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Berry quality, antioxidant compounds, antioxidant capacity and enzymes activity during storage of three local table grape cultivars growing in Saudi Arabia

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Abstract

Grapes are considered as good source for bioactive antioxidants intake that contribute to human health. Changes in berry quality, antioxidant compounds, antioxidant capacity and enzymes activity during storage (0°C ±1 and 90–95% RH plus 2 days of shelf life) of 'Hegazi', 'El-Bayadi' and 'Red Romy' table grape cultivars were evaluated. Total phenols concentration of 'Hegazi' remained stable after 25 days but was higher after 40 days of storage than initial. In 'Red Romy', it was higher after 25 and 40 days of storage than initial, but remained stable in 'El-Bayadi'. After 25 days of storage, 'Red Romy' showed higher total phenols than other cultivars. Total flavonoids concentration in 'Hegazi' and 'El-Bayadi' remained stable, but was higher after 25 and 40 days of storage in 'Red Romy' than initial. Initially, total flavonoids was similar among cultivars, but was higher in 'Red Romy' after 25 and 40 days of storage than initial *trans*-resveratrol concentration remained stable in 'Hegazi', fluctuated in 'Red Romy' and decreased in 'El-Bayadi' during storage. *trans*-piceid and vitamin C concentrations decreased during storage and were higher in 'El-Bayadi' than other cultivars. Antioxidant capacity (DPPH IC_{50}) decreased during storage compared to initial with no differences among cultivars. While, antioxidant capacity (ABTS IC_{50}values) was lower after 40 than after 25 days of storage and initial. 'Red Romy' showed higher antioxidant capacity than other cultivars. Peroxidase (POD), polyphenoloxidase (PPO) and polygalacturornase (PG) activities varied among cultivars and during storage. Such information might be useful for grape breeders, growers, nutritionists and consumers.

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Introduction

Grapes (*Vitis vinifera* L.), as many other fresh fruit, are considered important to human health owing to their nutritional and medicinal properties (Xia *et al*., 2010; Zhou and Raffoul, 2012). Epidemiological studies showed that the intake of fresh grapes and grape products associated with a lower risk of degenerative diseases caused by oxidative stress (Zhou and Raffoul, 2012).

Thus, nowadays, the attractiveness of fruit to consumers is determined not only by regular quality attributes such as appearance, size, color, total soluble solids content (TSS), titratable acidity and texture but also by their contents of health-promoting phytochemicals. Grapes contain considerable amounts of bioactive antioxidants such as polyphenols(phenolic acids, flavonols, anthocyanins, flavanols, and stilbenes) and vitamins (vitamin C) that largely contribute to both fruitquality and,via fruit consumption, to human health (Xia *et al.,* 2010; Zhou and Raffoul, 2012).

Therefore, it is very useful to study factors that affect the level of these substances with the aim of further improving the relevant fruit attributes. Resveratrol, its 3-glucopyranoside piceid, and their *cis* isomers are natural plant phenolics, representing the major active compound of stilbenephytoalexinsthat mainly occur in grapes, berries, and other dietary constituents and is presumed to be involved in defense system against plant pathogens and metabolic diseases in human (Adrian *et al*., 1997; Chen *et al*., 2016).

Therefore, induction of resveratrol and other phenolics biosynthesis and/or maintaining their level during storage is desirable for improving both postharvest disease control and nutraceutical properties of grapes (Sanchez-Ballesta *et al*., 2006). The ultimate objective of the production and handling chain of fruit is to satisfy consumer's needs. Cold storage is one of the most successful technology for delaying biochemical changes and quality deterioration of fresh grapes. However, the cold storage life of table grapes is limited by decay and weight loss as well as the decrease in other quality attributes as health-promoting phytochemicals (Romanazzi *et al*., 2012).

Additional pre and/or postharvest treatments such as UV-C irradiance, pre-storage high CO_2, packaging with SO_2 slow release sheets, edible coating and dipping in natural antioxidants such as resveratrol may,to some extent, improve and/or assure grapes quality during cold storage (Sanchez-Ballesta *et al*., 2006; Romanazzi *et al*., 2012; Freitas *et al.,* 2015; Al-Qurashi and Awad, 2016). Modified atmosphere packaging (MAP) alone or in combination with natural fungicides (Artes-Hernandez *et al*., 2006) and CAalso maintained grapes quality such as TSS, titratable acidity and vitamin C and reduced decayduring storage(Deng *et al*., 2005). However, rachis-browning development limits the use of this technique (Crisosto *et al*., 2002). In Saudi Arabia (SA), the grapes cultivated area reached about 13282 hectares producing 149847 tons in year 2013 (FAO, 2013). In this respect, 'Hegazy', 'Red Romy' and 'El-Bayadi' represent the most common local grape cultivars in SA.

There is relatively much published information on the level of phenolics (such as flavonoids and stilbenes), antioxidant capacity and enzymes activity in grapes at harvest time, but little on the changes of these parameters during storage, especially in the locally produced grape cultivars. This study, therefore, aim to explore the extent to which the antioxidant compounds concentration (total phenols, total flavonoids, resveratrol and vitamin C), antioxidant and hydrolytic enzymes activity and other quality attributes of table grapes varies among three cultivars growing in SA and how they change during storage and shelf life.

Materials and methods

Plant materials and experimental procedure

Grapes samples of three locally produced table grape cultivars namely 'Hegazy' (white seeded), 'Red Romy' (light red seeded) and El-Bayadi' (white seeded) were collected from commercial vineyard and directly transferred to the horticulture laboratory at King Abdulaziz University, Jeddah. For each cultivar, six cartons randomly collected from different lots (about 4.5-5.0Kg of each) were divided into 3 replicates (2 cartons of each).

The experimental design was a completely randomized with three replicates/cultivar. All the collected grape samples of the different cultivars were stored at 0 °C±1 and 90–95% relative humidity in perforated polyethylene bags inside perforated cartons for 10, 25 or 40 days upon cultivar plus 2 days of shelf life at 20 °C±2. At the beginning of cold storage and after25 and 40 days plus 2 days of shelf life at 20 °C±2, samples of 30 berries free of fungal diseases randomly collected from different bunches of each cultivar/replicate were withdrawn for direct quality measurements. Additional sample of 30 berries free of fungal diseases from each cultivar/replicate were peeled and the skin was kept at –80 °C until later biochemical analysis.

Decay incidence and weight loss determination

After 10, 25 and 40 days of storage, the weight of the decayed berries was calculated by subtracting healthy berries from the total clusters weight. Total decay was expressed as the percentage of decayed berries with respect to the original clusters weight. The total loss in weight was calculated on initial weight basis and expressed in percentage.

Firmness, TSS, titratable acidity and vitamin C measurements

At the beginning of cold storage and after 25 and 40 days plus 2 days of shelf life, berry firmness was recorded independently in each of the 30 berries per replicate by a digital basic force gauge, model BFG 50N (Mecmesin, Sterling, Virginia, USA) supplemented with a probe of 11 mm diameter that measure the compression force required to penetrate the berry and the results expressed in Newton. A homogeneous sample was prepared from these 30 berries per replicate for measuring TSS, titratable acidity and vitamin C. TSS content was measured as percentage in berry juice with a digital refractometer (Pocket Refractometer PAL-3, ATAGO, Japan). Titratable acidity was determined in distilled water diluted juice (1: 2) by titrating with 0.1N sodium hydroxide up to pH 8.2, using automatic titrator (HI 902, HANNA Instrument, USA) and expressed as percentage of tartaric acid.

Vitamin Concentration was measured by the oxidation of ascorbic acid with 2,6-dichlorophenol endophenol dye and the results expressed as mg L^{-1}juice (Ranganna, 1979).

Extraction and quantification of trans-resveratrol and its glycoside trans-piceid

Extraction and quantification of *trans*-resveratrol and *trans*-piceid were carried out according to Romero-Perez *et al*. (2001) with modifications. Two grams of frozen berry skin (randomly collected from 30 berries/replicate) were homogenized with 25 mL of ethanol/water (80:20 v/v) using a homogenizer and maintained at 60 °C for 30 min. The extract was filtered through a Whatmaninor-ganic 15 µm and concentrated to 3 mL by rotary evaporation (in vacuo) at room temperature (20 °C±2). The concentrated extracts were filtered through CA Syringe filters 0.2 µm and injected into a high-performance liquid chromatography (Shimadzu, Japan) coupled with ultraviolet-visible diode array detector (HPLC-UV-VIS-DAD) for *trans*-resveratrol and *trans*-piceid quantification. The system was equipped with a Tracer Agilent ZORBX Eclipse plus C18 Analytical column (4.6 × 150 mm), 5 micron particle size. The column temperature was kept at 30°C.

The mobile phase consisted of A and B where solvent A was glacial acetic acid in water mixture (0.1 glacial acetic acid: 70 water v:v) and solvent B 29.9 aceto-nitrile/acetic acid, with a flow rate of 1.0 mL/min. Injection volume was 20µL. Detection was performed at a 310 nm wavelength and run time was 15 min. Retention time was about 2 and 4.5 min for *trans*-piceid and *trans*-resveratrol, respectively. Quantification was based on the peak area.

The chromatogram peaks of individual compounds were identified by comparing their retention times with the retention times of pure standards. *Trans-resveratrol* standard was purchased from *Baoji Guokang Bio-Technology Co., Ltd (Baoji, China)*. *trans*-piceidstandard was purchased from Sigma Chemical Co., St. Louis, MO. (USA). Integrated peaks were calculated by comparison with standard solutions of known concentration and the results expressed as mg Kg^{-1}on a fresh weight (FW) basis.

Preparation of the methanol extract for total phenols, flavonoids and antioxidant activity determinations

Two grams of berries skin tissue (randomly collected from 30 berries/replicate) were extracted by shaking at 150 rpm for 12 h with 20 ml methanol (80%) and filtered through filter paper No. 1. The filtrate designated as methanol extract that will be used for total phenols and flavonoids and antioxidant activity estimations.

Estimation of total phenols by the Folin-Ciocalteu test

Total phenols concentration was measured according to Hoff and Singleton (1977). Fifty µL of the methanol extract was mixed with 100 µL Folin-Ciocalteu reagent, 850 µL of methanol and allowed to stand for 5 min at ambient temperature. A 500 µl of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured at 750 nm. Total phenols was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid and the results expressed as g Kg^{-1} FW gallic acid equivalent.

Estimation of total flavonoids

Total flavonoids concentration was determined using a modified colorimetric method described previously by Zhishen *et al.* (1999). Methanol extract or standard solution (250 µL) was mixed with distilled water (1.25 mL) and 5 % $NaNO_2$ solution (75 µL). After standing for 6 min, the mixture was combined with 10% $AlCl_3$ solution (150 µL), 1 M NaOH (0.5 mL) and distilled water (275 µL) were added to the mixture 5 min later. The absorbance of the solutions at 510 nm was then measured.

Total flavonoids was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of catechin and the results expressed as g Kg^{-1} FW catechin equivalent.

Evaluation of antioxidant capacity

DPPH radical scavenging assay

Free radical scavenging activity of methanol extract was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (Ao *et al.*, 2008). A methanol extract (0.1 ml) was added to 0.9 ml of freshly prepared DPPH methanol solution (0.1 mM). An equal amount of methanol was used as a control. After incubation for 30 min at room temperature in the dark, the absorbance (Abs) was measured at 517 nm using a spectrophotometer. Activity of scavenging (%) was calculated using the following formula:

DPPH radical scavenging % = [(Abs control – Abs sample)/Abs control] x 100.

The inhibition concentration (IC_{50}) was defined as µg phenolics of the test sample that decreases 50% of initial radical. The IC_{50} values were calculated from the dose responses curves.

ABTS radical cation decolorization assay

ABTS (2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) also forms a relatively stable free radical, which decolorizes in its non-radical form. The spectrophotometric analysis of $ABTS^{\bullet+}$ scavenging activity was determined according to the method of Re *et al.* (1999). In this method, an antioxidant was added to a pre-formed ABTS radical solution and after a fixed time period the remaining $ABTS^{\bullet+}$ is quantified spectrophotometrically at 734 nm. $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS in H_2O with 2.45 mM potassium per sulfate ($K_2S_2O_8$), store in the dark at room temperature for 16 h. The $ABTS^{\bullet+}$ solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in 0.1 M sodium phosphate buffer pH 7.4 (25 µL $ABTS^{\bullet+}$ solution was raised to 900 µL buffer). Then, 900 µL of $ABTS^{\bullet+}$ solution was added to 100 µL crude methanol extract. The absorbance was recorded 1 min after mixing and the percentage of radical scavenging was calculated relative to a blank containing no scavenger. The extent of decolorization was calculated as percentage reduction of absorbance. The scavenging capability of test compounds was calculated using the following equation:

$ABTS^{\cdot+}$ scavenging (%) = (1- AS/AC) x 100. AC is absorbance of a control (blank) lacking any radical scavenger and AS is absorbance of the remaining $ABTS^{\cdot+}$ in the presence of scavenger.

The results were plotted as the percentage of scavenging activity against concentration of the phenolic contents. The inhibition concentration (IC_{50}) was defined as µg phenolics of the test sample that decreases 50% of initial radical. The IC_{50} values were calculated from the dose responses curves.

Enzymes measurements

Crude extract

One gram of berry skin (randomly collected from 30 berries/replicate) was homogenized with 20 mMTris–HCl buffer, pH 7.2 using homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 ºC. The supernatant was designed as crude extract and stored at -20ºC for peroxidase, polyphenoloxidase, polygalacturonase and xylanase assay.

Peroxidase assay

Peroxidase (EC 1.11.1.7) activity (POD) was assayed according to Miranda *et al.* (1995). The reaction mixture containing in one ml: 0.008 mL of 0.97 M H_2O_2, 0.08 mL of 0.5 M guaiacol, 0.25 mL of 0.2 M sodium acetate buffer, pH 5.5 and least amount of enzyme preparation.

The change in absorbance at 470 nm due to guaiacol oxidation was followed for 1 min using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme which increases the O.D. 1.0 per min under standard assay conditions.

Polyphenoloxidase assay

Polyphenoloxidase (EC 1.14.18.1) (PPO) activity was assayed with catechol as a substrate according to the spectrophotometric procedure of Jiang *et al.* (2002). The extract (0.2 mL) was rapidly added to 2.8 ml of 20 mM catechol solution prepared in 0.01 M sodium phosphate buffer (pH 6.8).

The increase in absorbance at 400 nm was recorded for 3 min using a spectrophotometer. One unit of enzyme activity was defined as the amount of the enzyme that causes a change of 0.1 in absorbance per min under standard assay conditions.

Polygalacturonase and xylanase assay

Polygalacturonase (EC 3.2.1.15) (PG) and xylanase (EC 3.2.1.8), activities were assayed by determining the liberated reducing end products using galacturonic acid and xylose, respectively (Miller, 1959). The reaction mixture (0.5 mL) containing 5 mg substrate, 0.25 mL of 0.2 M sodium acetate buffer pH 5.5 and a suitable amount of crude extract. Assays were carried out at 37°C for 1 h. Then 0.5 mL dinitrosalicylic acid reagent was added to each tube and heated in a boiling water bath for 10 min.

After cooling to room temperature, the absorbance was measured at 560 nm. Substrates used were polygalacturonic acid and xylane for polygalacturonase and xylanase, respectively. One unit of enzyme activity was defined as the amount of enzyme which liberated 1 µM of reducing sugar per h under standard assay conditions.

Statistical analysis

The data were statistically analyzed as a completely randomized design with three replicates by analysis of variance (ANOVA) using the statistical package software SAS (SAS Institute Inc., 2000, Cary, NC., USA). Comparisons between means were made by the Duncan's multiple range test at $P \leq$ 5%. Correlations coefficient among the different parameters were also calculated by SAS.

Results

Decay and weight loss

Decay percentage significantly increased (from 1.49 to 7.3%) during storage and ranged from 2.70 to 5.30% among cultivars. 'El-Bayadi' showed significantly lower decay percentage than 'Hegazi' and 'Red Romy' cultivars (Table 1).

Table 1. Decay percentage of different table grape cultivars during storage.

	Decay (%)
	Storage period (SP days)
10	1.49c
25	3.85b
40	7.30a
F-test	***
Cultivar (C)	
Hegazi	5.30a
Red Romy	4.65a
El-Bayadi	2.70b
F-test	***
SP x C	
F-test	*NS*
NS	

Means within each column followed by the same letter are not significantly different at level $P \leq 0.05$. (***) significant at $P \leq 0.001$. (NS), not significant. Measurements were done after each cold storage period.

There were no significant interaction effects between storage period and cultivar on decay percentage. Berry weight loss significantly increased during storage in all cultivars. 'El-Bayadi' showed significantly lower weight loss percentage than 'Hegazi' and 'Red Romy' cultivars (Table 2). 'Hegazi' and 'Red Romy' cultivars showed similar weight loss percentage during storage. There was no significant difference in weight loss percentage between 'Hegazi' and 'El-Bayadi' cultivars after 10 days of storage (Table 2).

Table 2. The interaction effect between storage period and cultivar on weight loss percentage of different table grape cultivars during storage.

	Storage period (days)		
Cultivar	10	25	40
Hegazi	0.71de	1.48b	2.23a
Red Romy	0.82cd	1.53b	2.29a
El-Bayadi	0.48e	0.64de	1.02c

For each parameter, means within and between columns followed by the same letter are not significantly different at level $P \leq 0.05$. Measurements were done after each cold storage period.

Firmness, TSS, titratable acidity and TSS/acid ratio

TSS content, after 25 days of storage, was lower in 'Hegazi', higher in 'Red Romy' and similar in 'El-Bayadi' compared with initial level (Table 3). After 40 days of storage, TSS content was higher in both 'Hegazi' and 'Red Romy' than initial. At initial time, 'Hegazi' showed higher TSS content than 'Red Romy' and 'El-Bayadi' cultivars but with no significant differences after 25 days of storage (Table 3). After 40 days of storage, 'Hegazi', showed higher TSS content than 'Red Romy'. Titratable acidity in 'Hegazi' cultivar was lower after 25 and 40 days of storage than initial (Table 3). However, in 'Red Romy' cultivar it showed similar level after 25 but higher after 40 days of storage than initial. 'El-Bayadi' showed higher acidity level after 25 days of storage than initial. At initial time, 'El-Bayadi' showed lower acidity but, higher after 25 days of storage than other cultivars.

After 40 days of storage, 'Red Romy' showed higher acidity than 'Hegazi'. TSS/acid ratio remained stable in 'Hegazi', fluctuated in 'Red Romy' but significantly decreased in 'El-Bayadi'. At initial time, 'El-Bayadi' showed higher TSS/acid ratio but lower after 25 days of storage than other cultivars. After 40 days of storage, 'Hegazi' showed higher TSS/acid ratio than 'Red Romy'. Berry firmness was significantly lower after 25 and 40 days of storage than initial in all cultivars (Table 3). In this respect, 'El-Bayadi' showed the highest berry firmness followed by 'Red Romy' and then 'Hegazi' cultivar that showed the lowest berry firmness (Table 3).

Table 3. The interaction effect between storage period and cultivar on TSS content and titratable acidity concentration, TSS/acid ratio and firmness of different table grapes cultivars during storage and shelf life.

	Storage period (days)											
	TSS (%)			Acidity (%)			TSS/Acid (Ratio)			Firmness (N)		
Cultivar	0	25	40	0	25	40	0	25	40	0	25	40
Hegazi	22.0a	18.3b	21.7a	0.44ab	0.33d	0.38c	50.1bc	54.6b	56.2b	7.8d	6.3ef	6.0f
Red Romy	15.1d	18.2b	17.8bc	0.40bc	0.41bc	0.47a	37.7e	44.1cd	38.1de	17.2b	14.0c	7.0de
El-Bayadi	15.9cd	16.1bc	-	0.20e	0.44ab	-	79.1a	36.4e	-	25.6a	18.1b	-

For each parameter, means within and between columns followed by the same letter are not significantly different at level $P \leq 0.05$. (-), not calculated. Measurements were done after each cold storage period plus 2 days of shelf life.

Total phenols, Total flavonoids, trans-resveratrol, trans-piceid and vitamin C

Total phenols concentration in 'Hegazi' cultivar remained stable after 25 days but was higher after 40 days of storage than initial (Table 4). In 'Red Romy' cultivar, it showed higher level after 25 and 40 days of storage than initial. However, 'El-Bayadi' cultivar showed similar total phenols concentration after 25 days of storage to initial. At initial time and after 25 days of storage, 'Hegazi' showed lower total phenols concentration than other cultivars. In this respect, after 25 days of storage, 'Red Romy' gave higher total phenols concentration than other cultivars. After 40 days of storage, 'Red Romy' showed higher total phenols concentration than 'Hegazi'. Total flavonoids concentration in both 'Hegazi' and 'El-Bayadi' cultivars did not significantly change during storage. However, it showed higher level after 25 and 40 days of storage in 'Red Romy' cultivar (Table 4).

Table 4. The interaction effect between storage period and cultivar on total phenols, total flavonoids and *trans*-resveratrol concentration of different table grape cultivars during storage and shelf life.

	Storage period (days)								
Cultivar	Phenols (g Kg^{-1})			Flavonoids (g Kg^{-1})			*trans*-resveratrol (mg Kg^{-1})		
	0	25	40	0	25	40	0	25	40
Hegazi	0.84c	0.95c	1.59b	0.47cd	0.33cd	0.16d	0.09d	0.11d	0.11d
Red Romy	1.41b	2.65a	2.61a	0.48c	2.26a	1.17b	0.22b	0.11d	0.16c
El-Bayadi	1.34b	1.37b	-	0.38cd	0.41cd	-	0.26a	0.19bc	-

For each parameter, means within and between columns followed by the same letter are not significantly different at level $P \leq 0.05$. (-), not calculated. Measurements were done after each cold storage period plus 2 days of shelf life.

There were no significant differences in total flavonoids concentration among cultivars at initial time but after 25 and 40 days of storage 'Red Romy' showed higher level than other cultivars. *trans*-resveratrol concentration remained stable in 'Hegazi', fluctuated in 'Red Romy' and decreased in 'El-Bayadi' cultivar during storage.

At both initial time and after 25 days of storage 'El-Bayadi' showed higher *trans*-resveratrol concentration than other cultivars. After 40 days of storage, 'Red Romy' showed higher *trans*-resveratrol concentration than 'Hegazi' (Table 4). *trans*-piceid concentration significantly decreased during storage compared to initial (Table 5).

'El-Bayadi' cultivar showed the highest *trans*-piceid concentration followed by 'Hegazi' and then 'Red Romy' that gave the lowest level.

Vitamin C concentration significantly decreased during storage compared to initial (Table 5).

Table 5. *Trans*-piceid and vitamin C concentration of different table grape cultivars during storage and shelf life.

	trans-piceid (mg Kg^{-1})	Vitamin C (mg L^{-1} juice)
Storage period (SP days)		
0	0.67a	16.3a
25	0.48b	13.3b
40	0.49b	12.2b
F-test	***	*
Hegazi	0.49b	13.3b
Red Romy	0.14c	12.6b
El-Bayadi	1.28a	17.8a
F-test	***	*
SP x C		
F-test	*NS*	*NS*

Means within each column followed by the same letter are not significantly different at level $P \leq 0.05$. (*) and (***), significant at $P \leq 0.05$ and 0.001, respectively. (NS), not significant. Measurements were done after each cold storage period plus 2 days of shelf life.

In this respect, 'El-Bayadi' showed the highest vitamin C concentration followed by 'Hegazi' and 'Red Romy' cultivars that showed similar level.

Enzymes activities

POD activity in 'Hegazi' cultivar was higher after 25 and 40 days of storage than initial (Table 6). In 'Red Romy' cultivar, POD activity was similar after 25 days but higher after 40 days of storage compared to initial. However, it showed no significant change in 'El-Bayadi' cultivar after 25 days of storage. In this respect, 'Hegazi' cultivar showed significantly lower POD activity than other cultivars at initial time and after 25 days of storage.

There were no significant difference between 'Hegazi' and 'Red Romy' after 40 days of storage. PPO activity in 'Hegazi' cultivar was higher after 25 and 40 days of storage than initial but with a lower level after 40 days than after 25 days of storage (Table 6). In 'Red Romy' cultivar, PPO activity gradually increased during storage while, it did not change in 'El-Bayadi' cultivar. In this respect, at initial time, there were no significant differences in PPO activity among cultivars.

While, after 25 days of storage, 'Hegazi' cultivar showed the highest PPO activity followed by 'Red Romy' and then 'El-Bayadi' that showed the lowest. After 40 days of storage, 'Red Romy' gave higher PPO activity than 'Hegazi' cultivar (Table 6). PG activity in 'Hegazi' cultivar was higher after 25 and 40 days of storage than initial (Table 6). In 'Red Romy' PG activity was higher after 25 days but similar after 40 days of storage compared to initial. However, it showed no change in 'El-Bayadi' cultivar after 25 days of storage.

In this respect, 'El-Bayadi' showed significantly lower PG activity at initial time and after 25 days of storage than other cultivars. After 40 days of storage, 'Hegazi' gave higher PG activity than 'Red Romy' cultivar. Xylanase activity in 'Hegazi' cultivar was higher after 25 days but lower after 40 days of storage. In 'Red Romy' xylanase activity was similar after 25 days but lower after 40 days of storage than initial. However, it showed no change in 'El-Bayadi' cultivar after 25 days of storage. In this respect, 'El-Bayadi' showed significantly lower xylanase activity at initial time and after 25 days of storage than other cultivars. After 40 days of storage, 'Hegazi' and 'Red Romy' gave similar xylanase activity (Table 6).

Table 6. The interaction effect between storage period and cultivar on antioxidant and hydrolytic enzymes activities of different grape cultivars during storage and shelf life.

Cultivar	Storage period											
	POD (U min g FW)			PPO (U min g FW)			PG (U h g FW)			Xyl (U h g FW)		
	0	25	40	0	25	40	0	25	40	0	25	40
Hegazi	2.9d	6.9c	26.2a	0.61c	3.8a	2.7b	39.0c	60.2a	49.7b	22.3b	26.2a	16.6c
Red Romy	14.3b	15.1b	26.9a	0.63c	2.6b	3.4a	42.1c	59.4a	39.6c	22.1b	23.2b	18.7c
El-Bayadi	14.3b	14.3b	-	0.69c	0.75c	-	19.0d	19.0d	-	11.3d	11.3d	-

For each parameter, means within and between columns followed by the same letter are not significantly different at level $P \leq 0.05$. Measurements were done after each cold storage period plus 2 days of shelf life. (-), not calculated. POD, PPO, PG and Xyl refereeing to peroxidase, polyphenoloxidase, polygalacturornase and xylanase, respectively.

Antioxidant capacity

Antioxidant capacity (IC_{50}values) measured by DPPH assay gradually and significantly decreased (higher IC_{50}values) during storage compared to initial (Table 7). In this respect, there were no significant differences among cultivars in DPPH IC_{50}values. While, antioxidant capacity (IC_{50}values) measured by ABTS assay was significantly lower (higher IC_{50}values) after 40 than after 25 days of storage and initial (Table 7).

Table 7. Antioxidant capacity (IC_{50}values) measured by DPPH and ABTS methods of different table grape cultivars during storage and shelf life.

Storage period (SP, days)	DPPH (IC_{50})	ABTS (IC_{50})
0	1.98c	0.97b
25	3.73b	1.00b
40	6.10a	1.44a
F-test	***	***
Cultivar (C)		
Hegazi	3.57	1.51a
Red Romy	3.34	0.59b
El-Bayadi	4.28	1.25a
F-test		
SP x C		
F-test	*NS*	*NS*

Means within each column followed by the same letter are not significantly different at level $P \leq 0.05$. (***) significant at $P \leq 0.001$. (NS), not significant. Measurements were done after each cold storage period plus 2 days of shelf life.

Red Romy' showed significantly higher antioxidant capacity (lower IC_{50}values) than 'Hegazi' and 'El-Bayadi' cultivars. Total phenols and flavonoids were both highly correlated with each other ($r = 0.79^{***}$) (Table 8). Vitamin C concentration was positively correlated with both *trans*-resveratrol *and trans*-piceid and negatively with total phenols concentration. DPPH (IC_{50}values) was positively correlated with ABTS (IC_{50}values), total phenols, POD and PPO and negatively with xylanase activity. ABTS (IC_{50}values) was negatively correlated with total flavonoids concentration. Decay percentage was positively correlated with total phenols, POD, PPO and PG and negatively with *trans*-resveratrol *and trans*-piceid and vitamin C (Table 8).

Discussion

Although table grapes is known as a non-climacteric type of fruit that show a relatively low rate of physiological activity following harvest, berries are highly perishable due to decay and weight loss during storage and shelf life.

Weight loss is related to cooling delay, storage relative humidity, and susceptibility of bunch rachis to browning and dehydration. Bunch rachis dehydration and browning, therefore, is an important parameter to judge weight loss. Unfortunately, such parameter was not quantitatively measured in the current study. However, we observed that bunch rachis of 'El-Bayadi' retained more green color, showed lees browning and dehydration symptoms, especially after 25 days of storage, and gave lower total weight loss than other cultivars However, for a technical mistaken, there were no enough replicates to measure the other quality parameters after 40 days of storage. According to Deng *et al.* (2005), the normal acceptable limit for weight loss in table grapes during storage is up to 5%. However, after 40 days of storage, rachis of 'Hegazi' and 'Red Romy' cultivars exhibited severe dehydration and browning with about 10% berry drop.

Table 8. Pearson's correlation coefficients of total phenolics, total flavonoids, *trans*-resveratrol and *trans*-piceid, vitamin C, antioxidant and hydrolytic enzymes activates, antioxidant capacity and decay of different table grape cultivars during storage and shelf life.

Trait[a]	TPH	TF	*t*-Resver	*t*-Piceid	AA	POD	PPO	PG	XYL	DPPH	ABTS
TF	0.79***										
t-Resver	-0.05ns	-0.23ns									
t-Piceid	-0.32ns	-0.41*	0.36ns								
AA	-0.45*	-0.36ns	0.63***	0.42*							
POD	0.66***	0.15ns	0.18ns	-0.05ns	-0.15*						
PPO	0.40*	0.28ns	-0.49**	-0.57***	-0.47***	0.37*					
PG	0.21ns	0.38ns	-0.71***	-0.84***	-0.54*	-0.02ns	0.69*				
XYL	0.01ns	0.34ns	-0.61***	-0.83***	-0.43*	-0.35***	0.44*	0.82***			
DPPH	0.46*	-0.01ns	-0.02ns	0.16 ns	-0.20ns	0.73**	0.43*	-0.02ns	-0.43*		
ABTS	-0.28ns	-0.48*	-0.02ns	0.35ns	-0.08ns	0.16ns	0.11ns	-0.11ns	-0.32*	0.51**	
Decay	0.48*	0.11ns	-0.49**	-0.39*	-0.59***	0.67***	0.74***	0.49*	0.11ns	0.68***	0.30ns

[a]TPH = total phenols, TF = total flavonoids, *t*-Resver = *trans*-resveratrol, *t*-Piceid = *trans*-piceid, AA = vitamin C, POD = peroxidase, PPO = polyphenoloxidase, PG = polygalacturonase, XYL = xylanase, DPPH = antioxidant activity (IC_{50}values) measured in methanol extract based on DPPH assay, ABTS = antioxidant activity (IC_{50}values) measured in methanol extract based on ABTS assay. (*), (**) and (***), significant at level P = 0.05, 0.01 and 0.001, respectively; (NS), not significant. n = 24 except for decay and weight loss parameters in which n = 27.

The lowest decay incidence observed in 'El-Bayadi' cultivar after 25 days of storage might partly due to its higher concentration of both *trans*-resveratrol and *trans*-piceid (active stilbenephytoalexins). Indeed, using data of all cultivars, decay percentage was negatively correlated with *trans*-resveratrol and *trans*-piceid as well as vitamin C level. The accumulation of resveratrol was strongly associated with the resistance of table grapes to grey mould (Sbaghi *et al.*, 1995) and powdery mildew (Romero-Perez *et al.*, 2001). The higher levels of *trans*-resveratrol, *trans*-piceid and vitamin C in 'El-Bayadi' might reflect higher health value compared to other cultivars.

The higher firmness values of 'El-Bayadi' might partly due to lower activity of the hydrolytic enzymes PG (2.5-fold lower) and xylanase (2-fold lower) than other cultivars and suggesting a role of these enzymes in berry softening during storage and shelf life. In another study, PG showed similar activity in both skin and flesh and seems to be necessary and sufficient for pectin depolymerisation in the late stages of grapes ripening despite it was absent during early growth stages (Cabanne and Donèche, 2001). In addition, the activity of PG was much higher in the cultivar 'Thompson seedless' (less firm berries) than 'NN107' cultivar (firmer berries); meanwhile pectin methylesterase activity was similar in both cultivars during cold storage (Ejsmentewicz *et al.*, 2015).

Both POD and PPO are also considered as defensive enzymes (Campos-Vargas and Saltveit, 2002). Phenolics, especially (+)-catechin, gallic acid, chlorogenic acid, and ellagic acid as the most important PPO substrates, could be oxidized to quinones (highly toxic to pathogens) by the action of both PPO and POD (Campos-Vargas and Saltveit, 2002). In the current study, both POD and PPO were positively correlated with total phenols ($r = 0.66^{***}$ and 0.40^{*}, respectively). Both PPO and total phenols were involved in anthracnose resistance of mangoes and were suggested as indicators for cultivars resistant to postharvest diseases (Gong *et al*., 2013). The correlation between decay percentage and POD and PPO as well as antioxidant compounds seems to be complicated since fungus infection or other elicitors such as UV-irradiance might increase the accumulation of both antioxidant compounds and the activity of such enzymes (Sanchez-Ballesta *et al*., 2006; Al-Qurashi and Awad, 2016). The concentration of antioxidant compounds (total phenols and flavonoids, *trans*-resveratrol, *trans*-piceid and vitamin C) varied greatly among the different cultivars. It is known that the biosynthetic pathway of phenylpropanoid is genetically, developmentally and environmentally regulated in grapes as in other fruit (Versari, *et al*., 2001). However, the highest cultivar for each compound/group differed from compound/group to another. The *trans*-piceid level detected in berry skin is in agreement with previously published data indicating that the piceid level in grapes is mostly higher than that of resveratrol (Vincenzi *et al*., 2013).However, as a mean of all storage periods, 'Red Romy' cultivar showed almost similar levels of both *trans*-resveratrol (0.16 mg Kg^{-1})and *trans*-piceid (0.14 mg Kg^{-1}). It has been reported that the piceids could be more efficiently absorbed than the aglycons (Paganga and Rice-Evans, 1997). Thus, grape berries, in particular 'El-Bayadi' may be an alternative dietary source to wine to achieve the beneficial effect of resveratrol. The insignificant correlation between *trans*-resveratrol and its glycosylated form *trans*-piceid, suggests that the synthesis of the different stilbenes in grape skins could depend on different pathways (Vincenzi *et al*., 2013).

As a mean of all cultivars, total phenols and flavonoids concentration increased during storage, in contrast to *trans*-resveratrol, *trans*-piceid, vitamin C and TSS/acid ratio that were decreased. Takeda *et al*. (1983) reported an increase in total phenols concentration of 'Muscadine' grapes during storage at 20, 4.5 and 0 °C. However, they found no changes in TSS content, titratable acidity, individual sugars and organic acids during storage at the tested temperatures. Sanchez-Ballesta *et al*. (2006) found a sharp rise in *trans*-resveratrol level in non pre-storage CO_2-treated 'Cardinal' grapes after 33days of storage at 0 °C, in contrast to treated ones that remain stable. Doshi and Adsule (2008) reported that total phenols, flavonoids, procyanidin monomers and anthocyanins of grapes decreased during the first 10 days of storage at ambient temperature or 2-4 °C without bags and boxes, followed by a gradual increase, in contrast to those stored in bags and boxes at both temperatures that showed much less change. In many fruit, lipid-soluble antioxidants as total phenols and flavonoids are generally more stable or even increase during storage than water-slouble ones as vitamin C (Barden and Bramlage, 1994).In the current study, antioxidant capacity (IC_{50}values) measured by DPPH assay decreased during storage (higher IC_{50}values) while by ABTS assay, it showed an increase after 40 days of storage. In ABTS assay, 'Red Romy' showed the highest total phenols content and antioxidant capacity (lowest IC_{50}values) compared to other cultivars. The antioxidant compounds and capacity increased in strawberries and raspberries during storage due to the increase in anthocyanin in strawberries and in anthocyanin and total phenols in raspberries (Kalt *et al*., 1999). Several studies showed that the antioxidant activity of grapes is mainly attributed to phenolics content (Xia *et al*., 2010). However, our results, tacking all cultivars together, showed that DPPH IC_{50}values was positively correlated with total phenols ($r = 0.46^{*}$), while ABTS IC_{50}values was negatively correlated with total flavonoids ($r = -0.48^{*}$). Moreover, *trans*-resveratrol, *trans*-piceid and vitamin C concentration showed insignificant correlations with both DPPH and ABTS IC_{50} values.

However, in another study, the antioxidant activity (measured by ferric reducing antioxidant power, FRAP) correlated well with the total phenolics content of grapes during storage (Doshi and Adsule, 2008). The differences between various antioxidant assays may be attributed to differences in sensitivity/potential among antioxidant compounds such as phnolics and flavonoids classes and vitamin C toward a specific assay (Ou *et al.,* 2002; Ciz *et al.*, 2010). In this respect, the available literature information on the correlations among antioxidant compounds and antioxidant capacity of grapes are inconsistent. For example, Bozan *et al.* (2008) fond no correlations between individual flavanols or total phenols and antioxidant capacity of grape seed of several cultivars. Kallithraka *et al.* (2005) did not find significant correlation between total anthocyanin and antioxidant capacity of skin of several grape cultivars at harvest. However, considerable correlations were found between antioxidant capacity and total phenolics in skin and seeds of several grape cultivars (Xu *et al.,* 2010). Proanthocyanidin fractions were highly positively correlated with antioxidant capacity of grapes during maturation, in contrast to individual anthocyanins that were highly negatively correlated (Jordao *et al.*, 2012).Moreover, vitamin C and antioxidant capacity as determined by DPPH or FRAP was not correlated in peaches, nectarines, and plums (Gil *et al.,* 2002). Accordingly, each individual antioxidant compound might differentially contribute to the antioxidant activity assays upon its various possible mechanisms (free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity). Also, phenolic compounds are possibly not the only factor that contributes to antioxidant capacity of fruit but it might work synergistically with vitamins and minerals (Dani *et al.*, 2012).It has been found that grapes skin and flesh possessed equal amount of reactivity to hydroxyl radicals despite the great differences in phenolic content (Falchi *et al.*, 2006). It was suggested that the antioxidant capacity of phenolics possibly has a concentration saturation limit above which the activity could not increase further with the concentration (Dani *et al.*, 2012).

Thus, parallel several assays should be applied to investigate the principles of antioxidant/oxidation activity of a certain horticultural commodity (Ou *et al.*, 2002; Ciz *et al.*, 2010). Such information might be useful for grape breeders, growers, nutritionists and consumers.

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