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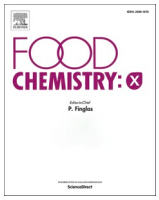

Effects of foliar applications of γ-polyglutamic acid and alginic acid on the quality and antioxidant activity of Marselan grapes and wines

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ABSTRACT

This study investigated the effects of γ-polyglutamic acid (PGA) and alginic acid (ALA) on grapes and wines. Marselan grapes were utilized to assess the accumulation and synthesis of phenolic compounds and antioxidant activity. The 0.35 % (*v*/v) PGA (PGA2) significantly enhanced the antioxidant activity of both grapes and wines in both years. Overall, treatments with 0.45 % (v/v) PGA (PGA3), 0.45 % (v/v) ALA (ALA3), and 0.25 % (v/v) ALA (ALA1) notably increased the total phenolic and anthocyanin content in both grapes and wines. Among these, PGA3 treatment significantly upregulated the levels of Delphinidin-3-*O*-(6-acetyl)-glucoside, Cyanidin-3-*O*-(6-acetyl)-glucoside, Peonidin-3-*O*-glucoside, and Malvidin-3-*O*-(trans-6-*O*-coumaryl)-glucoside in both years. Additionally, PGA3 treatment elevated the expression of the *VvPAL*, *VvCHS*, *VvDFR* and *VvLDOX* genes across both years. In contrast, ALA3 and ALA1 treatments increased anthocyanin content by upregulating the expression of *VvCHS*, *VvF3'H* and *VvUFGT* genes. In summary, PGA3 treatment significantly enhanced the phenolic compounds and antioxidant activity in both grapes and wines. These findings demonstrate the potential of PGA and ALA as biostimulants to significantly enhance grape and wine quality in viticulture.

1. Introduction

Grapes and wines are recognized for their rich phenolic content, which plays a crucial role in protecting grapes from environmental stressors like UV radiation while significantly influencing the color, flavor, and overall sensory qualities of wines (Matsuda et al., 2021). Phenolic compounds, which are secondary metabolites, can be broadly categorized into flavonoids and non-flavonoids based on their carbon structures (Cantu et al., 2021). Flavonoids, such as anthocyanins, flavonols, and flavanols, are directly linked to the color, astringency, and bitterness of wines (Duan et al., 2019). Non-flavonoid compounds, including phenolic acids and stilbenes, primarily contribute to the stabilization of red wine color (Zhao et al., 2023). Additionally, phenolic compounds are among the most abundant dietary antioxidants, known for their beneficial effects on health issues like osteoporosis and cardiovascular diseases, which has garnered them significant interest (Jiang et al., 2017).

The biosynthesis and accumulation of phenolic compounds are influenced by factors such as varieties, light, temperature, cultivation practices, and plant growth regulators (Degu et al., 2016; Gashu et al., 2020; Yue et al., 2022). For example, Ju et al. (2019) and Yang et al. (2020) found that regulated irrigation increases the content of non-acylated anthocyanins in Cabernet Sauvignon grapes and significantly enhances the endogenous hormone levels of abscisic acid (ABA) and indole-3-acetic acid (IAA). However, global warming leads to high temperatures during the ripening process, significantly reducing grape anthocyanin levels compared to the veraison and maturation stages (Pastore et al., 2017; Rienth et al., 2021). To counteract these challenges, viticultural management practices such as leaf removal, fertilization, and the application of exogenous hormones have been adopted to improve the quality of grapes and wines (Gutierrez-Gamboa et al., 2021). For instance, Li et al. (2021) found that the foliar application of antitranspirants increases fructose and total phenolic content in Riesling grapes. Applying nitrogen to grape leaves significantly enhances grape color by promoting phenylalanine metabolism and the expression of *VvUFGT* related anthocyanin biosynthesis (Cheng, Ma, et al., 2020).

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Similarly, Zhao et al. (2023) found that applying potassium dihydrogen phosphate at the end of veraison significantly increases anthocyanin contents in Cabernet Sauvignon. Despite these benefits, the long-term use of chemical treatments can negatively affect plants and soil microorganisms (Duan et al., 2019), highlighting the need for more sustainable agricultural practices.

Organic farming offers a solution to the drawbacks of modern chemical agriculture. In recent years, biostimulants have emerged as environmentally friendly organic fertilizers for sustainable agricultural practices (García-García et al., 2020). Biostimulants are formulated products of biological origin, including bacteria, algae, higher plants, and animals (Olavarrieta et al., 2022). Among these, polyglutamic acid and alginic acid have shown particular promise in viticulture. Polyglutamic acid is a biodegradable macromolecule that exists in two isomeric forms: α- polyglutamic acid and γ- polyglutamic acid, with γ-polyglutamic acid (PGA) being widely used in agriculture (Elbanna et al., 2024). Studies have reported that PGA significantly enhances the absorption of nitrogen, phosphorus, and potassium in plants, improving nutrient uptake (Olavarrieta et al., 2022). Additionally, PGA reduces salt and heavy metal stress in plants by increasing the activities of SOD, POD, and CAT (Zhang et al., 2017). Alginic acid (ALA), a natural polysaccharide mainly derived from kelp and seaweed, is used as a sustainable tool to enhance tolerance to abiotic stress (Deng et al., 2019; Deolu-Ajayi et al., 2022). It has been shown to improve photosynthesis, drought tolerance, and salt tolerance in grapevines (García-García et al., 2020). In Australia, the foliar application of alginate during the veraison stage increased wine grape yield by 14.7 % (Arioli et al., 2021). In table grapes, alginate stimulated the expression of genes involved in anthocyanin biosynthesis, thereby increasing berry anthocyanin content (Deng et al., 2019). However, there is limited research on the effects of these two biostimulants on the antioxidant activity and phenolic compounds in grapes and wines.

China, as a rapidly developing wine-producing country, has seen the emergence of Marselan as a widely cultivated wine grape variety across various regions (Lan et al., 2022). Therefore, it is crucial to improve the quality of grapes and wines efficiently and ecologically. This study investigates the effects of two biostimulants, polyglutamic acid and sodium alginate, on the quality of grapes and wines and analyzes the correlation between phenolic compounds and antioxidant activity. The research provides new insights into the role of polyglutamic acid and sodium alginate in wine grape production and offers strategies for sustainable grapes and wines production.

2. Materials and methods

2.1. Plant materials and experimental design

This study was conducted at Zhangyu Winery in Shaanxi, China (34°42 N, 108°82 E) in the 2023 and 2024 vintages. Marselan grapes (*Vitis vinifera* L., self-rooted), planted in 2014, were used as the plant material. The vineyard consists of 7-year-old grapevines, spaced 1.0 m apart within rows and 2.8 m between rows. Fertilization, irrigation, and pest management were performed according to local standard practices. The grapevines were oriented north to south.

Following previous studies, different concentrations of PGA (Zhang et al., 2017) and ALA (Gutiérrez-Gamboa, Garde-Cerdán, Rubio-Bretón, & Pérez-lvarez, 2020) were used. The experiment consisted of seven treatments. Tween 80 was added as a wetting agent to all solutions (0.1 % *v*/v): water as the control (CK); 0.25 % (*v*/v) PGA (PGA1); 0.35 % (v/v) PGA (PGA2); 0.45 % (v/v) PGA (PGA3); 0.25 % (v/v) alginate (ALA1); 0.35 % (v/v) alginate (ALA2); and 0.45 % (v/v) alginate (ALA3). Based on the recommendations of G. Gutiérrez-Gamboa, Garde-Cerdán, Martínez-Lapuente, et al. (2020), foliar applications were conducted twice on Marselan grapevines. The first application was at the veraison stage, and the second application was one week later. Each grapevine was sprayed with 200 mL of the respective treatment. The experiment was conducted in a completely randomized design with 7 treatments, each replicated three times, with 20 grapevines per replicate. Harvesting commenced when CK reached a soluble solid content of 19–22°Brix and a titratable acidity of 5–7 g/L tartaric acid. On September 14, 2023, for each biological replicate, three samples consisting of 10 kg grapes each were randomly selected from various positions within each cluster and from both the sun-exposed and shaded sides of the clusters. Some samples were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis. The remaining samples were pressed and subjected to alcoholic fermentation.

2.2. Winemaking protocol

At harvest, the grapes were manually destemmed and crushed, with each sample processed in triplicate. The grapes were fermented in 10 L containers containing 60 mg/L SO_2 (0.12 g/L potassium metabisulfite), 30 mg/L pectinase (Lallzyme Ex, Lallemand, France), and 0.20 g/L wine yeast strain (*Saccharomyces cerevisiae* strain XR; Lamothe Abiet, Can'e-jan, France). Alcoholic fermentation was conducted in a temperature-controlled environment at 20–25 °C. The vats were pressed twice daily, and specific gravity was measured three times a day. When the specific gravity fell below 0.997, the skins and seeds were removed to allow the fermentation to continue. Once the residual sugar level dropped below 4 g/L, the wine samples were bottled with 80 mg/L SO_2 in 750 mL bottles and stored at −4 °C for chemical analysis after three months (Cheng, Ma, et al., 2020).

2.3. Physicochemical indicators, soluble sugars, and organic acids

For each replicate, the hundred-grain weight (g) of the berries was recorded. For each biological replicate, three groups of samples consisting of 50 berries each were randomly selected. The berries were selected based on uniform coloration and consistent size. The berries were manually crushed, and the soluble solid content of the grape juice was measured using a handheld refractometer (Atago Co., Ltd., Japan). The pH of the grape juice was determined using a digital pH meter (PB-10; Sartorius, Germany). The titratable acidity (TA) was measured following the OIV method (OIV (International Organisation of Vine and Wine), 2017). Basic wine parameters, including alcohol content, residual sugar, and acid content, were determined using a Lyza 5000 Wine FTIR analyzer (Liu et al., 2022). Sixty grape berries were randomly selected, wrapped in gauze, and the juice was extracted. The juice was centrifuged at 10000 *g* for 10 min at 20 °C. The supernatant was filtered through a 0.45 μm filter and stored at 4 °C for further analysis of soluble sugars and organic acids. The wine samples were analyzed directly. Each treatment was performed in triplicate. Analyses were conducted using high-performance liquid chromatography (Nexera LC-30 A, Shimadzu Co., Ltd., Kyoto, Japan).

The conditions for soluble sugar separation were as follows: detector: refractive index detector (RID); column: Agilent ZORBAX SB-C18 (4.6 mm × 150 mm, 5 μm); mobile phase: acetonitrile/water = 80/20 (*v*/v); flow rate: 1.2 mL/min; injection volume: 10 μL; column temperature: 40 °C. Organic acids were detected using a diode array detector (DAD) under the following conditions: column: Thermo Hypersil GOLD AQ; column temperature: 20 °C; analysis time: 20 min; column: C18 (4.6 mm × 250 mm, 5 μm); mobile phase: 0.01 mol/L potassium dihydrogen phosphate (pH = 2.55)/methanol = 99/1 (v/v); flow rate: 0.5 mL/min; injection volume: 20 μL; column temperature: 45 °C; analysis time: 20 min (Liu et al., 2022).

2.4. Analysis of the phenolic compounds in grapes and wines

200 berries were randomly selected from each treatment group and manually peeled. The berries were selected based on uniform coloration and consistent size. The skins were ground in liquid nitrogen and freeze-dried using a vacuum freeze dryer (FD 5 series, GoldSIM, USA). Dry

powder (1.00 g) was accurately weighed and extracted with 20 mL of 60 % methanol containing 1 % formic acid. The extraction was performed at 30 °C for 30 min under ultrasonication at 40 Hz, followed by centrifugation at 10000 *g* for 10 min at 4 °C. The supernatant was collected, and the extraction process was repeated three times. The wine samples were analyzed directly. Total phenolic content (TPC) was determined using the Folin-Ciocalteu method with gallic acid as the standard (Lan et al., 2022). Total anthocyanin content (TAC) was measured by the pH differential method with malvidin-3-glucoside as the standard (Ren et al., 2023). Total tannin content (TTC) was determined using the methyl cellulose precipitation method with catechin as the standard (Duan et al., 2019).

2.4.1. Analysis of individual anthocyanins

Grape skins were ground into a powder using liquid nitrogen. A 2.00 g sample of the powder was dissolved in 30 mL of a hydrochloric acid-methanol solution (1 mol/L HCl/MeOH/H_2O, 1:80:19, *v*/v/v) and extracted with 100 mL of the same solution. The extraction process involved ultrasonication (100 % power, 35 °C, 20 min) performed six times. The extracts were then combined and the volume adjusted to 200 mL. The extracts were stored at 4 °C and protected from light. Wine samples were analyzed directly. Before HPLC analysis, wine samples were filtered through a 0.45 μm organic ultrafiltration membrane. The determination of anthocyanins were performed using a High-performance liquid chromatography (HPLC) system (LC-2030CD, Shimadzu, Japan). Detection conditions: Mobile phase A: water: formic acid: acetonitrile = 80:10:2.5; Mobile phase B: water: formic acid: acetonitrile = 40:2.5:50. The gradient elution program was 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; 51–55 min, 0 % B isocratic (Lu et al., 2022). Detection wavelength: 525 nm; injection volume: 30 μL; flow rate: 1 mL/min; column: Synergi Hydro-RP C18 column (250 × 4.6 mm, 4 μm, Phenomenex, Torrance, CA, USA); column temperature: 35 °C. The content of individual anthocyanin glycosides was quantified using an Mv-glu standard curve and expressed as mg/kg dry weight (DW) of grape skin and mg/L of wines (Table. S13).

2.4.2. Analysis of individual non-anthocyanins

A total of 0.50 g of dried skin powder was mixed with 50 mL of a water: ethyl acetate solution (9:1, *v*/v). The mixture was shaken for 30 min at 130 rpm and 30 °C, then centrifuged at 8000 *g* for 5 min. This extraction process was repeated four times. After centrifugation, the combined supernatant was evaporated to dryness at 33 °C. The residue was reconstituted in 5 mL of methanol. Wine samples were analyzed directly. Before analysis, all extracts were filtered through a 0.45 μm filter. The analysis was performed on an Agilent 1200 series LC/MSD Trap VL instrument equipped with a variable wavelength detector (VWD) and a reversed-phase column (Kromasil100-5C18, 250 × 4.6 mm i.d., 5 μm; Restek Co., Bellefonte, USA). at 25 °C. The detection wavelength was set at 280 nm, and the injection volume was 2 μL. Compounds were eluted using mobile phases A (acetic acid: water, 1:99, v/v) and B (acetic acid: acetonitrile, 1:99, v/v) at a flow rate of 1.0 mL/min according to the following gradient scheme: 0–15 min, 10–26 % B; 15–30 min, 26–40 % B; 30–50 min, 40–65 % B; 50–60 min, 65–95 % B; 60–63 min, 95–10 % B; and 63–66 min(Lu et al., 2022). The content of each compound in grapes and wines were calculated using standard curves of the respective standards and expressed as mg/kg dry weight (DW) of grape skins and mg/L of wines (Table. S13).

2.5. Antioxidant activity of grapes and wines

The skins were manually peeled from 50 frozen berries per replicate without thawing and then freeze-dried at −40 °C for 36 h. The berries were selected based on uniform coloration and consistent size. The dried skins were subsequently ground in liquid nitrogen using a chilled mortar and pestle. As previously described (Li et al., 2023), the antioxidant capacity of grapes and wines were evaluated using three methods: DPPH assay: 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity; ABTS assay: 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity; CUPRAC assay: Cupric reducing antioxidant capacity. The results were expressed as Trolox equivalent antioxidant capacity (mg TEAC/kg DW).

2.6. Expressions of anthocyanins biosynthesis genes

The expression levels of anthocyanin biosynthesis genes in grape skins were quantified using real-time quantitative PCR (qRT-PCR). Total RNA was extracted with a Total RNA Extraction Kit (Bioteke, RP3302, Beijing, China), and first-strand cDNA was synthesized using the Hiscript II Q RT SuperMix for qPCR (Vazyme, R223–01). Real-time PCR amplification was performed using ChamQ SYBR qPCR Master Mix (Vazyme, Q311) on an IQ5 System (Bio-Rad, Hercules, CA, USA). The primer sequences used are listed in Table S14, with *VvACTIN* serving as the internal reference gene. The reaction mixtures comprised 2 μL of cDNA, 0.8 μL of primers (10 μmol/L), 10 μL of 2× ChamQ SYBR qPCR Master Mix, and 7.2 μL of ddH_2O, totaling 20 μL. The thermocycling conditions were as follows: initial denaturation at 90 °C for 30 s, followed by 40 cycles of 95 °C for 10 s and 55 °C for 30 s. Data were analyzed using the $2^{-\Delta\Delta CT}$ method. Each sample was analyzed in triplicate to ensure technical accuracy.

2.7. Data processing and analysis

Analysis of variance (ANOVA) was performed using SPSS 22.0 (Tukey's test, $P < 0.05$). Histograms were prepared using Origin 2022. Correlation heatmaps and Principal component analysis (PCA) were generated using Chiplot v2.1 (https://www.chiplot.online). Chord diagrams were created using the R programming language within the R Studio integrated development environment.

3. Results and discussion

3.1. Physicochemical indicators, soluble sugars, and organic acids

In this study, the soluble solids (°Brix) content of grapes from the ALA3 group in 2023 and the ALA2 group in 2024 were significantly higher than CK, with increases of 15.54 % and 10.95 %, respectively (Table. S1, S2). Soluble sugars in grape berries primarily consist of glucose and fructose (Lan et al., 2022; Tian et al., 2024). For glucose, the PGA3 group exhibited 59.13 % and 15.10 % higher levels compared to the CK in 2023 and 2024, respectively (Fig. S1A, S2A). These results suggest that high concentrations of PGA application promote glucose accumulation in grape berries. Regarding fructose, the ALA3 group had the highest content in 2023, while the ALA2 group had the highest fructose content in 2024. This may be attributed to the exogenous application of ALA, which induces the upregulation of sugar metabolism genes, facilitating the conversion to fructose (Chen et al., 2020; Yang et al., 2020). For titratable acidity, the PGA2 treated grapes in both years exhibited significantly higher levels of acidity than CK (Fig. S1C, S2C). The main organic acids measured in grapes showed no significant increase in tartaric acid across treatments. However, in terms of malic acid, PGA2 increased its content by 18.94 % in 2023 compared to CK, while ALA2 increased malic acid content by 25.27 % in 2024.

To thoroughly evaluate the impact of PGA and ALA on wine quality, we vinified all groups using a uniform winemaking process. The detailed basic indicators of the wine results are presented in Table S3, S4. In 2023, wines from the PGA2 and PGA1 treatments exhibited increases in titratable acidity of 9.28 % and 7.17 %, respectively, compared to CK. In 2024, wines from the PGA2, PGA3 and ALA2 treatments showed significantly higher acidity than CK. Tartaric and malic acids are the primary organic acids in wines. In 2023, no significant differences in tartaric and malic acid content were observed across treatments

(Fig. S1D). However, in 2024, PGA2 significantly increased tartaric acid content, while PGA3 significantly increased malic acid content, thereby enhancing the wine's acidity and freshness (Fig. S2D). Overall, no significant differences were observed in the pH values of both the grapes and wines across treatments compared to CK in two years.

3.2. Differences in phenolic content of grapes and wines

3.2.1. Grapes

The grape skin contains abundant phenolic compounds, including flavonoids and non-flavonoids, which play a critical role in preventing oxidative damage and determining wine quality parameters (Cantu et al., 2021). The results from 2023 (Fig. 1A) and 2024 (Fig. S3A) demonstrate that PGA3 and PGA2 treatments significantly increased the total phenolic content (TPC) in grapes. Anthocyanins provide a crucial basis for the color of grapes and wines. In 2023, PGA1, PGA3, and ALA3 treatments significantly enhanced the total anthocyanin content (TAC) compared to CK (Fig. 1B). In 2024, all treatments increased TAC relative to the CK, with PGA3 and ALA1 treatments showing significant improvements (Fig. S3B). The increase in anthocyanin content suggests that the PGA3 treatment may enhance the expression of key genes or enzymes involved in anthocyanin biosynthesis.

Phenylalanine ammonia-lyase (PAL) activation has been widely studied as a key driver of anthocyanin accumulation, and previous research has shown that γ-PGA application can enhance PAL activity, leading to increased anthocyanin production (Fard & Hassanpour, 2023). This suggests that PGA could promote anthocyanin biosynthesis by upregulating this pathway. The mechanisms of flavonoid synthesis differ between exogenous hormones. Liu et al. (2022) discovered that the application of exogenous ABA, compared to strigolactone, upregulates *VvUFGT* expression, thereby promoting anthocyanin accumulation in 'Cabernet Sauvignon'. Tannins are crucial factors affecting the astringency or bitterness of wines. Except for the PGA1 and PGA2 treatments in 2023, which significantly reduced total tannin content (TTC) compared to CK, no significant differences in tannin levels were observed between the other treatments and CK (Fig. 1C, S3C).

3.2.2. Wines

Polyphenolic compounds in wines are derived from the maceration of grape skins and seeds, where tannins and other phenolics are extracted. The composition and concentration of polyphenols in grapes and wines are influenced by many factors, such as variety, ripeness, and environmental conditions. Moreover, various winemaking techniques and viticultural practices affect wine quality (Cheng, Wang, et al., 2020). In 2023, the PGA1 and PGA3 treatments significantly increased TPC in wines compared to the other treatments (Fig. 1D). In 2024, TPC was significantly enhanced by the PGA2 and ALA1 treatments (Fig. S3D). Over both years, the PGA3 treatment consistently resulted in a significant increase in anthocyanin content in wines (Fig. 1E, S3E). High-quality tannins are essential determinants of wine quality. Additionally, tannins contribute to color stability by forming complexes with anthocyanins (Lan et al., 2022). No significant differences in TTC were observed between the treatments and CK in 2023 (Fig. 1F). However, in 2024, TTC increased by 26.26 % under the PGA1 treatment compared to CK (Fig. S3F). Conversely, the ALA3 and ALA1 treatments in 2024 significantly reduced TTC in wines.

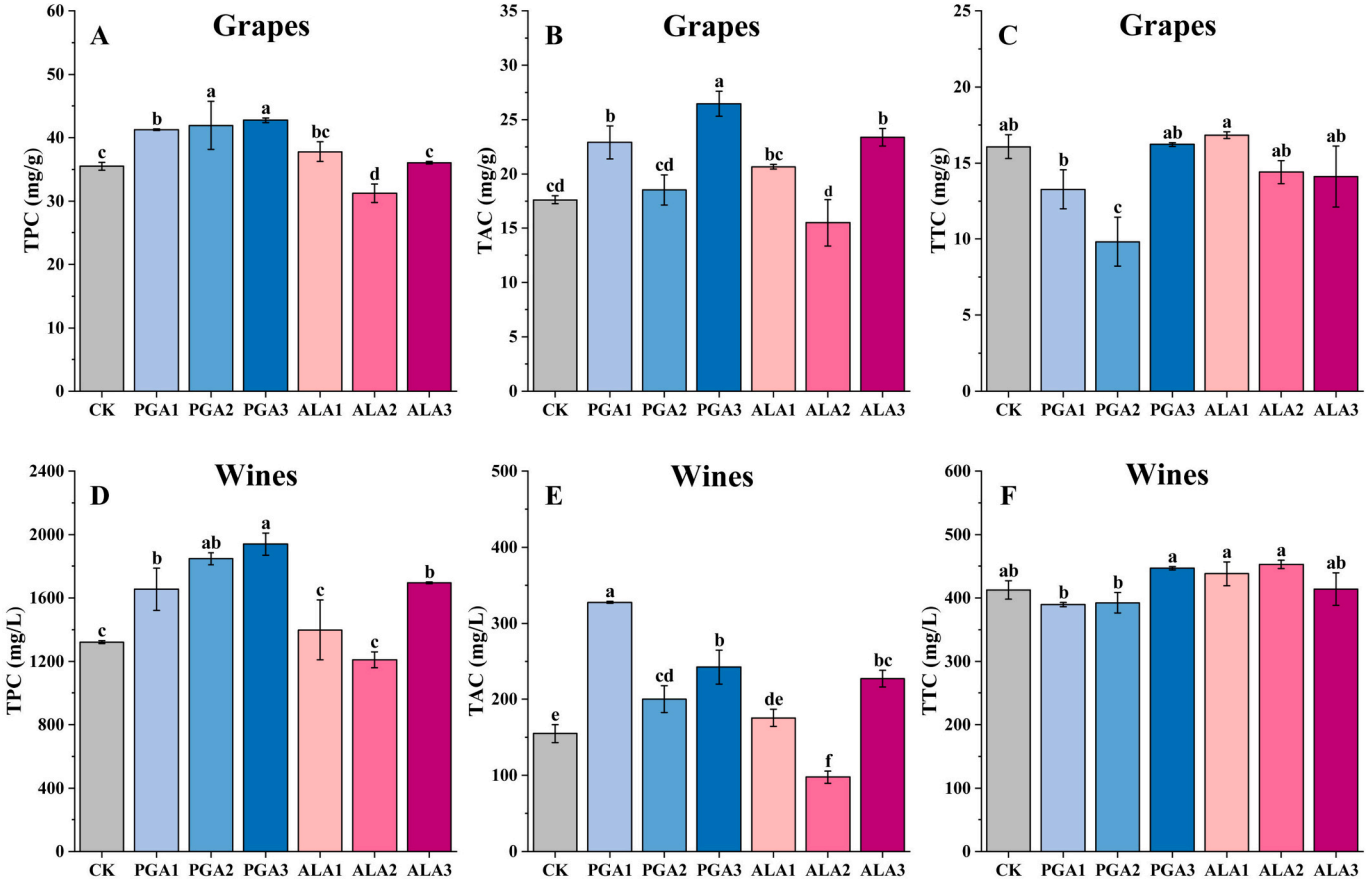


Fig. 1. Effects of different treatments on phenolic substances in the 2023 vintage. Contents of total phenolic compound (TPC) in grape skins (A) and wines (D). Contents of total anthocyanin (TAC) in grape skins (B) and wines (E). Contents of total tannin (TTC) in grape skins (C) and wines (F). 0.25 % (*v*/v) PGA (PGA1); 0.35 % (v/v) PGA (PGA2); 0.45 % (v/v) PGA (PGA3); 0.25 % (v/v) alginate (ALA1); 0.35 % (v/v) alginate (ALA2); and 0.45 % (v/v) alginate (ALA3). Data in bar is presented as mean ± SD ($n = 3$). Different letters indicate significant differences between treatments ($P < 0.05$).

3.3. Individual anthocyanins

3.3.1. Grapes

In this study, 19 types of individual anthocyanins were identified and quantified using HPLC analysis (Table. S5, S6, S7, S8). These included 5 non-acylated anthocyanins (TNAs), 5 acetylated anthocyanins (TAAs), 3 caffeic acylation anthocyanins (TCFAs), and 6 coumaric acylation anthocyanins (TCAs). Over the two-year period, TNAs comprised the highest proportion of anthocyanin content in the treated grapes, followed by TCAs (Fig. 2A, S4A). With the exception of the ALA2 treatment in 2023, all other treatments significantly promoted anthocyanin synthesis. Notably, the PGA3 treatment resulted in a substantial increase in anthocyanin content, with increases of 56.57 % and 44.27 % in 2023 and 2024, respectively. The increase in total anthocyanin content was primarily driven by elevated levels of TNAs and TCAs. Furthermore, the ALA3 treatment in 2023, as well as the PGA2 and ALA1 treatments in

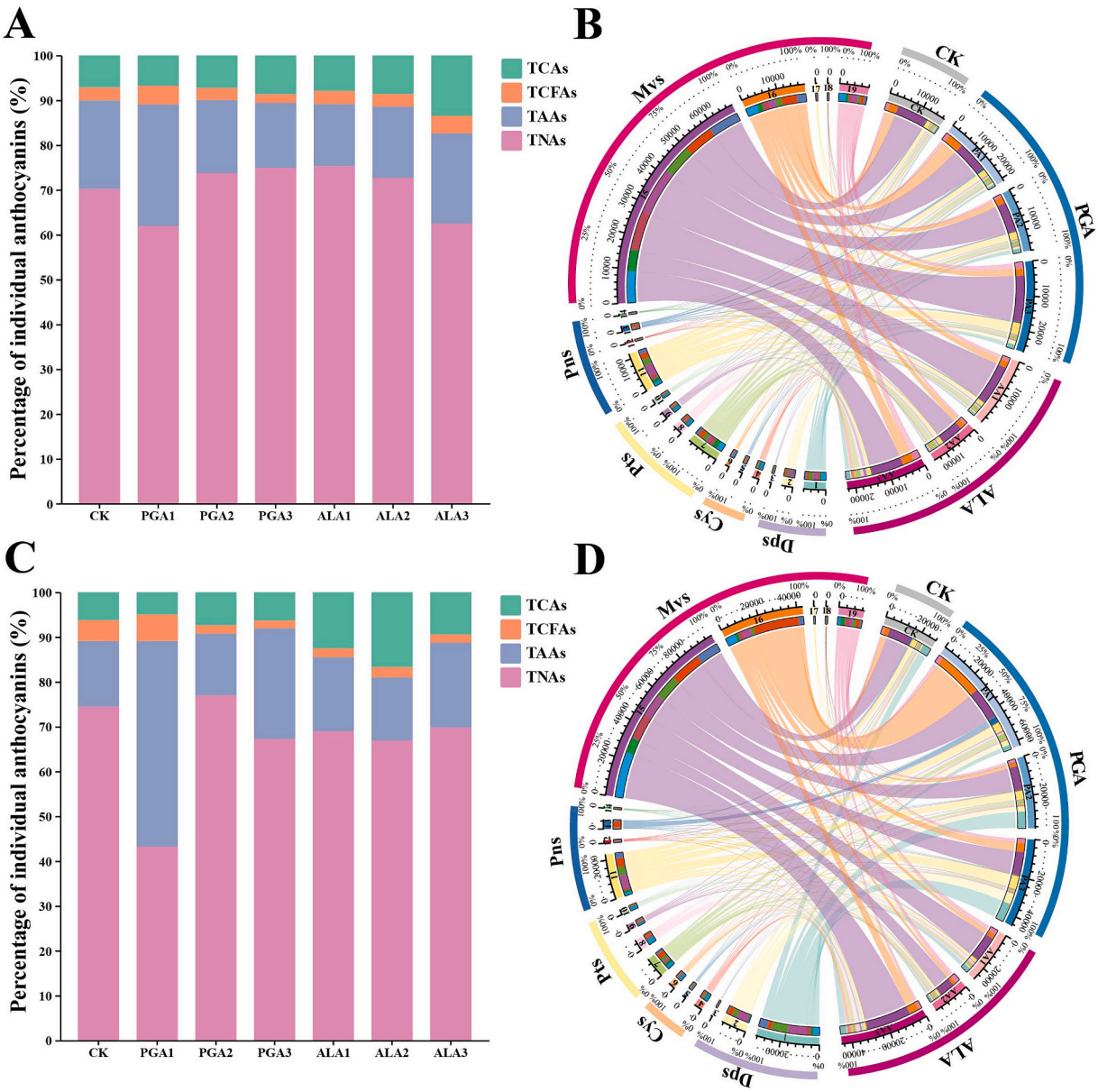


Fig. 2. Contents of individual anthocyanins in grapes and wines under different treatments in the 2023 vintage. Percentage of individual anthocyanins in grapes (A) and wines (C). Circos plot of individual anthocyanins in grapes (B) and wines (D). The circos plot illustrates relationships among groups, with each segment color-coded for a different group. Lines indicate connections, with thicker lines denoting stronger relationships. Line colors correspond to the groups they connect. The outer ring displays bar charts showing quantitative measurements, with different colors representing sub-categories or metrics. 0.25 % (v/v) PGA (PGA1); 0.35 % (v/v) PGA (PGA2); 0.45 % (v/v) PGA (PGA3); 0.25 % (v/v) alginate (ALA1); 0.35 % (v/v) alginate (ALA2); and 0.45 % (v/v) alginate (ALA3). TNAs, Contents of total non-acylation anthocyanins; TAAs, contents of total acetylation anthocyanins; TCFAs,contents of total caffeic acylation anthocyanins; TCAs, contents of total coumaric acylation anthocyanins. 1, Delphinidin-3-*O*-glucoside; 2, Delphinidin-3-*O*-(6-acetyl)-glucoside; 3, Delphinidin-3-*O*-(6-coumaryl)-glucoside; 4, Cyanidin-3-*O*-glucoside; 5, Cyanidin-3-*O*-(6-acetyl)-glucoside; 6, Cyanidin-3-*O*-(6-coumaryl)-glucoside; 7, Petunidin-3-*O* -glucoside; 8, Petunidin-3-*O*-(6-acetyl)-glucoside; 9, Petunidin-3-*O*-(6-coumaryl)-glucoside); 10, Petunidin-3-*O*-(trans-6-*O*-coumaryl)-glucoside; 11, Peonidin-3-*O*-glucoside; 12, Petunidin-3-*O* -(6-acetyl)-glucoside; 13, Peonidin-3-*O*-(6-caffeoyl)-glucoside; 14, Peonidin-3-*O* -(trans-6-*O*-coumaryl)-glucoside; 15, Malvidin-3-*O*-glucoside; 16, Malvidin-3-*O*-(6-acetyl) -glucoside; 17, Malvidin-3-*O*-(6-caffeoyl)-glucoside; 18, Malvidin-3-*O*-(cis-6-*O*-coumaryl) -glucoside; 19, Malvidin-3-*O*-(trans-6-*O*-coumaryl)-glucoside. Dps, contents of total delphinidin-3-*O*-glucoside derivatives; Cys, contents of total cyanidin-3-*O*-glucoside derivatives; Pts, contents of total petunidin-3-*O*-glucoside derivatives; Pns, contents of total peonidin-3-*O*-glucoside derivatives; Mvs, contents of total malvidin-3-*O*-glucoside derivatives.

2024, significantly enhanced anthocyanin content compared to CK. These findings align with those of previous studies, showing that the application of alginate enhances anthocyanin content in 'Pinot Noir'. (Frioni et al., 2018).

The identified anthocyanins were divided into five groups: delphinidin-3-*O*-glucoside derivatives (Dps), cyanidin-3-*O*-glucoside derivatives (Cys), petunidin-3-*O*-glucoside derivatives (Pts), peonidin-3-*O*-glucoside derivatives (Pns), and malvidin-3-*O*-glucoside derivatives (Mvs). Among these, Mvs (Mv3g, Mv3ag, Mv3cag, Mv3cgc) were the most prevalent in grapes. In both years, the highest anthocyanin content was observed in the Mv3g treated grapes, with concentrations ranging from 6369.53 to 13,651.35 mg/kg. As shown the chord diagram (Fig. 2B, S4B), the PGA2 and PGA3 treatments significantly increased the levels of Dps (Dp3g, Dp3ag, Dp3cgc) and Mvs. Conversely, the ALA treatments significantly enhanced the content of Pts (Pt3g, Pt3ag, Pt3cag, Pt3cgt). The observed increase in anthocyanin concentrations with higher PGA treatment doses highlights the dose-dependent nature of the effect, which is critical for optimizing treatment levels to improve wines quality. In contrast, ALA treatments exhibited more variability. ALA3 was more effective in 2023, whereas ALA1 showed better results in 2024. This discrepancy may be related to climatic conditions. In 2023, the higher rainfall compared to 2024 likely required higher concentrations of ALA to promote anthocyanin accumulation, while PGA treatments were less affected by environmental factors. Similarly, the application of seaweed extract during veraison in grapes effectively enhances tri-substituted anthocyanins, aligning with our results. Alginic acid has been shown to affect the expression of genes involved in secondary metabolism, particularly those related to anthocyanin biosynthesis (Guo et al., 2020; Tian et al., 2024). These studies suggest that the observed increases in anthocyanins could be linked to similar genetic

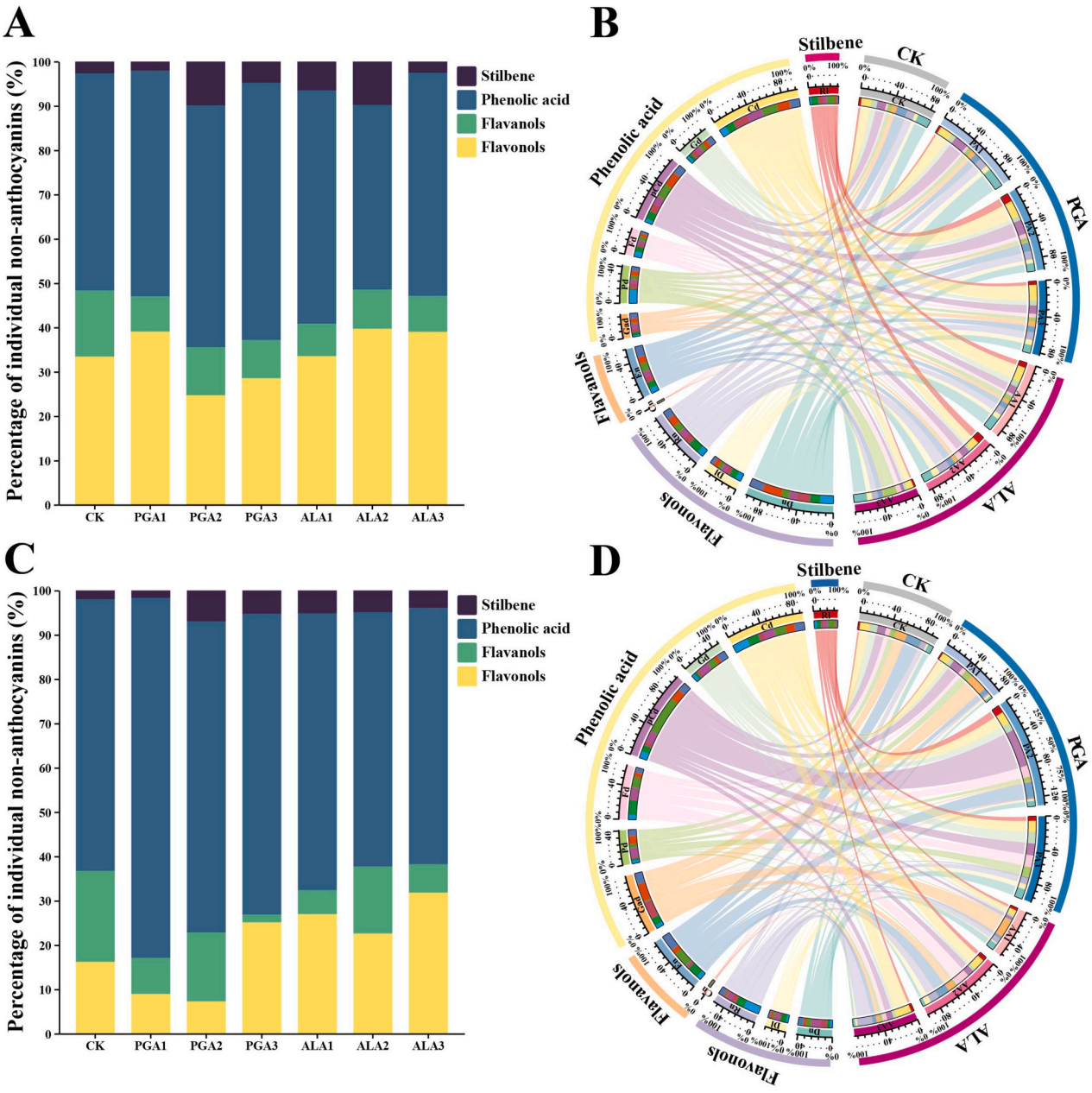


Fig. 3. Contents of individual non-anthocyanins in grapes and wines under different treatments in the 2023 vintage. Percentage of individual non-anthocyanins in grapes (A) and wines (C). Circos plot of individual anthocyanins in grapes (B) and wines (D). 0.25 % (v/v) PGA (PGA1); 0.35 % (v/v) PGA (PGA2); 0.45 % (v/v) PGA (PGA3); 0.25 % (v/v) alginate (ALA1); 0.35 % (v/v) alginate (ALA2); and 0.45 % (v/v) alginate (ALA3). Dn, Dihydroquercetin; Dl, Dihydrokaempferol; Rn, Rutin; Cn, Catechin; En, Epicatechin; Gad, Gallic acid; Pd, Protocatechuic acid; Fd, Ferulic acid; pCd, p-Coumaric acid; Gd, Gentisic acid; Cd, Caffeic acid; Rl, Resveratrol.

pathways, providing a basis for future exploration of these mechanisms in our treatments.

3.3.2. Wines

The results demonstrate that, compared to other treatments, PGA1 significantly increased anthocyanin content in wines in 2023, while PGA3 resulted in the highest anthocyanin content in 2024. Over the two years, PGA1 consistently enhanced TCFAs, with increases of 77.93 % in 2023 and 207.95 % in 2024 (Fig. 2C, S4C). The chord diagram (Fig. 2D, S4D) shows that PGA1 primarily increases anthocyanin content by elevating the levels of Mv3g and Dp3g. Specific anthocyanins, such as Cy3cg (trans), were significantly increased in grape skins under the influence of PGA3 and ALA3 over both years. Their concentrations were also notably higher in the wines. This indicates that these anthocyanins are retained during fermentation and may undergo conversion.

3.4. Individual non-anthocyanins

3.4.1. Grapes

In HPLC analysis, 12 types of individual non-anthocyanins were identified and quantified in all samples, including 3 flavonols, 2 flavanols, 6 phenolic acids, and 1 stilbene (Table. S9, S10, S11, S12). In grape berries, phenolic acids were the main component followed by flavonols. Resveratrol was the only stilbene detected, accounting for 2.08 %–9.86 % of the total stilbene content in 2023 and 1.55 %–12.94 % in 2024, consistent with previous studies (Fig. 3A, S5A) (Ren et al., 2023). Dihydroquercetin was the predominant flavonol. Among the treatments, PGA1 significantly increased dihydroquercetin content by 46.67 % in 2023 (Fig. 3B). In 2024, ALA1 treatment induced the most substantial increase in dihydroquercetin content, with an enhancement of 87.99 % (Fig. S5B). For flavan-3-ols, including catechin and epicatechin, all treatments led to a decrease in content compared to CK. However, in 2024, ALA treatments significantly increased catechin content. Leucoanthocyanidin reductase (LAR) is involved in converting leucoanthocyanidins to catechins (Tian et al., 2022). Previous research found that high doses of seaweed applied to Tempranillo grapevines activate LAR or AR enzymes, inducing catechin synthesis in grapes (Frioni et al., 2018; Gutiérrez-Gamboa, Garde-Cerdán, Martínez-Lapuente, et al., 2020). These findings align with our 2024 results, suggesting that the effect of ALA treatment on catechin content is influenced not only by concentration but also by climatic factors. Over the two years, the ALA3 treatment significantly increased protocatechuic acid content, with increases of 222.10 % in 2023 and 148.51 % in 2024. PGA3 significantly increased caffeic acid content in both years, with increases of 56.23 % in 2023 and 107.62 % in 2024.

3.4.2. Wines

In wines, PGA3 significantly increased monomeric phenol content over both years, followed by PGA2. As shown in the images, PGA3 significantly increased flavonol and phenolic acid content across both years (Fig. 3C, S5C). PGA2 had a significant enhancing effect on flavanols. In 2024, the ALA1 treatment resulted in a significant increase in flavonol content, with a 239.06 % elevation compared to CK. The chord diagram (Fig. 3D, S5D) results indicated that both PGA and ALA treatments significantly affected the levels of various individual non-anthocyanins compounds in wines, with specific concentrations producing the most significant effects. For example, PGA3 and ALA1 treatments significantly increased dihydroquercetin content over both years. Additionally, the ALA1 treatment significantly increased the rutin content in wine in 2024. Over both years, PGA2 and PGA3 significantly increased the levels of ferulic acid and p-coumaric acid.

3.5. Principal component analysis and partial least squares discriminant analysis

3.5.1. Grapes

Principal component analysis (PCA) revealed the significant impact of different concentrations of PGA and ALA on grape individual anthocyanins and non-anthocyanins (Fig. 4, S6). The PCA results in 2023 showed that the first principal component (PC1) and the second principal component (PC2) explained 35.97 % and 20.62 % of the data variance, respectively (Fig. 4A). These data highlighted significant differences in the chemical composition among the treatment groups. ALA3 was primarily separated from CK by PC1, while ALA2 and PGA groups were mainly separated from CK by PC2. The treatment groups were also distinctly separated from each other. In 2024, PC1 and PC2 accounted for 36.41 % and 16.68 % of the total data variation, respectively (Fig. S6A). ALA1, PGA2, and PGA3 treatments were clearly separated from CK along PC2.

The 2023 loading plot indicated that specific compounds such as Dp3ag, Mv3gc, Pn3g, Pt3ag, and Mv3g were positively correlated with PC1, whereas phenolic acids such as pCd, Cd, and Gd were negatively correlated with PC1 (Fig. 4C). PC2 was positively correlated with individual non-anthocyanins like Rl, Rn, and Fd. In 2024, pCd, Dp3cgc, Cy3ag, and Pn3ag exhibited a positive correlation with PC1, whereas Mv3cgc, Gad, and Cd were positively correlated with PC2 (Fig. S6C). This suggests that the expression levels of these compounds varied significantly among the different treatment groups, likely due to increased concentrations of PGA and ALA (Gutiérrez-Gamboa, Garde-Cerdán, Martínez-Lapuente, et al., 2020). Furthermore, the results revealed that ALA3 treatment had a more significant impact on anthocyanins and other phenolic compounds such as flavonols compared to PGA. This could be because high concentrations of ALA promote the activity of specific secondary metabolic pathways, thereby enhancing the accumulation of these compounds (Gutiérrez-Gamboa, Garde-Cerdán, Rubio-Bretón, & Pérez-lvarez, 2020).

3.5.2. Wines

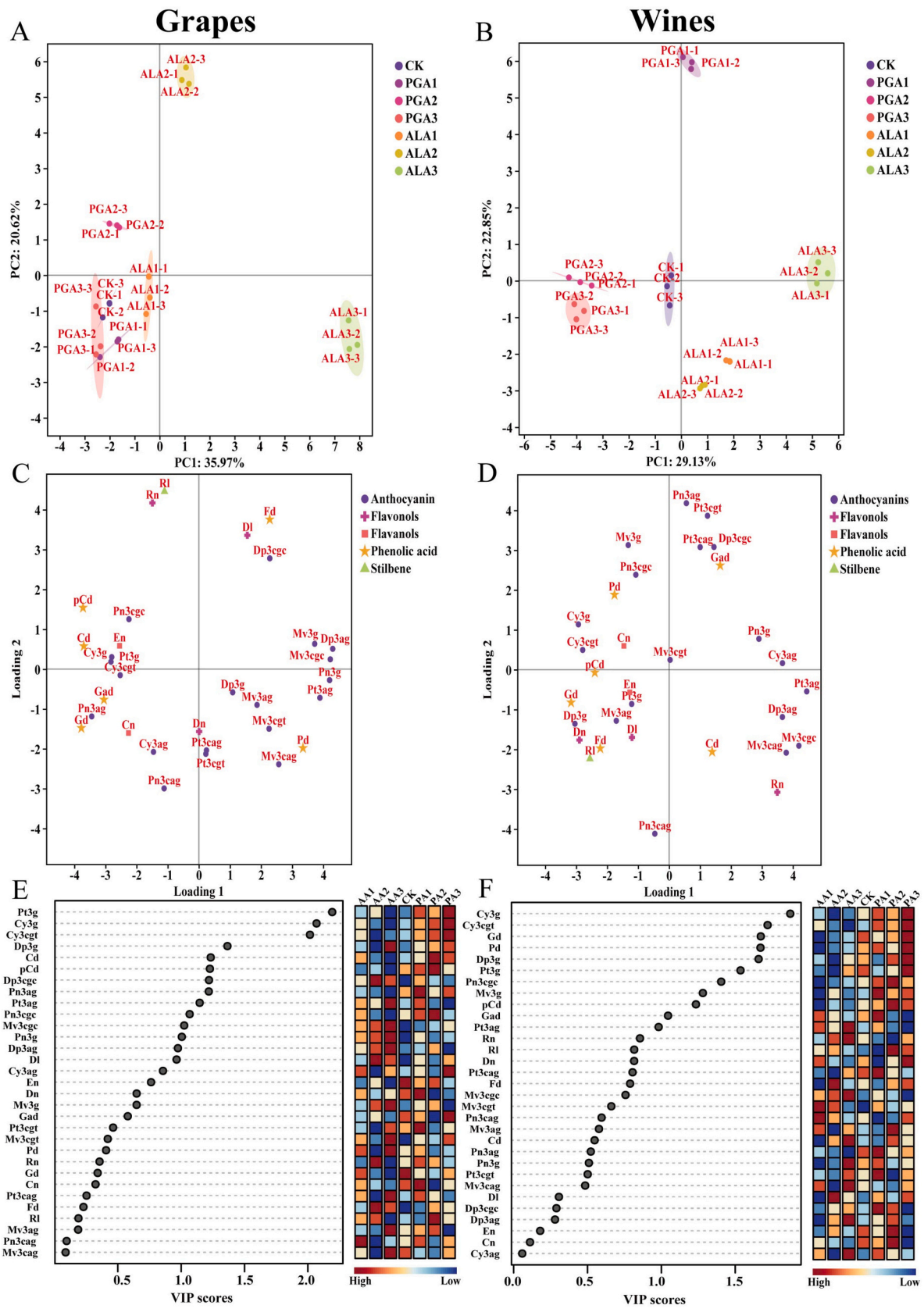
The wine score plot shown in Fig. 4B indicated that PC1 and PC2 explained 29.13 % and 22.85 % of the total variance in 2023, respectively. The treatment groups were distinctly separated, similar to the grape results, with ALA3 and CK mainly separated by PC1. The main characteristic phenolic compounds were Pt3ag and Mv3cgc. In 2023, PC2 primarily separated PGA1 and CK via Pn3ag, Pt3cgt, and Pn3cag (Fig. 4D). In 2024, the PC1 and PC2 explained 24.95 % and 20.44 % of the total variance, respectively (Fig. S6B, S6D).

To further elucidate the metabolic differences between the treatment and control groups, partial least squares discriminant analysis (PLS-DA) was conducted based on the PCA results. The most influential variables were identified using the variable importance in projection (VIP) scores. Compounds with VIP scores greater than 1 were considered the major contributors to the model. In 2023, 11 and 10 compounds in grapes and wines, respectively, had VIP scores greater than 1. Among them, Cy3g, Cy3cgt, Dp3g, Pt3g, pCd, and Pn3cgc were common differential metabolites. This highlights the impact of different treatments on the phenolic compounds in Marselan grapes and wines (Fig. 4E, F). In 2024, 14 and 10 compounds in grapes and wines, respectively, had VIP scores greater than 1, with pCd, Pn3g, Pd, Cd, and Cn as common differential metabolites (Fig. S6E. S6F). The compound consistently shared by both grapes and wines over two years, with VIP scores greater than 1, is pCd. This analysis is crucial for identifying biochemical markers that influence grape quality and wine flavor.

3.6. Antioxidant activity of grapes and wines

3.6.1. Grapes

This study employed three antioxidant evaluation systems—$ABTS^{+}$, DPPH, and CUPRAC—to assess the antioxidant activity of grape skins



(caption on next page)

Fig. 4. PCA and PLS-DA analysis of individual anthocyanins and non-anthocyanins in the 2023 vintage. 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). 0.25 % (v/v) PGA (PGA1); 0.35 % (v/v) PGA (PGA2); 0.45 % (v/v) PGA (PGA3); 0.25 % (v/v) alginate (ALA1); 0.35 % (v/v) alginate (ALA2); and 0.45 % (v/v) alginate (ALA3). Dp3g, Deplhinidin-3-*O*-glucoside; Dp3ag, Delphinidin-3-*O*-(6-acetyl)-glucoside; Dp3cgc, Delphinidin-3-*O*-(6-coumaryl)-glucoside; Cy3g, Cyanidin-3-*O*-glucoside; Cy3ag, Cyanidin-3-*O*-(6-acetyl)-glucoside; Cy3cgt, Cyanidin-3-*O*- (trans-6-*O*-coumaryl)-glucoside; Pt3g, Petunidin-3-*O*-glucoside; Pt3ag, Petunidin-3-*O*-(6-acetyl)-glucoside; Pt3cag, Petunidin-3-*O*-(6-coumaryl)-glucoside); Pt3cgt, Petunidin-3-*O*-(trans-6-*O*-coumaryl)-glucoside; Pn3g, Peonidin-3-*O*-glucoside; Pn3ag, Peonidin-3-*O*-(6-acetyl)-glucoside; Pn3cag, Peonidin-3-*O*-(6-caffeoyl)-glucoside; Pn3cgc, Peonidin-3-*O*-(cis-6-*O*-coumaryl)-glucoside; Mv3g, Malvidin-3-*O*-glucoside; Mv3ag, Malvidin-3-*O*-(6-acetyl)-glucoside; Mv3cag, Malvidin-3-*O*-(6-caffeoyl) -glucoside; Mv3cgc, Malvidin-3-*O*-(cis-6-*O*-coumaryl)-glucoside; Mv3cgt, Malvidin-3-*O*-(trans-6-*O*-coumaryl) -glucoside. Dn, Dihydroquercetin; Dl, Dihydrokaempferol; Rn, Rutin; Cn, Catechin; En, Epicatechin; Gad, Gallic acid; Pd, Protocatechuic acid; Fd, Ferulic acid; pCd, p-Coumaric acid;Gd, Gentisic acid; Cd, Caffeic acid; Rl, Resveratrol.

and wines, aiming for more comprehensive and reliable results (Fig. 5, S7). The $ABTS^+$ and DPPH assays measure antioxidant capacity based on free radical scavenging ability (Li et al., 2023). In 2023, treatments with PGA2, ALA2, and PGA3 enhanced $ABTS^+$ scavenging capacity by 26.25 %, 13.41 %, and 14.62 %, respectively, compared to CK (Fig. 5A). In 2024, PGA3 treatment significantly increased antioxidant activity by 20.97 % compared to CK (Fig. S7A). This enhancement may be due to increased NADPH availability, leading to higher $ABTS^+$ scavenging capacity (Olavarrieta et al., 2022). In 2023, ALA1 treatment, and in 2024, ALA2 treatment, significantly elevated DPPH radical scavenging activity relative to CK (Fig. 5B, S7B). Regarding the CUPRAC results, which reflect the reducing power of antioxidants, PGA2 and PGA3 were significantly higher than CK across both years, with PGA2 showing the the greatest increase (Fig. 5C, S7C). Over both years, ALA1 treatment enhanced CUPRAC activity compared to CK, with a significant 45.46 % increase observed in 2024.

3.6.2. Wines

The antioxidant activity results for wines were similar to those for grapes. For $ABTS^+$, PGA2 and PGA3 were significantly higher than CK in both years, with the high concentration of PGA3 being the most effective (Fig. 5D, S7D). The impact of the treatments on DPPH activity varied between the two years (Fig. 5E, S7E). In 2023, no significant differences were observed between the treatments and CK. However, in 2024, PGA2, PGA1, and ALA1 treatments significantly increased DPPH activity by 85.53 %, 46.33 %, and 28.40 %, respectively, compared to CK. In the CUPRAC assay, PGA2 demonstrated the strongest antioxidant capacity across both years, suggesting that PGA2 treatment can significantly enhance the reducing power of the wines (Fig. 5F, S7F).

By analyzing the free radical scavenging ability and reducing power, PGA treatment showed better effects in improving the antioxidant capacity of wines, especially at medium to high concentrations. The effects of ALA treatment were more complex and exhibited clear dose-dependence. Some literature has indicated that both PGA and ALA possess antioxidant capacities, but PGA may more effectively activate

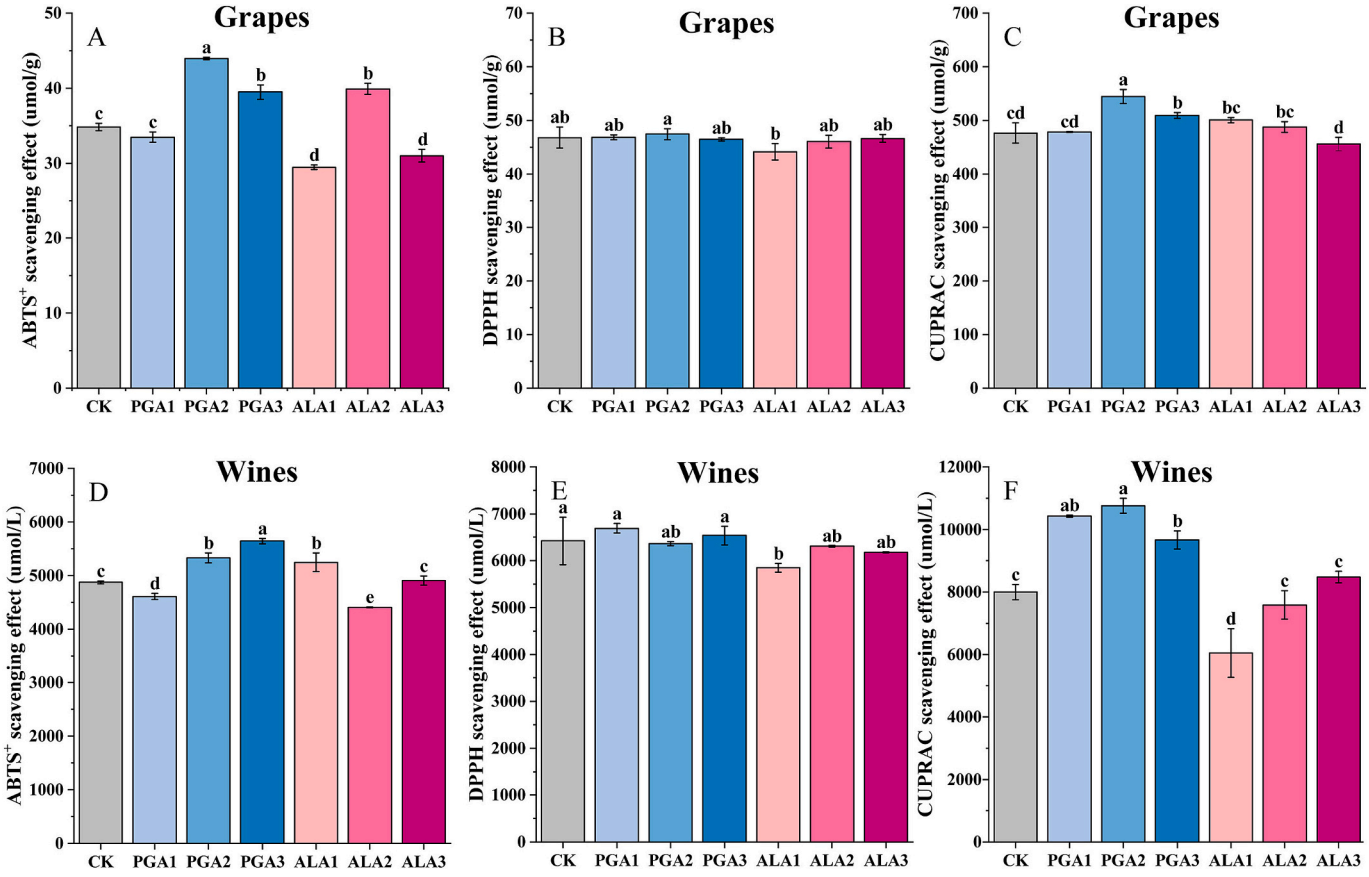


Fig. 5. Antioxidant capacity associated with grapes and wines in the 2023 vintage. $ABTS^+$ scavenging effect in grapes (A) and wines (D). DPPH scavenging effect in grapes (B) and wines (E). CUPRAC scavenging effect in grapes (C) and wines (F). 0.25 % (v/v) PGA (PGA1); 0.35 % (v/v) PGA (PGA2); 0.45 % (v/v) PGA (PGA3); 0.25 % (v/v) alginate (ALA1); 0.35 % (v/v) alginate (ALA2); and 0.45 % (v/v) alginate (ALA3). Data in bar is presented as mean ± SD (n = 3). Different letters indicate significant differences between treatments ($P < 0.05$).

antioxidant enzymes such as SOD and CAT in grapevines, defending against abiotic stress (Elbanna et al., 2024; Guo et al., 2020). It is also possible that medium to high concentrations of PGA enhance the accumulation of phenolic compounds, thereby increasing the antioxidant activity of grapes and wines (Fard & Hassanpour, 2023).

3.7. Correlation analysis of physicochemical properties, polyphenolic components, and antioxidant activities in grapes and wines

The quality and characteristics of wines are directly related to the chemical composition of grapes (Lu et al., 2022). This study explores the relationship between phenolic compounds and antioxidant activity in grapes and wines. We conducted a comprehensive analysis of physicochemical components, polyphenol content, and antioxidant activity, using Mantel test analysis to evaluate the overall relationship between these datasets (Fig. 6). Malic acid and citric acid exhibited a positive correlation in both 2023 and 2024, with a significant positive correlation observed in 2023 (Fig. 6A). This suggests that both compounds may share similar accumulation patterns during grape maturation. In 2024, malic acid showed a significant positive correlation with TPC (Fig. 6B). In 2023, both TAC and TPC exhibited positive correlations with the three antioxidant indicators, with TPC showing a high positive

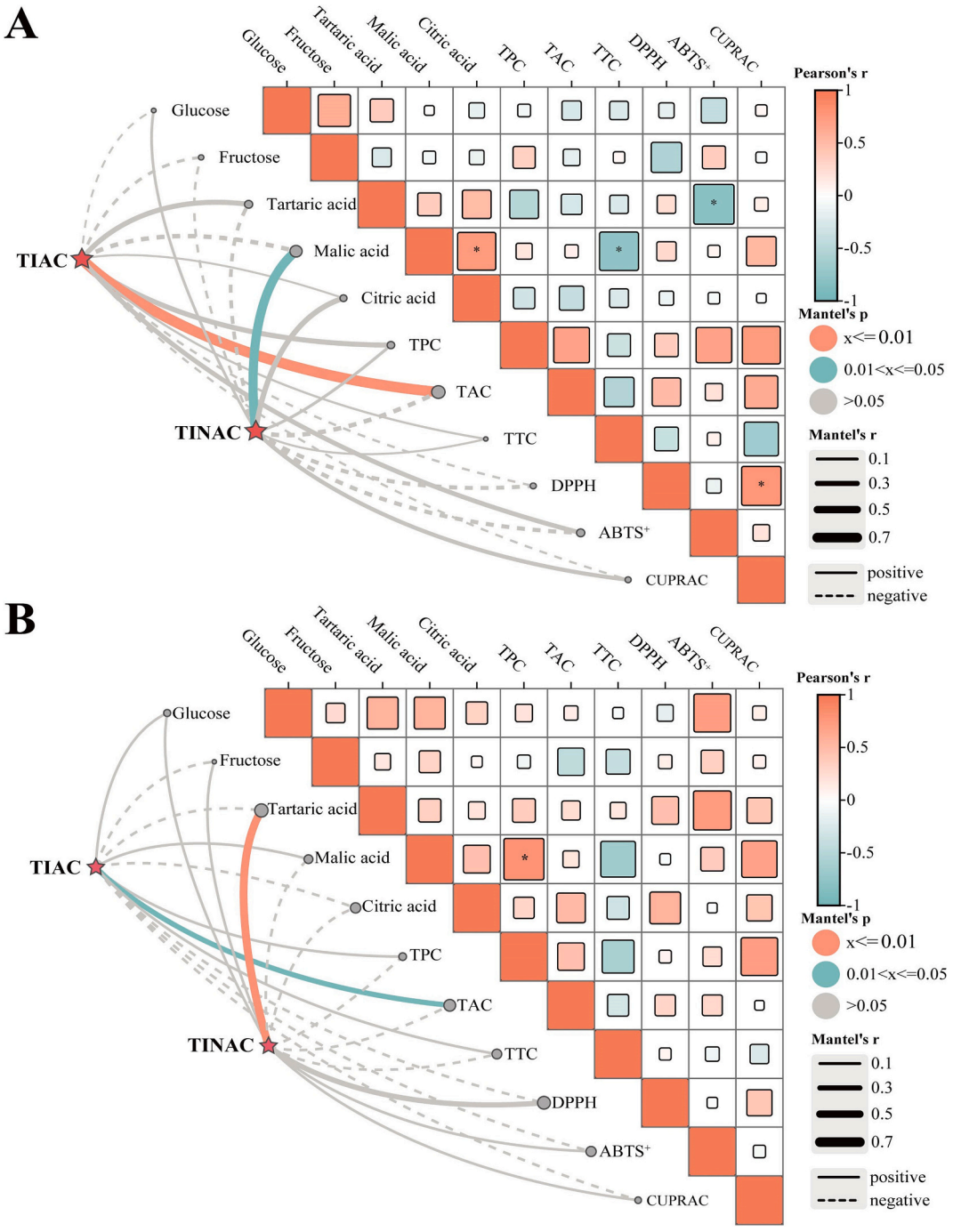


Fig. 6. Correlation analysis of physicochemical parameters, total phenolic content, and individual phenol profiles (including total individual anthocyanin (TIAC) and non-anthocyanin (TINAC) contents) in grapes and wines, as determined by the Mantel test using unweighted UniFrac distances in the 2023 (A) and 2024 (B) vintages. 0.25 % (v/v) PGA (PGA1); 0.35 % (v/v) PGA (PGA2); 0.45 % (v/v) PGA (PGA3); 0.25 % (v/v) alginate (ALA1); 0.35 % (v/v) alginate (ALA2); and 0.45 % (v/v) alginate (ALA3). * significant difference at $P < 0.05$ (Tukey's test).

correlation with CUPRAC in both years, further supporting the conclusion that higher phenolic content is associated with stronger antioxidant capacity. (Arioli et al., 2021; Cheng, Ma, et al., 2020). These correlations suggest that phenolic compounds are major contributors to the antioxidant power of grapes and wines. Therefore, increasing the content of phenols through viticultural methods can improve the health benefits and longevity of the wines.

Tartaric acid showed a significant negative correlation with $ABTS^{+}$, possibly due to its low reactivity and stability in antioxidant reactions. This negative correlation may indicate that although tartaric acid is abundant in grapes, it does not contribute significantly to antioxidant activity (Li et al., 2021). Over both years, Total individual anthocyanin content (TIAC) had a negative correlation with fructose, aligning with previous reports and possibly due to anthocyanin degradation during the later stages of ripening (Movahed et al., 2016; Xie et al., 2021). In 2023, TINAC correlated significantly with malic acid, and in 2024, with tartaric acid. These findings reveal a significant correlation between the phenolic content of grapes and wines, basic physicochemical parameters, and antioxidant capacity. Understanding these relationships offers valuable insights that can guide targeted interventions in vineyard management and winemaking processes, ultimately leading to enhanced wine quality.

3.8. Relative expression of anthocyanins biosynthesis genes

Previous studies have predominantly focused on evaluating the final composition of grapes and wines, often overlooking the gene expression of biosynthetic enzymes involved in the anthocyanin synthesis pathway (Xie et al., 2021). This study extends previous work by investigating the effects of various concentrations of PGA and ALA treatments on the expression of key genes within the anthocyanin biosynthesis pathway (Fig. S8, S9, S10). The genes *VvPAL*, *VvC4H*, and *VvCHS* are critical to the early stages of anthocyanin synthesis (Cheng, Ma, et al., 2020). Both PGA treatments significantly upregulated *VvPAL* gene expression across both years, with PGA1 and PGA2 exhibiting the most pronounced effects. These results are consistent with previous studies, which demonstrated that foliar application of glutamic acid in peaches promotes anthocyanin accumulation by upregulating the *PpPAL* gene and their tolerance to biotic stress (Kou et al., 2023). For *VvC4H*, both ALA1 and ALA3 treatments significantly downregulated gene expression in both years. With the exception of ALA2 in 2023, all treatments significantly upregulated the *VvCHS* gene relative to CK. Among them, PGA3 treatments consistently resulted in the highest *VvCHS* expression across both years. *VvCHS* catalyzes the formation of chalcones, a crucial step in the flavonoid biosynthesis pathway. The upregulation of *VvCHS* expression may contribute to the higher anthocyanin content observed in the PGA3 treated group compared to CK. *VvF3′H* and *VvF3′5′H* enzymes catalyze the transformation of flavonoid precursors into anthocyanins. Previous studies have demonstrated that these enzymes directly affect the 3′ and 5′ substitutions on the B-ring of anthocyanins, thereby altering the composition and relative proportions of individual anthocyanins (Cheng, Wang, et al., 2020). In both years, ALA3 treatment significantly upregulated *VvF3′H* gene expression, which may account for the notably higher Cy3g content observed in the ALA3 treated group compared to CK.

VvDFR and *VvLDOX* are essential enzymes in the anthocyanin biosynthesis pathway (Ju et al., 2019). Notably, both years of PGA treatments significantly upregulated DFR gene expression in comparison to CK. Various concentrations of ALA treatments promoted anthocyanin accumulation by upregulating *VvLDOX* gene expression. *VvUFGT* catalyzes the glycosylation of anthocyanins. Both ALA2 and ALA3 treatments significantly upregulated *VvUFGT* gene expression in both years. The VvGST gene is essential for facilitating the transport and accumulation of anthocyanins in plant tissues. The results indicate that, with the exception of PGA1 in 2024, the *VvGST* gene expression under other treatments did not differ significantly from CK. Our results suggest that PGA treatments have a more pronounced effect on berry color compared to ALA treatments, as reflected by higher gene expression levels in the PGA treated groups.

Previous studies have demonstrated that exogenous application of alginic acid induces a stress-like response in plants, leading to the activation of key transcription factors, such as MYB, bHLH, and WD40 (Matsuda et al., 2021). These factors regulate the expression of several structural genes in the anthocyanin biosynthesis pathway, including *VvUFGT*, *VvDFR*, and *VvANS*, which play critical roles in converting colorless flavonoid precursors into anthocyanin pigments. In this study, we observed that ALA3 treatment significantly increased anthocyanin content, primarily through the upregulation of TAAs. This effect is likely due to alginic acid's ability to regulate the expression of these key genes. Taken together, these findings suggest that exogenous PGA and ALA treatments influence gene transcription, thereby promoting anthocyanin accumulation.

4. Conclusion

This study evaluated the effects of the biostimulants PGA and ALA on the quality of Marselan grapes and wines. The results demonstrate that ALA3 and ALA2 treatments significantly increased grape soluble solids content by enhancing fructose levels in both 2023 and 2024. Furthermore, the two-year PGA2 treatment significantly increased titratable acid content in the berries. Moreover, PGA2 and PGA3 treatment more effectively enhanced antioxidant activity in both grapes and wines. Overall, PGA3, ALA3, and ALA1 treatments increased total phenol and anthocyanin content in both grapes and wines, with PGA3 showing the most pronounced effect. PGA3 increased total anthocyanin content by boosting Dps and Mvs anthocyanins, while ALA3 increased anthocyanin levels by elevating Cys content. Among these, PGA3 treatment significantly upregulated the levels of Dp3ag, Cy3ag, Pn3g, and Mv3cgt in both years. PCA and PLS-DA analyses identified pCd as a key biochemical marker affected by PGA and ALA treatments over two years, which in turn influenced grape quality and wine flavor. PGA3 increased anthocyanin content by upregulating *VvPAL*, *VvCHS*, *VvDFR*, and *VvLDOX* gene expression, while ALA3 and ALA1 treatments increased anthocyanin content by upregulating *VvCHS*, *VvF3′H*, and *VvUFGT*.

In summary, treatments with PGA2, PGA3, ALA3, and ALA1 all increased the phenolic compounds and antioxidant activity in both grapes and wines, with PGA3 showing the most pronounced effect. As environmentally friendly biostimulants, PGA and ALA hold significant potential for sustainable agriculture. This study establishes a foundation for further research on the regulation of phenolic compounds and antioxidant properties in grapes and wines by PGA and ALA, potentially advancing their application in improving grape and wine quality.

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CRediT authorship contribution statement

Huawei Chen: Writing – original draft, Data curation. **Lijian Zhang:** Methodology. **Bowei Yang:** Software. **Miaomiao Wang:** Writing – review & editing. **Litao Ma:** Resources, Investigation. **Jingjing Shi:** Methodology, Investigation. **Zhenwen Zhang:** Writing – review & editing, Funding acquisition. **Qingqing Zeng:** Writing – review & editing, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.102112.

Data availability

Data is contained within the article.

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