

1 **Effect of High Carbon Dioxide or Gaseous Ozone**
2 **Combined with MAP on the Chemical Composition of**
3 **Organic Late-Season Table Grapes Scarlotta Seedless[®]**
4 **during long-term storage**

5 Naouel Admane^{a,c,*}, Francesco Genovese^a, Giuseppe Altieri^a, Antonella Tauriello^a,
6 Antonio Trani^b, Giuseppe Gambacorta^b, Vincenzo Verrastro^c, Giovanni Carlo Di Renzo^a

7 ^a Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali (SAFE), Università
8 degli Studi della Basilicata (UNIBAS), Via Ateneo Lucano 10, 85100 Potenza, Italy

9 ^b Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli
10 Studi di Bari (UNIBA), Via Amendola 165/A, 70126 Bari, Italy

11 ^c Department of Mediterranean Organic Agriculture, Mediterranean Agronomic Institute
12 of Bari (CIHEAM-MAIB), Via Ceglie, 9, 70010 Valenzano, Bari, Italy

13 * Corresponding author: naouel.ad@gmail.com

14 **Abstract**

15 The aim of this study was to maintain the quality of organic table grapes and
16 extend their shelf life for long-term storage by using organically approved methods. The
17 effectiveness of the pretreatments with different concentrations of gaseous ozone
18 (varying from 5 to 20 $\mu\text{L L}^{-1}$) or carbon dioxide (at 50 and 70%) followed by storage
19 under modified atmosphere packaging (2% O₂: 5% CO₂: 93% N₂ MAP) were evaluated
20 on late-season organic Scarlotta[®] grapes as alternatives to usual commercial SO₂
21 application. After 45 days of cold storage (CS), pretreatments with O₃ increased
22 significantly total anthocyanins at the opposite of pretreatments with CO₂. Furthermore,

23 pretreatments with O₃ at 20 μL L⁻¹ controlled concentration of acetaldehyde, preserved
24 rachis chlorophyll content and skin color during CS.

25 Regarding cumulative decay incidence, it was reduced 5 to 6 fold by
26 pretreatments with O₃ at 20 μL L⁻¹ and CO₂, compared to control after shelf life (SL),
27 however, pretreatments with CO₂ caused also organoleptic quality loss with strong stem
28 browning and perceived off-flavor. The present experiment revealed the efficiency of
29 pretreatment with O₃ at 20 μL L⁻¹ to preserve initial sensory quality of organic
30 Scarlotta[®] grapes and to control efficiently grape decay after CS and SL. Our results
31 encourage confirming this postharvest alternative approach treatment in other cultivars
32 and under commercial conditions.

33 **Keywords:** quality loss, organic table grapes, decay control, SO₂ alternative, shelf life,
34 anthocyanin.

35

36 **1 Introduction**

37 Every year an important amount of table grapes is lost between harvest and
38 consumption. Table grapes (*Vitis vinifera L.*) as non-climacteric fruits, are highly
39 perishable after harvest and exposed to serious quality losses essentially due to water
40 loss, which results in stem drying and browning, berry softening and pathological
41 decay, mainly caused by gray mold (Valero et al., 2006; Baiano et al., 2007; Sanchez-
42 Ballesta et al., 2007).

43 Gray mold due to *Botrytis cinerea* is the most economically important
44 postharvest disease because of the damage caused in the harvest season and during
45 storage, it is particularly severe in years when heavy rainfall occurs during fruit
46 ripening, and can also develop at low temperature, shortening the duration of storage
47 and marketing (Ciccarese et al., 2013).

48 Berry decay is another post-harvest affection, visible as “slip-skin”, separation
49 of the skin from the flesh upon touch (Luvisi et al., 1992; Chervin et al., 2012).
50 Moreover, the maturity and storage period increase significantly berry’s susceptibility to
51 infection and decay symptoms during postharvest handling (Teles et al., 2014).

52 Commonly, the standard practice to control postharvest grape decay is achieved
53 by using sulfur dioxide gas (SO₂); the grapes are fumigated either by repeated
54 application of gas in storage room or by continuous release SO₂-generating pads in case
55 of shipment period longer than 10 days or long retail handling (Chervin et al., 2012).
56 This compound is registered as an adjuvant in different countries, and in spite of its
57 efficacy for controlling gray mold; several problems are associated with its application.
58 The main damages are: bleaching and other injuries to the rachis and berries, pitting of
59 berries, off-flavor, excessive sulfite residues, corrosion of the equipment within storage
60 facilities, worker safety, and air quality (Smilanick et al., 1990; Crisosto and Mitchell.

61 2002; Chervin et al., 2005; Zoffoli et al., 2008). For these reasons, this product has been
62 removed from the Generally Recognized as Safe (GRAS) compound list by US Food
63 and Drug Administration (US FDA) (Anon, 1986); whereas, it is not allowed as
64 postharvest treatment on organic grapes in Europe and USA by EU regulation (EC) No
65 889/2008 and National Organization Program (NOP-USDA) respectively.

66 The demand for this fresh product with immaculate appearance, high sensory
67 quality in terms of flavor, free of pathogens and chemical residue is a hard challenge
68 considering the difficulties to conserve them with alternative safe treatments to SO₂. In
69 order to fulfill this growing demand for fresh organic products, several efforts were
70 focused to develop alternative strategies to control postharvest decay of organic table
71 grapes; these strategies should be safe, effective, economical and compatible with
72 commercial handling. As the use of GRAS type decontaminating agents, physical
73 treatments and combined treatments (Romanazzi et al., 2012; Admane et al., 2015). The
74 integration of two or more alternative treatments/means can be worthwhile than the use
75 of single treatment (Wilson, 1997).

76 Ozone (O₃) was declared GRAS substance by the US FDA in 2001 (US FDA,
77 2001), and since that time it is being widely investigated and introduced into some
78 commercial applications in food industry such as table grapes storage. O₃ is a highly
79 reactive form of oxygen, naturally present in the atmosphere and one of the most potent
80 sanitizers against a wide spectrum of microorganisms (Khadre et al., 2001; Mlikota
81 Gabler and Smilanick, 2001; Von Gunten, 2003). It has been extensively tested for the
82 control of table grape decay (Cayuela et al., 2009; Sharpe et al., 2009; Mlikota Gabler et
83 al., 2010; Smilanick et al., 2010). Many cold storage facilities in California have
84 installed equipment that generates a constant low dose of O₃ (100 ppb day and 300 ppb
85 night cycle) and it reduced the spread of gray mold and prolonged the storage of grapes

86 for several weeks (Smilanick et al., 2010). The risk of injury to table grapes from O₃
87 have been reported for the rachis after a treatment of 30 min with very high
88 concentrations (5000 ppm) of O₃ (Mlikota Gabler et al., 2010). Therefore, ozone could
89 be considered as a promising antimicrobial agent for the sanitation of grape surfaces to
90 extent the storage period and shelf life.

91 In addition, postharvest treatment with short-term exposure to high carbon
92 dioxide (CO₂) concentrations is an effective treatment to maintain quality and to control
93 decay development in grapes (Crisosto et al., 2002b; Retamales et al., 2003; Sanchez-
94 Ballesta et al., 2006, 2007; Teles et al., 2014). Furthermore, low concentration of O₂
95 (below 1%) induces anaerobic respiration, which leads to undesirable metabolic
96 reactions, resulting in off-odors and off-flavors (Candir et al., 2012), while, high CO₂
97 concentration (equal or above 15%) results in stem and berry browning (Crisosto et al.,
98 2002b; Retamales et al., 2003). Moreover, Modified Atmosphere Packaging (MAP)
99 technique is considered as a non-toxic method for keeping quality of fruit and
100 vegetables (Artés, 1976; Kader et al., 1989) and could be an alternative methods which
101 control or avoid table grapes postharvest decay and maintains their visual and sensory
102 quality (Artés-Hernández et al., 2004). The application of MAP can result in reduction
103 of respiratory activity, retardation of softening and ripening and restraint of pathogens
104 and reduced incidence of various physiological disorders (Caleb et al. 2013). MAP, as a
105 semi-permeable coating with an adjusted ambience of CO₂/O₂ inside small storage
106 environment, has been proven to prolong the storability of perishable commodities like
107 grapes (Hagenmaier, 2005). Several authors consider MAP with 15% O₂ and 10% CO₂
108 such as a cheap and easy technique, which might be useful as an alternative to SO₂
109 (Crisosto et al., 2002b; Artés-Hernández et al., 2004).

110 However, these treatments vary in their effectiveness and lack enough support to
111 replace SO₂ as a commercial practice. Few studies have evaluated their effects on
112 common quality attributes as phenolic and aromatic compounds, in addition to decay
113 control (Sanchez-Ballesta et al., 2006, 2007; Romero et al., 2009; Ustun et al., 2012).
114 Therefore, in this study a detailed investigation was carried out to determine the effects
115 of MAP combined with superficial disinfectant, as high concentrations of gaseous O₃ or
116 CO₂, on decay incidence, sensorial quality maintenance, antioxidant capacity, total
117 phenolic compounds, total and individual anthocyanins of organic Scarlotta® table
118 grapes during cold storage (CS) period and after simulate commercial shelf life (SL).

119 **2 Material and methods**

120 **2.1 Plant material**

121 The experiment was undertaken in 2014 in an organic table grape vineyard
122 located in Gioia del Colle (Southeast of Italy) under Mediterranean climate conditions.
123 Four-year-old organic Scarlotta seedless® brand “Sugranineteen” table grapes grafted
124 onto 140 Ruggeri (*Vitis berlandieri* × *V. rupestris*), with historic and current high
125 incidence of gray mold. Vines were spaced 2 × 3.5 m (≈1428 vines/ha), trained to an
126 overhead trellis system (‘tendone’) and covered with plastic film to protect grapes from
127 rains and hailstorm, with drip irrigated.

128 Harvested clusters were transported to the laboratory and immediately
129 pre-cooled. The clusters were selected based on uniform berry size, color, firmness and
130 freedom from evident defects or diseases. The selected clusters met European Union
131 (“EU”) Class 1 and in agreement with Sun World Quality Specifications. Selected
132 bunches were at commercial maturity with sugar-acid ratio of 26:1; medium

133 symmetrical and well-filled bunches with a size of about 650 g; berries large, elongated
134 with diameter around 23 mm.

135 Selected grape clusters were randomly distributed into batches with five
136 replicates of one cluster per pretreatment. Clusters were placed inside plastic boxes
137 (carton Pack®) model CL1/135 (each box constituted a replicate) of 1 kg capacity.

138 **2.2 Pretreatments**

139 Grape boxes were placed inside sealed barrels provided with two pipes
140 connected to gaz analyser, the first one for removing the air and the second to treat the
141 grapes as follows:

- 142 i) Gaseous O₃ concentration at 5, 10 and 20 µL L⁻¹ mixed with air for 30 min;
143 gaseous O₃ was generated by OZAB-MF-A (Aeraque I.T. S.r.l., Stradella
144 (PV), Italy), and its concentration was monitored through OZOMAT-MP
145 (Anseros Ozone Gas Analyser MP, Germany);
- 146 ii) CO₂ concentration at 50 and 70% mixed with air for 24 h, the CO₂
147 concentration was monitored and adjusted throught SERVOPRO 1440 Gas
148 Analyser (SERVOMEX, USA).

149 The obtained results of all these pretreatments were compared to untreated grapes
150 (control). Except non-packed control, all the remaining grape boxes were packed in film
151 bags (85 µm thickness), made of polyamide (20 µm) / polyethylene (65 µm) (PA/PE),
152 under 2% O₂:5% CO₂:93% N₂ MAP, hermetically sealed using a heat sealer (MD,
153 Italy). Then, stored under simulated shipping conditions in container at 0±0.5 °C and
154 90-95% relative humidity (RH) for 55 days. At the end of storage, the temperature was
155 raised at 15±1.0 °C for one-week to simulate commercial shelf life (SL) as retail sale
156 period.

157 **2.3 Measurement of table grapes respiration rates**

158 Respiration rates were measured through apparent Michaelis constant ($K_{m_{appO_2}}$)
159 and maximal oxygen respiration rate (RR_{maxO_2}) by using the closed system method (Lee,
160 1987; Hagggar et al., 1992). Table grapes of known weight (≈ 270 g) were placed in a 1
161 L glass jar previously equilibrated in a temperature-controlled room. The initial gas
162 composition inside each jar was set by gas flushing. Initial CO_2 partial pressure was
163 constant and equal to atmospheric concentration 0.03% and initial O_2 partial pressures
164 were 5, 10, and 20%. At periodic intervals, gases were sampled through a silicone
165 septum set in the jar lid and analyzed using a CheckMateII O_2/CO_2 analyser (PBI
166 Dansensor, Ringsted, Denmark). Experiments were stopped when the change in CO_2
167 partial pressures became greater than 1.5%. Each experiment was done in triplicate. The
168 linear part of the Lineweaver plot ($1/RR_{O_2}$ against $1/O_2$) was extrapolated to estimate
169 the apparent Michaelis constant ($K_{m_{appO_2}}$) and the maximal oxygen respiration rate
170 ($RR_{max O_2}$).

171 **2.4 Film permeability**

172 Different pieces (130×125 mm) of previously mentioned film packaging PAPE
173 (85 μ m thickness) were sealed on two sides forming pouches, these pouches were
174 flushed with 4% O_2 : 15% CO_2 : 81% N_2 . During the sampling period, the pouches (three
175 replicates) were stored at 20 °C and 40 % RH. During a storage period of 10 days, the
176 changes in headspace gas composition were measured at interval times by using a
177 CheckMateII O_2/CO_2 analyser (PBI Dansensor, Ringsted, Denmark). In addition, the O_2
178 and CO_2 permeation process through the packaging film, were calculated by derived
179 Fick's law and the obtained result were expressed in $mol\ m^{-1}\ s^{-1}\ Pa^{-1}$ (Larsen et al.,
180 2000).

181 2.5 Modelling approach to simulate gas headspace composition

182 To predict gas atmosphere changes inside packaging during storage period, a
183 mathematical model considering simultaneously gas diffusion through film packaging
184 and grapes respiration rate, was developed and solved with Matlab[®] software (The
185 Mathworks Inc, Natick, Mass., U.S.A).

186 Gas exchanges through the plastic film were represented by the classic
187 permeability equation based on the 1st Fick's diffusion law for thin and infinite films
188 (Crank and Park, 1968):

$$J = \frac{Pe \times S}{e} \times \Delta P$$

189

190 where **J** is the gas flux per time unit through the film (mol s⁻¹); **Pe** is the gas
191 permeability coefficient of the film (mol m⁻¹ s⁻¹ Pa⁻¹); **S** is the surface area of film (m²);
192 **e** is the film thickness (m); and **ΔP** is the gas partial differential pressure between the
193 outside and inside of the package (Pa).

194 Respiratory activity is described by a Michaëlis-Menten-type equation with a
195 noncompetitive carbon dioxide inhibition (Lee et al., 1991; Fonseca et al., 2002):

$$RR_{O_2} = \frac{RR_{maxO_2} \times pO_2}{K_{mappO_2} \times pO_2}$$

196

197 where **RR_{O₂}** is the oxygen respiration rate (mmol kg⁻¹ h⁻¹); **RR_{maxO₂}** is the
198 maximum oxygen respiration rate; **pO₂** is the gase partial pressures (kPa); **K_{mappO₂}** is
199 the apparent constant of Michaëlis-Menten equation (kPa) defined as the amount of sub-
200 strate providing the reaction rate of **RR_{maxO₂}**/2.

201 In addition, the temperature quotient (**Q₁₀**) was calculated from the slope of the
202 regression line to obtain the temperature dependence of respiration. **Q₁₀** indicates the

203 increase in the respiration rate caused by a 10 °C increase in temperature. Q_{10} values
204 were calculated using the following equation, where R2 and R1 are relative respiration
205 rates at two temperatures, T2 and T1 ($T_2 > T_1$).

$$206 \quad Q_{10} = (R_2 / R_1)^{10 / (T_2 - T_1)}$$

207 **2.6 Decay incidence and weight loss**

208 Decay incidence was measured in naturally infected Scarlotta[®] organic grapes
209 after each sampling time (at harvest time, 15, 30 and 45 days) at 0 °C, and 55 days at 0
210 °C + SL. Decay incidence represented berries with visible “slip-skin” was calculated as
211 the weight of the decayed berries after removal from the entire cluster. Moreover,
212 cumulative decay incidence was expressed as a percentage of loss during all the storage
213 period at 0 °C and after SL.

214 Concerning weight loss, its percentage was determined according to the
215 following expression: $\% WL_t = (M_0 - M_t) \times 100 / M_0$, where $\% WL_t$ is the percentage mass
216 loss at time t, M_0 is the initial sample mass and M_t is the sample mass at time t. The
217 sample weight was determined by means of a digital precision balance (± 0.01 g).

218 **2.7 Mechanical attributes**

219 Mechanical characteristics expressed in Newton (N), were measured using
220 penetrometer (Digital Fruit firmness tester, TR Turoni, Italy). These attributes included;
221 force needed to detach berries from the rachis (berry detachment force), maximum force
222 necessary to puncture the skin of an individual berry with a 2 mm probe that penetrated
223 to a depth of 6 mm (skin firmness), and then the force required to compress a berry
224 through a flat cylinder probe of 8 mm diameter to reach a depth of 5 mm (berry
225 firmness).

226 **2.8 Chemical attributes**

227 Regarding the chemical analysis, berries were filtered through plastic bag
228 (BagPage[®]) fitted with filter to extract the juice. The Soluble Solid Content (SSC) was
229 determined with digital refractometer (Atago, Japan) and total titratable acidity (TA)
230 expressed in grams of tartaric acid per liter of table grape juice, was assessed by making
231 a titration with 0.1 N NaOH up to pH 7, (OIV-MA-AS313-01, 2009). Finally, the juice
232 pH was quantified by a pH-meter (Crison, Spain).

233 **2.9 Color analysis**

234 The berry skin color was measured through a spectrophotometer (Minolta CR
235 400 ChromaMeter, Japan), by assaying 50 berries for each replicate. Color parameters:
236 L^* (lightness) corresponding to a black-white scale (0, black; 100, white), a^* (red
237 tendency), b^* (yellow tendency) were recorded using the CIELAB color system. From
238 these values, different indices were calculated:

- 239 - Hue angle (h°) [$\tan^{-1} b/a$] which is the attribute of a visual sensation
240 according to which an area appears to be similar to one of the perceived
241 colors, red, yellow, green and blue, or a combination of two of them;
242 - Color index for red grapes (CIRG) as $CIRG = [(180 - h^\circ) / (L^* + C^*)]$ based on
243 the parameters L^* , a^* , b^* , and its characterized by showing a high
244 correlation with the external visual color of the fruit (Carreño et al., 1995).

245 **2.10 Rachis chlorophyll content**

246 Rachis chlorophyll was extracted by immersing 0.5 g of fresh chopped rachides
247 (grinded in cold liquid nitrogen) in 25 mL of pure methanol (99.9%) for 24 h at 4 °C in
248 darkness. The quantification of chlorophyll was carried out by using a
249 spectrophotometer (UV-1800, Shimadzu, Japan), then the samples were exposed to

250 visible light at a wavelength of (652.4, 665.2 and 470 nm; the content was expressed as
251 mg kg⁻¹ fresh weight using Lichtenthaler's formula (Lichtenthaler, 1987).

252 **2.11 Extraction of phenolic compounds**

253 From each replicate, 10 berries were divided into two sub-samples (five
254 berries/each). The extraction of antioxidant compounds from berry skins of sub-samples
255 was carried out on the skins removed manually from the pulp, dried with filter paper
256 and then macerated in 20 mL of ethanol/water/HCl solution (70/30/1 v/v) overnight at
257 room temperature (Gambacorta et al., 2011). Finally, the extract was filtered through
258 filter paper and immediately submitted to analysis. The separated pulp was
259 homogenized in a blender, successively centrifuged at 15,000 g for 5 min and the clear
260 juice was immediately submitted to analysis.

261 **2.12 Analysis of antioxidant activity**

262 Antioxidant activity (AA) in skin and flesh was analyzed with the ABTS [2,2'-
263 azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] assay, this assay is based on free-
264 radical-scavenging activity. The results were expressed as mmol Trolox (6-hydroxy-2,
265 5, 7, 8-tetramethylchromane-2-carboxylic acid) equivalent antioxidant capacity (TEAC)
266 for kg⁻¹ of skin or flesh. For the calibration process, Trolox standard solutions were
267 prepared at a concentration ranging from 10 to 800 µmol L⁻¹. Antioxidant activity was
268 measured in the ABTS assay through the ability of antioxidants to scavenge the ABTS
269 radical cation (ABTS^{•+}, a blue/green chromophore) by inhibiting its absorption at 732
270 nm. The ABTS antioxidant test was performed according to the method reported by Re
271 *et al.* (1999) with slight modifications. In order to produce ABTS^{•+}, 7 mmol L⁻¹ ABTS
272 solution was reacted with 2.45 mmol L⁻¹ potassium persulfate aqueous solution for 16 h
273 at room temperature and darkness conditions. The solution of ABTS^{•+} was then diluted

274 with ethanol to an absorbance of 0.90 ± 0.03 at 732 nm; after the addition of either 100
275 μL of skin extract (diluted at 1:20 with ethanol) or undiluted pulp juice to 3.9 mL of
276 diluted ABTS⁺ solution, the absorbance was measured after 5 min (Ferrara et al., 2015).

277 **2.13 Total phenolic content**

278 Total phenols content was determined on grape flesh and skins extract, the
279 phenolic content was determined by Folin-Ciocalteu method using an UV-visible
280 spectrophotometer (Beckman Coulter, USA). Sample solution of 100 μL of skin extracts
281 (diluted at 1:25 with ethanol/water/HCl solution (70/30/1 v/v)) or undiluted pulp juice
282 was added to 500 μL of H₂O and then to 100 μL of Folin-Ciocalteu's reagent;
283 homogenized and incubated at room temperature for 5 min, after 500 μL of 10% of
284 sodium carbonate was added, the mixture was then incubated for 90 min at room
285 temperature, following this incubation period, the absorbance was measured at 700 nm.
286 Total phenolic content was calculated on the basis of a calibration curve of gallic acid
287 and expressed as mg gallic acid equivalent (GAE) kg^{-1} of fresh weight. The standard
288 calibration curve was performed from 0.08 to 0.002 mg mL^{-1} of gallic acid pure
289 standard (Sigma Aldrich).

290 **2.14 Anthocyanins analysis**

291 Anthocyanin composition was determined on grape skins extract, by using a
292 Waters 600 E HPLC, (Waters Inc.), which included a quaternary pump, a PDA and an
293 injection valve with a 20 μL loop. Sample extracts, previously filtered on a 0.45 μm
294 nylon membrane, were injected into a NovaPack C18 (150 \times 3.9 mm, 4 μm particle
295 size, Waters Inc.) column maintained at 30 °C and eluted at a flow rate of 1 mL min^{-1}
296 with 10% formic acid (solvent A), and acetonitrile (solvent B). The gradient program
297 for solvent A was 0-1 min 95%, 1-22 min 60%, 22-27 min 30%, 27-35 min 30%. The

298 eluates were monitored at 520 nm, and quantitative analysis was made according to the
299 external standard method with a calibration curve obtained by injection of solutions at
300 different concentration of malvidin-3-glucoside (Sigma Aldrich) ($R^2 = 0.9991$).
301 Tentative identification of anthocyanins was achieved by combining the elution pattern
302 and data reported in literature (Revilla and Ryan, 2000; Singh Brar et al., 2008;
303 Acevado De la Cruz et al., 2012); the results were expressed as mg kg^{-1} malvidin-3-
304 glucoside equivalents.

305 Total anthocyanin content was determined using diluted skin extract 1:100 with
306 ethanol/water/HCl solution (70/30/1 v/v) in agreement with the method reported by
307 Gambacorta et al. (2011). The obtained results were expressed as mg kg^{-1} Malvidin-3-
308 glucoside.

309 **2.15 Extraction and analysis of acetaldehyde and ethanol by SPME-** 310 **GC/MS**

311 The extraction of volatile compounds (mainly used for acetaldehyde and ethanol
312 compounds) was carried out by headspace solid phase micro-extraction (SPME) using a
313 triphasic fibres DVB/Carboxen/PDMS 50/30 μm .

314 Frozen grapes were pounded in a mortar until obtaining a homogeneous compound. For
315 each measurement, fibre was exposed to the headspace of a 12 mL screw-capped vial,
316 which contained 2 g of puree with 2 mL of 0.1 mol phosphor-citrate buffer pH 5, 100
317 μL of pectolytic enzymes for oenological use Endozym (diluted 1000 times in water),
318 and 30 μL of internal standard solution (3-pentanone). The extraction was performed for
319 30 min at 50 ± 1 °C with an equilibration time of 5 min. All used fibres were conditioned
320 by keeping them in the GC injector following instructions from manufacturer. After the
321 extraction step, fibres were desorbed in a split/splitless injector at 220 °C, for 1.5 min
322 split ratio was 1:20. Gas chromatography/mass spectrometry (GC/MS) analysis was

323 performed on a Thermo Scientific ISQ™ QD Single Quadrupole GC-MS System.
324 Compounds were separated on a WAX MS capillary column (20 m × 0.1 mm i.d.; 0.1
325 μm film thickness), by applying the following temperature program: 50 °C for 0.1 min,
326 50-180 °C at 13 °C min⁻¹, 180 - 220 °C at 18 °C min⁻¹. Mass detector conditions were
327 electronic impact mode at 70 eV, source temperature 250 °C and mass scanning
328 acquisition range: 34 - 200 Da. Carrier gas was helium with a constant flow at 0.4 mL
329 min⁻¹.

330 Chromatographic data were analyzed with Xcalibur v2.0 program; moreover, the
331 identification of acetaldehyde and ethanol components was based on comparison of their
332 GC retention times and mass spectra with reference spectra contained in a library
333 (National Institute of Standards and Technology NIST) of reference data (matching
334 score P>80). Both components were expressed in relative quantities as 3-pentanone
335 equivalent; moreover, acetaldehyde was quantified as μg kg⁻¹ and ethanol as mg kg⁻¹
336 fresh weight.

337 **2.16 Sensory quality**

338 Clusters were evaluated by six untrained panelists and individually scored:
339 sourness, aroma, stem and berry browning and stem dehydration, using the following
340 five-point intensity scale of damage (1: none; 2: slight; 3: moderate and limit of
341 marketability; 4: severe; 5: extreme). Visual appearance, flavor, juiciness, sweetness and
342 crunchiness of berries were evaluated on a nine-point subjective scale (1: bad; 3: fair; 5:
343 moderate and limit of marketability; 7: good; 9: excellent) (Artés-Hernández et al.,
344 2004).

345 **2.17 Statistical analysis**

346 Data mean values have been separated analysing the data through the Matlab
347 software, the Tukey's honestly significant difference (HSD) post-hoc test has been used
348 with a familywise error rate (FWER) set to 0.05 significance level.

349 Decay incidence data were transformed (arcsin of the square root of the
350 proportion of affected fruit) before the analysis.

351 **3 Results and discussion**

352 **3.1 Film permeability**

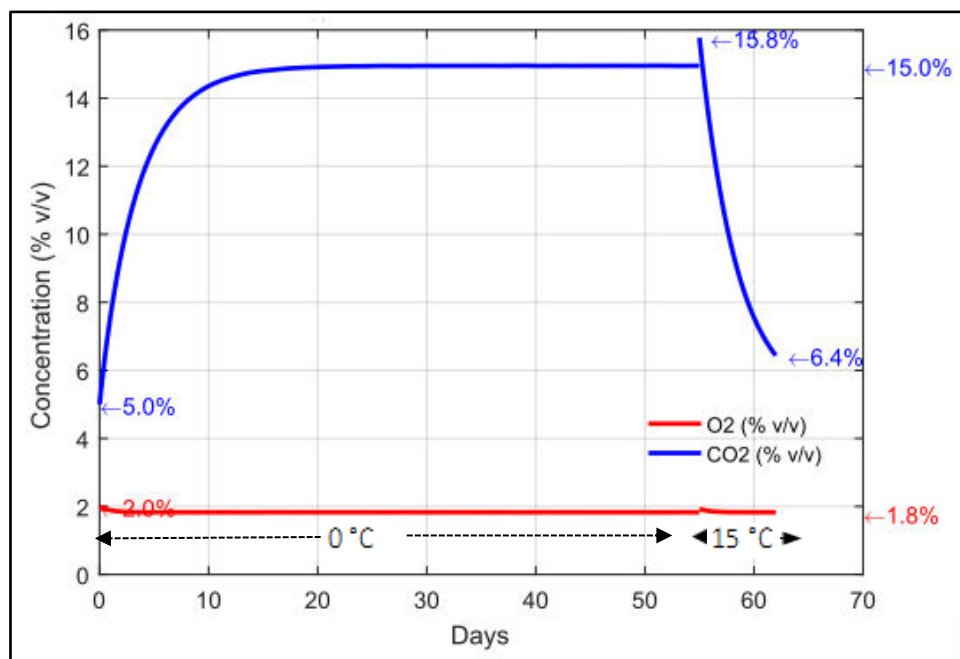
353 According to Lamikanra (2002), the commercial used film packaging PAPE
354 showed a low permeability against O₂ and CO₂ transmission rate (7.01×10^{-16} and $1.98 \times$
355 10^{-15} mol m⁻¹ s⁻¹ Pa⁻¹, respectively).

356 **3.2 Respiration rate**

357 Apparent Michaelis-Menten parameters of table grapes were estimated from
358 respiratory activity in the closed system, which allowed the RR_{O₂} determination through
359 the oxygen depletion. Then, the RR_{maxO₂} and the Km_{appO₂} were estimated on the
360 Lineweaver plot. The RR_{maxO₂} and the Km_{appO₂} of table grapes were 1.90 mmol h⁻¹ kg⁻¹
361 and 34.63 kPa respectively, demonstrating low physiological activity of table grapes.
362 Furthermore, the effect of temperature on respiration in fresh fruits and vegetables is
363 very significant (Cameron et al., 1994). A wide variety of enzymatic reactions are
364 involved in respiration. The rate of all of these reactions increases exponentially with
365 increasing temperature within the physiological temperature range (Exama et al., 1993).
366 The mathematical description of respiration increases (Q₁₀) of table grapes was around
367 2.12 such as main of fruits and vegetables.

368 **3.3 Predicted headspace O₂ and CO₂ concentration**

369 Fruit and vegetables consume oxygen and produce carbon dioxide while packed,
370 giving rise to a modification of the headspace gas composition (Jayas and Jeyamkondan,
371 2002). The respiration of the packed product and the gas permeability of the film
372 influence the change in gaseous composition of the environment surrounding the
373 product. As expected during cold storage, MAP was able to increase significantly CO₂
374 percentage with a tight decrease in O₂ concentrations in the surrounding of grapes
375 bunches. The gas equilibrium was reached after around 16 days for CO₂ and 3 days for
376 O₂ (15% and 1.8%, respectively) at 0 °C of storage (Figure 1). However during
377 simulated commercial shipping at 15 °C for one week, a fast increase in CO₂ and O₂
378 concentrations were recorded after one day reaching 15.8% and 2%, respectively, due to
379 the suddenly increase of temperature. The registred increase was followed by a severe
380 decrease in CO₂ concentration reaching 6.4% at the end of storage, while, O₂
381 concentration returned to the initial equilibrium (1.8%). Thus suggesting that headspace
382 gas condition in the package was affected by the increasing temperature, wich had an
383 impact on grapes respiration rate and film barrier properties.



384

385 **Figure 1.** Predicted evolution over storage of O₂ and CO₂ concentrations in the PAPE
386 bag headspace samples packed under active MAP.
387

388 **3.4 Weight loss**

389 The weight loss increased significantly in all samples as the storage time
390 increased; whereas, the effect of pretreatments were not significant at different sampling
391 time (Table 1). In all samples included packed control, the weight loss reached values
392 less than 1% after 45 days of cold storage and slightly higher than 1% after simulated
393 commercial shelf life (SL). However, in non-packed control weight loss reached 1.4%
394 after 15 days of cold storage (data not shown). These data confirmed the previous
395 reports on the effects of MAP and water permeation properties of the used film
396 regarding the preservation of table grapes moisture (Artés-Hernández et al., 2007,
397 Ngcobo et al., 2012).

398 **Table 1.** Effects of pretreatments and MAP on weight loss and decay incidence of organic table grapes cv. Scarlotta[®] during cold storage and
 399 after simulated commercial shelf life.
 400

Treatment	Weight loss (%)				Decay incidence (%)			
	15d	30d	45d	55d+SL	15d	30d	45d	55d+SL
Control-MAP	0,68a	0,65a	0,75b	1,19ab	0,02a	2,04a	2,51a	1,20b
O ₃ -5 µL L ⁻¹	0,47bc	0,54a	0,69bc	1,27a	0,55a	0,97ab	1,05ab	0,89b
O ₃ -10 µL L ⁻¹	0,51bc	0,56a	0,70bc	1,20ab	0,00a	0,02b	2,61a	2,90a
O ₃ -20 µL L ⁻¹	0,45c	0,60a	0,67c	1,20ab	0,00a	0,98ab	0,85b	0,06c
CO ₂ -50%	0,58b	0,63a	0,80ab	1,02b	0,00a	0,09b	1,03ab	0,00c
CO ₂ -70%	0,53bc	0,63a	0,84a	1,11ab	0,29a	0,58ab	0,45b	0,00c

Incidence data were transformed (arcsin of the square root of the proportion of affected fruit) before statistical analysis. Values presented are non-transformed means. Values followed by the same letter for each assesment time did not differ significantly according to HSD post-hoc test with FWER≤0.05.

401
 402
 403
 404
 405
 406
 407
 408

Table 2. Effects of pretreatments and MAP on physical and chemical qualities of organic table grapes cv. Scarlotta[®] after cold storage and simulated commercial shelf life.

Harvest time	Physical qualities						Chemical qualities					
	Berry detachment force 2.82 N		Berry firmness 11.83 N		Skin firmness 2.32 N		pH 3.23		SSC 15.34 %		TA 5.89 g tartaric acid L ⁻¹	
Treatment	45d	55d+SL	45d	55d+SL	45d	55d+SL	45d	55d+SL	45d	55d+SL	45d	55d+SL
Control-MAP	2.37a	2.31a	8.49ab	10.23ab	1.57ab	1.56ab	3.22a	3.29ab	15.32ab	14.75ab	5.99ab	5.44a
O ₃ -5 µL L ⁻¹	2.35a	2.15a	7.92b	9.26ab	1.19c	1.13c	3.22a	3.17c	14.94ab	13.40bc	6.26ab	5.52a
O ₃ -10 µL L ⁻¹	2.46a	1.99a	9.45a	10.50a	1.71a	1.55ab	3.22a	3.18c	15.53ab	15.54a	5.39bc	5.46a
O ₃ -20 µL L ⁻¹	2.18a	2.23a	7.88b	9.13b	1.71a	1.91a	3.25a	3.35a	15.95a	14.94a	6.53a	5.19a
CO ₂ -50%	2.31a	1.99a	9.05ab	10.32ab	1.39bc	1.48bc	3.28a	3.24ab	14.48b	14.70ab	5.58bc	5.16a
CO ₂ -70%	2.49a	2.21a	8.56ab	10.41ab	1.42ab	1.11c	3.27a	3.22bc	16.13a	13.14c	5.20c	5.21a

Mean values followed by the same letter for each assesment time did not differ significantly according to HSD post-hoc test with FWER≤0.05.

409

410 **Table 3.** Effects of pretreatments and MAP on color parameters of organic table grapes cv. Scarlotta[®] after cold storage and simulated
 411 commercial shelf life.
 412

Harvest time	<i>L</i> *		<i>h</i> [°]		CIRG	
	38.71		1.98		4.24	
Treatment	45d	55d+SL	45d	55d+SL	45d	55d+SL
Control-MAP	40.10a	38.65b	11.83a	17.72b	3.55c	3.58bc
O ₃ -5 μL L ⁻¹	40.70a	36.49c	-5.45bc	38.11a	3.97b	3.41bc
O ₃ -10 μL L ⁻¹	39.25a	38.90ab	-0.75ac	25.48ab	3.94bc	3.49bc
O ₃ -20 μL L ⁻¹	38.88a	36.80c	-12.24c	12.79b	4.43a	4.05a
CO ₂ -50%	40.44a	38.89ab	4.48ab	20.44ab	3.88bc	3.69b
CO ₂ -70%	41.51a	39.89a	5.27ac	35.41a	3.84bc	3.22c

413 Mean values followed by the same letter for each assessment time did not differ significantly according to HSD post-hoc test with FWER≤0.05.
 414
 415

416 **Table 4.** Effects of pretreatments and MAP on antioxidant activity, total phenolic compounds and total anthocyanins of organic table grapes cv.
 417 Scarlotta[®] stored at 0°C.
 418

Harvest time	Antioxidant activity (mmol kg ⁻¹)						Total phenolic content (mg GAE kg ⁻¹)						Total anthocyanins (mg Malvidin-3-glucoside kg ⁻¹) 110.89		
	Skin 78.32			Flesh 14.05			Skin 451.45			Flesh 141.97					
Treatment	15d	30d	45d	15d	30d	45d	15d	30d	45d	15d	30d	45d	15d	30d	45d
Control-MAP	75.44a	79.63a	78.18a	14.67b	14.63b	15.96ab	447.99ab	576.48a	417.05c	169.77ab	164.49ab	199.39a	103.38b	124.07ab	75.06c
O ₃ -5 μL L ⁻¹	53.60b	72.57ab	80.69a	15.02b	15.68ab	19.39a	434.88ac	418.32bd	422.25bc	166.10ab	193.35a	190.32a	106.03ab	127.42a	119.54a
O ₃ -10 μL L ⁻¹	69.03ab	75.28a	77.41a	12.08c	10.35c	17.31ac	399.81bc	451.48ad	563.93a	119.84b	139.69b	186.13a	112.84a	114.80c	116.59a
O ₃ -20 μL L ⁻¹	73.87a	84.2a	77.01a	14.58b	14.74b	17.94a	420.59ac	522.85abc	499.21ab	162.39ab	135.65b	187.88a	110.68ab	119.89b	125.49a
CO ₂ -50%	79.72a	86.24a	60.34b	18.94a	16.60a	13.83b	487.67a	411.05cd	377.49c	215.50a	160.96ab	171.53a	107.60ab	69.95d	62.57d
CO ₂ -70%	56.16b	57.64b	82.11a	11.62c	10.06c	14.80bc	372.42c	376.96d	535.79a	188.08a	170.37ab	156.08a	67.14c	67.90d	100.82b

419 Mean values followed by the same letter for each assessment time did not differ significantly according to HSD post-hoc test with FWER≤0.05.

420
421
422
423
424
425
426

Table 5. Effects of pretreatments and MAP on the concentration of individual anthocyanins of organic table grapes cv. Scarlotta[®] stored at 0°C.

Harvest time	Dp 0.96 mg kg ⁻¹			Cy 9.87 mg kg ⁻¹			Pt 1.56 mg kg ⁻¹			Pn 24.66 mg kg ⁻¹			Mv 11.62 mg kg ⁻¹			Ac-A 17.12 mg kg ⁻¹		
	15d	30d	45d	15d	30d	45d	15d	30d	45d	15d	30d	45d	15d	30d	45d	15d	30d	45d
Control-MAP	1.15a	1.34a	0.64a	7.85ab	5.83ac	10.44a	1.23a	1.90a	0.55a	20.54b	45.46a	24.15ab	13.64a	15.67a	5.53a	19.33a	21.55a	7.53b
O ₃ -5 μL L ⁻¹	0.88a	0.80ab	0.65a	10.57ab	12.87b	6.93ab	0.33b	0.89a	1.30a	12.18c	35.93ab	29.93ab	9.14a	6.66b	12.90a	12.91a	11.70b	18.33ab
O ₃ -10 μL L ⁻¹	0.80a	0.63ab	0.43a	9.68ab	9.50ab	7.72ab	0.62ab	0.77a	0.84a	23.08b	25.45bc	28.34ab	8.88a	6.14b	8.04a	14.35a	11.57b	17.24ab
O ₃ -20 μL L ⁻¹	0.70a	1.23ab	1.05a	10.69a	4.88ad	8.44ab	0.81ab	0.69a	1.58a	34.91a	20.85c	38.66a	6.39a	6.62b	11.60a	12.05a	10.18b	24.27a
CO ₂ -50%	0.64a	0.46ab	0.47a	4.74bc	4.38cd	6.08ab	1.05ab	0.66a	0.59a	34.40a	18.58c	14.74b	11.33a	5.28b	6.47a	17.12a	11.62b	12.81ab
CO ₂ -70%	0.47a	0.54ab	0.25b	1.08c	0.98d	4.44b	1.27a	0.98a	1.70a	21.54b	15.41c	25.28ab	12.52a	13.42a	14.76a	19.20a	21.28a	16.70ab

427
428
429
430
431
432
433
434
435
436
437
438

Dp, delphinidin-3-glucoside; Cy, cyanidin-3-glucoside; Pt, petunidin-3-glucoside; Pn, peonidin-3-glucoside; Mv, malvidin-3-glucoside, Ac-A, acylated
Mean values followed by the same letter for each assessment time did not differ significantly according to HSD post-hoc test with FWER≤0.05.

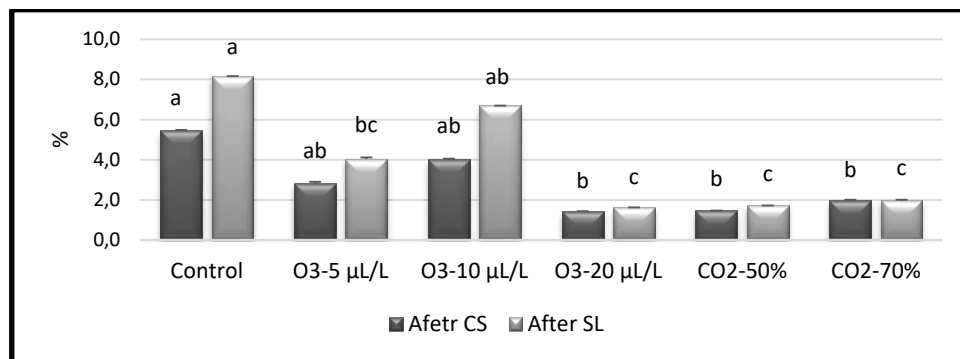
439 **3.5 Decay incidence**

440 The effects of pretreatments and storage time were investigated in naturally
441 infected clusters of organic table grapes cv. Scarlotta[®] (Table 1), where natural
442 incidence of decay was mostly caused by *B. cinerea*. The relative observations did
443 not show any significant differences between packed untreated and pretreated
444 samples until 15 days of cold storage with decay incidence around 0 to 0.55%, at the
445 opposite in non-packed control the decay attained 27% (data not shown).
446 Consequently, the non-packed control samples were excluded from the trial after the
447 first sampling time (15 days of CS). Moreover, the situation was worsening after 30
448 days of CS, where the packed control grapes reached 2.04% of decay; however, the
449 remaining pretreated grapes showed less than 1% decay. After 45 days of CS,
450 pretreated samples with O₃ at 20 µL L⁻¹ and CO₂ at 70% maintained significant high
451 control of decay (less than 1% decay) compared to packed control (2.51%) and a
452 remaining pretreatments. Furthermore, after 55 days of CS + SL, trend of decay
453 incidence decreased in samples pretreated with O₃ at 20 µL L⁻¹ and CO₂ reaching
454 values around 0% decay compared to packed control samples with 1.2% of decay.
455 Cumulative decay incidence confirmed also the efficiency of these pretreatments
456 (Figure 2); to control the decay after 45 days of CS and 55 days CS + SL by reducing
457 it 5 to 6 fold compared to packed control.

458 The effect of pretreatment with O₃ or CO₂ at high concentration to control
459 berries decay, was confirmed in previous work in which the decay decreased through
460 direct action against *B. cinerea*, including partial inhibition of conidia germination
461 (Mlikota Gabler et al., 2010, Karaca et al., 2012, Teles et al., 2014). The efficiency
462 of these gases in controlling decay could be due to the internal increase of ethanol

463 and acetaldehyde to fungal-toxic concentrations (Pesis, 2005) or the formation of
464 reactive oxygen species associated with stilbene synthase gene expression and
465 resveratrol accumulation (Sanchez-Ballesta et al., 2006; Romero et al., 2008; Minas
466 et al., 2010).

467 According to current E.U. marketing regulations EC N° 543/2011, the
468 maximum decay rate accepted is 1% by weight of table grapes bunches at the
469 receiving point for Class I EU grapes. During all storage period (CS and SL), all
470 samples packed under MAP, controlled decay incidence below this accepted
471 maximum rate (1%); moreover, samples pretreated with O₃ at 20 µL L⁻¹ and CO₂ at
472 50%, yielded cumulative decay incidence within this limit after CS and SL, by
473 reaching the minimal quality standards for commercial table grapes.



474
475
476
477
478
479
480
481

Figure 2. Effects of pretreatments and MAP on cumulative incidence of organic table grapes cv. Scarlotta® after cold storage (CS) and shelf life (SL). Cumulative incidence data were transformed (arcsin of the square root of the proportion of affected fruit) before statistical analysis. Values presented are non-transformed means. Values followed by the same letter for each assessment time did not differ significantly according to HSD post-hoc test with FWER≤0.05.

482 3.6 Physical and chemical qualities

483 During the 45 days of cold storage, no significant differences of physical and
484 chemical quality parameters were obtained between pretreated samples and control
485 on organic table grapes cv. Scarlotta® at different sampling time. However, after
486 simulated commercial shelf life (Table 2), some significant differences were
487 observed, such the decreases of skin firmness, in samples pretreated with CO₂ at 70%

488 (0.11 N) and O₃ at 5 μL L⁻¹ (0.12 N) compared to control (0.16 N). Likewise, pH and
489 SSC in samples pretreated with O₃ at 5 - 10 μL L⁻¹ (pH: 3.17 and 3.18, respectively)
490 and CO₂ at 70% (pH: 3.22 and SSC: 3.14%) compared to control (pH: 3.29 and SSC:
491 14.75). These registered variations remained very tiny and no relevant. Similar
492 results for changes in physical and chemical attributes were reported in “Red globe”
493 grapes stored at 0 °C under several controlled atmospheres for up to 3 months weeks
494 (Crisosto et al., 2002a), in “Superior seedless” grapes stored under different MAP
495 and after 7 days CS + SL (Artés-Hernández et al., 2006), and in “Flame Seedless”
496 grapes pretreated with 40% CO₂ and conserved under controlled atmosphere (Teles
497 et al., 2014).

498 **3.7 Berry color**

499 The obtained results showed that most pretreatments retained the L^* and h° of
500 berries over all sampling times with no relevant changes; however, after SL, L^*
501 decreased significantly regardless of pretreatments and the lower values were noted
502 in samples pretreated with O₃ at 5 and 20 μL L⁻¹ (36.49 and 36.8, respectively)
503 compared to control (38.65) (Table 3). In addition, h° increased significantly in
504 almost all samples except samples pretreated with O₃ at 20 μL L⁻¹ and packed control
505 (12.79 and 17.72) compared to value at harvest (1.98). These similar results were
506 already mentioned in unwrapped “Flame seedless” grapes after 18 days of CS
507 (Martínez-Romero et al., 2003), in organic “Crimson” grapes after 30 days of CS
508 immersed in hot ethanol (Mlikota Gabler et al., 2005), in “Red globe” grapes packed
509 in MAP bags during three months of CS (Candir et al., 2012). According to Mlikota
510 Gabler et al. (2005), the increases in h° and decrease in L^* values indicate a

511 progression in berry color toward brown, but the darker or deeper red color that they
512 reported are not visible to the naked eye.

513 In addition, CIRG permitted the objective definition of the external color in
514 all samples, based on this index, after SL only samples pretreated with O₃ at 20 μL L⁻¹
515 (4.05) maintained red skin color compared to the harvest time (4.24). Furthermore,
516 following the criterion of Carreno et al. (1996), the remaining samples presented
517 pink skin color with values ranging between 3.22 and 3.69.

518 **3.8 Rachis chlorophyll content**

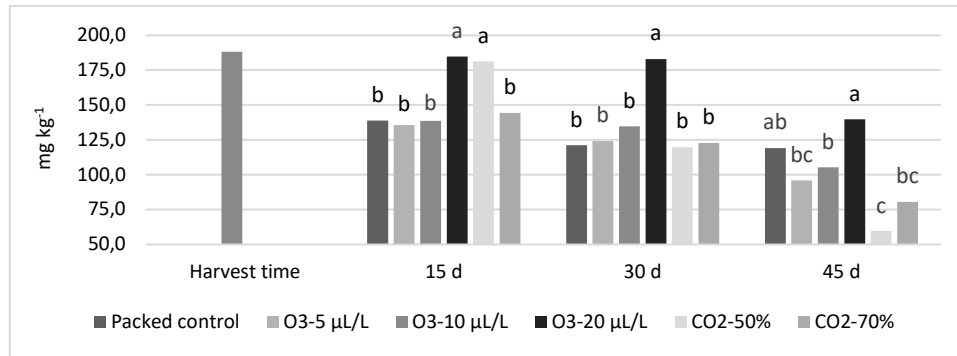
519 Rachis browning is considered the second most important postharvest
520 problem of table grapes after decay control, moreover for the consumer, a green
521 rachis is an indication of freshness, and hence, a brown rachis can be a major cause
522 of consumer rejection and eventually fruit waste (Lichter, 2016).

523 Compared to harvest values (188.09 mg kg⁻¹), trend of rachis chlorophyll
524 content decreased significantly during storage period, principally after 45 days of
525 cold storage where samples pretreated with CO₂ at 50 and 70% presented very low
526 concentration of chlorophyll (59.45 and 80.48 mg kg⁻¹, respectively) (Figure 3),
527 while, rachis green color was maintained in samples pretreated with O₃ at 20 μL L⁻¹
528 (139.89 mg kg⁻¹) followed by packed untreated samples (119.08 mg kg⁻¹). However,
529 only samples pretreated with O₃ at 20 μL L⁻¹ maintained chlorophyll content during
530 the whole period of CS. The obtained results concerning rachis chlorophyll content
531 confirmed the subjective results obtained by the panelist for rachis browning after 45
532 days of CS.

533 The green color loss or rachis browning affects overall cluster quality and has
534 been associated mainly to water loss (Valverde et al., 2005; Lichter et al., 2011) and

535 oxidation processes (Carvajal-Millán et al., 2001). However, several studies
536 suggested that other factors could be involved in rachis green color loss due to green
537 pigments degradation during the chlorophyll breakdown pathway and the consequent
538 formation of pheophytin-a by the putative enzyme Metal Chelating Substance (MCS)
539 (Shioi et al., 1996; Suzuki et al., 2005). Moreover, Hörtensteiner and Kräutler (2011)
540 reported that a non-enzymatic or a species-specific reaction generates a series of non-
541 colored catabolites that accumulates inside vacuole. Alternatively, results of the work
542 carried out by Carvajal-Millán et al. (2001) showed that clusters with severe rachis
543 browning symptoms had higher polyphenol oxidase (PPO) activity than clusters with
544 less rachis browning symptoms in “Flame Seedless” grapes. Additionally, Balic et al.
545 (2012) described a list of 30 senescence associative genes (SAGs). Suggesting that in
546 rachis of “Red Globe” grapes stored at 0 °C for 90 days, 10 genes increased the level
547 of transcript abundance, while another 7 did not show significant differences as
548 compared with harvest. Additionally, Silva-Sanzana et al. (2016) observed through
549 histological analysis of rachis that MAP storage increases rachis postharvest quality
550 of “Red Globe” grapes by reducing green color loss reported due to a combination of
551 processes involving a delay of green pigments degradation and a less accumulation
552 of brown compounds at the periderm and cortex tissues, thus preventing green
553 pigments masking.

554
555
556



557
558
559
560
561
562

Figure 3. Effects of pretreatments and MAP on rachis chlorophyll content of organic table grapes cv. Scarlotta® during cold storage.

Values followed by the same letter for each assessment time did not differ significantly according to HSD post-hoc test with $FWER \leq 0.05$.

563 3.9 Antioxidant activity and phenolic compounds

564 As expected, at harvest AA and total phenolic content in skin extract were
565 respectively more than 5 and 3 fold higher than those in flesh (Table 4) in accordance
566 with Pastrana-Bonilla et al. (2003).

567 Samples pretreated and untreated did not show the same trend for AA, total
568 phenolic content and total anthocyanins. During CS, AA in flesh berries increased in
569 all samples untreated and pretreated with O₃; this activity in skin berries was almost
570 maintained in all samples; while, in samples pretreated with CO₂ at 50%, AA
571 increased significantly in flesh and skin berries reaching high values after 15 and 30
572 days respectively, and decreased drastically at the end of storage. Regarding samples
573 pretreated with CO₂ at 70%, AA decreased significantly after 15 and 30 days of cold
574 storage and remained similar to those achieved in freshly harvested grapes. Sanchez-
575 Ballestra et al. (2007) and Romero et al. (2008) reported the same results in
576 “Cardinal” grapes treated with CO₂. The same observation was noted also regarding
577 the total phenol content, where the trend in flesh berries increased significantly in all
578 conserved samples, while in skin berries its concentration was maintained until the
579 end of storage in almost all samples, exception of those pretreated with CO₂. In

580 previous work it was demonstrated that the antioxidant capacity is dependent on the
581 level and type of phenolic compounds (Lutz et al., 2011).

582 Concerning the development of total anthocyanins in skin berries, the results
583 showed two distinguished trends. In samples pretreated with O₃ the concentration of
584 anthocyanins increased significantly, which was paralleled by an increase in
585 antioxidant activity during cold storage (Table 4), this correlation was also observed
586 in “Cardinal” grapes after 12 days of cold storage (Sanchez-Ballesta et al., 2007).
587 Which could be explained by the fact that O₃ reacted as elicitors for the biosynthesis
588 of phenolic compounds (Vincente et al., 2014).

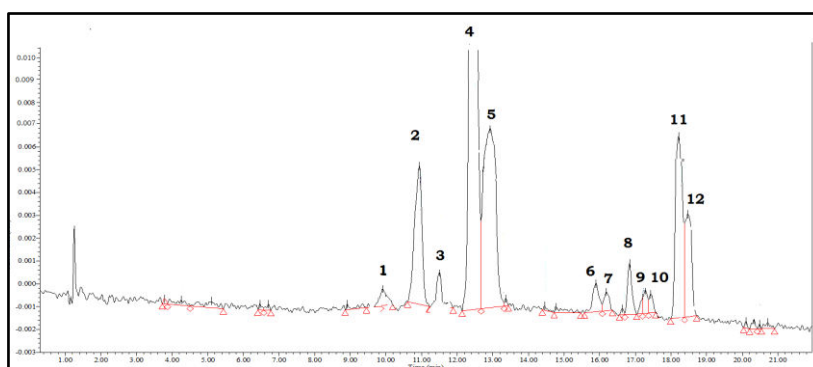
589 However, total anthocyanin decreased significantly in samples pretreated with
590 CO₂ during storage period to reach values of 100.82 and 62.57 mg Malvidin-3-
591 glucoside kg⁻¹ in samples pretreated with CO₂ at 70% and 50%, respectively. The
592 results obtained are in agreement with those of Artés-Hernandez et al. (2003) which
593 observed a decrease in total anthocyanin content in “Napoleon” grapes when using
594 an atmosphere of 15% CO₂ and 5% O₂. Even if, it is already known that anthocyanin
595 synthesis continues after harvest and also during long-term cold storage by activating
596 phenylpropanoid gene expression, total anthocyanin accumulation and antioxidant
597 activity, whereas the application of CO₂ treatment reduces/inhibits these responses
598 (Sanchez-Ballesta et al., 2007, Romero et al., 2008).

599 **3.10 Anthocyanins content**

600 The anthocyanin profile consisted of twelve compounds (seven of them
601 acylated), with the prevalence of peonidin (Pn) forms (Figure 4) in agreement with
602 what was reported by Cantos et al. (2002); Sanchez-Ballesta et al. (2007) and Ferrara
603 et al. (2015). The most abundant anthocyanin present was peonidin-3-glucoside

604 (37%), followed by malvidin-3-glucoside (18%), cyanidin-3-glucoside (15%), and in
605 lower quantities than other anthocyanins: petunidin-3-glucoside (2%) and
606 delphinidin-3-glucoside (1%) (Table 5). According to Liang et al. (2011), the most
607 abundant anthocyanin present in pink and red-colored cultivars was Pn forms,
608 whereas cyanidin, malvidin and petunidin forms were abundant in red-black
609 cultivars. This suggests that different proportions of individual anthocyanin
610 compounds, in addition to their absolute amount, can affect the skin color of grapes
611 (Ferrara et al., 2015). This result was confirmed at the end of cold storage where
612 pretreatment with O₃ at 20 μL L⁻¹ presented the highest concentration of Pn (38.66
613 mg kg⁻¹) and CIRG (4.43).

614 The individual anthocyanins in almost all samples showed not significant
615 variation during storage period, and their values remained similar to those achieved
616 in freshly harvested grapes. Except for cyanidin and delphinidin, where their
617 concentrations decreased significantly in samples pretreated with CO₂ at 70%.
618 Moreover, peonidin and acylated anthocyanins showed a similar trend with total
619 anthocyanin content in samples pretreated with O₃ at 20 μL L⁻¹.



620
621 **Figure 4.** Anthocyanin profile of a skin extract of berries at harvest from organic
622 table grapes cv. Scarlotta[®] berries [peaks: 1 = delphinidin-3-glucoside; 2 =
623 cyanidin-3-glucoside, 3 = petunidin-3-glucoside, 4 = peonidin-3-glucoside; 5 =
624 malvidin-3-glucoside; 6–12 = acylated anthocyanins].

625

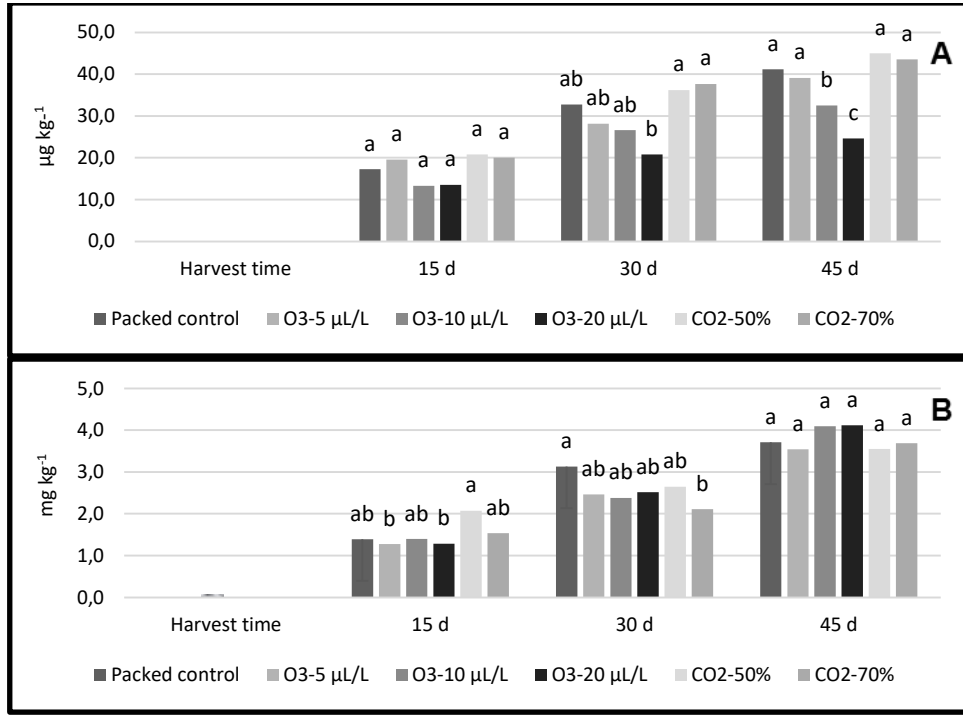
626 **3.11 Acetaldehyde and ethanol**

627 During sampling time, almost all samples presented similar chromatogram
628 profile with the same volatile compounds. The chromatogram of total volatile
629 compounds showed that during storage period, several aromatic peaks disappeared
630 and new peaks became more visible like ethanol or appeared as Acetaldehyde. The
631 presence of these compounds in table grapes are mainly associated with fermentative
632 and biochemical changes induced by progressive maturation, which could induce
633 perceived off-flavor (Candir et al., 2012, Teles et al., 2014, Piazzolla et al., 2015).

634 Compared to the acetaldehyde and ethanol values at harvest ($0 \mu\text{g kg}^{-1}$ and
635 0.08 mg kg^{-1} , respectively), trend of both components increased significantly during
636 storage period in all samples (Figure 5). Several not significant variation of
637 acetaldehyde and ethanol compounds were observed during conservation between
638 samples at different sampling time ($P < 0.01$). Except at the end of cold storage, where
639 samples pretreated with O_3 at 20 and $10 \mu\text{L L}^{-1}$ present significant low values of
640 acetaldehyde (24.59 and $32.51 \mu\text{g kg}^{-1}$) compared to packed untreated samples
641 ($41.16 \mu\text{g kg}^{-1}$). In addition, samples pretreated with O_3 at $20 \mu\text{L L}^{-1}$ presented
642 perceived low trend of acetaldehyde paralleled with trend of remaining samples.
643 Samples pretreated with CO_2 and packed untreated presented high concentration of
644 acetaldehyde during period of storage, similar result was also obtained by Teles et al.
645 (2014) on organic “Flame Seedless” grapes pre-stored with CO_2 at 40% for 24 - 48 h
646 and conserved for four weeks. According to Candir et al. (2012), a low concentration
647 of O_2 induces anaerobic respiration, which leads to undesirable metabolic reactions,
648 such as tissue breakdown and accumulation of acetaldehyde and ethanol in the tissue,
649 resulting in off-odors and off-flavors. The results concerning acetaldehyde and

650 ethanol content in berries at the end of cold storage confirmed the subjective results
 651 obtained by the panelist for the perceiving off-flavor after 45 days of CS.

652



653

654

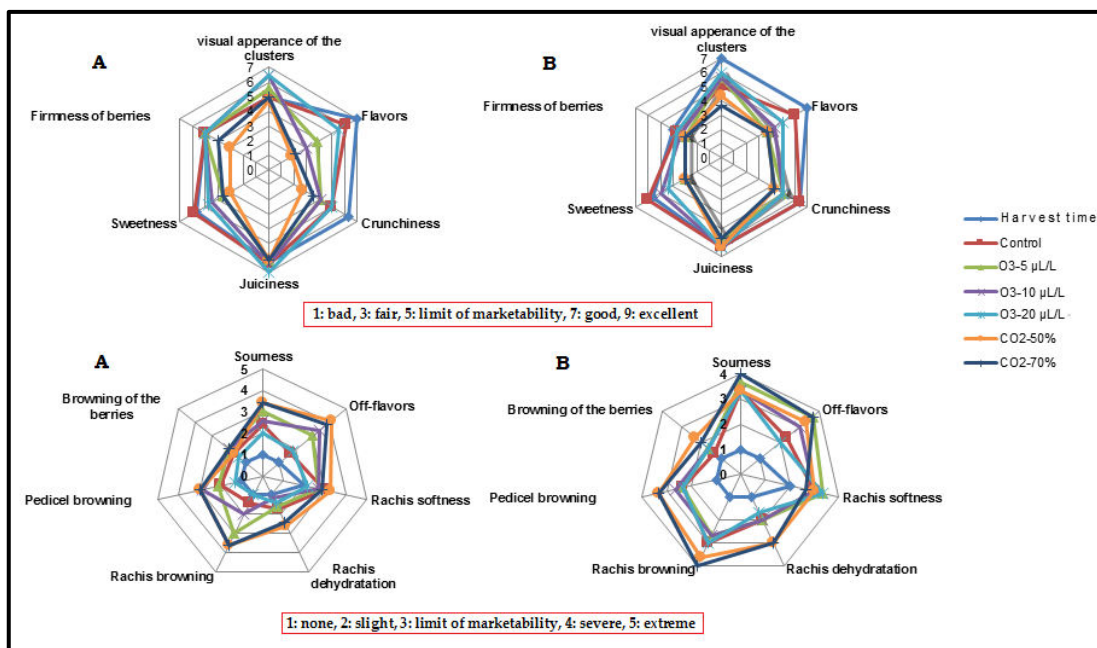
655 **Figure 5.** Effects of pretreatments and MAP on acetaldehyde (A) and ethanol (B)
 656 content on organic table grapes cv. Scarlotta[®] during cold storage.
 657 Values followed by the same letter for each assessment time did not differ significantly according to
 658 HSD post-hoc test with FWER_≤0.05

659 3.12 Sensory quality

660 After 45 days of cold storage, clusters pretreated with O₃ maintained good
 661 visual appearance compared to the control and good aroma was mainly conserved by
 662 untreated and treated samples with O₃ at 20 µL L⁻¹, while it decreased drastically in
 663 clusters pretreated with CO₂ at 50 and 70% (Figure 6). The same results were
 664 obtained after SL, except for good aroma, where also, samples pretreated with O₃ at
 665 5 and 10 µL L⁻¹ presented fair score. Furthermore, as described after 45 days of CS
 666 regarding juiciness and firmness of berries, also after SL almost all samples
 667 preserved juiciness of berries, for firmness untreated packed samples and pretreated
 668 with O₃ at 20 µL L⁻¹ were at limit of marketability while in remaining samples, it was

669 judged as fair. Concerning sweetness after SL; packed control and pretreated samples
670 with O₃ at 20 and 10 μL L⁻¹ obtained limit of marketability score, while in remaining
671 samples was refereed as very fair (Figure 6).

672 Equally, samples at both sampling time presented sourness in the taste, which
673 was considered as fair in almost all samples after 45 days of CS and fair in packed
674 control and pretreated samples with O₃ at 20 μL L⁻¹. Although after SL, in practically
675 all samples the score increased to reach limit marketable score and was perceived as
676 severe in samples pretreated with O₃ at 5 μL L⁻¹ and CO₂ at 70%. Moreover after SL,
677 the panelist detected severe off-flavor in berries pretreated with O₃ at 5 μL L⁻¹ and
678 CO₂ at 70%, limit of marketability in samples pretreated with O₃ at 10 μL L⁻¹ and
679 CO₂ at 50%, and fair in packed control and pretreated samples with O₃ at 20 μL L⁻¹.
680 The observed off-flavors could be due to an accumulation of fermentative volatiles
681 compounds in berries like ethanol and acetaldehyde (Candir et al., 2012; Teles et al.,
682 2014). Consequently, gas composition inside package did not influence grapes flavor;
683 our data confirm results obtained by Artés-Hernández et al., (2006) in “Superior
684 Seedless” grapes stored under MAP. Regarding rachis browning attribute, SL
685 increased the perceptibility of rachis browning to limit of marketability especially in
686 packed control and pretreated samples with O₃ at 10 and 20 μL L⁻¹, comparing to
687 samples stored for 45 days. This stem browning could be correlated to the
688 condensation of CO₂ inside package (Crisosto et al., 2002a, Chen et al., 2011, Candir
689 et al., 2012, Teles et al., 2014), produced by the accelerated respiration of the
690 products generated mainly by the stress of high temperature during SL.



691
692
693
694

Figure 6. Effects of pretreatments and MAP on organic table grapes cv. Scarlotta[®] sensory quality after CS (A) and SL (B).

695 4 Conclusion

696 The efficiency of modified atmosphere (MA) and film barrier properties was
697 highlighted to reduce water loss and maintain mechanical and chemical
698 characteristics on late-season organic table grapes (Scarlotta[®]) throughout a long
699 conservation according to market requirements for 55 days of CS and one week of
700 simulated commercial SL. O₃ could be a commercial alternative to the use of SO₂
701 generators for keeping an acceptable visual appearance of cluster close to that at
702 harvest and increased total anthocyanin accumulation, total phenol content and
703 antioxidant activity during CS. Moreover, pretreatment with O₃ at 20 µL L⁻¹ was the
704 most effective for controlling decay incidence by maintaining EU commercial
705 standards until 55 days of CS and one week of SL, preventing stem browning and
706 off-flavor production, in addition a good preserving of sensory quality and skin color
707 with positive influence on the pathways leading to the synthesis of the different
708 anthocyanins. Thus, combination of pretreatment with O₃ at 20 µL L⁻¹ and MAP

709 during storage, transportation or marketing could be a commercially practical
710 alternative for postharvest handling of organic grapes. Therefore, further trials with
711 other cultivars should be worth to validate the applied protocol.

712 **References**

713 Acevado De la Cruz, A., Hilbert, G., Rivière, C., Mengin, V., Ollat, N.,
714 Bordenave, L., Decroocq, S., Delaunay, J.C., Delrot, S., Mérillon, J.M., Monti,
715 J.P., Gomès, E., Richard, T., 2012. Anthocyanin identification and composition
716 of wild *Vitis* spp. accessions. *Anal. Chim. Acta.* 732, 145-152.

717 Admane, N., Altieri, G., Genovese, F., Di Renzo, G.C., Verrastro, V., Tarricone,
718 L, Ippolito, A., 2015. Application of High Carbon Dioxide or Ozone Combined
719 with MAP on Organic Late-Season ‘Scarlotta Seedless[®]’ Table Grapes. *Acta*
720 *Hort. (ISHS).* 1079, 193-199.

721 Anon., 1986. GRAS status of sulfating agents for use on fresh and frozen foods
722 revoked. *Fed. Regist.* 5, 25021.

723 Artés, F., 1976. Estudio y aplicacion de membranas de polimeros paragenerar y
724 estabilizar atmosferas modificadas. CEBAS-CSIC, Murcia, Spain, pp. 294.

725 Artés-Hernández, F., Aguayo, E., Artés, F., 2004. Alternative gas treatments for
726 keeping quality of ‘Autumn seedless’ table grapes during long term cold storage.
727 *Postharvest Biol. Technol.* 31, 59-67.

728 Artés-Hernández, F., Aguayo, E., Artés, F., Tomás-Barberán, F.A., 2007.
729 Enriched ozone atmosphere enhances bioactive phenolics in seedless table grapes
730 after prolonged shelf life. *J. Sci. Food Agric.* 87, 824-831.

731 Artés-Hernández, F., Artés, F., Tomás-Barberán, F.A., 2003. Quality and
732 enhancement of bioactive phenolics in cv. Napoleon table grapes exposed to
733 different postharvest gaseous treatments. *J. Agric. Food Chem.* 51, 5290-5295.

734 Artés-Hernández, F., Tomás-Barberán, F.A., Artés, F., 2006. Modified
735 atmosphere packaging preserves quality of SO₂-free 'Superior seedless' table
736 grapes. *Postharvest Biol. Technol.* 39, 146-154.

737 Baiano, A., Lamacchia, C., Previtali, M.A., Tufariello, M., Arace, E., Notte, E.L.,
738 2007. Influence of post-harvest treatments on the quality of table grape from
739 Apulia (Italy).
740 <http://www.oiv2007.hu/documents/viticulture/247_baiano_poster.pdf>

741 Balic, I., Moreno, A., Sanhueza, D., Huerta, C., Orellana, A., Defilippi, B.G.,
742 Campos-Vargas R., 2012. Molecular and physiological study of postharvest
743 rachis browning of table grape cv Red Globe. *Postharvest Biol. Technol.* 72, 47-
744 56.

745 Caleb, J.O., Mahajan, V.P., Al-Said, A.F., Opara, L.U., 2013. Modified
746 atmosphere packaging technology of fresh and fresh-cut produce and the
747 microbial consequences-a review. *Food Bioprocess Technol.* 6, 303-329.

748 Cameron, A.C., Beaudry, R.M., Banks, N.H., Yelanich, M.V., 1994. Modified
749 atmosphere packaging of blueberry fruit: modelling respiration and package
750 oxygen partial pressures as function of temperature. *J. Am. Soc. Hortic. Sci.* 119,
751 534-539.

752 Candir, E., Ozdemir, A.E., Kamiloglu, O., Soyulu, E.M., Dilbaz, R., Ustun, D.,
753 2012. Modified atmosphere packaging and ethanol vapor to control decay of
754 'Red Globe' table grapes during storage. *Postharvest Biol. Technol.* 63, 98-106.

755 Cantos, E., Espin, J.C., Barberan, F.A.T., 2002. Varietal differences among the
756 polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS.
757 *J. Agric. Food Chem.* 50, 5691-5696.

758 Carreño, J., Martínez, A., Almela, L., Fernández-López, J.A., 1996. Measuring
759 the color of table grapes. *Color Res. Appl.* 21, 50-54.

760 Carreño, J., Martínez, A., Almela, L., Fernández-López, J.A., 1995. Proposal of
761 an index for the objective evaluation of the colour of red table grapes. *Int. Food*
762 *Res. J.* 28, 373-377.

763 Carvajal-Millán, E., Carvallo, T., Orozco, J., Martínez, M., Tapia, I., Guerrero,
764 V., Rascón-Chu, A., Llamas, J., Gardea, A., 2001. Polyphenol oxidase activity,
765 color changes, and dehydration in table grape rachis during development and
766 storage as affected by N-(2-chloro-4-pyridyl)-N-phenylurea. *J. Agric. Food*
767 *Chem.* 49, 946–951.

768 Cayuela, J.A., Vazquez, A., Perez, A.G., Garcia, J.M., 2009. Control of table
769 grapes postharvest decay by ozone treatment and resveratrol induction. *Food Sci.*
770 *Tech. Inst.* 15, 495-502.

771 Chen, S., Zhang, M., Wang, S., 2011. Effect of initial hermetic sealing on quality
772 of ‘Kyoho’ grapes during storage. *Postharvest Biol. Technol.* 59, 194-199.

773 Chervin, C., Aked, J., Crisosto, C.H., 2012. Grapes, in: Rees, D., Farrell, G.,
774 Orchard, J. (Eds.), *Crop post-Harvest: Science and Technology*. Blackwell
775 Publishing Ltd., Oxford, OX4 2DQ, UK, pp. 187–211.

776 Chervin, C., Westercamp, P., Monteils, G., 2005. Ethanol vapours limit *Botrytis*
777 development over the postharvest life of table grapes. *Postharvest Biol. Technol.*
778 36, 319-322.

779 Ciccarese, A., Stellacci, A.M., Gentileco, G., Rubino, P., 2013. Effectiveness of
780 pre- and post-veraison calcium applications to control decay and maintain table
781 grape fruit quality during storage. *Postharvest Biol. Technol.* 75, 135-141.

782 Crank, J, Park, G.S., 1968. Diffusion in polymers. London, Academic Press, pp.
783 360.

784 Crisosto, C.H., Garner, D., Crisosto, G., 2002a. Carbon dioxide-enriched
785 atmospheres during cold storage limit losses from Botrytis but accelerate rachis
786 browning of 'Redglobe' table grapes. *Postharvest Biol. Technol.* 26, 181-189.

787 Crisosto, C.H., Garner, D., Crisosto, G., 2002b. High carbon dioxide atmospheres
788 affect stored 'Thompson Seedless' table grapes. *Hortscience.* 37, 1074-1078.

789 Crisosto, C.H., Mitchell, F.G., 2002. Postharvest handling systems: small fruits.
790 Table grapes, in: Kader, A. (Ed.), *Postharvest Technology of Horticulture Crops.*
791 University of California, Agriculture and Natural Resources, Oakland, pp. 357-
792 363.

793 Exama, A., Arul, J., Lencki, R.W., Lee, L.Z, Toupin, C., 1993. Suitability of
794 plastic Films for modified atmosphere packaging of fruits and vegetables. *J. Food*
795 *Sci.* 58, 1365-1370.

796 Ferrara, G., Mazzeo, A., Matarrese, A.M.S., Pacucci, C., Punzi, R., Faccia, M.,
797 Trani, A., Gambacorta, G., 2015. Application of abscisic acid (S-ABA) and
798 sucrose to improve colour, anthocyanin content and antioxidant activity of cv.
799 Crimson Seedless grape berries. *Aust. J. Grape Wine Res.* 21, 18-29.

800 Fonseca, S.C., Oliveira F.A.R., Brecht, J.K., 2002. Modelling respiration rate of
801 fresh fruits and vegetables for modified atmosphere packages: a review. *J. Food*
802 *Eng.* 52, 99-119.

803 Gambacorta, G., Antonacci, D., Pati, S., La Gatta, M., Faccia, M., Coletta, A., La
804 Notte, E., 2011. Influence of winemaking technologies on phenolic composition
805 of Italian red wines. *Eur. Food Res. Technol.* 233, 1057-1066.

806 Hagenmaier, R.D., 2005. A comparison of ethane, ethylene and CO₂ peel
807 permeance for fruit with different coatings. *Postharvest Biol. Technol.* 37, 56-64.

808 Hagggar, P.E., D.S. Lee, Yam, K.L., 1992. Application of an enzyme kinetics
809 based respiration model to closed system experiments for fresh produce. *J. Food*
810 *Proc. Eng.* 15,143-157.

811 Hörtensteiner, S., Kräutler, B., 2011. Chlorophyll breakdown in higher plants.
812 *BBA Bioenerg.* 1807, 977-988.

813 Jayas, D.S., Jeyamkondan, S., 2002. Modified atmosphere storage of grains
814 meats fruits and vegetables. *Biosyst. Eng.* 82, 235-251.

815 Kader, A.A., Zagory, D. Kerbel, E.L., 1989. Modified atmosphere packaging of
816 fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* 28, 1-30.

817 Karaca, H., Walse, S.S., Smilanick, J.L., 2012. Effect of continuous 0.3 µL L⁻¹
818 gaseous ozone exposure on fungicide residues on table grape berries. *Postharvest*
819 *Biol. Technol.* 64, 154-159.

820 Khadre, M.A., Yousef, A.E., Kim, J.G., 2001. Microbial aspects of ozone
821 applications in food: a review. *J. Food Sci.* 66, 1242-1252.

822 Lamikanra, O., 2002. *Fresh-cut fruits and vegetables: science, technology, and*
823 *market.* Boca Raton, Fla., CRC Press., pp. 480.

824 Larsen, H., Kohler, A., Magnus, E.M., 2000. Ambient Oxygen Ingress Rate
825 Method-An Alternative Method to Ox-Tran for Measuring Oxygen Transmission
826 Rate of Whole Packages. *Packag. Technol. Sci.* 13, 233-241.

827 Lee, D.S., Hagggar, P.E., Lee, J., Yam, K.L., 1991. Model for fresh produce
828 respiration in modified atmospheres based on principles of enzyme-kinetics. *J.*
829 *Food Sci.* 56,1580-1585.

830 Lee, J., 1987. The design of controlled or modified packaging systems for fresh
831 produce, in: Gray, J.I., Harte, B.R., J. Miltz (eds.), Food product-package
832 compatibility proceedings. Technomic Publishing, Lancaster, Pa, pp. 157.

833 Liang, Z., Owens, C.L., Zhon, G., Cheng, L., 2011. Polyphenolic profiles
834 detected in the ripe berries of *Vitis vinifera* germplasm. Food Chem. 129, 940-
835 950.

836 Lichtenthaler, H.K., 1987. Chlorophylls and Carotenoids: Pigments of
837 Photosynthetic Biomembranes. Methods in Enzymology. Academic Press. 147,
838 350-382.

839 Lichter, A., 2016. Rachis browning in tablegrapes. Aust. J. Grape Wine Res. 22,
840 161-168.

841 Lichter, A., Kaplunov, T., Zutahy, Y., Daus, A., Alchanatis, V., Ostrovsky, V.,
842 Lurie, S., 2011. Physical and visual properties of grape rachis as affected by
843 water vapor pressure deficit. Postharvest Biol. Technol. 59, 25-33.

844 Lichter, A., Mlikota Gabler, F., Smilanick, J.L., 2006. Control of spoilage in
845 table grapes. Stewart Postharvest Review. 2, 1-10.

846 Lutz, M., Jorquera, K., Cancino, B., Ruby, R., Henriquez, C., 2011. Phenolics
847 and Antioxidant Capacity of Table Grape (*Vitis vinifera* L.) Cultivars Grown in
848 Chile. J. Food Sci. 76, C1088-C1093.

849 Luvisi, D., Shorey, H., Smilanick, J.L., Thompson, J., Gump, B.H., Knutson, J.,
850 1992. Sulfur dioxide fumigation of table grapes. Bulletin 1932, University of
851 California, Division of Agriculture and Natural Resources, Oakland, CA, USA,
852 pp. 21.

853 Martínez-Romero, D., Guillén, F., Castillo, S., Valero, D., Serrano, M., 2003.
854 Modified Atmosphere Packaging Maintains Quality of Table Grapes. *J. Food Sci.*
855 68, 1838-1843.

856 Minas, I.S., Karaoglanidis, G.S., Manganaris, G.A., Vasilakakis, M., 2010. Effect
857 of ozone application during cold storage of kiwifruit on the development of stern-
858 end rot caused by *Botrytis cinerea*. *Postharvest Biol. Technol.* 58, 203-210.

859 Mlikota Gabler, F., Smilanick, J.L., 2001. Postharvest control of table grape gray
860 mold on detached berries with carbonate and bicarbonate salts and disinfectants.
861 *J. Enol. Vitic.* 52, 12-20.

862 Mlikota Gabler, F., Smilanick, J.L., Ghosop, J.M., Margosan, D.A., 2005.
863 Impact of Postharvest Hot Water or Ethanol Treatment of Table Grapes on Gray
864 Mold Incidence, Quality, and Ethanol Content. *Plant Dis.* 89, 309-316.

865 Mlikota Gabler, F., Smilanick, J.L., Mansour, M.F., Karaca, H., 2010. Influence
866 of fumigation with high concentrations of ozone gas on postharvest gray mold
867 and fungicide residues on table grapes. *Postharvest Biol. Technol.* 55, 85-90.

868 Ngcobo, M.E.K., Delele, M.A., Pathare, P.B., Chen, L., Opara, U.L., Meyer, C.J.,
869 2012. Moisture loss characteristics of fresh table grapes packed in different film
870 liners during cold storage. *Biosyst. Eng.* 113, 363-370.

871 Pastrana-Bonilla, E., Akoh, C.C., Sellappan, S., Krewer, G., 2003. Phenolic
872 content and antioxidant capacity of Muscadine grapes. *J. Agric. Food Chem.* 51,
873 5497-5503.

874 Pesis, E., 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol,
875 in fruit ripening, enhancement of fruit quality and fruit deterioration. *Postharvest*
876 *Biol. Technol.* 37, 1-19.

877 Piazzolla, F., Pati, S., Amodio, M.L., Colelli, G., 2015. Effect of harvest time on
878 table grape quality during on-vine storage. *J. Sci. Food Agric.* 96, 131-139.

879 Re, R., Pellegrini, N., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant
880 activity applying an improved ABTS radical cation decolorization assay. *Free*
881 *Radic. Biol. Med.* 26, 1231-1237.

882 Retamales, J., Defilippi, B.G., Arias, M., Castillo, P., Manriquez, D., 2003. High-
883 CO₂ controlled atmospheres reduce decay incidence in Thompson Seedless and
884 Red Globe table grapes. *Postharvest Biol. Technol.* 29, 177-182.

885 Revilla, E., Ryan, L.M., 2000. Analysis of several phenolic compounds with
886 potential antioxidant properties in grape extracts and wines by high-performance
887 liquid chromatography-photodiode array detection without sample preparation. *J.*
888 *Chromatogr. A* 881, 461-469.

889 Romanazzi, G., Lichter, A., Mlikota Gabler, F., Smilanick, J.L., 2012. Recent
890 advances on the use of natural and safe alternatives to conventional methods to
891 control postharvest gray mold of table grapes. *Postharvest Biol. Technol.* 63,
892 141-147.

893 Romero, I., Fernandez Caballero, C., Sanchez-Ballesta, M.T., Escribano, M.I.,
894 Merodio, C., 2009. Influence of the stage of ripeness on phenolic metabolism and
895 antioxidant activity in table grapes exposed to different CO₂ treatments.
896 *Postharvest Biol. Technol.* 54, 118-121.

897 Romero, I., Sanchez-Ballesta M.T., Maldonado, R., Isabel Escribano, M.,
898 Merodio, C., 2008. Anthocyanin, antioxidant activity and stress-induced gene
899 expression in high CO₂-treated table grapes stored at low temperature. *J. Plant*
900 *Physiol.* 165, 522-530.

901 Sanchez-Ballesta, M.T., Jiménez, J.B., Romero, I., Orea, J.M., Maldonado, R.,
902 Ureña, Á.G., Escribano, M.I., Merodio, C., 2006. Effect of high CO₂
903 pretreatment on quality, fungal decay and molecular regulation of stilbene
904 phytoalexin biosynthesis in stored table grapes. *Postharvest Biol. Technol.* 42,
905 209-216.

906 Sanchez-Ballesta, M.T., Romero, I., Jiménez, J.B., Orea, J.M., González-Ureña,
907 Á., Escribano, M.I., Merodio, C., 2007. Involvement of the phenylpropanoid
908 pathway in the response of table grapes to low temperature and high CO₂ levels.
909 *Postharvest Biol. Technol.* 46, 29-35.

910 Sharpe, D., Fan, L., McRae, K., Walker, B., Mackay, R., Doucette, C., 2009.
911 Effects of ozone treatment on *Botrytis cinerea* and *Sclerotinia sclerotiorum* in
912 relation to horticultural product quality. *J. Food Sci.* 74, 250-257.

913 Shioi, Y., Tomita, N., Tsuchiya, T., Takamiya, K.-I., 1996. Conversion of
914 chlorophyllide to pheophorbide by Mg-dechelating substance in extracts of
915 *Chenopodium album*. *Plant Physiol. Bioch.* 34, 41-47.

916 Silva-Sanzana, C., Balic, I., Sepúlveda, P., Olmedo, P., León, G., Defilippi, B.G.,
917 Blanco-Herrera, F., Campos-Vargas, R., 2016. Effect of modified atmosphere
918 packaging (MAP) on rachis quality of 'Red Globe' table grape variety.
919 *Postharvest Biol. Technol.* 119, 33-40.

920 Singh Brar, H., Singh, Z., Swinny, E., 2008. Dynamic of anthocyanin and
921 flavonol profiles in the 'Crimson Seedless' grape berry skin during development
922 and ripening. *Sci. Hortic.* 117, 349-356.

923 Smilanick, J.L., Hartsell, P.I., Henson, D., Fouse, D.C., Assemi, M., Harris,
924 C.M., 1990. Inhibitory activity of sulfur-dioxide on the germination of spores of
925 *Botrytis cinerea*. *Phytopathology.* 80, 217-220.

926 Smilanick, J.L., Mlikota Gabler, F., Margosan, D., 2010. Influence of continuous,
927 low concentration ozone during cold storage on postharvest decay and quality of
928 table grapes. In: Proceedings "6th International Table grape Symposium", Davis,
929 CA, USA, pp. 85-86.

930 Suzuki, T., Kunieda, T., Murai, F., Morioka, S., Shioi, Y., 2005. Mg-dechelation
931 activity in radish cotyledons with artificial and native substrates: mg-
932 chlorophyllin a and chlorophyllide a. *Plant Physiol. Biochem.* 43, 459-464.

933 Teles, C.S., Benedetti, B.C., Gubler, W.D., Crisosto, C.H., 2014. Prestorage
934 application of high carbon dioxide combined with controlled atmosphere storage
935 as a dual approach to control *Botrytis cinerea* in organic "Flame Seedless" and
936 "Crimson Seedless" table grapes. *Postharvest Biol. Technol.* 89, 32-39.

937 US Food and Drug Administration (US FDA), 2001. Title 21: Food and Drugs
938 Part 173. Secondary direct food additives permitted in food for human
939 consumption subpart D, specific usage additives. *Fed. Reg.*, 66, 33829.

940 Ustun, D., Candir, E., Ozdemir, A.E., Kamiloglu, O., Soylu, E.M., Dilbaz, R.,
941 2012. Effects of modified atmosphere packaging and ethanol vapor treatment on
942 the chemical composition of 'Red Globe' table grapes during storage. *Postharvest
943 Biol. Technol.* 68, 8-15.

944 Valero, D., Valverde, J. M., Martínez-Romero, D., Guillén, F., Castillo, S.,
945 Serrano, M., 2006. The combination of modified atmosphere packaging with
946 eugenol or thymol to maintain quality, safety and functional properties of table
947 grapes. *Postharvest Biol. Technol.* 41, 317-327.

948 Valverde, J.M., Valero, D., Martínez-Romero, D., Guillén, F., Castillo, S.,
949 Serrano, M., 2005. Novel edible coating based on Aloe vera gel to maintain table
950 grape quality and safety. *J. Agric. Food Chem.* 53, 7807-7813.

951 Vincente Ariel, R., Manganaris George, A., Ortiz Cristian, M., Sozzi Gabriel, O.,
952 Crisosto Carlos, H., 2014. Nutritional Quality of Fruits and Vegetables, in:
953 Von Gunten U., 2003. Ozonation of drinking water: Part II. Disinfection and by-
954 product formation in presence of bromide, iodide or chlorine. *Water Res.* 37,
955 1469-1487.

956 Wilson, C.L., 1997. Biological control and plant diseases- a new paradigm. *J.*
957 *Ind. Microbiol. Biotechnol.* 19, 158-159.

958 Zoffoli, J.P., Latorre, B.A., Naranjo, P., 2008. Hairline, a postharvest cracking
959 disorder in table grapes induced by sulfur dioxide. *Postharvest Biol. Technol.* 47,
960 90-97.