INTRODUCTION

The eastern foothills of Helan Mountain in Ningxia are among the best ecological regions for wine grapes in China. However, the fertilization method used for wine grape cultivation is inclined to supplement traditional macronutrient fertilizers, ignoring the importance of trace elements. The mineral nutrient availability in the alkaline soil in this area is low, the effective iron content is far lower than the national average, and the mobility of iron in plants is not strong, making it difficult for various elements that were originally insufficient to meet the nutritional requirements for the normal growth of grapes.

Foliar fertilization is one of the main methods used to improve the berry yield and quality during grape cultivation. Foliar application is the most rapid and effective method for satisfying the specific nutritional needs of plants (Fernandez et al., 2009). The effect of foliar iron application on the composition of grapes is highly dependent on the grape variety and the duration and form of iron used (Abadia et al., 2002; Alvarez-Fernández et al., 2006; Yunta...
Iron fertilizers can be divided into three categories: inorganic, organic compound, and chelated iron fertilizers. Inorganic iron fertilizer is inexpensive and has a fast fertilizer effect, but it is easily oxidized, resulting in an unsustainable fertilizer effect. Ferrous sulfate is the most common fertilizer. Organic compound iron fertilizers are relatively stable and easily degradable, and the most common ones are ferric gluconate and ferric sugar alcohol. The chelated fertilizers are relatively stable and easily degradable, and the most common chelating agents are EDTA-Fe, ethylenediamine-N,N′-bis(2-hydroxyphenylacetic acid) (EDDHA-Fe), and ferric citrate. Furthermore, studies have shown that the application of chelated iron can significantly increase the soluble sugar and phenolic acid contents of grapes under alkaline soil conditions (Karimi et al., 2019).

The quality of grapes is related to the balance between primary and secondary metabolites. Glucose and fructose are the main sugars in berries, and the contents of these primary metabolites are affected by variety, climate, and nutritional status, while the accumulation of sugar in berries can improve the volatile aromatic compound content (Ali et al., 2010; Zheng et al., 2009). Phenols are important components that determine the quality of grape berries and have various functions in plants, including as antioxidants, protecting against ultraviolet (UV) radiation, and combating pathogenic infections (Tian et al., 2019). Studies have shown that iron is related to the function of phenolic synthase; thus, iron deficiency affects the synthesis of phenolic compounds via the shikimate pathway (Bavaresco et al., 2005). In addition, iron concentration can affect pectin degradation and antioxidant capacity of phenolic compounds (Vidot et al., 2020). Many phenolic compounds are found in grapes at high concentrations. These phenolic compounds can be divided into flavonoids and nonflavonoids, where flavonoids comprise flavanols, flavonols, and anthocyanins, and nonflavonoids include resveratrol, benzoic acid, and cinnamic acid (Liang et al., 2013). The expression levels of genes related to anthocyanin biosynthesis are affected by developmental and environmental factors, including temperature, light, sugar content, and mineral content. Karimi et al. (2019) found that exogenous iron could regulate the anthocyanin content of berries. Zheng et al. (2009) showed that carbohydrates can promote the expression of flavonone 3-hydroxylase (EC 1.14.11.9), which is a key enzyme in anthocyanin synthesis, thereby increasing berry anthocyanin content. Flavonols are produced by the flavonoid biosynthesis pathway and mainly comprise quercetin, myricetin, kaempferol, andisorhamnetin (Mattiivi et al., 2006). Flavan-3-alcohol is a basic component of proanthocyanidins and condensed tannins, including catechin, epicatechin, gallocaicchin, epigallocatechin, and epigallocatechin gallate. Flavonoids and flavanols are mainly distributed in the grape seeds, peel, and berry stems. Flavonoids have important effects on astringency, bitterness, and structure of wine (Zimman et al., 2002). Foliar spraying with iron can increase the anthocyanin (Shi et al., 2017), flavonol, and flavanol (Shi et al., 2018) contents of grape berries. However, previous studies have not comprehensively investigated the effects of different forms of iron on the flavonoid content of grapes.

### 2 | MATERIALS AND METHODS

#### 2.1 | Test materials and experimental design

The experiment was conducted from April to October, 2021 at Lilan Winery (105°58′20″E, 38°16′38″N), which is in a wine grape-producing area at the eastern foothills of the Helan Mountain in China. The study site was located at an altitude of 1129m, with an annual average precipitation of 190mm and a frost-free period of 180days. Eight-year-old Cabernet Sauvignon grape vines were used in this study. The vine shape was “Y” and the row spacing was 0.6×3.5 m. The basal fertilizer, comprising organic sheep manure fertilizer, was ditched in early May and applied once at 10,500kg hm$^{-2}$ by drip irrigation. The soil type was calcareous gravel. Table 1 lists the chemical characteristics of the soil before the start of the experiment.

The experiment had a randomized block design with six treatments and three replicates for each treatment, with a total of 18 cells, and each cell area was 10.5 m$^2$. Each iron fertilizer treatment was sprayed three times (on July 12, July 27, and August 11) with an electric sprayer at the grape expansion and color-changing stages, where a 5 L solution of each treatment was applied. Table 2 shows the total amounts of iron received for each treatment after the three applications. Excluding the different forms of iron fertilizer, all other treatments and management measures were consistent with those used in the vineyard. During the optimal grape harvest period, 15 representative grapes were randomly selected from each treatment and transported rapidly to the laboratory. One hundred grapes were immediately frozen in liquid nitrogen and ground to determine berry quality. For each treatment, 30 grapes were randomly selected, washed with distilled water, peeled, frozen in liquid nitrogen, and stored at −80°C to determine the peel metabolites.

#### 2.2 | Determination of grape berry quality

Frozen grape berries were ground to determine the total soluble solids (TSS), reducing sugar, and titratable acid contents (TAC). The TSS
content was determined using a handheld glucose meter. The reducing sugar content was determined using the 3,5-dinitrosalicylic acid method. TAC was determined by titration using standard 0.1 mol L⁻¹ NaOH (endpoint pH 8.2) (Jin et al., 2016; Ma et al., 2019; Wang et al., 2019).

2.3 | Extraction of anthocyanin and flavonoid compounds from peel

Grape peel samples were vacuum dried and frozen for 24 h in a lyophilizer (Scientz-100F) and then ground (30 Hz, 1.5 min) into powder using a grinder (MM 400, Retsch). The powdered peel (100 mg) was extracted with 70% methanol solution (1.2 ml) before mixing six times in a vortex shaker for 30 s each at 30 min intervals and standing overnight at 4°C. The homogenate was centrifuged at 12,000 rpm (revolutions per minute) for 10 min and the supernatant was aspirated and filtered through a microporous membrane (pore size = 0.22 μm) for UPLC–MS/MS (ultrahigh performance liquid chromatography tandem spectrometry) analysis. Three independent extractions were performed for each treatment group.

2.3.1 | UPLC–MS/MS conditions

The sample extracts were analyzed using an UPLC–ESI–MS/MS system (ultrahigh performance liquid chromatography–electrospray ionization tandem mass spectrometry) (UPLC, SHIMADZU Nexera X2, www.shimadzu.com.cn; MS, Applied Biosystems 4500 Q TRAP, www.appliedbiosystems.com.cn/). The analytical conditions were as follows: UPLC column, Agilent SB-C18 (1.8 μm, 2.1×100 mm); mobile phase solvent A comprising pure water with 0.1% formic acid and solvent B comprising acetonitrile with 0.1% formic acid. Sample measurements were performed with a gradient program using starting conditions of 95% A/5% B. After 9 min, a linear gradient of 5% A/95% B was programmed, and a composition of 5% A/95% B was maintained for 1 min. Subsequently, the composition was adjusted to 95% A/5.0% B within 1.1 min and maintained for 2.9 min. The flow velocity was 0.35 ml per minute. The column oven temperature was set to 40°C. The injection volume was 4 μl. The effluent was alternatively connected to an ESI–triple quadrupole linear ion trap (QTRAP)–MS.

Linear ion trap (LIT) and triple quadrupole (QQQ) scans were acquired using a triple quadrupole–linear ion trap mass spectrometer (QTRAP; AB4500 Q TRAP UPLC/MS/MS System) equipped with an electrospray ionization (ESI) turbo ion-spray interface, which was operated in positive and negative ion modes and controlled by Analyst 1.6.3 software (AB Sciex). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature, 550°C; ion-spray voltage (IS), 5500 V (positive ion mode)/−4500 V (negative ion mode); ion source gas I (GSI), gas II (GSII), and curtain gas (CUR) were set to 50, 60, and 25.0 psi, respectively; collision-activated dissociation (CAD) was high. Instrument tuning and mass calibration were performed using 10 and 100 μmol L⁻¹ polypropylene glycol solutions in the QQQ and LIT modes, respectively. QQQ scans were acquired as multiple reaction monitoring (MRM) experiments with collision gas (nitrogen) set in the medium. Declustering potential and collision energy (DP and CE) for individual MRM transitions were calculated with further DP and CE optimization. A specific set of MRM transitions was monitored for each period according to the metabolites eluted in this period.

2.4 | Qualitative and quantitative analyses of anthocyanin and flavonoid compounds

The ion current intensities and retention times were compared with those of our self-developed “metware” database (MWDB database). Qualitative and quantitative analyses of the compounds were conducted according to the secondary spectrum information. The contents of different metabolites were analyzed according to the metabolite detection multimodal diagram.

2.5 | Statistical analysis

Microsoft Excel 2010 and SPSS 21.0 software were used to process and analyze the data. Origin2018 was used to plot the data. Significant differences were accepted at p < .05 (n = 5). All data are expressed as the mean± standard error.
3 | RESULTS AND DISCUSSION

3.1 | Effects of different iron treatments on the physicochemical properties of grape berries

Table 3 shows that the TSS and reducing sugar (RS) contents of grape berries increased under all of the iron treatments, where the highest TSS and RS contents were observed under FB3 (ferric citrate), which were 4.70% and 12.40% higher than those in the control, respectively. The TSS contents of grape berries did not differ significantly among the treatments, whereas the RS contents did. The RS contents were significantly higher under FB3 and FB5 (ferric sugar alcohol) than under the control and the other iron treatments, where the contents followed the order of: FB3 > FB5 > FB4 > FB2 > FB1. Except under FB3, the TAC values were lower under the iron treatments than under the control. The TAC value was the lowest under FB4 (ferric gluconate) (16.67% lower than that in the control). The iron treatments had significant effects on the sugar–acid ratio in grape berries. Compared with the control, the sugar–acid ratios were 14.63%, 8.12%, 4.47%, 20.28%, and 16.04% higher under FB1 (ferrous sulfate), FB2 (EDTA-Fe), FB3, FB4, and FB5, respectively. The iron treatments also significantly increased the 100- berry weight compared with the control, but the differences in the weights were not significant between the different iron treatments.

3.2 | Effects of different iron treatments on anthocyanin contents of grape peel

The UPLC–MS (ultrahigh performance–mass spectrometry) analysis detected 22 compounds (Table 4) in the grape peel, which mainly comprised cyanidins (five), paeoniflorins (four), petunidins (four), malvidins (five), delphinidins (three), and pelargonidin (one). The abbreviations used for these compounds are listed in Table 5 (only some representative data are presented in the table). Flavanoids included catechins, epicatechins, and gallo catechins, while flavanones included quercetin, myricetin, kaempferol, and 6-hydroxykaempferol derivatives. In particular, Ca, Hydro, Ga, Cid, and aCadih were the main flavanols detected. The content of most individual flavanols was higher in grape skin under FB3 than in the control, except for Hydro and Ga. However, the contents of Hydro and Ga were significantly higher under FB1, FB4, and FB5 compared with the control, where the Hydro content was the highest under FB1, i.e., 4.08% and 15.91% higher than those under the control and FB3, respectively. The Ga content was the highest under FB5, as it was 28.39% and 42.82% higher than those under the control and FB3, respectively. The total individual flavanol contents of the grape peel were lower under the iron treatments than under the control, except for FB3. The total individual flavanol content was the lowest under FB2, where it was 23.12% lower than that under the control. However, the total individual flavanol contents did not differ significantly under FB1, FB4, and FB5, where they were 9.31%, 4.80%, and 9.31% lower, respectively, than under the control. However, the contents of Hydro and Ga did not differ significantly under FB1, FB4, and FB5, where they were 12.35%, 4.80%, and 12.35% lower, respectively, than under the control.

Different iron treatments affected flavonol content. The contents of 12 monomer flavonoids, including Ka, Quglu, sQuglu, My, Dp. The contents of Cy, Pt, Dp, and their derivatives in grape peel were significantly lower under the different iron treatments than in the control, except for FB3 and FB4. The contents of most anthocyanins were significantly higher under FB3 than under the other treatments, except for Decoum. The different iron treatments had significant effects on the total anthocyanin content of berry peel, where the anthocyanin content was the highest under FB3, which was 14.20%, 32.37%, 29.06%, 16.20%, and 35.16% higher than that in the control, FB1, FB2, FB4, and FB5, respectively.

3.3 | Effects of different iron treatments on flavanols and flavonols in grape peel

The flavonoids detected in grape berry skin are shown in Table 5, where 19 flavanols and 42 flavonols were detected. The abbreviations for these compounds are listed in Table 5 (only some representative data are presented in the table). Flavonoids included catechins, epicatechins, and gallo catechins, while flavanones included quercetin, myricetin, kaempferol, and 6-hydroxykaempferol derivatives. In particular, Ca, Hydro, Ga, Cid, and aCadih were the main flavanols detected. The content of most individual flavanols was higher in grape skin under FB3 than in the control, except for Hydro and Ga. However, the contents of Hydro and Ga were significantly higher under FB1, FB4, and FB5 compared with the control, where the Hydro content was the highest under FB1, i.e., 4.08% and 15.91% higher than those under the control and FB3, respectively. The Ga content was the highest under FB5, as it was 28.39% and 42.82% higher than those under the control and FB3, respectively. The total individual flavanol contents of the grape peel were lower under the iron treatments than under the control, except for FB3. The total individual flavanol content was the lowest under FB2, where it was 23.12% lower than that under the control. However, the total individual flavanol contents did not differ significantly under FB1, FB4, and FB5, where they were 9.31%, 4.80%, and 9.31% lower, respectively, than under the control.

Different iron treatments affected flavonol content. The contents of 12 monomer flavonoids, including Ka, Quglu, sQuglu, My,
rKaglu, Kaneo, * rQuglu, Qu, Quneo, rQuglu, Se, and Me, were the highest in grape peel. The contents of the two monomeric flavonol compounds, rKaglu and Kaneo, were the highest, and their levels also differed significantly among the iron treatments. The rKaglu and Kaneo contents were the highest under FB3, where they were 72.41% and 70.67% higher than those of the control, respectively. The rKaglu and Kaneo contents were the lowest under FB5 (43.33% and 38.32% lower, respectively), compared with the control. The contents of other flavonol monomers also varied greatly among different treatments, where the levels of zero, 10, 14, two, zero, and 16 individual flavonols were the highest in FB1, FB2, FB3, FB4, FB5, and the control, respectively.

3.4 | Principal component analysis of grape berry flavonoids

The different iron treatments had significant effects on the content and proportion of sugar acids and flavonoids in grape berries. Principal component analysis (PCA) was conducted to determine the overall differences in flavonoids under different iron treatments. Figure 1 shows that the first two PCs accounted for 42.20% and 32.30% of the variance, respectively, and thus, 74.50% of the total variance. PC1 was mainly explained by flavanones such as Qu, Se, and Pt, and PC2 was mainly explained by anthocyanins and flavanols such as My and Qu. The scoring plot in Figure 1a shows that the results obtained under the different iron treatments and the control were clearly separated, with FB1, FB2, and FB5 in a single cluster, and the control, FB3, and FB4 in three separate clusters. The loading plot in Figure 1b shows that the sugar content and the contents of Mv, Pt, Ca, Ep, Qu, and their derivatives were well separated in the control and iron treatment groups, thereby indicating that the iron treatments affected the sugar, acid, and flavonoid contents of grape berries. However, FB1, FB2, and FB5 were grouped in the same cluster (A1), thereby indicating that the effects of these three iron treatments on the quality indices for grape berries and flavonoid contents were not significantly different, and that these treatments increased the contents of Qugal, Ga, Mymal, Mv, and their derivatives. FB4 clustered separately (A2) and TSS/TAC, WB, and the content of flavonols, such as gGoglu, increased under ferric gluconate (FB4) treatment. FB3 was within the confidence interval (A3), showing that RS and almost all flavonoids in grape berries were increased under ferric citrate treatment.

4 | DISCUSSION

Alvarez-Fernández et al., (2006) showed that iron deficiency decreases berry sugar content. The sugar-acid ratio is generally a measure of berry ripeness. In the present study, spraying with different forms of iron improved the quality of wine grapes, where TSS increased, and sugar content and TAC decreased, thereby increasing the sugar-acid ratio. The increase in the sugar-acid ratio in grape berries under the iron treatments indicates that iron can promote berry ripening, as also observed in pears ("Deveci" and "Santa Maria") (Ozturk et al., 2019), table grapes (cv. "Thompson Seedless") (Taghavi et al., 2020), and wine grapes (Vitis vinifera cv.) (Shi et al., 2017). Reductions in RuBisCO (ribulose-1,5-bisphosphate carboxylase-oxygenase) activity levels and lower chlorophyll and carotenoid contents in iron-deficient plants lead to a lower leaf CO2 exchange rate and photosynthetic efficiency, which may explain the higher sugar content under iron treatment in the present study (Chen et al., 2004). We found that the glycolic acid contents of grape berries differed significantly among the iron treatments, while the TSS contents were also higher and the sugar contents were lower under FB2, FB3, FB4, and FB5 than under FB1. Organic chelated iron is a small molecule that is absorbed by the leaves when chelated by sugar alcohols and amino acids, thereby avoiding the oxidation and precipitation of ferrous sulfate when sprayed alone (FB1), facilitating the absorption of nutrient iron (Fernández & Ebert, 2005). In addition, sugar alcohols and amino acids are small organic molecules with good moisture retention, permeability, and ductility characteristics; thus, they can reduce the surface tension and improve the capacity of leaf surfaces to absorb iron (Singh et al., 2013). Tartaric acid and malic acid are the main organic acids present in grape berries. We found that iron treatment decreased TAC, except for FB3 (ferric citrate), possibly because external application of iron promoted the accumulation of sugars and accelerated berry ripening, whereas the malic acid content of berries gradually decreased as the berries matured (Karimi et al., 2019). Malic and citric acids are the main substrates for plant respiration. The TSS and reducing sugar contents of berries increased under FB3 (ferric citrate), whereas TAC did not change compared with the control, probably because the externally applied ferric citrate was consumed by respiration to decrease the decomposition of malic acid; thus, TAC was higher under FB3 (ferric citrate) compared with other iron treatments (Chen et al., 2004; Schlegel et al., 2006). The weight of berries is determined by their size and density, which are important factors that affect the quality of grapes. The weights of the grape berries were significantly higher under the iron treatments than under the control, possibly because iron increased the metabolic activity of the plants. Iron deficiency during grape growth is known to reduce membrane integrity, leaf CO2 content, exchange rate, and chlorophyll photosynthetic efficiency to inhibit the accumulation of dry matter, which may explain why iron treatment significantly increased the weight of the berries in the present study (Bertamini & Nedunchezhian, 2005).

The types and quantities of anthocyanins detected in grape peel in the present study were generally consistent with those previously reported (Arozarena et al., 2002; Mattivi et al., 2006; Shi et al., 2017). Anthocyanins are important pigments in red grape. The proportions and quantities of anthocyanins were determined based on the specific variety and cultivation conditions. Studies have shown that iron is an important factor affecting anthocyanin synthesis (Ahmed et al., 1997). In the present study, we found that spraying different forms of iron increased the content of specific anthocyanins. In particular, foliar application of ferric citrate (FB3) and ferric gluconate
| Flavonoids       | FB1            | FB2            | FB3            | FB4            | FB5            |
|------------------|----------------|----------------|----------------|----------------|----------------|
| Cy               | 0.18 ± 0.02b   | 0.14 ± 0.02c   | 0.12 ± 0.01bc  | 0.11 ± 0.002b  | 0.09 ± 0.002c  |
| Pl               | 0.11 ± 0.02b   | 0.08 ± 0.02c   | 0.12 ± 0.01bc  | 0.11 ± 0.002b  | 0.09 ± 0.002c  |
| Cygluc           | 0.16 ± 0.02b   | 0.14 ± 0.02c   | 0.12 ± 0.01bc  | 0.11 ± 0.002b  | 0.09 ± 0.002c  |
| Pn               | 0.18 ± 0.02b   | 0.14 ± 0.02c   | 0.12 ± 0.01bc  | 0.11 ± 0.002b  | 0.09 ± 0.002c  |
| Cygluc           | 0.22 ± 0.02b   | 0.18 ± 0.02c   | 0.16 ± 0.01bc  | 0.15 ± 0.002b  | 0.13 ± 0.002c  |
| Pn               | 0.24 ± 0.02b   | 0.20 ± 0.02c   | 0.18 ± 0.01bc  | 0.17 ± 0.002b  | 0.15 ± 0.002c  |
| Cygluc           | 0.26 ± 0.02b   | 0.22 ± 0.02c   | 0.20 ± 0.01bc  | 0.19 ± 0.002b  | 0.17 ± 0.002c  |
| Pn               | 0.28 ± 0.02b   | 0.24 ± 0.02c   | 0.22 ± 0.01bc  | 0.21 ± 0.002b  | 0.19 ± 0.002c  |
| Cygluc           | 0.30 ± 0.02b   | 0.26 ± 0.02c   | 0.24 ± 0.01bc  | 0.23 ± 0.002b  | 0.21 ± 0.002c  |
| Pn               | 0.32 ± 0.02b   | 0.28 ± 0.02c   | 0.26 ± 0.01bc  | 0.25 ± 0.002b  | 0.23 ± 0.002c  |
| Cygluc           | 0.34 ± 0.02b   | 0.30 ± 0.02c   | 0.28 ± 0.01bc  | 0.27 ± 0.002b  | 0.25 ± 0.002c  |
| Pn               | 0.36 ± 0.02b   | 0.32 ± 0.02c   | 0.30 ± 0.01bc  | 0.29 ± 0.002b  | 0.27 ± 0.002c  |
| Cygluc           | 0.38 ± 0.02b   | 0.34 ± 0.02c   | 0.32 ± 0.01bc  | 0.31 ± 0.002b  | 0.29 ± 0.002c  |
| Pn               | 0.40 ± 0.02b   | 0.36 ± 0.02c   | 0.34 ± 0.01bc  | 0.33 ± 0.002b  | 0.31 ± 0.002c  |

Note: Lower case letters indicate significant differences between treatments, according to Tukey's HSD (honest significant difference) test (p < 0.05).

Abbreviations: Cy, cyanidin; Pl, peonidin; Cygluc, cyanidin-3-O-glucoside; Pn, peonidin; Cygluc, cyanidin-3-O-glucoside; Cy, cyanidin; Pn, peonidin; Cygluc, cyanidin-3-O-glucoside; Ptarab, petunidin-3-O-arabinoside; Ptcoum, petunidin-3-O-(6′′-O-p-coumaroyl)glucoside; Pneligl, peonidin-3-O-(6′′-O-p-coumaroyl)glucoside; Mvdi, malvidin-3-O-diglucoside; Pneligl, peonidin-3-O-(6′′-O-p-coumaroyl)glucoside; Mvgluc, malvidin-3-O-glucoside; Ptcoum, petunidin-3-O-(6′′-O-p-coumaroyl)glucoside; Dp, delphinidin-3-O-diglucoside; Mvdi, malvidin-3-O-diglucoside; Ptarab, petunidin-3-O-arabinoside; Pneligl, peonidin-3-O-(6′′-O-p-coumaroyl)glucoside; Mv, malvidin; Dp, delphinidin-3-O-diglucoside; Mvdi, malvidin-3-O-diglucoside; Mvgluc, malvidin-3-O-glucoside; Ptcoum, petunidin-3-O-(6′′-O-p-coumaroyl)glucoside; Mv, malvidin; Mvdi, malvidin-3-O-diglucoside; Mvgluc, malvidin-3-O-glucoside (malvin).
| Flavonoid      | Control     | FB1         | FB2         | FB3         | FB4         | FB5         |
|---------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Ca            | 3.73 ± 0.11b| 3.11 ± 0.16c| 2.58 ± 0.13d| 4.84 ± 0.10a| 2.98 ± 0.07cd| 3.36 ± 0.19bc|
| Hydro         | 2.45 ± 0.08ab| 2.55 ± 0.21a| 2.53 ± 0.06a| 2.20 ± 0.03b| 2.36 ± 0.08b | 2.33 ± 0.10b|
| Ga            | 4.65 ± 0.15cd| 5.42 ± 0.10b| 4.73 ± 0.13c| 4.18 ± 0.02d| 5.09 ± 0.32bc| 5.97 ± 0.07a |
| Cid           | 7.31 ± 0.62b| 5.87 ± 0.50bc| 4.30 ± 0.20d| 9.46 ± 0.47a| 7.00 ± 0.69b | 5.14 ± 0.23cd|
| αCadih        | 5.87 ± 0.21b| 4.28 ± 0.03c| 3.24 ± 0.06d| 7.16 ± 0.41a| 5.05 ± 0.49bc| 4.20 ± 0.13c|
| Total Flavan-3-ols | 33.30 ± 1.04b| 30.20 ± 0.68b| 25.60 ± 0.42c| 38.76 ± 1.04a| 31.70 ± 1.65b| 30.20 ± 0.82b|
| Ka            | 18.77 ± 0.87a| 17.5 ± 1.56a| 17.23 ± 0.74a| 16.43 ± 1.69ab| 12.05 ± 2.53b| 17.13 ± 0.03a |
| Qugluco       | 16.3 ± 0.00ab| 16.53 ± 1.21a| 17.23 ± 0.67a| 17.13 ± 0.87a| 13.60 ± 1.11b| 15.57 ± 0.56ab|
| sQuglu        | 20.63 ± 0.45a| 19.33 ± 0.58ab| 20.97 ± 1.14a| 20.00 ± 0.90ab| 16.43 ± 2.02b| 18.40 ± 1.31ab|
| My            | 17.5 ± 0.53b| 19.77 ± 0.79ab| 21.97 ± 1.42a| 19.73 ± 1.52ab| 17.73 ± 0.65b | 17.30 ± 0.66b |
| rKaglu        | 42.3 ± 0.60b| 25.63 ± 0.72d| 26.10 ± 1.72d| 72.93 ± 3.29a| 32.30 ± 2.86c | 23.97 ± 1.09d |
| Kaneo         | 40.37 ± 0.208b| 25.27 ± 1.17c| 25.27 ± 2.29c| 68.9 ± 6.29a | 34.53 ± 4.63bc| 24.9 ± 0.70c |
| *rQuglu       | 25.57 ± 1.25a| 16.53 ± 1.05c| 16.20 ± 0.55c| 22.43 ± 0.98b | 16.47 ± 0.24c | 18.60 ± 0.55c |
| Qu            | 24.57 ± 1.33a| 16.00 ± 0.79c| 16.33 ± 0.37c| 21.43 ± 0.98b | 16.50 ± 0.38c | 17.57 ± 0.24c |
| Quneo         | 21.90 ± 0.83a| 14.70 ± 1.10b| 15.53 ± 0.22b| 21.37 ± 0.59a | 15.33 ± 0.50b | 16.63 ± 0.77b |
| rQuglu        | 25.57 ± 0.76a| 16.67 ± 1.09c| 16.67 ± 0.79c| 21.67 ± 1.22b | 16.50 ± 0.56c | 17.80 ± 0.55c |
| Se            | 19.53 ± 0.83b| 16.3 ± 0.67c| 15.30 ± 0.93c| 24.50 ± 1.18a | 21.83 ± 1.12ab| 16.30 ± 0.79c |
| Me            | 15.40 ± 1.21bc| 13.37 ± 0.52c| 13.43 ± 0.12c| 23.57 ± 1.04a | 18.03 ± 1.26b | 13.17 ± 0.71c |
| Total flavonoids | 4394.1 ± 8.19b| 3491.16 ± 13.68c| 370.82 ± 8.02c| 491.88 ± 18.92a | 345.96 ± 17.98c| 351.03 ± 5.32c |

Note: Different lowercase letters indicate significant differences between treatments, according to Tukey’s HSD (honest significant difference) test (p < .05).

Ca, catechin; Hydro, 4′-hydroxy-5,7-dimethoxyflavanone; Ga, gallocatechin; Cid, cinchona Id; αCadih, catechin-(7,8-bc)-4′-(3,4-dihydroxyphenyl)-dihydro-2-(3H)-one. Ka, kaempferol-7-O-glucoside; Qugluco, quercetin-7-O-glucoside; sQuglu, quercetin-4′-O-glucoside (Spiraeoside); My, myricetin-3-O-gluconuronide; rKaglu, kaempferol-3-O-glucoside-7-O-rhamnoside; Kaneo, kaempferol-3-O-neohesperidoside; *rQuglu, quercetin-3-O-glucoside-7-O-rhamnoside; Qu, quercetin-7-O-rutinoside; Quneo, quercetin-3-O-neohesperidoside; rQuglu, quercetin-3-O-(4′″)-O-glucosylrhamnoside; Se, sexangularetin-3-O-glucoside-7-O-rhamnoside; Me, 6-C-methylquercetin-3-O-rutinoside.
(FB4) significantly increased the contents of some individual anthocyanins and the total anthocyanins in the grape peel, probably because glucose, fructose, and sucrose can induce the accumulation of anthocyanin in grape berries (Zheng et al., 2009). Indeed, the sugar content of grape berries increased significantly under these two treatments, thereby promoting anthocyanin synthesis in grape peel. However, we also found that under treatment with ferrous sulfate (FB1), EDTA-Fe (FB2), and ferric sugar alcohol (FB5), the levels of some individual anthocyanins (Cyacet, Dpacet, and Cycoum) were lower than those in the control, resulting in the total anthocyanin content being lower than that in the control as well. In contrast, previous studies found that anthocyanin content increased under iron treatment (Ahmed et al., 1997; Singh et al., 2013), where different iron treatments significantly increased the content of Mv and its morphological derivatives. Cy and Dp are considered the precursors of Pn, Mv, and Pt (He et al., 2010), respectively. Iron treatment led to an increase in the contents of Mv and its morphological derivatives, which may explain the decrease in the contents of Cy and Dp. Anthocyanin that contain more methoxy groups in the B ring may contribute to the redness of grapes. Methylated anthocyanins, including Pn, Mv, and Pt (He et al., 2010), respectively. Iron treatment led to an increase in the contents of Mv and its morphological derivatives, which may explain the decrease in the contents of Cy and Dp. Anthocyanins that contain more methoxy groups in the B ring may contribute to the redness of grapes. Methylated anthocyanins, including Pn, Mv, and Pt (He et al., 2010), respectively. Iron treatment led to an increase in the contents of Mv and its morphological derivatives, which may explain the decrease in the contents of Cy and Dp.

Anthocyanins are present in most higher plants and are products of flavonoid biosynthesis, while flavonoids are closely related to anthocyanin biosynthesis. We found that the different iron treatments had significantly different effects on the contents of specific flavonols in a manner similar to the changes in the anthocyanin content, possibly because the enzymes involved in the production of flavonols overlap greatly with those involved in the production of anthocyanins (Gonzalez-Manzano et al., 2009). In addition, these classes of compounds share the same skeleton and differ only in the oxidation state of the central pyran ring (Jaakola, 2013). Alvarez-Fernandez et al. (2011) found that iron treatment can improve the photosynthetic efficiency of grape vines and affect the synthesis of phenolic compounds or other secondary metabolites using precursors. However, further research is required to understand why different forms of iron can have different effects on the phenolic compound content in grape peel.

**FIGURE 1** Principal component analysis (PCA) results obtained based on the correlation matrix for the physical and chemical indexes and flavonoid components of grapes: (a) scoring plot and (b) loading plot. The abbreviations used in (b) are defined in Tables 3, 4, and 5.
5 | CONCLUSION

In this study, we found that five different foliar iron treatments affected the fructose, acid, and flavonoid contents of Cabernet Sauvignon grapes, and that the various iron treatments also had different effects. Spraying iron on the leaves could increase the sugar content and reduce the acid content of berries. However, spraying ferrous sulfate, EDTA-Fe, ferric gluconate, and ferric sugar alcohol on the leaves reduced the total anthocyanin, flavanol, and flavonol contents in the peel. In addition, the contents of specific flavonoid monomers were significantly higher in the grape peel under some iron treatments than in the control, as well as with the other iron treatments. However, our comprehensive study showed that foliar spraying with ferric citrate balanced the sugar–acid ratio in the berry and increased the anthocyanin, flavanol, and flavonol contents of the grape peel to further improve the quality of the grapes, thereby possibly enhancing the overall nutritional content of the berries and the final wine quality.

ACKNOWLEDGMENTS

This study was supported by the National Key Research and Development Project (2019YFD1002500) and Ningxia Natural Science Foundation (2020AAC03281). We thank our colleagues for their comments on this paper, and the journal’s editors and anonymous reviewers for their critical reviews and comments regarding this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Shu Zhang https://orcid.org/0000-0003-3267-5881

REFERENCES

Abadia, J., Lopez-Millan, A. F., Rombola, A., & Abadia, A. (2002). Organic acids and Fe deficiency: A review. Plant and Soil, 241, 75–86.
Ahmed, F. F., Akl, A. M., & El-Morsy, F. M. (1997). Yield and quality of ‘banaty’ grapes in response to spraying iron and zinc. Horticscience, 32, 516D.
Ali, K., Maltese, F., Choi, Y. H., & Verpoorte, R. (2010). Metabolic constituents of grapevine and grape-derived products. Phytochemistry Reviews, 9, 357–378.
Alvarez-Fernández, A., Abadia, J., & Abadia, A. (2006). Iron Deficiency, Fruit Yield and Fruit Quality. Iron Nutrition in Plants and Rhizospheric Microorganisms, 85–101.
Alvarez-Fernández, A., Carlos Melgar, J., Abadia, J., & Abadia, A. (2011). Effects of moderate and severe iron deficiency chlorosis on fruit yield, appearance and composition in pear (Pyrus communis L.) and peach (Prunus persica [L.] Batsch). Environmental and Experimental Botany, 71, 280–286.
Arozarena, I. I., Ayestarán, B., Cantalejo, M., Navarro, M., Vera, M., Abril, I., & Casp, A. (2002). Anthocyanin composition of Tempranillo, Garnacha and Cabernet Sauvignon grapes from high- and low-quality vineyards over two years. European Food Research & Technology, 214, 303–309.
Bavaresco, L., Civardi, S., Pezzutti, S., Vezzulli, S., & Ferrari, F. (2005). Grape production, technological parameters, and stilbene compounds as affected by lime-induced chlorosis. Vitis, 44, 63–65.
Bertamini, M., & Nedunchezhian, N. (2005). Grapevine growth and physiological responses to iron deficiency. Journal of Plant Nutrition, 28, 737–749.
Chen, L. S., Smith, B. R., & Cheng, L. L. (2004). CO₂ assimilation, photosynthetic enzymes, and carbohydrates of ‘Concord’ grape leaves in response to iron supply. Journal of the American Society for Horticultural Science, 129, 738–744.
De Gaulejac, N. V., Nonier, M. F., Guerra, C., & Vivas, N. (2001). Anthocyanin in grape skins during maturation of Vitis vinifera L. cv. Cabernet Sauvignon and Merlot Noir from different Bordeaux terroirs. Journal international des sciences de la vigne et du vin, 35, 149–156.
Fernández, V., & Ebert, G. (2005). Foliar iron fertilization: A critical review. Journal of Plant Nutrition, 28, 2113–2124.
Fernandez, V., Orera, I., Abadia, J., & Abadia, A. (2009). Foliar iron-fertilisation of fruit trees: Present knowledge and future perspectives – A review. Journal of Horticultural Science & Biotechnology, 84, 1–6.
Gonzalez-Manzano, S., Duenas, M., Rivas-Gonzalo, J. C., Escribano-Bailon, M. T., & Santos-Buelga, C. (2009). Studies on the copigmentation between anthocyanins and flavan-3-ols and their influence in the colour expression of red wine. Food Chemistry, 114, 649–656.
He, F., Liang, N. N., Mu, L., Pan, Q. H., Wang, J., Reeves, M. J., & Duan, C. Q. (2012). Anthocyanins and their variation in red wines I. Monomeric anthocyanins and their color expression. Molecules, 17, 1571–1601.
He, F., Mu, L., Yan, G. L., Liang, N. N., Pan, Q. H., Wang, J., Reeves, M. J., & Duan, C. Q. (2010). Biosynthesis of anthocyanins and their regulation in colored grapes. Molecules, 15, 9057–9091.
Jaakola, L. (2013). New insights into the regulation of anthocyanin biosynthesis in fruits. Trends in Plant Science, 18, 477–483.
Jin, Z., Sun, H., Sun, T., Wang, Q., & Yao, Y. (2016). Modifications of ‘gold finger’ grape berry quality as affected by the different rootstocks. Journal of Agricultural and Food Chemistry, 64, 4189–4197.
Karimi, R., Koulivand, M., & Ollat, N. (2019). Soluble sugars, phenolic acids and antioxidant capacity of grape berries as affected by iron and nitrogen. Acta Physiologica Plantarum, 41, 117.
Liang, N.-N., Pan, Q.-H., He, F., Wang, J., Reeves, M. J., & Duan, C.-Q. (2013). Phenolic profiles of Vitis davidii and Vitis quinquangularis species native to China. Journal of Agricultural and Food Chemistry, 61, 6016–6027.
Ma, J., Zhang, M., Liu, Z., Chen, H., Li, Y. C., Sun, Y., Ma, Q., & Zhao, C. (2019). Effects of foliar application of the mixture of copper and chelated iron on the yield, quality, photosynthesis, and microelement concentration of table grape (Vitis vinifera L.). Scientia Horticulatae, 254, 106–115.
Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., & Velasco, R. (2006). Metabolite profiling of grape: Flavonols and anthocyanins. Journal of Agricultural and Food Chemistry, 54, 7692–7702.
Ozturk, B., Karakaya, O., Erdem, H., Kucuker, E., Ozkan, Y., & Yildiz, K. (2019). The effects of foliar iron treatments (plus Fe) on fruit quality of different pear cultivars. Erwerbs-Ostbau, 61, 373–378.
Perron, N. R., & Brumaghim, J. L. (2009). A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochemistry and Biophysics, 53, 75–100.
Schlegel, T. K., Schönherr, J. R., & Schreiber, L. (2006). Rates of foliar penetration of chelated Fe(III): Role of light, stomata, species, and leaf age. Journal of Agricultural and Food Chemistry, 54, 6809–6813.
Shi, P., Li, B., Chen, H., Song, C., Meng, J., Xi, Z., & Zhang, Z. (2017). Iron supply affects anthocyanin content and related gene expression in berries of Vitis vinifera cv. Cabernet Sauvignon. Molecules, 22, 283.
Shi, P., Song, C., Chen, H., Duan, B., Zhang, Z., & Meng, J. (2018). Foliar applications of iron promote flavonoids accumulation in grape berry of Vitis vinifera cv. Merlot grown in the iron deficiency soil. *Food Chemistry*, 253, 164–170.

Singh, J., M. Singh, A. Jain, S. Bhardwaj & S. K. Dubey. 2013. *An introduction to plant nutrients and foliar fertilization: A review*. Daya Publishing Company.

Taghavi, T., Hoseinabadi, H., Solgi, M., Askari, M., & Rahemi, A. (2020). Influence of vinegar and chelated iron field sprays on mineral nutrients and fruit quality of grapes (cv. ‘Thompson seedless’). *Mitt Klosterneuburg*, 70, 75–86.

Tian, B., Harrison, R., Morton, J., & Jaspers, M. (2019). Changes in pathogenesis-related proteins and phenolics in Vitis vinifera L. cv. ‘Sauvignon Blanc’ grape skin and pulp during ripening. *Scientia Horticul aute*, 243, 78–83.

Vidot, K., Maury, C., Siret, R., & Lahaye, M. (2020). Phenolic compounds limit or promote oxidative degradation of pectin related to iron-H$_2$O$_2$ ratio. *LWT-Food Science and Technology*, 125, 7.

Wang, R., Qi, Y., Wu, J., Shukla, M. K., & Sun, Q. (2019). Influence of the application of irrigated watersoluble calcium fertilizer on wine grape properties. *PloS One*, 14(9), e0222104.

Yunta, F., Martin, I., Lucena, J. J., & Garate, A. (2013). Iron chelates supplied foliarly improve the iron translocation rate in tempranillo grapevine. *Communications in Soil Science and Plant Analysis*, 44, 794–804.

Zeng, X. Q., Du, Z. J., Sheng, Z. T., & Jiang, W. B. (2019). Characterization of the interactions between banana condensed tannins and biologically important metal ions (Cu$^{2+}$, Zn$^{2+}$ and Fe$^{2+}$). *Food Research International*, 123, 518–528.

Zheng, Y., Tian, L., Liu, H., Pan, Q., Zhan, J., & Huang, W. (2009). Sugars induce anthocyanin accumulation and flavanone 3-hydroxylase expression in grape berries. *Plant Growth Regulation*, 58, 251–260.

Zimman, A., Joslin, W. S., Lyon, M. L., Meier, J., & Waterhouse, A. L. (2002). Maceration variables affecting phenolic composition in commercial-scale cabernet sauvignon winemaking trials. *American Journal of Enology and Viticulture*, 53, 93–98.

How to cite this article: Zhang, S., Chen, H., Gao, M., Gu, C., & Wang, R. (2022). Effects of different iron treatments on wine grape berry quality and peel flavonoid contents. *Food Science & Nutrition*, 10, 3598–3607. [https://doi.org/10.1002/fsn3.2957](https://doi.org/10.1002/fsn3.2957)