Postharvest treatment with methyljasmonate affects the antioxidant system and the quality of wine grape during partial dehydration

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ABSTRACT

BACKGROUND - Postharvest partial dehydration is a widely used technique to produce important wines in Italy, dry and sweet. Accurate management of environmental parameters of dehydration permits to modulate berry metabolism to maintain/improve the enochemical quality of berries. As the water loss induces an oxidative process in berries, we have hypothesized that methyljasmonate (MeJA) and ozone (O_3) as postharvest treatment before partial dehydration, could be beneficial for grape berries.

RESULTS Grape bunches were treated with 10 or 100 µM MeJA at 20°C for 12 hours or with ozone gas (20 g h^{-1} with 6% w w^{-1} of ozone) at 10°C for 12 hours; control was untreated berries kept at 20°C for 12 hours. Partial dehydration was performed at 10°C, 70% relative himidity (RH) and air flow (1 m s^{-1}) until reaching 30% weight loss (w.l.). MeJA hastened water loss of grape berries. Total polyphenols and total flavonoids contents were increased by MeJA, immediately, and remained higher than

the concentration in control sample but, at the end of partial dehydration, the values were lower than the ozone-treated sample. Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacole peroxidase (GPX) increased in partial dehydration, especially in treated samples. Lipooxygenase (LOX) and polyphenoloxidase (PPO) showed lower activity in MeJA samples than in the control.

CONCLUSIONS - MeJA accelerates water loss but, in the same time, activates the antioxidant system. Ozone does not accelerate water loss but activates antioxidant system and increases polyphenol content.

Keyword: Methyl Jasmonate; Ozone; Grapes; Dehydration; Post-harvest.

INTRODUCTION

Postharvest partial dehydration of wine grape is a technique widely used in Italy and in Mediterranean Countries to produce Passito wines, sweet and dry. In the case of dry wine, this wine is considered as reinforced wine because the intensity of weight loss is not so high as for sweet Passito wine. Most of Passito wines in Italy are made from white varieties, e.g. Muscat of Alexandria, Malvasia, Trebbiano, Erbaluce, Picolit and more. Postharvest grape partial dehydration is a complex metabolic system[1]. Water stress and senescence interact when the environmental parameters of dehydration are well controlled in order to delay water loss, thus slowing down the dehydration process. Practically speaking, the obtained partial dehydration is a real postharvest technology where berry cells are still alive at the end. A cascade of metabolic responses through different and selective modulation of the expression of genes involved in pathways/processes (glycolysis/fermentation, phenylpropanoid, oxidative stress), affects berry composition[2]. Zenoni *et al.*[1] identified a group of transcripts that were modulated more slowly and gradually during the postharvest period, representing the common features of berries undergoing dehydration and/or commencing senescence. This included genes controlling ethylene and auxin metabolism as well as genes

involved in oxidative and osmotic stress, defense responses, anaerobic respiration, and cell wall and carbohydrate metabolism[2]. The role of oxidative stress, as consequence of water stress during postharvest partial dehydration of grapes, has not been studied but it has been postulated by studying an aerobic fermentation (or anaerobic respiration) taking place during water stress of grapes[3, 4, 5] where an increase of oxidized compounds such as acetaldehyde and acetic esters occurred. An up regulation of laccase and peroxidase genes has been observed in the last step of postharvest dehydration[1]. An increase of antioxidant activity of grapes during off-vine drying has been reported by Moreno *et al*.[6] due to Maillard compound formation. Recently, Panceri *et al*.[7] showed that, antioxidant activity determined by ABTS and DPPH methods, was higher in 40% dehydrated grape than in control or 30% dehydrated samples, and this result was attributed to the increase of polyphenol compounds. The interaction between water stress intensity and rate, seems to play a major role in modulating molecular responses involving not only transcriptional but also post-transcriptional and post-translational regulatory mechanisms[2] as well as the environmental parameters such as temperature, relative humidity and air flow[8].

Methyl jasmonate is the methylester of jasmonic acid and is synthesized by oxidation, via lipooxygenase, of unsaturated fatty acids of chloroplast membrane[9]. Jasmonate (JA) and methyl jasmonate (MeJA) are considered phytohormone[10]. Exogenous application of JAs on different plants under abiotic stresses particularly salinity, drought, and temperature (low/high) conditions, have been effective in improving plant stress tolerance but, also, a variety of JA-induced plant growth events (fertility, sex determination, storage organ formation, reproductive processes, root elongation, fruit ripening and senescence, oxidative defense, and interaction with other hormones) have been reported[11]. Several studies have shown as the treatment with MeJA increases antioxidant enzyme activity, such as ascorbate peroxidase, superoxide dismutase, glutathione peroxidase, ascorbate dehydroreductase, guaiacol peroxidase, glutathione reductase, and monodehydro ascorbate reductase[12, 13, 14, 15]. In addition, by

considering that this compound is already classified as Generally Recognise As Safe (GRAS) substance by the U.S. Food and Drug Administration[16], it may have a potential for enhancing the synthesis of bioactive compounds[17]. Preharvest and postharvest application of MeJA have produced a greater antioxidant capacity in Chinese red bayberries[16], strawberry[18] as well as have enhanced anthocyanin synthesis in apples[19] or in different berries[20, 21, 22, 23], in polyphenols in guava[24, 25], and in carotenoids, ascorbic acid and antioxidants in plum[26]. Treatment with (-) MeJA induced increase in resveratrol, quercetin-3-O-glycoside, quercetin-3-O-galactoside and quercetin 3-O-rutinoside whereas, a treatment with (+) MeJA provoked an increase in quercetin-3-O-glucoside and quercetin-3-O-rutinoside, without effects on resveratrol and quercetin-3-O-galactoside content[27]. Ozone (O_3) can be considered a postharvest elicitor because, at right concentration and application time, promote high levels of healthy phytochemicals in fruit and vegetables[28, 29, 30, 31] but, generally, as a well-known strong oxidizing agent it is used by the food industry as an antimicrobial agent[32]. Thus, its positive and negative effects on plant cells depend on the concentration and application time. O_3 as strong oxidant decomposes spontaneously producing a mass of reactive oxygen species (ROS)[33, 34]. If there is an imbalance in a cell compartment between the ROS and the antioxidant defense, oxidative stress and damage will occur[35]. Some studies have shown an increase in antioxidant enzymes superoxide dismutase, catalase, and ascorbate peroxidase in papaya[36] and pear[37] to protect fruits from oxidative damage following ozone treatment[38].

To our knowledge, the effects of treatment with MeJA on wine grapes subjected to postharvest partial dehydration, have never been investigated. As MeJA has the ability to protect against oxidation and it is known, as explained before, that postharvest water stress induces cell oxidation, our hypothesis has been that a postharvest treatment of wine grape with MeJA before postharvest partial dehydration, could protect or even enhance the quality characteristics of berry for wine production. As comparison we have used ozone postharvest treatment before than postharvest partial dehydration, at

a specific concentration we have studied before which resulted nontoxic but beneficial for grape berries; control grapes were untreated before postharvest partial dehydration.

EXPERIMENTAL

Fruit samples and experimental design

Bunches of white wine grapes cv. Romanesco (about 200 Kg) were harvested in the experimental vineyard of the University located in Montefiascone (VITERBO) in the Lazio region (Italy) (42°29'1.30"N latitude – 12°0'37.37E" longitude). Bunches were hand harvested at about 18 % TSS (total soluble solids) and sorted for color and absence of injuries prior to treatments. Bunches were divided in four lots (about 50 kg each lot). One lot was untreated (control) and bunches were kept at 20 (±1)°C and 70 (± 5) % RH for 12 hours in a cold room. Treatments with MeJA (Sigma–Aldrich. Milano, Italy) were performed at 20 (±1) °C for 12 hours in another temperature controlled room, placing the bunches in small airtight chambers (1 m^3) where the appropriated solution volume of MeJA to reach the desired gas concentration (10 or 100 µM), was deposited on filter paper and the chambers were tightly-sealed. A small fan was placed inside the chamber, to facilitate the MeJA solution evaporation. At the end of treatment, the CO_2 and O_2 concentrations inside the small chambers were 10% and 12%, respectively. Ozone gas (max 20 g h^{-1} with 6% w w^{-1} of ozone) with a flow rate at maximum 150 NL h^{-1} (NL= normal litre) rate (Ozone generator A series, PC Engineering, Uggiate Trevano, Italy) in a 9 m^3 cold room, was used. The treatment was performed at 10 (±1) °C for 12 hours.

After the treatments, all samples were kept in small dehydration tunnels with fans, placed in a cold room at 10 (±1)°C and 70 (± 5) % RH. Air flow of tunnels was fixed at 1 m s^{-1}. Analyses were performed on the first, second and third day after treatments and then at 10, 20 and 30% w.l..

Physical and Chemical Analyses

Weight loss of 10 bunches each sample was measured by using a technical balance (Adam Equipment Co. Ltd, Milton Keynes, England). Peel color was assessed using a

Minolta colorimeter (Minolta C2500. Konica Minolta, Ramsey, NY) to determine chromaticity values L* (Lightness), a* (green to red), and b* (blue to yellow). Initially, 50 berries each treatment were pen-marked and used to perform the colorimetric reading on the same berries for the whole duration of the test. The hue angle (h) was calculated from chromaticity values a* and b* as reported earlier by McGuire[39]. Peel resistance was evaluated by using an Instron Universal Testing Machine (model 3343; Instron Inc., Canton, MA) adapted with a needle probe with 1 mm diameter and performed at 1 mm s^{-1} bar speed. The maximum peel break force (Fsk) was expressed in N, corresponding to peel resistance to applied break force. For this test, at each sampling time, 60 berries per treatment from different bunches, were analyzed and then used for total soluble solid content using a digital refractometer (ATAGO, Palette PR-32). CO_2 production rate was monitored by the gas analyzer HELPY (Marvil engineering SRL, Bozen, Italy). Bunches were tightly closed for 2 hours into glass jars, adapted with a septum for sampling oxygen and carbon dioxide. One bunch in a glass jar, 3 jars each treatment. CO_2 production was expressed as ml kg^{-1} h^{-1}.

At each sampling time, berries from different bunches (three set of berries) were used for chemical and biochemical analyses. For the following chemical analyses, juices were extracted from the three sets, and used to measure total phenolic content by the Folin–Ciocalteu method[40] expressed as mg of gallic acid equivalents (GAE) per 100 g fresh weight (FW) and the total flavonoid content by using the aluminium chloride colorimetric method[41], expressed as mg of catechin equivalent (CE) per 100 g fresh weight (FW). For biochemical analyses, berries were immediately immersed in liquid nitrogen and ground to a fine powder in a mortar porcelain.

Extraction and measurement of total soluble protein

Total soluble proteins were extracted after re-suspending frozen fruit tissue powder in extraction buffer (2:5 wv) containing: 100 mM potassium phosphate buffer (pH 7.8), 100 mM sodium EDTA (pH 7), 1.25 mM polyethyleneglycol and 2 mM dithiothreitol. The sample was subsequently homogenized in the mortar with the addition of PVPP to

5% and transferred into transferred into a 2-mL Eppendorf tube. The samples were centrifuged at 14000 *g* for 30 minutes at 4°C. The supernatant was used for measurements of enzyme activities. Protein content was measured by the Bradford assay[42] using bovine serum albumin as a standard.

All the enzyme activities were assayed by using the method described by Pasquariello *et al*.[43] with slight modifications.

CAT activity

The reaction medium consisted of 50 mM potassium phosphate buffer (pH 7), 20 mM H_2O_2 and 100 µL of crude enzyme extract in a final volume of 1.5 mL. The reaction was started by adding H_2O_2, and the decrease in absorbance at 240 nm, caused by its breakdown, was monitored. The specific activity was expressed as nmol H_2O_2 g^{-1} fresh weight (FW).

APX activity

The reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7), 0.25 mM ascorbic acid, 0.70 mM H_2O_2, 0.66 mM sodium EDTA (pH 7) and 20 µL of crude enzyme extract in a final volume of 1.5 mL. The reaction was started by adding H_2O_2, and the oxidation of ascorbic acid was determined by the decrease at 290 nm. The specific activity was expressed as µmol H_2O_2 g^{-1} FW.

SOD activity

The reaction mixture consisted of 50 mM potassium phosphate buffer pH 7.8, 0.1 mM sodium EDTA, 13 mM methionine, 75 µM NBT, 2 µM riboflavin and 100 µL of crude enzyme extract in a total volume of 1.5 mL. The reaction was started by adding riboflavin, and after 15 min of incubation at room temperature under continuous light, the absorbance at 560 nm was measured. One SOD unit was defined as the amount of enzyme that inhibits the rate of NBT reduction by 50% under the above assay conditions. The specific activity was expressed as U g^{-1} FW.

Guaiacol peroxidase activity

The reaction mixture contained 100 mM potassium phosphate buffer pH 7, 0.20 mM sodium-EDTA pH 7.0, 13,2 mM H_2O_2, 32 mM guaiacol and 250 µL of crude enzyme extract in a final volume of 1 mL. Guaiacol peroxidase activity was detected spectrophotometrically by recording the formation of tetraguaiacol and the consequent increase in absorbance at 470 nm. The specific enzyme activity was expressed as as nmol tetraguiacol g^{-1} FW.

PPO activity

Crude enzyme extract (20 µL) was incubated with a buffered substrate (500 mM catechol in 100 mM sodium phosphate buffer pH 6.4) in a final volume of 1.5 mL and monitored by measuring the increase in absorbance at 398 nm. The specific activity for molar change in catechol was expressed µmol g^{-1} FW.

LOX activity

The reaction mixture consisted of 100 mM sodium phosphate buffer pH 6, 0.17 mM linoleic acid sodium salt, and 50 µL of crude enzyme extract in a final volume of 1.5 mL. The lipoxygenase activity was detected spectrophotometrically by recording the formation of hydroperoxides and the resulting increase in absorbance at 234 nm. LOX activity was expressed as the specific rate on a fresh weight basis of molar change of hydroperoxides in nmol g^{-1} FW.

Statistical analysis

All the data are expressed as the mean ± standard deviation (SD). To determine the difference between uncoated and treated fruit, one-way ANOVA and the least significant difference (LSD) test for mean comparisons were used. Differences at $p<0.05$ were considered significant and are indicated with different letters. Correlations among the evaluated parameters were analyzed using Pearson's correlations ($p<0.05$ and $p<0.01$). A principal component analysis (PCA), in order to identify the principal components contributing to the majority of the variation within the dataset, was applied to evaluate the effectiveness of different treatments on physical-chemical, nutraceutical

and enzymatic traits. All analyses were performed using the SPSS software package, Version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Partial dehydration process began on 28th of September and, plotting dehydration time versus weight loss (w.l.), two straight lines increased with R^2 of 0.9832 and 0.9677, respectively for ozone and control treatment (the former) and for MeJA treatments (the latter) (Figure 1). MeJA-treated grapes showed a more pronounced w.l. rate and the process lasted 27 days while, for the other two treatments, the process was of 50 days. Respiration rate, after 1 day, was significantly higher in MeJA and control samples while ozone showed the lowest value, but this response was to attribute to the different temperature used. Respiratory quotient, after 3 days, was 1.31, 1.08, 1.18, and 1.15, respectively for MeJA 100 µM, MeJA 10 µM, ozone, and control sample (data not shown).

TSS increased during partial dehydration process, without significant difference among the samples, reaching values between 22.3 and 23.4 (Table 1). Peel color values decreased progressively in all samples (Table 2); MeJA samples showed higher values at 10 and 20% w.l. but at 30%, only MeJA 10 µM had a lighter yellow color while the other samples appeared reddish, to indicate an oxidation of peel color. Berry peel resistance increased with the progress of weight loss without significant difference among samples (Table 3).

During the partial dehydration process, a significant increase in polyphenol content was observed (Table 4). Ozone treatment showed the highest values after one day of treatment and at the end of dehydration (30%). MeJA 10 µM induced an increase in polyphenol content on day 1 but, at the end, the concentration was the lowest one. MeJA 100 µM showed an increase in polyphenols on day 2 and, at the end, the value was slightly higher than MeJA 10 µM one. Total flavonoids raised, 1 day after the treatment, in all the treatments but overall in ozone and MeJA 10 µM (Table 5). In

control and MeJA 100 μM the value continued to rise until the end of experiment while, in ozone and in MeJA 10 μM, the values declined and rose again at 20% w.l.. At the end, the highest value was for ozone sample.

SOD activity increased after the treatment and during dehydration time. Ozone and MeJA-treated fruits exhibited higher activity than untreated ones (Fig. 2). All treatments stimulated CAT activity, whose values reached a peak in MeJA-treated sample (10 μM), on day 2 after treatment (Fig. 3). APX activity was also induced by treatments especially in the final stages of the partial dehydration process, with higher values in treated samples than in the control. APX activity reached the highest values at 10% w.l. and decreased later on (Fig. 4). During the partial dehydration process, GPX activity increased in all samples and reached higher values in the treated fruits (Fig.5). LOX and PPO activity showed a similar trend in all samples and the activities were higher in control fruit than in treated-samples. LOX activity was constant until the 3rd day after treatment and, then, increased in the partial dehydration process, but no statistical differences were registered among treated samples (Fig. 6). Similar to LOX activity, PPO activity (Fig. 7) increased in all treatments during the partial dehydration process.

DISCUSSION

The first surprising result has been that MeJA treatments facilitated water loss in grape during the partial dehydration process, reducing the dehydration time (27 days) vs 50 day of ozone-treated and untreated grapes. In another non climacteric fruit such as strawberry, postharvest application of MeJA provoked an induction of ethylene production[44] and stimulated ripening[45] and, in climacteric fruit, it has been shown the effect of MeJA in hastening ripening[26]. In contrast, it has been seen MeJA postharvest treatments (1 and 5 μM) on eggplant to delay postharvest senescence and consequently to reduce weight loss during 10 days of storage at 20°C[12]. No specific papers were found on the interaction of MeJA and water loss from plants. So, it is

unclear the role of MeJA in weight loss; in our case we can suppose that MeJA has stimulated ethylene production which is known to act on cell wall enzymes of grape berry, favoring the water loss[46]. In many cases only small amounts of enzyme activity are required to effect significant changes of cell wall polysaccharides during softening of grape berries[47] and cell wall enzymes are the very sensitive to ethylene and its activation is one of the first response to small amount of water loss[48]. Thus, we can speculate that an autocatalytic effect of water loss due to the response in ethylene production as consequence of MeJA treatment and then an induction of cell wall enzymes activity which facilitates the water loss which, as feedback, activates a further cell wall enzyme activity.

This effect of water loss acceleration did not affect ripening parameter as expected as grape is non climacteric fruit; at the end of experiment, TSS in MeJA samples were similar to the ones of the other samples as well the peel resistance and the hue angle.

Total polyphenols and total flavonoids, at the end of experiment, were lower in MeJA-treated berries than in control and ozone sample, the last one showing the highest value. This result of the ozone-treated sample is in agreement with those reported by Artés-Hernandez *et al*.[30] and Carbone and Mencarelli[31] who showed that treatments with ozone increase the concentration of phenolic substances on table and wine grapes. As regards methyl jasmonate and polyphenols, an increase of flavonols as a result of treatment with methyl jasmonate on table grapes has been reported[57]. In our case, we found a significant increase of polyphenols and flavonoids after 3 days from treatment, while, at the end of experiment, the values were lower than control. It is known that, during the postharvest partial dehydration process, the water stress causes an oxidative stress with increase of lipoxygenase and peroxidase[4,5]. Plant cells have a system of enzymatic and non-enzymatic antioxidants that maintains ROS at the right levels. The enzymes involved in ROS scavenging, such as CAT, APX, GPX and SOD , showed higher activity in the treated samples, overall during partial dehydration,

suggesting the antioxidant system is activated in berries as a consequence of water loss. APX activity increase confirms what it has been observed by a proteomic approach in a previous study[49] through 2D-DIGE (2-Dimensional Gel Electrophoresis), where an overregulation of this protein during postharvest partial dehydration process of grape (cv. 'Corvina') has been observed. The activity of CAT and APX is directly related to the stress level of samples and, probably, this rise allows for the reduction of H_2O_2 accumulation in tissue. Previous studies on other fruits such as peach, strawberry and apple have shown an increased activity of these enzymes during postharvest treatment with MeJA[12,50,51]. In our case, more than the single postharvest MeJA treatment, it is the combination of this treatment and partial dehydration which activates the antioxidant metabolism. The different performance of detoxifying enzymes of hydrogen peroxide during dehydration process, may be due to their different kinetic characteristics. CAT has a high turnover rate and low affinity for H_2O_2 compared to APX which has a lower K_m[52,53]. This suggests that CAT acts quickly to favour initial cell detoxification, while APX, a very labile enzyme inactivated at high H_2O_2 concentrations, acts in the advanced stages of partial dehydration process. As regards the stimulatory effect of ozone on antioxidant enzymes SOD, CAT, GPX and APX, the results confirm what observed in papaya[36] and in pear[37]. The effect of ozone is strongly dependent on concentration and time of application; in our case the concentration is low, and the time of application is short, this could have acted as a light oxidizing agent stimulating the activity of these enzymes for the berry cell protection.

In contrast, treatment with MeJA and ozone inhibited the LOX activity, an enzyme which plays an important role in lipid peroxidation process causing damage to cell membrane in plant tissues[54,55]. LOX has been reported as the first marker of postharvest water stress in Malvasia during postharvest partial dehydration[4]. In our experiment, already after 1 day, the treated fruits showed a lower level of activity and this difference remained with the progress of dehydration. Our hypothesis is that MeJA might protect cell membrane by inhibiting lipid peroxidation caused by water stress, as

demonstrated in other studies[56,57]. In fact, LOX is responsible for the biosynthesis of JA precursors, consequently MeJA treatment could regulate the synthesis of LOX enzyme. As regards ozone, the decrease of LOX activity is unexpected since ozone is a strong oxidizer but probably, as mentioned above, the low concentration and the short time of application, by activating the antioxidant system and increasing the phenol content, permitted the protection of membrane lipid peroxidation.

Wine grape partial dehydration leads to berry browning[58,59] and, in grape berry, the main cause of browning is the PPO activity[60]. As for LOX, PPO activity increased during partial dehydration and it was always higher in untreated fruits (control). The treatments inhibited PPO activity as reported in other fruits such as pears[37] and loquat[57].

CONCLUSIONS

A MeJA treatment before partial dehydration of wine grape, accelerates weight loss but, in the same time, protects grape from oxidation by activating the antioxidant enzymes, increasing polyphenols and flavonoids until 20% weight loss, and inhibiting LOX and PPO activities.

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TABLES

Table 1. Total soluble solids measured on the 1st, 2nd, and 3rd day and then at 10, 20, and 30% w.l. during postharvest partial dehydration. Data are the mean of 60 berry readings (± SD). Values with different letters are significantly different ($p < 0.05$).

TSS (%)						
	Day 1	**Day 2**	**Day 3**	**10% W. L.**	**20% W. L.**	**30% W. L.**

Control	18.1±0.6d	18.2±0.4d	18.5±0.3d	20.2±0.3c	20.8±0.3bc	22.3±0.4a
Ozone	18.0±0.4d	18.8±0.4d	20.2±0.5c	22.8±0.2a	22.9±0.3a	23.1±0.3a
MeJa 10 µM	18.0±0.7d	18.1±0.6d	18.5±0.6d	20.4±0..2c	20.9±0.1bc	23.4±0.4a
MeJa 100 µM	18.3±0.3d	19.0±0.6d	19.0±0.2d	20.6±0.4bc	21.6±0.4b	22.4±0.5a

Table 2. Hue angle measured on the 1st, 2nd, and 3rd day and then at 10, 20, and 30% w.l. during postharvest partial dehydration. Data are the mean of 50 berry readings (± SD). Values with different letters are significantly different ($p < 0.05$).

Hue Angle							
	Day 0	**Day 1**	**Day 2**	**Day 3**	**10% W. L.**	**20% W. L.**	**30% W. L.**
Control	92±6a	93±6a	93±5a	52±4d	53±5d	28±3f	26±3f
Ozone	86±4a	89±7a	97±6a	65±5c	57±5cd	28±3f	34±4ef
MeJa 10 µM	88±2ab	85±5ab	86±7ab	97±7a	77±4bc	54±4cd	39±5e
MeJa 100 µM	88±6ab	85±8ab	86±4ab	92±6a	77±3bc	66±6c	34±5ef

Table 3. Peel resistance measured on the 1st, 2nd, and 3rd day and then at 10, 20, and 30% w.l. during postharvest partial dehydration. Data are the mean of 60 berry readings (± SD). Values with different letters are significantly different ($p < 0.05$).

	Peel resistance (N)						
	Day 0	**Day 1**	**Day 2**	**Day 3**	**10% W. L.**	**20% W. L.**	**30% W. L**
Control	0.94±0.04cd	0.98±0.06cd	0.98±0.04cd	1.04±0.06ab	1.10±0.04a	1.04±0.03ab	1.04±0.04
Ozone	0.92±0.02cd	0.92±0.06cd	1.03±0.05ab	0.93±0.02cd	1.06±0.04ab	1.00±0.03bc	1.04±0.03
eJa 10 µM	0.94±0.05cd	0.91±0.05d	0.91±0.05d	0.98±0.03cd	1.05±0.02ab	1.05±0.02ab	1.06±0.01
eJa 100 µM	0.96±0.04cd	0.98±0.03cd	1.03±0.04ab	0.97±0.02cd	0.98±0.04cd	1.11±0.04a	1.04±0.03

Table 4. Total polyphenols measured on the 1st, 2nd, and 3rd day and then at 10, 20, and 30% w.l. during postharvest partial dehydration. Data are the mean of 60 berry readings (± SD). Values with different letters are significantly different ($p < 0.05$).

	Polyphenols (mg GAE/100 g FW)						
	Day 0	**Day 1**	**Day 2**	**Day 3**	**10% W. L.**	**20% W. L.**	**30% W. L.**
Control	130 ±5i	156 ±7h	178 ±6fg	116 ±5l	84 ±4m	189 ±9ef	325 ±11b
Ozone	128 ±7i	306 ±9c	192 ±8e	184 ±8ef	114 ±7l	190 ±4e	377 ±9a
MeJa 10 µM	132 ±5i	225 ±5d	187 ±5ef	167 ±7gh	103 ±5l	218 ±7d	187 ±6ef
MeJa 100 µM	125 ±6i	126 ±5i	216 ±9d	125 ±6i	127 ±6i	191 ±4e	291 ±10c

Table 5. Total flavonoids measured on the 1st, 2nd, and 3rd day and then at 10, 20, and 30% w.l. during postharvest partial dehydration. Data are the mean of 60 berry readings (± SD). Values with different letters are significantly different ($p < 0.05$).

Flavonoids (mg CE/ 100 g FW)							
	Day 0	**Day 1**	**Day 2**	**Day 3**	**10% W. L.**	**20% W. L.**	**30% W. L.**
Control	125 ±7o	200 ±11mn	315 ±9l	195 ±7n	325 ±12l	540 ±13f	1367 ±64b
Ozone	123 ±5o	490 ±20g	320 ±13l	416 ±16hi	318 ±10l	754 ±16e	1635 ±33a
MeJa 10 µM	129 ±5o	475 ±10g	223 ±8m	356 ±13i	378 ±9i	910 ±16d	1337 ±27b
MeJa 100 µM	125 ±8o	210 ±12mn	369 ±10i	390 ±11i	417 ±14hi	439 ±9h	1164 ±29c

FIGURES

Figure 1. Weight loss of grape bunches plotted vs dehydration times. Values are the mean of 3 boxes weights.

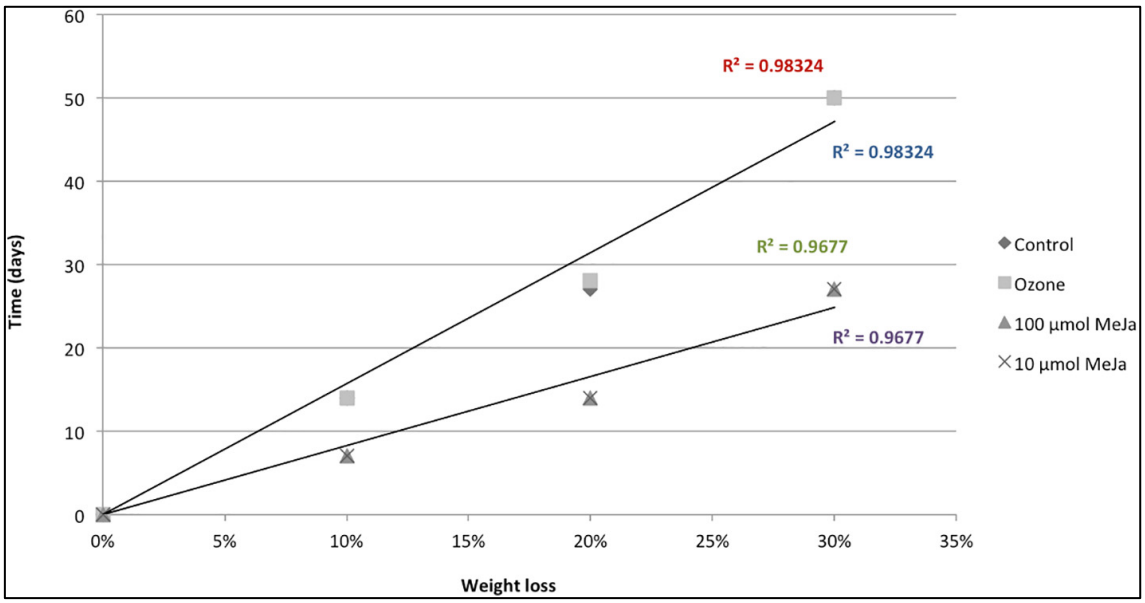

Figure 2. SOD activity in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).

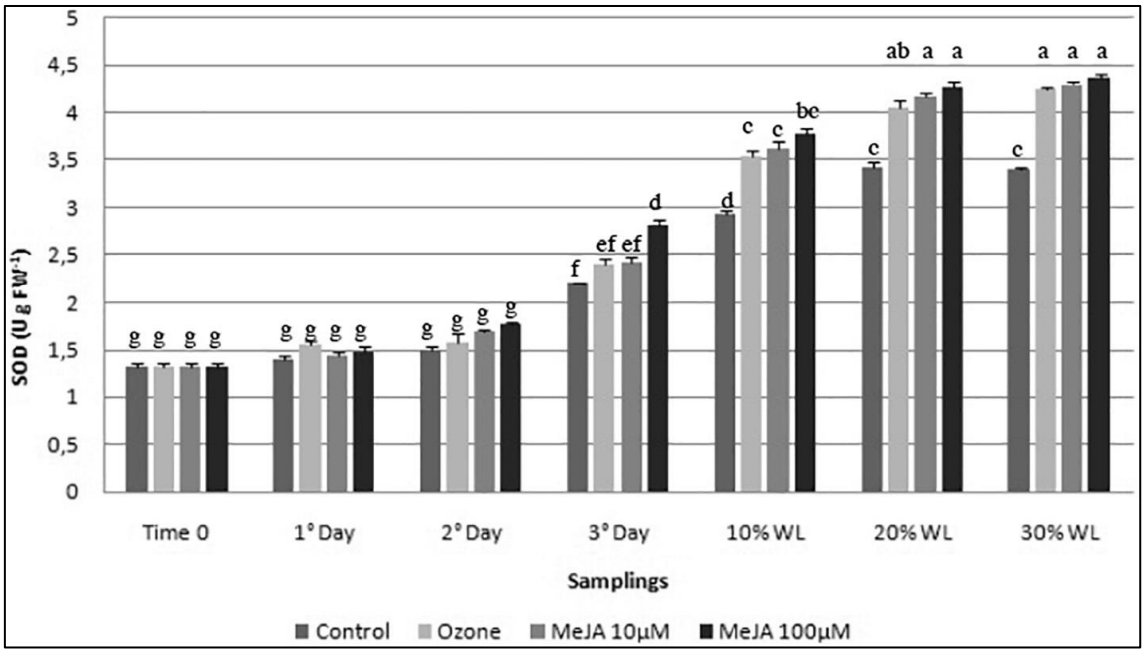

Figure 3. CAT activity in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).

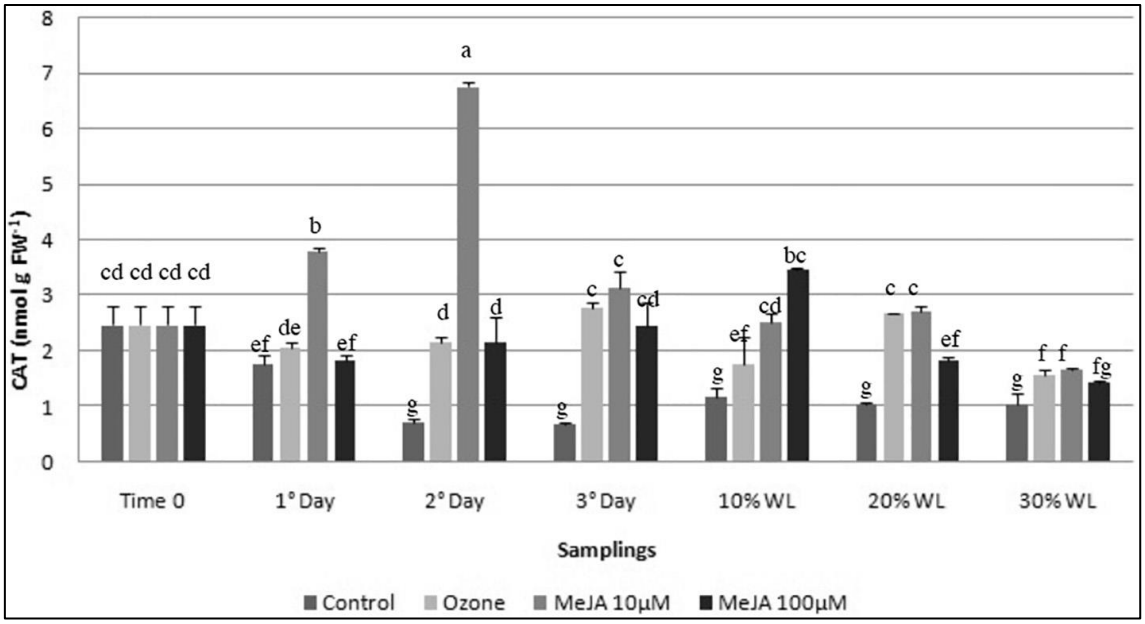

Figure 4. APX activity in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).

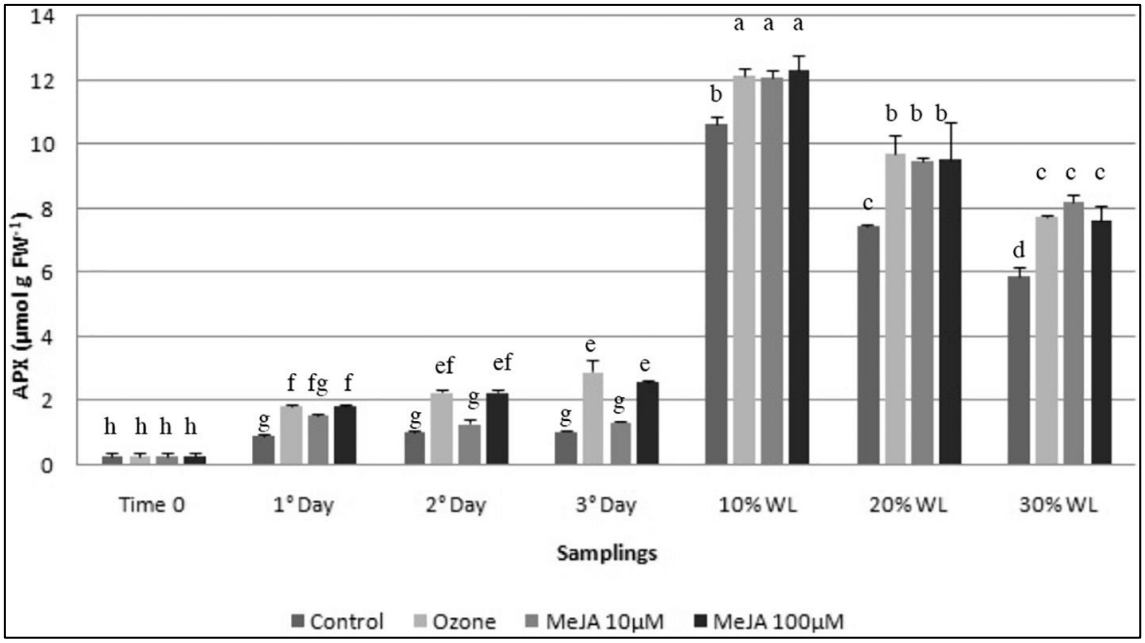

Figure 5. GPX activity in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).

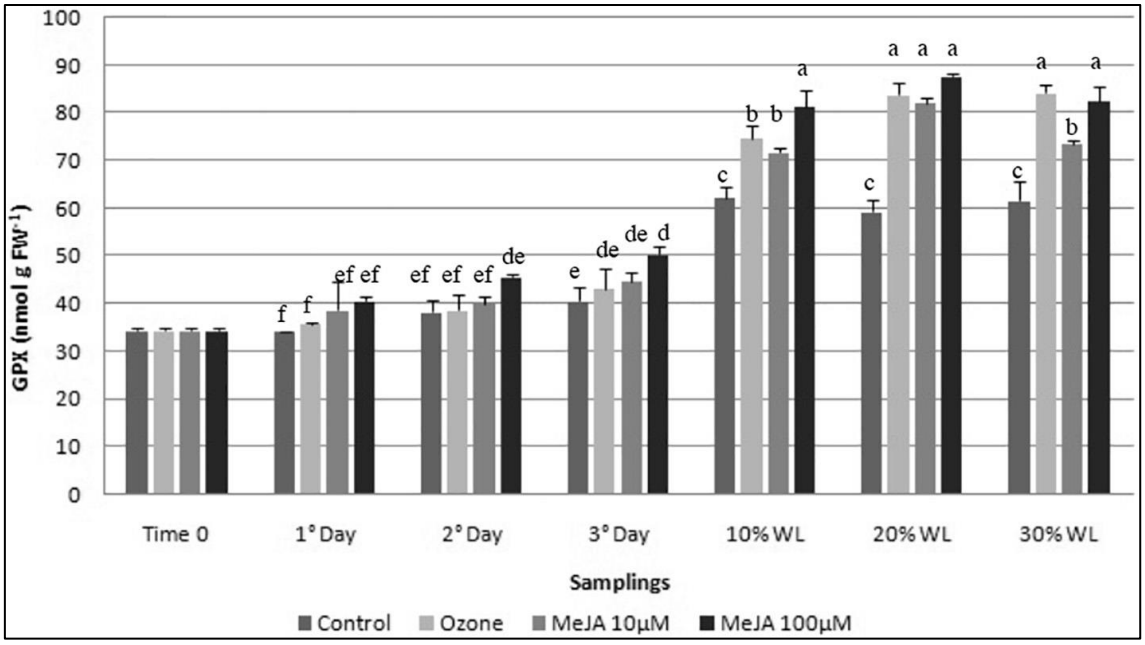

Figure 6. LOX activity in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).

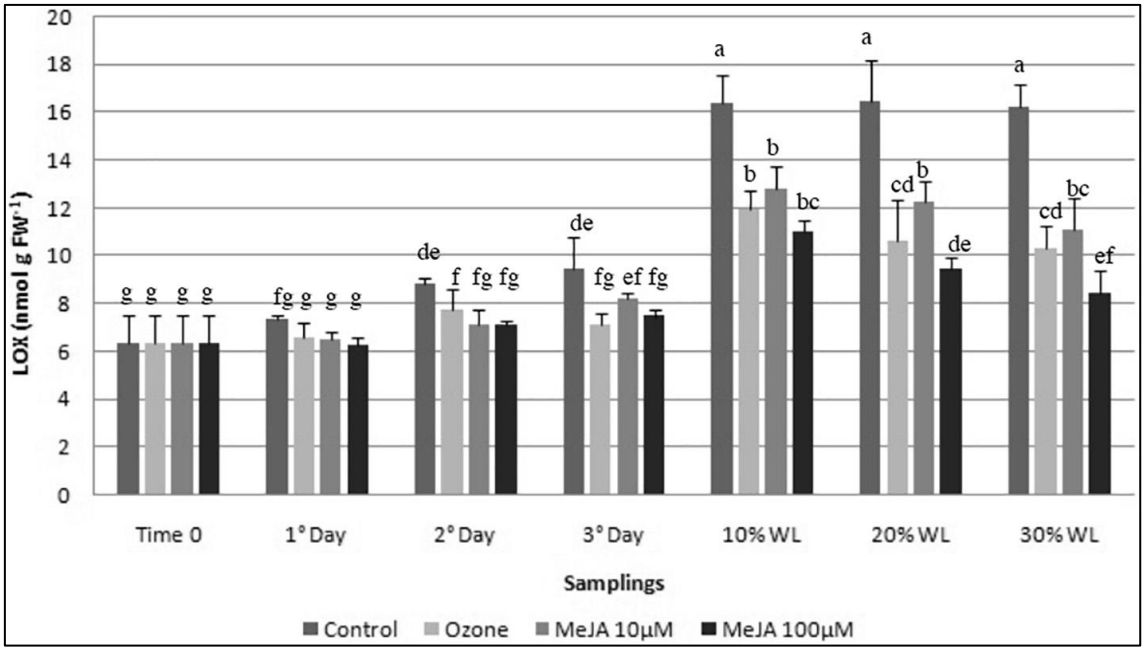

Figure 7. PPO activity in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).