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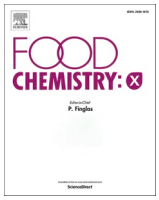

Metabolomics analysis based on UHPLC-QqQ-MS/MS to discriminate grapes and wines from different geographical origins and climatological characteristics

Lin Zhang [a,1], Zhaoxiang Wang [a,1], Cui Zhang [a,c], Shubo Zhou [a], Chunlong Yuan [a,b,*]

[a] College of Enology, Northwest A&F University, Yangling 712100, China
[b] Ningxia Helan Mountain's East Foothill Wine Experiment and Demonstration Station of Northwest A&F University, Yongning, Ningxia 750104, China
[c] Xinjiang Bainian Manor Wines & Spirits Co., Ltd, China

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Peonidin (PubChem CID: 441773)
Delphinidin (PubChem CID: 68245)
Petunidin (PubChem CID: 441774)
Malvidin (PubChem CID: 159287)
Resveratrol (PubChem CID: 445154)
Caffeic acid (PubChem CID: 689043)
(+)-Catechin (PubChem CID: 9064)
Gallic acid (PubChem CID: 370)

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ABSTRACT

With the proliferation of the consumer's awareness of wine provenance, wines with unique origin characteristics are increasingly in demand. This study aimed to investigate the influence of geographical origins and climatological characteristics on grapes and wines. A total of 94 anthocyanins and 78 non-anthocyanin phenolic compounds in grapes and wines from five Chinese viticultural vineyards (CJ, WH, QTX, WW, and XY) were identified by UHPLC-QqQ-MS/MS. Chemometric methods PCA and OPLS-DA were established to select candidate differential metabolites, including flavonols, stilbenes, hydroxycinnamic acids, peonidin derivatives, and malvidin derivatives. CCA showed that malvidin-3-*O*-glucoside had a positive correlation with mean temperature, and quercetin-3-*O*-glucoside had a negative correlation with precipitation. In addition, enrichment analysis elucidated that the metabolic diversity in different origins mainly occurred in flavonoid biosynthesis. This study would provide some new insights to understand the effect of geographical origins and climatological characteristics on phenolic compounds in grapes and wines.

1. Introduction

With the rapid development of new technical and scientific know-how, viticulture and winemaking technology tend to be more industrial and manufactured. In recent years, the phenomenon of over-reliance on modern technology has occurred (fertilizers, fungicides, commercial yeast, sulfurous acid, artificial deacidification or saccharification), which to some extent caused the wines to increasingly homogenization (Wei et al., 2022).

Studies have shown that consumers have more willingness to pay for wines that emphasize "local" (Eustice, McCole, & Rutty, 2019; Kustos, Goodman, Jeffery, & Bastian, 2019). Words such as terroir, regionality, and geographical typicality have become increasingly popular around the world (Slaghenaufi, Guardini, Tedeschi, & Ugliano, 2019). In this context, some studies have focused on the recognizable and unique chemical characteristics related to the geographical typicality of grapes and wines (Merkytė, Longo, Windisch, & Boselli, 2020). Arapitsas et al. (2020) conducted a study about the metabolome of 11 single-cultivar, single-vintage red wines from 12 regions across Italy, and concluded that flavan-3-ols were the biomarkers of Aglianico, Sangiovese, Nerello, and Nebbiolo, flavonols were the biomarkers of Sangiovese, and hydroxycinnamates were the biomarkers for Cannonau. Wang et al. (2023) selected flavonoids, organic acids, amino acids, terpenes, and fatty alcohols as the candidate differential metabolites to discriminate four geographical origins in China.

The term "wine-omics" was first proposed by Wohlgemuth (2008) in

* Corresponding author at: College of Enology, Northwest A&F University, Yangling 712100, China.
E-mail address: yuanchl69@nwsuaf.edu.cn (C. Yuan).
[1] These authors contributed equally to this work.

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the journal Nature, in search of key chemical components contributing to the wine body. With the continuous development of metabolomic analysis, metabolomics combined with powerful statistical techniques has been widely applied in wine research. For example, Zhang, Lan, Huang, Zhao, and Duan (2021) investigated anthocyanin derivatives and chromatic characteristics of 234 different-vintage red wines based on a targeted HPLC-MS/MS approach, coupled with K-means, PLSR, random forest, and support vector machine analysis. Crook et al. (2021) improved Pinot Noir wine classification by vineyard, region, and vintage based on combined analytical techniques NMR and differential sensing array. Therefore, metabolomics has been regarded as an effective method involving discriminative, predictive, and identification in grape and wine (Cavanna, Righetti, Elliott, & Suman, 2018; Hu, Zhang, Xing, Yu, & Chen, 2022; Sherman, Coe, Grose, Martin, & Greenwood, 2020).

Over the past decade, the Chinese wine market has expanded at a rapid rate and China has great vitality in wine production and consumption (Lu, Chi, & Zou, 2019). Over ten wine regions with their unique characteristics were divided in China (Wei et al., 2022). Most wine regions have continental monsoon climates, and the regions also contain a great diversity of ecological conditions (Li, Pan, Jin, Mu, & Duan, 2011). Therefore, finding the wine features of the origins and continuing to emphasize them in production practice of viticulture and winemaking seems a problem admits of no delay. However, most published research mainly focused on mediterranean-like climates, but the studies on the relationship between typical continental monsoon climates and phenolic profiles are relatively limited.

In order to preliminary investigate the above-mentioned queries, the chemical fingerprints of grapes and wines from five origins in China were characterized. Anthocyanin and non-anthocyanin phenolic compounds were analyzed by UHPLC-QqQ-MS/MS to investigate the unique features and differential performance in different origins. Chemometric approaches (PCA, OPLS-DA, CCA) and metabolite pathway enrichment analysis were performed to select the typical metabolites, examine the connection between meteorological parameters and phenolic metabolites, and elucidate the mechanisms of metabolic differences.

2. Material and methods

2.1. Chemicals and reagents

Cyanidin-3-*O*-glucoside chloride, peonidin-3-*O*-glucoside chloride, delphinidin-3-*O*-glucoside chloride, petunidin-3-*O*-glucoside chloride, malvidin-3-*O*-glucoside chloride, protocatechuic acid, chlorogenic acid, morin, rutin, resveratrol, caffeic acid, *p*-coumaric acid, (+)-catechin, gallic acid, (−)-epicatechin, quercetin, ferulic acid, kaempferol, syringic acid, salicylic acid, vanillic acid, and 4-methyl-2-pentanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (HPLC grade), acetonitrile (HPLC grade), and formic acid (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was obtained using a Milli-Q System (Millipore, Billerica, USA). 0.22 μm and 0.45 μm pore size syringe filters were purchased from Jinteng (Tianjin, China).

2.2. Grape and winemaking

2.2.1. Grape samples

Grapevine berries of *Vitis vinifera* L. cv Cabernet Sauvignon were collected from representative wine estates in Changji (CJ), Wuhai (WH), Qingtongxia (QTX) and Wuwei (WW), and Xianyang (XY) in 2021 (Fig. S1). The scaffolds in XY adopt vertical shoot-positioned trellis systems. While in CJ, WH, QTX, and WW, all the vines were trained to a slope trunk with a modified vertical shoot positioning trellis system, which were convenient to bury the main vines in winter. Representative samples were obtained by pooling bunches of different clusters collected by hand using a completely randomized design from the top, middle, and bottom of the vine at commercial maturity. Approximately 20 kg of berries were collected in each origin to conduct winemaking experiments. Macroclimatic condition data were collected from the China Meteorological Data network (Table S1).

2.2.2. Winemaking experiments and wine samples

The winemaking modalities were performed based on the published method with modifications (Yue et al., 2020). Grape berries were destemmed and crushed and then collected in 10 L fermenters in triplicate. 60 mg/L SO_2 was immediately added to grape juice to avoid oxidation. After adding 20 mg/L pectinase (Laffort, Bordeaux, France), fermentations were performed with the activated commercial *saccharomyces cerevisiae* strain of Actiflore F33 (Laffort, Bordeaux, France) at 200 mg/L at 25 °C (±1 °C). During fermentation, the residual sugar was measured by automatic system Analyzer Y15 (Biosystems S.A., Barcelona, Spain) to monitor the progress of fermentation until the fermentation was completed (residual sugar <2 g/L). Wines were stored in 750 mL bottles at 4 °C until analysis.

2.3. Extraction of anthocyanins

After being stripped off the berries, the grape skins were freeze-dried at −50 °C for over 24 h using a vacuum freeze drier (FD-1C-50, Eppendorf, Germany). Then they were ground to a powder in liquid nitrogen and stored at −80 °C until further use.

The method was based on Guo et al. (2021) with some modifications and all procedures were conducted under dark conditions. An amount of 0.250 g dried power samples and 5 mL formic acid-methanol solution (2% formic acid and 98% methanol) were added to a 50 mL centrifuge tube. Samples were subjected to 100 W ultrasonic extraction for 10 min at 20 °C in a water bath, agitated at the oscillation frequency of 130 rpm for 30 min at 25 °C, and centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant was collected, and the extraction process was repeated four times under the same conditions. The supernatants were pooled, condensed, and dry to solids by a concentrator. The residue solid at the bottom of the centrifuge tube was dissolved with a 5 mL solvent ratio of A: B = 9:1 (A represents a mixture of pure water: formic acid: acetonitrile = 92:2:6, B represents a mixture of pure water: formic acid: acetonitrile = 44:2:54). The samples were stored at −80 °C until analysis.

2.4. Extraction of non-anthocyanin phenolic compounds

An amount of 15 mL solvent mixture of CH_3OH/H_2O/HCOOH (50:48.5:1.5 *v*/v/v) was added to a 50 mL centrifuge tube containing 0.5 *g* skin powder to obtain the phenolic extracts following the method by Pérez-Navarro et al. (2019) with some modifications. The extraction was carried out by sonication at 4 °C for 20 min and centrifuged at 6000 rpm for 8 min at 4 °C. The supernatant liquid was separated and collected, and the second and third extractions were performed over the residue in the above-mentioned procedure. Three supernatants were combined and stored at −80 °C until analysis.

The wine samples were filtered with 0.22 μm polyethersulfone filters (Jinteng Experimental Equipment Co., Ltd., Tianjin, China) prior to anthocyanin and non-anthocyanin phenolic analysis.

2.5. UHPLC-QqQ-MS/MS analysis of anthocyanins

The analysis of anthocyanins and anthocyanin-derived compounds were evaluated by ultra-high performance liquid chromatography with triple-quadrupole mass spectrometry (UHPLC/QqQ-MS/MS) based on the method described by Zhang et al. (2020) with modifications. The chromatographic separation of anthocyanins and anthocyanin-derived compounds were performed using a Waters AcQuity BEH C18 (100 mm × 2.1 mm, 1.7 μm) column. Mobile phase A was 0.1% formic acid in ultrapure water and mobile phase B was 0.1% formic acid in 1:1 (*v*/v) methanol: acetonitrile. The elution gradient was as follows: 0–1 min: 5%

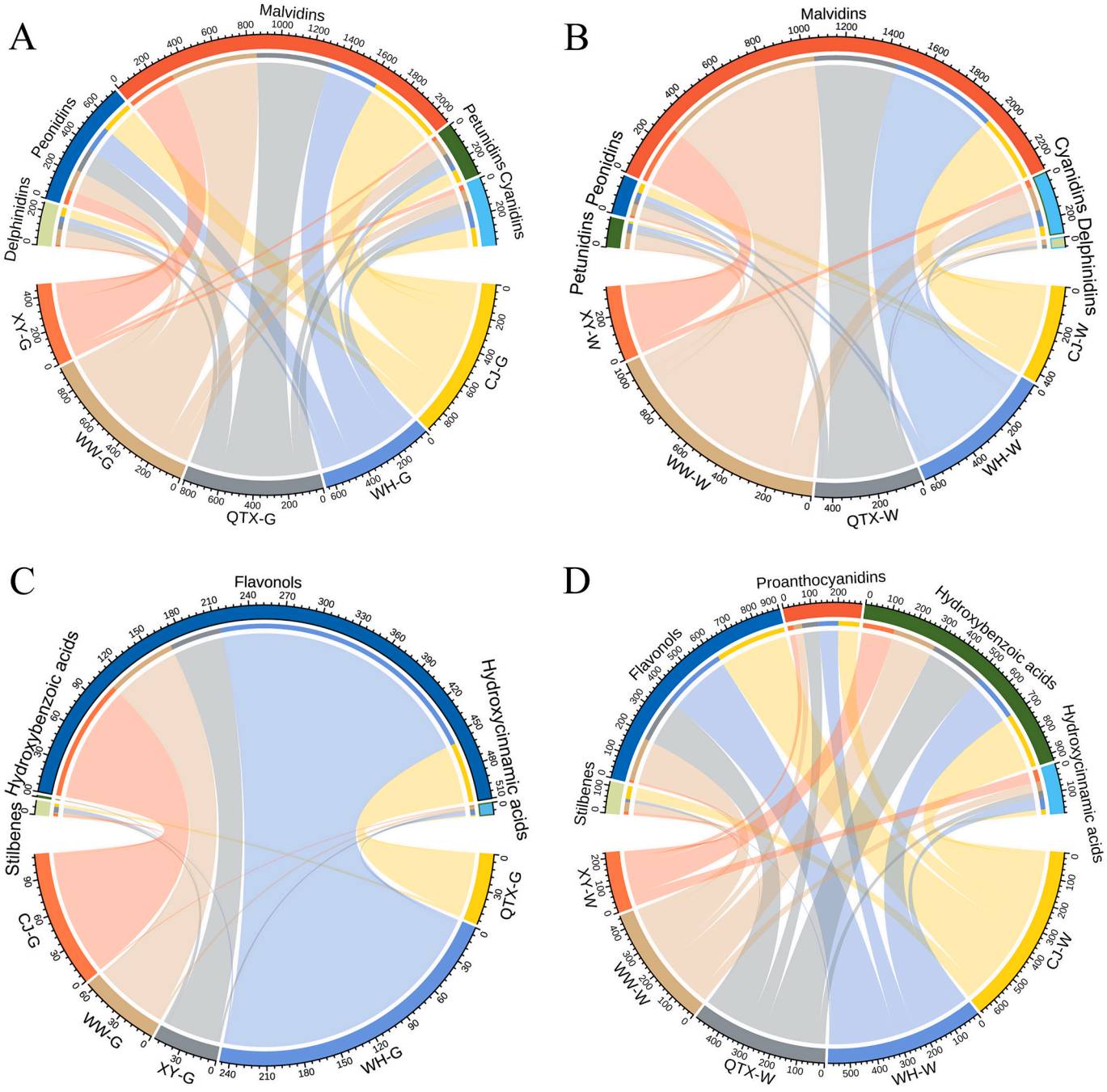

Fig. 1. The richness of anthocyanins in grape (A) and wine (B) samples and non-anthocyanin phenolic compounds in grape (C).

B; 1–8 min: 25% B; 8–20 min, 30% B; 20–25 min: 100% B; 25–30 min, 100% B; 30.1 min: 5% B; 30.1–35 min: 5% B. The flow rate was 0.3 mL/min and the injection volume was 3 μL. The oven temperatures and autosampler were respectively maintained at 55 °C and 4 °C.

The mass spectrometer (Triple Quad 5500+, AB Sciex, USA) was equipped with an electrospray ionization source (ESI Turbo V™ Source) with the following conditions: positive ion mode $[M - H]^+$, spray voltage +4.5 kV, source temperature 550 °C, GS1 50 psi and GS2 60 psi. High-purity nitrogen (>99.99% purity) was used as the source and collision gases. Multiple reaction monitoring (MRM) mode was selected for detection. The MRM transitions, including the individual declustering potential (DP) and collision energy (CE) for each anthocyanin, were shown in Table S2 in the supplementary material. The mixture of all the wine samples served as the quality control, and they were run between every nine wine samples to assess the stability of the instrument. Data acquisition was carried out using the Analyst TF 1.7.1 software (AB Sciex, USA).

Some of the anthocyanins were quantified using the calibration curves of their corresponding pure commercial standards, and the others were semi-quantified using the calibration curves of standards with similar chemical structures (Table S2).

2.6. UHPLC-QqQ-MS/MS analysis of non-anthocyanin phenolic compounds

The analysis of non-anthocyanin phenolics was evaluated by UHPLC/QqQ-MS/MS based on Royo, Ferradás, Martínez-Zapater, and Motilva (2021) with modifications. The mobile phase A was 0.1% formic

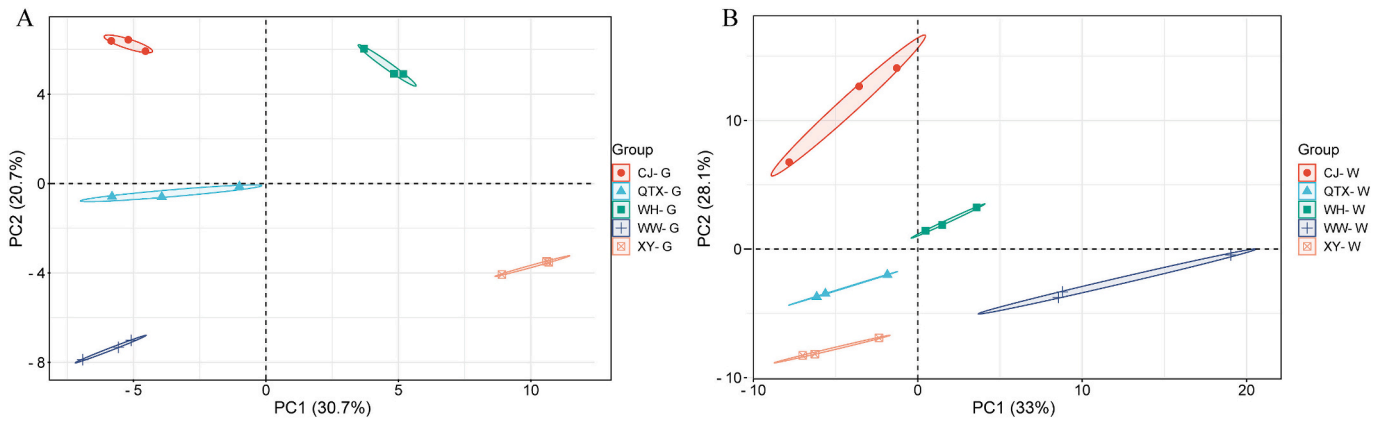

Fig. 2. PCA score plots of grape (A) and wine (B) samples from different origins.

acid in water, and the mobile phase B was 0.1% formic acid in acetonitrile. The elution gradient was as follows: 0–0.5 min, 1% B; 0.5–1.5 min, 1–8% B; 1.5–4 min, 8% B isocratic; 4–5 min, 8–12% B; 5–5.5 min, 12% B isocratic; 5.5–6 min, 12–14% B; 6–7 min, 14% B; 7–9 min, 14–22% B; 9–12 min, 22–30% B; 12–13.5 min, 30–90% B; 13.5–14.5 min, 90% B; 14.5–15 min, 90–1% B; 15–18 min, 1% B. The flow rate was 0.3 mL/min and the injection volume was 3 μL. The oven temperatures and autosampler were respectively maintained at 40 °C and 4 °C. The conditions of the mass spectrometer were as follows: negative ion mode $[M-H]^-$, spray voltage −4.5 kV, source temperature 650 °C, GS1 50 psi, and GS2 60 psi. MRM mode was selected for detection (Table S2). Data acquisition and quantification were the same as for anthocyanins.

2.7. Statistical analysis

IBM SPSS 26 was used for the Duncan test of ANOVA. Principal component analysis (PCA) was established to evaluate the origin classifications of grapes and wines by R package "ade4". Orthogonal partial least squares discrimination analysis (OPLS-DA) was performed by SIMCA 14.1 software to detect any cluster or separation of grape and wine samples. The differential metabolites from different origins were selected on the basis of the variable importance in projection value (VIP) > 1. R package "pheatmap" was used for hierarchical cluster analysis (HCA). Canonical correlation analysis (CCA) was performed by R package "CCA" to analyze the relationship between macroclimatic parameters and phenolic compounds. Metabolic pathways were analyzed by MetaboAnalyst 5.0 software, based on the KEGG database (https://www.metaboanalyst.ca) including chemical metabolite groups or lipid groups in the chemical structure. Then R package "ggplot2" was used for visualization.

3. Results and discussion

3.1. Anthocyanins and anthocyanin-derived compounds of grape and wine samples

In the present study, a total of 94 anthocyanins and anthocyanin-derived compounds were identified, including anthocyanins, glycosylated anthocyanins, acylated anthocyanins, pyanthocyanins, and polymerized anthocyanin (Fig. 1A and B). Anthocyanins and acylated anthocyanins were derived from the maceration procedure during winemaking and were a key factor in determining the red color of young wines. Pyanthocyanins and polymerized anthocyanin also showed the ability of anthocyanins to react with tannins or with certain by-products of yeast (e.g., acetaldehyde, pyruvate, and vinylphenol) during fermentation (Kumar, Tian, & Harrison, 2022). The total anthocyanin in grape and wine samples showed the highest concentration in WW but showed the lowest concentration in XY. Malvidin derivatives were the main anthocyanins quantified, for example, the concentrations of malvidin-3-*O*-glucoside and malvidin-3-*O*-(6-acetyl)-glucoside was the highest in the grape and wine samples, with 289.85 mg/L and 214.64 mg/L respectively (Table S3). WW is located in the northwest of China at an altitude of 2300 m asl, with an annual rainfall of about 200 mm and strong sunlight exposure, while XY has exactly the opposite climatic characteristics with a low altitude and high rainfall. de Oliveira et al. (2019) studied grapes from Morro do Chapéu (1100 m asl) and São Francisco Valley (350 m asl) and found that grapes grown at high altitudes were characterized by higher levels of anthocyanins. Liang et al. (2014) also got a similar result that total anthocyanins concentration rises with increased altitude.

3.2. Non-anthocyanin phenolic compounds of grape and wine samples

Seventy-eight non-anthocyanin phenolic compounds were identified and quantified in the grape and wine samples. These belonged to the chemical classes of flavonols, hydroxycinnamic acids, hydroxybenzoic acids, proanthocyanidins, stilbenes, etc. (Fig. 1C and D). Flavonols were the major non-anthocyanin phenolic compounds identified in grapes, and hydroxybenzoic acids also played a main role in wines, with >900 mg/L total concentration. The accumulation of hydroxybenzoic acids varied considerably in different wines, mainly related to grape varieties and growing conditions, such as climate, topography, vineyard management, etc. (Costa, da Silva, Cosme, & Jordão, 2015). In this study, the gallic acid had significant concentration differences with a range from 7.68 mg/L to 34.97 mg/L in five origins. Consistent with the results of Zhao et al. (2023), who studied six sub-regions in Eastern Foothills of the Helan Mountain. Many compounds could only be detected in wine samples rather than in grapes, including ethyl-gallate, epigallocatechin-gallate, and ethyl-caffeic acid (Table S4). The same situation also occurred in the study of Royo et al. (2021), which related to some hydrolysis and synthesis reactions during the fermentation or storage. According to reports, the esterification of gallic acid with ethanol to form ethyl gallate occurs during fermentation and aging conditions (Monagas, Bartolomé, & Gómez-Cordovés, 2005). Free caffeic acid mainly comes from the hydrolysis of the hydroxycinnamic acids present in grapes during the winemaking process (Lingua, Fabani, Wunderlin, & Baroni, 2016). Some hydroxycinnamic acids, including coutaric acid and caftaric acid, were significantly higher in WH, with the concentration of 0.22 mg/L and 0.62 mg/L. Quercetin-3-*O*-galactoside, kaempferol-glucuronide, myricetin-galactoside, and myricetin-glucoside in XY had a relatively lower concentration compared to other origins, with 0.43 mg/L, 2.05 mg/L, 2.30 mg/L, and 0.80 mg/L, respectively. Studies have reported that too high temperatures during berry ripening had a negative effect on flavonols accumulation (Degu, Ayenew, Cramer, & Fait, 2016; Pastore et al., 2017), which was consistent with the higher temperature of XY.

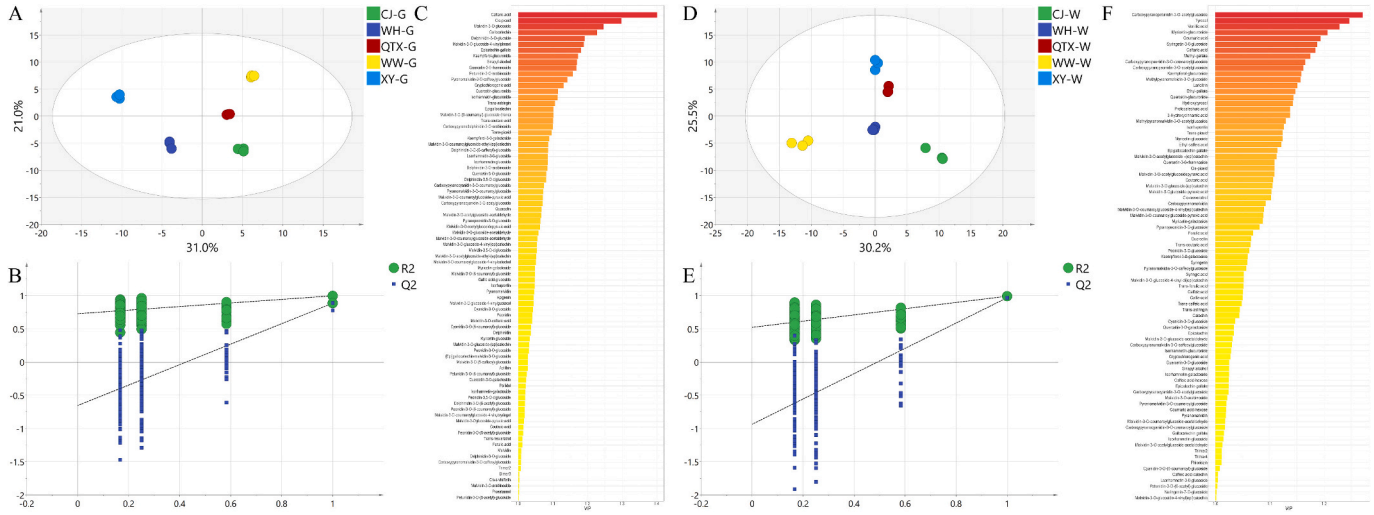

Fig. 3. OPLS-DA score plots of grape (A) and wine (D) samples, OPLS-DA model permutation test plots of grape (B) and wine (E) samples, and VIP values of grape (C).

3.3. Geographic origin discrimination of grape and wine samples from different origins

As reported, chemometric methods have been used for discriminating samples based on chemical parameters regarding their varieties, vintage, and geographical origin (Alañón, Pérez-Coello, & Marina, 2015; Valentin, Barroso, Barbosa, de Paulo, & Castro, 2020; L. Zhang et al., 2023). To reduce the data dimensions and obtain more detailed information, PCA and OPLS-DA were introduced into the processed data matrices.

After data correction, PCA models in the unsupervised mode were applied to the grape and wine samples from each origin to reveal the distribution trends among various samples (Fig. 2). The R^2X and Q^2 of PCA models corresponding to both grape and wine samples were > 0.8 and > 0.5, indicating the PCA models had good fitting effects. In Fig. 2A, PC1 and PC2 explained 51.4% of the grape samples together. In Fig. 2B, the two PCs explained 33.0% and 28.1% of the wine samples respectively. Every origin could be distinguished clearly. PCA as an unsupervised pattern recognition method, can maximize the differences between samples, but it cannot distinguish whether the differences come from within or among groups (Pan et al., 2022).

Supervised OPLS-DA models were established subsequently to maximize the differences among the groups and explain whether samples can be discriminated with some particular factors (Wang et al., 2023). As shown in Fig. 3A and D, the OPLS-DA score plots showed a clear separation among the origins, with excellent model parameters ($R^2Y = 0.994$, $Q^2 = 0.874$; $R^2Y = 0.989$, $Q^2 = 0.934$) in the two OPLS-DA models. Moreover, permutation tests were carried out and concluded that the models were not overfitting (Fig. 3B and E). These results strongly indicated the OPLS-DA models were reliable and had a good discriminating ability. The VIP value quantified the importance of each metabolite in the model. As shown in Fig. 3C, the top phenolic compounds ranked of grapes from different origins were caftaric acid, cis-piceid, malvidin-3-*O*-glucoside, gallocatechin, delphinidin-3-*O*-glucoside, etc. Caftaric acid had the highest concentration in WH but had the lowest concentration in WW. Both malvidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside had the highest concentration in WW but had the lowest concentration in WH and XY. The compounds associated with the geographical origin classification of wine were carboxypyranopetunidin-3-*O*-acetylglucoside, tyrosol, vanillic acid, myricetin-

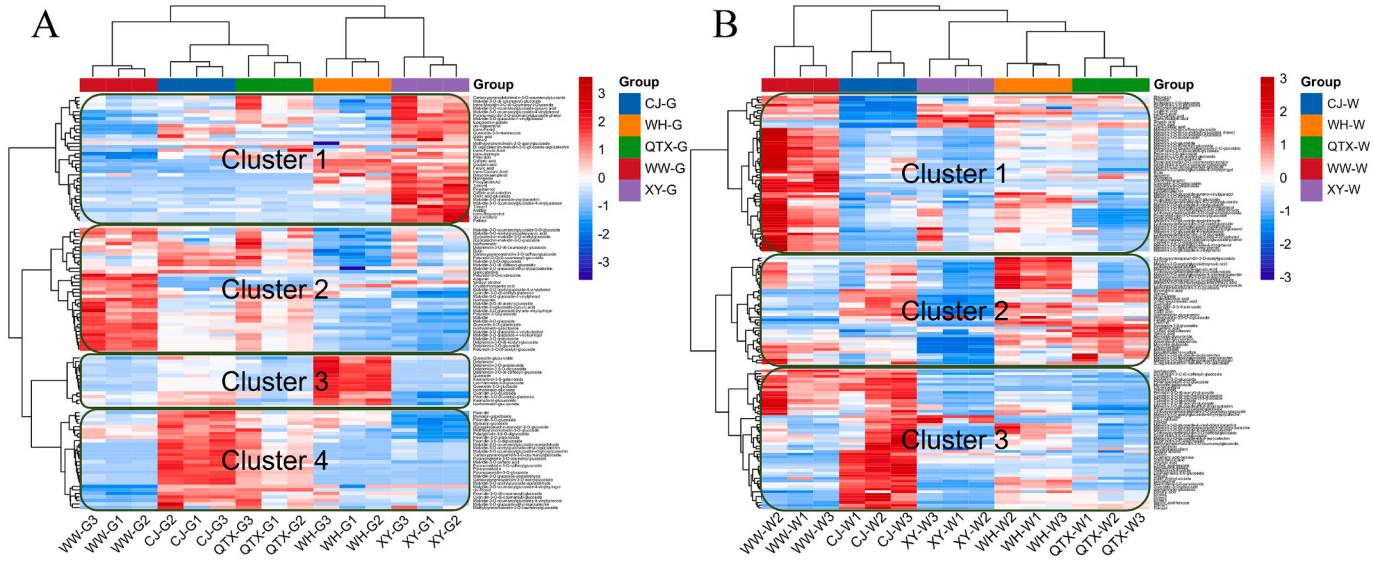

Fig. 4. Heatmaps of differential metabolites of grape (A) and wine(B) samples from different origins.

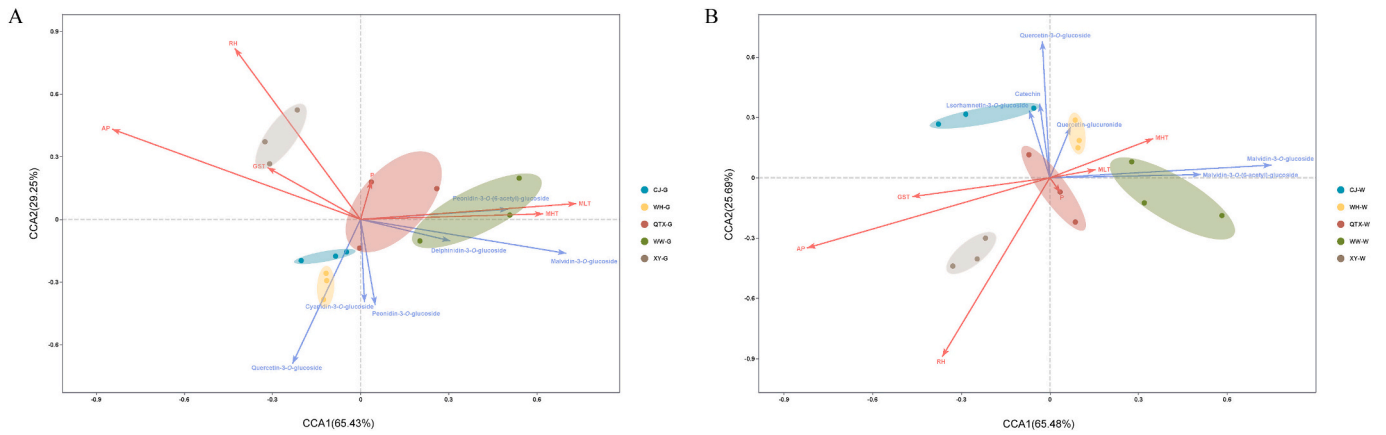

Fig. 5. CCA of grape (A) and wine (B) samples from different origins. MHD: mean high temperature; MLD: mean low temperature; RH: relative humidity; P: precipitation; AP:

glucuronide, coumaric acid (Fig. 3F). Tyrosol has the highest concentration in WH but has the lowest concentration in XY. The concentration of vanillic acid was highest in QTX but was lowest in XY. In general, these compounds in XY showed relatively lower concentrations, which indicated that the climate conditions in XY (high temperature, low altitude, and high rainfall) were closely related to grape secondary metabolites such as flavonoids, anthocyanins, and phenolic acids (Martínez-Lüscher, Chen, Brillante, & Kurtural, 2017; Wang et al., 2020). The composition of anthocyanins mainly determines the color of grape or wine, and flavonols can contribute to the color of the wine through their pigmentation with anthocyanins (Wang et al., 2022).

3.4. Selecting and analysis of candidate differential metabolites

In this study, pairwise OPLS-DA models were established among five regions for grape and wine samples, and then differential metabolites were selected with the criteria of VIP value >1 and p-value <0.05 (Wang et al., 2023; Zhang et al., 2021). Meanwhile, in order to unveil the diversity among the groups, the differential metabolites have been visualized in the clustered heatmaps (Fig. 4). Four distinct clusters were classified with unique features in grape samples (Fig. 4A). Overall, the components in cluster 1 showed a greater proportion of stilbenes (e.g., resveratrol, piceid, and astringin) and hydroxycinnamic acids (e.g., coumaric acid, ferulic acid, and caftaric acid), which displayed the highest concentrations in XY. Cluster 2 was mainly composed of malvidin derivatives with the highest concentration in WW. Cluster 3 was in the highest proportion in flavonols (e.g., quercetin-glucuronide, kaempferol-glucuronide, and isorhamnetin-glucuronide) and showed the highest concentrations in WH. Cluster 4 mainly contained malvidin and peonidin derivatives, which presented the highest concentration in CJ and QTX. For wine samples, the differential metabolites were divided to three clusters in the heatmap Fig. 4B. Cluster 1 was mainly comprised of malvidin derivatives with the highest concentration in WW. Cluster 2 included a larger proportion of flavonols and hydroxycinnamic acids, with the highest concentration in WH. Cluster 3 mainly contained proanthocyanidins, cyanidin derivatives, and peonidin derivatives, which showed the highest concentration in CJ. The four origins of CJ, WH, QTX, and WW are in the arid areas of northwest China, with low rainfall and large temperature differences, but with sufficient sunlight and strong solar radiation; but the XY is located in the middle of the Guanzhong Basin, where rain and heat occur at the same time. The high concentration of flavonols in WH was related to the strong solar radiation. Grapes exposed to sunlight increased the biosynthesis of all flavonoid compounds, and ultraviolet (UV) radiation, especially UV-B, stimulated the biosynthesis of flavonols (Zoratti, Karppinen, Luengo Escobar, Häggman, & Jaakola, 2014). Studies have shown that light, water deficit, and large temperature differences between day and night can up-regulate the expression of genes related to flavonoid metabolism, thereby significantly increasing the content of flavonoids (Li, He, Wang, Li, & Pan, 2014). In addition, inorganic ions in soil are also beneficial for the accumulation of flavonoids (Li et al., 2014; Reeve et al., 2005). Regardless of the mechanisms involved, environmental factors are thought to participate in the regulation of the content and composition of phenolic compounds in grape berries, thereby influencing the geographical characteristics of wines.

3.5. The relationship between phenolic metabolites and macroclimatic conditions

Canonical correlation analysis (CCA) between macroclimatic parameters and individual phenolics of grapes and wines from five origins was performed (Fig. 5). Some compounds were closely related to climatic factors. Some anthocyanins and flavonols (cyanidin-3-*O*-glucoside, peonidin-3-O-glucoside, quercetin-3-*O*-glucoside, lsorhamnetin-3-*O*-glucoside, and quercetin-glucuronide) had a negative correlation with precipitation, consistent with the results of Huang et al. (2022), which indicated precipitation exerted important effects on certain phenolics. In some studies, the concentrations of some anthocyanins were significantly enhanced in the rain shelter cultivated grapes compared with the open field (Li et al., 2014). Malvidin-3-*O*-glucoside showed a positive correlation with mean high temperature (MHT), mean low temperature (MLT), and a negative correlation with atmospheric pressure (AP) in both grape and wine samples. In addition, delphinidin-3-*O*-glucoside, peonidin-3-*O*-(6-acetyl)-glucoside, malvidin-3-*O*-(6-acetyl)-glucoside also showed a significant positive correlation with MHT and MLT. Studies have reported that temperature is an important environmental factor that affects anthocyanin biosynthesis, appropriate temperature and sufficient light can promote anthocyanin accumulation (Azuma, Yakushiji, Koshita, & Kobayashi, 2012; de Oliveira et al., 2019). Relative humidity (RH) had a negative correlation with most compounds, especially delphinidin-3-*O*-glucoside and quercetin-glucuronide, which could be explained by the occurrence of grape diseases. Previous research has shown that humidity could promote the development of grapevine diseases, including botrytis cinerea, anthracnose, powdery mildew, and black rot (Fedele, Brischetto, & Rossi, 2020; Li, Dos Santos, Gao, Chang, & Wang, 2021; Onesti, González-Domínguez, & Rossi, 2017). At the same time, temperature as another environmental condition also has a great impact on the spread of grape disease pathogens (Fedele et al., 2020). They can disrupt normal berry development and stress responses, thus inhibiting ripening pathways that influence sugar concentration, phenolics and volatile compounds in the grape berry and wine (Pereira et al., 2021).

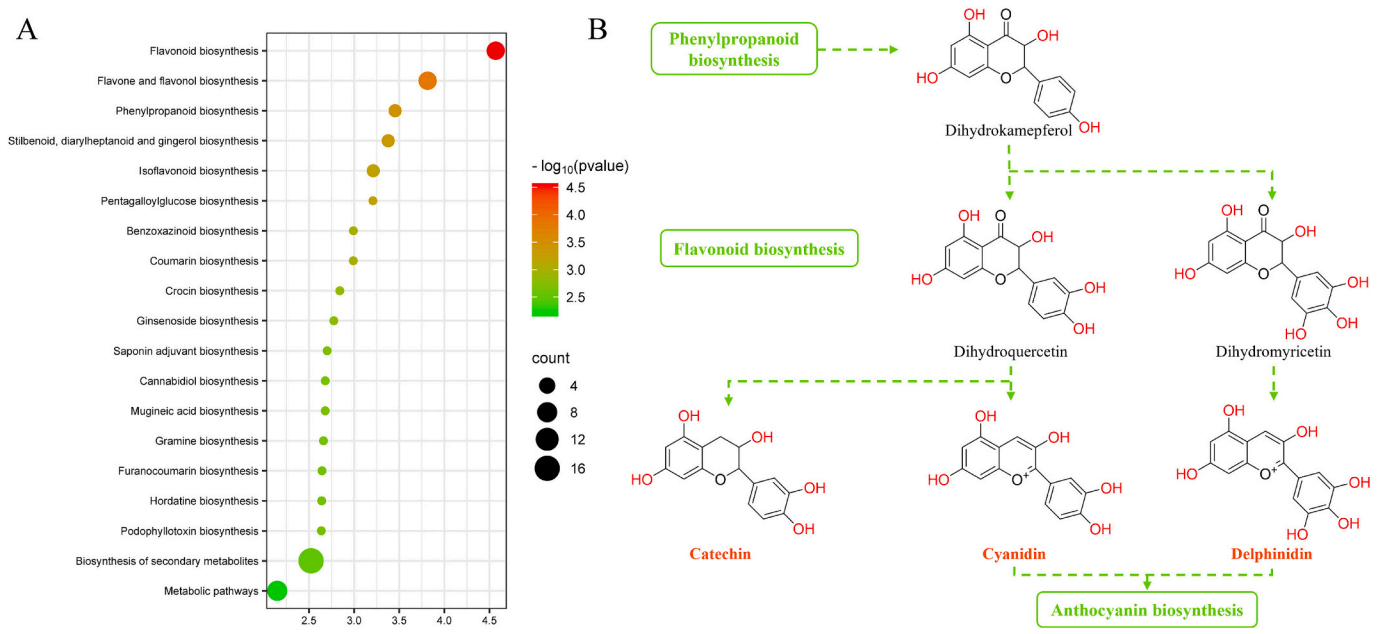

Fig. 6. Enrichment analysis of metabolic pathways (A) and the metabolic pathways related to flavonoids (B).

3.6. Analysis of metabolic pathways of grapes from different origin

In order to elucidate the mechanisms of metabolic diversity in different geographical origins, enrichment analysis was performed based on the KEGG database (Fig. 6A). In grape berry, phenylpropanoid metabolism and flavonoid metabolism are upstream steps in the biosynthesis of anthocyanins, flavonols, and flavan-3-ol (Fig. 6B). For example, dihydrokamepferol can flow to dihydroquercetin branch and dihydromyricetin branch (Castellarin, Matthews, Di Gaspero, & Gambetta, 2007). Cyanidin derivatives and delphinidin derivatives are respectively synthesized from the dihydroquercetin branch and dihydromyricetin branch (Ai, Wu, Battino, Bai, & Tian, 2021). In this study, WH exactly had the highest concentration of quercetin and cyanidin derivatives, while XY contained the highest concentration of myricetin and delphinidin derivatives. This phenomenon indicated that the different terroir could promote carbon flow to the quercetin synthetic branch or the myricetin synthetic branch. The vineyards in WH are located in a desert region at the altitudes of 1150 m asl, with an arid climate, a big temperature difference between daytime and nighttime, and an annual rainfall of 100 mm. The quercetin branch was probably with high activity in the flavonoid metabolism under that condition. XY is located at an altitude of about 300 m with a high temperature, semi-humid climate, and an annual rainfall of about 900 mm. This terroir condition might promote myricetin and delphinidin derivatives accumulated at a higher level. In addition, catechin and cyanidin derivatives share a series of steps from leucoanthocyanidin to cyanidin, which leads to a competitive mechanism between catechin and cyanidin derivatives (Li et al., 2011). The grapes from WH presented the highest level of the cyanidin derivatives and a low level of catechin, suggesting that more carbon flowed to the cyanidin synthetic branch rather than the catechin synthetic branch.

4. Conclusion

This study investigated the metabolic differences of grapes and wines from five origins in China. A total of 94 anthocyanins and 78 non-anthocyanin phenolic compounds were identified and quantitated by UHPLC-QqQ-MS/MS. Flavonols, stilbenes, hydroxycinnamic acids, and anthocyanin derivatives, including peonidin and malvidin were selected as candidate differential metabolites. Mean high temperature and mean low temperature had a positive correlation with some phenolic compounds, while precipitation, relative humidity, and atmospheric pressure had a negative correlation with some anthocyanins and flavonols. This study also elucidated the metabolic diversity in different geographical origins mainly occurring in the flavonoid biosynthesis pathway, enhancing the understanding of Chinese wine terroir, thus providing a scientific basis for viticulture and winemaking with distinct geographic characteristics.

CRediT authorship contribution statement

Lin Zhang: Writing - original draft, Methodology, Data curation, Conceptualization.**Zhaoxiang Wang:** Methodology, Formal analysis. **Cui Zhang:** Writing – original draft, Funding acquisition, Methodology, Data curation, Conceptualization. **Shubo Zhou:** Software. **Chunlong Yuan:** Resources, Funding acquisition.

Declaration of competing interest

The author declares no competing financial interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101396.

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