The key role of vineyard parcel in shaping flavonoid profiles and color characteristics of Cabernet Sauvignon wines combined with the influence of harvest ripeness, vintage and bottle aging

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ABSTRACT

Recently, revealing the *terroir* influence on wine chemical features has drawn increasing interest. This study aimed to explain how wine flavonoid signatures were altered by vineyard parcel, harvest ripeness, vintage and bottle aging. Six commercial Cabernet Sauvignon vineyards were selected in the Manas region to produce wines at three harvest ripeness in three seasons (2019–2021) and aged for three years. The six vineyards had little difference in mesoclimate conditions while varying greatly in soil composition. Results showed high vineyard pH (> 8.5) could accelerate grape ripening rate and increase wine flavonol concentration. Vineyards with moderate nutrition produced wines with abundant anthocyanin derivatives and maintained color characteristics during aging. The role of detailed anthocyanin derivatives in regulating wine color was clarified. As the harvest ripeness elevated, wine's flavonoid profiles were altered and gained a higher red color intensity. This work provides chemical mechanisms underlying single-vineyard wines and a theoretical basis for targeted wine production.

1. Introduction

Cabernet Sauvignon is one of the most widely planted varieties in the world because of its delightful flavors and adaptability to environmental variables (Robinson et al., 2013). According to the International Organisation of Vine and Wine (OIV) statistics, the planting area of Cabernet Sauvignon reached 341,000 ha in 2015, which was mainly grown in France, China, Chile, the United States, Australia, Spain, Argentina, Italy and South Africa. The large environmental variations in different regions lead to the variability of grape and wine flavor, which usually leaves the fingerprints of specific regions. For years, the wine industry has turned its focus on the so-called *terroir* effect, which means a confluence of factors including climate, landscape, soil, geology, viticultural and oenological technologies (de Andres-de Prado et al., 2007; Ubalde et al., 2010). All these *terroir* parameters could be reflected in the wine, which was described as a collection of complex chemical features (Roullier-Gall et al., 2014). These chemical features involved volatile and non-volatile compounds and contributed to the wine's sensory quality. Thanks to the development of analytical technologies, the

chemical mechanisms underlying the wine's color and taste have been gradually understood.

The secondary metabolites in grapes, especially flavonoid compounds, play crucial roles in affecting wine's sensory aspects. Anthocyanins are the main compounds that contribute to the color of dry red wines. During aging, the grape-derived anthocyanins in wines could be declined by 75% within two years while the color of wines did not fade (Waterhouse, 2002; Zhang et al., 2020). Recently, the chemical bases under wine color gradually revealed and more and more anthocyanin-derived pigments were identified (Dipalmo et al., 2016; He et al., 2012; Zhang et al., 2021). In general, most studies regarding the behavior of anthocyanin derivatives focused on the wine aging stage and ignored the viticulture stage. However, the phenolic compounds for forming anthocyanin derivatives were accumulated in grapes and these compounds are susceptible to environmental variables and water stress, such as climate changes, soil types and deficit irrigation (de Andres-de Prado et al., 2007; Lu et al., 2022; Torres et al., 2022). Besides, the proper choice of harvest date is also essential to maintain the sustainability of the final wine products (Bindon et al., 2014; Bindon et al.,

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2013; Gao et al., 2019).

The concept of single-vineyard wine was deemed to reflect a particular terroir that was increasingly being promoted as offering the consumer a distinctive wine experience (White, 2015). In fact, single-vineyard wines were not rare in the wine industry since the term 'Single Vineyard' often appeared on a wine bottle label to represent the wines made in a certain vineyard were superior to other vineyards. Within a specific region, the soil usually plays a dominant role in affecting vine development and wine quality (Ubalde et al., 2010). Soil texture directly affects water retention and irrigation strategies, even the choice of varieties. The optimum soil pH is also essential for grapevines since it affects the solubility of metal ions and the availability of nutritional cations and anions (Oliver et al., 2013). The soil organic matter and nutrient elements provide essential nutrients to the grapevine, directly influencing the balance between vegetative vigor and grape production. Ubalde et al. (2010) suggested that correlations between soil minerals and wine quality could not be established until serious deficiencies affecting vineyard growing occurred. However, excessive nutrient amounts can be deleterious to grape composition, increasing vine vigor and yield, stimulating rot development, and reducing wine quality (de Andres-de Prado et al., 2007).

Recently, choosing the proper grape harvest date seemed to be more challenging for winemakers as the profound impact brought by climate change. Besides the well-known 'anthocyanin-sugars decoupling', the decoupling phenomenon was also found in other relevant metabolites, such as organic acids and proanthocyanidins (Gutiérrez-Gamboa et al., 2021). Multiple biochemical processes occur at various speeds throughout grape ripening and grape-derived chemicals that favorably or unfavorably affect wine chemistry and sensory qualities may increase, decrease, or maintain constant at a specific stage of grape development (Bindon et al., 2013). When making decisions for commercial winemaking, winemakers need to consider multiple factors, not only the traditional sugar maturity but also technical maturity and phenolic maturity. Usually, decisions concerning the harvest timing aim to maximize the wine's positive attributes, minimize negative attributes, and optimize resources during the season (Bindon et al., 2014). The changes in metabolites at different harvested ripeness levels should be evaluated in a certain region, which seemed to vary in different climate conditions.

The north foot of Tianshan mountain was a major wine-producing region in Xinjiang, which occupied 45.6% of the total viticultural area of Xinjiang. The soils in this region are formed through the alluvial soil of the Manas River, which lack homogeneity and are accompanied by salinization. The saline soils were commonly distributed in northwest China, Australia and Europe, which had extensive viticultural areas. Even in adjacent vineyards with the same variety, their corresponding wines can show varied styles according to the winemaker's experience. Besides, the semi-arid climate here is characterized by abundant sunlight resources and high temperatures, which leads to a rapid ripening rate after grape veraison (Lu et al., 2022). So, the decoupling of sugar and phenolic compounds is evident. It is hypothesized that soil changes are the leading cause of differences in wine chemicals on a mesoclimate scale, and some vineyards can maintain their wine flavonoid signatures among vintages, harvest ripeness and aging. In the present study, we selected six vineyards in the Manas region and produced their corresponding single-vineyard wines at three harvest ripeness in three consecutive seasons (2019–2021). Besides, the wines made in 2019 were aged from one to three years to investigate whether the wine's flavonoid characteristics brought by soils could be maintained. Our study not only provides the chemical mechanisms underlying the concept of single-vineyard wines but also provides a theoretical basis for targeted wine production and vineyard soil management.

2. Materials and methods

2.1. Chemicals

The analytical grade chemicals including hydrochloric acid, sulfuric acid, potassium metabisulphite, sodium hydroxide, phenolphthalein and copper sulfate, were used to determine the chemical parameters of must and wine and purchased from Tianjin Chemical Factory (Tianjin, China). For the determination of phenolic compounds, ethanol, methanol, formic acid and acetonitrile were HPLC grade and were purchased from Honeywell (Marris, NJ, USA). Phenolic standards of malvidin-3-*O*-glucoside, quercetin-3-*O*-glucoside, (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-*O*-gallate, and (−)-epigallocatechin, gallic acid and caffeic acid with a purity of ≥ 98% were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Field experiment layout

The three years study (2019–2021) was performed in six commercial vineyards selected from the Manas region, the north foot of Tianshan mountain, Xinjiang. The basic information about each vineyard is presented in Supplementary Table 1. Among all six vineyards, one was located at the Yuanyi farm (named Y1) and others belonged to the Zijian farm (named Z2-Z6). The distance between the vineyards ranged from 200 m to 9 km (Supplementary Fig. 1). These vineyards were selected not only due to their widespread geographical locations but also their possible unique wine styles based on the winemaker's experience. These vineyards consisted of three row orientations: northeast-southwest (Y1, Z2, Z3, Z5), northwest-southeast (Z4), and east–west (Z6). All the vineyards were managed according to local winery rules. Briefly, own-rooted *Vitis vinifera* cv. Cabernet Sauvignon vines were trained to the uniformly modified vertical shooting positioning system with 17–23 shoots and 24–32 clusters per meter row. The cluster zone was distributed within 0.5–0.8 m above the ground. The vines had similar ages, within 8–10 years. The limited-yield strategy was applied to all the vineyards with 9000–12,000 kg/ha. Vines were drip irrigated twice a month from May to August with 750 m³·ha⁻¹ each time. In each vineyard, nine adjacent rows with uniform vines were selected for the subsequent cluster sampling. We tried to cover the whole vineyard to represent the vineyard characteristics. The randomized block design was applied in these rows with nine blocks randomly distributed. All the blocks consisted of three replicates, and three blocks were regarded as one replicate.

2.3. Mesoclimate determination

Three weather stations (H21-002, Onset, Bourne, MA, USA) were set up individually to determine the mesoclimate of Yuanyi farm (Y1), the north part of Zijian farm (Z2) and the south part of Zijian farm (Z6). The weather stations were installed within the vineyard, which was 0.5 m above the canopy top, with a temperature (S-THB-M002, Onset) and solar radiation (S-LIB-M003, Onset) sensor. The data were recorded at the 5 min interval. Besides, the rainfall and sunshine duration of the Manas region were obtained from China Meteorological Data Service Centre (https://cdc.cma.gov.cn/).

2.4. Soil analysis

In 2020, the soil samples were collected in all six vineyards before anthesis (flowering). For each vineyard, nine sample points were evenly distributed among nine rows. The sample position was in the middle of two adjacent rows which was 1.5 m away from the trunk. The sample depth was 0–30, 30–60 and 60–90 cm below the ground. Each vineyard consisted of three replicates and each replicate was a mixture of three random sampling points. Soil physical and chemical parameters were measured according to previous studies (Cheng et al., 2014; Qi et al.,

2019), including texture, pH, particle size, cation exchange capacity (CEC), electrical conductivity (EC), and organic matter content.

Soil nutrient elements, including total elements (P, K), available or effective elements (P, K, Ca, Mg, Fe, Mn, Cu, Zn), were determined using inductively coupled plasma-optical emission spectroscopy (Agilent 5110 ICP-OES) after extraction and digestion. The plasma gas flow was 12.0 L/min and the auxiliary airflow was 1.00 L/min. The observation mode was radial and the observation height was 8 mm. The determination wavelength of individual elements was as follows: Ca, 422.67 nm; Cu, 327.39 nm; Fe, 238.20 nm; K, 766.49 nm; Mg, 285.21 nm; Mn, 257.61 nm; P, 213.61 nm; Zn, 213.85 nm. The total N was determined using an elemental analyzer (Element Vario MAX cube). The carrier gas of the gas mass spectrometer is high-purity helium with 0.38 MPa pressure. The primary combustion tube temperature was 1140 °C and the secondary combustion tube temperature was 800 °C. The oxygen flow was 150 mL/min and the oxygenation time was 60 s. The available N was determined using the alkali diffusion method (Qi et al., 2019).

2.5. Maturity gradient harvest, small-scale vinification and bottle aging

The harvest period usually occurs in early September and lasts nearly one month in the Manas region. In 2019, the grapes were harvested in early September when the total soluble solids reached about 24 °Brix. In 2020 and 2021, the gradient harvest was performed in the experimental vineyards based on grape maturity. The first, second and last harvest was around the beginning, middle, and end of September, with the grape TSS ranging from 23°Brix to 28°Brix from R1 to R3. The clusters were manually harvested and transported to the workshop. The vinification method was according to a previous study (Lu et al., 2022). After the malolactic fermentation was finished, potassium metabisulfite was added to the wines to maintain the free SO$_2$ at around 40 mg/L. Then the wines were filled into 750 mL brown bottles and stored in the cellar at 10–15 °C. In all three seasons, the new wines were stored for one month before subsequent composition analysis. Besides, the wines made in 2019 were bottle aged for one year and three years to determine the flavonoid composition in wines.

2.6. Determination of basic chemical parameters of must and wine

The total soluble solids of the must were determined using a digital hand-held refractometer (PAL-1, Atago, Tokyo, Japan). The pH of the must was determined using the pH meter (Sartorius PB-10, Germany). The titratable acidity of the must was measured by titrating 0.05 mol/L NaOH to a pH 8.2 endpoint and was represented as tartaric acid (g/L).

Wine's alcohol content, residual sugar, pH, total acidity and volatile acidity were determined following the OIV standard (OIV, 2014).

2.7. Color determination and HPLC-MS analysis of flavonoid composition

The color characteristics of the wines were measured using a UV spectrophotometer (Shimadzu, Tokyo, Japan), as described by Gao et al. (2019). The following parameters were calculated based on the spectrophotometric results: lightness (*L**), red/green color coordinate (*a**), yellow/blue color coordinate (*b**), saturation (*C**$_{ab}$) and hue angle (*H**).

The phenolic compounds in wines, including monomeric anthocyanins, flavonols, flavanols and phenolic acids, were determined using an Agilent 1200 series HPLC system equipped with a 6410 triple quadrupole mass spectrometer (HPLC-QqQ-MS/MS). The detailed instrument parameters and procedures were described in a previous study (Li et al., 2016). The anthocyanin derivatives were determined according to a previous method (Zhang et al., 2020). The malvidin-3-*O*-glucoside was used as the quantitative standard for anthocyanin and their derivatives. The quercetin-3-*O*-glucoside was the quantitative standard for flavonols. The (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-*O*-gallate and (−)-epigallocatechin were the quantitative standard for flavanols. Gallic acid and caffeic acid were the quantitative standards for phenolic acids.

2.8. Statistical analysis

Statistical calculations were performed using Microsoft Excel 2019 software. The SPSS version 22.0 was used for all significance analysis at *p* < 0.05 (Duncan's multiple range test). The figures were drawn using GraphPad Prism 8.0.2 and SIMCA 14.1.

3. Results and discussion

3.1. Weather data and soil composition

Regarding the vineyard environmental conditions, two aspects were the main focus of the present study: the climate above ground and the soil composition below the ground. As shown in Supplementary Fig. 1, all the vineyards had similar climate conditions with little difference in GDD (growing degree days) and solar radiation during the whole growing season. Besides, the changes in daily temperature and solar radiation were also similar among all vineyards, indicating that the mesoclimate variability was little within the 15 km scale in this region. However, the soil composition varied significantly among the six vineyards, with all detected parameters reaching significant levels (Duncan's multiple range test at *p* < 0.05, Supplementary Table 1). According to the soil texture classes of the United States Department of Agriculture, vineyards Y1, Z2, Z3 and Z5 belonged to silty clay loam, while Z4 and Z6 belonged to silty loam. The soils in Z4 and Z6 had a lower clay content and higher sand content. In contrast, the soils in Z2 and Z5 had a lower sand content and higher clay content. The soil salinity, as measured by the electrical conductivity (EC), has been suggested to have an optimum range of lower than 180 mS/m (Oliver et al., 2013). Only Z4 (197.89 mS/m) exceeded the optimum range among all vineyards. The soil pH ranged from 7.69 (Y1) to 8.70 (Z5) in all vineyards, which belonged to alkaline soils. The cation exchange capacity (CEC) was significantly higher in Y1 than in other vineyards. Similarly, Y1 also had a higher organic matter content and higher concentrations of most elements except for effective Ca and Mg, indicating that the vineyard Y1 was more fertile than other vineyards. For the rest of the five vineyards, Z3 was the most fertile with higher concentrations of organic matter, total N, P, K, available N, effective P, K, Mg, Fe, Zn than other vineyards. Z6 was lower in organic matter, total N, K, available N, effective K, Fe, Mn, and Cu while having a higher effective Ca concentration than other vineyards. Z4 had lower effective P and Zn than other vineyards.

3.2. General oenological parameters of must and wines

The grape ripening rate varied greatly in selected vineyards, which led to variations in general oenological parameters of must and wines. As shown in Fig. 1, the harvest period in 2019 could last around three weeks when harvesting the grapes at a similar ripeness level. In 2020 and 2021, the first harvest period (R1) could also last at least one week. Notably, grapes of Z5 were always harvested earliest in three vintages which indicated that grapes in Z5 had the fastest maturity rate. Although the distance between Z4 and Z5 was only around 300 m, the R1 harvest date of Z5 was advanced 4–14 d compared to Z4 in three vintages. The phenomenon was rare in previous studies, especially at such a close distance. For example, de Andres-de Prado et al. (2007) found that the grapes from two vineyards 500 m apart showed no difference in harvest date or TSS level. Ubalde et al. (2010) found that the variations in soil type from two vineyards 600 m apart significantly affected grape ripening while the effect seemed more vintage-dependent. In the present study, Z5 maintained a higher ripening rate than other vineyards and was irrelevant to vintage. As shown in Fig. 2, even harvesting 14 d earlier than Z4, Z5 showed no higher grape TSS in 2019. In 2020 and 2021, the grapes in Z5 were still harvested earlier than other vineyards and had a high TSS level. It was speculated that the higher soil pH in Z5 accelerated the ripening of grapes during growth. The pH of Z5 grapes exceeded the optimum pH range (5.5–8.0) suggested by a previous study

Fig. 1. Daily temperature changes and harvest dates in the ripening period of six experimental vineyards in the 2019–2021 growing seasons.

Fig. 2. Oenological parameters of the must (a) and the wines (b) of Cabernet Sauvignon obtained from six experimental vineyards and three ripeness levels in the 2019–2021 growing seasons. TSS, total soluble solids; TiA, titratable acidity; TA, total acidity. Different letters within a plot indicate significant differences among vineyards (Duncan's multiple range test at $p < 0.05$).

(Oliver et al., 2013), leading to a lower vine vigor with an open canopy. Exposed clusters were more likely to ripen, especially in the Manas region with a semi-arid climate, and abundant light and temperature resources. Besides, higher vineyard soil pH could reduce the assimilation of plant micronutrients and lead to a lower crop level and smaller berries. Then the sugar content was concentrated with a higher accumulation rate. Notably, Y1 and Z3 grapes had a lower ripening rate and TSS content than other vineyards, especially in 2020 and 2021. As mentioned above, the Y1 and Z3 were the two most fertile vineyards with higher organic matter and nitrogen contents. Previous studies showed that higher organic matter and nitrogen contents could lead to vigorous nutritional growth and shaded canopy conditions, both of which were detrimental to the berry ripening process (Iland et al., 2011). Interestingly, the latter ripening rate in Y1 and Z3 was not associated with higher titratable acidity (TiA). Z6 grapes had the highest TiA in all vineyards from 2019 to 2021, followed by Z4. Supplementary Table 1 showed that Z4 and Z6 had the lowest soil K content, which

might be the cause of higher TiA. As Mpelasoka et al. (2003) described, the high K content in vineyards could easily cause excess K levels in grape berries which decreased free tartaric acid. However, the higher juice TiA in Z6 was not always associated with lower pH in grape juice. As shown in Supplementary Table 2, Z6 had the lowest juice pH in 2019 and 2020, while Y1 had the lowest juice pH in 2021. Compared to different harvest stages, the grape juice TSS content and pH increased as the harvest was delayed, which was shown in all vineyards. However, juice TiA showed different trends among vintages, with a decreasing trend from R1 to R3 in 2020 and a decreasing trend from R1 to R2 while increasing from R2 to R3 in 2021. During the last ripening period, two possible reasons might lead to an increased TiA in 2021: i) The lower temperature in the R3 period could inhibit the breakdown of malic acid (Sadras et al., 2013; Sweetman et al., 2009); ii) The overripe berries risked in dehydration with a smaller volume which caused the concentrated acids.

Regarding the wine's general parameters, wine alcohol degree

showed an increasing trend from R1 to R3. Similar to juice TSS levels, Z3 wines had a lower alcohol degree than other vineyards, especially in 2021. Nowadays, lower alcohol degrees seem more in line with consumer preferences due to enhanced awareness of alcohol-related health problems (Herrera et al., 2015; Palliotti et al., 2014). The higher TiA in Z4 and Z6 juice did not reflect in the resultant wine total acidity (TA), which indicated the complex changes in tartaric and malic acids during fermentation. As for the wine pH, no consistent result was found regarding the vineyard effect. Similar to must pH, the wine pH increased from R1 to R3 when the harvest was delayed.

3.3. Phenolic profiles of fresh wines

Table 1 shows the total phenolic concentration of each type obtained from different single-vineyard wines combined with different ripeness levels. The detailed phenolic compounds identified by HPLC-QqQ-MS/MS were shown in Supplementary Table 3, including fifteen monomeric anthocyanins, four kinds of anthocyanin derivatives, nine flavonols, six flavanols and nine phenolic acids. In terms of the vineyard effect, Z5 wines had the highest concentration of monomeric anthocyanin among all vineyards in three vintages except for R1 in 2020. As mentioned above, the Z5 soils had a high pH which might lead to the augmented exposure of clusters and smaller crop size. In general, light is a fundamental requirement for color formation in grapes and moderate cluster exposure could promote the synthesis of anthocyanins (Li et al., 2023; Matus et al., 2009). Besides, the smaller crop size could also cause the enrichment of anthocyanins in berries. The ripeness effect on wines monomeric anthocyanin reached a significant level in Z2, Z3 and Z5, and the results consistently showed that wines harvested in higher ripeness stages had lower monomeric anthocyanin concentrations in these vineyards. Interestingly, the ripeness effect on anthocyanin derivatives also reached a significant level in the same three vineyards while showing an opposite trend to monomeric anthocyanins. During grape ripening, the anthocyanin changes seemed varied in previous reports. Bindon et al. (2013) found that grape anthocyanin concentrations increased as ripening progressed (22.5 °Brix-26 °Brix) and the same result was found in monomeric anthocyanins in the corresponding wines. However, Iland et al. (2011) proposed that the decline of anthocyanin content towards the latter stages of berry ripening could occur at any time when the grape TSS level reached above 22.5 °Brix due to the breakdown of anthocyanins by glucosidase and peroxidase activity, which was in agreement with our findings. Regarding the vineyard effect, Z3 wines had a higher concentration of total anthocyanin derivatives than other vineyards in 2019 and R3 in 2020 and 2021.

Flavonols were believed to be synthesized through a light-dependent process and play a fundamental role during winemaking (Malacarne et al., 2015). As expected, Z5 wines had the highest flavonol concentration among all vineyards due to the exposure of clusters caused by high soil pH, which had been discussed above. Compared to the three harvest periods, the total flavonol concentration continuously reduced from R1 and R3, especially in 2021, when this phenomenon showed in all vineyards. The same result was also found by Martínez-Lüscher et al. (2019) that the total flavonol content declined when the grapes' TSS exceeded 22 °Brix. It was considered that the flavonoid losses were associated with fruit senescence and were exacerbated by high temperature or severe water stress in the ripening period (Brillante et al., 2017; Martínez-Lüscher et al., 2019). In the present study, the losses were observed not only in monomeric anthocyanins and flavonols but also in flavanols and phenolic acids (Table 1). Although the flavanol concentration varied significantly in all vineyards, there were no consistent results among vintages. Z5 wines had a lower flavanol concentration in 2019 while Y1 wines had a lower flavanol concentration in 2020 and 2021 compared to other vineyard wines.

3.4. Evaluating the role of vintage, vineyard parcel and harvested ripeness on wine phenolic composition through multivariate analysis

To figure out how the detailed wine phenolic composition was affected by multiple factors, the principal component analysis was used to classify the different wine samples based on detailed compounds, as shown in Fig. 3a. The first three principal components were extracted and explained 64.2% of the total variance, which could separate the wines aged at different vintages. In general, the vintage effect on wine metabolic profiles might be profound due to the varying climate conditions in different vintages. From the loading plot, it could be seen that mono anthocyanins were more abundant in 2019 and 2021. In 2020, the grapevines had an advanced phenological stage which could be two weeks earlier than the normal vintage (Lu et al., 2022). So, 2020 could be regarded as a typical vintage characterized by the effect of climate change, which led to the reduced accumulation of anthocyanins.

Furthermore, the two-way orthogonal partial least square with discriminant analysis was used to separate wine samples from different vineyards, as shown in Fig. 3b. The six vineyards could be grouped into four parts in the 3D score plot based on the phenolic composition: Y1, Z2 & Z3, Z4 & Z6, Z5. Interestingly, their positions in the score plot were highly correlated with their geographical locations, indicating that wine phenolic profiles could be fingerprints to reflect a certain *terroir* even on a mesoclimate scale. The Z5 wines were characterized by more abundant flavanols than other vineyards, reflecting the link between soil characteristics (pH), exposed climate condition and the final wine composition. Among all four types of poly anthocyanins (anthocyanin derivatives), three of them had higher concentrations in Z3 wines. However, for mono anthocyanins, their concentrations were not abundant in Z3 wines. As discussed in a previous study (Lu et al., 2022), the anthocyanin derivatives usually showed opposite trends to monomeric anthocyanins, leading to unpredictable wine colors through their concentrations. Except for Z3 and Z5 vineyards, the rest vineyard wines could be also clearly separated in the two-way orthogonal partial least square with discriminant analysis, indicating that the wine phenolic profiles were easily altered by vineyard parcels.

Regarding the effect of harvested ripeness levels, the two-way orthogonal partial least square with discriminant analysis could also separate different wine samples from R1 and R3 while R2 wines located at their transitioning position in the score plot (Fig. 3c). The R1 wines were characterized by more abundant phenolic compounds, especially mono anthocyanins, flavonols, and flavan-3-ols. The degradation of flavonoids in the latter period of grape ripening was observed commonly in previous reports (Holt et al., 2010; Iland et al., 2011). The decline in anthocyanin content in berries can occur at any time after the juice sugar level has reached 22.5 °Brix during the ripening process (Iland et al., 2011). However, the poly anthocyanins (anthocyanin derivatives) were more abundant in R2 and R3 wines.

3.5. Color characteristics of fresh wines and their correlations with phenolic composition

Using CIELAB parameters (Gao et al., 2019; Zhang et al., 2021), the wines' color characteristics after bottling for one month were presented in Fig. 4a. Compared to six vineyards, Z3 wines were characterized by high red coordinate (a^*) and color saturation (C^*_{ab}), which was shown in all three vintages (Supplementary Table 5). While for other vineyard wines, their color intensities only showed high values in certain vintages, such as Z5 wines in 2020, Y1 wines in 2021 and Z2 wines in 2020 and 2021. Z4 and Z6 wines were characterized by low color intensities and high yellow coordinates, regardless of vintage or ripeness level. So the color performance of wines showed sustainability and plasticity in terms of vineyard effect as some of the vineyards could maintain their wine color character while the rest varied with the vintages. Compared to different ripeness levels, the wines color intensity increased from R1 and R3.

Table 1

Phenolic compounds of Cabernet Sauvignon wines obtained from six experimental vineyards and three ripeness levels in the 2019–2021 growing seasons (mg/L).

Parameters	Vintage	Vineyard						Vineyard sig.
		Y1	Z2	Z3	Z4	Z5	Z6	
Monomeric anthocyanin	2019	371.76 ± 17.72a	360.06 ± 46.92a	332.44 ± 58.08ab	320.83 ± 28.41ab	383.05 ± 37.09a	271.18 ± 28.70b	*
	2020 (R1)	316.60 ± 9.71b	430.51 ± 30.70a	346.89 ± 26.61b	246.22 ± 6.22c	348.77 ± 14.05b	231.99 ± 31.68c	*
	2020 (R2)	301.07 ± 12.29b	218.13 ± 21.87c	390.04 ± 19.75a	224.85 ± 32.14c	363.60 ± 23.43a	274.73 ± 15.08b	
	2020 (R3)	200.65 ± 14.53b	325.05 ± 16.13a	228.81 ± 34.34b	281.63 ± 12.37a	281.28 ± 10.77a	228.12 ± 47.82b	
	2021 (R1)	165.47 ± 18.28d	394.31 ± 17.45a	282.56 ± 18.66c	361.64 ± 20.95b	411.22 ± 13.96a	352.32 ± 3.84b	*
	2021 (R2)	106.95 ± 5.11c	153.25 ± 27.25c	236.57 ± 101.56b	305.15 ± 6.99ab	313.89 ± 22.79ab	324.84 ± 10.92a	
	2021 (R3)	318.75 ± 20.60ab	133.68 ± 71.63c	163.64 ± 23.82c	282.26 ± 34.36ab	351.88 ± 46.40a	250.85 ± 54.48b	
	Vintage sig.	*	ns	*	*	ns	*	
	Ripeness sig.	ns	*	*	ns	*	ns	
Anthocyanin derivatives	2019	61.26 ± 6.52ab	50.53 ± 4.44bc	74.16 ± 13.85a	42.03 ± 5.74c	37.27 ± 9.91c	62.45 ± 12.47ab	*
	2020 (R1)	25.33 ± 2.09b	34.62 ± 1.53a	28.92 ± 0.89ab	29.18 ± 1.27ab	28.54 ± 0.74ab	30.56 ± 4.60ab	*
	2020 (R2)	31.12 ± 0.24c	54.86 ± 5.33a	37.56 ± 1.88bc	43.84 ± 5.16b	36.90 ± 1.14bc	33.42 ± 7.14c	
	2020 (R3)	56.94 ± 1.90b	45.67 ± 4.52bc	61.31 ± 6.17a	28.65 ± 0.02c	43.02 ± 0.23bc	31.87 ± 2.86c	
	2021 (R1)	71.24 ± 5.83a	17.55 ± 0.63c	31.47 ± 11.58b	16.01 ± 1.13c	16.63 ± 0.23c	11.52 ± 1.18c	*
	2021 (R2)	69.65 ± 4.40a	68.45 ± 4.74a	33.54 ± 22.49b	12.19 ± 1.27c	24.04 ± 7.70c	14.10 ± 2.65c	
	2021 (R3)	67.71 ± 13.34b	71.40 ± 1.90ab	80.52 ± 2.88a	19.60 ± 1.09c	21.49 ± 1.72c	16.21 ± 0.41c	
	Vintage sig.	*	ns	*	*	*	*	
	Ripeness sig.	ns	*	*	ns	*	ns	
Flavonols	2019	28.98 ± 2.81c	17.71 ± 1.54d	49.71 ± 1.62a	37.74 ± 3.34b	46.91 ± 1.87a	27.38 ± 2.44c	*
	2020 (R1)	30.27 ± 2.21de	33.47 ± 0.69 cd	43.99 ± 0.29b	34.58 ± 4.29c	72.81 ± 1.37a	26.74 ± 0.64e	*
	2020 (R2)	34.03 ± 3.21bc	29.41 ± 4.93 cd	36.29 ± 1.29c	28.40 ± 3.96 cd	62.49 ± 5.23a	23.80 ± 0.49d	
	2020 (R3)	24.05 ± 0.90d	25.31 ± 0.75d	38.09 ± 2.86b	29.64 ± 1.18c	46.80 ± 2.02a	26.40 ± 0.76d	
	2021 (R1)	26.09 ± 1.80e	34.70 ± 0.60d	30.02 ± 2.71e	45.01 ± 0.59b	50.42 ± 2.30a	37.78 ± 13.68c	
	2021 (R2)	18.01 ± 1.39d	29.54 ± 0.29b	21.09 ± 0.81c	28.06 ± 1.67b	44.06 ± 3.63a	28.05 ± 1.76b	*
	2021 (R3)	16.95 ± 1.74d	24.31 ± 0.58b	12.94 ± 0.20e	19.57 ± 1.08c	33.22 ± 2.01a	16.65 ± 1.07d	
	Vintage sig.	ns	*	*	*	*	ns	
	Ripeness sig.	*	*	*	*	*	*	
Flavanols	2019	134.17 ± 10.82abc	146.34 ± 6.14a	111.52 ± 17.64bc	139.54 ± 8.59ab	54.76 ± 7.12d	105.29 ± 31.65c	*
	2020 (R1)	129.92 ± 5.21c	165.95 ± 0.23b	184.89 ± 1.25a	162.01 ± 5.29b	161.60 ± 0.80b	166.42 ± 6.31b	
	2020 (R2)	121.10 ± 4.30 cd	130.70 ± 11.20bc	152.36 ± 5.66a	137.28 ± 5.58b	116.54 ± 2.90d	126.24 ± 6.60bcd	*
	2020 (R3)	75.04 ± 4.16e	104.95 ± 6.64c	94.41 ± 8.46d	109.37 ± 2.29bc	116.91 ± 2.08b	130.76 ± 5.44a	
	2021 (R1)	80.82 ± 5.44d	157.35 ± 4.75ab	113.15 ± 24.21c	161.31 ± 10.33ab	174.40 ± 7.16a	147.80 ± 4.79b	
	2021 (R2)	52.45 ± 5.69c	80.08 ± 8.73b	133.35 ± 25.94a	134.63 ± 7.69a	129.40 ± 7.07a	137.16 ± 6.26a	*
	2021 (R3)	87.06 ± 4.00 cd	46.04 ± 24.57e	64.85 ± 12.98de	115.90 ± 12.49ab	135.23 ± 1.29a	99.96 ± 5.29bc	
	Vintage sig.	*	*	ns	ns	*	*	
	Ripeness sig.	ns	*	*	*	*	*	
Phenolic acids	2019	23.32 ± 3.32a	26.89 ± 1.20a	15.88 ± 4.98b	22.38 ± 3.34a	16.95 ± 1.86b	13.10 ± 1.48b	*
	2020 (R1)	17.11 ± 0.52d	21.20 ± 0.99b	23.95 ± 0.32a	22.47 ± 1.50ab	18.85 ± 0.78c	16.47 ± 0.27d	
	2020 (R2)	15.97 ± 0.77c	19.47 ± 0.67a	18.69 ± 2.52ab	18.95 ± 0.23ab	16.96 ± 0.56bc	15.21 ± 0.41c	*
	2020 (R3)	12.53 ± 0.54d	17.72 ± 0.97a	15.66 ± 0.68b	14.21 ± 0.52c	17.86 ± 0.61a	16.96 ± 0.24a	
	2021 (R1)	17.95 ± 0.48c	18.64 ± 0.33bc	17.67 ± 0.81c	20.34 ± 0.55a	18.99 ± 0.36b	14.94 ± 0.48d	
	2021 (R2)	14.93 ± 0.40de	15.31 ± 0.57c	17.38 ± 0.14a	15.68 ± 0.62bc	16.15 ± 0.45b	14.39 ± 0.20e	*
	2021 (R3)	13.27 ± 0.23c	13.51 ± 0.11c	16.27 ± 0.38a	14.61 ± 0.33b	16.35 ± 0.18a	12.60 ± 0.53d	
	Vintage sig.	*	*	ns	*	ns	*	
	Ripeness sig.	*	*	ns	*	*	ns	

Values represent means ± SD (n = 3) separated by Duncan's multiple range test. Values in the same row followed by a different letter are significantly different (*p* < 0.05).
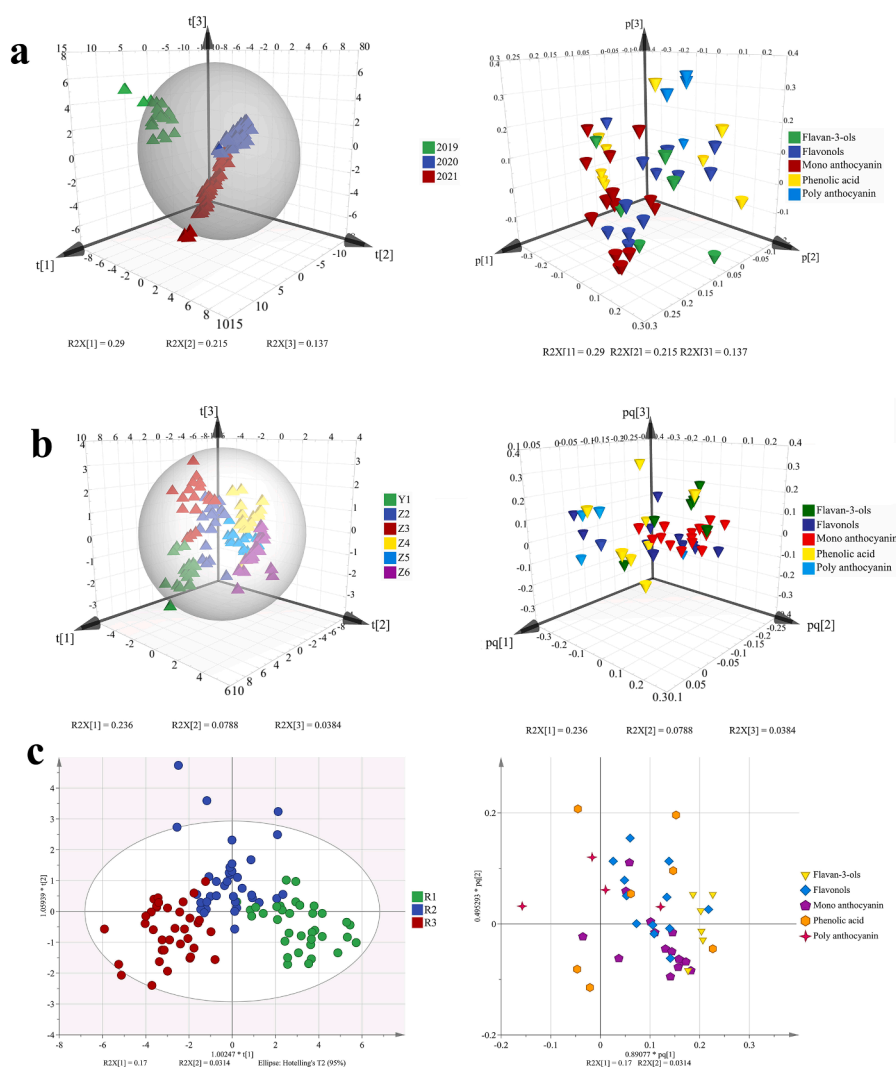
Fig. 3. Multivariate analysis based on wine phenolic compounds to evaluate the influence of vintage (a, 3D principal component analysis), vineyard parcel (b, 3D two-way orthogonal partial least square with discriminant analysis) and harvested ripeness (c, 2D two-way orthogonal partial least square with discriminant analysis). The left parts of figures a, b and c were score plots and the right parts were loading plots.

To figure out how the phenolic compounds affect the wines' color properties, the Pearson correlation analysis was performed between all the phenolic compounds and CIELAB parameters of wines, as shown in Fig. 4b. It showed that all four types of anthocyanin derivatives and their total concentrations were significantly and highly correlated with wines lightness (L^*), red color coordinate (a^*) and saturation (C^*_{ab}). As shown in Fig. 4c, Y1, Z2 and Z3 wines had significantly higher anthocyanin derivatives than other vineyards. Besides, the R2 and R3 wines had significantly higher anthocyanin derivatives than R1. In general, anthocyanin derivatives were formed since fermentation and played essential roles in maintaining color characteristics during wine aging. In this case, most studies regarding the behavior of anthocyanin derivatives focused on the wine aging stage, and few focused on the early stages of vinification, let alone in viticulture (Zhang et al., 2021). The significant discrepancy in wines' anthocyanin derivatives seemed to originate from the viticulture stage instead of vinification in the present study since certain vineyard characteristics could maintain across vintages, which therefore determined the wines' color characteristics. The Y1 and Z3 vineyard had abundant nutrient elements, which provided an efficient soil base for the accumulation of phenolic compounds in grapes (Iland et al., 2011). Besides, for anthocyanin derivatives, their formation was easily affected by environmental factors, and lower pH in wines was

beneficial for the formation of vitisins and pinotins (Zhang et al., 2022). So the vineyards with lower soil pH and higher nutrient elements could accumulate more abundant anthocyanin derivatives in wines due to the positive correlations between soil pH and wines pH. As for the ripeness effect, the same result was also found in previous studies that delayed harvest increased wine color and anthocyanin pigments (Bindon et al., 2013; Pérez-Magariño & González-San José, 2006). Besides the anthocyanin derivatives, eight flavonols and their total concentration also showed significantly high correlations with wines' color characteristics (Fig. 4b). Flavonols appeared to be the best copigmentation cofactors with anthocyanins, and the concentration of quercetin 3-*O*-glucoside was found to correlate with the strength of copigmentation (Rustioni et al., 2012). In the present study, all the quercetin and kaempferol-based flavonols were highly and positively correlated with wines' a^* and C^*_{ab}.

3.6. Changes in wine phenolic profiles during aging and the role of anthocyanins derivatives in maintaining color characteristics

In fresh wines, the phenolic composition and color parameters showed vineyard-related characteristics. However, commercial wines usually experience aging in oaks or bottles. So, it was essential to
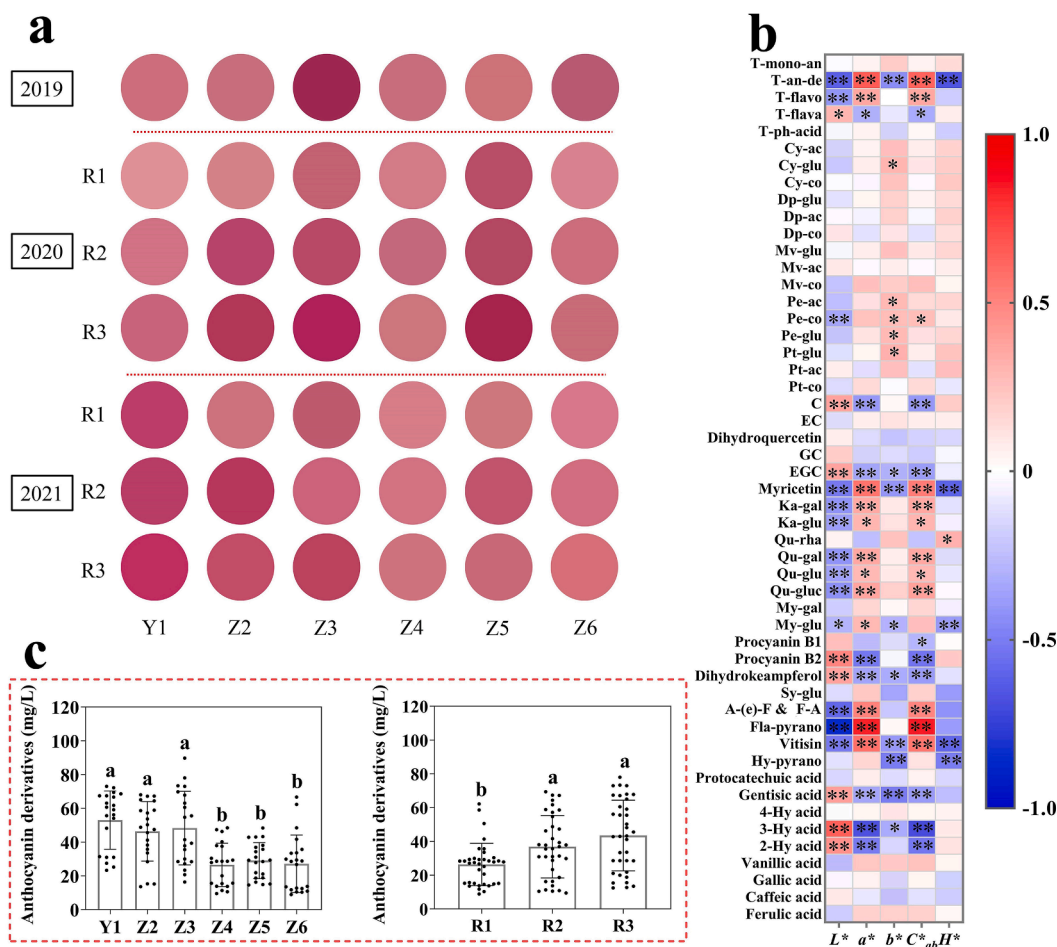
Fig. 4. Color characteristics (a) and their correlations with phenolic compounds (b) and concentrations of anthocyanin derivatives (c) in Cabernet Sauvignon wines obtained from six experimental vineyards and three ripeness levels in the 2019–2021 growing seasons. T-mono-an, total concentration of monomeric anthocyanins; T-an-de, total concentration of anthocyanin derivatives; T-flavo, total concentration of flavonols; T-flava, total concentration of flavanols; T-ph-acid, total concentration of phenolic acids. The abbreviations of detailed phenolic compounds in (b) are shown in Supplementary Table 3. *, the correlation reaches a significant level at $p < 0.05$ (Pearson correlation); **, the correlation reaches a significant level at $p < 0.01$ (Pearson correlation). Different letters in (c) indicate significant differences among vineyards or ripeness levels (Duncan's multiple range test at $p < 0.05$).

investigate whether the vineyard-related characteristic could be maintained during wine aging. The phenolic profiles of wines after 12 months and 36 months of bottle aging were shown in Fig. 5. The wines' mono anthocyanins decreased rapidly when aging 12 months, then followed a slow decline from 12 months to 36 months. Z6 wines maintained the lowest mono-anthocyanin concentration during aging, followed by Z4 and Z3. Besides, the stable vineyard effect could also show in flavonol and flavanol concentrations. However, for anthocyanin derivatives, the variations in different vineyard wines changed when bottled for 12 months and 36 months. Z3 wines had the highest anthocyanin derivatives concentration when bottled for 1 month while declining rapidly in the 36 months. In contrast, the Y1 wines had the highest anthocyanin derivatives concentration in the 12 months and become more significant in the 36 months. For phenolic acids, only Z5 maintained the lowest concentration during aging compared to other vineyards.

It was believed that anthocyanin derivatives were critical in imparting color to aging red wine (Zhang et al., 2020). Their concentration changes during wine aging involved complex and inevitable chemical reactions, which seemed unpredictable from the fresh wines (He et al., 2012). In the fresh wines, we have analyzed that Z3 wines were characterized by high red coordinate (a^*) and color saturation (C^*_{ab}) which originated from higher concentrations of anthocyanins derivatives. However, after aging for 12 and 36 months, the total

concentration of anthocyanins derivatives in Z3 wines declined below Y1 wines. Interestingly, as shown in Fig. 5c, Z3 wines still maintained the highest red color intensity among all vines, which seemed incompatible with the result found in the total concentration of anthocyanins derivatives. Thus, we divided the anthocyanins derivatives into four types according to their structures and calculated their concentrations individually, as shown in Fig. 5b. The A-(e)-F & FA type, which included condensation products between anthocyanin and flavan-3-ols (Zhang et al., 2021), and the vitisin type, which included condensation products between anthocyanins and pyruvic acid (vitisin A) and acetaldehyde (vitisin B) (Bakker & Timberlake, 1997), declined rapidly from the new wines to aging for 36 months. As reported by Zhang et al. (2021), the A-(e)-F & F-A type concentration followed a decreased trend during wine aging, which was in agreement with our study. However, for vitisins, they found that the vitisin A followed a rising and then falling trend during aging (Zhang et al., 2021). As shown in Supplementary Table 4, the vitisin A kept stable during the first 12 months of aging while vitisin B declined rapidly since bottling. Quaglieri et al. (2017) suggested that in dry red wines, the vitisin A was very stable pigments, reaching the maximum concentration within the first aging year and then a slow decline. While vitisin B seemed to be intermediate in the polymerization reaction to form more stable structures, a decrease is observed through aging (Ivanova-Petropulos et al., 2015; Quaglieri et al., 2017). As for flavanol-pyranoanthocyanins, their total concentration reached the
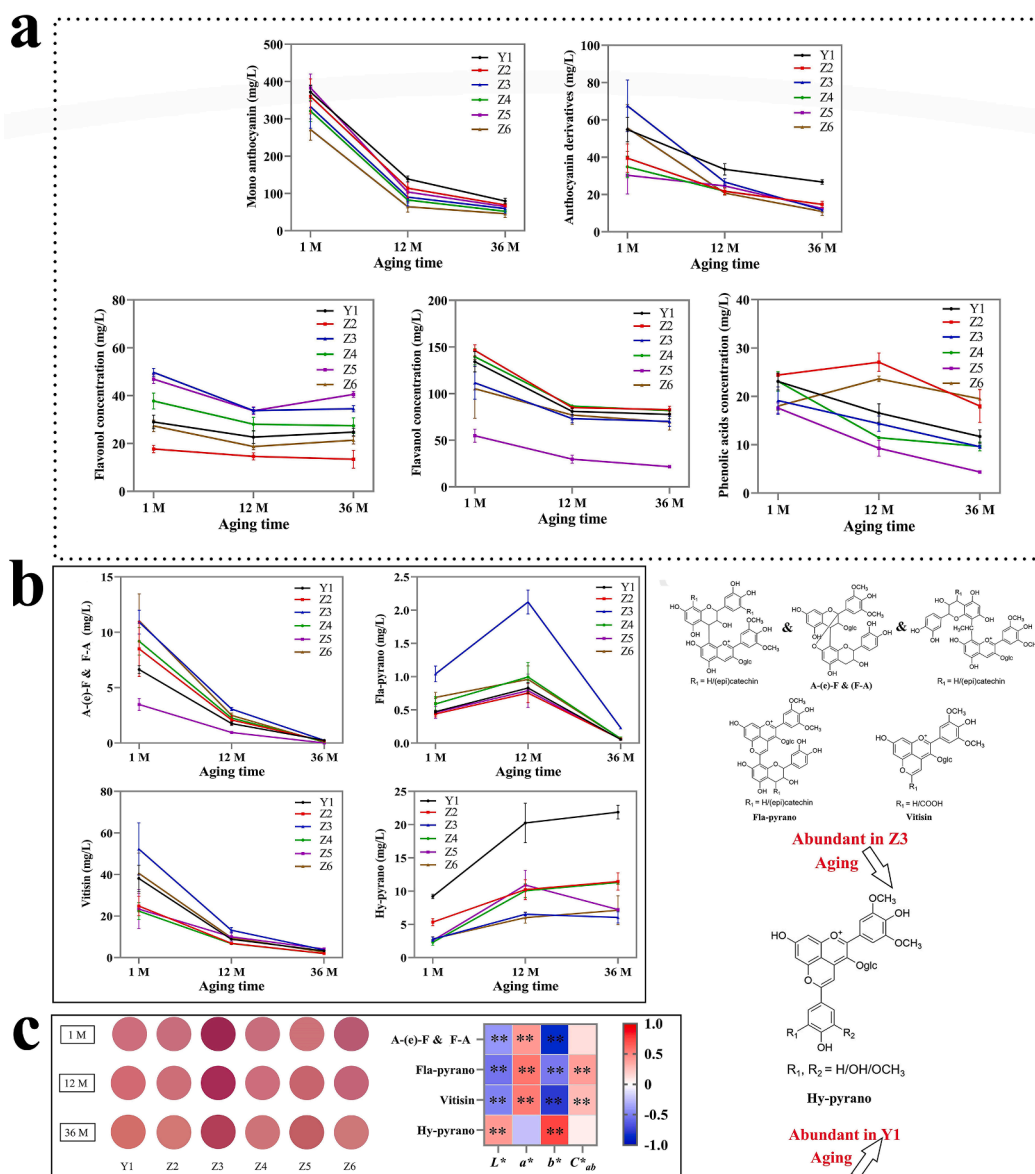
Fig. 5. Changes in phenolic profiles (a), four types of anthocyanins derivatives (b) and their roles in maintaining color characteristics (c) of different vineyard wines during aging.

highest when bottling for 12 months and declined thereafter (Fig. 5b). Interestingly, Z3 wines had the highest concentrations of all three types (A-(e)-F & FA, vitisin, flavanol-pyranoanthocyanins) during aging compared to other vineyards, which might be the reason for their high red color intensity. So it was shown that the different vineyard wines could also maintain their color characteristics during three years of aging, which was in agreement with the results found in mono anthocyanins, flavonols, flavanols and the above-mentioned three types of anthocyanin derivatives. However, for hydroxyphenyl-pyranoanthocyanins, Y1 wines had the highest concentration which caused a higher yellow color coordinate than other vineyard wines. Hydroxyphenyl-pyranoanthocyanins, known as pinotins, followed an increased trend as the aging went on (Fig. 5b) (Blanco-Vega et al., 2014; Schwarz et al., 2004). Similar to our study, Zhang et al. (2021) found that wine aging tawny characteristics is especially related to pinotins.

4. Conclusion

The present study demonstrated that wine flavonoid chemistry was

significantly altered by vineyard soils and grape harvest ripeness levels. We showed the chemical mechanisms underlying the concept of single-vineyard wines and certain vineyards could maintain their phenolic profiles in different vintages and aging. While for some vineyards, their wines showed high plasticity across vintages. High soil pH led to the rapid grape ripening rate and the early harvest date. The results of flavonols reflected the link between soil characteristics (pH), exposed climate condition and the final wine composition. The vineyards with moderate nutrition without elemental deficiency had abundant anthocyanin derivatives, especially A-(e)-F & FA, vitisins and flavanol-pyranoanthocyanins. The observations from this research draw a clear picture of the sustainability and plasticity of wine flavonoid features.

CRediT authorship contribution statement

Hao-Cheng Lu: Formal analysis, Data curation, Investigation, Writing – original draft, Visualization. **Meng-Bo Tian:** Software, Investigation. **Xiao Han:** Investigation. **Ning Shi:** Investigation. **Hui-Qing Li:** Investigation. **Chi-Fang Cheng:** Resources. **Wu Chen:** Resources. **Shu-De Li:** Resources. **Fei He:** Supervision. **Chang-Qing Duan:** Supervision. **Jun Wang:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100772.

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