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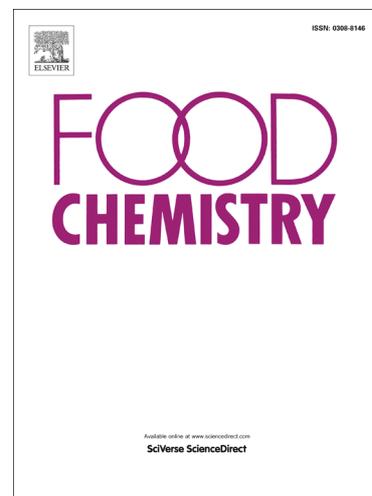
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2 **Ozone fumigation for safety and quality of wine grapes in postharvest dehydration**

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10
11 **Abstract**

12 This paper proposes postharvest ozone fumigation (as a method) to control microorganisms and
13 evaluate the effect on polyphenols, anthocyanins, carotenoids and cell wall enzymes during the
14 grape dehydration for wine production. Pignola grapes were ozone-treated (1.5 g/h) for 18h
15 (A=shock treatment), then dehydrated or ozone-treated (1.5 g/h) for 18h and at 0.5 g/h for 4 hours
16 each day (B=long-term treatment) during dehydration. Treatment and dehydration were performed
17 at 10°C. No significant difference was found for total carotenoids, total phenolics and total
18 anthocyanin content after 18 hours of O₃ treatment. A significant decrease in phenolic and
19 anthocyanin contents occurred during treatment B. Also carotenoids were affected by B ozone
20 treatment. Pectin methylesterase (PME) and polygalacturonase (PG) activities were higher in A-
21 treated grapes during dehydration. Finally, ozone reduced fungi and yeasts by 50%. Shock Ozone
22 fumigation (A treatment) before dehydration can be used to reduce the microbial count during
23 dehydration without affecting polyphenol and carotenoid contents.

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

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27 1. Introduction

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

38 To prevent the development of moulds, sulfur bentonite is commonly spread over grape
39 bunches, resulting in a white, powdery blanket. This powder absorbs water vapour from the surface
40 of the berry, facilitating water loss, but, at the same time, when absorbent capacity is exhausted, it
41 creates a barrier to prevent the water vapour from escaping (Mencarelli & Bellincontro, 2013).
42 Aside from this, the sulfur residues remaining on the berries are released into wine, increasing the
43 risks it poses to human health. High-concentration exposure to sulfur can induce asthma, while a
44 low concentration, together with other air contaminants, can provoke cardiovascular disease (Vally,
45 Misso, & Madan, 2009). Among the sanitizing techniques for grapes which have been proposed in
46 the last decade, ozone fumigation is one of the most prominent, producing safer wines without
47 compromising quality. Ozone (O₃) is a naturally-occurring gas in the atmosphere and one of the
48 most potent sanitizers against a wide spectrum of food microorganisms (Khadre, Yousef, & Kim,
49 2001), used even in fruits and vegetables (Carletti et al. 2013). Ozone is generated by the passage of
50 air, or oxygen gas, through a high-voltage electrical discharge or by ultraviolet light irradiation
51 (Mahapatra, Muthukumarappan, & Julson, 2005). It can be applied either as a gas or dissolved in
52 water. For commercial use, ozone must be produced on site and it is classified as GRAS (generally

53 recognized as safe) for food contact applications in the USA (U.S. Food and Drug Administration,
54 2001). The product of ozone degradation is oxygen; therefore, it leaves no residues on treated
55 commodities. There are other conceivable benefits to ozone, such as depuration of mycotoxins
56 (Karaca & Velioglu, 2007), pesticide residues (Ikehata & El-Din, 2005) and control of microbes of
57 concern to food safety (Selma, Ibanez, Cantwell, & Suslow, 2008). Ozone has been extensively
58 tested for use in controlling table grape decay (Mlikota Gabler, Smilanick, Mansour, & Karaca,
59 2010). Postharvest ozone treatment enhances the synthesis of resveratrol and of other bioactive
60 phenolics in grapes (Artés-Hernández, Aguayo, Artés, & Tomás-Barberán, 2007), confirming
61 earlier work on this subject (Sarig et al., 1996). However, it is unclear if the increase of these
62 compounds is really biosynthesis or simply the result of an easier extraction. In viticulture, cell
63 wall degradation is considered to be another potential index of berry maturity, as are technological
64 and phenol maturities. This is because the extraction of important enological compounds, such as
65 anthocyanins, or polyphenols in general, is easier when the berry's cell wall is broken. Cell wall
66 enzymes, mainly PME (PG is almost undetectable), are responsible for cell wall degradation and
67 these enzymes are strongly activated during berry ripening (Deytieux-Belleau, Vaillet, Donèche, &
68 Geny, 2008). In 2011, Botondi, Lodola & Mencarelli observed that PME activity increased
69 throughout the postharvest dehydration process for Aleatico wine grapes while PG activity rose to a
70 point and then dropped off again. Vicenzi et al (2012) also observed PME activity in Erbaluce
71 grapes even at the end of the dehydration process. Very recently, Zoccatelli et al. (2013) showed
72 that, during dehydration, six PME genes were strongly induced in Corvina berries and, to a lesser
73 extent, in Sangiovese and Oseleta berries. Regarding the cell wall, it has been observed in
74 tomatoes that short-term treatment (10 min) with high concentration (10 ppm) of ozone did not
75 alter the activity of the pectin-degrading enzymes polygalacturonase and β -Galactosidase (β -Gal),
76 though it did decrease pectin methyl esterase activity (Rodoni, Casadei, Concellòn, Chaves, &
77 Vicente, 2010).

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

83 **2. Materials and methods**

84 *2.1. Experimental procedure and treatment*

85 The red wine grape bunches were carefully harvested (sound and uniformly sized berries) at
86 26°Brix. After 1 h of transport under shaded conditions, the bunches (arrival temperature at lab: 21
87 °C) were placed in a single layer in perforated boxes (60 x 40 x 15 cm). For each treatment, 6
88 perforated boxes with 6 kg (± 500 g) of bunches each were placed in a small metallic tunnel (45 x
89 45 x 100 cm) fitted with an exhaust fan with air-flow regulation (1.5 ± 0.3 m/s). The small tunnels
90 were placed in three thermohygro-metric controlled rooms at 10 (± 1)°C and 70% (± 5 %) RH for the
91 treatments; at the end of treatments, RH was reduced to 50% using a dehumidifier. The following
92 treatments were performed:

- 93 - A (shock treatment): O₃ fumigation, 1.5 g/h in continuous flow (Ozone generator A series,
94 PC Engineering srl, Uggiate Trevano, Como, Italy) for 18 hours followed by dehydration in
95 normal atmosphere;
- 96 - B (long term treatment): O₃ fumigation, 1.5 g/h in continuous flow followed by dehydration
97 in normal atmosphere with 0.5 g/h of O₃ fumigation for 4 hours each day ;
- 98 - Ck (control treatment): no ozone fumigation, but similar treatment conditions and followed
99 by dehydration in normal atmosphere.

100 The berry sampling was performed at the beginning, after the 18h treatment, and at 20 and
101 35% weight loss (w.l.).

102 *2.2. Physical and physiological analyses*

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

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

112 Carbon dioxide (CO₂) production was measured by placing a few bunches inside a glass jar
113 (three jars per treatment), tightly capped with a lid, for 2 hours. CO₂ production rate was monitored
114 by gaschromatographic method using a GC Clarus 400 (shincarbon ST 80/100 column, TC detector,
115 Perkin Elmer Inc., MA, USA). The activity was expressed as ml of CO₂/kg h.

116 2.3. Chemical analyses

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

129 at 700 nm. Total anthocyanins were determined by filtering the previous extract using the same
130 Sep-pak C18 column and reading at 520 nm (Di Stefano & Cravero, 1991). All chemical data are
131 expressed on a dry weight basis to avoid 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 $\mu\text{mol}/\text{min g}_{\text{dw}}$.

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 $\mu\text{mol}/\text{min g}_{\text{dw}}$.

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

155 80 (Sigma-Aldrich, Milan, Italy) for 30 min at 25°C. Rinses were serially diluted in 0.1%
156 Bacteriological Peptone solution and 100 µl were spread onto Malt Extract Agar (MEA) and Yeast
157 Extract Peptone Dextrose (YEPD) (Lai, Siti Murni, Fauzi, Abas Mazni, & Saleh, 2011). The latter
158 was used to count yeasts, while MEA was used to enumerate general fungal community. All media
159 were supplemented with 100 mg/l chloramphenicol (Sigma-Aldrich, Milan, Italy) to inhibit
160 bacterial growth. Plates were incubated at 28 °C for 5 days for colony development and counted.

161 2.6. Statistical analysis

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

168 3. Results

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

181 A significant increase of CO₂ production was detected at 20% w.l. in the ozone-treated
182 grapes while, in the Ck grapes, the increase occurred only at 35% w.l. (Table 1).

183 After 18 hours of the O₃ treatment, no significant difference was found in the total
184 carotenoids, total phenolic and total anthocyanin contents. The initial value of the polyphenol
185 content was 10372 mg catechins/kg_{dw} and, at 20% w.l., a significant decrease in the phenolic
186 content was observed in the Ck- and B-treated berries (3127 mg catechins/kg_{dw}) while the A-treated
187 ones kept a higher polyphenol content (Fig. 1). No changes occurred at 35% w.l.. At 20% w.l.,
188 anthocyanin content in the Ck- and B-treated grapes decreased significantly: the anthocyanins fell
189 from 746 mg malvidin/kg_{dw} to 512 and 626 mg malvidin/kg_{dw}, respectively (Fig. 2). At 35% w.l.,
190 the final values were 477, 411 and 407 mg malvidin/Kg_{dw}, respectively, for the grapes that received
191 Ck, A and B treatment. The total carotenoid content was unaffected by ozone treatment but, at 35%
192 w.l., it declined significantly in the B sample (Table 2). The berry colour as expressed by hue angle
193 increased slightly but significantly in sample A, from 290 up to 335, indicating a drift to redness
194 and loss of blueness (Table 2); significant difference was observed also in control samples but not
195 in B one. PME and PG activities were unaffected by the ozone treatment but, at 20% w.l., a
196 significant decrement of PME activity was observed in all samples, especially in the untreated
197 berries; a further decrease occurred at 35% for the Ck and B samples (Table 3). PG activity declined
198 significantly at 20% and 35% w.l. in the Ck sample whereas it remained constant or even increased
199 slightly in the ozone-treated ones.

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

207 significantly lower when the grape berries were treated with the ozone for 18h (ANOVA- Tukey's
208 Test; Ck treatment $P=0.01$) and did not increase at 35% w.l. (ANOVA-Tukey's Test; $P=0.04$) and
209 at 20% w.l. (ANOVA-Tukey's Test $P=0.00$). According to the Tukey's Test, no significant
210 differences were found between shock and long ozone treatments.

211 **4. Discussion**

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

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

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

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

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

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

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

305 **5. Conclusions**

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

311 dehydration process. Ozone shock treatment is also able to preserve the polyphenol and anthocyanin
312 content. Moreover, shock treatment keeps the PME and PG activities, which are important in
313 improving metabolite extractability, high. In contrast, the long-term ozone treatment greatly reduces
314 the polyphenol content through a supposed oxidant activity.

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318

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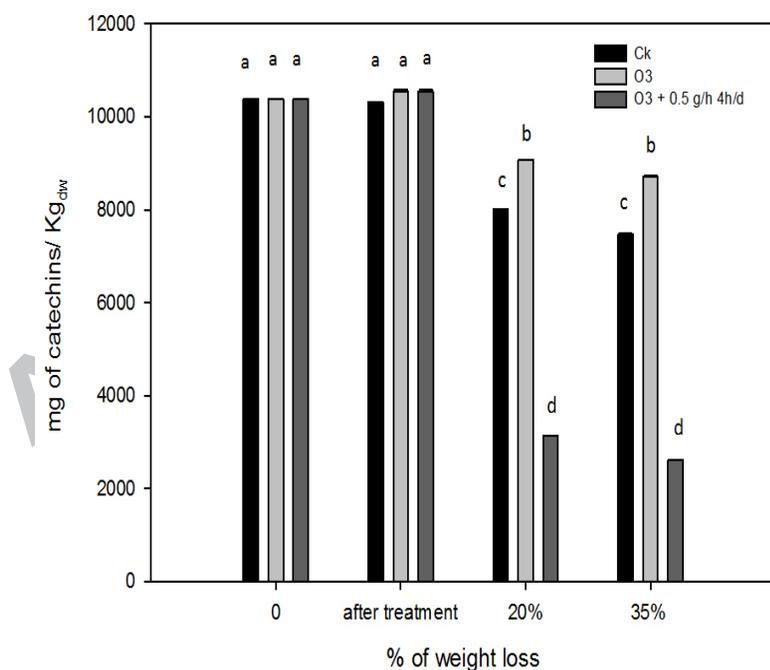
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445 Figures

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447 Figure 1. Changes in total polyphenols (mg of catechins $\text{Kg}^{-1}_{\text{dw}}$) after postharvest ozone treatments
 448 and during dehydration at 20 and 35% w.l.. O3=ozone fumigation for 18 h; O3 + 0.5 g/h
 449 4h/d=ozone fumigation for 18 h + 0.5 g/h ozone fumigation for 4 h every day during dehydration;
 450 Ck = untreated but kept in cold room at the same temperature. Data are the mean (\pm SD) of three
 451 sets of juice (30 ml each) from different bunches from each treatment group and sampling time. Means
 452 followed by the same letter are not statistically different ($p < 0.05$). Where no bar (SD) appears, no
 453 difference occurred among the reps.

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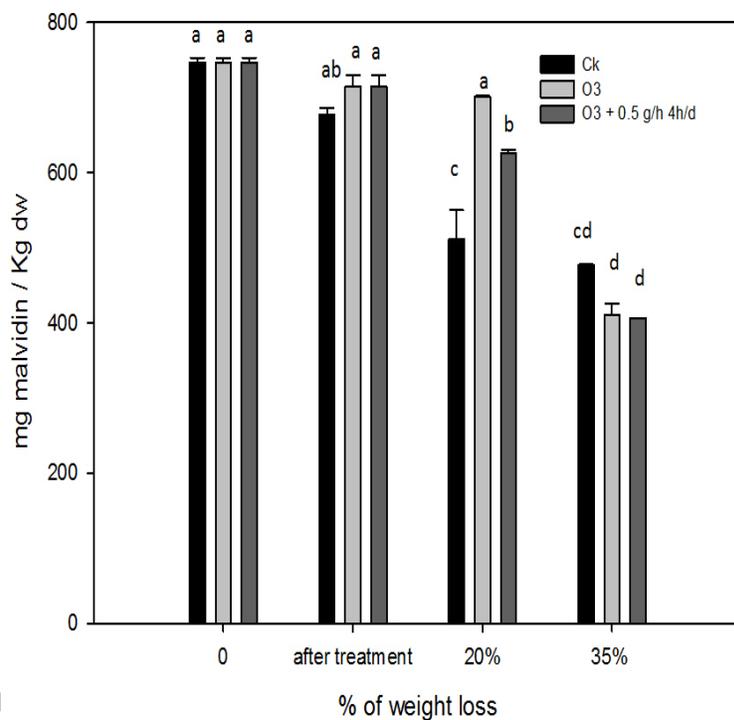
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459 Figure 2. Changes in total anthocyanins (mg of malvidin Kg^{-1} dw) after postharvest ozone
 460 treatments and during dehydration at 20 and 35% w.l. O₃=ozone fumigation for 18 h; O₃ + 0.5 g/h
 461 4h/d=ozone fumigation for 18 h + 0.5 g/h ozone fumigation for 4 h every day during dehydration;
 462 Ck = untreated but kept in cold room at the same temperature. Data are the mean (\pm SD) of three
 463 sets of juice (30 ml each) from different bunches from each treatment group and sampling time. Means
 464 followed by the same letter are not statistically different ($p < 0.05$). Where no bar appears, no
 465 difference occurred among the reps.



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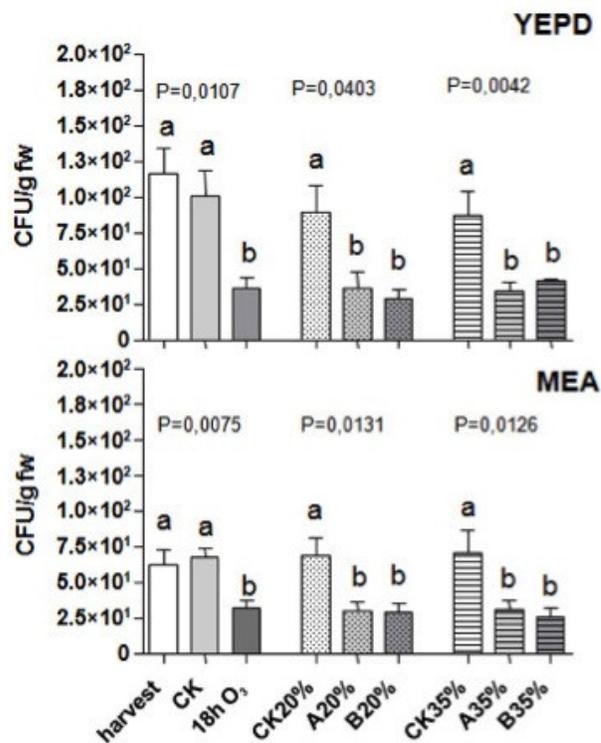
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475 Figure 3. Yeast (up) and fungi (down) count after postharvest ozone treatments and during
 476 dehydration at 20 and 35% w.l.. 18hO₃=ozone fumigation for 18 h; CK20% and CK35%=untreated
 477 sample, respectively, at 20% and 35% w.l.; A20% and B20%=respectively, shock (ozone
 478 fumigation for 18 h) and long-term ozone (ozone fumigation for 18 h + 0.5 g/h ozone fumigation
 479 for 4 h every day during dehydration) treatment at 20% w.l.; A35% and B35%=respectively, shock
 480 and long-term ozone treatment at 35% w.l.. Data are the mean (\pm SD) of three mycological analyses
 481 from three sets of berries from different bunches from each treatment group and sampling time.
 482 Means with the same letter are not statistically different from the indicated *p*.



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Tables

Table 1 Changes in titratable acidity (g/L tartaric acid_{dw}), SSC (°Brix), and CO₂ production (ml/kg h) after postharvest ozone treatments and during dehydration at 20 and 35% w.l..

	Titratable acidity			SSC			CO ₂ production		
	A ^A	B	Ck	A	B	Ck	A	B	Ck
Harvest	32±3ab ^B	32±1ab	32±2ab	26±1d	26±1d	26±1d	0.8±0.1cd	0.9±0.1cd	0.9±0.0cd
After treatment	32±3ab	31±4ab	33±3a	27±1cd	27±1cd	26±0d	0.7±0.1d	0.7±0.1d	0.9±0.1cd
20% weight loss	35±2a	21±1e	26±2d	30±2b	29±1bc	28±2bcd	1.3±0.3ab	1.1±0.1bc	0.8±0.2cd
35% weight loss	29±1bc	20±2e	27±2cd	36±1a	38±2a	36±2a	1.4±0.3a	1.2±0.2ba	1.3±0.2ab

^AA = O₃ fumigation for 18 h; B = O₃ fumigation for 18 h + 0.5 g/h of O₃ fumigation for 4 hours each day during dehydration; Ck = untreated but kept in cold room at the same temperature

^BData are the mean (± SD) of analyses of 30 berries (SSC), three sets of berry juice from different bunches for the analysis of the titratable acidity and three glass jars for CO₂ production. Means, in the same group of analysis, followed by the same letter are not statistically different (p<0.05).

Table 2. Changes in total carotenoid content (μg of xanthophylls and carotenes/ mg_{dw}) and hue angle before and after ozone treatment and during grape dehydration at 20 and 35% w.l..

	Total carotenoids			Hue angle		
	A ^A	B	Ck	A	B	CK
Harvest	1.15 \pm 0.03ab ^B	1.10 \pm 0.03bc	1.10 \pm 0.02 bc	290 \pm 22b	300 \pm 30ab	285 \pm 20b
After treatment	1.22 \pm 0.06a	1.18 \pm 0.06ab	1.15 \pm 0.03ab	303 \pm 15ab	285 \pm 20b	290 \pm 13b
20% weight loss	1.15 \pm 0.03ab	1.00 \pm 0.06c	1.10 \pm 0.04bc	318 \pm 12ab	304 \pm 16ab	300 \pm 18ab
35% weight loss	1.15 \pm 0.03ab	0.84 \pm 0.04d	1.00 \pm 0.03c	335 \pm 15a	320 \pm 21a	320 \pm 15a

^AA = O₃ fumigation for 18 h; B = O₃ fumigation for 18 h + 0.5 g/h of O₃ fumigation for 4 hours each day; Ck = untreated but kept in cold room at the same temperature.

^BData are the mean (\pm SD) of three sets of berry juice from different bunches from each treatment group and sampling time (total carotenoids) and 30 berries from different bunches (hue angle). Means in the same group of analysis followed by the same letter are not statistically different ($p < 0.05$).

Table 3. Changes in PME ($\mu\text{mole}/\text{min g}_{\text{dw}}$) and PG ($\mu\text{mole}/\text{min g}_{\text{dw}}$) activities before and after ozone treatment and during grape dehydration at 20 and 35% w.l..

	Harvest	after 18h treatment	20% weight loss	35% weight loss
PME				
Ck (control) ^A	0.75 \pm 0.05a	0.76 \pm 0.02a	0.55 \pm 0.06bc	0.45 \pm 0.02c
A treatment	0.73 \pm 0.03a	0.75 \pm 0.02a	0.63 \pm 0.03b	0.59 \pm 0.03b
B treatment	0.75 \pm 0.05a	0.75 \pm 0.05a	0.60 \pm 0.04b	0.47 \pm 0.05c
PG				
Ck (control)	0.24 \pm 0.02ab	0.23 \pm 0.03ab	0.17 \pm 0.01c	0.15 \pm 0.02c
A treatment	0.22 \pm 0.03ab	0.25 \pm 0.01ab	0.23 \pm 0.02ab	0.27 \pm 0.03a
B treatment	0.25 \pm 0.05ab	0.25 \pm 0.02ab	0.20 \pm 0.03bc	0.22 \pm 0.03ab

^AA = O₃ fumigation for 18 h; B = O₃ fumigation for 18 h + 0.5 g/h of O₃ fumigation for 4 hours each day; Ck = untreated but kept in cold room at the same temperature.

^BData are the mean (\pm SD) of three sets of berry juice from different bunches from each treatment group and sampling time. Means in the PME or PG rows followed by the same letter are not statistically different ($p < 0.05$).

HIGHLIGHTS

1. Wine grape dehydration, an useful technique to increase wine quality, can be compromised by fungi
2. Ozone has been applied as shock or long term treatment during dehydration
3. In both cases, ozone reduced the fungi and yeasts contamination of about 50%.
4. Ozone shock treatment preserved polyphenols, anthocyanins and carotenoids
5. Ozone long term treatment reduced the acitivity of PME and PG