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Foliar-sprayed manganese sulfate improves flavonoid content in grape berry skin of Cabernet Sauvignon (*Vitis vinifera* L.) growing on alkaline soil and wine chromatic characteristics

Huangzhao Chen[a,b], Jun Yang[a], Xiaoxu Deng[b], Yujuan Lei[a], Sha Xie[a], Shuihuan Guo[a], Ruihua Ren[a], Junnan Li[a], Zhengwen Zhang[a,*], Tengfei Xu[c,*]

[a] College of Enology, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] College of Food and Biological Engineering, Henan University of Animal Husbandry and Economy, Zhengzhou, Henan 450046, China
[c] State Key Laboratory of Crop Stress Biology for Arid Areas, College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100, China

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ABSTRACT

Flavonoids are key determinants of grape quality and wine color. Grapevines growing in alkaline soil are prone to manganese deficiency, which can decrease the contents of secondary metabolites, including flavonoids. We determined the effects of a foliar Mn treatment ($MnSO_4 \cdot H_2O$) of Cabernet Sauvignon grapevines (*V. vinifera* L.) growing in alkaline soil on the flavonoid contents in grape skin, and the quality of wine. The Mn treatments were applied in 2017 and 2018, and tended to increase the grape sugars, berry weight, and the contents of phenolic compounds from veraison until harvest. The Mn treatments increased the amounts of acetylated, methylated, and total anthocyanins, as well as the total flavonol contents in grape berry skin at harvest. The wines prepared from these grapes had a higher color intensity than those prepared from grapes from control vines. Foliar-applied $MnSO_4 \cdot H_2O$ can promote flavonoid biosynthesis in grape berries, and improve the color of wine.

1. Introduction

Manganese (Mn) is a necessary microelement for plants. Manganese ions act as an activator for about 35 enzymes in plants, notably those involved in phenylpropanoid metabolism pathways leading to the synthesis of various secondary metabolites (e.g. flavonoids, lignin) and auxin (Burnell, 1988). Only a fraction of enzymes contains Mn as part of their structure, for example, the Mn-protein in PS II and the Mn-containing superoxide dismutase (MnSOD), which play vital roles in the Hill Reaction (oxygen evolution) and in maintaining the structure of chloroplast membranes (Schmidt, Jensen, & Husted, 2016). Manganese can induce protective mechanisms and enhance the host plant's resistance (Burnell, 1988). Consequently, exogenous Mn can activate the synthesis of soluble phenolic compounds and induce some defense responses; this effect occurs both locally at the application site and systemically in distant organs (Burnell, 1988; Ruiz-García et al., 2013; Yao

* Corresponding authors at: College of Enology, Northwest A&F University, No. 22 Xinong Road, Yangling, Shaanxi 712100, China.
E-mail addresses: guaiwuclever1986@nwafu.edu.cn (H. Chen), leiyujuan@nwsuaf.edu.cn (Y. Lei), xiesha@nwafu.edu.cn (S. Xie), zhangzhw60@nwsuaf.edu.cn (Z. Zhang), tengfei.xu@nwafu.edu.cn (T. Xu).

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et al., 2012). Many biochemical reactions that involve Mn cannot function properly in Mn-deficient plants.

Manganese deficiency often occurs in alkaline soils. Interveinal chlorosis on young leaves is the most obvious symptom of severe Mn deficiency. However, in recent years, hidden Mn deficiency (no visible symptoms) has become widespread, and this has affected the yields and quality of many crops, leading to substantial economic losses. Grapevine is particularly sensitive to Mn deficiency (Alloway, 2008; Schmidt et al., 2016). In China, soil surveys have revealed that 20.3 M ha (21.3% of cultivated land) is latent Mn-deficient, and that the contents of available Mn in soil are higher in South China than in North China (Chunqin Zou, 2008). However, the main vintage areas are located in northwest China. Among them, the Eastern Helan Mountain of Ningxia is one of the premium vintage areas (Song et al., 2015).

In previous studies, the effects of Mn application on physicochemical indexes (i.e. total soluble sugars, acids, berry weight) have been studied extensively in diverse fruits, e.g. table grape (Batukaev, Magomadov, Chavanov, Sushkova, & Deryabkina turina, 2018), apple (ElShazly, 2004) and pomegranate (Hasani, Zamani, Savaghebi, & Fatahi, 2012). However, few studies have focused on the effects of Mn application on the accumulation of phenolic compounds in fruits. Hasani et al. (2012) showed that a foliar application of Mn increased the total anthocyanins content in pomegranate, compared with the control. Acuna-Avila, Vasquez-Murrieta, Hernandez, and Lopez-Cortez (2016) found a close relationship between Mn content in soil and catechin content in wine grapes. To our knowledge, the effect of a foliar Mn treatment on the flavonoid profile of grape berries has not been determined in any previous studies.

The types of phenolic compounds, especially flavonoids, in grapes have technological importance for wine making (Sun et al., 2020). In this sense, anthocyanins have a crucial role in determining the color of red grapes and wines (Cheng, He, Yue, Wang, & Zhang, 2014). Among the non-anthocyanin phenolics, flavonols contribute to wine color as copigments and are related to the health-promoting properties of wine (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007); Flavanols (flavan-3-ols or proanthocyanidins) are important for the organoleptic properties of wine (astringency or bitterness) (Yang, He, et al., 2020; Yang, Yao, et al., 2020; Ma et al., 2019) and for color stability (Kennedy, Saucier, & Glories, 2006).

In this study, we tested the hypothesis that a foliar application of Mn to grapevines growing in alkaline soil will increase biosynthesis of the flavonoid compounds in grape berries and improve the chromatic characteristics of wine prepared from those grapes.

2. Materials and methods

2.1. Plant material and field treatments

The field experiment was conducted in a commercial vineyard of the wine company "Guanlan" (38°43′1″N, 106°3′43″E, 1220 m altitude), located about 50 km northwest of Yinchuan (Ningxia, China). The treatment was applied during two consecutive vintages, 2017–2018, and the field had not received any Mn fertilizer previously. The grapevines were 4-year-old Cabernet Sauvignon (*Vitis vinifera* L.). The vines were self-rooted, north–south oriented, spaced at 3.0 m × 0.8 m, and were selected on the basis of uniform vigor. All the vines were trained to a slope trunk with a vertical shoot-positioning trellis system, drip-irrigated, and managed using standard vinicultural procedures.

Three biological replicates per treatment were established with a randomized complete-block design, with 24 vines per biological replicate and excluding marginal rows and vines. Isolation vines were placed between treatments in the same row. The vines were sprayed using a backpack hand-pressure sprayer (16 L), filled with an aqueous solution of manganese sulfate monohydrate ($MnSO_4{\cdot}H_2O$) with 1% Tween 80 as a wetting agent. The control consisted of tap water containing 1% Tween 80. The Mn^{2+} was applied to leaves at three concentrations: 300 mg/L (low, L), 1200 mg/L (medium, M), and 2400 mg/L (high, H). The applied Mn^{2+} concentration was chosen according to previously published papers, in which the concentration in the range of 500 mg/L-2700 mg/L was used (Hasani et al., 2012; Moosavi & Ronaghi, 2011; Tariq, Sharif, Shah, & Khan, 2007). The pH was adjusted to 7.0 with 0.1 M NaOH or HCl (Reisenauer, 1988; Yao et al., 2012). The Mn^{2+} solution was sprayed twice on both sides of the canopy, 1 week before and 1 week after first bloom, respectively. Treatments were done late in the afternoon (5–7p.m.) and during favorable weather conditions where rainfall or winds were not forecasted for the following 24 h. Vines sprayed until most of the leaves got wet and each vine received about 100 ml Mn^{2+} solution. The Mn^{2+} solution was sprayed twice on both sides of the canopy, 1 week before and 1 week after first bloom, respectively. Each vine received about 100 ml Mn^{2+} solution.

The climate conditions markedly affected berry growth and development (Supplementary Table 1). The 2017 season had higher precipitation and lower temperatures than the 2018 season during vegetative stage (April to July), so the date of first bloom differed by 10 days between the two years (May 25 in 2017, May 15 in 2018).

2.2. Plant and soil samples analysis

On one hand, we conducted a full nutrient diagnosis of the grapevines before treatment by analyzing soil and plant tissue (grape petiole) (Alloway, 2008), sampled as described by Cheng et al. (2014) and Song et al. (2015), respectively. On the other hand, twenty-four complete, disease-free and non-senescent leaves from the middle part of previously treated shoots (old leaves) were collected per replicate for chemical analysis at harvest stage in 2018. All samples were sent to the College of Resource and Environment (Northwest A & F University, Yangling, China) for analysis.

2.3. Berry sampling and general parameters

Three 100-berry samples per biological replicate were randomly selected from different positions within each cluster and from clusters on the sunny and shady side. Berries were collected by cutting the pedicle using scissors, and then stored in ziplock bags. Sampling was conducted at 45, 70, 86, and 110 DAA (days after anthesis) in 2017 and at 45, 68, 92, and 123 DAA in 2018. Veraison was at 70 and 68 DAA and harvest was at 110 and 123 DAA in 2017 and 2018, respectively. The harvest time was when the mean Brix value was 23° to 24°. Maximum temperatures were 1.7 °C higher and precipitation was lower (76.2 mm *vs.* 228.1 mm) in 2017 than in 2018 during the grape ripening period (July to September). Hence, the harvest date was 13 days later in 2018 (September 22nd) than in 2017 (Supplementary Table 1).

The samples were kept in an icebox to avoid dehydration, then brought back to the laboratory and stored at −40 °C. For each replicate, before removal of skins, berries were weighed (g) (100-berry weight). After skins were removed, both skins and seeds were rinsed three times with deionized water and weighed, the pulp weight was calculated by their subtraction from the individual-berry weight, and the pulp was put in nylon mesh to press juice manually. The juice was analyzed to determine total soluble solids (TSS), titratable acidity content (TAC), and pH as described by Shi et al. (2018).

2.4. Vinification

Wine was made as described in previous articles (Pérez-Magariño & González-San José, 2004; Ruiz-García et al., 2012). Grapes (about 20 kg from each replicate) were crushed and destemmed, and potassium metabisulfite and pectinase (Lallzyme Ex, Lallemand, France) were added to a final concentration of 60 mg/L SO_2 and 30 mg/L, respectively. Yeast (Lalvin D254, Lallemand, France, 200 mg dry yeast/L must) was added soon afterwards. All vinification procedures were

conducted at 22 ± 1 °C. About 1 week later, when specific gravity had decreased to 1.000, the pomace was racked at 1.0 bar in a 50-L atmosphere presser. Free-run and press wines were combined, and then alcohol fermentation proceeded until dryness (reducing sugars < 4 g/L). Aliquots of each wine were stored at −20 °C until analysis.

2.5. Pretreatment of grape skins

Skins were peeled by hand from 100 frozen berries per replicate without thawing and then freeze-dried at −40 °C for 36 h. The dried skin was then ground in liquid nitrogen with a chilled mortar and pestle. The weights of fresh berries, fresh skins, and dried skins were weighed and recorded.

2.6. Spectrophotometric characterization

Total phenols (TP), total flavonoids (TFO), proanthocyanidins (PA), and total flavanols (TFA) were extracted at four developmental stages, and total monomer anthocyanins (TMA) were extracted after veraison in both years (Ma et al., 2019). All these compounds were extracted from dried skin powder as described previously (Meng, Fang, Qin, Zhuang, & Zhang, 2012; Song et al., 2015). The absorbance of each extract at particular wavelengths was measured using a multiplate reader (Infinite M200 Pro, Tecan, Mannedorf, Switzerland). Two extractions were conducted for each biological replicate. The data are expressed as mg equivalents of the respective standard (i.e. gallic acid for TP, rutin for TFO, catechin for PA & TFA, cyanidin-3-monoglucoside for TMA) per kg grapes fresh weight.

2.7. HPLC determination of individual flavonoids in skin and wine

The monomer flavonoid compounds were extracted from skins independently twice per replicate as described by Shi et al. (2018). Flavonoids in wine and skin were detected and identified as described by Li, Pan, Jin, Mu, and Duan (2011). The relative amounts of anthocyanins, flavonols, and flavanols are expressed as equivalents of malvidin-3-*O*-glucoside, quercetin-3-*O*-glucoside, and (+)-catechin, respectively. All flavonoid contents are expressed as mg/kg grapes fresh weight, μg/g skin fresh weight, or mg/L wine.

2.8. Determination of wine color

Wine color was analyzed as described by Pérez-Magariño and González-San José (2006) using a UV-2450 spectrophotometer (Shimadzu, Japan). The parameters defining the CIELAB space are L* (lightness), a* (green/red), b* (blue /yellow), and C (chroma) = $(a^{*2} + b^{*2})^{1/2}$.

2.9. Statistical analyses

Univariate ANOVA was conducted using SPSS 20.0 software for Mac. Significant variations were detected by Tukey's test at $P \leq 0.05$. Data are shown as mean ± SD.

3. Results and discussion

3.1. Mn deficiency diagnosis

The results of the soil analyses are shown in Supplementary Table 2. The contents of organic matter, available P, and Mn and Fe in soil were below the normal values (Shi et al., 2018; Song et al., 2015), while the pH and contents of available Ca and Cu exceeded the critical range. The contents of available N and K as well as Mg and Zn were within the standard range. These results confirm that the soil was highly calcareous and alkaline, which results in Mn deficiency, and that the concentration of available Mn was very low. Unlike most higher plants, the petiole rather than the leaf blade accurately reflects the element status of grapevines. The critical range of Mn in petioles is 30–650 mg/kg dry weight (Li gangli, 1987), and the mean Mn content of petioles was at the lower end of that range (59.3 mg/kg dry weight). There were no visible Mn deficiency symptoms on grapevines at the experimental site. Therefore, the test vines were under latent Mn deficiency.

3.2. Effects of Mn foliar fertilizer on Mn concentration of grape leaves

Compared to applying Mn by soil fertilization, foliar application has been considered a more short-term yet efficient approach to increasing Mn concentrations in plants (Burnell, 1988). In the present study, all treatments markedly increased the Mn concentrations within grape leaves compared with control at harvest (Supplementary Table 3). However, with the increase of spraying concentration, leaf Mn contents were not continuously enhancing, perhaps because of the positive correlation existed in the range of certain amount between foliage sprayed Mn concentration and its absorption rate (Yan-ting, Xiu-ying, Yan, Bing-qiang, & Li-xia, 2009). Therefore, the leaves from M treatment has the greatest absorption efficiency, the Mn concentration reaches about 179 mg/kg (1.73-fold increased).

3.3. General analytical parameters

The total soluble solids, titratable acidity contents, pH of the berries and skin/pulp ratio are presented in Table 1. Some physicochemical indexes of grape berries are known to be related to flavonoid content. The Mn treatment tend to increase TSS, 100-berry weight and the skin to pulp ratio from veraison to harvest. The differences in TSS between the treatments and control were significant only in 2017, the 100-berry weight was significantly higher in Mn-treated vines than in control vines only in 2018. And there was a similar trend in a marked enhancement in skin to flesh ratio both years as foliage-sprayed manganese sulfate at harvest. The Mn treatments did not affect the TAC, pH of grape berries.

The rainfall was abnormally heavy in August 2018 (Supplementary Table 1), resulting in a sharp increase in berry weight from 68 to 92 DAA. This amplified the effect of the Mn treatments. However, the lower sunlight hours in September 2018 severely affected dry matter accumulation, and correspondingly weakened the effects of the Mn treatment on TSS at harvest. Manganese is a cofactor for indole acetic acid oxidase systems (Burnell, 1988). Hence, Mn nutrition affects the level of auxin, which regulates cell division in plants (Eskandari, Khoshgoftarmanesh, & Sharifnabi, 2018). Hasani et al. (2012) found that a foliar spray of Mn sulfate positively affected the 100-aril weight of pomegranate. Similar findings have been reported for apple (ElShazly, 2004). Manganese also plays a fundamental role in photosynthesis (Schmidt et al., 2016). Thus, Mn deficiency has strong adverse impacts on many biological processes. Under Mn deficiency, the amount of chloroplast pigments decreases and so does the net photosynthesis rate (Sherman, Heerema, VanLeeuwen, & St. Hilaire, 2017). These negative effects directly affect the accumulation of photosynthetic products (sugars). In previous studies, foliar Mn spray treatments of pomegranate (Hasani et al., 2012) and grape (Batukaev, Magomadov, Chavanov, Sushkova, & Deryabkina turina, 2018) growing in alkaline soil increased the TSS contents in their fruits.

3.4. Phenolic composition in skin of grape berries at different developmental stages

The TP, TFO, PA, TFA, and TMA are crucial quality indicators of grapes and wines. The total phenolic parameters in skins of grapes at different developmental stages in 2017 and 2018 are shown in Figs. 1 and 2, respectively. These values were mostly consistent with the range of values reported in other studies (Fanzone, Zamora, Jofré, Assof, & Peña-Neira, 2011; Meng et al., 2012).

Table 1
Physicochemical analyses of developing berries in 2017 and 2018.

parameter	year	DAA[d]	L	M	H	CK
total soluble solids (°Brix)	2017	45	4.0 ± 0.3 a[a]	3.7 ± 0.1 a	3.6 ± 0.1 a	3.6 ± 0.2 a
		70[b]	10.7 ± 0.3 b	11.2 ± 0.4 b	12.1 ± 0.2 a	9.8 ± 0.1 c
		86	19.0 ± 0.1 a	18.9 ± 0.1 a	19.2 ± 0.1 a	18.3 ± 0.2 b
		110[c]	24.0 ± 0.2 a	23.8 ± 0.1 a	24.2 ± 0.3 a	23.0 ± 0.1 b
	2018	45	3.4 ± 0.2 a	3.7 ± 0.1 a	3.4 ± 0.1 a	3.5 ± 0.2 a
		68[b]	11.4 ± 0.1 a	10.8 ± 0.1 a	11.0 ± 0.4 a	10.4 ± 0.2 b
		92	21.7 ± 0.4 a	20.5 ± 0.4 b	20.5 ± 0.2 b	19.3 ± 0.1 c
		123[c]	23.0 ± 0.2 a	23.2 ± 0.1 a	23.0 ± 0.4 a	22.8 ± 0.3 a
titratable acidity (g/L of tartaric acid)	2017	45	30.81 ± 0.51 a	29.79 ± 0.94 a	28.80 ± 0.86 a	29.84 ± 0.78 a
		70[b]	18.33 ± 0.66 a	17.70 ± 0.54 a	17.39 ± 0.35 a	18.69 ± 0.41 a
		86	5.70 ± 0.32 a	5.82 ± 0.11 a	5.91 ± 0.14 a	5.57 ± 0.45 a
		110[c]	4.47 ± 0.13 a	4.55 ± 0.12 a	4.36 ± 0.14 a	4.73 ± 0.18 a
	2018	45	29.72 ± 0.81 a	30.38 ± 0.97 a	28.82 ± 0.76 a	28.18 ± 0.19 a
		68[b]	12.34 ± 0.87 a	11.68 ± 0.71 a	10.77 ± 0.90 a	10.27 ± 0.82 a
		92	5.36 ± 0.18 a	5.33 ± 0.02 a	5.14 ± 0.16 a	5.12 ± 0.17 a
		123[c]	4.55 ± 0.21 a	4.82 ± 0.14 a	4.71 ± 0.25 a	4.53 ± 0.12 a
pH	2017	45	2.62 ± 0.03 a	2.61 ± 0.01 a	2.65 ± 0.02 a	2.59 ± 0.01 a
		70[b]	2.90 ± 0.03 a	2.99 ± 0.05 a	3.02 ± 0.12 a	2.92 ± 0.02 a
		86	3.62 ± 0.03 a	3.69 ± 0.04 a	3.75 ± 0.08 a	3.68 ± 0.02 a
		110[c]	4.03 ± 0.03 a	4.01 ± 0.04 a	4.04 ± 0.05 a	4.14 ± 0.07 a
	2018	45	2.84 ± 0.01 b	2.83 ± 0.01 b	2.94 ± 0.01 a	2.94 ± 0.02 a
		68[b]	3.25 ± 0.02 a	3.27 ± 0.06 a	3.33 ± 0.02 a	3.28 ± 0.01 a
		92	3.94 ± 0.02 a	3.95 ± 0.04 a	4.02 ± 0.01 a	3.96 ± 0.05 a
		123[c]	4.05 ± 0.02 a	4.09 ± 0.04 a	3.99 ± 0.06 a	3.98 ± 0.05 a
100-berry weight (g)	2017	45	67.3 ± 2.2 a[a]	69.1 ± 2.0 a	68.4 ± 2.7 a	72.0 ± 4.9 a
		70[b]	95.2 ± 1.1 a	94.9 ± 1.3 a	97.2 ± 2.9 a	94.1 ± 1.8 a
		86	110.0 ± 2.3 a	112.1 ± 2.4 a	107.4 ± 1.2 a	105.0 ± 3.8 a
		110[c]	122.0 ± 4.5 a	118.4 ± 3.2 a	116.1 ± 3.8 a	113.9 ± 2.6 a
	2018	45	52.9 ± 2.4 a	54.8 ± 1.5 a	59.5 ± 3.2 a	57.5 ± 1.2 a
		68[b]	79.9 ± 2.3 a	77.6 ± 1.4 a	80.0 ± 2.2 a	72.1 ± 2.1 b
		92	137.2 ± 1.5 a	130.5 ± 2.4 b	127.2 ± 1.3 bc	121.7 ± 2.9 c
		123[c]	128.7 ± 2.8 ab	129.9 ± 1.5 a	132.6 ± 3.9 a	121.8 ± 2.3 b
skin/pulp ratio (%)	2017	45	10.4 ± 0.2 a	10.3 ± 0.3 a	10.1 ± 0.1 a	10.6 ± 0.4 a
		70[b]	11.4 ± 0.3 a	11.7 ± 0.2 a	11.3 ± 0.1 a	11.1 ± 0.4 a
		86	13.2 ± 0.2 a	13.3 ± 0.4 a	13.1 ± 0.4 a	12.7 ± 0.3 a
		110[c]	14.8 ± 0.4 a	14.7 ± 0.1 a	14.5 ± 0.3 ab	13.9 ± 0.2 b
	2018	45	13.2 ± 0.1 a	13.1 ± 0.4 a	13.5 ± 0.2 a	13.8 ± 0.3 a
		68[b]	25.5 ± 0.4 a	25.2 ± 0.7 a	25.3 ± 0.2 a	24.3 ± 0.5 a
		92	19.0 ± 0.3 ab	19.2 ± 0.4 a	18.7 ± 0.2 ab	18.2 ± 0.4 b
		123[c]	22.7 ± 0.7 ab	23.3 ± 0.6 a	22.2 ± 0.2 ab	21.6 ± 0.4 b

[a]Different lowercase letters in the same row indicate significant differences between treatments as calculated by Tukey's test ($p < 0.05$). [b]Veraison. [c]Harvest time. [d]DAA, days after anthesis.

During development in 2017, the TP and TMA contents in grape skins increased progressively, while the TFO, PA, and TFA contents decreased until the third sampling and then slightly increased at harvest. Similar general trends were observed in 2018, but because of the large differences in climate conditions between the two years, the atypical vintage in 2018 had certain differences in phenolic composition compared with that of the 2017 vintage. Firstly, the phenolic compounds contents at all sampling dates were lower in 2018 than in 2017, and the changes in phenolic compounds contents over time were relatively small. Secondly, the changes in TMA contents showed the largest difference between 2017 and 2018. The TMA content peaked at 92 DAA and then decreased until harvest in 2018. A similar trend was reported by Fanzone and n., Zamora, F., Jofré, V., Assof, M., & Peña-Neira, A. l. (2011), who found that the anthocyanin content decreased at the late developmental stage when precipitation was higher and air temperature was lower in the first test year.

Various physiology and biochemistry changes happen in grape berries around veraison, such as the initiation of sugar accumulation and berry coloring, and the remarkable change of proportions of different plant hormones and phenolics. In the present study, noticeable differences of phenolics contents occurred between the Mn-treated groups and the control group after veraison, especially in M in 2017 and L in 2018.

At harvest, the effects of Mn treatments on phenolic compounds in berry skins mainly reflected their respective accumulation patterns during development. The most significant promoting effects were in M in 2017 and L in 2018. The contents of TP, TFO, PA, TFA were increased by 12.3%, 17.6%, 25.3%, and 16.5%, in M in 2017, and by 11.4%, 18.8%, 41.5%, and 15.4%, respectively, in L in 2018, compared with their respective controls. The TMA contents were significantly higher in all Mn-treated groups than in the control in both 2018 and 2017 (increased by 17.0%–20.4% and 16.0%, respectively, compared with the control). Because flavonoids account for a large proportion of phenolic compounds in berry skin (Meng et al., 2012), the TFO content is often correlated with the TP content. Manzoor, Anwar, Mahmood, Rashid, and Ashraf (2012) also found that the higher the Mn concentration in peach peel, the greater the TP and TFO contents. Few studies have focused on the relationship between Mn content and PA content in fruit, but Mn content is known to be positively related to PA content in leaves (Santos, Fortes, Ferri, & Santos, 2011). As for the relationship between Mn and TMA, Boussaa et al. (2019) reported that a stronger red hue of arils was correlated with high Mn and TMA contents in pomegranate fruits.

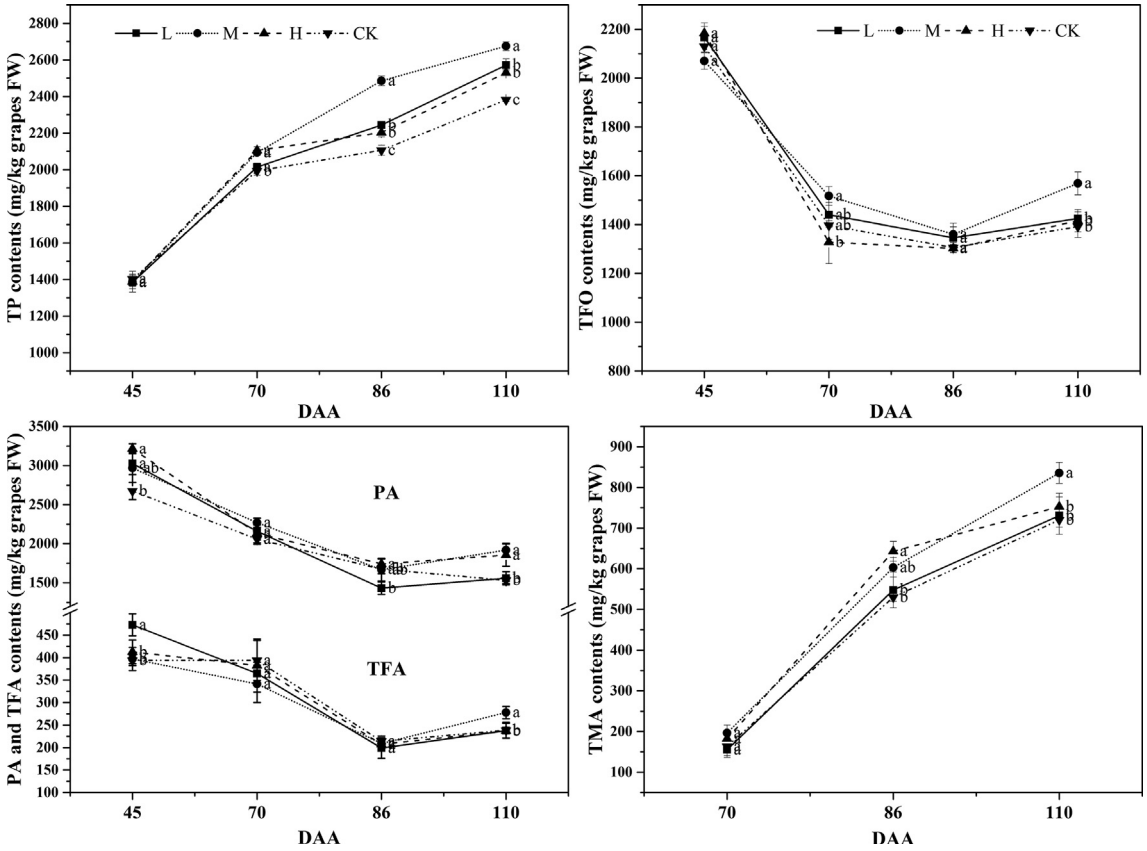

Fig. 1. Contents (mg/kg grapes fresh weight) of total phenols (TP), total flavonoids (TFO), proanthocyanins (PA), total flavanols (TFA), and total monomer anthocyanins (TMA) in skin of grape berries at different developmental stages in control and Mn treatments in 2017. Sampling date corresponds to days after anthesis (DAA). Data are mean ± SD (n = 3).

3.5. Maturity index

Supplementary Table 4 shows the results of maturity index in different treatments at harvest both years. The ripeness of grapes at the harvest time is one of the most important parameters for obtaining high quality red wines. Two types of maturity can be distinguished: technological and phenolic maturities. Technological maturity corresponds to the stage when sugar accumulation in the pulp is maximum, low acidity and high sugar/acidity ratio. Phenolic maturity is defined as the stage when the anthocyanin compounds reach maximum concentration in the skins (Meléndez, Ortiz, Sarabia, ÍÑiguez, & Puras, 2013). And the balance between technological maturity parameters and phenolic maturity plays a crucial role in wine quality. In this study, the ratio between TSS and grape acidity was significantly modified by foliar Mn fertilizer applied only in 2017. On the other hand, the TMAC/TSS was significantly higher than that of the control in two years. From a sensory standpoint, the higher TSS and TMAC produced wines with less green or vegetative attributes and more dark fruit attributes.

3.6. Flavonoid profiles in grape skin as determined by HPLC

3.6.1. Anthocyanin composition

In the HPLC analyses, 18 anthocyanins were identified and quantified in all samples (Table 2): glycosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin; their acetyl (3-acglc) and *trans-p*-coumaroyl (tr-3-cmglc) derivatives (except for delphinidin tr-3-cmglc); and the *cis-p*-coumaroyl (*cis*-3-cmglc) and caffeoyl (3-cfglc) derivatives of peonidin and malvidin. The concentrations of monomer anthocyanins in grape skins differed markedly between the two years, but the types and quantities detected were within the range reported elsewhere (Wang et al., 2018). Malvidin derivatives were the most abundant anthocyanins in the grape skin, accounting for 70%–80% of total anthocyanins. Acylated anthocyanins accounted for about 40% of total anthocyanins. Of these, acetyl derivatives were dominant (75% of all acylated anthocyanins).

Anthocyanin synthesis was enhanced by the foliar Mn treatments in both years. The total individual anthocyanin contents were higher in 2017 than in 2018. The results of HPLC analyses were consistent with those obtained by spectrophotometric analysis. The total monomer anthocyanin contents were, on average, 24.9% higher in the Mn treatments than in the control (on a fresh weight basis) in 2017. Specifically, there were significant increases mainly in non-acylated, Mv-acglc, and Mv-tr-cmglc anthocyanins in all Mn treatments, as well as in Dp-acglc, Cy-acglc, and Pt-acglc in the M and H treatments. In 2018, the Mn treatments resulted in an average increase of 12.1% (on a fresh weight basis) in total anthocyanin contents. The increases in monomer contents in 2018 were similar to those in 2017, the main differences among treatments were in the contents of acetylated/methylated anthocyanins (all increased in M; peonidin- and petunidin-type anthocyanins increased in L; petunidin-type anthocyanins increased in H), in addition, L and M treatments increased the contents of Mv-cfglc.

To further analyze the potential effects of foliar Mn treatments on anthocyanin profiles in grapes and wine, it is important to analyze different types of anthocyanins. The Mn treatments significantly increased the contents of acetylated and methylated anthocyanins by 20.53% and 25.20%, respectively, in 2017, and by 7.16% and 10.92%, respectively, in 2018, compared with their respective controls (Table 3).

The promoting effects of Mn treatments could be the consequence of increased soluble sugars content, which has been shown to activate the expression of several genes and transcription factors in the flavonoid pathway (Zheng et al., 2009). Aside from its essential role in

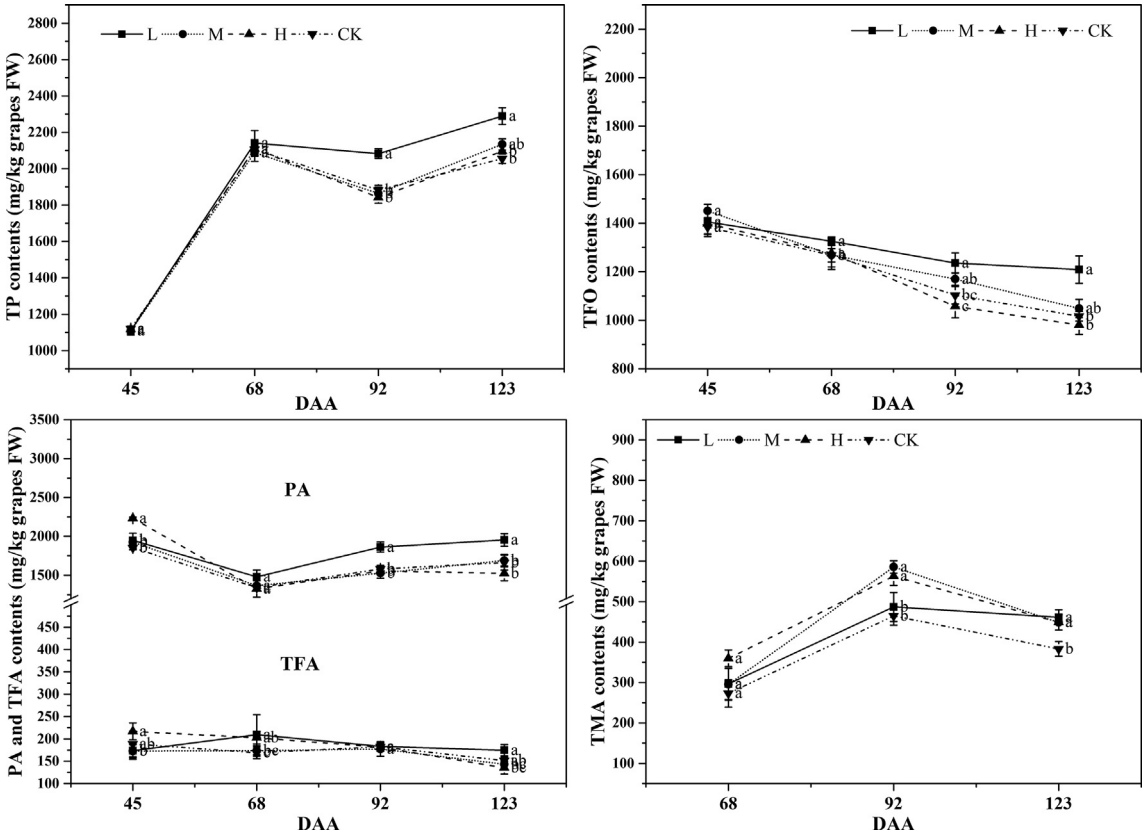

Fig. 2. Contents (mg/kg grapes fresh weight) of total phenols (TP), total flavonoids (TFO), proanthocyanins (PA), total flavanols (TFA), and total monomer anthocyanins (TMA) in skin of grape berries at different developmental stages in control and Mn treatments in 2018. Sampling date corresponds to days after anthesis (DAA). Data are mean ± SD (n = 3).

photosynthesis, Mn is an indispensable cofactor for a series of reactions in the biosynthesis of secondary metabolites in higher plants. For example, phenylalanine ammonia lyase (PAL), the enzyme that catalyzes the first reaction in the phenylpropanoid pathway, is stimulated by Mn as a cofactor (Burnell, 1988). A previous study detected a strong positive correlation between grape skin anthocyanin content and PAL activity (Ruiz-García et al., 2012).

Excess Mn has been shown to increase the contents of salicylic acid (SA) and PR-like proteins, both of which are related to resistance, in grape leaves (Yao et al., 2012). Hence, exogenous Mn application can induce systemic acquired resistance (SAR), which triggers plant secondary metabolic pathways to synthesize secondary metabolites including flavonoids (Eskandari et al., 2018; Ruiz-García et al., 2012).

As for the types of individual anthocyanins, a higher proportion of methylated anthocyanins increases chemical stability and redness (He et al., 2010). Acetylated anthocyanins have been shown to contribute to high color stability, redder hues, and better color quality (Pea-Neira, Cceres, & Pastenes, 2007).

3.6.2. Flavonol composition in grape berry skins

We detected 11 flavonols in the HPLC analyses, including five flavonol glycosides, mainly 3-*O*-glucosides (Table 3). The total individual flavonol contents ranged from 36.45 to 54.76 mg/kg fresh berry weight in 2017 and from 33.21 to 44.68 mg/kg fresh berry weight in 2018. The identity and quantity of flavonols were within the ranges reported previously (Shi et al., 2018; Wang et al., 2018).

In 2017, the contents of all monomer flavonols in berry skin were higher in the M treatment except for Sy-gal and Ka-gal, and in the L treatment except for Ka-glc, Qu-glc, and Ir-glc, compared with the control. The total monomer flavonol contents were also higher in the M and L treatments than in the control (50.25% and 21.69% higher, respectively). In 2018, the increases in the contents of individual flavonols in the M treatment were similar to those detected in 2017, but there were also significantly increased contents of Qu-glc, Ir-glc, Sy-glc, My-gal, and Qu-gal in the H treatment compared with the control (increased by 34.53% and 21.51%, respectively). The total monomer flavonol contents in H (2017) and L (2018) were higher than those in the control, but the differences were not statistically significant. However, when the results were expressed as μg/g skin, the total monomer flavonol contents were higher in L (2018) than in the control.

Flavonols and anthocyanins share the same biosynthetic pathway prior to the formation of dihydroflavonols. Therefore, Mn treatments will have equal promoting effects on enzyme activity in the anthocyanin and flavonol pathways. Our results were consistent with this idea, since the flavonol contents were promoted by the Mn treatments in both years. When the total contents were expressed as μg/g skin, which removed the effects of the weather conditions on berry fresh weight, the anthocyanin content was increased by Mn treatments more in 2018 than in 2017 (mean increase of 14.24% *vs.* 11.10%), and the same trend was observed for flavonols (mean increase of 25.25% *vs*, 19.81%).

The lower temperatures and higher humidity in 2018 provided better conditions for pathogen development (Supplementary Table 1). As stated above, Mn can function as an elicitor to prime plants to respond quickly to pathogen attack. This could be one reason why anthocyanin and flavonols contents in grape skins increased more in 2018 than in 2017.

3.6.3. Flavanol compounds

The flavanol composition in grape berries is shown in Table 3. The pattern and contents of flavanols were within the ranges reported elsewhere (Portu, Santamaría, López-Alfaro, López, & Garde-Cerdán,

Table 2
Concentrations of individual anthocyanins in grape skin at harvest in the control and Mn-treated groups in 2017 and 2018.

Anthocyanins (mg/kg grapes fresh weight)	2017				2018			
	L	M	H	CK	L	M	H	CK
Dp-glc [a]	110.99 ± 2.56 a [b]	118.87 ± 5.22 a	112.79 ± 2.01 a	89.00 ± 1.38 b	26.66 ± 0.11 a	24.00 ± 1.47 b	23.50 ± 1.02 b	17.78 ± 0.42 c
Cy-glc	15.27 ± 0.45 a	16.08 ± 0.79 a	13.50 ± 0.31 b	13.67 ± 0.38 b	4.72 ± 0.17 a	3.78 ± 0.43 b	2.74 ± 0.16 c	2.36 ± 0.47 c
Pt-glc	74.16 ± 0.90 a	75.42 ± 2.47 a	78.17 ± 6.01 a	55.89 ± 0.19 b	27.75 ± 0.07 a	26.04 ± 0.66 b	24.86 ± 0.82 b	21.89 ± 0.38 c
Pn-glc	58.17 ± 1.56 a	56.04 ± 1.11b	52.71 ± 0.28 c	45.61 ± 0.61 d	43.54 ± 0.23 a	43.81 ± 1.07 a	37.61 ± 0.69b	35.87 ± 0.26 c
Mv-glc	484.23 ± 1.84 ab	490.85 ± 15.14 a	468.12 ± 0.35b	375.51 ± 2.64 c	254.67 ± 0.31b	269.76 ± 4.71 a	256.42 ± 3.76b	236.15 ± 6.46 c
Dp-acglc	25.61 ± 0.06b	28.84 ± 1.40 a	28.43 ± 1.24 a	25.74 ± 0.32b	4.76 ± 0.16 a	4.61 ± 0.11 a	4.72 ± 0.45 a	3.63 ± 0.71 a
Cy-acglc	2.66 ± 0.10 ab	3.91 ± 0.54 a	4.02 ± 0.11 a	2.00 ± 0.09b	1.28 ± 0.11 a	1.27 ± 0.10 a	1.22 ± 0.13 a	1.49 ± 0.22 a
Pt-acglc	25.37 ± 0.52 ab	28.02 ± 1.93 a	27.99 ± 1.72 a	24.01 ± 0.30b	9.41 ± 0.03 a	8.77 ± 0.92 a	8.55 ± 0.78 a	6.50 ± 0.43b
Pn-acglc	26.05 ± 3.82 a	26.25 ± 2.80 a	23.87 ± 0.73 a	24.72 ± 3.31 a	19.36 ± 0.95 ab	20.77 ± 0.31 a	17.47 ± 1.09 bc	17.08 ± 0.56 c
Mv-acglc	265.58 ± 7.82 a	280.00 ± 10.94 a	278.47 ± 16.01 a	220.35 ± 2.20b	150.53 ± 1.01 c	165.07 ± 5.10 a	160.54 ± 4.34 ab	151.65 ± 1.09 bc
Cy-cmglc	1.21 ± 0.17 a	1.29 ± 0.23 a	1.19 ± 0.20 a	1.06 ± 0.09 a	1.07 ± 0.13 a	1.03 ± 0.18 a	0.95 ± 0.16 a	0.85 ± 0.07 a
Pt-cmglc	8.06 ± 0.84 a	8.33 ± 1.34 a	8.57 ± 0.36 a	6.50 ± 0.64 a	1.96 ± 0.19 a	2.60 ± 0.37 a	2.41 ± 0.92 a	1.28 ± 0.10 a
Pn-*cis*-cmglc	0.47 ± 0.24 a	0.63 ± 0.38 a	1.13 ± 0.21 a	0.98 ± 0.30 a	0.57 ± 0.36 a	0.82 ± 0.06 a	0.65 ± 0.05 a	0.81 ± 0.05 a
Pn-*trans*-cmglc	28.39 ± 2.07 a	23.73 ± 2.76 a	28.09 ± 1.93 a	27.18 ± 1.06 a	10.32 ± 0.21 a	10.67 ± 0.47 a	10.92 ± 0.80 a	11.12 ± 0.8 a
Mv-*cis*-cmglc	4.53 ± 0.49 a	4.15 ± 0.16 a	3.90 ± 0.18 a	3.93 ± 0.22 a	2.79 ± 0.04 a	3.55 ± 0.84 a	3.46 ± 0.45 a	2.43 ± 0.05 a
Mv-*trans*-cmglc	86.41 ± 5.37 a	82.57 ± 5.26 a	85.64 ± 5.51 a	65.06 ± 5.84b	38.22 ± 1.27 a	36.54 ± 1.15 a	35.89 ± 1.41 a	30.18 ± 1.73b
Pn-cfglc	0.34 ± 0.18 a	0.40 ± 0.30 a	0.61 ± 0.25 a	0.16 ± 0.03 a	1.26 ± 0.25 a	1.84 ± 0.32 a	1.49 ± 0.21 a	1.03 ± 0.11 a
Mv-cfglc	4.70 ± 1.59 a	2.75 ± 0.63 a	3.48 ± 0.75 a	3.64 ± 0.35 a	9.86 ± 1.01 a	10.47 ± 0.83 a	9.01 ± 0.62 ab	7.38 ± 0.33b
Σmethylated	1066.46 ± 5.64 a	1078.78 ± 35.27 a	1060.75 ± 13.23 a	853.55 ± 7.98b	571.46 ± 2.14b	600.73 ± 14.05 a	569.26 ± 11.68b	523.36 ± 3.52 c
Σacetylated	345.27 ± 3.86b	365.66 ± 14.13 a	362.77 ± 18.74 a	296.82 ± 1.25 c	185.34 ± 1.84 bc	199.64 ± 5.57 a	191.62 ± 5.32 ab	179.36 ± 2.10 c
Sum (mg/kg grapes fresh weight)	1223.01 ± 7.65 a	1246.47 ± 42.3 a	1219.49 ± 16.60 a	983.96 ± 6.51b	607.65 ± 3.12 ab	633.53 ± 15.03 a	600.56 ± 12.09b	547.62 ± 3.37 c
Sum (μg/g skinfresh weight)	9475.49 ± 65.30b	10643.56 ± 361.45 a	9482.59 ± 128.39b	8881.73 ± 58.73 c	3682.54 ± 18.88b	4360.77 ± 103.46 a	3727.57 ± 75.04b	3434.60 ± 21.11 c

[a]Abbreviations: D, delphinidin; C, cyanidin; Pt, petunidin; Pn: peonidin; Mv, malvidin; glc, glucoside; acglc, acetyl glucoside; cmglc, *trans*-*p*-coumaroyl glucoside; cfglc, caffeoyl glucoside. [b]Different lowercase letters in the same row indicate significant differences among treatments (Tukey's test, $p < 0.05$).

Table 3
Concentrations of individual flavonols and flavanols in grape skin at harvest in control and Mn-treated groups in 2017 and 2018.

Compounds (mg/kg grapes fresh weight)	2017				2018			
	L	M	H	CK	L	M	H	CK
My-gal[a]	1.24 ± 0.03 b[b]	1.99 ± 0.12 a	1.15 ± 0.002 b	1.30 ± 0.09 b	0.57 ± 0.05 b	0.73 ± 0.03 a	0.77 ± 0.04 a	0.38 ± 0.05 c
My-glc	6.90 ± 0.11 c	9.84 ± 0.66 a	7.21 ± 0.17 b	7.25 ± 0.57 c	3.17 ± 0.13 b	3.63 ± 0.09 a	3.76 ± 0.14 a	2.25 ± 0.19 c
Qu-gal	2.16 ± 0.07 b	2.65 ± 0.22 a	1.70 ± 0.08 c	1.73 ± 0.18 c	2.52 ± 0.16 bc	3.02 ± 0.12 a	2.76 ± 0.18 ab	2.23 ± 0.17 c
Qu-glc	12.50 ± 0.37 a	13.59 ± 0.85 a	9.58 ± 0.42 b	9.78 ± 0.91 b	12.70 ± 0.34 b	14.69 ± 0.56 a	13.12 ± 0.52 b	11.07 ± 0.62 c
Qu-glcU	10.52 ± 0.18 b	13.65 ± 0.91 a	9.21 ± 0.19 bc	8.15 ± 0.76 c	6.59 ± 0.56 b	8.31 ± 0.63 a	6.05 ± 0.51 b	6.20 ± 0.53 b
Qu-rha	0.13 ± 0.005 a	0.14 ± 0.01 a	0.08 ± 0.002 b	0.07 ± 0.01 b	0.08 ± 0.01 b	0.12 ± 0.01 a	0.08 ± 0.01 b	0.09 ± 0.01 b
Ir-glc	3.24 ± 0.12 b	4.04 ± 0.29 a	2.80 ± 0.12 bc	2.38 ± 0.23 c	4.55 ± 0.36 ab	5.06 ± 0.34 a	4.97 ± 0.40 a	3.80 ± 0.43 b
Sy-gal	0.01 ± 0.001 a	0.01 ± 0.002 a	0.02 ± 0.001 a	0.01 ± 0.001 a	0.01 ± 0.001 a	0.01 ± 0.001 a	0.01 ± 0.001 a	0.01 ± 0.002 a
Sy-glc	2.92 ± 0.13 c	4.30 ± 0.32 a	3.75 ± 0.19 b	2.73 ± 0.27 c	2.88 ± 0.31 b	3.07 ± 0.19 ab	3.67 ± 0.28 a	2.84 ± 0.33 b
Ka-gal	0.83 ± 0.01 a	0.50 ± 0.03 b	0.52 ± 0.03 b	0.57 ± 0.08 b	0.61 ± 0.08 b	0.80 ± 0.05 a	0.62 ± 0.05 b	0.51 ± 0.04 b
Ka-glc	3.90 ± 0.11 a	4.13 ± 0.27 a	1.94 ± 0.12 b	2.47 ± 0.27 b	4.31 ± 0.24 b	5.24 ± 0.36 a	4.54 ± 0.24 ab	3.85 ± 0.36 b
Sum (mg/kg grapes fresh weight)	44.35 ± 1.04 b	54.76 ± 3.68 a	37.82 ± 1.31 bc	36.45 ± 3.30 c	37.98 ± 2.24 bc	44.68 ± 2.38 a	40.36 ± 2.37 ab	33.21 ± 2.73 c
Sum (μg/g skin fresh weight)	400.31 ± 9.42 a	424.95 ± 28.53 a	294.22 ± 10.16 b	311.45 ± 28.24 b	261.45 ± 15.41 a	270.79 ± 14.42 a	250.48 ± 14.69 a	208.31 ± 17.10 b
C	36.24 ± 3.27 a	35.16 ± 3.01 a	33.65 ± 1.06 a	31.37 ± 1.17 a	54.57 ± 4.96 b	71.17 ± 1.77 a	47.91 ± 2.67 bc	45.75 ± 2.59 c
EC	3.53 ± 0.34 a	3.38 ± 0.69 a	3.31 ± 0.03 a	3.35 ± 0.62 a	0.52 ± 0.06 a	0.37 ± 0.03 a	0.54 ± 0.13 a	0.35 ± 0.01 a
GC	10.79 ± 0.16 a	10.69 ± 0.56 a	10.47 ± 0.24 a	10.12 ± 0.40 a	2.47 ± 0.08 a	2.64 ± 0.08 a	2.11 ± 0.41 a	1.75 ± 0.05 a
EGC	9.82 ± 0.61 a	10.49 ± 0.51 a	9.37 ± 0.33 a	9.83 ± 0.53 a	2.94 ± 0.05 a	2.51 ± 0.12 a	2.90 ± 0.09 a	2.71 ± 0.27 a
PB1	59.74 ± 3.57 a	61.66 ± 5.04 a	62.34 ± 2.02 a	60.05 ± 0.24 a	32.56 ± 2.38 a	18.58 ± 0.79 b	21.71 ± 1.18 b	18.55 ± 0.87 b
PC1	3.02 ± 0.36 a	3.49 ± 0.27 a	3.30 ± 0.11 a	3.06 ± 0.11 a	1.49 ± 0.16 a	1.36 ± 0.05 a	1.45 ± 0.09 a	1.55 ± 0.15 a
Sum (mg/kg grapes fresh weight)	123.13 ± 8.31a	124.87 ± 10.09 a	122.43 ± 3.80 a	117.78 ± 3.38 a	94.55 ± 7.77 a	95.51 ± 2.01 a	75.82 ± 3.56 b	71.12 ± 3.88 b
Sum (μg/g skin fresh weight)	955.55 ± 64.52 a	1067.15 ± 86.19 a	952.33 ± 29.56 a	1063.10 ± 30.55 a	591.38 ± 48.75 a	644.17 ± 13.81 a	466.13 ± 22.07 b	431.01 ± 23.52 b

[a]Abbreviations: My, myricetin; Qu, quercetin; Ir: isorhamnetin; Sy, syringetin; Ka: kaempferol; gal, galactoside; glc, glucoside; glcU, glucuronide; rha, rhamnoside; C, catechin; EC, epicatechin; GC, gallocatechin; EGC, epigallocatechin; PB1, procyanin B1; PC1, procyanin C1. [b]Different lowercase letters in the same row indicate significant differences among treatments (Tukey's test, $p < 0.05$).

2015; Shi et al., 2018). In all treatments, catechins (C) and procyanidin dimers (PB1) accounted for the highest proportion of flavanols in grape skin, and EC was a minor compound.

Although the total individual flavanol contents in grape skin were higher in the Mn treatments than in the control in 2017, the monomer composition and gross amount did not differ significantly between the Mn treatments and control. In 2018, the Mn foliar spray significantly increased C and PB1 in the L treatment, but only C in the M treatment. The total flavanol contents were significantly higher in the L and M treatments than in the control, whether expressed as mg/kg fresh grape weight or μg/g skin. However, the total flavanol contents in the H treatment were almost the same as that in the control.

In this study, the total flavanol content differed significantly between the Mn treatments and the control only in 2018, providing further evidence that Mn-treated vines were able to mount a response to pathogen attack more promptly and strongly. Although Mn application had positive effects on the biosynthesis of individual anthocyanins and flavonols in berry skin, it had limited effects on monomer flavanols. In contrast, Acuna-Avila et al. (2016) detected a direct relationship between the Mn concentration in soil and the contents of catechin and epicatechin in grape berry skin at harvest.

3.7. Wine flavonoid compounds and chromatic properties

From a practical perspective, it is important to determine whether improvements in the quality of grapes are similarly embodied in the resulting wine, especially the wine flavonoid composition and color characteristics. The color indexes (L*, a*, b* and C) and the flavonoid contents (anthocyanins, flavonols, and flavanols) of wine prepared using grapes from Mn-treated and control vines are shown in Table 4. These results were consistent with those of previous studies (Pérez-Magariño & González-San José, 2006; Portu et al., 2015). The values of a* and C and the total anthocyanin and flavonol contents in wine were higher in the 2017 vintage than in the 2018 vintage.

The Mn treatments resulted in increased a* and C values of wine, and lower L* values, although this was not statistically significant in 2017. In 2018, the L* and b* values were lower and the C (Ma et al., 2019) and a* values of wine were higher in the Mn-treatments than in the control (except for C in the H treatments). These results show that the red color of wine was stronger (higher a*) in the Mn treatments than in the control in both years, resulting in greater color intensity (higher C) in both years. In 2018, the wine had a darker color (lower L) and had a stronger blue hue (lower b*) in the Mn treatments than in the control.

The flavonoid compounds contents in wine were higher in the Mn treatments than in the control, except for the anthocyanin and flavonol contents in H in 2017 and the flavanol contents in H in 2018. Deserved to be mentioned, the differences in flavonoid concentration of the wines between the control and the Mn treatments were higher compared to the differences between the berries. One explanation of these findings might be the discrepancy in skin to pulp ratio between the treatments and control (Table 1), As abovementioned, the Mn treatments increased the skin/pulp of the berry compared with the control. Especially, L and M treatments reached significant level. Since the most decisive compounds for berry and wine quality, such as aromas and phenols, are located in the skin, the larger the skin-to-pulp ratio is, the higher the potential quality of the final wine will be.

The flavonoid composition dramatically affects the chromatic characteristics of wine. In the current study, the wine in the Mn treatments had higher flavonoid contents, consistent with its deeper color (lower L*) and stronger color intensity (higher C). Similar results have been reported in other studies (Pérez-Magariño & González-San José, 2006). In addition, the higher anthocyanin and flavonol concentrations contributed to the stronger red hue of wine made from Mn-treated grapes. Anthocyanins are strongly related to the a* CIELab (red/green) index (Figueiredo-González, Cancho-Grande, & Simal-Gándara, 2013).

Table 4
Concentrations of flavonoids in wine and chromatic characteristics of wine made from grape berries from Mn treatments and control in 2017 and 2018.

parameters	2017				2018			
	L	M	H	CK	L	M	H	CK
L*[b]	25.15 ± 1.00 a[a]	24.99 ± 0.43 a	24.88 ± 0.67 a	26.57 ± 0.52 a	32.64 ± 0.88 b	31.75 ± 0.43 bc	30.70 ± 0.43 c	35.60 ± 0.49 a
a*	64.62 ± 0.88 a	62.79 ± 0.87 ab	61.60 ± 0.56 b	56.39 ± 0.45 c	51.45 ± 0.73 a	51.25 ± 0.37 a	49.10 ± 0.31 b	46.04 ± 0.28 c
b*	25.20 ± 0.48 a	24.66 ± 1.32 a	26.52 ± 0.24 a	25.25 ± 0.25 a	28.71 ± 0.88 b	28.96 ± 0.20 b	28.16 ± 0.39 b	31.52 ± 0.45 a
C	69.36 ± 0.57 a	68.16 ± 0.68 ab	67.07 ± 0.61 b	61.78 ± 0.73 c	58.95 ± 0.04 a	58.87 ± 0.35 a	56.48 ± 0.45 b	55.64 ± 0.47 b
Total anthocyanins (mg/L)	432.85 ± 11.06 a	430.33 ± 12.71 a	398.48 ± 8.35 b	378.01 ± 11.90 b	135.41 ± 7.60 b	163.57 ± 11.08 a	120.04 ± 8.70 b	81.41 ± 6.20 c
Total flavonols (mg/L)	119.23 ± 1.98 b	134.95 ± 2.34 a	101.61 ± 1.24 c	102.95 ± 2.82 c	51.53 ± 1.76 ab	53.16 ± 2.61 a	46.67 ± 3.06 b	37.82 ± 1.07 c
Total flavanols (mg/L)	101.84 ± 1.48 ab	106.72 ± 2.50 a	96.64 ± 2.80 b	84.82 ± 3.61 c	158.33 ± 1.14 b	197.52 ± 2.67 a	137.01 ± 5.81 c	131.57 ± 3.22 c

[a]Different lowercase letters in same row indicate significant differences among treatments (Tukey's test, $p < 0.05$). [b]L*, clarity; C, chroma.

Flavonols are better copigments than other flavonoids like flavanols (Heras-Roger, Díaz-Romero, & Darias-Martín, 2016), and can facilitate the extraction of anthocyanins during the vinification process to produce wine with a stronger red hue (Castillo-Muñoz et al., 2007). Notably, only the wines made in 2018 showed a constant hypochromatic shift. According to Cáceres et al. (2012), a stronger blue color can result in greater condensation of anthocyanins with flavanols, which stabilizes the violet tone of wine. The total individual flavanol content was significantly increased by the Mn treatments only in 2018; this may be one reason for the stronger blue color of wine in 2018 than in 2017.

4. Conclusion

We explored in detail the influence of foliar-sprayed $MnSO_4{\cdot}H_2O$ on global parameters (physicochemical indexes and total phenolic compounds) and flavonoid profiles (anthocyanins, flavonols, and flavanols) of Cabernet Sauvignon grape skin, as well as the chromatic properties of wine prepared from the grapes. These Mn treatments, which were conducted over two years (2017 and 2018), tended to promote the accumulation of TSS, berry weight, and phenolic compounds from veraison to harvest. As for individual flavonoids, the Mn treatments markedly increased the contents of acetylated, methylated, total anthocyanins, and total flavonols in grape skins in both years, compared with the control. The foliar application of Mn only slightly affected the total monomer flavanol content. The Mn treatments increased the flavonoid compounds contents and color intensity of wine. The results of this study show that foliar $MnSO_4{\cdot}H_2O$ application is a simple agronomic practice that can improve the nutritional value of grapes and the quality of wine prepared from them.

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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Author Contributions Section

Huangzhao Chen, and Zhengwen Zhang designed the study, performed the research, analysed data, and wrote the paper.

The remaining authors contributed to refining the ideas, carrying out additional analyses and finalizing this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.126182.

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