Cold storage effects on oxidative stress of cv. Red Globe table grape rachises

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Abstract

R. Campos-Vargas, P. Zamora, R. Contreras, H. Köhler, G.E. Zuñiga, A. Pérez-Donoso, and B.G. Defilippi. 2012. Cold storage effects on oxidative stress of Red Globe table grape rachises. Cien. Inv. Agr. 39(1): 91-104. Table grape (*Vitis vinifera* L.) quality includes the condition of both the berries and the rachis. In the present report, physiological parameters of Red Globe rachises from fully elongated inflorescences (RFEI) and from mature clusters were studied after storage at 0 or 20 °C for different durations. To understand changes in rachis physiology as a result of changes in temperature conditions and storage time, the activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were measured. In addition, hydrogen peroxide content, membrane lipoperoxidation (TBARS), total phenolic compounds and antioxidant capacity (FRAP) were assayed. TBARS was higher in mature rachises than in RFEI. This parameter remained constant throughout storage, indicating a change presumably associated with ontogeny or senescence processes. Short-term storage (0-96 h) increased SOD, CAT and APX activities in RFEI, while in mature rachises, no changes were observed in enzyme activities or in hydrogen peroxide content. Longer cold storage (25 or 53 days at 0 ºC) of mature rachises reduced CAT activity, but SOD and APX activities did not change under these conditions. At 0 h, the FRAP and total phenolic contents of mature rachises were three and 20 times higher than in immature rachises, respectively.

Key words: Fruit quality, rachis, storage, table grape, *Vitis vinifera* L.

Introduction

Fruit quality is a complex mixture of different attributes related to flavor and appearance. Although there are cultural differences concerning

the appropriate quality of table grapes, the overall characteristics of the cluster (berries plus rachis) are important conditions for consumers. In the literature, several papers describe table grape berry changes during development and post-harvest life. However, the rachis, the non-edible but structurally important component of the cluster, has not been studied with similar attention. During long-term

storage or extended shipping conditions, the rachis undergoes a loss of quality resulting in a poor condition and lack of fresh appearance. This loss of rachis quality reduces the value and selling potential for the receiver or retail market, even though the quality of the berries can be normal.

It was hypothesized that the reduced quality of the rachis in cold storage is associated with water loss (Crisosto *et al.*, 2001; Valverde *et al.*, 2005a) and oxidation processes (Carvajal-Millán *et al.*, 2001); however, rachis deterioration is apparently due to a combination of circumstances, especially considering that under conditions of high relative humidity, rachis quality varies during prolonged storage (Retamales *et al.*, 2003; Valverde *et al.* 2005b). In addition, Crisosto *et al.* (1994) discussed the possibility of other factors in addition to water loss involved in the deterioration of Thompson Seedless rachises. Cantin *et al.* (2007) applied abscisic acid (ABA) at veraison, obtaining a better quality of rachis after cold storage due to an effect on the parameters of harvest that allowed cluster storage in a less senescent state. Therefore, rachis metabolism is an important issue to address to understand the loss of quality post-harvest. This work is focused on understanding the physiological processes, other than dehydration, related to oxidative stresses that table grape rachises undergo during post-harvest cold storage.

Materials and methods

Plant material

Red Globe table grape clusters (*V. vinifera*) were harvested from a commercial vineyard located in the Aconcagua Valley. The maturity parameters and packaging were similar to the requirements for long-term storage or export purposes. At harvest, the soluble solids berry content was 17.9%, with an acidity level of 0.4% expressed as tartaric acid. In short-term experiments, clusters or fully developed inflorescences (80% anthesis) of Red Globe grapes were stored at 0 or 20 °C and were removed from such conditions at 0, 9, 24, 48 or 96 h. The rachises

of mature bunches were frozen in liquid nitrogen and stored at -80 °C until analysis. In the case of inflorescences, only the rachis tissue was assayed, not the flowers. In long-term experiments, boxes of Red Globe grapes were packed following industry standards and stored at 0 °C for 0, 25 or 53 days and then kept for up to 5 days at 20 °C to simulate shelf-life conditions. After each sampling time, the rachis was frozen as stated above.

Membrane lipoperoxidation assay

Lipid peroxidation was estimated by measuring the concentration of thiobarbituric acid reactive substances (TBARS) (Ederli *et al.*, 1997). First, 100 mg of rachis was homogenized with 2 mL of 1% w/v tricarboxylic acid (TCA) and centrifuged at 10,000 x g for 5 min. After centrifugation, 250 µL of the supernatant was mixed with 1 mL 0.5% w/v thiobarbituric acid (TBA) in 20% w/v TCA. The mixtures were incubated in boiling water for 30 min and then cooled at room temperature. The absorbance was measured at 532 nm, and the non-specific absorbance was measured at 600 nm (Hodges *et al.*, 1999). The malondialdehyde (MDA) content was determined using a molar extinction coefficient of 155 mM $^{-1}$ cm^{-1}.

Hydrogen peroxide content

The hydrogen peroxide content was determined by a reflectometric method using Rqflex (Merck) and applying a sensitivity range of 0.2-20 mg L^{-1}. Fresh rachis tissue (0.1 g) was macerated with 2 mL of 50 mM sodium phosphate (pH 7.0) and immediately used for analysis (Ombódi *et al.*, 2000).

Reactive oxygen species (ROS) scavenging enzymes

The protocol used for extracting proteins from plant tissues (1 g) started with mortar-ground material homogenization in an extraction buffer A (0.5 M Tris-HCl, pH 7.5, 7.5% glycerol, 1 mM

MgCl$_2$, 5 mM DL-dithiothreitol, 1 mM ethyl-enediaminetetraacetic acid, 2% Triton and 1% polyvinylpyrrolidone) in liquid nitrogen. After centrifugation, the recovered supernatant was used to quantify ascorbate peroxidase, catalase and superoxide dismutase activities as explained below. The protein content of the samples was determined using a modified Bradford method (1 mL of Bradford reagent, 80 μL of 0.15 M NaCl and 20 μL of supernatant) (Bradford, 1976). Protein concentration was calculated using a standard calibration curve made with bovine serum albumin (1 mg mL^{-1}).

Ascorbate peroxidase (APX) activity assay

APX (EC 1.11.1.11) activity was determined by measuring the decomposition of ascorbate at 290 nm for 45 sec. The reaction mixture contained 1 mL of extraction buffer A, 5 μL of 30% H$_2$O$_2$, 40 μL of 10 mM ascorbic acid and 20 μL of the supernatant. Enzyme activity was calculated using a molar extinction coefficient of 2.8 mM^{-1} cm^{-1} (Zhao and Blumwald, 1998).

Catalase (CAT) activity assay

Measurements of CAT (EC 1.11.1.6) activity were obtained using the decomposition of hydrogen peroxide at 240 nm for 45 sec. The reaction mixture contained 1 mL of extraction buffer A, 3 μL of 30% H$_2$O$_2$ and 20 μL of the supernatant. Enzyme activity was calculated using a molar extinction coefficient of 39.4 mM cm^{-1} (Pinhero et al., 1997).

Superoxide dismutase (SOD) activity assay

SOD (EC 1.15.1.1) activity was measured using photochemical inhibition of nitroblue tetrazolium (NBT). The mixture consisted of 700 μL of 50 mM potassium phosphate buffer at pH 8.5, 10 μL of 10 mM EDTA, 10 μL of 1.3 M MET, 10 μL of 2 mM riboflavin, 200 μL of 3 mM NBT and 100 μL of crude extract. The tubes were illuminated (fluorescent light, 40W) for 15 min, and the absorbance was read at 560 nm. Tubes without illumination were used as the control. A calibration curve with commercial SOD (Sigma) was utilized to calculate SOD activity. One unit of SOD was defined as the amount of enzyme required to cause a 50% reduction of NBT (Donahue et al., 1997).

Total phenolic compounds assay

The total phenol concentration was measured with a modified Folin-Ciocalteu assay (Asami et al., 2003). Crude extract (40 mL) was mixed with 100 mL of Folin-Ciocalteu reagent and 560 mL of water, and the mixture was incubated for 15 min at room temperature. After incubation, 300 μL of 7% sodium carbonate was added, and the absorbance of the mixture was read at 660 nm. The total phenol concentration was determined using a 0-25 mg mL^{-1} gallic acid (Sigma) calibration curve.

Determination of total antioxidant activity: ferric reducing-antioxidant power (FRAP) assay

The total antioxidant activities of aqueous extracts taken from rachis tissue and from standard solutions were evaluated by the FRAP assay (Pellegrini et al., 2003). Test or control solution (0.1 mL) was transferred into a test tube, and 3.0 mL of freshly prepared FRAP reagent (25 mL of 300 mmol L^{-1} acetate buffer, pH 3.6, 2.5 mL of 10 mmol L^{-1} TPTZ in 40 mmol L^{-1} HCl and 2.5 mL of 20 mmol L^{-1} FeCl$_3$•6H$_2$O) was added. After a 4 min incubation at 37 °C, the absorbance at 593 nm was recorded against a blank solution containing 0.1 mL of solvent. Relative activities were calculated using a calibration curve of L-ascorbic acid standard solutions (0.1-1 mmol L^{-1}) under the same experimental conditions and expressed as ascorbic acid equivalents (AAE) per gram of fresh plant material.

The data reported are means of at least four replicates. ANOVA analysis and comparisons between means were made with Tukey tests at $p \leq 0.05$ using JMP statistical software (SAS Institute, Inc., Cary, NC, USA).

Results

The general approach was to study the effects of short- and long-term low-temperature storage on rachis tissue from mature table grape clusters and on young rachises from fully elongated inflorescences.

Effect of short-term low-temperature treatments on rachises from mature clusters and fully elongated inflorescences (RFEI, 80% full bloom)

The level of membrane lipoperoxidation of mature rachises was close to 60 nmol MDA g^{-1} FW (Figure 1), remaining constant for the next 96 h with no significant differences at either temperature. Because it is generally recognized that young tissue responds differently to stress than mature tissue, we studied the effect of low temperature on fully elongated (80% full bloom, RFEI) rachises of Red Globe table grapes. The lipid peroxidation of membranes in RFEI stored at 0 or 20 °C is presented in Figure 1. The TBARS value of RFEI at time 0 h was less than half of the value registered for mature table grape clusters (P = 0.05, Figure 1). The lower TBARS content in RFEI compared to mature rachises was significant at every time point analyzed. After 48 h, the TBARS of RFEI was almost twice the level recorded at 0 h. At 96 h, for both temperature conditions, lipid peroxidation was significantly below the level measured at 0 h.

The content of H_2O_2 in mature rachises stored at 20 °C (Table 1) did not change significantly with storage duration. In rachises stored at 0 °C, however, H_2O_2 initially decreased, and only after 48 h was it possible to observe an increase when the H_2O_2 content reached a level similar to the one measured at harvest. The H_2O_2 content of RFEI stored at 0 or 20 °C (Table 2) remained steady throughout the experiment relative to the initial value.

When mature rachises were stored at 0 ºC, there was a major reduction in SOD activity (Table
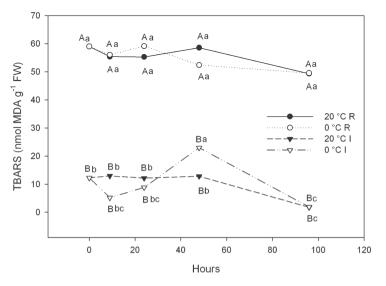

Figure 1. Determination of membrane lipid peroxidation (TBARS) in rachises of mature clusters (R, circles) and fully elongated inflorescences (I, triangles) of Red Globe table grapes stored for 0, 9, 24, 48 and 96 h at 0 and 20 °C. Different lower case and capital letters indicate significant differences (P ≤ 0.05) based on Tukey's comparison test using samples of the same tissue type (R or I) or from the same sampling time point, respectively. FW, fresh weight; MDA, malondialdehyde.

Table 1. Determination of the hydrogen peroxide content (H_2O_2) and superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities of mature rachises of Red Globe table grapes stored for 0, 9, 24, 48 and 96 h at 0 and 20 °C.

Treatment	H_2O_2 μg g^{-1} FW		SOD U (mg total protein)$^{-1}$		CAT nmol min^{-1} (mg total protein)$^{-1}$		APX nmol min^{-1} (mg total protein)$^{-1}$	
0 h	8.4	a[1]	10.19	b	8.57	bc	29.62	c
9 h 0 °C	5.5	bc	0.21	d	3.82	c	74.42	ab
9 h 20 °C	7.0	abc	6.77	bc	9.58	bc	71.18	ab
24 h 0 °C	4.5	c	0.28	d	11.67	ab	46.16	abc
24 h 20 °C	8.0	ab	4.82	cd	12.55	ab	56.85	abc
48 h 0 °C	7.0	ab	8.19	bc	18.79	a	43.24	abc
48 h 20 °C	6.5	abc	11.50	b	7.17	bc	87.43	a
96 h 0 °C	7.5	abc	16.86	a	13.32	ab	37.81	bc
96 h 20 °C	7.0	abc	9.49	bc	6.74	bc	40.42	abc

[1]Values within a column followed by the same letter are not significantly different (P0.05) according to Tukey's multiple range test. FW, fresh weight.

Table 2. Determination of the hydrogen peroxide content (H_2O_2) and superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities of rachises of fully elongated inflorescences of Red Globe table grapes stored for 0, 9, 24, 48 and 96 h at 0 and 20 °C.

Treatment	H_2O_2 μg g^{-1} FW		SOD U (mg total protein)$^{-1}$		CAT nmol min^{-1} (mg total protein)$^{-1}$		APX nmol min^{-1} (mg total protein)$^{-1}$	
0 h	7.3	abc[1]	5.42	c	60.20	bc	241.86	cd
9 h 0 °C	6.0	c	7.41	bc	32.68	bc	98.95	d
9 h 20 °C	7.3	abc	4.41	c	18.39	c	114.20	d
24 h 0 °C	6.0	bc	6.40	bc	24.52	c	125.79	d
24 h 20 °C	7.5	ab	7.08	bc	61.53	bc	618.55	ab
48 h 0 °C	5.0	c	6.29	bc	75.31	bc	849.31	a
48 h 20 °C	7.5	ab	13.44	bc	65.80	bc	389.97	bc
96 h 0 °C	8.8	a	17.52	ab	207.67	a	538.22	b
96 h 20 °C	8.8	a	27.63	a	103.37	b	864.29	a

[1]Values within a column followed by the same letter are not significantly different (P0.05) according to Tukey's multiple range test. FW, fresh weight.

1) in the first 9 to 24 h of storage, followed by a sustained rise between 48 and 96 h of storage. This pattern differed from rachises stored at 20 °C, which exhibited stable SOD activity, except at 24 h, when the activity was statistically lower than at 0 h. The activity of SOD in RFEI (Table 2) at both temperatures in the first 48 h was similar to that observed at 0 h; only at 96 h did SOD activity significantly increase.

The CAT activity in mature rachises stored at 20 °C (Table 1) was similar to the activity measured at 0 h. For rachises stored at 0 °C, only at 48 h was CAT activity significantly lower than at 0 h. CAT activity of RFEI stored at 0 °C for 96 h (Table 2) showed a statistically significant increase, reaching a value that was more than three times the one registered at 0 h. However, samples stored at 20 °C were not significantly different from samples measured at 0 h.

The APX activity of mature rachises (Table 1) stored at 0 or 20 °C significantly increased at 9 h, with more than twice the activity measured at 0 h. After 9 h of storage, the APX activity of rachises stored at 0 °C remained at statistically similar levels through 96 h. A different pattern was recorded for the 20 °C samples, which exhibited higher APX activity after 48 h followed by a reduction at 96 h, when, for both temperature conditions, levels were similar to the ones observed at 0 h. The APX activity of RFEI (Table 2) stored at 0 or 20 °C in the first 9 h was similar to the level at 0 h. This plateau was followed by a second stage of increasing activity between 48 and 96 h, when the APX activity of rachises at both temperatures was statistically higher than the activity at 0 h.

The total antioxidant activity (Figure 2) of mature rachises was high at harvest, exhibiting FRAP values close to 100 µmol g^{-1} FW. The FRAP content between 9 and 96 h was higher than at 0 h for both temperatures, and FRAP was 60% higher at the end of the experiment compared with 0 h. The total antioxidant capacity of RFEI is described in Figure 2. The RFEI samples stored at 20 °C for

48 h had significantly lower FRAP values than at 0 h, and the samples stored for 9 h at 0 °C had the most significantly different value of antioxidant capacity. In all cases studied, mature rachises had statistically higher FRAP levels than RFEI samples.

The total phenolic content of the rachises is shown in Figure 3. The mature rachises stored at 20 °C for 24 h showed a 75% increase of the phenolic content compared to the level at 0 h, followed by a decrease to values similar to those observed at 0 h. The behavior of total phenolic compounds in rachises stored at 0° C was similar. However, the maximum amount was observed after 9 h and was almost 100% higher than at harvest; then, total phenolic content decreased sharply after 24 h to values observed at 0 h. The total phenolic content of RFEI stored at 20 °C (Figure 3) did not show significant changes in the first 48 h; only after 96 h did it reduce significantly, reaching approximately 58% of the 0 h values. Rachises stored at 0 °C significantly increased from 0 to 9 h; then, after 96 h, the values decreased to levels below those observed at 0 h. At all times evaluated, the samples from RFEI had significantly lower total phenolic compounds than the mature rachises.
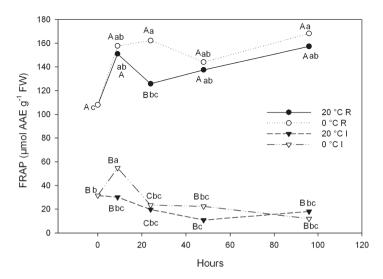

Figure 2. Determination of the total antioxidant capacity (FRAP) in rachises of mature clusters (R, circles) and fully elongated inflorescences (I, triangles) of Red Globe table grapes stored for 0, 9, 24, 48 and 96 h at 0 and 20 °C. Different lower case and capital letters indicate significant differences (P≤0.05) based on Tukey's comparison test using samples of the same tissue type (R or I) or from the same sampling time point, respectively. FW, fresh weight; AAE, ascorbic acid equivalent.

Effect of long-term low temperature on rachises

Analyses similar to those previously described were carried out on mature rachises of table grape clusters stored at 0 °C and exposed to 20 °C, with conditions analogous to commercial procedures normally used for table grapes.

The lipid peroxidation of membranes (TBARS), total antioxidant capacity (FRAP) and total phenolic content of rachises at harvest are shown in Table 3. The values of these parameters after cold storage for 25 or 53 days plus shelf life conditions (20 °C) were not significantly different relative to 0 h

H_2O_2, SOD and APX activities (Table 4) at each time of measurement were not significantly different from the values at 0 days (harvest) or after

cold storage plus shelf life. CAT activity (Table 4) at 0 d was significantly higher than at other sampling times regardless of storage time.

Discussion

As mentioned earlier, fruit product appearance and freshness are important attributes that motivate consumer decisions (Bruhn, 2002). Both quality attributes are especially important for table grapes grown for export because quality should be maintained at optimum levels after several days of cold storage and transport. Therefore, our study focused on understanding the effect of cold on 'Red Globe' rachis tissue in order to inform strategies for extending table grape postharvest quality.

Table 3. Determination of membrane lipid peroxidation (TBARS), the antioxidant capacity (FRAP) and the total phenolic content from mature rachises of Red Globe table grapes stored at 0 ˚C for 25 or 53 days followed by 5 or 3 days of shelf life at 20 ˚C.

Treatment	TBARS nmol MDA g^{-1} FW		FRAP umol AAE g^{-1} FW		Total Phenolics mg GAE g^{1} FW	
0 d	59.69	a[1]	107.94	ab	13.57	ab
25 d 0 ˚C	71.76	a	112.38	a	15.84	a
25 d 0 ˚C + 5 d 20 ˚C	53.63	a	109.52	ab	12.97	ab
53 d 0 ˚C	69.49	a	120.33	a	12.69	ab
53 d 0 ˚C + 3 d 20 ˚C	61.81	a	85.69	b	9.80	b

[1]Values within a column followed by the same letter are not significantly different (P≤0.05) according to Tukey's multiple range test. FW, fresh weight; MDA, malondialdehyde; AAE, ascorbic acid equivalent ; GAE, gallic acid equivalent.

Table 4. Determination of the hydrogen peroxide content (H2O2) and superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities of mature rachises of Red Globe table grapes stored at 0 ˚C for 25 or 53 days followed by 5 or 3 days of shelf life at 20 ˚C.

Treatment	H_2O_2 µg g^{-1} FW		SOD U mg total protein^{-1}		CAT nmol min^{-1} mg total protein^{-1}		APX nmol min^{-1} mg total protein^{-1}	
0 d	8.4	a[1]	10.19	a	8.57	a	29.62	a
25 d 0 ˚C	8.0	a	9.71	a	4.84	b	21.82	a
25 d 0 ˚C + 5 d 20 ˚C	9.8	a	6.41	a	6.61	b	33.59	a
53 d 0 ˚C	9.3	a	10.93	a	6.06	b	19.66	a
53 d 0 ˚C + 3 d 20 ˚C	7.5	a	8.09	a	6.75	b	24.79	a

[1]Values within a column followed by the same letter are not significantly different (P≤0.05) according to Tukey's multiple range test. FW, fresh weight.
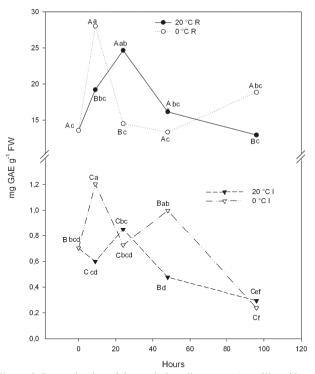
Figure 3. Determination of the total phenolic content (as gallic acid equivalent, GAE) in rachises of mature clusters (R, circles) and fully elongated inflorescences (I, triangles) of Red Globe table grapes stored for 0, 9, 24, 48 and 96 h at 0 and 20 °C. Different lower case and capital letters indicate significant differences (P≤0.05) based on Tukey's comparison test using samples of the same tissue type (R or I) or from the same sampling time point, respectively. FW, fresh weight.

The collected data show that the level of membrane lipid peroxidation of the mature rachis at harvest (0 h) in both short- and long-term storage experiments was 3-fold less than in rachises from fully extended inflorescences. In addition, the TBARS content in mature rachises at harvest was higher than that described for *V. vinifera* leaf samples (Gunes *et al*., 2006; Toumi *et al*., 2008) but less than that reported for grape berries (Xu *et al*., 2009; Wen *et al*., 2008; Zhang *et al*., 2008). One possible explanation for this difference is that the material in those studies did not match the standard level of quality in terms of fruit age (over mature), but the clusters used in the experiments had quality indexes similar to those used commercially. Cantín *et al*. (2007) reported that rachises of Crimson Seedless grapes harvested at normal maturity (16.5% soluble solids) could begin to senesce prior to cold storage, which leads to a significant loss in rachis quality during post-harvest storage. Because table grapes cor-

respond to a non-climacteric fruit, all the sensorial characteristics must be developed at harvest. However, the development of senescence and berry ripening as concomitant processes cannot be ruled out because lipid peroxidation has been described in senescent vegetative tissue (Dhindsa *et al*., 1981; Munne-Bosch and Penuelas, 2003; Zhuang *et al*., 1995). Nelson (1979) mentioned that due to cultural practices, it is possible to have mature grape berries attached to senescent vascular tissue, which could explain our results. The TBARS content observed in mature rachises was relatively constant in short- and long-term storage (Figure 1, Table 3), indicating that either the stimuli associated with lipid peroxidation at harvest were not maintained post-harvest or that mechanisms to avoid lipid peroxidation were sufficiently intact. This situation is different in rachises of inflorescences, where there was a marked response at low temperature following 48 h of incubation, characterized by a reduction

in TBARS. This value was below that observed at time 0 h and less than the values observed for mature rachises, demonstrating that tissue ontogeny is relevant to the membrane stress process, at least under cold conditions.

It is known that lipoperoxidation of membranes can be associated with ROS action (Apel and Hirt, 2004; Moon and Shibamoto, 2009; Shulaev and Oliver, 2006; Thompson *et al.*, 1987) and that ROS are present at leaf senescence (Lim *et al.*, 2007). In short- and long-term experiments with mature rachises, there were no significant changes in the H_2O_2 content after harvest and incubation at either warm or cold temperatures, suggesting that processes inducing H_2O_2 occur at, or prior to, harvest. The dynamic values of SOD, CAT and APX in mature rachises stored at 0 °C in short-term experiments did not show significant changes in response to cold treatment, indicating that this tissue is not affected by cold storage in this time frame. This conclusion is reinforced by the results of long-term cold storage experiments in which SOD and APX activity did not show significant differences compared to 0 d values and in which post-harvest storage consistently reduced CAT activity. An analogous reduction of enzymes associated with ROS has been described in senescence of detached chrysanthemum florets (Chakrabarty et al., 2007). Although CAT resides in peroxisomes (Buchanan *et al.*, 2000), it could be speculated that, in the case of mature rachises, peroxisomes are more susceptible to degradation by senescence compared with the locations of SOD or APX within the cell.

In RFEI, the activities of SOD, CAT and APX are apparently affected by low temperature at 96 h, but the magnitude of the response was quite different compared with that in mature rachises (Table 1 versus Table 2). The lower responsiveness in mature tissue might be due to senescence, as reported in other studies (Thompson et al., 1987). The concomitant high activities of SOD, CAT and APX in RFEI in cold conditions have been described in other

ROS-associated stress experiments (Cao *et al.*, 2009; González *et al.*, 1998).

Although there was a rapid increase in the FRAP content in the mature rachises following harvest (Figure 2), the FRAP value eventually reached a steady level that was maintained in prolonged storage, which was higher than the one reported for avocados (Corral-Aguayo *et al.*, 2008), peaches and nectarines (Gil *et al.*, 2002), pomegranate juice (Gil *et al.*, 2000) and blueberries (Taruscio *et al.*, 2004). The increase in the antioxidant capacity of rachises under similar low temperature conditions has also been reported in grape berries (Sánchez-Ballesta *et al.*, 2007), suggesting that although the table grape is not chilling-sensitive (Kader, 2002), it could respond to short-term storage at 0 °C. This characteristic is more evident in rachises from inflorescences in which the FRAP content was observed to rise sharply after exposure to cold temperatures (Figure 2). It is possible to speculate that a sudden increase in antioxidant compounds in young rachises could be useful to reduce the impact of ROS as a result of a sudden change in temperature (Romero et al., 2008) and to complement the increase of SOD, CAT and APX after prolonged cold storage (96 h), as discussed previously. Similar results of low temperature storage (accumulation of compounds linked with antioxidant capacity) have been reported for other non-chilling species (Leja *et al.*, 2001; McKown *et al.*, 1996; Oh *et al.*, 2009).

Another relevant issue derived from this study is the considerable level of phenolic compounds (approximately 12 mg GAE g^{-1} FW) in mature rachises relative to young rachises (Figure 3), grape berries (Yang *et al.*, 2009) and other species such as strawberries (Aaby *et al.*, 2007), apples and pears (Imeh and Khokhar, 2002), cereals (Perez-Jimenez and Saura-Calixto, 2005), spinach (Bottino *et al.*, 2009) and blueberries (Taruscio *et al.*, 2004). Pinelo *et al.* (2006) mentioned that grape stems are rich in phenolic compounds, mainly flavonols and tannins (Souquet *et al.*, 2000). These tannins are present in the parenchyma and

ray cells in *Vitis* species (Esau, 1965) and were also observed in anatomical analyses of rachis tissue (data not shown).

The rapid increase in the phenolic content of mature rachis and inflorescence tissue after harvest could be induced by a combination of temperature change and water loss because similar patterns were observed in both temperature conditions. In addition, water loss and phenolic compounds have been linked in a report studying the up-regulation of the phenylalanine ammonia lyase (PAL) gene, the committed step in phenylpropanoid metabolism, in grape berry tissue under dehydration post-harvest (Rizzini *et al.*, 2009). Some phenolic compounds have antioxidant properties (Heredia and Cisneros-Zevallos, 2009) that could be complemented by the high FRAP content found in mature rachises (approximately 3 times higher than in RFEI); therefore, antioxidants may be responsible in part for the steady state of membrane-lipid peroxidation of mature rachises in long- and short-term storage. These data are supported by results from eugenol, menthol and thymol treatments, which reduced the rate of rachis deterioration and suggest that such effects could be produced in part by an antioxidant effect of these compounds (Valverde *et al.*, 2005a). In addition, Guillén *et al.* (2007), using essential oils and modified atmospheric conditions to maintain the quality of table grapes, concluded that the antioxidant capacity of the essential oils helped to prevent the deterioration by oxidation that occurs in senescence. Therefore, the possibility that the phenolic compounds naturally found in rachises participate in a ROS defense process is plausible.

Another explanation for the high content of total phenolic compounds in mature versus young rachises is the possibility that the mature rachis may undergo anatomical change described as ripening of the cane (Mullins *et al.*, 1992). It is known that table grape canes suffer anatomical modifications, such as formation of the periderm

with tissue separation (epidermis, cortex and primary phloem) (Mullins *et al.*, 1992). Rachises could undergo an analogous process. Carvajal-Millán *et al.* (2001) discussed an increase in lignin and suberin together with a reduction in water content as rachises ripen. Development of the periderm on the rachis (central axis) of Red Globe clusters was observed (data not shown). Therefore, some amount of the phenolic compounds in the mature rachis could be useful in the processes of suberization and lignification.

Although the approach of the present work was to study processes related to ROS and rachis deterioration other than water loss, it is evident that dehydration occurred during table grape cold storage. Water loss has been correlated with an increase in the ABA content when plant tissues are kept under drying conditions (Zeevaart and Creelman, 1988). In addition, the amount of ABA in berry flesh increases after veraison and grape ripening (Coombe and Hale, 1973). ABA can induce lipoxygenase activity involved in lipid oxidation in grape berries (Costantini *et al.*, 2006), which could partially explain the membrane degradation level found in mature rachises. Regarding senescence, ABA could additionally induce H_2O_2 formation, and the basal content found throughout the described experiments could be useful to fuel other processes involved in rachis physiology, such as lignification.

There was a noticeable level of rachis deterioration at harvest, compared to young tissue, which was maintained by low temperature conditions in prolonged storage. Although rachises showed responsiveness to cold conditions, the pattern from this study did not suggest damage due to either cold storage or ROS involvement in short or prolonged storage. The high antioxidant capacity found in mature rachises, if it is available, may be sufficient to avoid tissue damage by ROS induced by the senescence process.

In the mature rachis, concomitant processes could be developing along with senescence, such as anatomical changes similar to those

described during grape cane maturation. It is necessary to study changes in rachises in earlier stages of development, for example, between veraison and harvest, to understand the processes that rachises undergo pre-harvest. It is also important to understand the combined effects of cultural practices and post-harvest conditions on rachis tissues and how senescence modulates such events.

Acknowledgments

Resumen

R. Campos-Vargas, P. Zamora, R. Contreras, H. Köhler, G.E. Zúñiga, A. Pérez-Donoso y B.G. Defilippi. 2012. Efecto de las condiciones de almacenamiento refrigerado sobre el estrés oxidativo en raquis de uva de mesa, cv. Red Globe. Cien. Inv. Agr. 39(1): 91-104. La calidad de uva de mesa (*Vitis vinifera* L.) involucra tanto la condición de bayas y el raquis. En el presente trabajo, los parámetros fisiológicos de raquis de ´Red Globe´ de inflorescencias completamente elongadas (RFEI) y de racimos maduros fueron estudiados después de almacenamientos por diferentes tiempos a 0 ó 20 ºC. Para entender los cambios en la fisiología del raquis debido a variaciones de temperatura y condiciones de almacenamiento, se midieron las actividades de superoxido dismutasa (SOD), catalasa (CAT), ascorbato peroxidasa (APX). Además fueron analizados el contenido de peróxido de hidrógeno, lipoperoxidación de membranas (TBARS), compuestos fenólicos totales y capacidad antioxidante (FRAP). TBARS de los raquis maduros a la cosecha fue mayor que el de inflorescencias completamente elongadas (RFEI). Este parámetro permaneció constante a través del almacenamiento, indicando cambios presuntamente asociados a ontogenia o procesos de senescencia. Almacenamiento cortos (0-96h) incrementaron la actividad de SOD, CAT y APX en RFEI, sin embargo no se observaron cambios en la actividad de estas enzimas o contenido de peróxido de hidrogeno en raquis maduros. Almacenamiento refrigerado prolongado (25 ó 30 días 0 ºC) de raquis maduros redujo la actividad de CAT, pero SOD y APX no mostraron cambios bajo estas condiciones. A 0 h el contenido de FRAP y fenólicos totales de raquis maduros fueron tres y 20 veces mayores que en raquis inmaduro respectivamente.

Palabras clave: Calidad de fruta, raquis, uva de mesa, *Vitis vinifera* L.

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