Effects of strigolactone and abscisic acid on the quality and antioxidant activity of grapes (Vitis vinifera L.) and wines

Bochen Liu\textsuperscript{a}, Yang Zhang\textsuperscript{a}, Shu Wang\textsuperscript{a}, Wanni Wang\textsuperscript{a}, Xuelei Xu\textsuperscript{a}, Jinren Wu\textsuperscript{a}, Yulin Fang\textsuperscript{a,b,c,*}, Yanlun Ju\textsuperscript{a,b,c,*}
\textsuperscript{a} College of Enology, Northwest A\&F University, Yangling 712100, PR China
\textsuperscript{b} Shaanxi Engineering Research Center for Viti-Viniculture, Yangling 712100, PR China
\textsuperscript{c} Heyang Viti-viniculture Station, Northwest A \& F University, Heyang, Shaanxi 715300, PR China

\textbf{A R T I C L E I N F O}

\textbf{A B S T R A C T}

This study employed various methods, including recording of phenological phenomena and analysis of physicochemical indicators, to scrutinize effects of strigolactone and abscisic acid on indicators of ripeness, phenolic compounds, and antioxidant activity. 50 \( \mu \)M GR24 (strigolactone analog), 200 \( \mu \)M ABA (abscisic acid), 50 \( \mu \)M GR24 + 200 \( \mu \)M ABA, and 200 \( \mu \)M ABA + 10 \( \mu \)M TIS108 (strigolactone-biosynthesis inhibitor) were employed in E-L34 and E-L35. Samples were collected from E-L34 to E-L38. Each treatment could improve sugar contents and reduce acid contents, especially in the ABA + TIS group whose contents were 1\textdegree brix higher and 1.11 g/L lower than the control group. Additionally, the ABA and ABA + TIS groups could significantly contribute to phenolic accumulation, especially anthocyanins which were increased by at least 1.5 mg/g at each stage. However, the ABA + GR group had some inhibitory effects on ripening. Therefore, this study can lay a foundation for precisely applying exogenous ABA and GR24.

1. Introduction

Changes in the sugar, acid, and phenolic content of the fruit are related to grape ripening and wine quality. In order tool gain high-quality fruit, plant growth regulators play an indispensable role in the plants’ life. Many studies have been conducted on the effects of plant growth regulators on grapes. Abscisic acid (ABA) is one of them. Some pieces of research suggest that it can facilitate seed germination, fruit softening, and anthocyanin content throughout plant growth (Ali et al., 2022; Bai et al., 2020). The strigolactone analog GR24 is involved in parasitic seed germination, plant branching inhibition, and anthocyanin accumulation promotion (Ali et al., 2022; Ilhoi et al., 2021; Wang et al., 2020). It is common to observe different interactions existing among various plant growth regulators. Additionally, the relationship between GR and ABA has been reported in several studies, such as the symbiotic relationship between these two regulators and arbuscular mycorrhizal fungi on plants under salt stress, the accumulation of strigolactone (SL) increased by exogenous ABA under stress conditions, and ABA regulated by SL promoting germination under high-temperature conditions (Omoarelojie et al., 2019).

Grapes and wines are popular among customers because of their health benefits. Furthermore, the antioxidant activity of wine is intimately associated with the grapes’ raw material and phenolics. The phenolic compounds in grapes are transferred to the wine during fermentation and maceration, providing it with antioxidant potential (Gutierrez-Escobar et al., 2021; Jara-Palacios, 2019).

Although there are numerous reports on the effects of ABA on fruit aspects, and Ferrero et al. (2018) also studied the effect of GR on fruit anthocyanins, the studies of GR on fruit development, fruit quality, and antioxidant activity are still deficient, especially in the development phenomena of fruits when the two regulators are combined and sprayed at the same time. As a result, this study analyzed physicochemical indicators. Moreover, it implied that strigolactone and ABA might have a competitive relationship, which delivered a fresh perspective on the role of ABA and GR regulators on the fruit, as well as a new approach to harvesting wine grapes properly and making high-quality wine.
2. Materials and methods

2.1. Plant materials and experimental design

This study was carried out at a commercial vineyard in China (Chateau Lilan in Yinchuan, at 38°28′ N, 105°970 E, and an altitude of 1170 m, with an arid and semiarid continental climate) in 2021. Vines of ‘Cabernet Sauvignon’ (Vitis vinifera L., self-rooted) were planted in 2012 at a level of 3538 vines ha−1, with a spacing of 0.6 m between the vines and 3.0 m between rows. Rows of vines were oriented from north to south. The experimental design and the period of sample collection were determined according to the E-L system of grape growing stages modified by Coombe (1995). The experiment consisted of 5 treatments. As a wetting agent, Tween 80 was added to the five solutions (0.1 % v/v): Aqueous solution as the control group (CK); 50 μM GR24 (Beijing Coolaber Technology Co., Ltd.); 200 μM ABA (Shanghai Yuanye Biotechnology Co., Ltd.); 50 μM GR24 + 200 μM ABA; 200 μM ABA + 10 μM TIS108 (Beijing Coolaber Technology Co., Ltd.) as the treated groups. Five treatments were carried out in a completely randomized design with three repetitions, and every replicate included eight lines of 20 grapevines. The treatment solutions were evenly sprayed in two periods with three repetitions, and every replicate included eight lines of 20 grapevines. The treatment solutions were evenly sprayed in two periods (E-L34 and E-L35). A total of 6 times sampling were taken from E-L 34 to E-L 38. When the sampling time coincided with the spraying time, the sampling was performed first and then the hormones spraying were performed. Two bunches of grapes were selected from each plant, and five berries were selected from each bunch. The berries were randomly sampled at the bottom of the bunch, both sides in the middle of the bunch, and both sides on the top of the bunch. Then we collected and frozen samples under liquid nitrogen and stored them at −80 °C for further analysis.

2.2. Physicochemical indicators, soluble sugars, and organic acids

The berries were crushed by hand, and grape juice was then collected to analyze the maturity degree. Titratable acidity (TA) was measured (g/L tartaric acid) referring to the OIV method (OIV, 2017). The pH was detected by a digital pH meter (PB-10; Sartorius, Germany). And a digital refractometer (PAL-1; Atago Co. Ltd., Japan) was used to determine the total soluble solids (TSS) and expressed it as Brix.

2.2.1. Sugar content determination

Standard solution preparation: A total of 1.00 g each of glucose and fructose was accurately weighed in a 10 mL volumetric flask, and 100, 50, 40, 30, 20, 10, 5, and 2.5 g/L standard solutions were prepared with pure water in sequence; the solutions were filtered through a 0.45 μm filter membrane and stored at −20 °C.

Extraction: The seeds and skin were extracted. Liquid nitrogen was used to freeze the flesh and then it was weighed (3.00 g) in a 15 mL centrifuge tube precisely; the samples were mixed with 6 mL of 80 % ethanol and sonicated for 20 min (35 °C). The mixture was centrifuged (RC-5C-PLUS, Kendro, USA) at 8000 g for 10 min (20 °C). The extraction was repeated 3 times, and the supernatants were combined, concentrated under reduced pressure (35 °C), diluted to 10 mL, and filtered (0.45 μm).

Analysis and quantification: A high-performance liquid chromatography (HPLC) instrument (Nexera LC-30A; Shimadzu Co., Ltd., Kyoto, Japan) equipped with both a differential refractometer detector (RID) and a reversed-phase column (Zorbax SB-C18, 150 mm × 4.6 mm, 5 μm; Agilent, Santa Clara, CA) were used for the analysis at 40 °C. The compounds were eluted by acetone-water: (water: 80:20 v/v) at a flow rate of 1.2 mL/min, and the injection volume was 20 μL. Quantitative calculations were performed using the standard curves.

2.2.2. Organic acid

Sample pretreatment: Grape juice/wine was diluted 5 times with 10 % phosphoric acid solution, filtered with 0.45 μm microporous membrane, and loaded.

Chromatographic conditions: Column: CAPCELL PAK C18 (250 mm × 4.6 mm, 5 μm). All 11 standard materials (oxalic acid, tartaric acid, quinic acid, malic acid, shikimic acid, lactic acid, citric acid, fumaric acid, succinic acid, and propionic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mobile phase A: 0.02 M dipotassium phosphate (pH2.3), 4.56 g dipotassium hydrogen phosphate trihydrate to dilute 1 L and then adjust pH to 2.3 with phosphoric acid; mobile phase B: pure methanol; mobile phase A:B = 99:1. Column temperature: 45 °C; wavelength: 210 nm; isocratic elution, flow rate: 0.5 mL/min.

2.3. Extraction and analysis of phenolic compounds in grapes and wine

Berries (200 in total) were randomly selected from each treated group and then peeled by hand. The skins were ground with liquid nitrogen and freeze-dried using a vacuum freeze dryer (FD5 series, Gold-SIM, USA). The dry powder weighed accurately (1.00 g) was extracted with 60 % methanol (20 mL) containing 1 % formic acid, and then extracted under 40 Hz ultrasound for 30 min (30 °C), followed by being centrifuged at 10,000 g for 10 min (4 °C), and finally, the supernatant was collected. The extraction process was repeated three times. An analysis of the wines was carried out directly. The photometric analysis methods and corresponding quantitative standards (Sigma-Aldrich, St Louis, MO) were as follows. Total phenolic compound content (TPC) was using the Folin-Ciocalteu method (gallic acid); total flavonoid content (TFC) was adopting the aluminum chloride colorimetric method (rutin); total anthocyanin content (TAC), the pH differential method (malvidin-3-glucoside); And total flavan-3-ol content (TFOC), p-dimethyl-laminocinnamaldehyde-HCl method, (catechin).

Monomeric anthocyanins. Extraction and high-performance liquid chromatography (HPLC) analysis of monomeric anthocyanins in grapes and wines: the grape skin was ground into a powder with liquid nitrogen. The 2.0 g powder was dissolved with 30 mL HCl-methanol (1 mol/L HCl/MOH/H₂O, 1:80:19, v/v/v). The following step was extracted with the ultrasonic (power 100 %, 35 °C, 20 min) 6 times, combined with the extracts, and fixed the volume to 200 mL. After obtaining the extracts, 4 °C was supposed to store and they were protected from light. The wines were analyzed directly. Before HPLC measurement, the wines were filtered with 0.45 μm ultrafiltration membrane (organic). Detection conditions: mobile phase A: water:formic acid:acetoniitrile = 80:10:2.5; mobile phase B: water:formic acid:acetoniitrile = 40:2:5.5. The mobile phase elution procedures were as follows: 0–45 min, 0 %–35 % B; 45–46 min, 35 %–100 % B; 46–50 min, 100 % B isocratic; 50–51 min, 100 %–0 % B; and 51–55 min, 0 % B isocratic. The wavelength was 520 nm; the sample volume was 30 μL; the flow rate was 1 mL/min; the column was Syngery Hydro-RP C18 column (250 × 4.6 mm, 4 μm, Phenomenex, Torrance, CA, USA); the column temperature was 35 °C. Malvidin 3-O-glucoside was used as the external anthocyanin standard for the quantification of all the anthocyanins in the wine. Measuring instruments: Shimadzu LC-20AT. The concentration range of the standard curve for anthocyanin was from 0.27 to 400 mg/L with a coefficient of determination of 0.9999. Based on the retention time of anthocyanin. The maximum absorption wavelength and the experimental anthocyanin mass spectrometry database were used for the qualitative analysis of anthocyanin (Yang et al., 2018).

2.4. Vinification and enological indicators and their basic indicators

The grapes were crushed and de-stemmed mechanically, and then the total mass of each treated group was divided into two batches and transferred to 50 L stainless steel barrels. Potassium metabisulfite was added under the condition of total SO₂ content of 35 mg/L. Then 200 mg/L dry active commercial yeast (Saccharomyces cerevisiae strain XR; Lamothe Abiet, Câtejan, France) was added after 24 h of immersion. Alcohol was fermented for 10 d to dry at 20–25 °C. The vats were
pressed twice daily and their density was monitored every day. The pomace was separated from the wine at the end of alcoholic fermentation, the sulfur dioxide concentration was adjusted to 50 mg/L, and the wine samples were bottled and stored at 10–15 °C. All wine indicators were measured in duplicate and repeated three times.

Determination of basic wine indicators. Alcohol, residual sugar, and acid content of wine were determined using the FTIR analyzer Lyza 5000 Wine.

2.5. Chromatic characteristics in wine

The NF333 spectrophotometer (Nippon Denki Kogyo Co., Ltd., Japan) was used to check the CIELab indicators of the wines, directly obtaining L* (brightness), a* (green/red), b* (blue/yellow), C* (saturation) values, h° (hue angle) and ΔE. After obtaining the CIELab parameter index, the CIELab feature color map was obtained using the method of Li et al. (2017).

2.6. Antioxidant activity of grapes and wine

Phenolic extraction solutions from the skin were also used to determine the antioxidant properties of the extract, see Section 2.3 for the extraction method.

2.6.1. ABTS

The removal effect of ABTS was determined according to Apak et al. (2004). Briefly, 0.1 mL of the extraction solutions (wine diluted 10-fold) were added to 3.9 mL of ABTS solution, and the absorbance of the mixture was measured at 734 nm after 8 min of reaction under dark conditions. The results were expressed as Trolox equivalent antioxidant capacity (mg TEAC/kg DW).

2.6.2. DPPH

The scavenging effect on DPPH was measured by the Blois (1958) method. To summarize, the DPPH solution was prepared by dissolving 12.5 mg of DPPH in 100 mL of methanol. Then, 0.1 mL of grape skin extraction solution and wine diluted 10 times were added to 3.9 mL of DPPH solution and mixed well. The absorbance of the mixed solution was measured at 517 nm after 20 min of reaction under dark conditions. The results were expressed as Trolox equivalent antioxidant capacity (mg TEAC/kg DW).

2.6.3. CUPRAC

Cupric reducing antioxidant capacity (CUPRAC) was determined according to Choi and Lee (2009). After diluting the grape skin extraction solution 5 times, 0.1 mL of grape skin extraction solution and wine diluted 10 times were dropped into a 10 mL centrifuge tube. Then, 1 mL of 5 mmol copper sulfate solution, 1 mL of 3.75 mmol of new cuprous, 1 mL of 1 mol of acetic acid-ammonium acetate buffer (pH = 7.0), and 1 mL of distilled water were added to the test tube. The absorbance of the mixed solution was measured at 450 nm after 30 min of reaction under dark conditions. The results were expressed as Trolox equivalent antioxidant capacity (mg TEAC/kg DW).

2.7. Data processing and analysis

Statistical analysis of all data performed was using SPSS 24.0 (IBM, Armonk, NY, USA). One-way ANOVA and Duncan’s multiple polar difference tests were used for statistical treatment (P < 0.05). GraphPad Prism 9.2.0 was used to visualize data. Pearson correlation analysis of the antioxidant activity of grapes and wines with different types of phenolics was performed using R studio (P < 0.05; P < 0.01).

3. Results and discussion

3.1. Grape ripeness indicators, monomeric sugar, and organic acid indicators at maturity

The rise in sugar concentration and reduction in acid content are essential indicators for monitoring berry maturity, which determines the wine’s quality. The trends in titratable acid (TA), pH, and soluble solids (TSS) content were similar among all treatments, but the contents in each treatment were drastically varied. When compared to the untreated group, all treatments dramatically reduced TA content (Fig. 1A) and increased TSS content (Fig. 1C) during berry ripening. The ABA groups had the most substantial impacts on the content of TSS, as well as TA (about 0.5 °brix and 1.49 g/L lower than CK in E-L 38 respectively), which corresponded to the previous studies (D. Li et al., 2021; J. Li et al., 2021; Ma et al., 2022). Interestingly, the ABA + TIS group also increased the contents of sugars (1.04 times higher than CK), whereas the GR + ABA group had smaller effects on TSS and TA than the previous two groups (1.02 and 0.81 times higher than CK) (p < 0.05). Similar to the research (Ferrero et al., 2018), the GR treatment exhibited the smallest impact among the treated groups, although differentiating from the control group in terms of TSS and TA contents. These phenomena could be explained by some evidence that exogenous ABA could induce endogenous ABA, and it could regulate sugar-signaling during veraison so that the increasing contents of sugars were the results (Alferez et al., 2021, Li et al., 2006). In E-L37, the application of GR24, a strigolactone analog, had the most significant effect on pH elevation (3.55) (Fig. 1B). In E-L36.5 and E-L37, however, the GR + ABA group displayed a smaller pH elevation than the CK group (3.20, 3.33, and 3.39, 3.43, respectively). The most significant effects were observed in the ABA and ABA + TIS groups in the rest of the periods.

At maturity, this study measured monomeric sugars and organic acids in all samples. The amount of malic acid and citric acid among organic acids was significantly reduced in all treated groups. The ABA group had the most significant effect on malic acid content (0.96 g/L, 21 % lower than CK). The most apparent effect on citric acid concentration was observed in the GR and the GR + ABA groups (0.12 g/L, 17 % lower than CK). In terms of tartaric acid concentration, the GR group had little impressive performance in reducing the content and its impact was similar to the control group (p < 0.05) (Fig. 1D). Since previous reports had proved that exogenous ABA contributed to the conversion of sucrose to fructose and glucose, as well as the conversion of glucose to fructose, by upregulating sets of genes from sugar catabolism, the ABA group, the GR + ABA group, and the ABA + TIS group had remarkable effects on the accumulation of fructose and glucose content (both were 1.10, 1.09, 1.11 times higher than CK), which were consistent with the performance of TSS indices in the E-L38 period (p < 0.05) (Fig. 1E) (D. Li et al., 2021; J. Li et al., 2021). The GR treatment, on the other hand, made a slight contribution to the concentration of the two monomeric sugars. In conclusion, similar to prior research, ABA and GR applied alone could cause maturing to reach sooner, with the most significant impact being observed in the ABA treatment (Lin et al., 2018; Murcia et al., 2017). It appeared that utilizing ABA and TIS, GR inhibitors, simultaneously had more favorable effects on fruit ripening than applying ABA and GR separately.

3.2. Differences in phenolic content of grapes

The phenolic content of fruit is also an essential measurement of maturity, and it plays important role in contributing to antioxidant capability. Total phenolic compounds (TPC), total flavonoids (TFC), and total flavan-3-ol (TFOC) all showed a similar downward, upward, and downward pattern (Fig. 2A, B, D). During the E-L34 to E-L36 phases, the ABA + TIS group showed the tendency of deterioration of TPC content. The ABA, GR, and GR + ABA groups significantly increased TPC content during the E-L36 period (19 %, 15 %, and 14 % higher than CK), as a
result, the rising stage was attained ahead of schedule \((p < 0.05)\) (Fig. 2A). Furthermore, the CK group did not begin to decline until E-L37 due to the delayed process of accumulating TPC content. At this time, the TPC contents in the other groups were already at a lower level. Under the application of ABA, TFC had a higher content level than the CK group during the maturity process \((1.28, 1.52, 1.28, \text{ and } 1.22 \text{ times higher than CK from E-L35 to E-L37})\). The contents of TFC in the ABA + TIS group were always at a decreasing stage and did not show a rising trend (Fig. 2B). As maturity progressed, TFOC content in all treated groups was lower than in the CK group \((p < 0.05)\) (Fig. 2D). Moreover, during the harvest period, the high accumulation in the control group over the previous period resulted in greater TPC, TFC, and TFOC concentrations than in the treated groups \((p < 0.05)\).

Anthocyanins are the most important water-soluble pigments in grapes and provide an important basis for the color of grapes and wines. Due to the fact that sugar can promote anthocyanin accumulation, combining the data of TSS, all hormone treatments dramatically increased anthocyanin contents when fertility advanced \((p < 0.05)\) (Fig. 2C, E) (Dai et al., 2014). Undoubtedly, since exogenous ABA could increase sugar contents and upregulate the expression of \(VvUFGT\), the ABA group showed an increase in anthocyanin content, which was consistent with some research (Moriyama et al., 2020; Peppi et al., 2015). Similar to the experimental results of Ferrero et al. (2018), it was noticeable that after two times of applying GR (E-L35 and E-L36), the contents of anthocyanins did not significantly increase compared with the control group. In contrast, the application of ABA two times had more significant effects on anthocyanins accumulation (an increasing of 43, 48, 9, and 9 % over the control group from E-L35 to E-L37). Interestingly, combining ABA with GR did not have a cumulative effect compared with the application of GR only and ABA only. However, it was the combined use of ABA and TIS that significantly increased the accumulation of TAC, with its contents were 1.32, 0.93, 1.23, 1.13, and 1.02 times higher than GR + ABA, ensuring that the content always remained at a high level and sometimes even higher than the ABA group \((p < 0.05)\). Previous pieces of the literature showed (Ferrero et al., 2018; López-Ráez et al., 2010) that GR could interfere with the ABA signal, especially the activation of ABA catabolism and its membrane transport, resulting in decreasing endogenous ABA and the lower concentration of free ABA in the cell; and then the decreasing expression of anthocyanin-biosynthesis genes, especially \(VvMybA1\) and \(VvUFGT\) mediated by ABA, which was the reason why combining TIS, the strigolactone-biosynthesis inhibitor, with ABA had more effects on TAC than the GR + ABA group.
However, there existed decreasing trends in all groups during E-L38, which was accordant with some results (Li, Pang, et al., 2021; Li, Liu, et al., 2021; Ferrero et al., 2018).

HPLC was the method to measure the contents of monomeric anthocyanins in the mature samples (Table 1). The results showed that the content of cyanidin 3-glucoside in the CK group was lower than the others, and the contents of other monomeric anthocyanins were basically higher than that in the treated groups ($p < 0.05$). The application of GR only seemed to have a contribution to the contents of monomeric anthocyanins except for malvidin-3-O-glucoside and malvidin-3-O-(6-O-acetyl)-glucoside. Since exogenous ABA could upregulate the expression of VvF3′H and VvF3′5′H which were the precursors for cyanidin-based anthocyanins and delphinidin-based anthocyanins, the ABA group illustrated a lower concentration of malvidin-3-O-glucoside, but higher concentrations of delphinidin-3-O-glucoside and cyanidin-3-O-glucoside, which were accordant with the research (Sun et al., 2019). Malvidin 3-O-glucoside is the most important monomeric anthocyanin in the grape skin. When the contents of anthocyanins were low in all treatments, its performance in the ABA + TIS group seemed to be the best among all treatments, with no discernible difference compared to the CK group. However, the low contents in the GR and the GR + ABA groups were significantly different from that of the CK group ($p < 0.05$).

In previous studies, it was shown that anthocyaninase, strong light, and temperature could lead anthocyanins to degrade remarkably in the late mature stage. Temperature is one of the vital factors to accelerate the degradation and slows down the synthesis. (Liu et al., 2018; Xie, Liu, Chen, Zhang, & Ge, 2021; Zhao, Wang, Huang, & Hu, 2021). As the harvest approached, the data on temperature and humidity revealed that there were high and low temperatures in the vineyard. At the same time, it had several rainy days in the local vineyards before the harvest (Figs. S1 and 3). Combining with these data, it seemed to be able to explain the following phenomenon: during the harvest period, the fruit of the treated groups ripened earlier and reached the late stage of

| Table 1 | Monomeric anthocyanin content in grapes and wine. |
|---------|--------------------------------------------------|
| **Monomeric anthocyanin profiles** | **Grape (mg/g DW)** | **Wine (mg/L)** |
| CK | GR | ABA | GR + ABA | ABA + TIS | CK | GR | ABA | GR + ABA | ABA + TIS |
| Delphinidin-3-O-glucoside | 1.32 ± 0.01c | 1.49 ± 0.02a | 1.45 ± 0.01a | 1.34 ± 0.01b | 1.23 ± 0.01c | 18.08 ± 0.04a | 11.36 ± 0.04a | 12.52 ± 0.04a | 14.15 ± 0.07b | 10.11 ± 0.07b |
| Cyanidin-3-O-glucoside | 0.34 ± 0.00d | 0.47 ± 0.01a | 0.41 ± 0.02b | 0.38 ± 0.02c | 0.32 ± 0.03d | 0.17 ± 0.00a | 0.63 ± 0.00b | 0.74 ± 0.00b | 0.82 ± 0.00c | 0.53 ± 0.00c |
| Petunidin-3-O-glucoside | 1.16 ± 0.00d | 1.20 ± 0.00a | 1.24 ± 0.00b | 1.11 ± 0.02c | 1.09 ± 0.03d | 20.16 ± 0.01a | 13.29 ± 0.04a | 14.73 ± 0.05b | 15.20 ± 0.07b | 11.53 ± 0.07b |
| Peonidin-3-O-glucoside | 1.13 ± 0.00d | 1.25 ± 0.02a | 1.21 ± 0.01a | 1.14 ± 0.02b | 1.12 ± 0.03c | 10.73 ± 0.01a | 6.23 ± 0.01a | 8.58 ± 0.06b | 10.16 ± 0.12b | 11.29 ± 0.12b |
| Malvidin-3-O-glucoside | 0.09b ± 0.00b | 0.02a ± 0.01a | 0.01a ± 0.00b | 0.07b ± 0.00a | 0.01b ± 0.01b | 0.18ab ± 0.05d | 0.54d ± 0.06c | 0.25b ± 0.07c | 0.36a ± 0.09b |
| Peonidin-3-O-(6-O-acetyl)-glucoside | 0.37 ± 0.04c | 0.37 ± 0.02b | 0.38 ± 0.00b | 0.37 ± 0.06a | 0.37 ± 0.04a | 8.28 ± 0.03b | 4.74 ± 0.02b | 5.81 ± 0.02c | 6.38 ± 0.07b | 5.90 ± 0.07b |
| Malvidin-3-O-(6-O-acetyl)-glucoside | 3.24 ± 0.02a | 2.90 ± 0.00c | 3.01 ± 0.00b | 3.12 ± 0.02b | 3.12 ± 0.03c | 79.74 ± 0.07a | 59.59 ± 0.08a | 62.53 ± 0.09b | 63.31 ± 0.17b | 76.76 ± 0.17b |
| Trans-Peonidin-3-O-(6-O-p-coumaryl)-glucoside | 0.02a ± 0.01b | 0.02c ± 0.02c | 0.00b ± 0.00a | 0.02b ± 0.00a | 0.02b ± 0.00b | 3.07a ± 0.06a | 0.97b ± 0.04b | 2.04b ± 0.15c | 0.42b ± 0.17b | 0.88b ± 0.17b |
| Trans-Malvidin-3-O-(6-O-p-coumaryl)-glucoside | 1.09 ± 0.00a | 1.01 ± 0.01a | 1.08 ± 0.01a | 1.09 ± 0.01a | 1.17 ± 0.01a | 15.46 ± 0.01a | 10.21 ± 0.01a | 10.73 ± 0.01a | 11.67 ± 0.01a | 13.22 ± 0.01a |
| Sum of anthocyanins | 15.27 ± 0.08a | 14.80 ± 0.12b | 15.17 ± 0.08a | 14.90 ± 0.13ab | 15.00 ± 0.13ab | 357.04 ± 4.54a | 269.58 ± 4.70d | 281.45 ± 8.19d | 283.38 ± 8.16c | 334.37 ± 4.29b |
| 0.08a | 0.11b | 0.08a | 0.36ab | 0.13ab | 4.54a | 7.28 | 8.03 | 8.11 | 8.27 |
ABA groups. Thus, there was a hypothesis that could be made: because thesis pathways and their precursor was B-Carotene (Bhoi et al., 2021); and co-combination ABA and TIS groups rather than the GR and GR was still obvious that the higher contents could be witnessed in the ABA the fact that anthocyanin contents were reduced in the treated groups, it 1.21 times in ABA fertility (1.08 times in GR, 1.10 times in ABA, 1.07 times in GR 2013). According to the above results, it could be seen that the relative treatments of GR and ABA resulted in early ripening of the fruit at different stages. Consequently, harvesting and terminating fermentation in time can assist to manage sugar content and alcohol degree, which will accelerate the manufacturing stage and obtain higher wine quality.

The balance between acid and sugar contents affects the quality of the wine. Only the ABA + TIS group had a lower titratable acid concentration shown in Table S1. Malic and tartaric acids are often considered bitter and astringent on the palate, but the addition of lactic acid gives the wine a gentler taste (Zhang et al., 2018). The CK, ABA, and ABA + TIS groups all reduced the levels of malic and tartaric acid, whereas the control group did not reach this period, thus the anthocyanin content in CK group was not significantly declined. In order to comprehend relative analyses.

3.4. Phenolic content and color index of wine

The phenolics in wine are directly related to not only the antioxidant activity but also the stability and aging potential of the wine. Overall, the differences between treatments regarding TPC, TAC, TFC, and TFOC indicators were similar to those results seen on fruits (Fig. 3). Except for TFC, the other three indicators in the CK group were apparently higher maturity earlier, resulting in a decrease of the anthocyanin contents, whereas the control group did not reach this period, thus the anthocyanin content in CK group was not significantly declined. In order to obtain the specific reasons more accurately, it was necessary to measure the contents of related enzymes such as glycosidase, phenol oxidases, and polyphenol oxidases, which were also a focus of our future research. On the other hand, there existed another hypothesis that exogenous ABA might delay the appearance of postharvest anthocyanin peaks in wine grapes, and some genes from downstream flavonoids biosynthesis were upregulated simultaneously, such as VvFLS, leading to the increasing of flavonoid content but the decreasing of anthocyanin content (Li, Liu, et al., 2021). However, the flavonoid content did not increase significantly in the ABA-related groups, we suspected that there might be other secondary metabolites generated, which needed our further examination. In addition, R2R3-MYB and R3-MYB had been proven to suppress the expression of genes regarding anthocyanin-biosynthesis by competing with MYB positive regulators that promote anthocyanin accumulation and response to the ABA signal (Liu et al., 2018). Although the content of anthocyanins in CK was higher in than CK). Therefore, the wines made from the CK, ABA, and ABA + TIS groups all reduced the levels of malic and tartaric acid contents, while the GR and GR + ABA groups had the opposite effects (p < 0.05). In terms of lactate content, only the GR + ABA group was at a relatively lower level and was significantly different from other groups (p < 0.05). However, a high alcohol level in wine reduces fruity and flowery aromas while increasing fermentation odors such as pepper (King et al., 2013). In the above results, it could be seen that the relative treatments of GR and ABA resulted in early ripening of the fruit at different stages. Consequently, harvesting and terminating fermentation in time can assist to manage sugar content and alcohol degree, which will accelerate the manufacturing stage and obtain higher wine quality.

Fig. 3. Content of phenolic compounds in wine under different treatments. (A) Contents of total phenolic compound (TPC), (B) Contents of total flavonoid (TFC), (C) Contents of total anthocyanin (TAC), (D) Contents of total flavan-3-ol (TFOC). Data in bar graphs is presented as mean ± SD (n = 3). Different letters represent a significant difference at each sampling point (p < 0.05).

In order to comprehensively investigate the effects of GR and ABA on wine quality, we brewed all groups under the same conditions, and the specific results were provided in Table S1. Since the increasing sugar content encourages yeast to convert more sugar to alcohol, the ABA and ABA + TIS groups were 0.38% and 0.59% of alcohol degrees higher than CK, which were significantly different from other groups (p < 0.05). However, a high alcohol level in wine reduces fruity and flowery aromas while increasing fermentation odors such as pepper (King et al., 2013). In the above results, it could be seen that the relative treatments of GR and ABA resulted in early ripening of the fruit at different stages. Consequently, harvesting and terminating fermentation in time can assist to manage sugar content and alcohol degree, which will accelerate the manufacturing stage and obtain higher wine quality. The balance between acid and sugar contents affects the quality of the wine. Only the ABA + TIS group had a lower titratable acid concentration shown in Table S1. Malic and tartaric acids are often considered bitter and astringent on the palate, but the addition of lactic acid gives the wine a gentler taste (Zhang et al., 2018). The CK, ABA, and ABA + TIS groups all reduced the levels of malic and tartaric acid contents, while the GR and GR + ABA groups had the opposite effects (p < 0.05). In terms of lactate content, only the GR + ABA group was at a relatively lower level and was significantly different from the control group (58% lower than CK). Therefore, the wines made from the CK, ABA, and ABA + TIS groups had a softer texture than the GR and GR + ABA groups, which meant the combination of GR and ABA seemed to have a certain negative impact on the flavor of the wine.

3.4. Phenolic content and color index of wine

The phenolics in wine are directly related to not only the antioxidant activity but also the stability and aging potential of the wine. Overall, the differences between treatments regarding TPC, TAC, TFC, and TFOC indicators were similar to those results seen on fruits (Fig. 3). Except for TFC, the other three indicators in the CK group were apparently higher
than the treated groups \((p < 0.05)\). Among the treated groups, the ABA + TIS group showed higher amounts in all four indicators on wines.

The relevant indicators for anthocyanins were shown in Fig. 4C and Table 1. The application of ABA and the combination of ABA and TIS had a more contribution to the total anthocyanins of wine than other treatments, even though anthocyanins were degraded in the mature stage of the fruit. (Fig. 3C). Malavidin 3-O-glucoside and malavidin 3-O-(6-O-acetyl)-glucoside are accounted for relatively large proportions in monomeric anthocyanins. The ABA + TIS group also had significantly higher content in these two substances compared with other treated groups \((p < 0.05)\), which indicated that these substances could keep the color of wine more stable than other groups \((Sroka, 2005)\). In the majority of the indicators, the combination of GR and ABA did not appear to have a favorable influence on the phenolic indicators of wine, whereas the opposite results were witnessed in the group of ABA + TIS, which were similar to the data on grapes.

Subsequently, we used the CIELab method to identify the color of the wine (Fig. 4A). The \(L^*\) (lightness), \(a^*\) (green/red), \(b^*\) (blue/yellow), \(C^*\) (chroma), \(h^*\) (hue angle), and \(\Delta E\) values were obtained directly (Ioannidis et al., 2013). All exogenous hormone treatments dramatically enhanced \(L^*\) values as compared to CK, indicating that these treatments boosted the brightness of wines. However, the \(a^*\), \(b^*\), \(C^*\), and \(h^*\) values were significantly declined \((p < 0.05)\). The performances of the GR, ABA + TIS, GR + ABA and ABA groups in \(a^*\), \(b^*\), and \(C^*\) were arranged in descending order, with the red tone decreasing, the blue tone increasing, and the saturation decreasing \((p < 0.05)\) (Fig. 4B). Combining this with the analysis of the anthocyanin contents, it could be found that \(L^*\) was negatively correlated with anthocyanin content, whereas \(a^*\), \(b^*\), \(C^*\), and \(h^*\) were positively correlated (Cheng et al., 2022). This also explained that although the relevant ABA and GR treatments prematurely ripened the fruit, the color performance of the wine was not ideal.

3.5. Antioxidant activity of grape and wine and its correlation analysis

Grapes and wine have strong antioxidant activity. Three generally used indicators, ABTS\(^+\) scavenging effect, DPPH scavenging effect, and CUPRAC, were utilized to assess the influence of GR and ABA on antioxidant activities. The findings revealed that CK had a greater contribution to the antioxidant capacity (Fig. 5A, B). In terms of grape skins, the ABA and GR + ABA groups had lower antioxidant capacity regarding all three indices, while ABA + TIS had the opposite effect \((p < 0.05)\) (Fig. 5A), which was contrary to the results of some studies (Sandhu et al., 2011; Zhu et al., 2016). Some literature had illustrated that strignolactone had a relative antioxidant capacity including activating antioxidant enzymes, such as SOD and CAT, to defend against abiotic stresses (Garcia-Caparros et al., 2020). In our study, from the perspective of antioxidant evaluation in vitro, GR had some scavenging capacity against ABTS\(^+\) and DPPH among the treated groups. Although all phenolic compounds in the treated groups were lower than in the control group, the ABA + TIS group had the highest level of all three antioxidant activity indicators and was not apparently different from the control group, which indicated that this application had strong effects on the ability to scavenge free radicals (Fig. 5B). However, since the phenolic compounds remained at low levels in the maturity, the wine made from the ABA group remained at the same low level of antioxidant capacity, which was consistent with the antioxidant indicators of grape skins. Similarly, the combination of GR and ABA did not significantly increase the antioxidant capacity and consistently exhibited almost opposite effects with the combination of ABA and TIS group.

To interpret the results, we then analyzed the correlations of three indicators of antioxidant activity with secondary metabolites, phenolics, that have the antioxidant capacity (Fig. 5C). All three antioxidant indicators had a strong correlation with TPC, followed by malavidin 3-O-(6-O-acetyl)-glucoside, trans-malvidin 3-O-(6-O-p-coumaryl)-glucoside, malvidin 3-O-glucoside, and TFC. In the ABA group, the TPC concentrations of both grapes and wines were at the lowest level, which might explain their poor antioxidant capacity. In the ABA + TIS group, the contents of three monomeric anthocyanins mentioned above which were highly related to the antioxidant activity were all higher and had no significant difference with the control group, thus, ABA + TIS had the great antioxidant ability. Since the contents of TPC and TFC in the group of the GR-treated wine were not at a low level, it seemed to explain the higher ability to scavenge ABTS\(^+\) in the GR group. According to the results of Cheng et al. (2020), the indicators of monomeric phenols and NADPH could give a solid explanation for the antioxidant activity, which was also an important direction to scrutinize the effects of exogenous hormones on the antioxidant capacity of grapes and wine. Endogenous ABA not only affected the antioxidant activity but also revealed the relationship between GR and ABA when they were used together. And it might lay the foundation for the rational application of

---

**Fig. 4.** Color-related images and index of wine. (A) Images of different treatments of wine, (B) Chroma CIELab representation. Abbreviation: \(L^*\) (lightness), \(a^*\) (green/red), \(b^*\) (blue/yellow), \(C^*\) (chroma), \(h^*\) (hue angle). Data in radar chart is presented as mean ± SD \((n = 3)\). Different letters represent a significant difference at each sampling point \((P < 0.05)\). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
hormones in the future.

4. Conclusion

ABA and GR applied only, as well as ABA and TIS in combination, could accelerate fruit ripening and boost the contents of phenolics, especially anthocyanins, and sugar as well as acid substances during the development process. In contrast, the combined application of GR and ABA had inhibitory effects on fruit development and the accumulation of phenolic substances. However, there was a slight degradation of phenolics in the ABA and GR relative groups at maturity, thus, the antioxidant activity was less or equal to that of the untreated group. Although the ABA + TIS group experienced degradation of the phenolic components, its antioxidant activity was comparable to the CK group.

Author contribution statement

YLF and YLJ designed the research and provided materials; BCL and YZ conducted the experiments; WNW and SW analyzed the data; BCL, XXL and JRW wrote the manuscript; YLF promoted the manuscript. All the authors have read and approved the final manuscript.

CRediT authorship contribution statement

Bochen Liu: Investigation, Writing – original draft. Yang Zhang: Conceptualization, Visualization. Shu Wang: Data curation. Wanni Wang: . Xuelei Xu: Formal analysis. Jinren Wu: Software. Yulin Fang: Funding acquisition, Project administration, Supervision, Writing – review & editing. Yanlun Ju: Methodology, Resources, Validation.

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

Data will be made available on request.

Acknowledgments

This work was supported by the research and demonstration of key technologies for wine style solidification at the eastern foothills of Helan Mountain in Ningxia (2020BCF01003); the Key R&D project of Shaanxi Province (2018ZDXM-NY-053) ; and China Agriculture Research System of MOF and MARA (CARS-29-zp-6).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100496.

References

Allerrez, F., de Carvalho, D. U., & Boakye, D. (2021). Interplay between abscisic acid and gibberellins, as related to ethylene and sugars, in regulating maturation of non-climacteric fruit. International Journal of Molecular Sciences, 22(2), 669. https://doi.org/10.3390/ijms22020669

Ali, F., Qanmber, G., Li, F., & Wang, Z. (2022). Updated role of ABA in seed maturation, dormancy, and germination. Journal of Advanced Research, 35, 199–214. https://doi.org/10.1016/j.jare.2021.03.011

Apak, R., Güçlü, K., O, M., zyürek, & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry, 52, 7970–7981. https://doi.org/10.1021/jf048741x

Bai, Q., Huang, Y., & Shen, Y. (2020). The physiological and molecular mechanism of abscisic acid in regulation of fleshy fruit ripening. Frontiers in Plant Science, 11, Article 619553. https://doi.org/10.3389/fpls.2020.619553

Bhoi, A., Yadu, B., Chandra, J., & Keshavkant, S. (2021). Contribution of strigolactone in plant physiology, hormonal interaction and abiotic stresses. Planta, 254(2), 28. https://doi.org/10.1007/s00425-021-03675-1

Blois, M. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181, 1199–1200. https://doi.org/10.1038/1811199a0

Cheng, X., Wang, X., Zhang, A., Wang, P., Chen, Q., Ma, T., & Fang, Y. (2020). Foliar Phenylalanine Application Promoted Antioxidant Activities in Cabernet Sauvignon
