Postharvest treatment with chitosan affects the antioxidant metabolism and quality of wine grape during partial dehydration

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ABSTRACT

The effectiveness of postharvest chitosan treatments on qualitative traits and antioxidant biochemical system during postharvest partial dehydration of "Sagrantino" grape has been studied, compared with ozone postharvest treatment. One % and 2 % chitosan coatings delayed water loss but no difference in berry color or peel resistance during partial dehydration up to 30 % mass loss (m. l.), was found among samples. The reducing sugar content rose straightly from 275 g L^{-1} (harvest), up to 445, 428, 411, and 390 g L^{-1}, in 2 % chitosan, 1 % chitosan, water, and ozone, respectively. Malic acid, and consequently total acidity, increased progressively in all samples with higher values in ozone- and in chitosan-

treated berries. In all samples, total polyphenol content rose already at 10% m.l., and 1% chitosan sample had the highest value. Postharvest treatments enhanced the activity of antioxidant enzymes, superoxide dismutase (SOD) and ascorbate peroxidase (APX), during partial dehydration process, whereas inhibited polyphenoloxidase (PPO) and lipoxygenase (LOX) activity, preventing polyphenol loss and avoiding membrane oxidation, as shown by lower malondialdehyde (MDA) accumulation.

***Keywords*:** Quality, ozone, antioxidant enzymes, principal component analysis

1. Introduction

Chitosan (poly-β(1-4)N-acetyl-D-glucosamine), a natural polysaccharide with a polycationic nature, known for its antifungal and eliciting properties, is considered an ideal coating to control decay of fresh fruit and vegetables due to its biocompatibility, biodegradability and bioactivity properties (Bautista-Banos et al., 2003; Bautista-Baños et al., 2006; Romanazzi et al., 2012; Romanazzi et al., 2013; Shiekhet al., 2013; Xu et al., 2007; Wu et al., 2005). Just recently a book on chitosan and agricultural product preservation has been published where a numerous functional features of chitosan are reported in agriculture, food and environmental engineering (Bautista-Banos et al., 2016). Chitosan coating forms a semipermeable film that regulates gas exchange and reduces the transpiration rate, which is generally determined by the gradient of water vapor pressure between the fruit and the surrounding air (Bautista-Banos et al., 2006). Gao et al. (2013) showed that chitosan coating on table grapes reduced weight loss, and inhibited gas exchange and decreased nutrient loss; an induction of peroxidase and superoxide dismutase activities was also found. The effect of chitosan on weight loss by 1% chitosan postharvest treatment of table grape was confirmed by Al-Qurashi and Awad (2015) who measured a preservation of berry firmness, polyphenol

content and antioxidant activity. Pre and postharvest treatment with chitosan on table grapes, controlled decay due to an induction of the activities of defense-related enzymes (polyphenoloxidase and phenylamoniolyase); in the same time, a decrease in soluble solid content (SSC)/acidity ratio and in weight loss was observed (Meng et al., 2008). Furthermore, chitosan postharvest treatment on table grapes to control *Botrytis cinerea* showed a positive effects on treated fruits with an increase in hydrogen peroxide, and in quercetin, myricetin, and resveratrol contents (Feliziani et al., 2013). In contrast, the same postharvest treatment on table grapes did not affect respiration and resveratrol content (Freitaset al., 2015) and these results were confirmed by Tessarin et al. (2016) in chitosan-treated wine grapes and derived wine. In conclusion, conflicting results on table grapes have been found and the reason is due to different form of application, different concentration, different application time. One thing seems to be confirmed: chitosan controls decay and decreases the weight loss.

Ozone has been tested for postharvest pest control in table grapes (Romanazzi et al., 2012; Feliziani et al., 2014) but, in the last few years, some publications have been done also on wine grapes (Carbone and Mencarelli, 2015; Botondi et al., 2015; Bellincontro et al., 2016; Laureano et al., 2016). Ozone is known as sanitizing agent but its effect as stressor has been also investigated (Heath, 2008). A significant increase in polyphenols in table and wine grapes has been observed (Artes-Hernandez et al., 2007; Carbone and Mencarelli, 2015; Bellincontro et al., 2016). Up to day it is unknown if this increase in grape polyphenols is due to a new synthesis or to an induction of antioxidant system. Yaseen et al. (2014) found on postharvest treated table grapes with ozone gas, a decrease in polyphenols, and an increase in catalase and no effect on lipoxygenase.

In Italy, the technique of partial dehydration is used for Amarone, one of the most worldwide famous dry wine. This technique induced a slow and long water stress on grape

berry followed by a berry senescence (withering process). Just recently, an exhaustive survey in berries on six grapevine genotypes subjected to postharvest dehydration under identical controlled conditions has showed that this technique is not only a simple concentration process of the some substances due to water loss but a complex process that involved different transcriptomic and metabolomic responses (Zenoni et al., 2016).

If chitosan is effective in delaying water loss, then it could be useful for the withering process of wine grape berries during postharvest partial dehydration, and affecting positively the quality features of berry, e.g. increase of polyphenols. As the increase in polyphenols is a common result of chitosan or ozone treatment on grapes, then the study of the antioxidant biochemical system could be useful. In this paper results are reported on an experimental work on wine grape postharvest-treated with chitosan or ozone and then subjected to partial dehydration. Beyond important metabolic and quality features of wine grape, SOD, APX, PPO, LOX, and MDA were analysed.

2. Materials and methods

2.1. Experimental procedure and treatment

Grapes var. Sagrantino (*Vitis vinifera*) were harvested manually in the Terre della Custodia vineyard (Montefalco), sorting bunches with sound and turgid berries; SSC was 27 ± 1 %. 123 kg of grapes were harvested and divided in four lots, about 40 kg each, consisting in: control (water), ozone, 1 % chitosan, 2 % chitosan.

Grape bunches, after berry sampling for chemical and physical initial analyses, were treated with chitosan (Iko Hydro, Rutigliano, Italy) with 90 % deacetylation and a molecular weight of 360 kDa prepared at two different concentrations, 1 % and 2 % (w v^{-1}) in an

aqueous solution of acetic acid (0.5 % v v^{-1}). The solution was warmed to 45 °C and stirred on a magnetic stirrer for complete dissolution of chitosan, adjusting its pH to 5.2 with NaOH. After cooling at 15 °C, the fruits were dipped in the chitosan solution for 10 min and dried at room temperature.

Another lot of grape bunches was fumigated, overnight (10 hours), with ozone (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, at 10 °C, relative humidity (RH) 80 %. Control bunches (water) were immersed in water for 10 min as done for chitosan. After chitosan and control treatments, grape bunches were left to superficially dry in another cold room at 10 °C, RH at 80 %, for 10 h, as for ozone treatment. Then, 6 perforated boxes, each one with 6 ± 0.5 kg of bunches for each treatment, were placed in small metallic tunnels (45 x 45 x 100 cm) fitted with an exhaust fan with airflow regulation (1.5 ± 0.3 m s^{-1}). The small tunnels were placed in a thermohygrometric controlled room at 20 ± 1 °C and 60 % ± 5 % RH for the partial dehydration treatment. The experiment lasted until grape bunches lost 40 % of their mass but berry sampling for analyses was done initially, then at 10 (±1), 18 (±1), and 30 (± 1) % m.l. but not at 40 %, because of the impossibility to extract juice from chitosan-treated berries.

2.2. Physical and chemical analyses

The mass of bunches (3 bunches per each box, total 18 bunches) was carefully measured using a technical balance (Adam Equipment Co.Ltd., Milton Keynes, U.K.). The color of 20 berries, sampled by cutting the berry with pedicel from different bunches (5 berries from 4 bunches of each treatment and sampling time), was assessed, at the indicated sampling times, with a CM-2600d colorimeter (Konica Minolta Inc., Ramsey, NY) set at SCE (specular component excluded), measuring CIELAB coordinates "L", "a", and "b". After color reading,

121 the same berries were analysed for peel resistance. Instron Universal Testing Machine mod.
122 3343 (Instron Ltd, High Wycombe, UK) was adapted with a 1 mm diameter flat probe and the
123 bar speed was fixed to 10 mm min^{-1}. Berries were punched in the equatorial part, until the
124 peel broke; two punches each berry. Data were expressed in terms of applied force (N), to
125 break the peel resistance, to peel deformation (mm) until the time of peel break (N mm^{-1}).
126 These berries were used to measure SSC by a digital refractometer (Atago CO. Ltd., Tokyo,
127 Japan) and the values were expressed as %.
128 Juices from three set of berries of different bunches, each sampling time and each sample,
129 were analyzed for reducing sugars, titratable acidity, malic acid, and total polyphenol content
130 by following the OIV procedures (Organisation International de la Vigne et du Vin, 2009).
131 MDA content was evaluated following the modified method of Health and Packer (1968).
132 Tissue powder was homogenized in ice bath by adding 10% (w v^{-1}) trichloroacetic acid
133 (TCA) in 1:10 ratio (w v^{-1}). The homogenate was centrifuged at 15000 *g* for 10 min and
134 supernatant was collected. To aliquot of the supernatant (500 µL), 1.5 ml of 15 % TCA
135 containing 0.5 % thiobarbituric acid were added. The solution was heated in a boiling water
136 bath at 95 °C for 18 min and immediately cooled. The absorption of 1 mL aliquots of
137 supernatant was read at 450, 532 and 600 nm. The MDA content was expressed as nmol (g
138 $DW)^{-1}$ (DW = dry weight) and calculated in agreement with Bao et al. (2009).
139 Total soluble proteins were extracted by resuspending 1 g of frozen fruit tissue powder in
140 5 ml of extraction buffer 100 mM potassium phosphate buffer (pH 7.8), 1 mM sodium EDTA
141 (pH 7), 5 % (w v^{-1}) PVPP supplemented with 2 mM DTT, 1 mM PMSF, 0.2 % Triton X-100.
142 The homogenate was centrifuged at 18000 *g* for 10 min at 4 °C and supernatant used for
143 enzymatic activities.

144 Protein content for all examined crude enzyme extracts was measured by the Bradford
145 assay (Bradford, 1976) using bovine serum albumin as a standard.
146 *2.3. Biochemical analyses*
147 PPO (EC.1.10.3.1) activity was determined following the modified method described by
148 Chen et al.(2009). First, 2.5 g of fruit were homogenized in 5 mL of 100 mM sodium
149 phosphate buffer pH 6.4 containing 0.125 g PVPP. Crude enzyme extract (100 µL) was
150 incubated with a buffered substrate (500 mM catechol in 100 mM sodium phosphate buffer
151 pH 6.4) in a final volume of 1.5 mL and monitored by measuring the increase in absorbance
152 at 398 nm. The specific activity for molar change in catechol was expressed nmol $(g\ DW)^{-1}$
153 LOX (EC 1.13.11.12) activity was quantified following the method described by Pérez et
154 al.(1999) with slight modifications. The enzyme was extracted by resuspending 1 g of frozen
155 fruit tissue powder with 3 mL of extraction buffer (50 mM potassium phosphate buffer pH
156 7.8, 1 mM sodium-EDTA pH 7,2 % PVPP). The reaction mixture consisted of 0.093 M
157 sodium phosphate buffer pH 6, 0.17 mM linoleic acid sodium salt, and 50 µL of crude
158 enzyme extract in a final volume of 1.5 mL. LOX activity was detected
159 spectrophotometrically by recording the formation of hydroperoxides and the resulting
160 increase in absorbance at 234 nm. LOX activity was expressed as the specific rate on a fresh
161 weight basis of molar change of hydroperoxides in µmol $(g\ DW)^{-1}$.
162 APX (EC 1.11.1.11) activity was assayed according to Garcìa-Limones et al. (2002) with
163 some modifications. The reaction mixture consisted of 100 mM potassium phosphate buffer
164 (pH 7), 0.33 mM ascorbic acid, 0.35 mM H_2O_2, 0.66 mM sodium EDTA (pH 7) and 50 µL of
165 crude enzyme extract in a final volume of 1.5 mL. The reaction was started by adding H_2O_2,
166 and the enzyme activity was determined by monitoring the ascorbate oxidation rate at 290

nm. The specific activity was expressed as the specific rate of molar change in ascorbate, μmol $(g\ DW)^{-1}$.

SOD (EC 1.15.1.1) activity was determined from the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) in presence of riboflavin, as described by modified method of Garcìa-Limones et al. (2002). The reaction mixture consisted of 50 mM potassium phosphate buffer pH 7.8, 0.1 mM sodium EDTA pH 7.0, 13 mM methionine, 75 μM NBT, 2 μM riboflavin and 400 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 $(g\ DW)^{-1}$.

2.4 Statistical analysis

The chemical, physical and biochemical data were subjected to ANOVA and significance was evaluated for $p<0.05$. Mean values were compared by Tukey's test using GRAPHPAD PRISM 3.05 (GraphPad Software, La Jolla, CA, USA) and LSD was used for mean separation.

A principal component analysis (PCA) was applied to describe the relationship between the different analyzed traits and to identify the principal components that accounted for the majority of the variation within the dataset. All analyses were performed using the SPSS software package, version 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results

190 The daily rate of mass loss was 2.2 % for water (control) and ozone-treated grapes while
191 was 1.8 % for chitosan-treated ones. The water and ozone-treated grapes reached 40 % m. l.
192 after 21 days while 1 and 2 % chitosan-treated grapes after 26 days (Figure 1). Berry color did
193 not change during partial dehydration and no significant difference was found among
194 samples. This result is due the very dark color of Sagrantino grape. Indeed, while the values
195 of "a" were close to 0, ranging between - 0.72 (harvest sample) and - 0.06 (untreated sample
196 at 30% m.l.), the "b" parameter was negative with a range between - 5.69 (harvest) and - 4.64,
197 - 5.37, - 4.26, and - 4.13, respectively for water, ozone, 1 and 2 % chitosan sample. The peel
198 resistance values are reported in Table 1. An unusual high ratio was found at 10 % m. l. in 2
199 % chitosan but successively the values decreased significantly. At 20 % m.l. the highest value
200 was for 1 % chitosan, followed by that of ozone sample. At the end of test, the lowest value
201 was for 1 % chitosan.
202 The reducing sugar content rose straightly from 275 g L^{-1} at harvest up to 445, 428, 411,
203 and 390 g L^{-1}, in 2 % chitosan, 1 % chitosan, water, and ozone, respectively. The increase
204 rate was 7.3 g d^{-1} of 2 % chitosan vs 6.4 g d^{-1} of ozone-treated grapes. Theoretically speaking,
205 in a controlled environmental condition of partial dehydration, by 30% m. l. from berry, the
206 sugar content, which is mainly due to a concentration effect, starting from 275 g L^{-1} it should
207 become 357 g L^{-1}; this value is close to the measured 380 and 390 g L^{-1} in ozone and water-
208 treated berries, respectively, but much lower than the content in chitosan-treated ones, 418
209 and 434 g L^{-1} for 1 and 2 % chitosan.
210 Total acidity increased in all samples but, overall, in ozone and in 1 % chitosan, reaching
211 6.5 and 6.0 g L^{-1} respectively, at 20 % m.l.; at 30 % m.l., ozone sample lost acidity (5.6 g L^{-1})
212 while the acidity of the other samples continued to rise, reaching 6.7 g L^{-1} in 1 % chitosan.
213 Malic acid, which is very sensitive to all kind of stress, increased in ozone sample until 18 %

214 m. l. and then declined, by pointing out its role in the pattern of total acidity (Table 2); malic
215 acid in 1 % and 2 % chitosan samples raised less than in ozone one but more than in water-
216 treated sample; no decline occurred at the end of test.

217 In all the samples, total polyphenol content rose at 10 % m.l. and 1 % chitosan sample
218 had the highest value (Table 3); the rise lasted until 18 % m.l. and then the values declined; 2
219 % chitosan sample maintained the highest value. Ozone sample had intermediate values
220 between chitosan and water-treated samples.

221 Analysis of SOD activity revealed an increase especially in treated berries with higher
222 values than the ones in untreated samples (Figure 2). Two % chitosan sample showed the
223 greatest increase. Similar pattern was observed for APX (Figure 3), a peroxidasic enzyme
224 which oxidises hydrogen peroxide to water; 2 % chitosan-treated grapes reached the highest
225 values but, at the end (30 % m.l.), no significant difference in APX activities in all treated
226 samples was observed but the values doubled than the one in untreated sample.

227 PPO increased during grape partial dehydration (Figure 4), with the highest values in
228 untreated sample. In chitosan-treated berries, PPO activity was lower than the one of ozone-
229 treated berries. Also lipooxygenase (LOX) activity showed similar pattern (Figure 5); ozone
230 and chitosan treatment delayed the increase in LOX activity during grape partial dehydration
231 compared to untreated sample. Furthermore, malondialdehyde, the metabolite of lipid
232 oxidation, was significantly higher in treated berries than that of untreated sample (Figure 6).

233 Principal component analysis has been used to explain the multifactorial effect of our
234 postharvest treatments on quality and enzymatic changes of grapes during partial dehydration.
235 Using the cross-validation technique, two principal components were necessary to explain the
236 total variability of the analysed traits. The eigenvalues of the covariance matrix showed that
237 two PCs, PC1 and PC2, were extracted, accounting for the total of 83.43% of the variances

238 (57.65% and 25.78%, respectively, for PC1 and PC2). SSC, titratable acidity, malic acid
239 content, LOX, APX, and SOD activities as well as MDA content, were positively correlated
240 with PC1. PPO activity was positively correlated with PC2 while polyphenol content was
241 negatively correlated.

242 A plot illustrating the effectiveness of chitosan and ozone for the partial dehydration of
243 grapes compared to untreated ones, is shown in Figure 7. With the progress of water loss,
244 control berries increased oxidative stress and a lower polyphenol content as pointed out by
245 higher shift in PC scores than the ones in chitosan and ozone samples. A lower oxidative
246 stress occurred in chitosan and ozone samples, thus suggesting that these treatments reduced
247 oxidative stress during partial dehydration.

248 The associations among different analyzed quality traits were evaluated by a correlation-
249 based approach using the Pearson coefficient. Matrix illustrates similar correlation trends
250 during partial dehydration, with positive and negative values (Table 4).

251 A positive correlation between titratable acidity and malic acid (r = 0.945; $p \leq 0.01$) was
252 observed whereas, as expected, MDA correlated significantly with LOX activity (r = 0.945; p
253 ≤ 0.01) and PPO (r = 0.813; $p \leq 0.01$).

254 SOD activity showed a positive correlation with APX activity (r = 0.872; $p \leq 0.01$).

255

256 **4. Discussion**

257

258 Our data confirm what is known on chitosan regarding the surface barrier effect which
259 halts the water transfer, delaying water loss from grape bunch partial dehydration, as it has
260 been observed in table grapes (Gao et al., 2013). The observed lower mass loss in chitosan
261 samples is not due to an increase of peel resistance. The significant rise in reducing sugars

262 measured in chitosan-treated berries is not only a concentration effect and/or the response of
263 tissue degradation but, also, the presence of glucose in chitosan molecule which affects
264 refractometer reading and reducing sugar analysis. This is particularly true for 2% chitosan
265 sample. The rise of malic acid content (which influences total acidity) during portharvest
266 partial dehydration of samples is the result of a concentration effect which is rapid at 20 °C.
267 At lower temperature (e.g. 10 °C), malic acid is respired during postharvest partial
268 dehydration of berry (Costantini et al., 2006; Chkaiban et al., 2007; Cirilli et al., 2012) but
269 when water loss is very rapid, the concentration effect overbears on respiration. One % and 2
270 % chitosan seem to stimulate malic acid synthesis and this has been observed also in 'Huang
271 guan' pear where chitosan stimulated NAD-dependent malate dehydrogenase (NAD-MDH)
272 (Kouet al., 2014). The stimulation of malic acid by ozone is unusual and there is no apparent
273 explanation. Ozone, as stressing agent, alters completely the antioxidant system and activates
274 cell defense system (Heath, 2008) thus the synthesis of malic acid from oxalacetic acid might
275 occur in order to supply further respiration substrate. Other explanation could refer to
276 cytoplasmic acidosis occurring in condition of hypoxia and anoxia (Perata and Alpi, 1993).
277 As during postharvest partial dehydration of grape berries, an aerobic fermentation (or
278 anaerobic respiration) takes place, this cytoplasmic acidosis might take place.
279 As regards polyphenols, the concentration increase in ozone-treated berries has been
280 found by other Authors (Artes-Hernandez et al., 2007; Carbone and Mencarelli, 2015;
281 Bellincontro et al., 2016). The effect of chitosan on polyphenols is different because chitosan
282 delays the polyphenol loss in postharvest (Meng et al., 2008; Shiri et al., 2013). Preharvest
283 treatment with chitosan on wine grapes, Sangiovese and Cabernet Sauvignon, did not change
284 the concentration in flavonols in berry peel of Sangiovese but a significant increase of (+)-
285 catechin, (-)-epicatechin and procyanidin B2 was found in Cabernet Sauvignon (Tessarin et

al., 2016). Thus, the response of polyphenol metabolism of grapes to chitosan seems to be dependent from different factors: concentration (Al-Qurashi and Awad, 2015), time of application (Meng et al., 2008; Feliziani et al., 2013), variety (Tessarin et al., 2016). The reason of different response is probably to attribute to two factors: first, the coating effect which changes gas concentration into the berry (Romanazzi et al., 2009), second the eliciting effect of plant defence (Feliziani et al., 2013). The behaviour of these two enzyme confirms what observed by Di Carli et al. (2011) about the formation of ROS during postharvest withering of Corvina grapes and the over expression of APX. In our case, a higher activities of SOD and APX in treated berries than the ones in control sample, suggest a further production of ROS due to chitosan and ozone treatment. The action between H_2O_2-generating SOD and the H_2O_2-metabolizing APX rather than the individual antioxidant enzyme can contribute to increase the oxidant resistance in grape.

Chitosan accumulates in plant cell wall, cytoplasm, and nucleus charging positively the cell along with its high affinity for negatively charged DNA, suggesting that it has a direct effect on the regulation of plant defense responses, with influences on mRNA and protein synthesis (Hadwiger and Loschke, 1981). The response of plant tissue to this chemical mechanism is, beyond the overexpression of genes of PR proteins, the regulation of ROS levels, antioxidant enzymes, and the ascorbate–glutathione cycle, the earliest events that correlate plant resistance to pathogens (Romanazzi and Feliziani, 2016).

As regards ozone it is well known that beyond hydrogen peroxide, more-reactive species such as superoxide, hydroxyl radical and peroxyl radical can be formed by ozone in plant cell (Heath, 2008). Thus the SOD increase is expected as well as of APX. Gupta et al. (2005) found an increase of gene expression of SOD and glutathione S-transferase, beyond other genes stress-related. Yaseen et al. (2014) found an increase in catalase but not in SOD in

table grape stored with ozone while in beans treated with ozone (Peters et al., 1988), extracellular ascorbate peroxidase (APX) and guaiacol-peroxidase (G-PO) increased significantly. Ozone as chitosan show a clear stress response with induction of SOD and APX for hydrogen peroxide detoxification, and the increase is progressive with water loss from berries, confirming a double stress response. At the same time, wine grape partial dehydration leads to berry browning and, in grape berry, the main cause of browning is the PPO activity (Macheix et al., 1991) that is particularly true in grape berry during partial dehydration (Antelmiet al., 2010; Mencarelli et al., 2010). In our study, chitosan inhibits PPO activity confirming what observed in strawberry and cherry (Wang and Gao, 2013; Petriccione et al., 2015; Dang et al., 2010; Pasquariello et al., 2015) but also ozone, moderately, inhibits PPO activity during berry partial dehydration as it has been shown on minimally processed longan fruits (Whangchai et al., 2006; Zhang et al., 2005). LOX activity rises during grape partial dehydration (Costantini et al., 2006; Chkaiban et al., 2007) as well as during pepper drying (Maaleku et al., 2006). The ability of chitosan to pass through cell membranes apparently depends on membrane composition, where high content of polyunsaturated fatty acids makes more sensitive to chitosan. The cationic nature of chitosan leads to a strong interaction with lipids having an opposite charge. This event could alter the binding activity of LOX on fatty acids thus reducing its activity. To confirm, in another non climacteric fruit such cherry, chitosan treatment maintained membrane integrity by delaying LOX activity and malondialdehyde accumulation (Pasquariello et al., 2015).

The induction of antioxidant enzymes and the inhibition of oxidant ones would explain the increase of polyphenol concentration in treated fruits during postharvest partial dehydration .

5. Conclusions

Postharvest dipping of wine grapes in chitosan appears to be a good potential tool to preserve the antioxidant system of berry cell during partial dehydration, by increasing the polyphenol content and inducing higher activity of SOD, APX, while reducing PPO and LOX activities. Ozone fumigation showed similar effect on wine grapes but with less production of polyphenols.

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TABLES

Table 1. Force to deformation ratio (N mm^{-1}) as index of berry peel resistance after treatment and during mass loss (%) Data are the mean 20 berries of different bunches at each mass loss percentage. Values with different letters are significantly different ($p < 0.05$)

	after treatment	**10 %**	**18 %**	**30 %**
water	46.0±0.8b	44.6±1.0bc	32.2±0.8h	33.4±1.2gh
ozone	45.0±1.2b	42.1±1.4cd	41.4±1.3d	35.7±1.6fg
1 % Chitosan	44.3±1.7bc	44.0±1.5bc	44.6±1.6bc	32.7±0.9h
2 % Chitosan	46.5±1.4b	53.2±1.7a	38.7±1.1e	35.7±1.3fg

Table 2 Malic acid concentration of grapes after treatment and during mass loss (%). Data are the mean 5 analyses from berries of different bunches at each mass loss percentage. Values with different letters are significantly different ($p < 0.05$)

	after treatment	**10 %**	**18 %**	**30 %**
water	2.4±0.3efg	2.1±0.1bc	2.9±0.2cd	2.7±0.1de
ozone	2.2±0.2fg	2.8±0.3d	3.3±0.3ab	2.8±0.1de
1 % Chitosan	2.3±0.2fg	2.1±0.2g	2.9±0.1cd	3.0±0.2bcd
2 % Chitosan	2.5±0.2ef	2.5±0.1ef	2.7±0.2de	3.0±0.3bcd

Table 3 Total polyphenol concentration of grapes after treatment and during mass loss (%). Data are the mean 5 analyses from berries of different bunches at each mass loss percentage. Values with different letters are significantly different ($p < 0.05$)

	after treatment	**10 %**	**18 %**	**30 %**
water	2056±83i	2534±80h	2611±91gh	2536±99h
ozone	2125±97i	2808±87ef	2880±87d	2756±97fg
1 % Chitosan	2099±71i	3085±97cd	3486±101a	2756±89fg
2 % Chitosan	2060±87i	2851±92ef	3156±87b	2918±99de

Table 4 Pearson's correlation matrix of the qualitative traits, antioxidant enzyme activities and malondialdehyde content in the treated grape during partial dehydration process.

Traits	APX	SOD	PPO	LOX	MDA	RS	TA	MA	POL	SSC
APX	1	.872**	-.154	.289	.362	.737**	.628**	.321*	.288	.769**
SOD	.872**	1	-.095	.282	.336	.770**	.543**	.276	.318*	.828**
PPO	-.154	-.095	1	.813**	.756**	.382	.363	.377	-.681**	.307
LOX	.289	.282	.813**	1	.945**	.707**	.702**	.642**	-.479*	.660**
MDA	.362	.336	.756**	.945**	1	.725**	.759**	.650**	-.476*	.720**
RS	.737**	.770**	.382	.707**	.725**	1	.771**	.537**	-.058	.946**
TA	.628**	.543**	.363	.702**	.759**	.771**	1	.688**	.014	.765**
MA	.321*	.276	.377	.642**	.650**	.537**	.688**	1	-.145	.501**
POL	.288	.318*	-.681**	-.479*	-.476*	-.058	.014	-.145	1	-.066
SSC	.769**	.828**	.307	.660**	.720**	.946**	.765**	.501**	-.066	1

Correlation levels significant at *$p<0.05$; **$p<0.01$, respectively.

APX: ascorbate peroxidase; SOD: superoxide dismutase; PPO: polyphenoloxidase; LOX: lipoxygenase; MDA: malondialdehyde content; RS: reducing sugars; TA: titratable acidity MA: malic acid; POL: total polyphenol content; SSC: soluble solid content.

FIGURES

Figure 1. Mass loss of grape bunches during partial postharvest dehydration. Values are the mean of the mass of 18 bunches. Values with different letters are significantly different ($p < 0.05$).

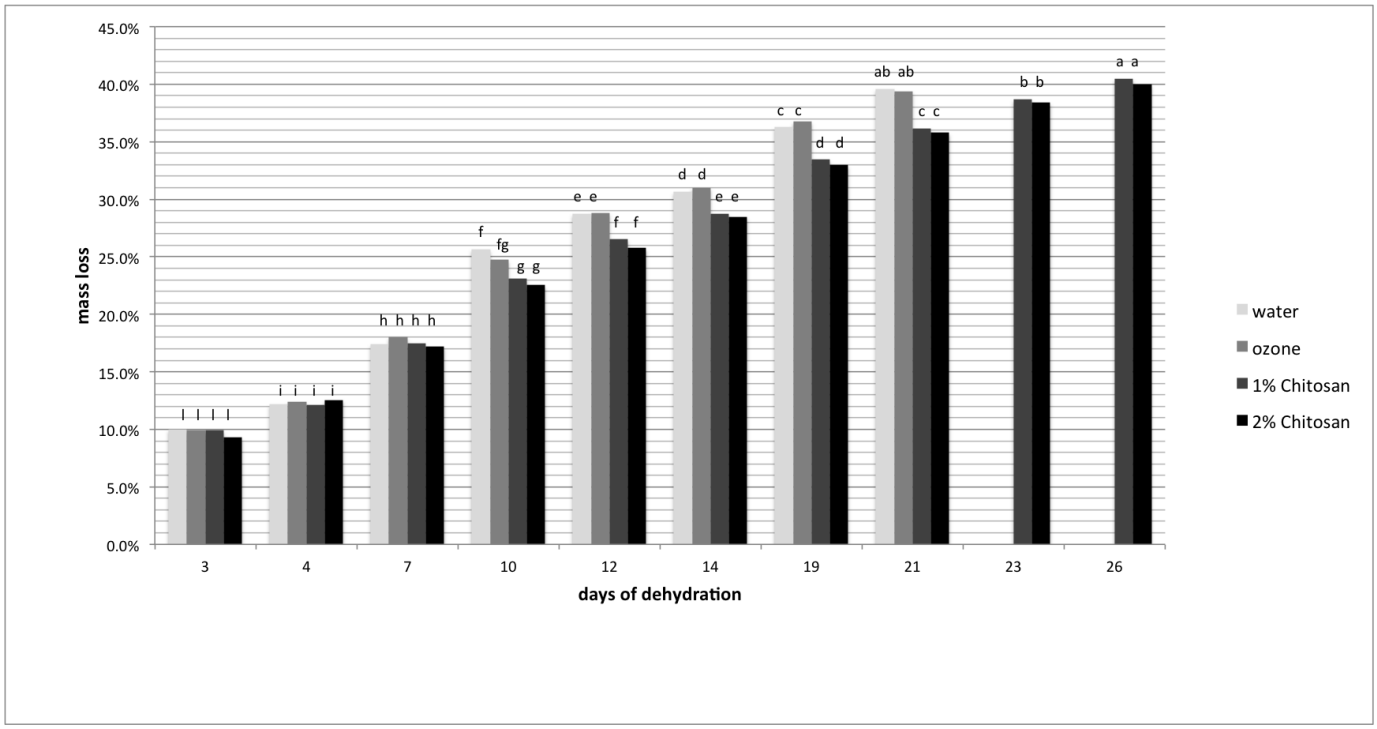


Figure 2. Superoxide dismutase activity in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).

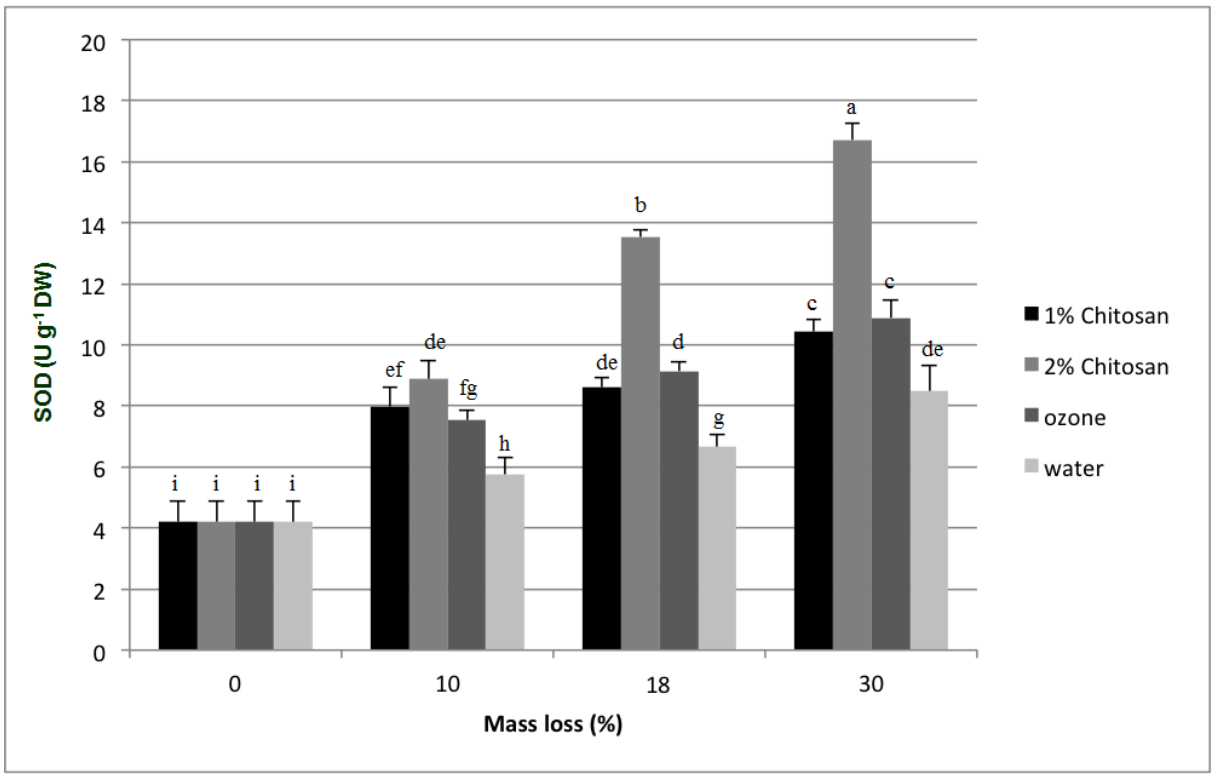


Figure 3. Ascorbate peroxidase activity in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).

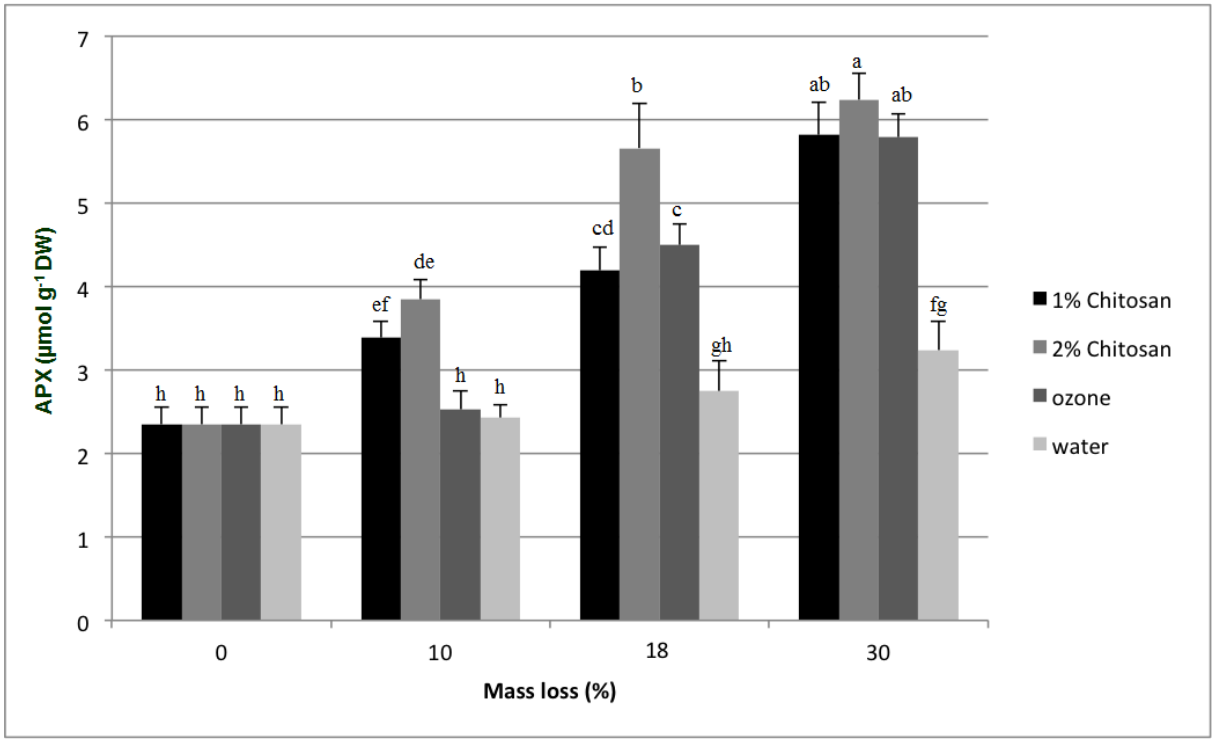


Figure 4. Polyphenoloxidase activity in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).

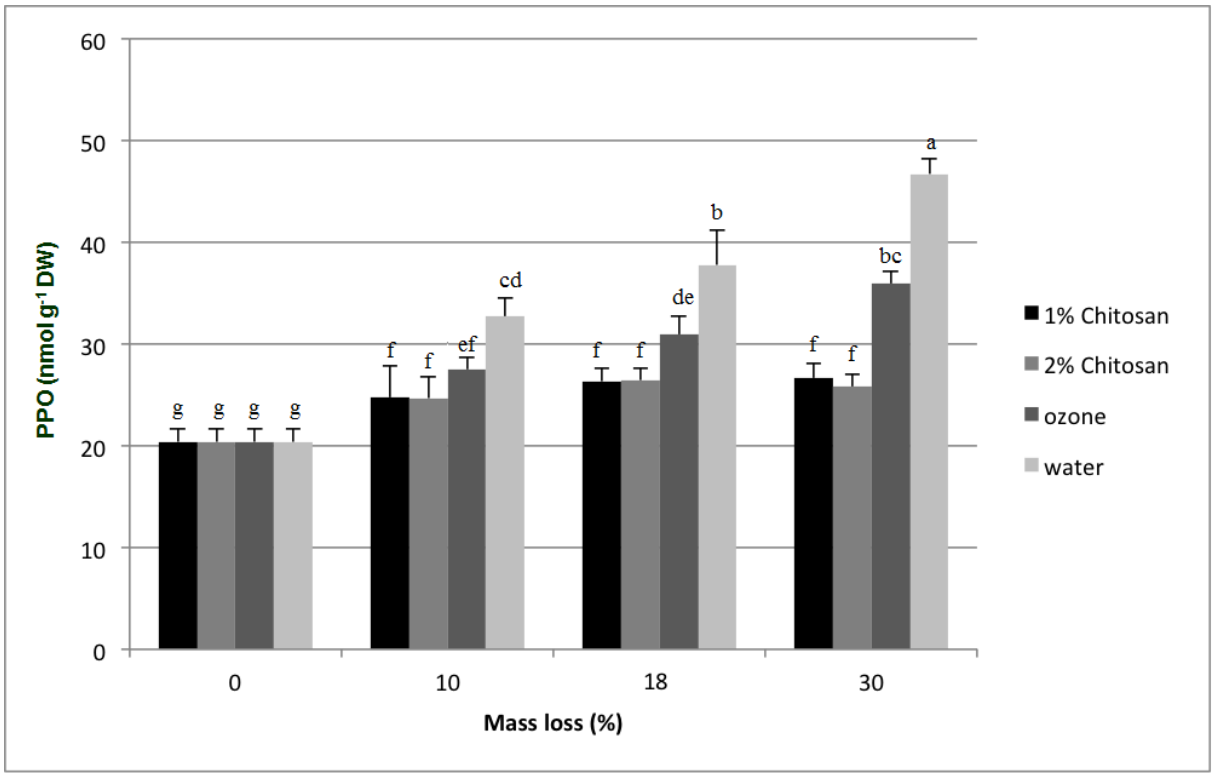


Figure 5. Lipooxygenase activity in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).

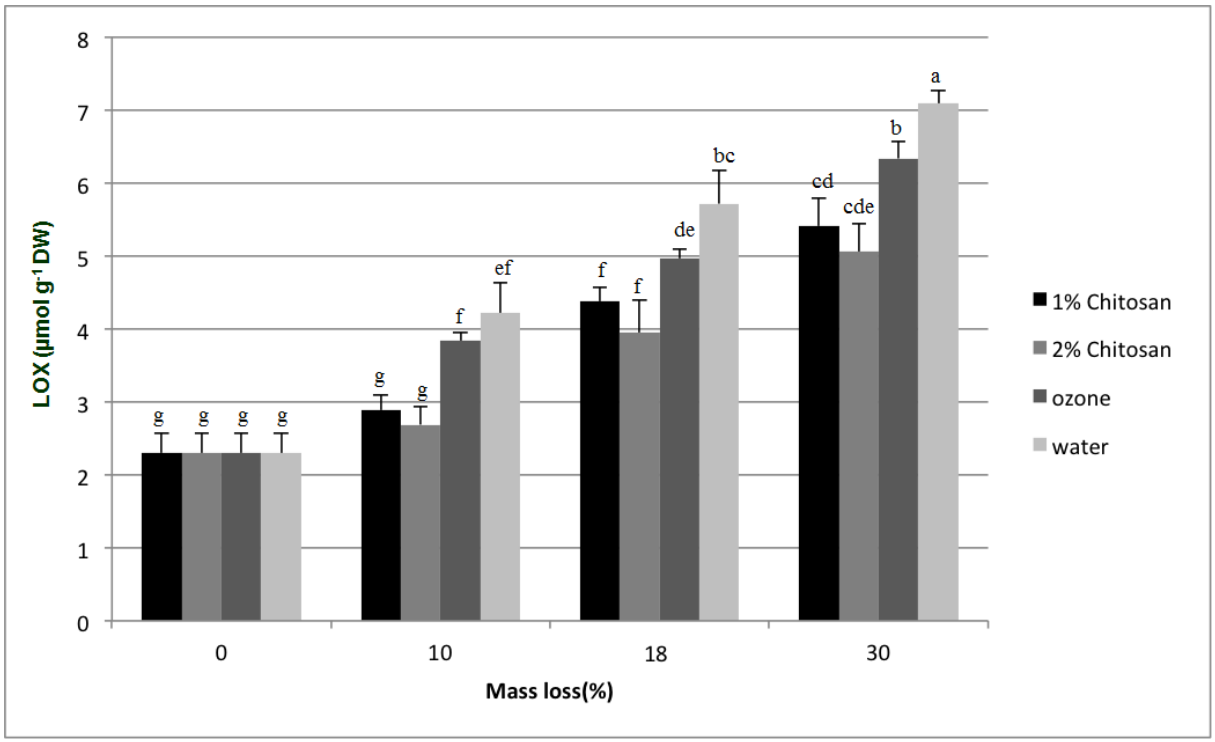
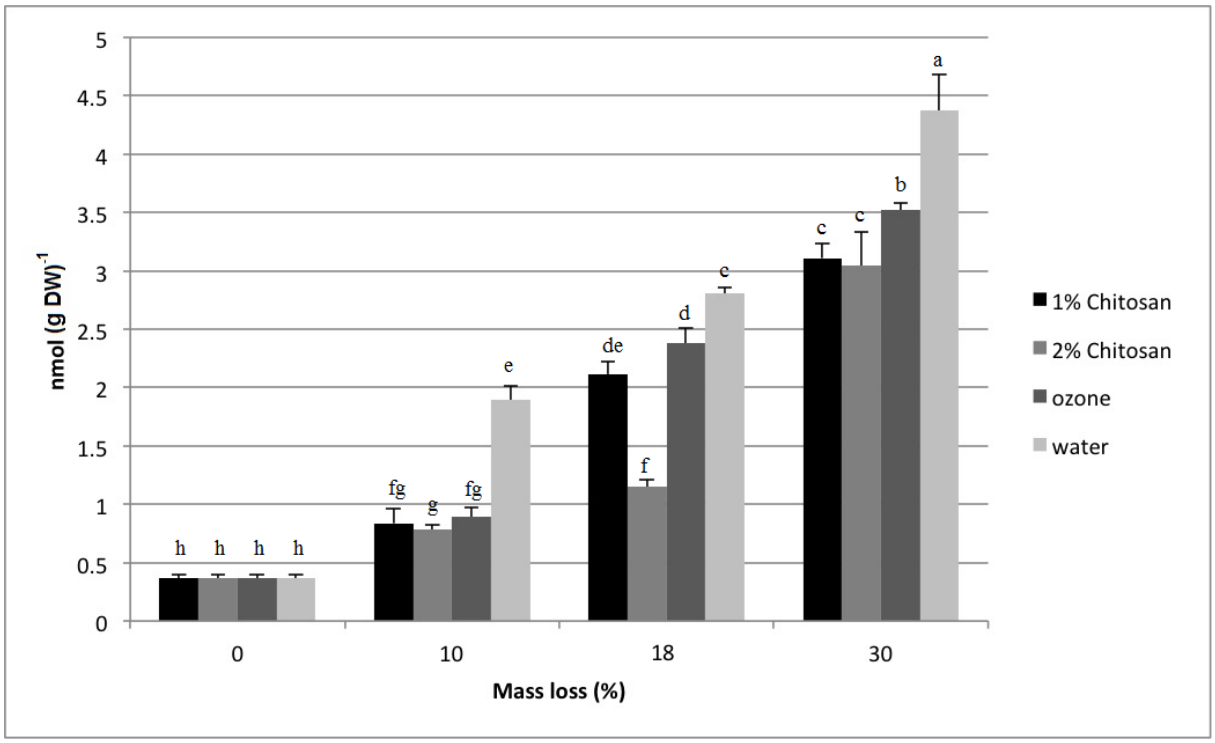
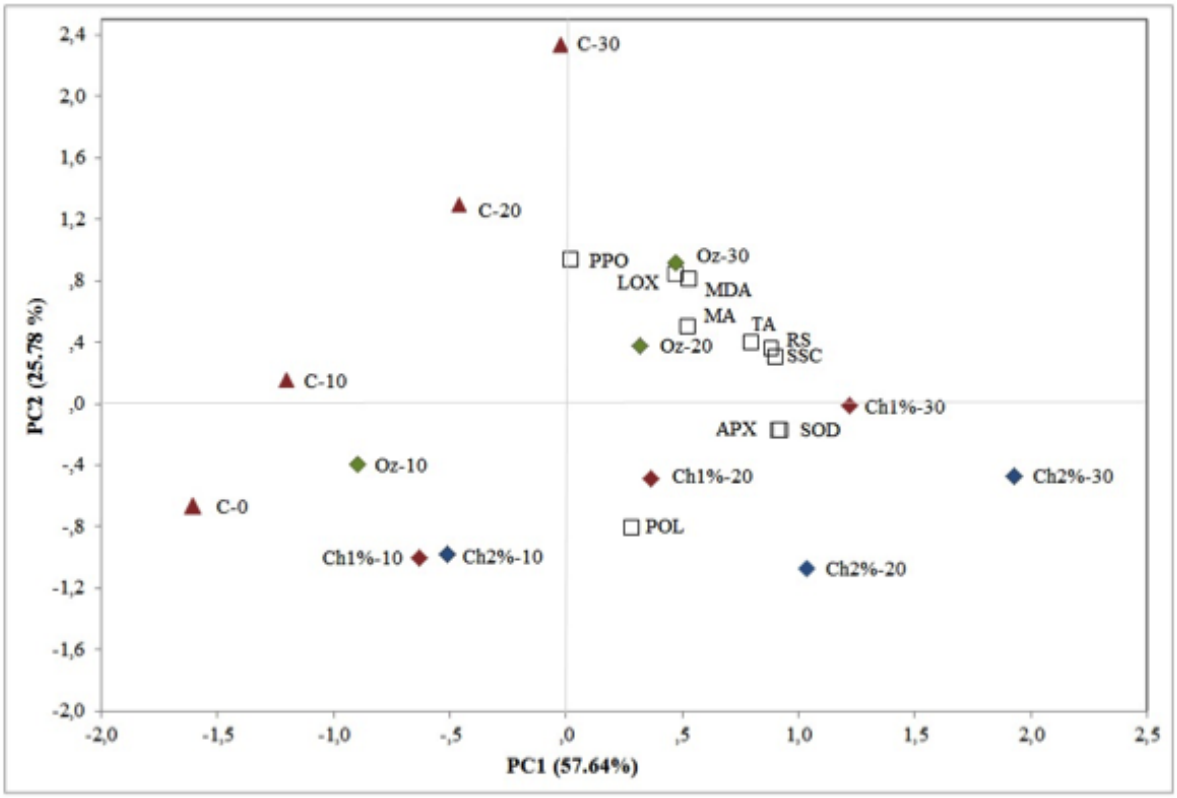


Figure 6. Malondialdehyde content in grape berries versus mass loss. Values are the mean (± SD) of three enzymatic analyses of three lots of berries from different bunches. Values with different letters are significantly different ($p < 0.05$).



602 Fig. 7. 2D principal component analysis plot of qualitative traits, antioxidant enzyme
603 activities and MDA content in treated grape (chitosan 1% (Ch1%), chitosan 2% (Ch2%),
604 ozone (Oz), water (C) at harvest (0) and at 10, 18 and 30% mass loss (RS: reducing sugars;
605 TA: titratable acidity; MA: malic acid; POL: total polyphenol content; SSC: soluble solid
606 content; APX: ascorbate peroxidase; SOD: superoxide dismutase;: polyphenol oxidase; LOX:
607 lipoxygenase; MDA: malondialdehyde).
608


C-30
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