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Anthocyanin profiles and color parameters of fourteen grapes and wines from the eastern foot of Helan Mountain in Ningxia

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ABSTRACT

To identify wine grape cultivars (*Vitis vinifera*) with superior color properties for wine production, the anthocyanin composition and color characteristics of fourteen cultivars and their wines were investigated. Grapes and wines from 'Dornfelder', 'Dunkelfelder', and 'Malbec' cultivars exhibited significantly higher total phenolic contents. At harvest, 'Dornfelder' grapes and wines exhibited the highest total anthocyanin contents, with values of 249.94 mg/kg in grapes and 1686.76 mg/L in wines, significantly higher than other cultivars. PCA and PLS-DA analyses identified malvidin-3-*O*-(trans-6-*O*-coumaryl)-glucoside as a common differential metabolite in both grapes and wines across all cultivars. Correlation analysis revealed that non-acylated anthocyanins ($r = 0.68$) have a more significant impact than acylated anthocyanins ($r = 0.28$) on color chromatic diversity in grapes and wines ($P < 0.05$). These findings suggest that wines produced from 'Dornfelder' grapes are particularly valuable for their superior color properties and high individual anthocyanin contents. This study addresses a gap in comparative anthocyanin analysis among cultivars and provides valuable insights for grape growers and winemakers seeking to optimize grape selection for improved wine quality.

1. Introduction

Anthocyanins are the primary pigments responsible for the color of grapes and wines and significantly affect the sensory properties of wine (Tian et al., 2022). These compounds are vital phenolics known for their antioxidant, anti-inflammatory, and cardioprotective properties (Chen et al., 2020). Anthocyanins are synthesized through the flavonoid pathway and are primarily found in grape skins. In teinturier grapes, however, anthocyanins also accumulate in the flesh and other tissues (Xie et al., 2019). The synthesis of anthocyanins in grape skins begins at the veraison stage. In *Vitis vinifera* grapes, the primary anthocyanins are: delphinidin, cyanidin, petunidin, peonidin, and malvidin, along with their derivatives (Yin et al., 2022). Among these, malvidin-3-*O*-glucoside is the most abundant in the majority of *Vitis vinifera* cultivars, particularly those used for wine production (Ren et al., 2023; Zhao et al., 2023).

The composition and content of anthocyanins in grapes and wines are influenced by factors such as cultivar, light exposure, temperature, water availability, and cultivation practices (Lu et al., 2023). For instance, high temperatures during ripening decrease anthocyanin levels at grape maturity (Xie et al., 2021). However, the genotype plays a more crucial role in determining anthocyanin accumulation. Global warming poses new challenges because cultivars that performed well under previous climatic conditions may not be optimal in the future (Gouot et al., 2019). Therefore, understanding the genetic basis of anthocyanin synthesis in different cultivars is crucial for future viticulture strategies. Cultivars with unique traits, such as the absence of acylated anthocyanins in 'Pinot Noir', provide valuable insights into the metabolism and regulation of anthocyanins (Ren et al., 2023). Such knowledge can support grape breeding programs aimed at developing varieties with enhanced quality and resilience. Additionally, identifying cultivars with superior anthocyanin profiles expands the tools available to winemakers, facilitating the development of new wine products (Tian et al., 2024). Consequently, exploring the genetic diversity among grape cultivars is essential to comprehend their phenotypic variations and chemical compositions.

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Grape cultivars display considerable genetic complexity, leading to phenotypic diversity that significantly affects morphological traits and chemical composition (Gao et al., 2024; Ren et al., 2023). Ju et al. (2021) found that 'Junzi#2' wine had the highest total phenolic and anthocyanin content, while 'Xiangzhenzhu' wine showed the highest total flavonoid content. In Argentina, Cabernet Sauvignon exhibits higher malvidin content than Merlot and Tempranillo, attributed to the elevated activities of flavonoid-3′-hydroxylase and *o*-dihydroxyphenol-*o*-methyltransferase (Fanzone et al., 2012). Some studies have found that 'NW196', a hybrid of *Vitis vinifera* grapes, exhibiting the highest total anthocyanin content and relatively high levels of diglucosides (Zhu et al., 2023). Additionally, phenolic compounds are commonly used as chemical fingerprints to verify the authenticity and provenance of wine varieties (Gao et al., 2024; Lukic et al., 2019). Similarly, Han et al. (2024) utilized mid-infrared and ultraviolet spectroscopy combined with chemometrics to successfully trace and identify the origin of garlic, demonstrating the effectiveness of spectral data fusion and machine learning algorithms in agricultural product authentication. Therefore, analyzing these chemical components is essential for comparing quality differences among grape and wine varieties.

The eastern foothills of Helan Mountain in Ningxia are recognized as one of China's most prominent wine regions. This area has gained significant international recognition for producing high-quality wines with distinctive sensory profiles (Zhao et al., 2022). 'Cabernet Sauvignon' and 'Merlot' are among the most renowned wine grape varieties worldwide, recognized for their high anthocyanin content and complex flavor profiles (Zhao et al., 2023). 'Meili' is a new red grape variety developed by the College of Enology at Northwest A&F University (Yang et al., 2019). This variety shows strong resistance to fungal diseases and demonstrates good adaptability and cultivation potential (Lan et al., 2021). 'Beibinghong' a hybrid of *Vitis amurensis* and *Vitis vinifera*, is notable for its significant cold resistance (Lan et al., 2019). This variety does not require vine burial before winter, making it valuable for cultivation in cold regions (Duan et al., 2019).

Despite the extensive grape-growing regions in China, research on the chemical and sensory properties of wine has predominantly focused on commonly cultivated varieties, such as 'Cabernet Sauvignon,' 'Cabernet Franc,' and 'Merlot'. In contrast, less common varieties grown in the Eastern Foot of Helan Mountain in Ningxia are comparatively less studied. To our knowledge, detailed studies on the anthocyanin composition of various grape varieties cultivated in this region and their corresponding wines are lacking. This study addresses this gap by systematically analyzing the anthocyanin profiles of fourteen grape varieties from the Eastern Foot of Helan Mountain to understand how anthocyanin composition influences wine color characteristics and overall quality. This comprehensive analysis will elucidate the anthocyanin profiles and color attributes of these grape varieties, offering a theoretical foundation for their commercial development and providing valuable insights for grape growers and winemakers in Ningxia and similar semi-arid regions.

2. Materials and methods

2.1. Experimental site and plant material

The grape varieties used in this study were sourced in 2022 year from two vineyards in Ningxia, China. Both vineyards are located within the Helan Mountain East Foothill region, characterized by similar climatic conditions, soil types, and elevation. The Chateau GreatWall Terroir (38°37′ N, 105°95′ E) provided the following varieties (*Vitis vinifera*): 'Cabernet Sauvignon' (CS), 'Pinot Noir' (PN), 'Malbec' (MB), 'Marselan' (MS), 'Syrah' (SY), 'Cabernet Franc' (CF), 'Dornfelder' (DF), 'Merlot' (ML), 'Petit Verdot' (PV), and 'Sangiovese' (SG). The Xige Estate (38°72′ N, 106°09′ E) supplied 'Beibinghong' (BBH), 'Cabernet Gernischet' (CG), 'Meili' (ME), and 'Dunkelfelder' (DK). The grapes were planted as own-rooted vines in 2016 and trained on a single trellis system with vertical shoot positioning, at a spacing of 1 m × 3.5 m. Drip irrigation was utilized, and consistent vineyard management practices were followed across both sites, including pruning, canopy management, pest and disease control, irrigation scheduling, and harvesting protocols. Based on Coombe et al. (1995), five-point sampling was performed at stages *E*-L 34, E-L 35, E-L 36, E-L 37, and E-L 38 (Table. S1). Approximately 350 berries were collected for each variety, with three replicates per grape variety. All samples were immediately transported on ice to the laboratory and stored at −80 °C for further analysis.

2.2. Winemaking protocol

For the winemaking experiments, 45 kg of grapes from each variety per repeat were collected during the harvest period. The winemaking process was conducted according to the laboratory-scale micro-vinification method described by Gao et al. (2024). Briefly, 45 kg of berries from each variety were destemmed and crushed, then divided into three replicates, which were each transferred into a 10 L glass fermenter. The samples were treated with 50 mg/L sulfur dioxide. Active commercial yeast (200 mg/L, *Saccharomyces cerevisiae* CECA, Angel Yeast Co., Ltd., China) was added, and alcoholic fermentation began after a 24 h soak. Fermentation was maintained at 25 °C–28 °C. To ensure effective maceration and prevent juice overflow, the cap was punched down three times daily. When the residual sugar dropped below 4 g/L, the wine was separated from the pomace, transferred to sterile containers, treated with 50 mg/L sulfur dioxide, clarified, and stored at 4 °C for three months for further analysis.

2.3. Physicochemical parameters of grapes and wines

Berries were manually crushed, and the soluble solids content of the grape juice was measured using a handheld refractometer (Atago Co., Ltd., Japan). Titratable acidity in grapes and wines were measured using acid-base titration and expressed in grams per liter (g/L) of tartaric acid (Duan et al., 2019; Fanzone et al., 2012). Alcohol content, pH, and volatile acidity of the wine were measured according to the National Standard of the People's Republic of China (*GB/T* 15037–2006, Wine, 2006). Each sample was analyzed using three biological replicates.

2.4. Analysis of the phenolic compounds in grapes and wines

200 berries were randomly selected from each sample group and manually peeled. The skins were ground in liquid nitrogen and freeze-dried using a vacuum freeze dryer (FD 5 series, GoldSIM, USA). 1.00 g of dried powder was accurately weighed and extracted with 20 mL of 60 % methanol containing 1 % formic acid. The extraction was sonicated at 40 Hz for 30 min at 30 °C, followed by centrifugation at 10,000*g* for 10 min at 4 °C. The supernatant was collected, and the extraction process was repeated three times. Wine samples were analyzed directly. Phenolic compounds were measured according to the method described by Chen et al. (2020). Specifically, total phenolic content (TP) was determined using the Folin-Ciocalteu method, expressed as gallic acid equivalents. Total tannin content (TTA) was determined using the methyl cellulose precipitation method, expressed as catechin equivalents. Total anthocyanin content (TAN) was determined using the pH differential method, expressed as malvidin-3-*O*-glucoside equivalents. Results were calculated using standard curves of gallic acid for TPC and catechin for TTC (Table. S2).

2.5. Analysis of monomeric anthocyanin in grapes and wines

Monomeric anthocyanins in grapes were prepared as described in Section 2.4. For monomeric anthocyanin extraction, 0.50 g of skin powder was sonicated in 10 mL of extraction buffer (formic acid/methanol, 2:98, *v*/v) for 10 min, followed by shaking in the dark at 130 rpm and 25 °C for 30 min. The mixture was centrifuged at 4 °C and 8000

×*g* for 10 min, and the supernatant was collected. The residue was extracted twice more, and the combined supernatants (30 mL) were concentrated to dryness. The dried residue was redissolved in 10 mL of mobile phase A (formic acid: acetonitrile, 2:6:92, v/v/v) and filtered through a 0.45 μm polypropylene syringe filter (Jinteng, Tianjing, China) for anthocyanin quantification.

High-performance liquid chromatography (HPLC) was performed as described by Yin et al. (2022), using an LC-20 A system (Shimadzu, Japan) equipped with a Synergi Hydro-RP 80 A column (250 × 4.6 mm, 4 μm, Phenomenex). The mobile phases were (A) distilled water/acetonitrile/formic acid (32:4:1, v/v) and (B) distilled water/acetonitrile/formic acid (16:20:1, v/v). The gradient elution program was: 0–15 min, 0–10 % B; 15–30 min, 10–20 % B; 30–45 min, 20–35 % B; 45–46 min, 35–100 % B; and 50–51 min, 100–0 % B. The flow rate was set at 1.0 mL/min. Anthocyanin content was quantified using a malvidin-3-*O*-glucoside standard curve (Table. S2) and expressed as mg/kg dry weight (DW) for grape skins and mg/L for wine.

2.6. Color parameters

Grape samples were analyzed following the method of Yin et al. (2022), with grape skins collected after sampling. During the harvest period, 50 frozen grape berries from each variety were randomly selected and manually peeled. The grape skins were ground into powder using liquid nitrogen. The samples (1.00 g) were extracted with 5 mL of methanol-water solution in an ultrasonic bath in the dark for 10 min. The extracts were then incubated at 25 °C and shaken at 130 rpm for 30 min. The extract was centrifuged at 7156 ×*g* for 5 min. The precipitate was subjected to a second extraction, and the supernatants were combined for analysis. Wine samples were analyzed directly (Han et al., 2017). Color parameters were measured using a CM-5 spectrophotometer (Konica Minolta, Inc., Japan) and recorded as L* (lightness), a* (red-green), and b* (yellow-blue) values. Chroma (C^*_{ab}), tone (h_{ab}), and color difference ($\triangle E^*_{ab}$) were calculated according to the method of Ju et al. (2021). Each sample was analyzed in triplicate.

2.7. Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS 23.0 with the Tukey test ($P < 0.05$). Histograms were generated using Origin 2022. Heatmaps and principal component analysis (PCA) plots were created using ChiPlot v2.1 (https://www.chiplot.online). Feature color map was drawn using Color Express software. Chord diagrams were generated in R using the RStudio integrated development environment.

3. Results and discussion

3.1. Physicochemical parameters

Sugar concentration and acid content are key indicators of berry ripeness, which directly influence wine quality (Yang et al., 2019) The trends in soluble solids content (TSS) and titratable acidity (TA) are similar across all varieties, with sugars gradually reaching a plateau and acids steadily decreasing. However, notable differences exist among the varieties (Table. S3). Among the 14 tested varieties, 'Marselan' and 'Sangiovese' had significantly higher soluble solids content at harvest, at 28.00°Brix and 27.93°Brix, respectively. 'Pinot Noir' and 'Meili' had low soluble solid content, at 20.71°Brix and 21.06°Brix, respectively. The titratable acidity of the varieties ranged from 3.47 g/L to 10.91 g/L. 'Beibinghong' showed the highest titratable acidity. In summary, these differences are primarily attributed to the inherent characteristics of the grape varieties.

The chemical parameters of wines are influenced by grape berry characteristics and the winemaking process (Tian et al., 2022). The results indicate that 'Marselan' had a significantly higher alcohol content than the other varieties, at 15.89 % vol (Table. S4). Except for 'Pinot Noir', all other varieties had an alcohol content exceeding 11.00 % vol. The pH values of the wines ranged from 3.22 to 3.85, indicating slight differences among the various wine samples. The volatile acidity in wine can indicate its health status and any changes in quality. According to the Chinese National Standard (GB/T 15037–2006), volatile acidity in wine should not exceed 1.2 g/L. As shown in the Table. S4, the volatile acidity of the varieties ranged from 0.41 g/L to 0.83 g/L. Acidity is a crucial component of a wine's flavor profile, contributing to a fresh and vibrant palate in wines with balanced acidity. In red wines, titrable acidity aids in stabilizing anthocyanins, while maintaining an optimal acid level supports the preservation of color over time (Zhang et al., 2021). 'Beibinghong' wine had the highest titratable acidity, reaching 8.32 g/L. 'Meili' wine had the lowest titratable acidity, also at 4.97 g/L. Acids in wine originate from grapes and are essential for balancing flavor and ensuring microbial stability. In semi-arid regions such as Ningxia, high temperatures and low humidity often lead to grapes with reduced acidity, posing challenges for winemakers (Tian et al., 2022). In this study, the high titratable acidity observed in 'Beibinghong' wine corresponds to the acid content of its grapes. Therefore, selecting grape varieties such as 'Beibinghong' that naturally maintain higher acidity levels is crucial for addressing wine quality issues related to low acid content in semi-arid regions.

3.2. Differences in phenolic content of grapes and wines

Phenolic compounds are significant secondary metabolites that reflect the quality of grape berries and wine, playing a crucial role in their antioxidant activity. The total phenolic content in grape skins at various developmental stages is shown in Fig. 1 (A-E). These values align closely with those reported in other studies (Allegro et al., 2021; Van Leeuw et al., 2014). The TP value in grape skins gradually increases, while TTA value decreases over time. At the *E*-L 34 stage, The TP value was higher in the 'Dunkelfelder', 'Malbec', 'Petit Verdot', and 'Syrah' varieties. At harvest, 'Malbec', 'Dornfelder', and 'Dunkelfelder' exhibited significantly higher TP value than other varieties. 'Beibinghong' had the lowest TP value, with 'Malbec' having 2.99 times that of 'Beibinghong'. Tannins play a significant role in the perception of bitterness and mouthfeel. The changes in TTA value during the growth and development stages of various grape varieties are shown in Fig. S1 (A-E). At harvest, 'Malbec' had significantly TTA value than other varieties, while 'Beibinghong' had significantly lower TTA value. Song et al. (2022) found that 'Beibinghong' had lower TTA value, consistent with our findings.

Anthocyanins are crucial in determining the color of grapes and wines. The total anthocyanin content (TAN) changes during the growth stages of various grape varieties are shown in the Fig. 2 (A-E). The TAN value in grape skins initially increases and then stabilizes or slightly decreases. In varieties such as 'Dunkelfelder', 'Syrah', 'Sangiovese', 'Merlot', 'Cabernet Gernischet', 'Petit Verdot', and 'Meili', TAN value peaks at the E-L 37 stage. Xie et al. (2021) reported that in the late ripening stage of grapes, increased POD and β-glucosidase activity leads to anthocyanin degradation, which may explain the decrease in anthocyanin content observed in some grapes during ripening. Our results align with these findings, as we observed a decline in TAN values from E-L 37 to harvest in these varieties, suggesting that enzymatic degradation may be a contributing factor. However, 'Dornfelder' and 'Dunkelfelder' had significantly higher TAN value than other varieties at harvest, measuring 57.13 mg/kg and 53.59 mg/kg, respectively. This indicates that these varieties may exhibit lower POD and β-glucosidase activities or possess anthocyanin structures that are more resistant to enzymatic degradation. In contrast, 'Meili', 'Syrah', 'Sangiovese', 'Merlot', and 'Pinot Noir' had lower TAN value, ranging from 13.57 mg/kg to 17.25 mg/kg, which is consistent with expected anthocyanin degradation. These observations highlight varietal differences in anthocyanin stability during ripening, possibly due to genetic factors affecting enzyme activity levels or variations in anthocyanin composition. Fanzone et al.

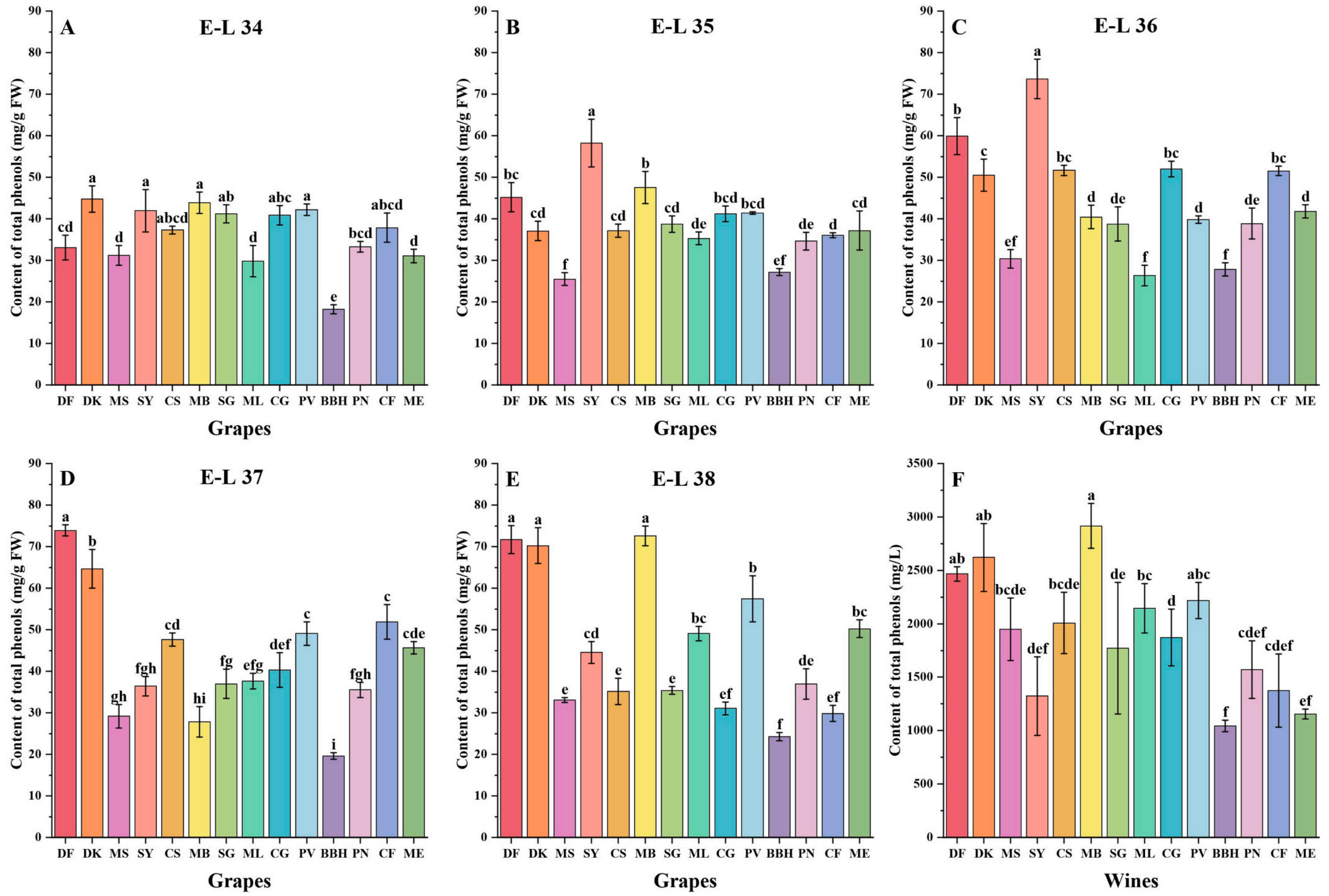

Fig. 1. Analysis of total phenols contents in 14 kind of grapes (A, B, C, D, E) and wines (F). Data in bar is presented as mean ± SD ($n = 3$). Different letters indicate significant differences between treatments ($P < 0.05$).

(2012) reported that Cabernet Sauvignon contains higher anthocyanin levels than Merlot and Tempranillo. The polyphenolic composition of grapes is primarily influenced by varietal differences (Van Leeuw et al., 2014; K. Zhang et al., 2021), which aligns with our findings.

Polyphenolic compounds in wine are partially derived from the maceration of grape skins, while others are synthesized during fermentation and aging through complex physiological and biochemical reactions (Lu et al., 2023). The TP value in 'Malbec', 'Dunkelfelder', and 'Dornfelder' wines were higher than in other varieties, aligning with the polyphenol content found in their corresponding grapes (Fig. 1F). Higher TP values contribute to increased antioxidant capacity, enhanced flavor complexity, and a fuller body, positively affecting the wine's taste and mouthfeel. Tannins form the structural backbone of wine, contributing to astringency and balancing acidity and alcohol. 'Dornfelder' and 'Cabernet Sauvignon' wines have significantly higher TTA value compared to other varieties, whereas 'Pinot Noir' and 'Meili' exhibit the lowest levels (Fig. S1F). Extended maceration times and higher temperatures generally increase the extraction of tannins and anthocyanins, leading to wines with deeper color and more robust tannin structure (Tian et al., 2024). Regarding anthocyanins, TAN value in 'Petit Verdot' and 'Dornfelder' wines were significantly higher than in other varieties, exceeding 500 mg/L (Fig. 2F). The TAN value in 'Meili' wines is significantly lower than in other varieties, measuring only 58.12 mg/L. Higher TAN values contribute to deeper color intensity and improved color stability, important factors influencing the visual appeal and perceived quality of the wine. Anthocyanin contents in wines were significantly lower than in grapes, with a similar decreasing trend observed in other red wines. Previous studies have reported that anthocyanins in red wine change during aging and storage due to degradation and condensation with other phenolic compounds, resulting in more stable polymeric pigments or proanthocyanidins (Tian et al., 2022; Wang, Yang, et al., 2022). Reactions between anthocyanins and hydroxycinnamic acids have been shown to reduce anthocyanin content in wine (Lu et al., 2022). These factors may explain the observed differences in anthocyanin content between grapes and wines in this study.

3.3. Monomeric anthocyanins

Anthocyanins are crucial for the coloration of grapes and wines. During wine fermentation, anthocyanins in grape skins undergo transformation (Heras-Roger et al., 2016). Ren et al. (2023) reported that different clones of the same grape variety exhibit similar anthocyanin profiles but vary in composition. The primary anthocyanins in grapes are delphinidin, cyanidin, petunidin, peonidin, and malvidin, including their derivatives. A total of 9 kinds of anthocyanins were detected in grape and wine samples in our study (Table. S5, S6).

At the *E*-L 34 stage, 'Malbec' and 'Cabernet Sauvignon' had higher anthocyanin content than other varieties, mainly due to elevated levels of malvidin-3-*O*-glucoside (Mv) and deplhinidin-3-*O*-glucoside (Dp) (Table. S5). At the E-L 36 stage, 'Dunkelfelder' exhibited significantly higher levels of Mv, malvidin-3-*O*-(trans-6-*O*-coumaryl) -glucoside (tMv-cou), and malvidin-3-*O*-(6-acetyl)-glucoside (Mv-ac) compared to other varieties. At E-L 38 stage, 'Dornfelder' had the highest anthocyanin levels. The levels of Dp, cyanidin-3-*O*-glucoside (Cy), petunidin-3-*O*-glucoside (Pt), peonidin-3-*O*-glucoside (Pn), and Mv in 'Dornfelder' were significantly higher than in other varieties, indicating a notable

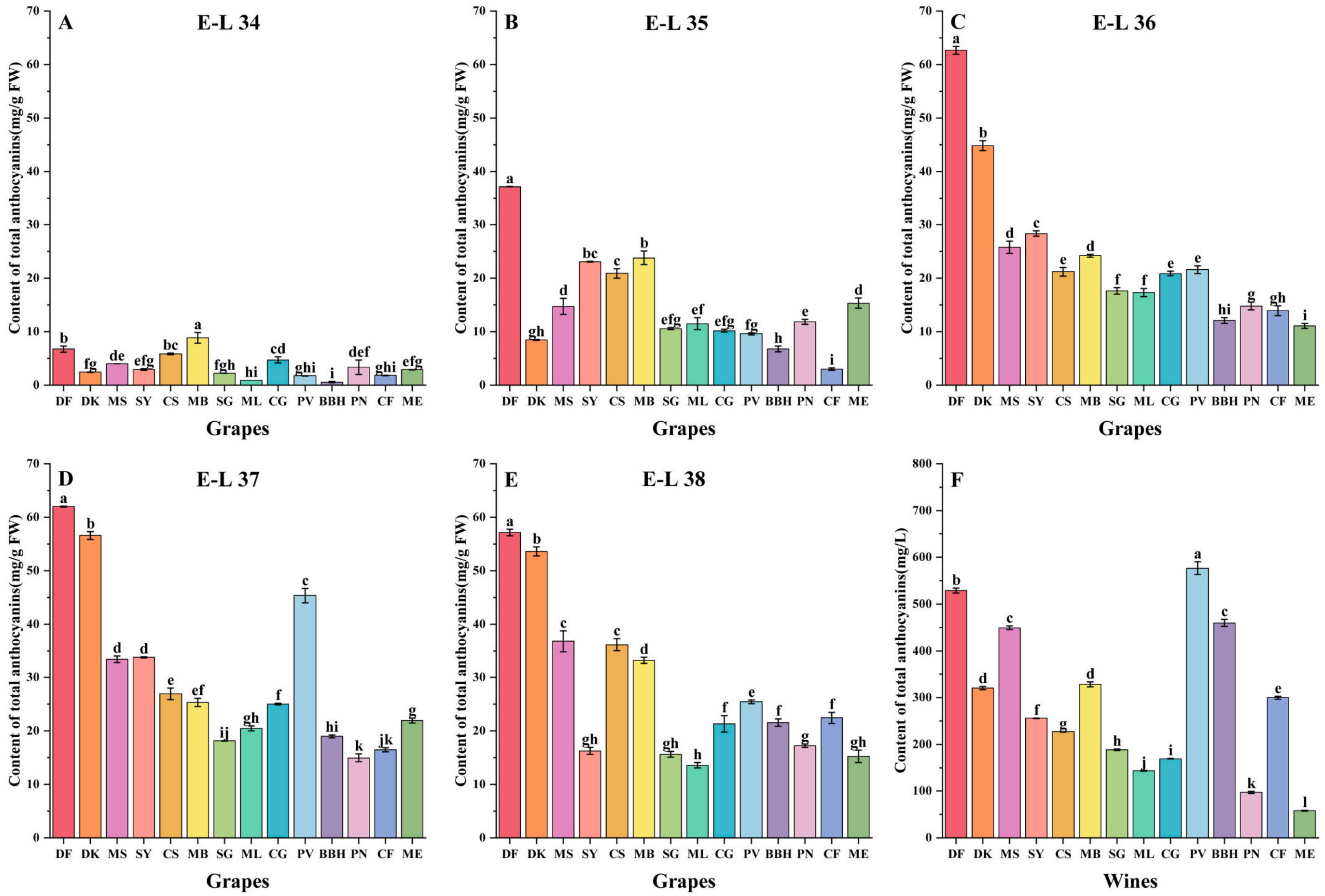

Fig. 2. Analysis of total anthocyanins contents in 14 kind of grapes (A, B, C, D, E) and wines (F). Data in bar is presented as mean ± SD (n = 3). Different letters indicate significant differences between treatments ($P < 0.05$).

advantage in anthocyanin biosynthesis pathways. 'Meili' and 'Merlot' had overall lower anthocyanin content, primarily due to significantly lower Mv levels compared to other varieties. Interestingly, 'Meili' had significantly higher levels of peonidin-3-*O*-(6-acetyl)-glucoside (Pn-ac) compared to other varieties. These variations may be related to different gene expression levels of flavonoid 3′-hydroxylase (*F3′H*), flavonoid 3′,5′-hydroxylase (*F3′5′H*), and *O*-methyltransferase (*OMT*) in the anthocyanin biosynthesis pathway (Arrizabalaga et al., 2018; Drappier et al., 2019). Cluster heatmap analysis grouped Pn, Cy, and Pn-ac together; Mv and tMv-cou into another group; and Mv, Dp, and Pt into a third group, suggesting similar evolutionary patterns during growth, as shown in Fig. 3A. Total anthocyanins are classified into non-acylated anthocyanin (TNAA) and total acylated anthocyanin (TAA). Acylation enhances anthocyanin stability and promotes color deepening under adverse temperature and light conditions (Xie et al., 2019).

Chord diagrams (Fig. 3C) and hierarchical clustering (HCA) cluster (Fig. 3E) analysis grouped 'Dunkelfelder' and 'Dornfelder' as high-anthocyanin varieties, while the remaining 12 varieties formed a separate cluster. For non-acylated anthocyanins, 'Dunkelfelder', 'Syrah', and 'Malbec' primarily contained Mv and Pt. In 'Merlot' and 'Pinot Noir', Mv and Pn were predominant, while in 'Meili', Mv and Cy were the dominant anthocyanins. In the remaining varieties, Mv and Dp were the main anthocyanins. For acylated anthocyanins, 'Meili' predominantly contained Pn-ac, 'Pinot Noir' primarily contained Mv-ac, and the other varieties mainly contained tMv-cou. This similarity may reflect shared gene expression patterns, resulting in comparable anthocyanin accumulation. Future research could examine the enzyme activity and genes involved in anthocyanin synthesis across different grape varieties to further elucidate the differences in monomeric anthocyanin components among the varieties.

In this study, the anthocyanin content in 'Dornfelder' wine is significantly higher than in other varieties, particularly for Dp, Cy, Pt, Pn, Mv, and Pn-ac (Fig. 3B). 'Petit Verdot' also exhibits high anthocyanin levels, primarily due to significantly higher concentrations of Mv-ac and peonidin-3-*O*-(trans-6-*O*-coumaryl)-glucoside (tPn-cou) compared to other varieties. The higher levels of acylated anthocyanins in 'Petit Verdot' enhance the color stability of the wine, as acylated anthocyanins are more resistant to degradation and contribute to long-term color retention (Xie et al., 2021). 'Dornfelder' has a significantly higher total monomeric anthocyanin content than 'Petit Verdot'. However, 'Petit Verdot' possesses a significantly higher total anthocyanin content than the other varieties (Fig. 2F). This difference could be attributed to the limited range of anthocyanins detected in this study. Tian et al. (2022) detected 19 types of monomeric anthocyanins, which may explain why 'Petit Verdot' had higher levels of other monomeric anthocyanins and their derivatives compared to 'Dornfelder'. In addition, 'Cabernet Franc' contains significantly higher levels of tMv-cou than the other varieties.

Chord diagrams (Fig. 3D) and HCA cluster (Fig. 3F) analysis group 'Malbec', 'Dunkelfelder', 'Syrah', 'Marselan', 'Petit Verdot', and 'Dornfelder' into one category; 'Meili' and 'Pinot Noir' into another; and the remaining varieties into a third category. In 'Dornfelder', 'Malbec', 'Merlot', 'Petit Verdot', 'Beibinghong', and 'Cabernet Franc', TNAA are predominantly Mv and Dp. In the other varieties, TNAA are primarily Mv and Pn. In 'Sangiovese', 'Cabernet Gernischet', 'Cabernet Franc', and 'Meili', TAA are primarily tMv-cou, whereas in the other varieties, TAA

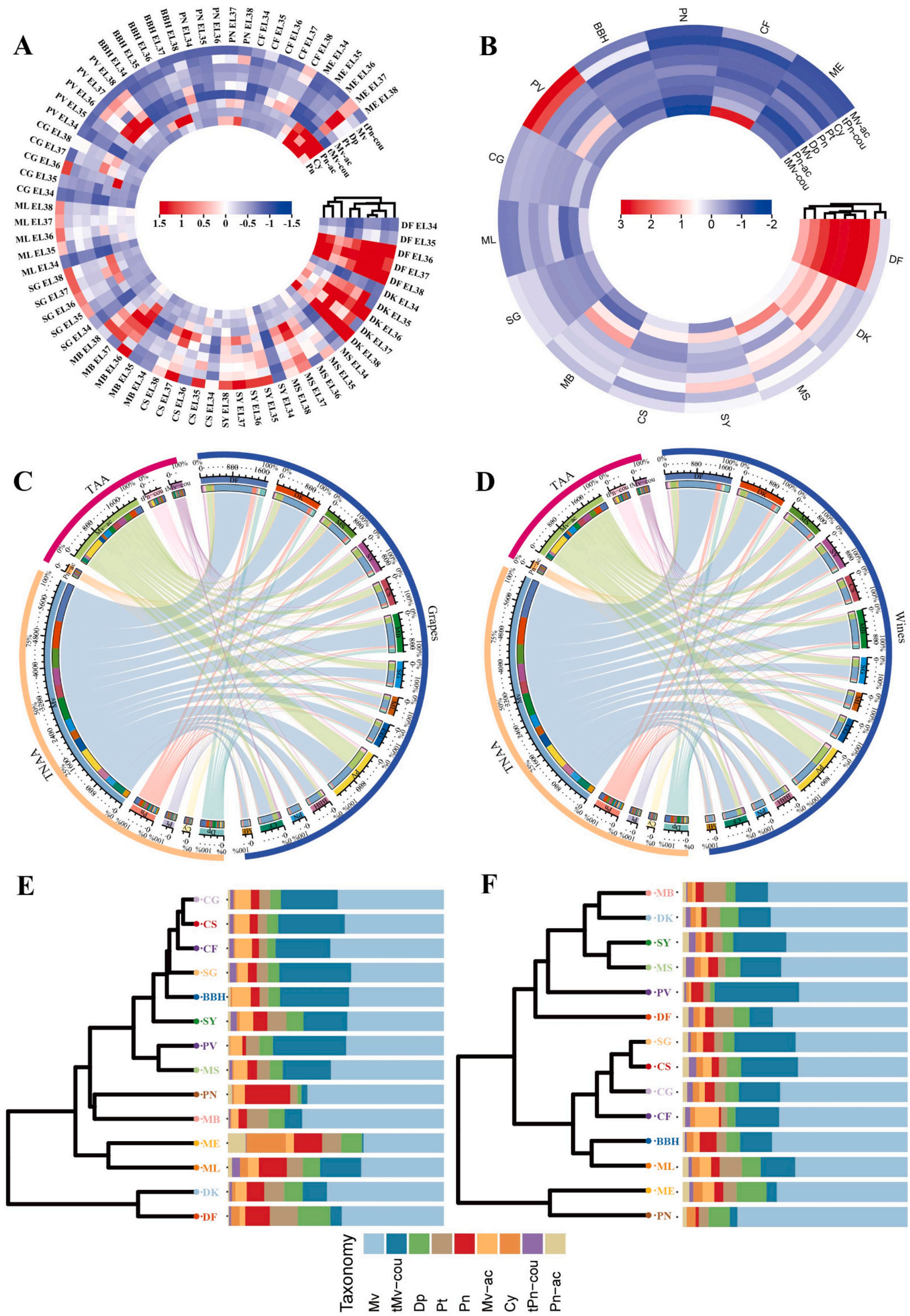

Fig. 3. Evolutionary heatmaps showing monomeric anthocyanins in grapes (A) and wines (B). Circos plot of monomeric anthocyanins in grapes (C) and wines (D). Hierarchical clustering and relative abundance% of monomeric anthocyanins in 14 kind of grapes (E) and wines (F). (Consult the online version of this article for an elucidation of the color codes mentioned in the figure legend.)

are mainly Mv-ac. These results suggest that different grape varieties may influence the monomeric anthocyanin profile of wines. When associated with changes in their concentration and the wine's nutritional and sensory characteristics, monomeric anthocyanin compounds may serve as putative biomarkers, effectively distinguishing the quality of wines produced from various grape varieties (Zhang et al., 2024; Zhao et al., 2022). In addition, these differences in anthocyanin profiles have important implications for winemaking practices and grape breeding programs. For winemakers, selecting grape varieties like 'Dornfelder' with high levels of monomeric anthocyanins can produce wines with vivid color intensity, appealing to markets that favor richly colored young wines. This study was conducted in a semi-arid region. Future research should investigate these grape varieties across diverse regions and environmental conditions to validate and further expand upon the current findings.

3.4. Principal component analysis and partial least squares discriminant analysis

The synthesis and accumulation of anthocyanins are influenced by grape genetics, environmental factors, and cultivation conditions (Drappier et al., 2019). Principal component analysis (PCA) was employed to visualize the differences in anthocyanin components among grape varieties. In addition, PCA was performed on normalized data for anthocyanin indices. Fig. 4 illustrates changes in monomeric anthocyanins during fruit development of various grape varieties, as analyzed by PCA. The PCA results indicate that the first principal component (PC1) and the second principal component (PC2) account for 53.93 % and 19.21 % of the variance, respectively (Fig. 4A). 'Dornfelder' and 'Dunkelfelder' varieties are mainly separated from other varieties by

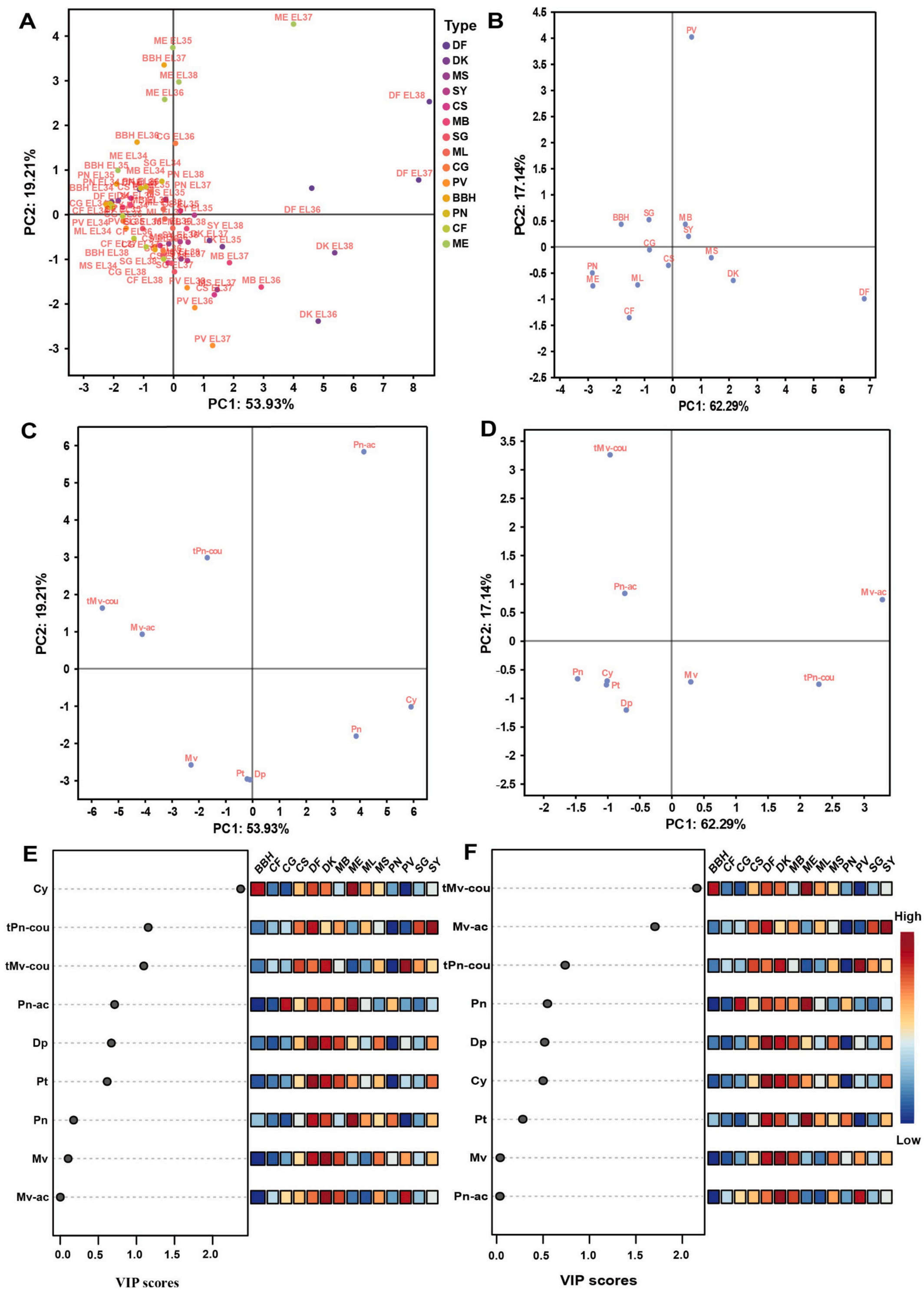

Fig. 4. PCA and PLS-DA analysis of monomeric anthocyanins. PCA scores for grapes (A) and wines (B). PCA loadings for grapes (C) and wines (D). VIP values from PLS-DA for grapes (E) and wines (F). (Consult the online version of this article for an elucidation of the color codes mentioned in the figure legend.) Dp, Deplhinidin-3-*O*-glucoside; Cy, Cyanidin-3-*O*-glucoside; Pt, Petunidin-3-*O*-glucoside; Pn, Peonidin-3-*O*-glucoside; Mv, Malvidin-3-*O*-glucoside; Pn-ac, Peonidin-3-*O*-(6-acetyl)-glucoside; Mv-ac, Malvidin-3-*O*-(6-acetyl)-glucoside; tPn-cou, Peonidin-3-*O*-(trans-6-*O*-coumaryl)-glucoside; tMv-cou, Malvidin-3-*O*-(trans-6-*O*-coumaryl) -glucoside.

PC1, indicating significant differences in anthocyanin content and composition. Similarly, the 'Meili' variety is mainly separated from other varieties by PC2, highlighting unique characteristics in anthocyanin content and composition. Additionally, the analysis of monomeric anthocyanin profiles reveals significant differences between 'Dornfelder', 'Dunkelfelder', 'Meili', and other varieties during the *E*-L 36, E-L 37, and E-L 38 stages. These differences serve as clear chemical indicators for classifying and identifying grape varieties. The loading plot indicates that monomeric anthocyanins, such as Pn, Pn-ac, and Cy, are positively correlated with PC1. PC2 is positively correlated with Pn-ac and tPn-cou (Fig. 4C). These monomeric anthocyanins may significantly contribute to the color and flavor profiles of the 'Meili' variety. To further elucidate the differential anthocyanin metabolites among grape varieties, partial least squares discriminant analysis (PLS-DA) analysis was performed based on PCA. The most influential variables were identified through VIP scores (Fig. 4E). Compounds with VIP scores greater than 1 were considered significant contributors to the model, with three compounds being identified. Among these, Cy, tPn-cou, and tMv-cou were identified as key differential anthocyanin metabolites at various developmental stages of the grape varieties.

The wine score plot is presented in Fig. 4B, where PC1 and PC2 explain 62.29 % and 17.14 % of the total variance, respectively. Wines from different grape varieties were distinctly separated. 'Dornfelder' and other varieties were mainly separated by PC1, which were characterized by higher levels of the anthocyanins Mv-ac and tPn-cou (Fig. 4D). PLS-DA analysis identified two compounds with VIP scores greater than 1, tMv-cou and Mv-ac, which are the primary differential anthocyanin metabolites in wine (Fig. 4F). The common differential metabolite between grape and wine varieties was found to be tMv-cou. This finding underscores the significance of tMv-cou in differentiating anthocyanin profiles among cultivars, which is crucial for grape classification and quality assessment. This aligns with previous studies that emphasize the importance of determining critical quality parameters to enhance traceability and transparency in agricultural products (Han et al., 2024; Xiao et al., 2017).

For winemakers, determining tMv-cou concentrations in grape must is crucial for decisions on maceration duration and temperature management, optimizing acylated anthocyanin extraction and preservation. Refining these practices enhances wine color intensity and stability, aligning with consumer preferences and market demands (Tian et al., 2024). Similarly, Xiao et al. (2017) utilized real-time monitoring and correlation analysis to identify critical quality parameters in table grapes, our study highlights the use of chemometric techniques to identify key anthocyanin compounds that can serve as quality indicators in winemaking. For grape breeders, tMv-cou serves as a marker of grape quality and a selection criterion in breeding programs for developing varieties with improved anthocyanin profiles. Using tMv-cou facilitates breeding cultivars that yield wines with superior anthocyanin content, thus improving quality. Han et al. (2024) demonstrated that combining spectroscopic data with machine learning algorithms effectively traced the origin of garlic, enhancing product authentication. Our use of multivariate analyses like PCA and PLS-DA similarly contributes to the authentication and classification of grape varieties based on their anthocyanin profiles, thereby improving traceability in the wine industry.

3.5. Color parameters

In the CIELab color space, color is defined by three orthogonal parameters: L*, a*, and b* (Han et al., 2017). The L* value indicates the lightness of the grape color, and the b* value reflects the degree of yellowness or blueness. No significant differences were observed in L* and b* values among the grape varieties (Table. S7). The a* value reflects the degree of redness or greenness, with positive values indicating redness and negative values indicating greenness. As shown in the Table. S7, the a* values of 'Pinot Noir' and 'Sangiovese' were significantly higher than those of other varieties, at 14.52 and 13.60, respectively. Additionally, the C^*_{ab} and ΔE^*_{ab}, values of 'Pinot Noir' and 'Sangiovese' were significantly higher than those of other varieties.

In wine, color is a critical sensory attribute and a key factor influencing consumer appeal (Cheng et al., 2020). The L* values of 'Meili' (62.81) and 'Cabernet Franc' (56.26) wines were significantly higher than those of other varieties, indicating a brighter appearance (Table. S8). 'Malbec' (56.20) wine exhibited the highest a* value, indicating the strongest red hue, while 'Dornfelder' (34.76) wine had the lowest a* value, indicating a weaker red hue. 'Beibinghong' (42.31) wine had the highest b* value, indicating the strongest yellow hue, while 'Dornfelder' (1.92) and 'Dunkelfelder' (4.43) wines had significantly lower b* values than other varieties, suggesting a color more inclined towards blue. The C^*_{ab} value represents color intensity. 'Beibinghong' and 'Malbec' wines had significantly higher C^*_{ab} values than other varieties. The h_{ab} value represents the hue angle, indicating color orientation. 'Beibinghong' (43.82) wine had the highest h_{ab} value. The total color difference, ΔE^*_{ab}, incorporates contributions from L*, a*, and b* components, representing the overall color difference between wine samples (Perez et al., 2007). This parameter is very important for the wine industry because it reflects the human eye's ability to distinguish between the colors of two wines (Han et al., 2017). A higher ΔE^*_{ab} value signifies a greater overall color difference, with values above 6 indicating a strong perceptible color difference.

The color characteristics of wine are influenced by factors such as grape variety, region of origin, and vineyard management practices (Ren et al., 2023). However, genotype directly affects anthocyanin synthesis potential and the wine's color profile (Ju et al., 2021). Brighter colors and purer hues are generally more appealing to consumers. The traditional CIELab color analysis method provides numerical parameters without visually representing the characteristic colors of wine samples. The L*, a*, and b* values of grapes and wines were used in Color Express software to identify corresponding colors, creating characteristic color maps for the tested varieties. Each sample is represented by a numbered do. The overall color of grapes is black or purple (Fig. S2). 'Dornfelder' and 'Malbec' wine samples exhibit significantly lower brightness than other varieties, appearing nearly black-red or purple-red, which indicates a deeper color (Fig. 5). In contrast, 'Pinot Noir', 'Meili', and 'Cabernet Franc' wine samples show relatively higher brightness, displaying pink or light pink hues on the color characteristic map. The color parameter map effectively distinguishes the 14 tested varieties.

3.6. Correlation analysis of polyphenolic components and color parameters in grapes and wines

The quality and characteristics of wine are directly linked to the quality of the grapes (Liu et al., 2022). In this study, Mantel test correlation analysis was conducted to investigate the relationship between grape and wine quality. In the heatmap, color gradients represent the strength and direction of correlations. Darker or more intense colors indicate stronger correlations, while lighter colors signify weaker correlations. Shades of orange denote positive correlations, whereas shades of blue indicate negative correlations. The correlation matrix in the dynamic heatmap indicates a positive correlation between TSS and TAN (Fig. 6A). Previous studies have shown that sugar accumulation positively regulates anthocyanin synthesis in many plants through metabolic and signaling pathways (Lu et al., 2023). TP, TTA, and TAN are positively correlated with each other, while all three indicators are negatively correlated with six color parameters. Specifically, TP and TTA are significantly negatively correlated with L* and ΔE^*_{ab}. The six color parameters are positively correlated. TAA and TNAA are significantly positively correlated with L*. TNAA is significantly positively correlated with TP, TAN, and TTA. Except for a* and C^*_{ab}, TNAA is significantly positively correlated with the other 4 color parameters. Wang, Wang, et al. (2022); Wang, Yang, et al. (2022) reported that acylation anthocyanins may be associated with changes in color and variation.

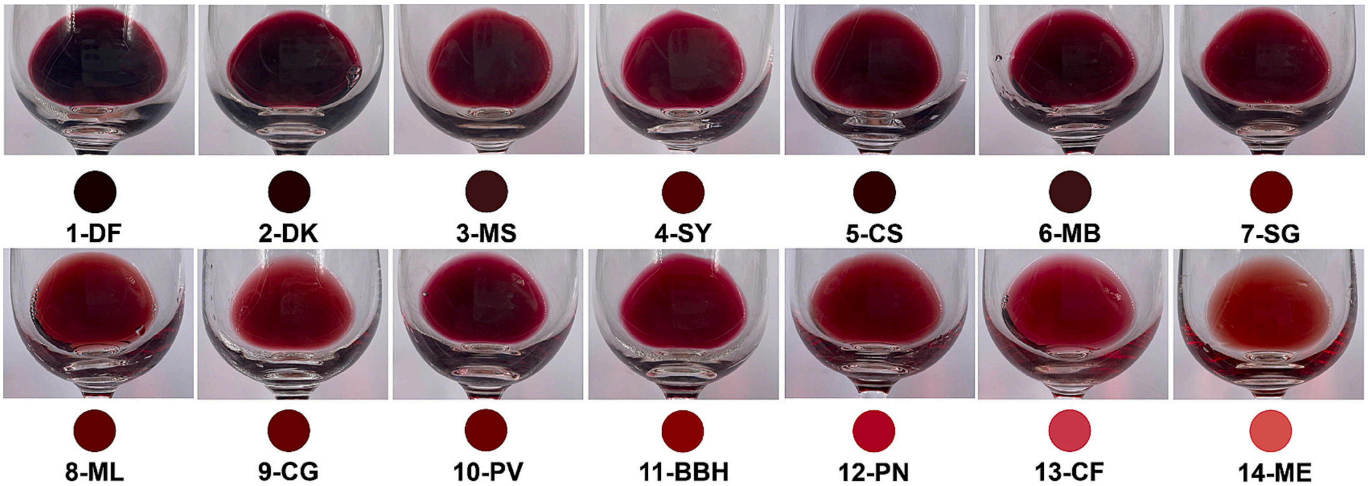

Fig. 5. The colored circles beneath each glass represent the CIELAB characteristics of the corresponding wine samples. The numbers below the glasses indicate the specific wine samples.

However, our study found that TNAA have a more substantial impact on wine color diversity than TAA. This discrepancy may be due to the distinct chemical structures and interactions of these anthocyanins with other wine components (Xie et al., 2019, 2021). Non-acylated anthocyanins, which lack acyl groups, are more reactive in wine matrices, allowing them to form a wider variety of pigments through copigmentation and other reactions (Yin et al., 2022).

The content and composition of anthocyanins significantly influence the color of wine. To elucidate the contribution of anthocyanin compounds to color, pearson correlation analysis was performed to investigate the relationship between color properties and anthocyanin compounds. As shown in Fig. 6B, the L* values of all samples are significantly negatively correlated with all anthocyanin types. These findings are consistent with those reported by Ju et al. (2021). The a* value is inversely correlated with total anthocyanins. Notably, a* is positively correlated with Dp, Mv, Pn-ac, Mv-ac, and tMv-cou, suggesting that these monomeric anthocyanins may significantly contribute to the red hue of grapes and wines (Han et al., 2023; Li et al., 2024). The b* value is negatively correlated with Cy, Pt, Pn, Mv, Pn-ac, and tMv-cou, indicating that these anthocyanins impart a blue tint to the wine. Except for Mv-ac and Pn-cou, all other anthocyanins are negatively correlated with h_{ab} and ΔE^*_{ab}. These findings indicate that monomeric anthocyanin components play a significant role in determining the color and chromatic diversity of wine. Understanding these correlations aids winemaking by guiding the selection of grape varieties rich in specific anthocyanins to achieve desired wine colors. The focus on a single region and the limited number of cultivars meant that the observed trends in anthocyanin accumulation and color parameters might vary under different climatic conditions or with other grape varieties. As a future direction, we recommend conducting multi-regional studies involving a broader range of grape cultivars and multiple growing seasons to validate these findings and expand their applicability. Such studies would help understand how different environmental factors interact with genetic traits to influence anthocyanin content and wine quality.

4. Conclusion

This study provides a comprehensive analysis of the anthocyanin content and color characteristics of fourteen grape varieties and their corresponding wines cultivated at the eastern foot of Helan Mountain in Ningxia. Our results demonstrated significant differences among cultivars, with 'Beibinghong' exhibiting the highest titratable acidity. Total phenolic content in 'Dornfelder', 'Dunkelfelder', and 'Malbec' grapes and wines was significantly higher than in other varieties. At harvest, 'Dornfelder' grapes and wines exhibited the highest total anthocyanin contents, with values of 249.94 mg/kg in grapes and 1686.76 mg/L in wines, significantly higher than other cultivars. Non-acylated anthocyanins, predominantly malvidin-3-*O*-glucoside, were the primary contributors to wine color, while acylated anthocyanin compositions varied among varieties. HCA cluster analysis grouped all varieties into different subclasses based on their monomeric anthocyanin profiles. PCA and PLS-DA analyses identified tMv-cou as the common differential metabolite across both grape and wine varieties. Correlation analysis revealed that non-acylated anthocyanins ($r = 0.68$) have a more significant impact than acylated anthocyanins ($r = 0.28$) on color chromatic diversity in grapes and wines ($P < 0.05$). These results suggest that wines made from 'Dornfelder' grapes are notable for their distinct color characteristics and high anthocyanin content. These findings enhance our understanding of how genetic differences among grape cultivars influence anthocyanin profiles and wine quality in the eastern foot of Helan Mountain in Ningxia. These findings provide valuable insights for grape growers and winemakers in selecting appropriate grape varieties to enhance wine quality through improved color attributes and phenolic composition. Future research should explore these relationships across diverse regions and environmental conditions to validate and extend our findings.

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CRediT authorship contribution statement

Huawei Chen: Writing – original draft, Methodology, Investigation. **Miaomiao Wang:** Writing – review & editing, Funding acquisition. **Lijian Zhang:** Software, Methodology. **Fuxian Ren:** Writing – review & editing, Funding acquisition. **Yutao Li:** Investigation. **Yong Chen:** Software, Investigation. **Yunqi Liu:** Writing – original draft, Software, Methodology. **Zhenwen Zhang:** Writing – review & editing, Funding acquisition. **Qingqing Zeng:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

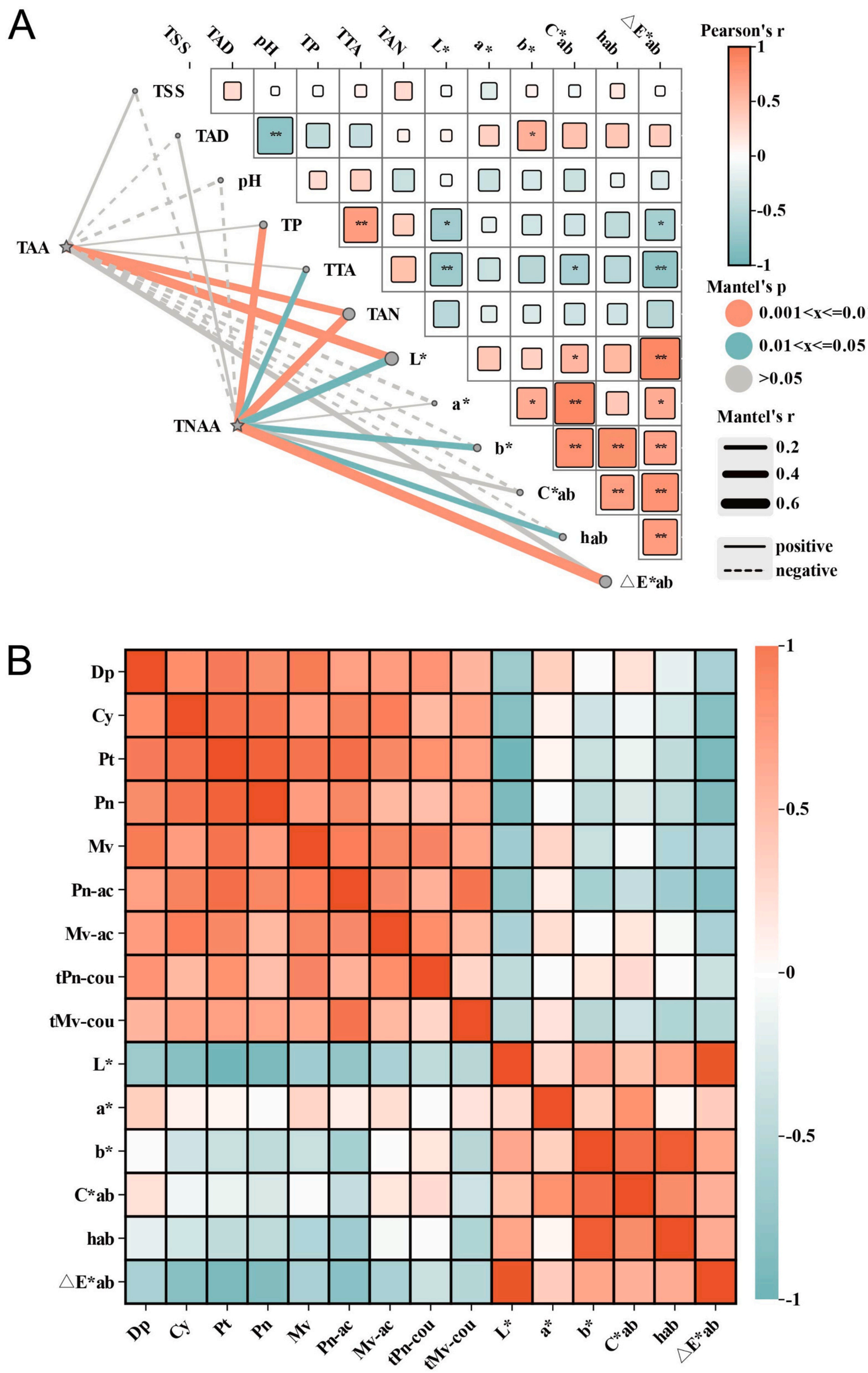

Fig. 6. Correlation analysis of physicochemical parameters, phenolic component, color properties, and monomeric anthocyanins (including total non-acylated anthocyanins (TNAA) and cylated anthocyanins (TAA) contents) in grapes and wines, as determined by the Mantel test using unweighted UniFrac distances (A). The line and square color and represent the significance differences level (*P*-values). Heatmap of the correlation analysis between color properties and monomeric anthocyanins in grapes and wines (B). * significant difference at $P < 0.05$; ** significant difference at $P < 0.01$ (Tukey's test). Dp, Deplhinidin-3-*O*-glucoside; Cy, Cyanidin-3-*O*-glucoside; Pt, Petunidin-3-*O*-glucoside; Pn, Peonidin-3-*O*-glucoside; Mv, Malvidin-3-*O*-glucoside; Pn-ac, Peonidin-3-*O*-(6-acetyl)-glucoside; Mv-ac, Malvidin-3-*O*-(6-acetyl)-glucoside; tPn-cou, Peonidin-3-*O*-(trans-6-*O*-coumaryl)-glucoside; tMv-cou, Malvidin-3-*O*-(trans-6-*O*-coumaryl) -glucoside.

the work reported in this paper.

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.2024.102034.

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