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Pre-harvest Application of Potassium and Iron Promotes Phenolic Acids and Anthocyanidin Accumulation and Boosts Antioxidant Capacity in Raisin Produced from 'Red Sultana' Grape (*Vitis vinifera L.*)

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ABSTRACT

Raisins are good sources of bioactive compounds with beneficial effects on human health. Mineral nutrition is one of the main viticultural practices affecting grape and raisin phytochemical compositions. In this study, the effect of preharvest foliar application of potassium (K_2SO_4; 0, 1.5 and 3%) and iron (Fe-EDDHA; 0, 0.5 and 1%) was evaluated on some nutritional and biochemical properties of raisins produced from ripped 'Red Sultana' grape (*Vitis vinifera* L.). The experiment laid on a factorial arrangement of variables using a completely randomized block design. The highest soluble sugars of fructose and glucose were related to raisin produced from the vines treated with 3% potassium in combination with 0.5% iron fertilizers. However, 3% K_2SO_4-treated vines in combination with 1% Fe- EDDHA showed a considerable increase in raisin sucrose and also putrescine concentration. The raisin organic acids of succinic acid, fumaric acid, citric acid, and malic acid increased significantly in treated vines with both fertilizers at final doses; however, tartaric acid showed the highest amount in 3% potassium in combination with 0.5% iron treatments. The vines treated with a high level of potassium in combination with moderate level of iron produced raisin with the highest phenolic acids of kaempferol, quercetin, chlorogenic acid and resveratrol and also showed the lowest polyphenol oxidase activity. Furthermore, raisin cinnamic acid, rutin and catechin concentration showed a peak in vines sprayed with a high level of potassium and iron and also most anthocyanidins such as petunidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-glucoside and delphinidin-3-glucoside reached their highest concentration by this treatment. Likewise, the highest antioxidant capacities (measured by FRAP, DPPH and ABTS methods) were achieved in 3% potassium–treated vines in combined with iron at a moderate level. In conclusion, results indicated that preharvest application of potassium and iron are highly effective to improve the Red Sultana raisin bioactive compositions.

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Introduction

Grapes are cultivated throughout the world (over than 90 countries) and consumed mainly as table grape, juice, wine, raisins, and other grape byproducts (Keller, 2015; Karimi and Mirzaei, 2018). Generally, the dried seedless grape cultivars where the internal moisture is reduced to around 15% are known as raisins (Karimi and Mirzaei, 2018). Raisins are a good source of nutritive elements, including of potassium, iron, flavonoids and organic acids, and their health benefits have been documented by many researchers (Karadeniz et al., 2000; Sério et al., 2014; Restani et al., 2016). Today the global production of industrial dried grape as raisins reached 1.21 million tons based on the final report of the United States Department of Agriculture (USDA, 2019). The four top raisins producing countries worldwide in 2018/2019 are Turkey (280,000 tons), USA (263,000 tons), China (190,000 tons) and Iran (150,000 tons) according to dry production weight (USDA, 2019).

Unlike the other fruits that are sliced for drying (*i.e.* apricot, apple), during the processing of grapes into raisins, the outer skin is not destroyed entirely, and depending on the different treatments, only some tiny pores are formed on the waxy cuticle (Simal et al., 1996). For this reason, its phytochemical compositions can be preserved for 9-12 months (Karimi and Mirzaei, 2018). These compounds are rich in anthocyanidins, phenolic acids, and flavonoids, which play an important role in deactivating free radicals and preventing the development of various diseases including cancer in humans (Spiller et al., 2003). However, during raisin processing and storage there is the possibility of losses in polyphenols and anthocyanins by polyphenol oxidase (PPO) activity or non-enzymatic oxidation (Maillard reaction; Lante et al., 2016). Moreover, different drying methods may negatively change or decrease some of the valuable internal composition of raw materials of raisins throughout production (Carranza-Concha et al., 2012; Karimi and Mirzaei 2018). Therefore, the uses of proper pre-harvest vineyard operations to increase these compounds are necessary to compensate for this deficiency (Jogaiah et al., 2014).

Among the pre-harvest operations, mineral nutrition is a determining factor affecting the yield and quality of raisins (Wang et al., 2006). The quality of grapes and processed raisins are a consequence of the balance between the amount of primary and secondary metabolites accumulation in berries. Proper applications of fertilizers have played a major role in increasing the production of phenolic acids and flavonoids in different grape cultivars (Karimi and Mirbagheri 2018; Karimi 2019); however, the relationship between foliar nutrients spray and raisins secondary metabolites biosynthesis is not clear and there is no indication of the role of mineral nutrition during the growing season on the accumulation of polyphenols in processed raisin.

Among the essential nutrients involvement in grape berries' growth and development, two elements of potassium and iron play a key role in fruit quality improvement (Karimi and Mirbagheri, 2018). Physiologically potassium has a main role in activating different enzymes, helping with photosynthesis, regulating the opening and closing of stomata, and the biosynthesis of polyphenols involvement in fruit natural antioxidants (Brunetto et al., 2015, Karimi, 2017; Norozi et al., 2019). Potassium would increase yield, berry size and total soluble solids of fertilized grapevine plants (Martin et al., 2004; Abd El-Razek et al., 2011; Karimi and Mirbagheri, 2018). Potassium could influence berry soluble sugars, titratable acidity, polyamines and phenolic acids of grape berries' and improve overall fruit quality (Karimi and Mirbagheri, 2018). Iron is the most abundant micronutrient in grape tissues. It mediates chlorophyll biosynthesis and activates enzymes involved in photosynthesis, respiration and also some antioxidant enzymes (Curie et al., 2008; Ghrairi et al., 2013). Iron deficiency has a negative effect on the total anthocyanin, polyphenol and resveratrol content of grapes and harvested raisins (Bavaresco et al., 2005). The useful effects of iron spray on grape berries sugar content and their phytochemical compositions were reported by different researchers (Bacha et al., 1995; Shi et al., 2017; Karimi et al., 2019).

'Red Sultana' is a main grape (*Vitis vinifera* L.) cultivated variety in Iran and many grape-producing countries of the world and consumed mainly as table grape, juice and raisins (Ghrairi et al., 2013; Karimi and Mirzaei, 2018). However, improper fertilization condition could negatively influence its raisin phenolic compounds and antioxidant capacity (Sério et al., 2014; Ghrairi et al., 2013). Therefore, application of proper treatments that increase the nutritional value and further accumulation of secondary metabolites in this cultivar may increase the quality of raisins. During the past 20 years, extensive research emphasis has been placed on the preharvest application of mineral nutrition on fruit yield and fruit quality (Bavaresco, 1993; Delgado et al., 2006; Abd El-Razek et al., 2011; Brunetto et al., 2015; Karimi and Mirbagheri, 2018). However, there is no information about the pre-harvest iron and potassium nutrition on the amount of raisins phytochemical parameters. We hypothesize that

the raisin quality is a function of grape quality as raw material and for this, any increase in the level of phytochemical compounds in grape berries can affect these metabolites subsequently in raisins. Therefore, this study was aimed to obtain the best concentration of potassium sulfate in combination with iron chelate to improve the phenolic acids, organic acid, soluble sugars and antioxidant capacity of 'Red Sultana' raisins. We explored whether 'Red Sultana' raisin nutritional and biochemical compounds could be affected by the pre-harvest application of potassium and iron in the vineyard.

Materials and methods

Plant material and treatments

In this study, a commercial vineyard of *Vitis vinifera* L. Cv. 'Red Sultana' (17-year old; own-rooted vines) was chosen in Malayer city of Iran with latitude, longitude and altitude of 34° 52′ N, 48° 84′ E and 1780 m respectively. This region's minimum, maximum and average annual temperatures were -17.5 ˚C, 39.4 ˚C, and 13.3 ˚C, respectively, with mean rainfall per annum of 328 mm. The vines were grown with a density of 2 × 4 m and non-trellising canopies which were managed according to conventional local viticultural practices (Karimi and Mirbagheri, 2018). In this experiment, potassium sulfate 52% (K_2SO_4; 0, 1.5 and 3%) and iron chelate 6% (Fe-EDDHA; 0, 0.5 and 1%) were sprayed on vines canopy at three main stages on cluster development including a week before bloom (1 June), two weeks after bloom (22 June) and five weeks after bloom (13 July) during the growing seasons of 2016 and 2017. All nine combined foliar treatments were named as K_1F_1 (control, 0% K_2SO_4 + 0% iron chelate); K_1F_2 (0% K_2SO_4 + 0.5% iron chelate); K_1F_3 (0% potassium sulfate + 1% iron chelate); K_2F_1 (1.5% potassium sulfate + 0% iron chelate); K_2F_2 (1.5% potassium sulfate + 0.5% iron chelate); K_2F_3 (1.5% potassium sulfate + 1% iron chelate); K_3F_1 (3% potassium sulfate + 0% iron chelate); K_3F_2 (3% potassium sulfate + 0.5% iron chelate); K_3F_3 (3% potassium sulfate + 1% iron chelate). Fruits were hand-picked on September based on total soluble solid index (°Brix) transported to laboratory for raisin preparation.

Raisin preparation

In the present investigation, chemical pre-drying treatments that hasten drying were applied for raisin processing. Briefly, 5 kg of freshly grape bunches were washed carefully with tap water and dipped into an alkaline oil-in-water emulsion known to grapevine growers as 'cold dip' (50 gr potassium carbonate + 2 mL ethyl oleate + 1L distilled water; pH 9.5) for 5 min (Karimi and Mirzaei, 2017; Yari et al., 2017). After this treatment, grape bunches were spread on a drying rack with wire mesh sheets under direct sunlight and left on the rack until they reached 15% moisture content after 5 days (Karimi and Mirzaei, 2017). Thereafter, raisins were boxed immediately after shaking and kept in a cool place for biochemical analysis of reducing sugars, polyamines, organic acids, phenolic acids, anthocyanidins, PPO activity and antioxidant capacity.

Extraction and analysis of soluble sugars

The raisin samples (0.5 g) were powdered, dissolved in 10 mL ethanol (80%), and centrifuged at 8000 rpm for 15 min. The solution was filtered (0.2 µM pore size Filter) and then the supernatants were taken from the tubes. A Crystal 200 series HPLC pump (ATI Unicam, UK) equipped with a SPD UV –Vis detector and a Spherisorb ODS-2 Column (0.3 µm, 150 mm × 4.6 mm i.d.) was applied for measurement of soluble sugar such as fructose, glucose and sucrose (Shin et al., 2002) using external standard solution calibrations of these soluble sugars (Sigma, Australia). Sodium citrate (pH 5.5) and ultrapure acetonitrile (1:99, V/V) at a flow rate of 0.1 mL^{-1} min was used as the mobile phase. The injection volume was 10 µL (Comis et al., 2001). Sugar concentrations were expressed as µmol g^{-1} fresh weight (FW).

Extraction and analysis of polyamines

The free polyamines were detected by direct dansylation and HPLC according to previous description by Walter and Geuns (1987). Briefly, 250 mg of frozen raisin samples were solved in 2 mL internal standard solution (4% $HClO_4$ containing 1, 7 diaminoheptane-2HCl), homogenized for 1 h at 4 °C and then filtrated (0.45 µ filter) carefully. A volume of 0.2 mL of obtained supernatant dissolved in 1 mL of carbonate buffer (pH 9) and 1 mL of dansyl chloride solution (10 mg mL^{-1} acetone). The solution was heated for 1 h at 60 °C and 3 mL of toluene added to it for extraction of dansylated polyamines. After loading of the obtained raisin dansylated polyamines extraction on silica gel (0.5 g), it was washed toluol (3 mL) and a volume of 5 mL toluol- triethyl amine (10/0.3 v/v) and reduced under N2 after elution with 3 mL of ethyl acetate. An octadecyl silica column with 10-cm long was applied for isocratic HPLC analysis using acetonitrile/H_2O (72/28, v/v) for 7 min with the solvent flow of 2 mL min^{-1} and dansylated polyamines (putrescine, spermidine and spermine) injected as references.

Organic acids analysis

Analysis of organic acids (tartaric acid, malic acid, citric acid, succinic acid and fumaric acid, ascorbic acid) was performed according to methods previously described by Zheng et al. (2009). Raisin samples (5 g) were dissolved in 10 mL acetonitrile 0.01 mL L^{-1} KH_2PO_4 (5:95, pH 2.7) buffer, homogenized with this buffer up to a volume of 25 ml and centrifuged for 10 min at 12,000×g under refrigerator temperature (4°C). The upper phase was diluted 1:5 with extraction buffer and passed through a 0.22-µm filter for water solvents (tetrafluoroethylene filter; Gelman/ Pall Life Sciences, Michigan, MI, USA). Liquid chromatography (LC) analysis was done by injection of filtered samples (10 µL) to a WondaSil C18 column (4.6×250 mm i.d., 5 µm). The LC system was equipped with a LC-15C series pumping system (Shimadzu) with an auto-sampler (SIL-10AF) and a SPD-15C series UV double wavelength detector (Shimadzu). Ultrapure acetonitrile (5%) and 0.01 mol L^{-1} potassium dihydrogen phosphate (95%, pH 2.7) with a flow rate of 0.8 mL/min were used as solvents (mobile phase). L-ascorbic acid was detected at 243 nm and other organic acids at 210 nm wavelengths.

Extraction and determination of phenolic acids and anthocyanidins

Raisin phenolic acids (kaempferol, quercetin, chlorogenic acid, resveratrol, cinnamic acid, rutin, catechin) and anthocyanidins (petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, pelargonidin-3-O-glucoside, cyanidin-3-glucoside, delphinidin-3-glucoside) analysis was started with boiling of each sample powders (1 g) in HCL (0.1 N) for 27 min and homogenate subsequently filtered. The obtained solution was separated with ethyl acetate, which soluble and insoluble portions dissolved in water and 80% methanol respectively. Samples were filtered again through a Millex HA 0.45 µm filter (Millipore Crop.) before injection to a HPLC pump (Crystal 200 series, Unicam, Cambridge, UK) under room temperature (Koponen et al. 2007). Separations were done with a UV-Vis detector, 4.6 × 250 mm, 5 µm ODS column (HiChrom, USA) with a flow rate of 1 mL min^{-1} and potassium di-hydrogen phosphate and acetonitrile (80:20, v: v) as mobile phase. Stock solutions of the standard acids (E. Merck) were prepared in a concentration of 1 g 100 mL^{-1} in pure methanol (Vekiari et al., 2008).

Analysis of polyphenol oxidase activity

Raisin samples (5 g) were homogenized in 0.2 M KH_2PO_4 buffer (10 mL, pH 7.0) and centrifuged at 6000× *g* for 12 min at 5° C. For PPO activity measurement, 0.2 M sodium hydrogen phosphate and 0.1 M citrate monohydrate (2/1, v/v, PH 6.5) were used as McIlvaine buffer according to Haplin and Lee (1987). Afterward, catechol (1.4 g) was added to McIlvaine buffer (25 mL) and its volume reached 250 mL with this buffer and shaken for 30 minutes. The activity of PPO was measured at 420 nm after adding enzyme extract (200 mL) to the obtained solution (2.8 mL). The change in absorbance of 0.1 per minute per mL of the enzyme extract was expressed as one unit of PPO activity.

Determination of antioxidant capacity

DPPH

Raisin antioxidant activities based on DPPH (1, 1-diphenyl-2- picrylhydrazyl) were performed according to Bozin et al. (2008). Briefly, methanolic raisin extracts at different concentrations were added to one mL DPPH solution (90 µM) and its volume reached 3 mL with methanol (95% v/v) and immediately shaken for 60 min at 23°C in a dark place. The absorbance of prepared samples was read at 517 nm compared to the blank solution with no raisin extract. The following equation was applied for calculating DPPH radical scavenging capacity (RSC):

(1) $\text{DPPH RSC (\%)} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$

FRAP

The ferric reducing ability of plasma (FRAP) of raisin samples was determined based on the method described by Benzie and Strain (1996). The reactive solution contained 25 mL 0.3 M L^{-1} sodium acetate (as buffer solution with pH 3.6), 2.5 mL TPTZ (tripyridyl-s-triazine; 10 mM/L), 2.5 mL $FeCl_3$ (20 mM/L) and 3 mL water. Diluted raisin (30 µL), which was extracted with solving raisin in water (1:10), was added to 970 µL of the reactive solution and heated to 36 ºC for half hour and used for analysis at 593 nm based on the formation of tripyridyl-s-triazine complexes with iron (II) (TPTZFe(II)) in the presence of a reductive agent. Linear calibration was fitted to different concentrations of $FeSO_4$ and was used to express of the result as mM FeII.

ABTS

The raisin antioxidant capacity also was performed based on ABTS (2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) assay according to Re et al. (1999). This assay is based on the increased absorbance recorded at 734 nm due to the color change resulted from the reduction of ABTS+ to ABTS. This reaction was

started when 7 Mm ABTS was added to potassium persulphate (2.45 mM, 1:1) solution in water under darkness place at 23°C. After 16 hours in this condition, the assay was completed by adding ABTS+ solutions (980 µL) to diluted raisin extract samples (20 µL) in water (1:50). After 15 min of reaction time, the absorbance measurements were recorded at 734 nm.

Statistical analysis

This experiment consisted of a factorial arrangement of variables using a completely randomized block design with three replications per treatment (nine combined foliar treatments) and two vines per replication. Data analysis of variance (ANOVA) were performed by GLM procedures of SAS (SAS Institute Inc., USA), and Duncan's multiple range test at a confidence level of 5% was used for mean comparisons.

Results

Soluble sugars

Potassium, iron, and their interactions significantly ($P<0.001$) influenced the 'Red Sultana' raisin soluble sugars concentration (Fig.1). Across all treatments, the highest concentrations of fructose and glucose were related to raisin produced from the vines treated with 3% K_2SO_4 in combination with 0.5% chelated iron fertilizers (K_3F_2), indifferent with glucose values of K_3F_3- treated vines (Fig. 1A, 1B). The lowest raisin glucose and fructose were related to the control untreated vines with no significant difference from the vines treated with 1.5% potassium sulfate alone (K_2F_1; Fig. 1A, 1B). Moreover, the highest raisin sucrose concentration was found in 3% K_2SO_4 treated vines in combination with 1% Fe- EDDHA (K_3F_3; Fig. 1C).

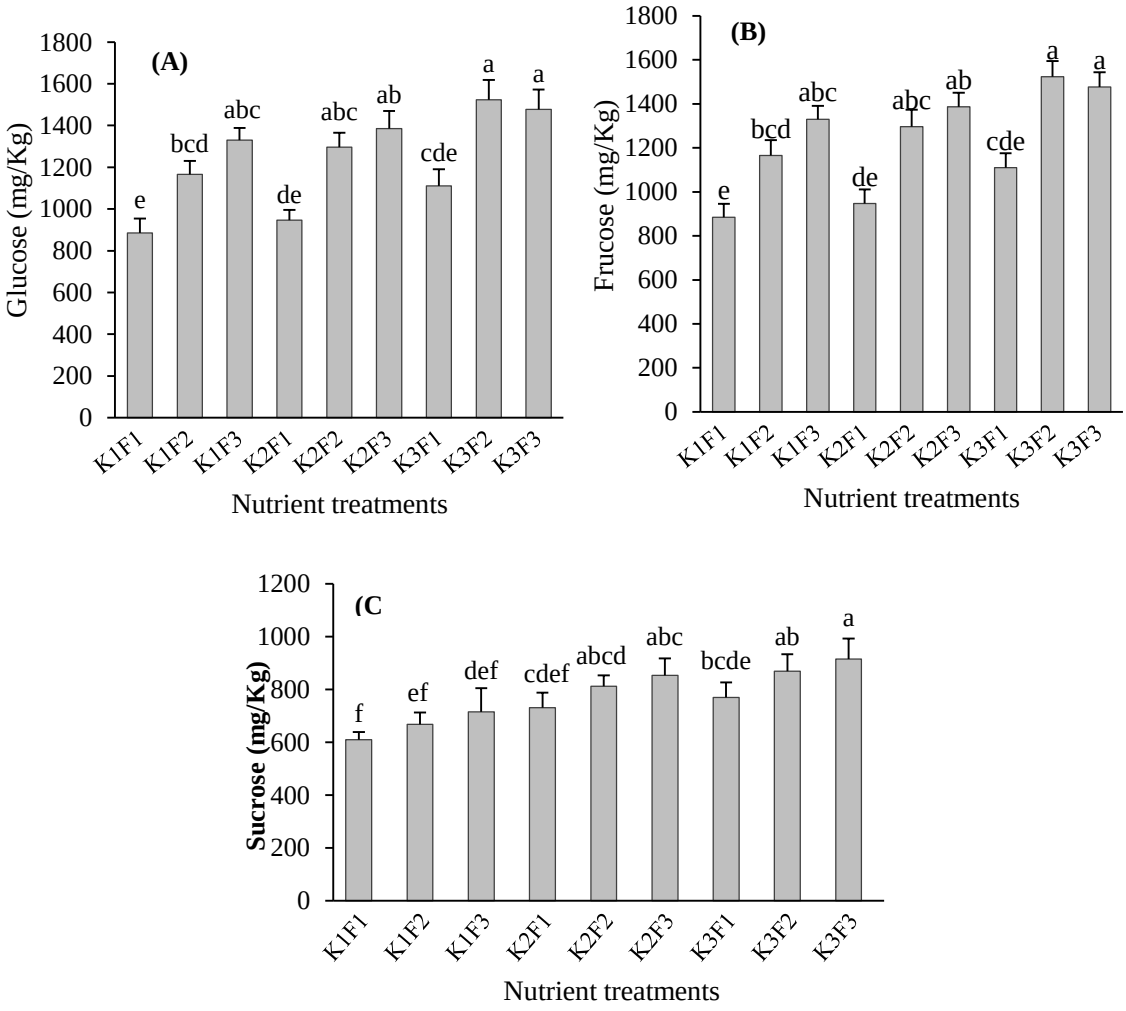

Fig. 1. Interaction effect of pre-harvest foliar application of potassium sulfate and iron chelates on produced 'Red Sultana' raisin glucose (panel A), fructose (panel B) and sucrose (panel C). The means showing same letters in each column are not different statistically ($P\leq 0.05$). K_1, K_2 and K_3 are indicating potassium sulfate at 0, 1.5 and 3% doses respectively and F_1, F_2 and F_3 indicating iron chelate at 0, 0.5 and 1% doses respectively

Polyamines

Raisin polyamines concentration was affected significantly ($P<0.001$) by the pre-harvest spray of the vines with potassium, iron, and their interactions. Data on raisin polyamines were given in Table 1. Based on the results of all treatments, the highest putrescine concentration was found in raisin samples of K_3F_3-treated vines. Likewise, the highest raisin spermidine and spermine values were detected in grape from K_3F_2- treated vines (Table 1). The lowest of all polyamines contents were related to raisin samples obtained from the control untreated vines (Table 1), although it did not differ significantly with raisin spermidine and spermine of 0.5% iron chelate (K_1F_2) treated vines (Table 1).

Table 1. Interaction effect of pre-harvest foliar application of potassium sulfate and iron chelates on produced Red Sultana raisin polyamines (nmol g^{-1} of raisin)

Nutrient treatments	Putrescine	Spermine	Spermidine
K_1F_1	6.41e	14.08e	9.15e
K_1F_2	6.77de	19.88c	10.15e
K_1F_3	7.82cd	23.68b	11.38d
K_2F_1	7.59d	16.16de	10.69de
K_2F_2	9.46bc	18.44cd	13.07bc
K_2F_3	10.24b	24.09ab	14.46b
K_3F_1	8.73c	18.25cd	12.68c
K_3F_2	10.61b	27.37a	16.10a
K_3F_3	12.24a	20.10c	14.77b

*The means showing same letters in each column are not different statistically ($P\leq 0.05$).
K_1, K_2 and K_3 are indicating potassium sulfate at 0, 1.5 and 3% doses respectively and F_1, F_2 and F_3 indicating iron chelate at 0, 0.5 and 1% doses respectively.

Organic acids

Raisin organic acid concentrations were affected significantly ($P<0.001$) by the pre-harvest spray of the vines with potassium, iron, and their interactions. As shown in Table 2, the predominant raisin organic acid was tartaric acid, followed by malic acid, citric acid, ascorbic acid, succinic acid and fumaric acid. Succinic acid, fumaric acid, citric acid and malic acid of raisins obtained from the fruits grown on the vines treated with 3% potassium in combination with 1% Fe-EDDHA fertilizers were found to be consistently higher than other treatments (Table 2), although it did not differ significantly with this organic acid measured of raisin of K_3F_2-treated vines (Table 2). Ascorbic acid concentration of raisin samples produced from the pre-harvest sprayed vines with K_2F_3 treatment was found to be higher in comparison to other combined treatments (Table 2). Raisin tartaric acid concentration of K_3F_2- treated vines had the highest value compared to other treatments although its value did not differ significantly with raisin obtained from the K_3F_3-treated vines (Table 2). Although the lowest concentration of all organic acids was related to control vines, but, their values did not differ with same level of treatments as reported in Table 2.

Table 2. Interaction effect of pre-harvest foliar application of potassium sulfate and iron chelates on produced Red Sultana raisin organic acid (mg 100 g^{-1} of raisin)

Nutrient treatments	Succinic acid	Fumaric acid	Citric acid	Ascorbic acid	Tartaric acid	Malic acid
K_1F_1	14.79f	9.485f	63.45f	32.23f	131.40f	94.5f
K_1F_2	15.73ef	10.12ef	69.45e	39.91de	143.4ef	100.3ef
K_1F_3	16.33ef	12.26de	73.30d	45.49b	158.1de	106.3e
K_2F_1	18.41de	14.085d	65.50ef	34.93ef	152.45def	121.2d
K_2F_2	19.07cd	14.66cd	79.65c	36.32e	165.2de	129.15c
K_2F_3	21.39bc	17.20bc	91.6b	49.74a	187.45bc	141.2b

Nutrient treatments	Succinic acid	Fumaric acid	Citric acid	Ascorbic acid	Tartaric acid	Malic acid
K_3F_1	22.10b	17.51b	77.35cd	37.75def	169.3cd	127.3c
K_3F_2	23.46ab	18.33ab	99.30a	43.19bc	205.4a	150.7ab
K_3F_3	25.56a	20.19a	101.40a	41.46cd	196.3ab	162.4a

The means showing same letters in each column are not different statistically (P≤ 0.05).
K_1, K_2 and K_3 are indicating potassium sulfate at 0, 1.5 and 3% doses respectively and F_1, F_2 and F_3 indicating iron chelate at 0, 0.5 and 1% doses respectively.

Phenolic acids

The concentration of raisin phenolic acids including kaempferol, quercetin, chlorogenic acid, resveratrol, cinnamic acid, rutin and catechin were affected significantly (P≤0.01) by potassium, iron, and their interactions (Table 3). The kaempferol, quercetin, chlorogenic acid and resveratrol were found to be highest in raisin obtained from the grape grown on the vines treated with 3% potassium in combination with 0.5% iron (Table 3), although their values did not differ significantly with these phenolic acids measured of raisin of K_3F_3- treated vines (Table 3). Raisin cinnamic acid, rutin and catechin concentrations reached a peak in treated vines with 3% K_2SO_4 in combination with 1% chelated iron (Table 3). The raisin quercetin concentration of the vines sprayed with chelated iron alone at 0.5% was found to be the lowest among all treatments with no significant difference with raisin of control vines (Table 3). Moreover, the raisin chlorogenic concentration of the vines sprayed with K_2F_1 was found to be the lowest among all treatments with no significant difference with raisin of control vines (Table 3). For all treatments, the raisin phenolic acid levels increased with enhancement in iron doses in treated vines. However, this increment was dramatically higher in the raisins obtained from the vines treated with iron in combination with potassium (Table 3).

Table 3. Interaction effect of pre-harvest foliar application of potassium sulfate and iron chelates on produced Red Sultana raisin phenolic acid concentration (μg g^{-1} of raisin).*

Nutrient treatments	Kaempferol	Quercetin	Chlorogenic acid	Resveratrol	Cinnamic acid	Rutin	Catechin
K_1F_1	3.11f	6.72f	9.07ef	3.21f	0.85f	1.39f	4.29d
K_1F_2	4.09def	8.14e	11.1de	4.78de	0.92ef	1.51ef	4.07d
K_1F_3	4.96de	10.4d	13.4cd	5.79cd	1.30e	1.96e	4.88d
K_2F_1	3.51f	6.52f	8.41f	3.83ef	1.70d	2.49d	5.69c
K_2F_2	5.11cd	10.2d	13.1cd	6.01c	1.80d	2.55d	5.81c
K_2F_3	6.10bc	12.2c	15.1bc	6.63bc	2.31c	3.08c	6.36bc
K_3F_1	3.96ef	7.95e	11.3de	4.60e	2.44c	2.96cd	6.51bc
K_3F_2	7.47a	15.8a	17.8a	8.22a	2.95b	3.81b	6.96b
K_3F_3	6.81ab	15.1a	16.3ab	7.55ab	3.39a	4.59a	7.69a

*The means showing same letters in each column are not different statistically (P≤ 0.05).
K_1, K_2 and K_3 are indicating potassium sulfate at 0, 1.5 and 3% doses respectively and F_1, F_2 and F_3 indicating iron chelate at 0, 0.5 and 1% doses respectively.

Anthocyanidins

The raisin concentrations of petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, pelargonidin-3-o-glucoside, cyanidin-3-glucoside and delphinidin-3-glucoside were affected significantly (P≤0.01) by potassium, iron, and their interactions (Table 4). Based on the results, the petunidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-glucoside and delphinidin-3-glucoside concentration of raisin were found to be higher in raisin obtained from the grapes grown on the K_3F_3-treated vines than other treatments (Table 4). The lowest anthocyanidin was related to raisin produced from control vines (K_1F_1; Table 3), but not different with raisin produced from K_1F_2-treated vines (Table 3).

The highest concentration of malvidin-3-glucoside and pelargonidin-3-o-glucoside was obtained in K_3F_2- treated vines (Table 3). The lowest concentration of these two anthocyanidins was acquired with raisin produced from control vines (K_1F_1; Table 3).

Table 4. Interaction effect of pre-harvest foliar application of potassium sulfate and iron chelates on produced Red Sultana raisin anthocyanidins concentration (mg kg^{-1} of raisin).*

Nutrient treatments	Delphinidin-3-glucoside	Cyanidin-3-glucoside	Pelargonidin- 3-O-glucoside	Malvidin-3-glucoside	Peonidin-3-glucoside	Petunidin-3-glucoside
K_1F_1	98.10g	39.70f	44.40f	186.1f	65.2f	93.60f
K_1F_2	116.1fg	49.85ef	49.50cd	288.2c	77.97ef	101.7f
K_1F_3	142.4ef	58.70de	50.75c	316.0b	97.12e	123.6e
K_2F_1	169.2de	62.05de	46.60e	210.4e	128.1cd	118.8e
K_2F_2	180.5d	83.06c	48.55cd	260.6cd	120.4d	169.5c
K_2F_3	219.0c	96.20b	54.60bc	322.2b	155.5b	184.5b
K_3F_1	229.3c	74.21cd	47.70de	248.5d	148.6bc	144.1d
K_3F_2	269.2b	95.50b	62.65a	349.2a	145.4bc	185.3b
K_3F_3	307.5a	110.7a	55.90b	326.3b	189.1a	202.7a

*The means showing same letters in each column are not different statistically ($P \leq 0.05$).
K_1, K_2 and K_3 are indicating potassium sulfate at 0, 1.5 and 3% doses respectively and F_1, F_2 and F_3 indicating iron chelate at 0, 0.5 and 1% doses respectively.

Polyphenol oxidase

As shown in Figure 2, preharvest foliar applications of potassium, iron, and their interactions on PPO activity were significant ($P \leq 0.05$). Accordingly, the lowest PPO activity (0.45 EU mL^{-1} min^{-1}) of raisin samples was achieved for 3% potassium combined with 0.5% Fe-EDDHA, although its value did not differ significantly with PPO activity (0.48 EU mL^{-1} min^{-1}) of raisin obtained from the K_2F_3- treated vines (Fig. 2). However, the highest PPO activity (0.72 EU mL^{-1} min^{-1}) was found in raisin of control untreated vines.

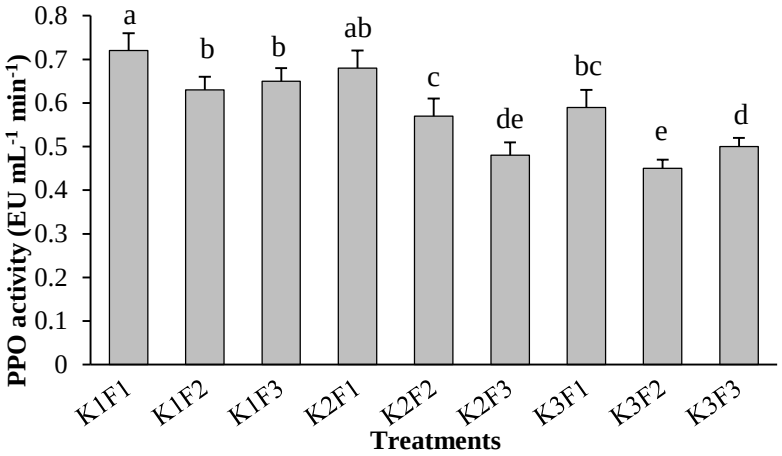

Fig. 2. Interaction effect of pre-harvest foliar application of potassium sulfate and iron chelates on produced 'Red Sultana' raisin polyphenol oxidase activity (PPO). The means showing same letters in each column are not different statistically ($P \leq 0.05$). K_1, K_2 and K_3 are indicating potassium sulfate at 0, 1.5 and 3% doses respectively and F_1, F_2 and F_3 indicating iron chelate at 0, 0.5 and 1% doses respectively

Antioxidant capacity

Pre-harvest foliar applications of potassium, iron, and their interactions significantly ($P \leq 0.05$) influenced Red Sultana raisins antioxidant capacity based on ABTS, FRAP and DPPH methods. Based on data measured by FRAP, the highest raisin antioxidant capacity was related to those vines pre-harvest treated with K_3F_2 fertilizers levels (Fig. 3A), although their values did not differ significantly with FRAP-measured antioxidant capacity of raisin of K_3F_3-treated vines (Fig. 3A). Moreover, DPPH-measured antioxidant capacity was found to be higher in raisin obtained from the grape grown on the vines treated with combined treatment of K_3F_2 than other fertilizers levels (Fig. 3B), although their values did not differ significantly with K_2F_3- treated vines (Fig. 3B). Among all applied treatments, the raisins of K_3F_2- treated vines were significantly higher from ABTS method measured antioxidant capacity (Fig. 3C), although their values did not differ significantly

with K_2F_3- and K_3F_3 treated vines (Fig. 3C). The raisin samples produced from the control vines (K_1F_1) also had a lower value of antioxidant capacity based on all three methods (Fig. 3A, B, C).

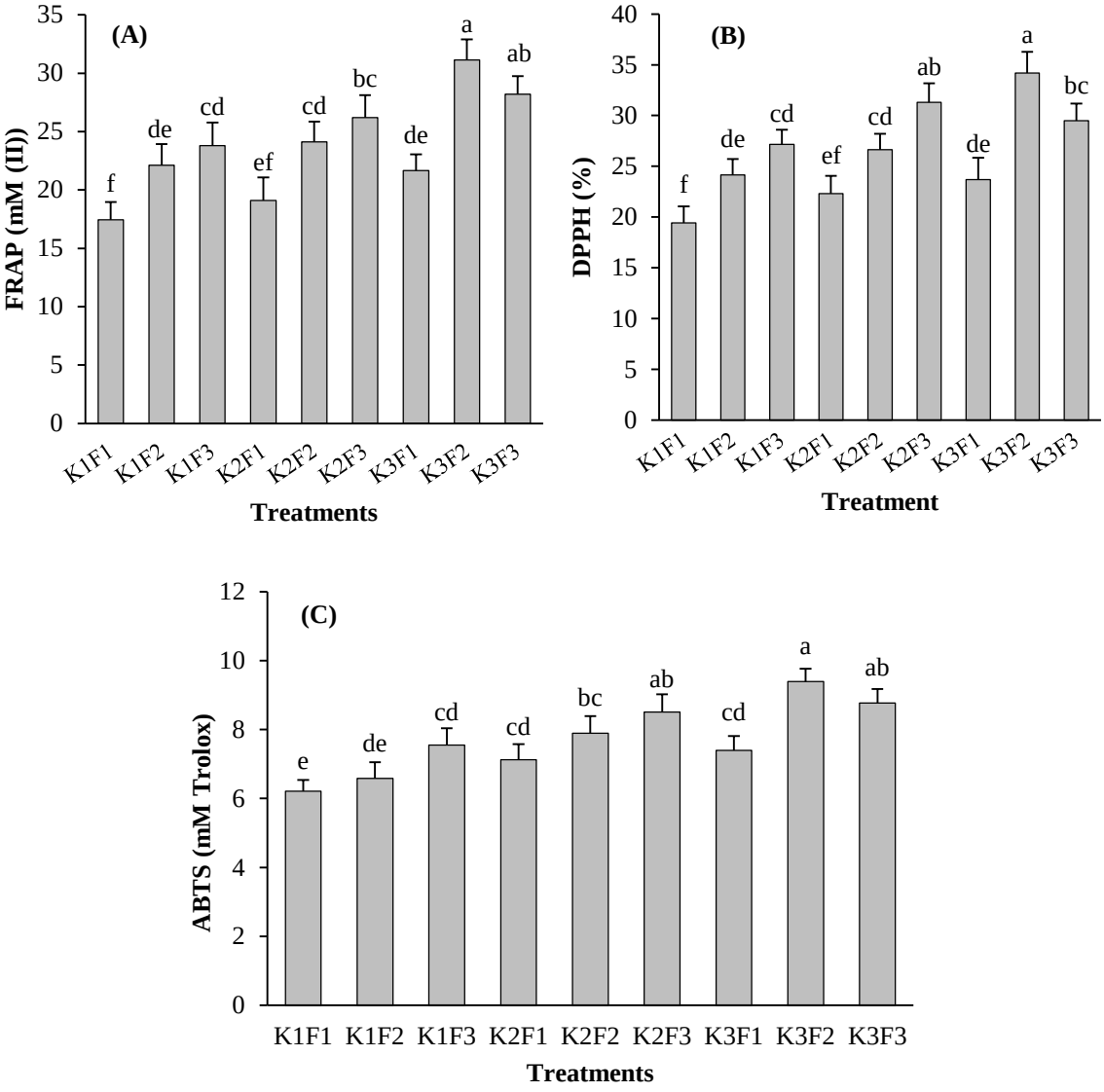

Fig. 3. Interaction effect of pre-harvest foliar application of potassium sulfate and iron chelates on produced 'Red Sultana' raisin antioxidant capacity measured by FRAP (panel A), DPPH (panel B) and ABTS (panel C). The means showing same letters in each column are not different statistically ($P \leq 0.05$). K_1, K_2 and K_3 are indicating potassium sulfate at 0, 1.5 and 3% dozes respectively and F_1, F_2 and F_3 indicating iron chelate at 0, 0.5 and 1% doses respectively

Discussion

Pre-harvest growing conditions of vineyards such as proper application of fertilizers especially at critical developmental stages can affect the phytochemical compositions of both ripen grape and raisin production. Raisin soluble sugars and polyamines content were higher in sample raisin of grapevine plans treated with high levels of potassium combined with 0.5 and 1% concentrations of iron fertilizers compared to vines without supplemental fertilizers. Mineral nutrition such as potassium and iron has a main impact on soluble sugars accumulation in grapes (Keller, 2015; Karimi et al., 2019). In 'Bezel Naka' local grape cultivar, foliar treatment with micronutrient such as Fe improved soluble sugars which are confirmed in this investigation (Abdel-Salam, 2016). Iron has a key role in tree fruit carbohydrate metabolism and quality. Glucose and fructose are two main soluble sugars in raisin (Ghrairi et al., 2013) with an inherent low glycemic index which can enter in

blood stream without digestion and provide energy immediately for those persons subjected to stress (Sério et al., 2014). Moreover, potassium and iron have different physiological functions in grape nutritional profile (Karimi and Mirbagheri, 2018) and in our study their application improved the quality of raisins as a functional food.

In this study, 'Red Sultana' raisin organic acids (tartaric acid, malic acid, citric acid, ascorbic acid, succinic acid and fumaric acid) reached a peak for the moderate levels of fertilizers treatments. In a previous research on cherry (*Prunus avium*), foliar application of potassium as KNO_3 increased fruits citric acid, malic acid, and fumaric acid (Nagy et al., 2010), which is supportive of our results. Organic acids are one of the main factors which affect grape and raisin quality via regulation of the acid/sugar ratio (Karimi and Mirzaei, 2017). Raisin tartaric acid had maximum concentration among the other organic acids. It was reported that tartaric acid had potential health benefits in decreasing the colorectal cancer risk (Spiller et al., 2003). The organic acid can enter the berries by oxidation of glucose by transferring from the leaves phloem and sometimes they may even be produced inside the phloem (Curie and Briat, 2003; Keller, 2015). The mediating of potassium and especially iron in the activation of many enzymes involved in cellular metabolism and their role in biosynthesis, connection, or degradation of sugars in different metabolic pathways (Curie and Briat, 2003) is one of the suggested topics which explains the higher soluble sugars or organic acid in the fruit grown on vines treated with these nutrients compared to control plants. However, this phenomenon warrants further investigation.

Foliar application of potassium sulfate especially in combination with iron chelate significantly, increased anthocyanidins in raisin production which is consistent with the results of other researchers (Karimi and Mirbagheri, 2018). It is important to note that raisin samples obtained from vines treated with K_3F_3 displayed a greater anthocyanidin concentration compared to samples from the control untreated vines (K_1F_1). Two anthocyanidins of Malvidin 3- glucoside and delphinidin 3- glucoside were the prevalent compounds compared to other anthocyanidins. In grape, the amount of anthocyanin increased during the color change phase when supplied by foliar potassium (Delgado et al., 2006). The application of iron chelates in grapes of 'Banaty' cultivar would increase accumulation of total anthocyanin in berries according to Ahmed et al. (1997). In another study, application of iron at moderate levels increased anthocyanin accumulation of berries of Cabaret Sauvignon grapevine cultivar in comparison to its low and high concentrations of this microelement (Shi et al., 2017). Anthocyanins are among the secondary metabolites of grapes in red berry skin cultivars from the stage of color change to ripening and can be measured in white berry skin cultivars in the ripening stage. During the growing season, viticultural practices could change the phenolics profile of grape and obtained raisins (Jogaiah et al., 2014). Among the various garden operations, mineral nutrition is one of the important factors which can affect the biochemistry of anthocyanins. Potassium and iron are two nutrients affecting polyphenol profiles as an antioxidant of raisins. Since glucose, fructose and raffinose can stimulate the accumulation of anthocyanins in grapes (Karimi et al., 2019), the positive effect of the iron application on photosynthesis and accumulation of sugars may increase the accumulation of anthocyanins. In the current study, foliar application of potassium sulfate and iron chelate increased anthocyanin concentration and improved raisin quality. Since the combined effects of potassium and iron through the impact and regulation of osmotic pressure causes better transfer of elements to different parts of the plant, thereby resulting, in the amount of raisin anthocyanins to increase in treated vines (Delgado et al., 2006; Karimi et al., 2019). The results of the present study indicated that in addition to the inherent difference between the concentrations of anthocyanins in the berries of different grape cultivars, the amount of anthocyanins can also increase by using the optimum combinations of mineral nutrients.

The produced raisin of treated vines with a high level of potassium sulfate in combination with a moderate or high level of iron chelate was shown to be at a higher concentration in the case of most phenolic acids. Conversely, the PPO activity of raisin samples was found to be the lowest in these treatments, which confirmed the involvement of these two elements in the biosynthesis or stability of polyphenol compounds. Phenolic compounds are a group of secondary metabolites in grape and produced raisin which has antioxidant and anticancer properties in human health (Braidot et al., 2008). Several physiological and environmental factors can affect the amount of production and transport of phenolic acids in grape and raisin (Jogaiah et al., 2014). All of these factors are interconnected in a network

and only when they are at optimal concentration they can improve the production of raisin phenolic acids (Karimi and Mirbagheri, 2018). A decrease in the biosynthesis of phenolic acids has been observed under the influence of a decrease or increase in internal (plant hormones, rootstocks; Jogaiah et al., 2014; Karimi, 2017) or external factors (irrigation, temperature stress, light, fertilization, etc.; Braidot et al., 2008; Karimi et al., 2019). Based on the results of this study, the application of balanced potassium ratios in combination with iron increased the accumulation of secondary metabolites as highly beneficial bioactive compounds in Red Sultana raisins (Williamson and Carughi, 2010). Accordingly, this increase was associated with lower activity of PPO in raisin produced from the vines treated with combined treatment of these fertilizers especially at moderate levels.

In grapevine, the ratio and content of phenolic acids in berries are affected directly or indirectly by proper nutrition during the growing season (Bavaresco et al., 1993, 2005; Karimi and Mirbagheri, 2018). These changes in the amount of berries phenolic acids can be reflected and seen in the raisins produced. In current work, the concentration of raisin phenolic acids was improved dramatically in response to pre-harvest mineral nutrition and reached a peak for vines treated with K_2SO_4 at 3% in combination with Fe -EDDHA at 1.0% (K_3F_2; kaempferol, quercetin, chlorogenic acid, resveratrol) or 0.5% (K_3F_3; for cinnamic acid, rutin and catechin) than control produced raisins. In research on grapes, foliar application of iron chelates increased the amount of berries phenolic compounds (Ghrairi et al., 2013; Shi et al., 2017). Strawberries (Valentinuzziet al., 2015) grown in calcareous soils confirmed the results of the present study. In another investigation, soil application of potassium increased grape phenolic compounds (Delgado et al., 2006). Moreover, foliar application of potassium sulfate during the pre- and post-flowering stages improved the amount of polyphenols in all grape berries (Karimi, 2017; Karimi and Mirbagheri, 2018), which is in agreement with the results of the present study. Also, nutrition with sufficient potassium has been known to improve grape berry's color and amount of polyphenols (Mohammed et al., 1993). Potassium stimulates photosynthetic activity and increases the transfer of sugars to berries allowing sugars to accumulate indirectly, thereby improving the biosynthesis of phenolic compounds during ripening, which is closely related to the presence of carbohydrates (Mohammed et al., 1993; Delgado et al., 2006). An increase in raisin total phenol concentration determined in this experiment under the influence of iron and potassium treatments, indicated the key role of these ions indirectly in the biosynthesis of phenolic compounds or directly by affecting the PPO activity.

Resveratrol are among the most important stilbene phytoalexins produced in plants such as grapes, and their presence in vegetative tissues increases the defense system and in fruit juice leads to an increase in its nutritional and medicinal properties (Moreno et al., 2008). The biosynthesis of stilbene is under genetic control and their accumulation varies among different grape cultivars (Moreno et al., 2008; Keller, 2015). However, managerial factors such as the use of nutrients may affect the expression of this feature (Keller, 2015). In the present study, resveratrol concentrations increased in raisin produced from the vines treated with balanced levels of potassium and iron. Bavaresco (1993) has indicated that the optimal application of potassium plays an important role in grape berries phenolic acids biosynthesis that our research findings are in agreement and therefore consistent with their results. Therefore, balanced concentrations of iron, especially in combination with moderate levels of potassium can increase the biosynthesis of stilbenes but this requires further investigation.

Pre-harvest foliar application of potassium sulfate and iron chelates, and their interaction, significantly ($P \leq 0.05$) improved antioxidant capacity of Red Sultana raisin as measured by FRAP, DPPH and ABTS methods, which are consistent with the results of Valentinuzzi et al. (2015). Raisin higher antioxidant capacity of nutrient-treated vines can be explained by higher phenolic and anthocyanidins contents as well as lower PPO activity in this treatment (Sério et al., 2014). Due to the role of potassium in the photosynthetic apparatus and the production of sugars and their transfer from leaf to fruit, it can increase the antioxidant capacity by indirectly affecting the biosynthesis of phenolic compounds and anthocyanins (Bavaresco, 1993; Marschner, 2012). Many grape phenolic compounds can be effective in scavenging oxygen-reactive species, however; their ability for scavenging free radicals of oxygen depends on cultivar and viticultural operations such as tree nutrition (Bozan et al., 2008). In this study, the balanced application of potassium and iron increased antioxidant capacity through improvement in biosynthesis or accumulation of phenolic compounds in

produced raisin of treated vines. The antioxidant activity of grape varieties which is related to the higher accumulation of phenolic compounds include anthocyanidins, flavonols, flavonoids and phenolic acids in their berries (Ghrairi et al., 2013; Sério et al., 2014). The amount of grape and raisin antioxidant capacity and their anthocyanins depend on the amount and type of nutrition of microelements, thus emphasizing the importance of establishing a rigid nutritional plan to achieve raisins with high antioxidant value and its use as an herbal medicine in human health improvement (Williamson and Carughi, 2010). In the current study, the raisin color and flavor preferences were not affected by pre-harvest foliar application of potassium sulfate and iron chelate treated vines compared to control untreated vines (data not shown).

In conclusion, pre-harvest foliar application of potassium and iron considerably improved the phytochemical compositions of raisin produced from the treated vines. The highest fructose and glucose and also spermidine and spermine were related to raisin produced from the vines treated with a high level of potassium in combination with a moderate level of iron. The raisin organic acids increased in treated vines with both fertilizers at final doses; however, tartaric acid concentration showed the highest in 3% potassium in combination with 0.5% iron treatments. The vines treated with a high level of potassium in combination with a moderate level of iron produced raisin with the highest phenolic acids and anthocyanidins. Likewise, the highest antioxidant capacities were achieved in potassium –treated vines at 3% in combination with iron at a moderate level. In fact, optimum concentrations of potassium sulfates and iron chelates increased the anthocyanidins content and phenolic compounds in the Red Sultana raisins. These results indicate the importance of pre-harvest potassium and iron application to achieve enriched grapes and produce raisin with beneficial function and utilization as a herbal anti-cancer drug.

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Conflict of interest

There is no conflict of interest for this study.

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