OZONE FUMIGATION FOR SAFETY AND QUALITY OF WINE GRAPES IN POSTHARVEST DEHYDRATION

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Abstract

This paper proposes postharvest ozone fumigation (as a method) to control microorganisms and evaluating the effect on polyphenols, anthocyanins, carotenoids and cell wall enzymes during the grape dehydration for wine production. Pignola grapes were ozone-treated (1.5 g/h) for 18h (A=shock treatment), then dehydrated or ozone-treated (1.5 g/h) for 18h and at 0.5 g/h for 4 hours each day (B=long-term treatment) during dehydration. Treatment and dehydration were performed at 10°C. No significant difference was found for total carotenoids, total phenolics and total anthocyanin content after 18 hours of O_3 treatment. A significant decrease in phenolic and anthocyanin contents occurred during treatment B. Also carotenoids were affected by B ozone treatment. Pectin methylesterase (PME) and polygalacturonase (PG) activities were higher in A-treated grapes during dehydration. Finally, ozone reduced fungi and yeasts by 50%. Shock Ozone fumigation (A treatment) before dehydration can be used to reduce the microbial count during dehydration without affecting polyphenol and carotenoid contents.

Keywords: Ozone, Dehydration, Grape, Polyphenols, Enzymes.

1. **Introduction**

Italy is the country with the highest number of wines produced by drying or dehydration techniques according to the description given by Mencarelli and Tonutti (2013). Beyond the special characteristics of wine obtained with these techniques, one of the major problems of grape dehydration is berry decay due to the development of fungi such as *Botrytis cinerea* as well as *Aspergillus* spp and *Fusarium* spp, well-known as micotoxin producers. Indeed, it has been reported that wines produced from dried grapes are the most contaminated by ochratoxin (Valero, Marìn, Ramos, & Sanchis, 2008). The occurrence of moulds and yeasts varies strongly with the habitat, climatic conditions during the grape-growing period, the grape cultivar and degree of maturity, plant-protecting agents, farming systems and most of all the phytosanitary status of the grape berries and cellar equipment (Martins et al, 2014).

To prevent the development of moulds, sulfur bentonite is commonly spread over grape bunches, resulting in a white, powdery blanket. This powder absorbs water vapour from the surface of the berry, facilitating water loss, but, at the same time, when absorbent capacity is exhausted, it creates a barrier to prevent the water vapour from escaping (Mencarelli & Bellincontro, 2013). Aside from this, the sulfur residues remaining on the berries are released into wine, increasing the risks it poses to human health. High-concentration exposure to sulfur can induce asthma, while a low concentration, together with other air contaminants, can provoke cardiovascular disease (Vally, Misso, & Madan, 2009). Among the sanitizing techniques for grapes which have been proposed in the last decade, ozone fumigation is one of the most prominent, producing safer wines without compromising quality. Ozone (O_3) is a naturally-occurring gas in the atmosphere and one of the most potent sanitizers against a wide spectrum of food microorganisms (Khadre, Yousef, & Kim, 2001)**,** used even in fruits and vegetables (Carletti et al. 2013). Ozone is generated by the passage of air or oxygen gas through a high-voltage electrical discharge or by ultraviolet light irradiation (Mahapatra, Muthukumarappan, & Julson, 2005). It can be applied either as a gas or dissolved in water. For commercial use, ozone must be produced on site and it is classified as GRAS (generally

recognized as safe) for food contact applications in the USA (U.S. Food and Drug Administration, 2001). The product of ozone degradation is oxygen; therefore it leaves no residues on treated commodities. There are other conceivable benefits to ozone, such as depuration of mycotoxins (Karaca & Velioglu, 2007), pesticide residues (Ikehata & El-Din, 2005) and control of microbes of concern to food safety (Selma, Ibanez, Cantwell, & Suslow, 2008). Ozone has been extensively tested for use in controling table grape decay (Mlikota Gabler, Smilanick, Mansour, & Karaca, 2010). Postharvest ozone treatment enhances the synthesis of resveratrol and of other bioactive phenolics in grapes (Artés-Hernández, Aguayo, Artés, & Tomás-Barberán, 2007), confirming earlier work on this subject (Sarig et al., 1996). However, it is unclear if the increase of these compounds is really biosynthesis or simply the result of an easier extraction. In viticulture, cell wall degradation is considered to be another potential index of berry maturity, as are technological and phenol maturities. This is because the extraction of important enological compounds such as anthocyanins, or polyphenols in general, is easier when the berry's cell wall is broken. Cell wall enzymes, mainly PME (PG is almost undetectable), are responsible for cell wall degradation and these enzymes are strongly activated during berry ripening (Deytieux-Belleau, Vaillet, Donèche, & Geny, 2008). In 2011, Botondi, Lodola & Mencarelli observed that PME activity increased throughout the postharvest dehydration process for Aleatico wine grapes while PG activity rose to a point and then dropped off again. Vicenzi et all (2012) also observed PME activity in Erbaluce grapes even at the end of the dehydration process.Very recently, Zoccatelli et al. (2013) showed that, during dehydration, six PME genes were strongly induced in Corvina berries and, to a lesser extent, in Sangiovese and Oseleta berries. Regarding the cell wall, it has been observed in tomatoes that short-term treatment (10 min) with high concentration (10 ppm) of ozone did not alter the activity of the pectin-degrading enzymes polygalacturonase and β-Galactosidase (β-Gal), though it did decrease pectin methyl esterase activity (Rodoni, Casadei, Concellòn, Chaves, & Vicente, 2010).

In this paper we present the results of an innovative experimental study conducted using two postharvest ozone treatments before and during the postharvest dehydration of var. Pignola wine grape. To our knowledge, no papers have been published with regard to ozone application in wine grapes, especially during the dehydration process. Changes in total polyphenols, anthocyanins, carotenoids, respiration and PME and PG activities were measured beside the mycological analysis.

2. Materials and methods

2.1 Experimental procedure and treatment

The red wine grape bunches were carefully harvested (sound and uniformly sized berries) at 26°Brix. After 1 h of transport under shaded conditions, the bunches (arrival temperature at lab: 21 °C) were placed in a single layer in perforated boxes (60 x 40 x 15 cm). For each treatment, 6 perforated boxes with 6 kg (±500 g) of bunches each were placed in a small metallic tunnel (45 x 45 x 100 cm) fitted with an exhaust fan with air-flow regulation (1.5 ± 0.3 m/s). The small tunnels were placed in three thermohygrometric controlled rooms at 10 (±1)°C and 70%(±5%) RH for the treatments; at the end of treatments, RH was reduced to 50% using a dehumidifier. The following treatments were performed:

- A (shock treatment): O_3 fumigation, 1.5 g/h in continuous flow (Ozone generator A series, PC Engineering srl, Uggiate Trevano, Como, Italy) for 18 hours followed by dehydration in normal atmosphere;
- B (long term treatment): O_3 fumigation, 1.5 g/h in continuous flow followed by dehydration in normal atmosphere with 0.5 g/h of O_3 fumigation for 4 hours each day ;
- Ck (control treatment): no ozone fumigation, but similar treatment conditions and followed by dehydration in normal atmosphere.

The berry sampling was performed at the beginning, after the 18h treatment, and at 20 and 35% weight loss (w.l.).

2.2 Physical and physiological analyses

The weight of the bunches (2 bunches per each crate) was carefully measured using a technical balance (Adam Equipment Co. Ltd., Milton Keynes, U.K.). The total sugars (°Brix) of 30 berries from different bunches (10 berries from 3 bunches from each treatment group and sampling time) were measured using a digital refractometer (ATAGO CO. Ltd., Tokyo, Japan).

The colour of 30 berries from different bunches (10 berries from 3 different bunches per treatment, the same berries until the end of the experiment) was assessed at the beginning of the experiment and at 20% and 35% w.l. with a CM-2600d colorimeter (Konica Minolta Inc., Ramsey, NY) set at SCE (specular component excluded) measuring CIELAB coordinates L, a, and b. The hue angle (h°) was calculated as h° = arctan (b/a).

Carbon dioxide (CO_2) production was measured by placing few bunches inside a glass jar (three jars per treatment), tightly capped with a lid, for 2 hours. CO_2 production rate was monitored by gaschromatographic method using a GC Clarus 400 (shincarbon ST 80/100 column, TC detector, Perkin Elmer Inc., MA, USA). The activity was expressed as ml of CO_2/ kg h.

2.3 Chemical analyses

For the following chemical analyses, three sets of juice (30 ml each) from different bunches of each treatment and each sampling time were used. Titratable acidity (TA) was measured by titration of 5 g of juice to pH 8.1 with 0.1 N NaOH, using phenolphthalein as a colorimetric indicator. Total carotenoids were extracted ~~by~~ using a solution of acetone with 20% (v/v) water (Lichtenthaler, 1987). After 30 min in the dark, the samples were centrifuged using a BECKMAN JA-21 at 13000 *g* for 15 min at 10°C and subsequently spectrophotometrically analyzed by a 25 UV-Vis (Perkin Elmer Instruments Ltd., Seer Green, Beaconsfield, U.K.). The extraction of total phenolics (total polyphenol index) was carried out by mixing the berries with water, and 1 mL was filtered through a Sep-pak C18 column (Bakerbond spe™ Columns, J.T. Baker, USA) to avoid the interference of sugars, while polyphenols were eluted with MeOH. After washing the column, first Folin–Ciocalteu reagent (1 ml) was added to the column eluate and then, after 5 min, sodium carbonate (10% w/v). Catechin was used as the reference standard. After 90 min, phenols were read at 700 nm. Total anthocyanins were

129 determined by filtering the previous extract using the same Sep-pak C18 column and reading at 520
130 nm (Di Stefano & Cravero, 1991). All chemical data are expressed on a dry weight basis to avoid
131 the interference of water loss on data discussion.
132 *2.4 Enzymatic assays*
133 For pectinmethylesterase, PME (EC 3.1.1.11) analysis, extraction was performed on three
134 sets of 15 g of berries after seed removal; berries were manually ground in a mortar by adding
135 liquid nitrogen to obtain a fine powder and extracted with 0.2 M phosphate buffer at pH 7.5 with the
136 addition of 1 mM EDTA, 5% PVPP (polyvinylpolypyrrolidone), and 2 M NaCl, up to a final
137 volume of 10 ml. The homogenate was centrifuged at 39800 *g* for 1 h at 4°C. The supernatant was
138 filtered through a Sephadex G25 column to remove polyphenols. PME activity was assayed using
139 Hagerman and Austin's procedure (1986) with modifications for use in grapes. 0.4 ml of extract
140 were incubated with 0.01% bromothymol blue, 0.5% w/v pectin in water at pH 7.5, 0.1 M NaOH,
141 and 0.01 M phosphate buffer. A spectrophotometric reading was taken at 620 nm for 6 min.
142 Enzyme activity is expressed as μmol/min g_{dw}.
143 For polygalacturonase (PG, EC 3.2.1.15) extraction, the same procedure was used as for
144 PME but with 0.5 M NaCl and 2% PEG (polyethylene glycol). The procedure of Lohani, Trivedi, &
145 Nath (2004)**,** adapted to grapes, was used for the assay: 0.4 ml of extract were incubated with 1.2
146 ml of 0.2 M sodium acetate (pH 4.5) buffer, to which 1.2 ml of 0.5% polygalacturonic acid was
147 added. The sample was incubated at 37°C for 1 h, then boiled (100°C for 5 min) after the addition
148 of 400 μl of 0.1% DNS (3,5 dinitrosalicylate) prepared in 30% potassium sodium tartrate and 0.4
149 M NaOH. PG reading was taken at 540 nm. Enzyme activity is expressed in μmol/min g_{dw}.
150 *2.5 Mycological analyses*
151 After each treatment, the total density of moulds and yeast on the grapes' skin was
152 determined by media serial dilution plating. Individual grape berries were randomly and aseptically
153 removed from each grape bunch to get samples of about 50 g. Grape berries were suspended in 90
154 ml of sterile 0.1% bacteriological peptone solution (Oxoid, Milan, Italy) containing 0.01% Tween

80 (Sigma-Aldrich, Milan, Italy) for 30 min at 25°C. Rinses were serially diluted in 0.1% Bacteriological Peptone solution and 100 µl were spread onto Malt Extract Agar (MEA) and Yeast Extract Peptone Dextrose (YEPD) (Lai, Siti Murni, Fauzi, Abas Mazni, & Saleh, 2011). The latter was used to count yeasts, while MEA was used to enumerate general fungal community. All media were supplemented with 100 mg/l chloramphenicol (Sigma-Aldrich, Milan, Italy) to inhibit bacterial growth. Plates were incubated at 28 °C for 5 days for colony development and counted.

2.6 Statistical analysis

All chemical and biochemical values are reported on a dry weight basis and represent the means of replicate samples (± SE). Normality and homogeneity of variances were checked with the Shapiro–Wilk and the Bartlett's tests, respectively. Analysis of variance was performed by ANOVA and significance evaluated for $p<0.05$. Mean values were compared by Tukey's test ($p<0.05$) and significant differences are indicated on the figures and tables with letters. Calculations were performed with the statistical software Graphpad Prism 3.05 (San Diego, CA, USA).

3. Results

The weight loss (w.l.) increased with a rising straight line during dehydration in all the samples (R^2 = 0.9615, 0.9685 and 0.9503), respectively**,** for the Ck (untreated), A (shock treatment) and B (long-term treatment) samples (data not shown). To reach 20% weight loss, the Ck and B samples took 17 days while the A sample took 19 days; another 24 days were needed for the A sample to reach 35% w.l. and 22 days were required for the Ck and B samples. As expected, the SSC increased from 26°Brix to 36-38°Brix during dehydration without a significant difference among the samples (Table 1). The titratable acidity, expressed on a dry weight basis and thus unaffected by weight loss, did not change after the 18 h ozone treatment; at 20% w.l., the acidity decreased by 35% and 19%, respectively, for the B-treated and untreated grapes and similar values were also kept at 35% w.l. The A-treated grapes maintained their acidity at 20% w.l., at 35% w.l., it declined to the level found in the Ck sample (Table 1). Dehydration caused the acidity to decrease by 16% at 20% w.l..

A significant increase of CO_2 production was detected at 20% w.l. in the ozone-treated grapes while, in the Ck grapes, the increase occurred only at 35% w.l. (Table 1)

After 18 hours of the O_3 treatment, no significant difference was found in the total carotenoids, total phenolic and total anthocyanin contents. The initial value of the polyphenol content was 10372 mg catechins/kg_{dw} and, at 20% w.l., a significant decrease in the phenolic content was observed in the Ck- and B-treated berries (3127 mg catechins/kg_{dw}) while the A-treated ones kept a higher polyphenol content (Fig. 1). No changes occurred at 35% w.l.. At 20% w.l., anthocyanin content in the Ck- and B-treated grapes decreased significantly: the anthocyanins fell from 746 mg malvidin/kg_{dw} to 512 and 626 mg malvidin/kg_{dw}, respectively (Fig. 2). At 35% w.l., the final values were 477, 411 and 407 mg malvidin/Kg_{dw}, respectively, for the grapes that received Ck, A and B treatment. The total carotenoid content was unaffected by ozone treatment but, at 35% w.l., it declined significantly in the B sample (Table 2). The berry colour as expressed by hue angle increased slightly but significantly in sample A, from 290 up to 335, indicating a drift to redness and loss of blueness (Table 2); significant difference was observed also in control samples but not in B one. PME and PG activities were unaffected by the ozone treatment but, at 20% w.l., a significant decrement of PME activity was observed in all samples, especially in the untreated berries; a further decrease occurred at 35% for the Ck and B samples (Table 3). PG activity declined significantly at 20% and 35% w.l. in the Ck sample whereas it remained constant or even increased slightly in the ozone-treated ones.

A quantitative approach based on counting cultivable populations indicated higher values of colonization for the yeast community both in harvested and dehydrated grape berries. Dehydration time did not significantly influence the density of either fungi (ANOVA- Tukey's Test; Ck treatment $P<0.05$; 35% w.l. $P<0.05$; 20% w.l. $P<0.05$) or yeasts (ANOVA- Tukey's Test Ck treatment; 35% w.l $P<0.05$; 20% w.l. $P<0.05$). The fungal colonization of grape berries ~~s~~ decreased significantly after the ozone applications in all the dehydration treatments (ANOVA- Tukey's Test; Ck treatment $P=0.00$; 35% w.l. $P=0.01$; 20% w.l. $P=0.01$). The yeast-counts were significantly lower when the grape berries were treated with the ozone for 18h (ANOVA- Tukey's

206 Test; Ck treatment P=0.01) and did not increase at 35% w.l. (ANOVA-Tukey's Test; P=0.04) and
207 at 20% w.l. (ANOVA-Tukey's Test P=0.00). According to the Tukey's Test, no significant
208 differences were found between shock and long ozone treatments.

209 **4. Discussion**

210 To our knowledge, this study is the first attempt at describing the changes caused by ozone
211 treatments in the metabolic compounds and total microorganism flora colonizing the fruits during
212 the dehydration of grapes in wine production.

213 The ozone treatment affected slightly how long it took the berries to lose weight; shock-
214 treated bunches took 2 days more to reach 35% weight loss than long-term ozone-treated bunches
215 and control ones. Anyway, in all samples, there was a rising, linear trend of weight loss with time.
216 This confirms previous findings (Barbanti, Mora, Ferrarini, Tornielli, & Cipriani, 2008) and thus
217 also the importance of constant environmental conditions for dehydration in achieving the regular
218 water loss that is key to maximizing grape quality. The increase in sugar in response to dehydration
219 was not influenced by ozone, while the acidity, expressed on a dry weight basis, declined
220 significantly in the Ck grapes but even more so in the long-term ozone-treated grapes. The addition
221 of the ozone increased acid loss. Barboni, Cannac, & Chiaramonti (2010) observed a decline in non-
222 volatile organic acids in kiwifruit during the ozone gas storage while, in Autumn seedless table
223 grapes stored in a macro-perforated polypropylene container and treated with 0.1 μl/l of O_3 (Artés-
224 Hernández, Aguayo, & Artés, 2004), no change in acidity was observed. Heath (2008) reported that
225 multiple sets of metabolic pathways are stimulated by ozone exposure, depending on different doses
226 or time regimes: a short pulse of ozone activates wounding and ethylene dependent genes/pathways
227 while longer periods of ozone treatment allows a shift of other types of metabolism, such as the
228 synthesis of cell wall components and secondary products. Malic acid, among grape acids, is the
229 most susceptible to abiotic stress; thus the observed acidity decreased could be due to malic acid
230 oxidation as consequence of ozone treatment. CO_2 production rate does not confirm this
231 hypothesis because its rise occurs both in shock and long-term treated berries but malic degradation

232 could be transformed in sugar (gluconeogenesis process) but it could be hypothesized the malate
233 catabolism could be affected by an oxidative burst which triggers an antioxidant response with
234 higher TCA (tricarboxilic acid) cycle flux (Sweetman et al., 2009). Our supposition is a double
235 stress response (water stress and ozone) which affects the malate catabolism due to water stress and
236 ozone treatment especially in the case of long-term treatment. The effect of cumulative stress
237 (ozone and water loss) is the cause of the different pattern of the total polyphenols during
238 dehydration, depending on the ozone treatment. We have expressed data on a dry weight basis so
239 the concentration is unaffected by water loss. The initial shock treatment (18 h) does not affect the
240 polyphenol concentration**,** though a slight increase was observed. Subsequently, the dehydration
241 process significantly lowered the polyphenol content and the long-term treatment with ozone
242 caused a further loss. The shock treatment reduced the polyphenol loss. In 2003, Artés-Hernández,
243 Artés, & Tomás-Barberán reported that var. Napoleon table grapes postharvest fumigated with
244 ozone increased greatly in resveratrol but lost anthocyanins and in 2007 Artés-Hernández, Aguayo,
245 Artés & Tomás-Barberán reported a significant increase in flavan-3-ols when grapes were
246 postharvest fumigated with 0.1 μl/l or 0.8 μl/l for 60 days at 0°C, though the total polyphenols did
247 not increase significantly. As indicated by Heath (2008), the line between the reaction to ozone
248 stress with the activation of secondary metabolisms such as the polyphenol pathway, and the shift to
249 a developmental response such as senescence, is dose- and time-dependent, and we must add
250 species- and variety-dependent. Postharvest grape dehydration has been shown to increase
251 polyphenols in some varieties depending on the temperature and the percentage of weight loss (De
252 Sanctis et al., 2012; Panceri et al., 2013), while in others a decrease of specific fractions was
253 observed (Moreno et al., 2008). However, a general activation of the phenylpropanoid pathway in
254 dried samples has also been confirmed by the up-regulation of cinnamate 4-hydroxylase (C4H) and
255 4-coumarate-CoA ligase (4CL) genes (Zamboni et al., 2010; Bonghi et al., 2012). Anthocyanins
256 were less sensitive to ozone compared to the dehydration at 20% w.l. , while at 35% w.l., the ozone
257 effect added on to water stress (dehydration) by producing a great loss of anthocyanins. An

important aspect of polyphenols is their extractability. A higher concentration of polyphenols could be the result of a greater extractability due to cell wall enzymes activities and/or physical cell wall modification. The significant decline of polyphenol content in the long term ozone-treated grapes is due, most likely , to oxidation caused by ozone's well-known oxidant activity. This supposition seems to be confirmed by the analytical response of the total carotenoids, which decreased significantly only at 35% w.l. in the long-term ozone-treated samples. Recently, in papaya fruit fumigated with ozone for 14 days, a significant increase in carotenoids has been reported (Ali, Ong, & Forney, 2014). However, Chauhan, Raju, Ravi, Singh, & Bawa (2011) reported that total carotenoid content of ozone-treated fresh-cut carrots decreased when compared to that of the control. Meanwhile, the reduction of ß-carotene and lycopene in fruit and vegetables subjected to high concentrations of and long exposure to ozone may be triggered by the oxidative cleavage of carotenoids that leads to the production of abscisic acid (ABA).

The contrasting behaviour of antioxidant compounds such as polyphenols, including anthocyanins, and carotenoids in different commodities, is a consequence not only of different experimental procedures (concentration, time of exposure, temperature of treatment) but also of different plant tissues, ripening stages and growing conditions. The metabolic pathways of these compounds are very sensitive to all kind of stress, as mentioned above, because they are involved in plant cell protection, but the increase in the phenolic content of the fruit might also have been caused by the cell wall modification that occurred during ozone exposure. Cell wall modification may release some of the conjugated phenolic compounds in the cell wall. The level of secondary metabolites in our sample, mainly polyphenols, depends on the ripening stage of growing condition of the grapes, and thus on the state of their cell walls. Biosynthesis and extractability play an important role in the final concentration of these compounds. The analysis of PME revealed no effect from ozone immediately after the treatment, but instead showed a general decline in the activities of all samples at 20% and 35% w.l., more marked for the long-term ozone-treated and untreated berries, the latter also showing declining PG activity with dehydration. In a previous

study, Botondi et al. (2011) observed an increase of PME and PG activities during Aleatico grape dehydration at 20°C, a-trend opposite to what was observed here in var. Pignola. Recently, Zoccatelli et al. (2013) have shown that pectin degradation during postharvest dehydration is strictly dependent on the variety as well as the activities of PME and PG; in var. Corvina, pectin degradation is correlated with the activity of two enzymes during dehydration, but the same does not occur with var Sangiovese and Oseleta. Rodoni et al. (2010) in tomatoes fumigated with 10 µl/l for 10 min found that ozone reduced pectin solubilization and that the effect was stronger in uronic acids than in sugars; PG activity was unaffected while PME activity declined by 50%. The relatively higher PME and PG activities in the shock ozone-treated berries compared to the other groups, could explain the higher polyphenol content by favouring higher extractability.

Our study shows that the growth of fungi and yeasts is controlled by the dehydration process but does not guarantee the maintenance of berry integrity during dehydration because it depends on the initial concentration and dehydration environment. In agreement with previous studies, in this experiment, ozone was significantly active in reducing the viability of microorganisms (Bataller, González, Veliz, & Fernández, 2012). However, no significant difference was observed between treatments A and B, probably due to a positive effect on host resistance. Sarig et al. (1996) showed that in addition to its sterilizing effect in control of *Rhizopus stolonifer* on table grapes, ozone also induced the resveratrol and pterostilbene phytoalexins in grape berries, making them more resistant to subsequent infections.

5. Conclusions

Pignola is a local red variety grown in Valtellina area of the Alps. Postharvest dehydration of wine grapes is a well-known technique in the area because it is used to make Sfurzat wine from Chiavennasca (local name of Nebbiolo) variety. The innovative postharvest ozone treatment has been effective as a sanitizing agent for Pignola grape dehydration and a gas shock treatment is sufficient to reduce the initial pathogen concentration which is kept low, later on, by the dehydration process. Ozone shock treatment is also able to preserve the polyphenol and anthocyanin content. Moreover,

shock treatment keeps the PME and PG activities, which are important in improving metabolite extractability, high. In contrast, the long-term ozone treatment greatly reduces the polyphenol content through a supposed oxidant activity.

Acknowledgment

Research supported by Italian Ministry of Education and University, PRIN Project, coordinator: Fabio Mencarelli. We thank Cantina di Tirano to providing the grapes.

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Figures

Figure 1. Changes in total polyphenols (mg of catechins Kg^{-1}_{dw}) after postharvest ozone treatments and during dehydration at 20 and 35% w.l.. O3=ozone fumigation for 18 h; O3 + 0.5 g/h 4h/d=ozone fumigation for 18 h + 0.5 g/h ozone fumigation for 4 h every day during dehydration; Ck = untreated but kept in cold room at the same temperature. Data are the mean (± SD) of three sets of juice (30 ml each) from different bunches from each treatment group and sampling time. Means followed by the same letter are not statistically different ($p<0.05$). Where no bar (SD) appears, no difference occurred among the reps.

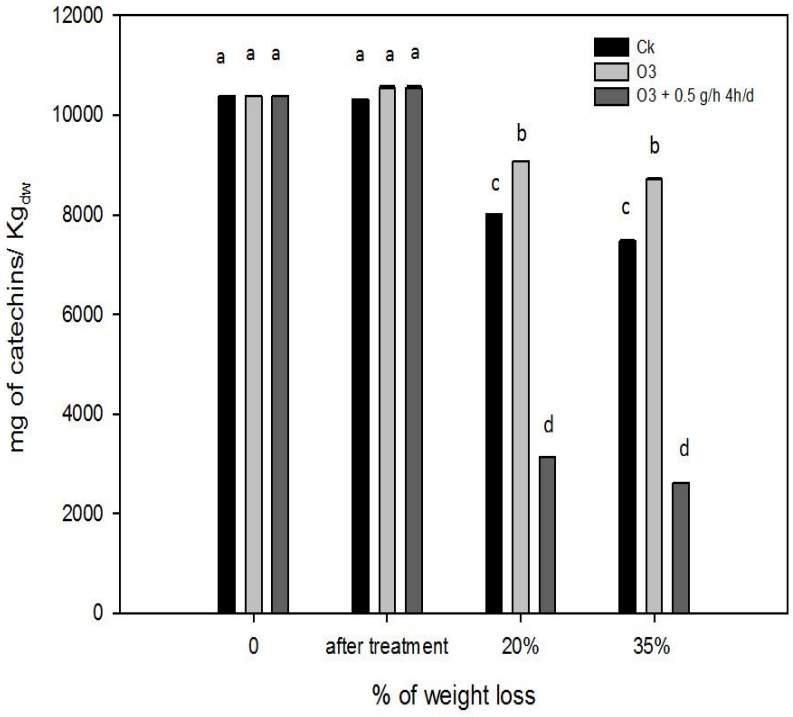


Figure 2. Changes in total anthocyanins (mg of malvindin $Kg^{-1}{}_{dw}$) after postharvest ozone treatments and during dehydration at 20 and 35% w.l.. O3=ozone fumigation for 18 h; O3 + 0.5 g/h 4h/d=ozone fumigation for 18 h + 0.5 g/h ozone fumigation for 4 h every day during dehydration; Ck = untreated but kept in cold room at the same temperature. Data are the mean (± SD) of three sets of juice (30 ml each) from different bunches from each treatment group and sampling time. Means followed by the same letter are not statistically different ($p<0.05$). Where no bar appears, no difference occurred among the reps.

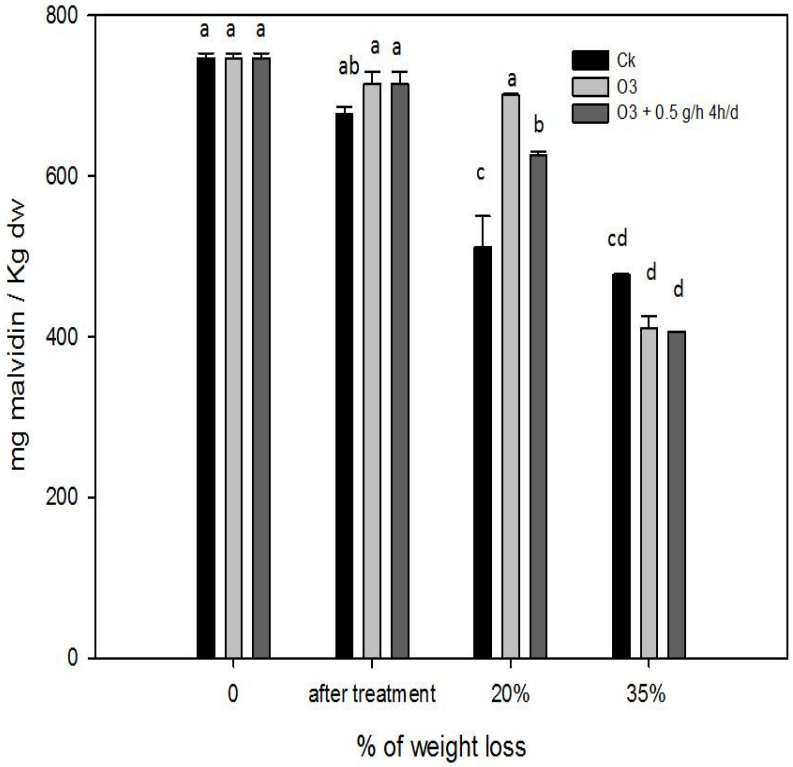
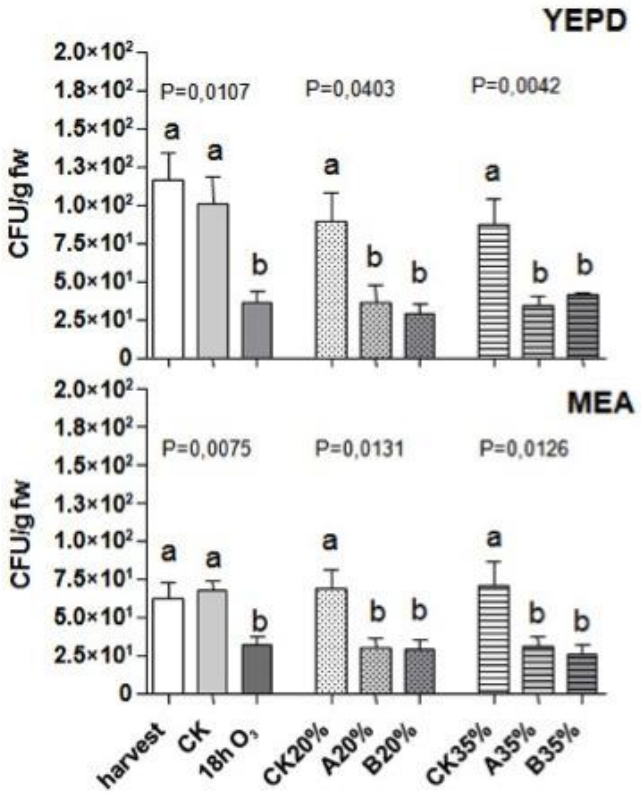


Figure 3. Yeast (up) and fungi (down) count after postharvest ozone treatments and during dehydration at 20 and 35% w.l.. 18hO_3=ozone fumigation for 18 h; CK20% and CK35%=untreated sample, respectively, at 20% and 35% w.l.; A20% and B20%=respectively, shock (ozone fumigation for 18 h) and long-term ozone (ozone fumigation for 18 h + 0.5 g/h ozone fumigation for 4 h every day during dehydration) treatment at 20% w.l.; A35% and B35%=respectively, shock and long-term ozone treatment at 35% w.l.. Data are the mean (± SD) of three mycological analyses from three sets of berries from different bunches from each treatment group and sampling time. Means with the same letter are not statistically different from the indicated *p*.



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