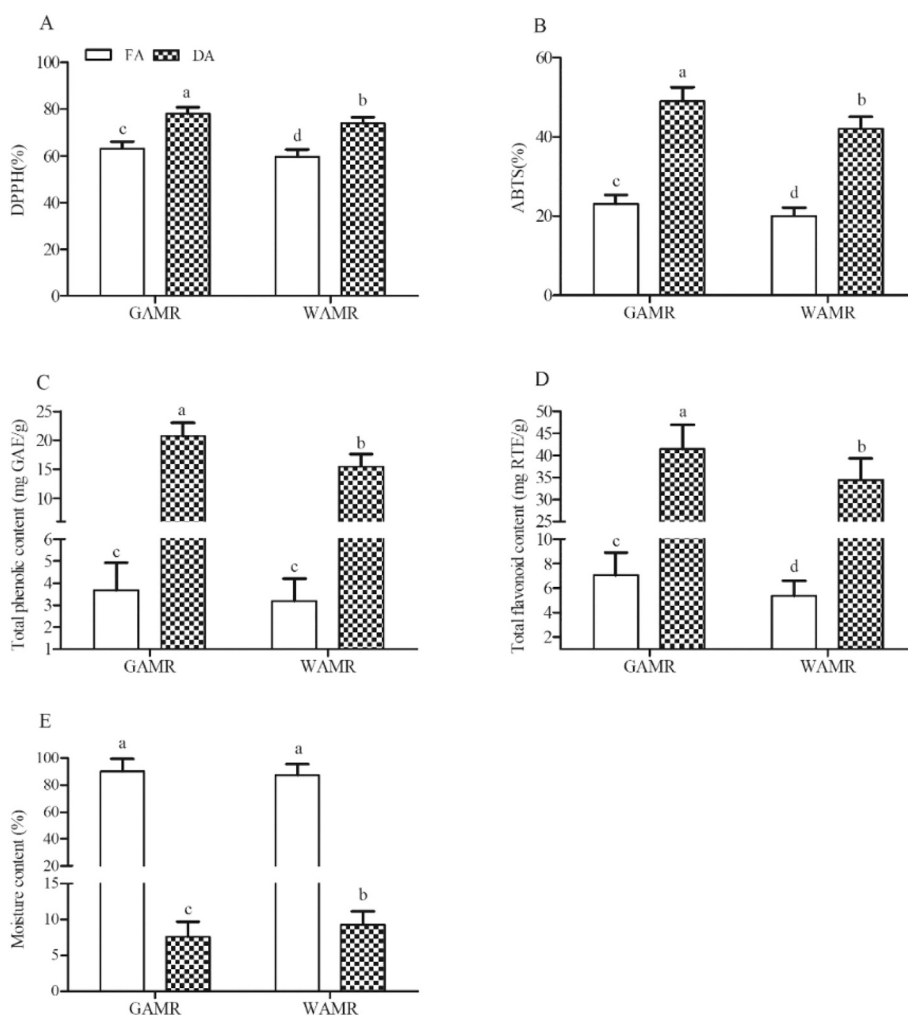

Fig. 1. Comparison of antioxidant activity and main active ingredients between the two varieties of FA and DA. (A) DPPH scavenging activity; (B) ABTS scavenging activity; (C) total phenolic content; (D) total flavonoid content; (E) moisture content of *A. mongolicum* Regel. The data are expressed on a dry weight basis for *A. mongolicum* Regel leaf samples and are the means of ten biological replicates. a, b, c and d: mean values with different letters are significantly different at $P < 0.05$. FA leaves, fresh *A. mongolicum* Regel leaves; DA leaves, dried *A. mongolicum* Regel leaves; GAMR, *A. mongolicum* Regel grown in a greenhouse; WAMR, *A. mongolicum* Regel grown in the Gobi Desert.

A

B

C

- Phytohormones
- Terpenoids
- Alkaloids and derivatives
- Phenols and its derivatives
- Flavonoids
- Lipids
- Organic acid and its derivatives
- Carbohydrates and its derivatives
- Amino acid and derivatives
- Phenylpropanoids
- Organoheterocyclic compounds
- Purines and purine derivatives
- Amines
- Cinnamic acids and derivatives
- Nucleotide and its derivates
- Others

D

E

(caption on next page)

Fig. 2. Identification and analysis of differentially abundant metabolites in fresh and dried leaves of GAMR and WAMR. (A) Heatmap of total differentially abundant metabolite clustering in positive and negative ion mode. (B) Principal component analysis graph. (C) Pie chart of the differentially abundant metabolite classification in two varieties of *A. mongolicum* Regel leaves in the dried and fresh states. (D) Partial least squares discrimination analysis. (E) Venn diagram showing the numbers of differentially abundant metabolites in FAGA vs. DAGA (FA-GAMR vs. DA-GAMR), FAWA vs. DAWA (FA-WAMR vs. DA-WAMR), FAGA vs. FAWA (FA-GAMR vs. FA-WAMR) and DAGA vs. DAWA (DA-GAMR vs. DA-WAMR). FA leaves, fresh *A. mongolicum* Regel leaves; DA leaves, dried *A. mongolicum* Regel leaves; GAMR, *A. mongolicum* Regel grown in a greenhouse; WAMR, *A. mongolicum* Regel grown in the Gobi Desert.

and their derivatives (7.43%), phenols and their derivatives (6.31%), organic acids and their derivatives (6.17%), phenylpropanoids (6.03%), and sugars and their derivatives (4.77%). Of these, flavonoids, phenols and their derivatives are the main secondary metabolites, and amino acids and derivatives, lipids, organic acids and their derivatives and carbohydrates and their derivatives are the main primary metabolites. Amino acids and their derivatives, flavonoids and lipids are the top three major metabolites, of which flavonoids can be further classified as 10 flavanones, 21 flavones and flavonols, 50 flavonoids, 3 isoflavonoids, 6 anthocyanins, and 11 other flavonoids. Among these metabolites, 634, 630, 320 and 383 metabolites were detected from FAGA vs. DAGA (FA-GAMR vs. DA-GAMR), FAWA vs. DAWA (FA-WAMR vs. DA-WAMR), FAGA vs. FAWA (FA-GAMR vs. FA-WAMR) and DAGA vs. DAWA (DA-GAMR vs. DA-WAMR), respectively (Fig. 2E). Overall, the results indicated that the distribution of metabolites is different between DA and FA.

3.3. Differential metabolite-based enrichment of KEGG pathways

KEGG is a powerful tool for metabolic analysis and metabolic network studies in living organisms. The enrichment results are presented in KEGG pathway units and are based on the principle of the hypergeometric distribution test (Jia et al., 2016). The KEGG pathway enrichment analysis allowed for the identification of the main biological functions performed by the differential metabolites. A bubble diagram of the top 20 enriched metabolic pathways is shown in Supplementary Fig. S1. The secondary metabolites (Supplementary Table S1) and primary metabolites (Supplementary Table S2) enriched in the Top-20 metabolic pathway were rearranged and analysed to better investigate changes in the major metabolites of *A. mongolicum* Regel leaves after drying. We observed that these differential metabolites were eventually enriched in lipid metabolism, amino acid metabolism, sugar metabolism and flavonoid synthesis pathways, and these results suggested that drying had a significant effect on the relevant metabolic pathways.

3.4. Secondary metabolites in DA and FA leaves

A total of 34 secondary metabolites were detected in two varieties of FA and DA by LC–MS/MS, including 16 flavonoids, 6 phenolics, and several other metabolites (Supplementary Table S3). The total relative contents of flavonoid, phenolic, other metabolites, and secondary metabolites in the DA of both varieties were significantly higher than those in FA (Fig. 3 A-D). Compared with those in FA, the relative contents of flavonoids, phenolics, other metabolites and total secondary metabolites in DA increased by an average of 12.20-, 21.51-, 35.44- and 23.31-fold, respectively. Isorhamnetin and protocatechuic acid were the most significantly upregulated flavonoid and phenolic in *A. mongolicum* Regel, respectively, demonstrating that DA had a significantly higher accumulation of isorhamnetin and protocatechuic acid. Moreover, GAMR and WAMR showed the most and least significant increases in flavonoid, phenolic, other metabolites, and secondary metabolites, respectively (Supplementary Table S1). In summary, flavonoids and phenolics were the dominant secondary metabolites, among which isorhamnetin and protocatechuic acid were the most abundant in *A. mongolicum* Regel. The contents of isorhamnetin and protocatechuic acid increased significantly after drying, varying greatly between different varieties.

Hierarchical cluster analysis was carried out to divide different

varieties of *A. mongolicum* Regel before and after drying based on secondary metabolite composition. The results showed that the 34 secondary metabolites were clearly divided into two groups (DA and FA) and were all upregulated in DA (Fig. 3 E). The shikimate pathway is one of the important pathways for the synthesis of secondary metabolites in plants, while the phenylpropane pathway is the main pathway for the production of plant flavonoids and phenolics (Abdelrahman et al., 2020). Of these metabolites, shikimic acid is the precursor for the start of the shikimate pathway and is further reacted by phosphorylation to produce phenylalanine and tyrosine, the two essential amino acids that are the starting molecules of the phenylpropane pathway. Esculetin (19.62 vs. 11.68-fold), ferulic acid (18.14 vs. 10.71-fold), neochlorogenic acid (3.73 vs. 1.62-fold), naringenin chalcone (10.60 vs. 3.68-fold), and butein (3.82 vs. 3.02-fold) are all important intermediates in the shikimate pathway and were significantly upregulated upon drying between GAMR and WAMR. In particular, downstream metabolites of phenylpropanoid metabolism, including isorhamnetin, protocatechuic acid, kaempferol, quercetin, and eriodictyol, were all significantly upregulated in DA. The isorhamnetin content of GAMR was upregulated by 65.86-fold, and the protocatechuic acid of WAMR was upregulated by 182.06-fold in DA. The above results suggest that the metabolic pathway of phenylpropane pathway may be enhanced during the drying of *A. mongolicum* Regel leaves, leading to the accumulation of flavonoids and phenolic compounds, while the increase in content of these secondary metabolites may suggest a decrease in some primary metabolites.

3.5. Primary metabolites differences between DA and FA leaves

A total of 74 primary metabolites were identified by LC–MS/MS in all *A. mongolicum* Regel leaf samples, including 24 amino acids and derivatives, 22 organic acids and their derivatives, 13 lipids, 7 sugars and their derivatives and 8 other metabolites (Supplementary Table S4). Compared with FA, DA had higher total relative contents of amino acid metabolites (Fig. 4 A), lipid metabolites (Fig. 4 B), organic acid metabolites (Fig. 4 C), and primary metabolites (Fig. 4 E) but a lower total relative content of sugar metabolites (Fig. 4 D). The 74 primary metabolites were divided into two groups by hierarchical clustering: one group included metabolites whose content decreased after drying, including most of the sugar and its derivatives, and the other group included the compounds that increased significantly upon drying, including most of the amino acid, lipid, and organic acid derivatives (Fig. 4 F). The significantly downregulated sugars and lipids mainly included dihydroxyacetone phosphate, 6-phosphogluconic acid, d-glucuronic acid and S7p, 2-alpha-linolenoyl-glycerol, and MAG (18:2). The relative amounts of these metabolites were significantly lower in DA than in FA of both varieties, with the most obvious decline being found for 2-alpha-linolenoyl-glycerol and S7p in GAMR (40.63-fold) and WAMR (52.59-fold), respectively (Supplementary Table S2). 6-Phosphogluconic acid and S7p are involved in the pentose phosphate pathway, while dihydroxyacetone phosphate, 2-alpha-linolenoyl-glycerol, MAG (18:2) is an intermediate in the glycolytic pathway, and the downregulation of these metabolites suggests that the content of precursors in *A. mongolicum* Regel for the synthesis of secondary metabolites decrease after drying. The primary metabolites with significant increases in content included most organic acids and their derivatives, except for O-phosphorylethanolamine. *cis*-aconitic acid, citramalate, (2*S*)-2-isopropylmalate, succinic acid, citric acid, and isocitrate, which have all
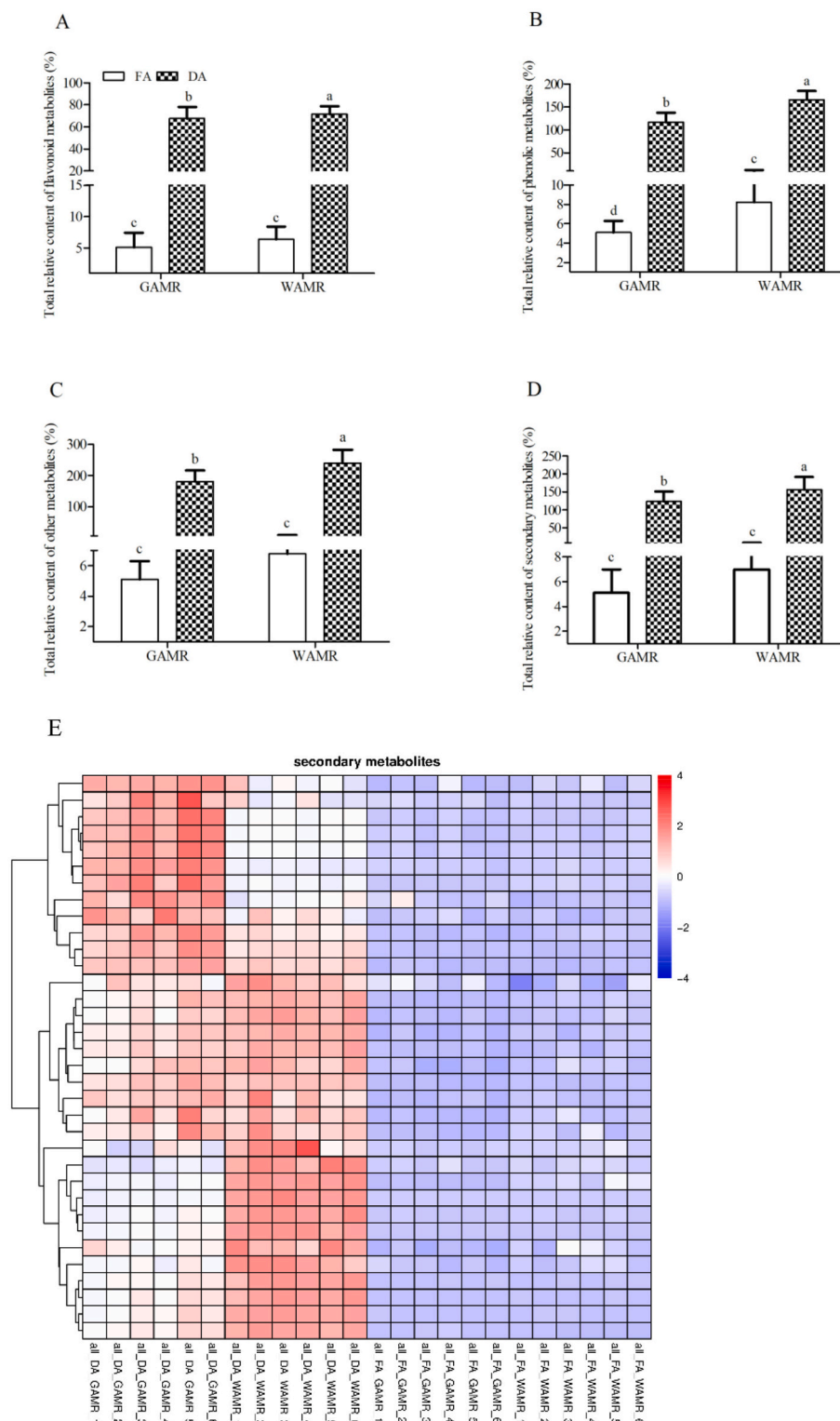
Fig. 3. Secondary metabolite analysis in two varieties of FA and DA. (A) Total relative content of flavonoid metabolites. (B) Total relative content of phenolic metabolites. (C) Total relative content of other metabolites. (D) Total relative content of secondary metabolites. The data are the means from six biological replicates. a, b, c and d: mean values with different letters are significantly different at $P < 0.05$. (E) Hierarchical cluster analysis of secondary metabolites from two varieties of FA and DA. The scale in the colour bar was standardized using the scale function of raw abundance for the compounds. The differences in metabolites are indicated by different colours: blue indicates lower abundance, while red indicates higher abundance. The data were obtained from six biological replicates. FA, fresh *A. mongolicum* Regel leaves; DA, dried *A. mongolicum* Regel leaves; GAMR, *A. mongolicum* Regel grown in the greenhouse; WAMR, *A. mongolicum* Regel grown in the Gobi Desert. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
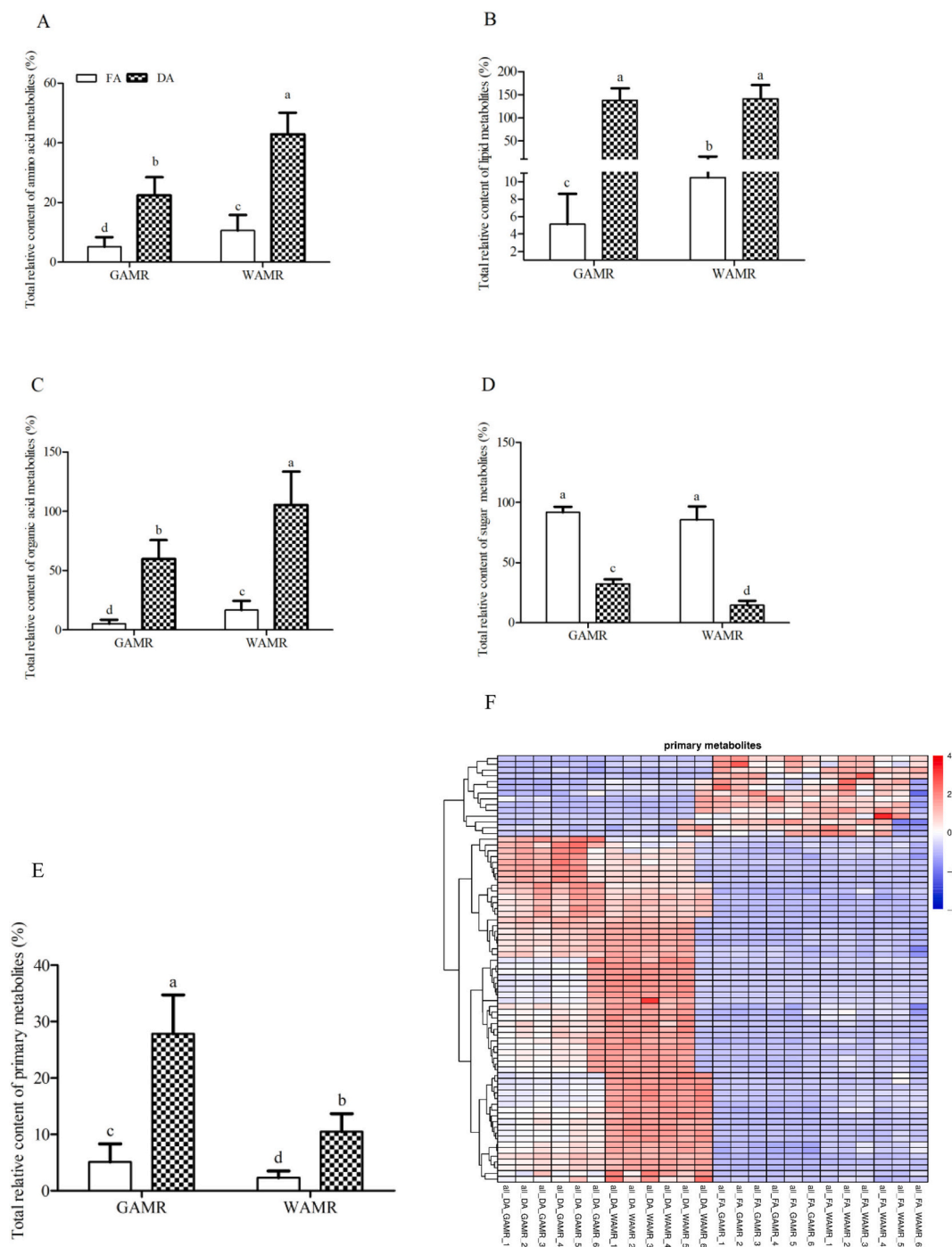
Fig. 4. Primary metabolite analysis of two varieties of FA and DA. (A) Total relative content of amino acid metabolites. (B) Total relative content of lipid metabolites. (C) Total relative content of organic acid metabolites. (D) Total relative content of sugar metabolites. (E) Total relative content of primary metabolites. The data are the means from six biological replicates. a, b, c and d: mean values with different letters are significantly different at $P < 0.05$. (F) Hierarchical cluster analysis of the primary metabolites of two varieties of FA and DA. The scale in the colour bar was standardized using the scale function of raw abundance for the compounds. The differences in metabolites are indicated by different colours: blue indicates lower abundance, while red indicates higher abundance. The data were obtained from six biological replicates. FA, fresh *A. mongolicum* Regel leaves; DA, dried *A. mongolicum* Regel leaves; GAMR, *A. mongolicum* Regel grown in the greenhouse; WAMR, *A. mongolicum* Regel grown in the Gobi Desert. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

been shown to be involved in the tricarboxylic acid cycle. p-Hydroxy-cinnamic acid, 2-hydroxycinnamate, 3-hydroxycinnamic acid, p-cou-maric acid, hydroxycinnamate, sinapinic acid, and caffeic acid and their derivatives have been shown to be involved in the phenylpropane pathway to produce flavonoids, and these metabolites were both significantly upregulated during the drying process. In addition, malonic acid and methylmalonate were both upregulated in the DA samples, and these compounds were shown to be involved in the acetate-malonate (AA-MA) pathway for the synthesis of phenolic compounds. At the same time, a comparative analysis showed that metabolites associated with both amino acid and fatty acid synthesis, particularly unsaturated fatty acid (UFA) synthesis, were significantly upregulated, suggesting a

possible increase in fatty acid content in DA.

3.6. Fatty acid profiles of DA and FA leaves

We used targeted metabolomics to detect the changes in fatty acid composition in two varieties of FA and DA and the corresponding impact of drying (Fig. 5 E). In the *A. mongolicum* Regel samples of the present study, a total of 44 fatty acids were detected and identified. DA had a significant increase in the total content of fatty acids relative to that of FA (2.68-fold), while the total amount of fatty acids in WAMR was 1.68-fold that in GAMR (Supplementary Table S5). Gamma-linolenic acid (c18:3 n-6) and alpha-linolenic acid (c18:3 n-3) were the most abundant fatty acids in *A. mongolicum* Regel. In DA, the contents of c18:3 n-6 and c18:3 n-3 in WAMR were significantly higher than those in GAMR. Drying significantly promoted the accumulation of the mono-unsaturated fatty acids (MUFAs) (t15:1, c18:1 n-9, c18:1 n-7, t16:1, t14:1, c14:1; Supplementary Fig. S2 A-F) and the trace PUFAs (c18:2 n-6, c20:5; Supplementary Fig. S2 G and H) in both *A. mongolicum* Regel varieties. Interestingly, some fatty acids (t18:2 n-6, c17:1, t22:1; Supplementary Fig. S2 I—K) had lower contents in DA than in FA. This result is consistent with the results of primary metabolite analysis. c18:2 n-6 [(7S,8S)-DiHODE, Supplementary Table S4] was a primary metabolite that was significantly downregulated after drying.

UFAs are usually used to estimate the nutritional value of *A. mongolicum* Regel. In this study, UFAs in FA and DA accounted for >63% of the total fatty acids on average (Fig. 5B). The proportions of UFAs and PUFAs in DA were lower than those in FA (Fig. 5B, and C) of two varieties, but the opposite was true for saturated fatty acid (SFA) proportions (Fig. 5A). Interestingly, for MUFAs, there was no significant change in the content before and after drying in GAMR, while the content of MUFAs decreased significantly after drying in WAMR (Fig. 5D). These results indicated that the accumulation rate of MUFAs during the drying process was different in the two varieties. In FA, the proportion of PUFAs was 71.83% (GAMR) ~ 80.50% (WAMR), the proportion of SFAs was 13.06% (WAMR) ~ 22.80% (GAMR), and that of MUFAs was 5.40% (GAMR) ~ 6.40% (WAMR). In DA, PUFAs accounted for over 61.50% of fatty acids in WAMR and 59.90% in GAMR. SFAs accounted for over 36.08% of fatty acids in WAMR and 34.30% in GAMR, although there were no significant differences between the two *A. mongolicum* Regel varieties, and MUFAs accounted for >5.17% of fatty acids in GAMR and only 2.45% in WAMR. These results suggested that drying may have a significant impact on the UFA and SFA contents of *A. mongolicum* Regel, particularly that of PUFAs. The decrease in the PUFA content was significantly higher than that in the MUFA content, which indicated that PUFAs were oxidized to form MUFAs and SFAs first in the drying stage. Moreover, MUFAs are produced via the oxidation of PUFAs, and the oxidation of MUFAs contained in *A. mongolicum* Regel during the drying process results in less reduction in the total content of MUFAs.

3.7. Relationship between key differential metabolites and antioxidant activity

We selected target primary and secondary metabolites with Pearson correlation coefficients higher or lower than ±0.90 with at least one of the parameters MC, TPC, TFC, or ABTS and DPPH scavenging activity to understand the relationship between the key differential metabolites and antioxidant activity. As a result, 22 metabolites were finally selected (Table 1). As shown in Table 1, 7 metabolites had direct and significant effects on the results of ABTS assays of *A. mongolicum* Regel, including four flavonoids [naringenin ($\rho = 0.93$), quercetin ($\rho = 0.91$), naringenin chalcone ($\rho = 0.92$), eriodictyol ($\rho = 0.92$)], one phenolic [ferulic acid ($\rho = 0.94$)], and two other metabolites [GA7–1 ($\rho = 0.96$), esculetin ($\rho = 0.97$)]. Interestingly, only two metabolites had a direct and significant effect on DPPH scavenging activity: quercetin ($\rho = 0.91$) and ferulic acid ($\rho = 0.92$). Except for (7S,8S)-DiHODE, the other primary metabolites were all significantly and positively correlated with TPC and TFC,

including amino acids and their derivatives, lipids, organic acids and their derivatives, and other metabolites, among which 3-hydroxycin-namic acid and TPC had the lowest correlation coefficient ($\rho = 0.90$), and malonic acid and TFC had the lowest correlation coefficient ($\rho = 0.90$). Among the secondary metabolites, GA7–1 ($\rho = 0.98$), esculetin ($\rho = 0.98$) and TPC had the highest correlation coefficient. The TFC also showed the same results. The above results suggested that the key primary metabolites were directly and significantly correlated with the synthesis of phenolics and flavonoids, while the secondary metabolites were directly correlated with the antioxidant activity of *A. mongolicum* Regel.

4. Discussion

The antioxidant activity of *A. mongolicum* Regel is one of the most important indicators of its nutritional value, and the level of antioxidant activity depends mainly on the method of processing, the way it is grown and its origin. This study was the first to investigate the effect of drying treatment on the antioxidant activity of different varieties of *A. mongolicum* Regel. The results showed that the antioxidant activity of FA was significantly lower than that of DA, indicating that drying could improve the antioxidant activity of *A. mongolicum* Regel. Recent studies have shown that different drying methods have a significant effect on the degree of retention of the volatile components of *A. mongolicum* Regel (Zhang et al., 2022), but there is no literature on the effect of drying on the antioxidant activity of *A. mongolicum* Regel. Interestingly, we found similar findings in the cash crop walnut, where drying increased the TPC and TFC content of walnut kernels and improved their antioxidant activity (Wang et al., 2022; Wu et al., 2021). TPC and TFC are important indicators of the antioxidant activity of *A. mongolicum* Regel (Wang et al., 2019). The TPC in FA was not significantly different between the two varieties, and the TPC in DA was significantly different, but the results of the DPPH and ABTS assays and the TFC were significantly different, indicating that drying had a greater effect on the antioxidant activity of *A. mongolicum* Regel than the variety.

Active ingredients are usually plant secondary metabolites (quinones, alkaloids, flavonoids, terpenoids, phenylpropanoids, polysaccharides, etc.), flavonoids and phenolic acids are important antioxidant secondary metabolites under the action of adversity stress, is an important material basis for the plant's adversity (insect pests, water, temperature or heavy metals, and other environmental stress due to) to enhance the ability of its resistance to external stress, and the drying for the role of the harvested fresh roots and stems of plants in the process of the process of the substance that is under the environment of loss of water on the plants to produce a stressful effect (Abascal, Ganora, & Yarnell, 2005; Song et al., 2016). Drying can timely remove the free water within the *A. mongolicum* Regel to avoid mould, insects and the decomposition and destruction of the active ingredients, which is conducive to storage and transport, and extend the shelf life, while many studies have confirmed that the drying process of the plant body is subjected to water loss of the adversity of the stress, which will cause intracellular changes in metabolites such as proteins, amino acids, sugars, and so on, and the plant body to cope with the adversity of water loss of the stress of the response mechanism involves a variety of metabolites and metabolic pathways (Liang et al., 2021). A broadly targeted metabolomics study to reveal the transformation mechanism of active compounds in Cistanche during the drying process revealed that the biosynthesis of key metabolites, such as phenylalanine and flavonoids, was significantly enhanced during the drying process, and the production of amino acids and unsaturated fatty acids increased in Cistanche, suggesting that there is a reciprocal transformation relationship between the primary and secondary metabolites of Cistanche (Ai, Zhang, Li, Sun, & Liu, 2021). Similarly, Hou et al. (2023) used metabolomics and transcriptomics analyses to reveal the metabolic pathways of endocarp during segmental drying of 'Dayagan' hybrid citrus, and the changes in the endocarp were correlated with the loss of
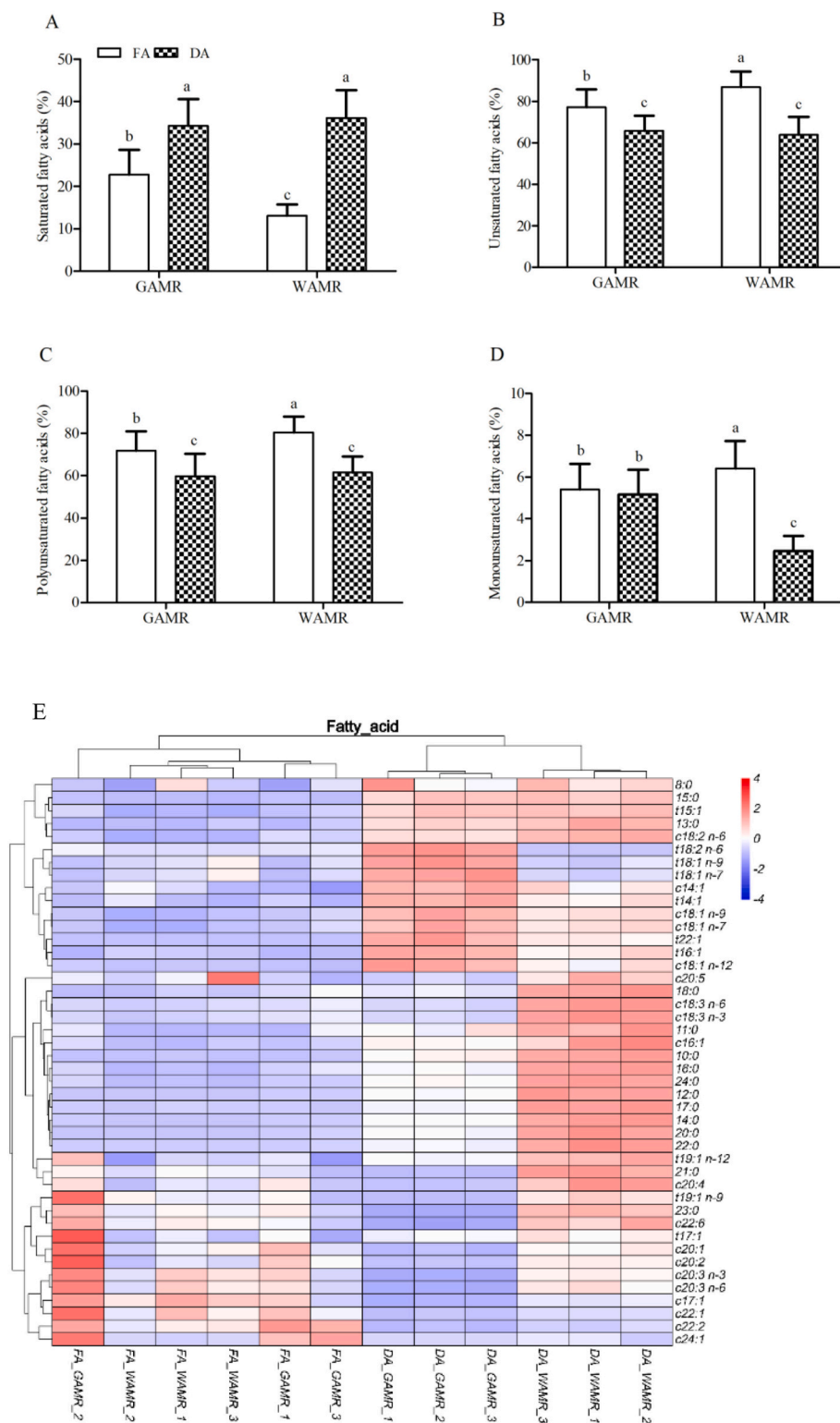
Fig. 5. Fatty acid composition of the two varieties of FA and DA. (A) Percentage of saturated fatty acids; (B) percentage of unsaturated fatty acids (UFAs); (C) percentage of polyunsaturated fatty acids (PUFAs); (D) percentage of monounsaturated fatty acids (MUFAs). (E) Hierarchical cluster analysis of fatty acids in two varieties of FA and DA. The scale in the colour bar was standardized using the scale function of raw abundance for the compounds. The differences in metabolites are indicated by different colours: blue indicates lower abundance, while red indicates higher abundance. The fatty acid content was calculated using the dry weight of *A. mongolicum* Regel from three biological replicates. a, b, c and d: mean values with different letters are significantly different at $P < 0.05$. *c*, cis; *t*, trans; FA, fresh *A. mongolicum* Regel leaves; leaves, dried *A. mongolicum* Regel leaves; GAMR, *A. mongolicum* Regel grown in a greenhouse; WAMR, *A. mongolicum* Regel grown in the Gobi Desert. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Correlation analysis between key differentially abundant metabolites and antioxidant activity.

Metabolites	TPC		TFC		ABTS		DPPH	
	ρ[1]	P Value[2]	ρ	P Value	ρ	P Value	ρ	P Value
Primary metabolites								
Methylenesuccinic acid	0.92	1.8×10^{-10}	0.91	5.9×10^{-10}	0.87	2.7×10^{-8}	0.62	1.2×10^{-3}
L-leucyl-*L*-phenylalanine	0.94	1.6×10^{-11}	0.96	1.1×10^{-13}	0.84	2.2×10^{-11}	0.71	8.6×10^{-5}
9,10,13-Trihydroxyoctadec-11-enoic acid	0.91	4.1×10^{-10}	0.91	9.7×10^{-10}	0.86	4.5×10^{-8}	0.61	1.7×10^{-3}
L-alanyl-*L*-phenylalanine	0.96	8.2×10^{-14}	0.98	1.1×10^{-12}	0.86	2.7×10^{-13}	0.72	6.7×10^{-5}
N-acetyl-L-glutamic acid	0.91	1.1×10^{-9}	0.92	2.4×10^{-10}	0.87	2.5×10^{-8}	0.64	7.8×10^{-4}
3-Hydroxycinnamic acid	0.90	1.5×10^{-9}	0.89	8.0×10^{-9}	0.85	1.5×10^{-7}	0.59	2.2×10^{-3}
Isoferulic acid	0.94	9.5×10^{-12}	0.97	2.7×10^{-14}	0.85	2.7×10^{-14}	0.74	3.8×10^{-5}
Eicosadienoic acid	0.91	6.7×10^{-10}	0.95	3.2×10^{-12}	0.71	4.9×10^{-10}	0.70	1.6×10^{-4}
Tyramine	0.93	3.7×10^{-11}	0.96	1.1×10^{-11}	0.85	2.3×10^{-12}	0.73	4.3×10^{-5}
Malonic acid	0.91	8.7×10^{-10}	0.90	1.9×10^{-9}	0.85	1.1×10^{-7}	0.56	4.9×10^{-3}
(7S,8S)-DiHODE	−0.90	1.9×10^{-9}	−0.92	1.5×10^{-10}	−0.81	4.6×10^{-10}	−0.71	9.8×10^{-5}
Secondary metabolites								
GA7–1	0.98	4.4×10^{-16}	0.98	1.8×10^{-9}	0.96	9.5×10^{-14}	0.72	7.4×10^{-5}
Aesculetin	0.98	1.9×10^{-9}	0.98	1.8×10^{-9}	0.97	1.8×10^{-15}	0.73	5.5×10^{-5}
Ferulic Acid	0.94	6.9×10^{-12}	0.97	1.8×10^{-15}	0.94	8.3×10^{-12}	0.92	8.9×10^{-5}
Jasmonic acid	0.87	3.2×10^{-8}	0.92	2.2×10^{-10}	0.87	2.8×10^{-8}	0.65	5.5×10^{-4}
Naringenin	0.90	1.3×10^{-9}	0.87	3.3×10^{-8}	0.93	3.7×10^{-8}	0.66	4.6×10^{-4}
Quercetin	0.92	2.9×10^{-10}	0.93	5.1×10^{-11}	0.91	1.1×10^{-9}	0.91	3.5×10^{-4}
Naringenin chalcone	0.90	1.8×10^{-9}	0.86	5.2×10^{-8}	0.92	4.6×10^{-8}	0.65	5.4×10^{-4}
Vitexin	0.92	1.4×10^{-10}	0.90	3.6×10^{-9}	0.88	1.1×10^{-8}	0.62	1.1×10^{-3}
Eriodyctiol	0.96	2.4×10^{-13}	0.95	2.0×10^{-12}	0.92	5.9×10^{-11}	0.67	3.6×10^{-4}
Afzelechin	0.88	2.2×10^{-8}	0.92	1.7×10^{-10}	0.89	5.7×10^{-9}	0.66	4.0×10^{-4}
Magnoflorine	0.87	3.7×10^{-8}	0.90	1.7×10^{-9}	0.87	3.9×10^{-8}	0.66	4.6×10^{-4}

[1] ρ: Spearman's rank correlation coefficient analysis.

[2] The data were considered significantly different at a P value <0.05.

water during drying. Metabolomics results confirmed that differential metabolites were enriched in metabolic pathways related to amino acids and their derivatives, lipids and flavonoids synthesis, and up-regulated genes confirmed that the phenylpropane pathway was activated during drying to participate in the accumulation of flavonoids and other active constituents. In addition, the significant increase in the content of phenolic acid polymers in walnut kernels during drying is thought to be related to the increase in the phenylpropane metabolic pathway and aldol reaction activity, and furthermore changes in the content of phenolic acid metabolites are the main reason for the difference in antioxidant activity between fresh and dried walnut kernels, with different varieties accumulating metabolites associated with different rates of antioxidant activity during drying (Wang et al., 2022), which is in agreement with the the results of this study.

A. mongolicum Regel is a species of plants in the *lily* family, *Allium*, that have relatively high antioxidant activity due to the richness and diversity of flavonoids and polyphenols found in them (Liu et al., 2022). In addition, our previous research found that *A. mongolicum* Regel and its extracts could be serve as a source of natural bioactive compounds in lambs' diets, improving the total antioxidant capacity of lamb meat and prolonging its storage time (Liu et al., 2022). In this study, it was found that isorhamnetin was the flavonoid with the highest content in *A. mongolicum* Regel, followed by kaempferol, and protocatechuic acid was the phenolic with the highest content, followed by ferulic acid. Kaempferol is the most abundant flavonol compound in *Camellia sinensis*, derived directly from naringenin, and these compounds help protect the plant from herbivore and abiotic stresses (Tong et al., 2022). In addition, the apigenin detected in this assay was also derived from naringenin, and all of these metabolites were significantly upregulated after drying. Previous reports demonstrated that *A. mongolicum* Regel contain 16 flavonoids, including aglycone and glycosylated products of quercetin, kaempferol, and isorhamnetin, while quercetin glucosides are the main flavonol present in *A. mongolicum* Regel (Abdelrahman et al., 2019). The same results were obtained for flavonoids and polyphenols in other *Allium* species, and these metabolites have high antioxidant activity (Sobolewska, Podolak, & Makowska-Was, 2015). Combined with multifactorial analysis, these results suggest that the differences in antioxidant activity between FA and DA are mainly attributable to changes in the flavonoid and phenolic profiles.

By elucidating the correlation between potential key metabolites and antioxidant activity, flavonoids and phenolic compounds were found to be the main metabolites influencing the antioxidant activity of *A. mongolicum* Regel. Five of the most relevant key differential metabolites had significant antioxidant activity. For example, quercetin (quercetin-4′-glucoside and quercetin-3,4′-diglucoside) is a flavonoid that has been proven to be the core functional component of the antioxidant activity of *Allium cepa* L., which is a red onion cultivar (Metrani, Singh, Acharya, Jayaprakasha, & Patil, 2020). Ferulic acid, naringenin, naringenin chalcone, and eriodictyol are considered important phenolic and flavonoid substances of *A. mongolicum* Regel, which were also identified in other species, such as corn fibre (Valério et al., 2021), citrus (Kicinska et al., 2020), tomato fruit (Tomas et al., 2017), and *Thymus sibthorpii* Bentham (Kontogiorgis et al., 2016). In many previous studies, these metabolites have been proven to have strong antioxidant activity (Williamson, Kay, & Crozier, 2018). Plants preferentially accumulate dihydroxy B-ring substituted flavonoids, which are effective scavengers of reactive oxygen species, under severe stress conditions (Agati et al., 2011). It was found that the drying process caused a strong stressful effect on rhubarb, thereby promoting the synthesis and accumulation of phenolic acids and anthraquinone in rhubarb (Liang et al., 2021). Respiration plays a very important role in the carbon cycle and energy metabolism of plants, which can provide sufficient energy for plant life activities and substrates for the synthesis of secondary metabolites. Respiration consists of glycolysis, tricarboxylic acid cycle cycle and the pentose phosphate pathway, etc. (Van et al., 2011). We speculate that the vacuum freeze-drying method used in this experiment may affect the respiration of *A. mongolicum* Regel tissue cells, which in turn affects the accumulation of secondary metabolites, and the specific mechanism needs to be further investigated.

A detailed analysis of the differential metabolites between FA and DA revealed that the metabolism of the phenylpropane pathway in the shikimic acid pathway and the reaction of the AA-MA pathway may be the main reasons for the elevated levels of active antioxidant metabolites such as flavonoids and polyphenols in DA. Ferulic acid, naringenin, quercetin, naringenin chalcone, and eriodictyol levels, which are highly correlated with the antioxidant activity of *A. mongolicum* Regel,

accumulated significantly in DA. These metabolites are all flavonoids and polyphenols, suggesting that the increase in these substances may be the main reason for the increased antioxidant activity of DA. It can be speculated that the increase in secondary metabolites and the decrease in primary metabolites may be due to several reasons. During drying, the downregulation of primary metabolites such as 6-phosphogluconic acid and dihydroxyacetone phosphate and metabolites suggests that the pentose phosphate pathway, as well as the glycolytic pathway, are being strengthened and that the metabolites of glycolytic pathway and pentose phosphate pathway are the basis for the initiation of tricarboxylic acid cycle, which is the hub linking the metabolism of sugars, lipids and amino acids. In our results, intermediate metabolites of tricarboxylic acid cycle, such as cis-aconitic acid, were found to be upregulated. Tricarboxylic acid cyclecan provide energy, reducing power and intermediates necessary for secondary metabolism for plant life activities. Genes associated with the tricarboxylic acid cycle have been reported to be significantly up-regulated in Arabidopsis under desiccation and senescence stress, and the accumulation of end products of its metabolic pathways is significantly increased (Urano et al., 2009). Immediately following D-erythrose-4-phosphate (from the pentose phosphate pathway) and phosphoenolpyruvate (from the glycolytic pathway), mangiferous acid, a precursor substance for the synthesis of aromatic amino acids (tryptophan, phenylalanine and tyrosine), was synthesized, and both shikimic acid and phenylalanine were significantly upregulated during the drying process in our study. These two substances then enter the phenylpropane pathway, where phenylalanine is deaminated by phenylalanine deaminase to form cinnamic acid, which is further converted into coumarins via intermediates such as coumaric, ferulic and mustardic acids and chlorogenic and caffeic acids for the final synthesis of flavonoids. In detail, flavonoids are all synthesized via the phenylpropane pathway, a branch of the phenylpropane metabolic pathway to be precise, starting with the polymerization of 1 molecule of coumaroyl-CoA and 3 molecules of malonyl-CoA catalysed by chalcone synthase to produce chalcones, followed by flavonoids, isoflavonoids and flavonols (Agati, Azzarello, Pollastri, & Tattini, 2012; Austin & Noel, 2003; Kumar & Pandey, 2013).

Another study found that the metabolites phenylalanine and valine were also involved in establishing the antioxidant defence system of the plant. Phenylalanine and valine were able to significantly increase glutathione levels and copper/zinc superoxide dismutase, catalase and glutathione peroxidase activities and improve the antioxidant activity of the plant itself by upregulating the mRNA expression of Nrf2 and downregulating the mRNA expression of Keap1 (Fan, Jia, Du, & Shi, 2022). At the same time, we found an upregulation of the intermediate metabolites malonic acid and methylmalonic acid involved in the acetate-malonate pathway, the enhancement of which was the main reason for the increase in phenolic compounds. Additionally, we found an upregulation of the intermediate metabolites malonic acid and methylmalonic acid involved in the AA-MA pathway, the enhancement of which was the main reason for the increase in TPC. It has previously been reported that abiotic stress can lead to increased production of phenolic compounds, that treatment with appropriate exposure conditions such as drought or high temperatures may stimulate phenolic metabolism, and when aerobic or photosynthetic metabolism is disrupted by environmental stress, it can greatly restrict CO_2 diffusion to carboxylation sites and reduce the efficiency of carboxylation and avoid oxidative stress in the plant itself, so phenolic compounds in *Allium* are involved in defence against reactive oxygen species (Agati et al., 2012). The results of a transcriptomic study of *A. mongulicum* Regel root under drought stress conditions explained that differential metabolites are mainly enriched in secondary metabolic transport, photosynthesis and lipid metabolism, which can be used to synthesise flavonoids and phenolic acids using both signalling and secondary metabolic pathways as an adaptive response to drought stress, and this study and this study indirectly confirms that the WAMR in the present experiments differed in the rate of accumulation of metabolites related to the antioxidant

activity of the GAMR, because of their specific growth environment (Tang, Chen, Zhang, Yang, & Wang, 2022). However, whether other unknown compounds contribute to the antioxidant activity of *A. mongolicum* Regel during the drying process remains to be determined. Additionally, whether there are other metabolic pathways and chemical reactions that change the metabolites during drying of *A. mongolicum* Regel and how they relate to antioxidant activity should be investigated in the future.

5. Conclusion

The antioxidant activity of DA was significantly higher than that of FA in the two varieties. The antioxidant activity of DA was significantly higher than that of FA leaves in the two varieties. Interestingly, in FA, there were no significant differences in TPC between GAMR and WAMR, but the results of DPPH and ABTS assays were significantly different, indicating that drying had a greater effect on the antioxidant activity of *A. mongolicum* Regel leaves than the variety. The key primary metabolites were directly and significantly correlated with the synthesis of TPC and TFC, including amino acids and their derivatives, lipids, and organic acids and their derivatives, while the secondary metabolites (flavonoids and phenolics) were directly correlated with the antioxidant activity of *A. mongolicum* Regel leaves. DA had significantly higher contents of flavonoids and polyphenols than FA, which can be attributed to the metabolism of the phenylpropane pathway and the enhancement of the AA-MA pathway during the drying process. However, *A. mongolicum* Regel drying is a very complex process and different drying methods may also lead to changes in its antioxidant activity, which needs to be further investigated in future research. In this article, the drying process of *A. mongolicum* Regel was investigated for the first time using targeted metabolomics, and it provided new insights into the biosynthesis of active components occurring during *A. mongolicum* Regel drying.

CRediT authorship contribution statement

Wangjing Liu: Writing – original draft, Validation, Resources, Methodology, Investigation, Data curation. **Aihuan Yu:** Resources. **Yaodi Xie:** Resources. **Haibo Yao:** Resources. **Chenxu Sun:** Resources. **Huixia Gao:** Resources. **Jianjian He:** Resources. **Changjin Ao:** Writing – review & editing, Visualization, Conceptualization. **Defu Tang:** Writing – review & editing, Visualization, Conceptualization.

Declaration of competing interest

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101436.

References

Abascal, K., Ganora, L., & Yarnell, E. (2005). The effect of freeze-drying and its implications for botanical medicine: A review. *Phytotheraphy Research, 19*(8), 655–660. https://doi.org/10.1002/ptr.1651

Abdelrahman, M., Ariyanti, N. A., Sawada, Y., Tsuji, F., Hirata, S., Hang, T. T. M., … Shigyo, M. (2020). Metabolome-based discrimination analysis of shallot landraces and bulb onion cultivars associated with differences in the amino acid and flavonoid profiles. *Molecules (Basel, Switzerland), 25*(22), 5300. https://doi.org/10.3390/molecules25225300

Abdelrahman, M., Burritt, D. J., & Tran, L. P. (2018). The use of metabolomic quantitative trait locus mapping and osmotic adjustment traits for the improvement of crop yields under environmental stresses. *Seminars in Cell and Developmental Biology, 83*, 86–94. https://doi.org/10.1016/j.semcdb.2017.06.020

Abdelrahman, M., Hirata, S., Sawada, Y., Hirai, M. Y., Sato, S., Hirakawa, H., … Shigyo, M. (2019). Widely targeted metabolome and transcriptome landscapes of *Allium fistulosum*-A. Cepa chromosome addition lines revealed a flavonoid hot spot on chromosome 5A. *Scientific Reports, 9*(1), 3541. https://doi.org/10.1038/s41598-019-39856-1

Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science, 196*, 67–76. https://doi.org/10.1016/j.plantsci.2012.07.014

Agati, G., Biricolti, S., Guidi, L., Ferrini, F., Fini, A., & Tattini, M. (2011). The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in *L. vulgare* leaves. *Journal of Plant Physiology, 168*(3), 204–212. https://doi.org/10.1016/j.jplph.2010.07.016

Ai, Z. P., Zhang, Y., Li, X. Y., Sun, W. L., & Liu, Y. H. (2021). Widely targeted metabolomics analysis to reveal transformation mechanism of Cistanche Deserticola active compounds during steaming and drying processes. *Fronties in Nutrition, 8*, Article 742511. https://doi.org/10.3389/fnut.2021.742511

Austin, M. B., & Noel, J. P. (2003). The chalcone synthase superfamily of type III polyketide synthases. *Natural Product Reports, 20*(1), 79–110. https://doi.org/10.1039/b100917f

Dean, L. L. (2018). Targeted and non-targeted analyses of secondary metabolites in nut and seed processing. *European Journal of Lipid Science and Technology, 120*(11), 1700479. https://doi.org/10.1002/ejlt.201700479

Ding, H., Liu, W. J., Erdene, K., Du, H. X., & Ao, C. J. (2021). Effects of dietary supplementation with *Allium mongolicum* regel extracts on growth performance, serum metabolites, immune responses, antioxidant status, and meat quality of lambs. *Animal Nutrition, 7*(2), 530–538. https://doi.org/10.1016/j.aninu.2021.04.001

Dong, Y., Ruan, J., Ding, Z., Zhao, W., Hao, M., Zhang, Y., … Wang, T. (2020). Phytochemistry and comprehensive chemical profiling study of flavonoids and phenolic acids in the aerial parts of allium mongolicum regel and their intestinal motility evaluation. *Molecules, 25*(3), 577. https://doi.org/10.3390/molecules25030577

Fan, Z., Jia, W., Du, A., & Shi, L. (2022). Pseudo-targeted metabolomics analysis of the therapeutic effect of phenolics-rich extract from se-enriched green tea (*Camellia sinensis*) on LPS-stimulated murine macrophage (RAW264.7). *Food Research International, 159*, Article 111666. https://doi.org/10.1016/j.foodres.2022.111666

Fu, M., Qu, Q., Yang, X., & Zhang, X. (2016). Effect of intermittent oven drying on lipid oxidation, fatty acids composition and antioxidant activities of walnut. *LWT - Food Science and Technology, 65*, 1126–1132. https://doi.org/10.1016/j.lwt.2015.10.002

Han, Y., Zheng, Y., Li, S., Mo, R., Long, X., & Liu, Y. (2019). Effects of drying process with different temperature on the nutritional qualities of walnut (*Juglans regia* L.). *Food Science and Technology Research, 25*(2), 167–177. https://doi.org/10.3136/fstr.25.167

Hithamani, G., & Srinivasan, K. (2016). Bioaccessibility of polyphenols from onion (*Allium cepa*) as influenced by domestic heat processing and food acidulants. *The Indian Journal of Nutrition and Dietetics, 53*(4), 391. https://doi.org/10.21048/ijnd.2016.53.4.8398

Hossain, M. B., Lebelle, J., Birsan, R., & Rai, D. K. (2018). Enrichment and assessment of the contributions of the major polyphenols to the total antioxidant activity of onion extracts: A fractionation by flash chromatography approach. *Antioxidants (Basel, Switzerland), 7*(12), 175. https://doi.org/10.3390/antiox7120175

Hou, J., Yan, D. D., Liu, Y. C., Wang, W., Hong, M., He, M. Y., … Yao, S. X. (2023). Global changes in metabolic pathways in endocarp of "Dayagan" hybrid citrus fruit during segment drying revealed by widely targeted metabolomics and transcriptomics analysis. *Postharvest Biology and Technology, 198*, Article 112255. https://doi.org/10.1016/j.postharvbio.2023.112255

Jia, S., Wang, Y., Hu, J., Ding, Z., Liang, Q., Zhang, Y., & Wang, H. (2016). Mineral and metabolic profiles in tea leaves and flowers during flower development. *Plant Physiology and Biochemistry, 106*, 316–326. https://doi.org/10.1016/j.plaphy.2016.06.013

Kennedy, D. O., & Wightman, E. L. (2011). Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. *Advances in Nutrition, 2*(1), 32–50. https://doi.org/10.3945/an.110.000117

Kicinska, A., Kampa, R. P., Daniluk, J., Sek, A., Jarmuszkiewicz, W., Szewczyk, A., & Bednarczyk, P. (2020). Regulation of the mitochondrial BKCa channel by the citrus flavonoid naringenin as a potential means of preventing cell damage. *Molecules (Basel, Switzerland), 25*(13), 3010. https://doi.org/10.3390/molecules25133010

Kontogiorgis, C., Ntella, M., Mpompou, L., Karallaki, F., Athanasios, P., Hadjipavlou-Litina, D., & Lazari, D. (2016). Study of the antioxidant activity of *Thymus sibthorpii* Bentham (Lamiaceae). *Journal of Enzyme Inhibition and Medicinal Chemistry, 31* (sup4), 154–159. https://doi.org/10.1080/14756366.2016.1222583

Kortei, N. K., Odamtten, G. T., Obodai, M., Appiah, V., Akuamoa, F., Adu-Bobi, A. K., … Acquah, S. A. (2014). Evaluating the effect of gamma radiation on the total phenolic content, flavonoids, and antioxidant activity of dried *Pleurotus ostreatus* ((Jacq. ex. Fr) Kummer) stored in packaging materials. *Advances in Pharmaceutics, 2014*, 1–8. https://doi.org/10.1155/2014/262807

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal, 2013*, Article 162750. https://doi.org/10.1155/2013/162750

Lakhani, N., Kamra, D. N., Lakhani, P., & Alhussien, M. N. (2019). Immune status and haemato-biochemical profile of buffalo calves supplemented with phytogenic feed additives rich in tannins, saponins and essential oils. *Tropical Animal Health and Production, 51*(3), 565–573. https://doi.org/10.1007/s11250-018-1727-z

Liang, W., Chen, Y., Li, X., Guo, F. X., Sun, J. C., Zhang, X. M., … Gao, W. Y. (2021). Label-free proteomic analysis of smoke-drying and shade-drying processes of postharvest rhubarb: A comparative study. *Frontiers in Plant Science, 12*, Article 663180. https://doi.org/10.3389/fpls.2021.663180

Liu, W., & Ao, C. (2021). Effect of dietary supplementation with *Allium mongolicum* regel extracts on growth performance, carcass characteristics, and the fat color and flavor-related branched-chain fatty acids concentration in ram lambs. *Animal Bioscience, 34* (7), 1134–1145. https://doi.org/10.5713/ajas.20.0246

Liu, W., Fu, Y., Zu, Y., Kong, Y., Zhang, L., Zu, B., & Efferth, T. (2009). Negative-pressure cavitation extraction for the determination of flavonoids in pigeon pea leaves by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A, 1216*(18), 3841–3850. https://doi.org/10.1016/j.chroma.2009.02.073

Liu, W., Tang, D., & Ao, C. (2022). Adding of *Allium mongolicum* regel extracts to lamb feedlot diets influences 4-alkyl-branched fatty acids deposition and the meat quality during storage. *Meat Science, 193*, Article 108951. https://doi.org/10.1016/j.meatsci.2022.108951

Liu, Y. H., Li, Y. M., Liu, Z., Wang, L., Bi, Z. Z., Sun, C., … Zeng, Y. T. (2023). Integrated transcriptomic and metabolomic analysis revealed altitude-related regulatory mechanisms on flavonoid accumulation in potato tubers. *Food Research International, 170*, Article 112997. https://doi.org/10.1016/j.foodres.2023.112997

Luo, P., Dai, W., Yin, P., Zeng, Z., Kong, H., Zhou, L., … Xu, G. (2015). Multiple reaction monitoring-ion pair finder: A systematic approach to transform nontargeted mode to pseudotargeted mode for metabolomics study based on liquid chromatography-mass spectrometry. *Analytical Chemistry, 87*(10), 5050–5055. https://doi.org/10.1021/acs.analchem.5b00615

Metrani, R., Singh, J., Acharya, P., Jayaprakasha, G. K., & Patil, B. S. (2020). Comparative metabolomics profiling of polyphenols, nutrients and antioxidant activities of two red onion (*Allium cepa* L.) cultivars. *Plants (Basel), 9*(9), 1077. https://doi.org/10.3390/plants9091077

Qu, Q., Yang, X., Fu, M., Chen, Q., Zhang, X., He, Z., & Qiao, X. (2015). Effects of three conventional drying methods on the lipid oxidation, fatty acids composition, and antioxidant activities of walnut (*Juglans regia* L.). *Drying Technology, 34*(7), 822–829. https://doi.org/10.1080/07373937.2015.1081931

Sobolewska, D., Podolak, I., & Makowska-Was, J. (2015). Allium ursinum: Botanical, phytochemical and pharmacological overview. *Phytochemistry Reviews, 14*(1), 81–97. https://doi.org/10.1007/s11101-013-9334-0

Song, X., Diao, J., Ji, J., Wang, G., Guan, C., Jin, C., & Wang, Y. (2016). Molecular cloning and identification of a flavanone 3-hydroxylase gene from Lycium chinense, and its overexpression enhances drought stress in tobacco. *Plant Physiology and Biochemistry, 98*, 89–100. https://doi.org/10.1016/j.plaphy.2015.11.011

Tang, N., Chen, Q. Z., Zhang, B. J., Yang, P., & Wang, L. K. (2022). Root transcriptome of Allium mongolicum regel under drought stress conditions. *International Journal of Agriculture and Natural Resources, 49*(1), 23–35. https://doi.org/10.7764/ijanr.v49i1.2357

Tomas, M., Beekwilder, J., Hall, R. D., Sagdic, O., Boyacioglu, D., & Capanoglu, E. (2017). Industrial processing versus home processing of tomato sauce: Effects on phenolics, flavonoids and in vitro bioaccessibility of antioxidants. *Food Chemistry, 220*, 51–58. https://doi.org/10.1016/j.foodchem.2016.09.201

Tong, Q., Zhang, C., Tu, Y., Chen, J., Li, Q., Zeng, Z., Wang, F., Sun, L., Huang, D., Li, M., Qiu, S., & Chen, W. (2022). Biosynthesis-based spatial metabolome of *Salvia miltiorrhiza Bunge* by combining metabolomics approaches with mass spectrometry-imaging. *Talanta, 238*(Pt 2), Article 123045. https://doi.org/10.1016/j.talanta.2021.123045

Urano, K., Maruyama, K., Ogata, Y., Morishita, Y., Takeda, M., Sakurai, N., & Shinozaki, K. (2009). Characterization of the ABA-regulated global responses to dehydration in Arabidopsis by metabolomics. *The Plant Journal, 57*(6), 1065–1078. https://doi.org/10.1111/j.1365-313X.2008.03748.x

Valério, R., Serra, A. T., Baixinho, J., Cardeira, M., Fernández, N., Bronze, M. R., … Brazinha, C. (2021). Combined hydrothermal pre-treatment and enzymatic hydrolysis of corn fibre: Production of ferulic acid extracts and assessment of their antioxidant and antiproliferative properties. *Industrial Crops and Products, 170*, Article 113731. https://doi.org/10.1016/j.indcrop.2021.113731

Van, D. J. T., Gupta, K. T., Ramirez-aguilar, S. J., Araujo, W. L., Nunes-Nesi, A., & Fernie, A. R. (2011). Regulation of respiration in plants: A role for alternative metabolic pathways. *Journal of Plant Physiology, 168*(12), 1434–1443. https://doi.org/10.1016/j.jplph.2010.11.004

Wang, J. B., Pu, S. B., Sun, Y., Li, Z. F., Niu, M., Yan, X. Z., … Xiao, X. H. (2014). Metabolomic profiling of autoimmune hepatitis: The diagnostic utility of nuclear

magnetic resonance spectroscopy. *Journal of Proteome Research, 13*(8), 3792–3801. https://doi.org/10.1021/pr500462f

Wang, P., Zhong, L., Yang, H., Zhu, F., Hou, X., Wu, C., Zhang, R., & Cheng, Y. (2022). Comparative analysis of antioxidant activities between dried and fresh walnut kernels by metabolomic approaches. *LWT - Food science and Techonlogy, 155*, Article 112875. https://doi.org/10.1016/j.lwt.2021.112875

Wang, W., Li, J., Zhang, H., Wang, X., Fan, J., & Zhang, X. (2019). Phenolic compounds and bioactivity evaluation of aqueous and methanol extracts of *Allium mongolicum* Regel. *Food Science & Nutrition, 7*(2), 779–787. https://doi.org/10.1002/fsn3.926

Want, E. J., Masson, P., Michopoulos, F., Wilson, I. D., Theodoridis, G., Plumb, R. S., … Nicholson, J. K. (2013). Global metabolic profiling of animal and human tissues via UPLC-MS. *Nature Protocols, 8*(1), 17–32. https://doi.org/10.1038/nprot.2012.135

Williamson, G., Kay, C. D., & Crozier, A. (2018). The bioavailability, transport, and bioactivity of dietary flavonoids: A review from a historical perspective. *Comprehensive Reviews in Food Science and Food Safety, 17*(5), 1054–1112. https://doi.org/10.1111/1541-4337.12351

Wu, S., Shen, D., Wang, R., Li, Q., Mo, R., Zheng, Y., Zhou, Y., & Liu, Y. (2021). Phenolic profiles and antioxidant activities of free, esterified and bound phenolic compounds in walnut kernel. *Food Chemistry, 350*, Article 129217. https://doi.org/10.1016/j.foodchem.2021.129217

Zhang, L., Cao, S., Li, J., & Wang, G. (2022). Effects of drying methods on the volatile compounds of *Alliummongolicum* Regel. *Foods (Basel, Switzerland), 11*(14), 2080. https://doi.org/10.3390/foods11142080

Zhao, L., Sun, X., Wu, J., Su, L., Yang, F., Jin, Y., … Ao, C. (2022). Effects of *Allium mongolicum* regel and its extracts on the quality of fermented mutton sausages. *Food Science & Nutrition, 10*(1), 169–178. https://doi.org/10.1002/fsn3.2657

Zhou, X., Gao, H., Mitcham, E. J., & Wang, S. (2018). Comparative analyses of three dehydration methods on drying characteristics and oil quality of in-shell walnuts. *Drying Technology, 36*(4), 477–490. https://doi.org/10.1080/07373937.2017.1351452