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

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Abstract

The aim of this study was to maintain the quality of organic table grapes and extend their shelf life for long-term storage by using organically approved methods. The effectiveness of the pretreatments with different concentrations of gaseous ozone (varying from 5 to 20 µL L⁻¹) or carbon dioxide (at 50 and 70%) followed by storage under modified atmosphere packaging (2% O₂: 5% CO₂: 93% N₂ MAP) were evaluated on late-season organic Scarlotta® grapes as alternatives to usual commercial SO₂ application. After 45 days of cold storage (CS), pretreatments with O₃ increased significantly total anthocyanins at the opposite of pretreatments with CO₂. Furthermore,
pretreatments with O$_3$ at 20 µL L$^{-1}$ controlled concentration of acetaldehyde, preserved rachis chlorophyll content and skin color during CS.

Regarding cumulative decay incidence, it was reduced 5 to 6 fold by pretreatments with O$_3$ at 20 µL L$^{-1}$ and CO$_2$, compared to control after shelf life (SL), however, pretreatments with CO$_2$ caused also organoleptic quality loss with strong stem browning and perceived off-flavor. The present experiment revealed the efficiency of pretreatment with O$_3$ at 20 µL L$^{-1}$ to preserve initial sensory quality of organic Scarlotta® grapes and to control efficiently grape decay after CS and SL. Our results encourage confirming this postharvest alternative approach treatment in other cultivars and under commercial conditions.

**Keywords:** quality loss, organic table grapes, decay control, SO$_2$ alternative, shelf life, anthocyanin.
1 Introduction

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

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

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

Commonly, the standard practice to control postharvest grape decay is achieved by using sulfur dioxide gas (SO$_2$); the grapes are fumigated either by repeated application of gas in storage room or by continuous release SO$_2$-generating pads in case of shipment period longer than 10 days or long retail handling (Chervin et al., 2012). This compound is registered as an adjuvant in different countries, and in spite of its efficacy for controlling gray mold; several problems are associated with its application. The main damages are: bleaching and other injuries to the rachis and berries, pitting of berries, off-flavor, excessive sulfite residues, corrosion of the equipment within storage facilities, worker safety, and air quality (Smilanick et al., 1990; Crisosto and Mitchell.
2002; Chervin et al., 2005; Zoffoli et al., 2008). For these reasons, this product has been removed from the Generally Recognized as Safe (GRAS) compound list by US Food and Drug Administration (US FDA) (Anon, 1986); whereas, it is not allowed as postharvest treatment on organic grapes in Europe and USA by EU regulation (EC) No 889/2008 and National Organization Program (NOP-USDA) respectively.

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

Ozone (O₃) was declared GRAS substance by the US FDA in 2001 (US FDA, 2001), and since that time it is being widely investigated and introduced into some commercial applications in food industry such as table grapes storage. O₃ is a highly reactive form of oxygen, naturally present in the atmosphere and one of the most potent sanitizers against a wide spectrum of microorganisms (Khadre et al., 2001; Mlikota Gabler and Smilanick, 2001; Von Gunten, 2003). It has been extensively tested for the control of table grape decay (Cayuela et al., 2009; Sharpe et al., 2009; Mlikota Gabler et al., 2010; Smilanick et al., 2010). Many cold storage facilities in California have installed equipment that generates a constant low dose of O₃ (100 ppb day and 300 ppb night cycle) and it reduced the spread of gray mold and prolonged the storage of grapes.
for several weeks (Smilanick et al., 2010). The risk of injury to table grapes from O₃ have been reported for the rachis after a treatment of 30 min with very high concentrations (5000 ppm) of O₃ (Mlikota Gabler et al., 2010). Therefore, ozone could be considered as a promising antimicrobial agent for the sanitation of grape surfaces to extent the storage period and shelf life.

In addition, postharvest treatment with short-term exposure to high carbon dioxide (CO₂) concentrations is an effective treatment to maintain quality and to control decay development in grapes (Crisosto et al., 2002b; Retamales et al., 2003; Sanchez-Ballesta et al., 2006, 2007; Teles et al., 2014). Furthermore, low concentration of O₂ (below 1%) induces anaerobic respiration, which leads to undesirable metabolic reactions, resulting in off-odors and off-flavors (Candir et al., 2012), while, high CO₂ concentration (equal or above 15%) results in stem and berry browning (Crisosto et al., 2002b; Retamales et al., 2003). Moreover, Modified Atmosphere Packaging (MAP) technique is considered as a non-toxic method for keeping quality of fruit and vegetables (Artés, 1976; Kader et al., 1989) and could be an alternative methods which control or avoid table grapes postharvest decay and maintains their visual and sensory quality (Artés-Hernández et al., 2004). The application of MAP can result in reduction of respiratory activity, retardation of softening and ripening and restraint of pathogens and reduced incidence of various physiological disorders (Caleb et al. 2013). MAP, as a semi-permeable coating with an adjusted ambience of CO₂/O₂ inside small storage environment, has been proven to prolong the storability of perishable commodities like grapes (Hagenmaier, 2005). Several authors consider MAP with 15% O₂ and 10% CO₂ such as a cheap and easy technique, which might be useful as an alternative to SO₂ (Crisosto et al., 2002b; Artés-Hernández et al., 2004).
However, these treatments vary in their effectiveness and lack enough support to replace SO\textsubscript{2} as a commercial practice. Few studies have evaluated their effects on common quality attributes as phenolic and aromatic compounds, in addition to decay control (Sanchez-Ballesta et al., 2006, 2007; Romero et al., 2009; Ustun et al., 2012). Therefore, in this study a detailed investigation was carried out to determine the effects of MAP combined with superficial disinfectant, as high concentrations of gaseous O\textsubscript{3} or CO\textsubscript{2}, on decay incidence, sensorial quality maintainence, antioxidant capacity, total phenolic compounds, total and individual anthocyanins of organic Scarlotta\textsuperscript{®} table grapes during cold storage (CS) period and after simulate commercial shelf life (SL).

2 Material and methods

2.1 Plant material

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

Harvested clusters were transported to the laboratory and immediately precooled. The clusters were selected based on uniform berry size, color, firmness and freedom from evident defects or diseases. The selected clusters met European Union (“EU”) Class 1 and in agreement with Sun World Quality Specifications. Selected bunches were at commercial maturity with sugar-acid ratio of 26:1; medium
symmetrical and well-filled bunches with a size of about 650 g; berries large, elongated with diameter around 23 mm.

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

2.2 Pretreatments

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

i) Gaseous \( \text{O}_3 \) concentration at 5, 10 and 20 \( \mu \text{L L}^{-1} \) mixed with air for 30 min; gaseous \( \text{O}_3 \) was generated by OZAB-MF-A (Aeraque I.T. S.r.l., Stradella (PV), Italy), and its concentration was monitored through OZOMAT-MP (Anseros Ozone Gas Analyser MP, Germany);

ii) \( \text{CO}_2 \) concentration at 50 and 70\% mixed with air for 24 h, the \( \text{CO}_2 \) concentration was monitored and adjusted through SERVOPRO 1440 Gas Analyser (SERVOMEX, USA).

The obtained results of all these pretreatments were compared to untreated grapes (control). Except non-packed control, all the remaining grape boxes were packed in film bags (85 \( \mu \text{m} \) thickness), made of polyamide (20 \( \mu \text{m} \)) / polyethylene (65 \( \mu \text{m} \)) (PA/PE), under 2\% \( \text{O}_2 \):5\% \( \text{CO}_2 \):93\% \( \text{N}_2 \) MAP, hermetically sealed using a heat sealer (MD, Italy). Then, stored under simulated shipping conditions in container at 0±0.5 °C and 90-95\% relative humidity (RH) for 55 days. At the end of storage, the temperature was raised at 15±1.0 °C for one-week to simulate commercial shelf life (SL) as retail sale period.
2.3 Measurement of table grapes respiration rates

Respiration rates were measured through apparent Michaëlis constant ($K_{m_{\text{appO}_2}}$) and maximal oxygen respiration rate ($R_{\text{RmaxO}_2}$) by using the closed system method (Lee, 1987; Haggar et al., 1992). Table grapes of known weight (≈ 270 g) were placed in a 1 L glass jar previously equilibrated in a temperature-controlled room. The initial gas composition inside each jar was set by gas flushing. Initial CO$_2$ partial pressure was constant and equal to atmospheric concentration 0.03% and initial O$_2$ partial pressures were 5, 10, and 20%. At periodic intervals, gases were sampled through a silicone septum set in the jar lid and analyzed using a CheckMateII O$_2$/CO$_2$ analyser (PBI Dansensor, Ringsted, Denmark). Experiments were stopped when the change in CO$_2$ partial pressures became greater than 1.5%. Each experiment was done in triplicate. The linear part of the Lineweaver plot ($1/R_{R_{O_2}}$ against $1/O_2$) was extrapolated to estimate the apparent Michaelis constant ($K_{m_{\text{appO}_2}}$) and the maximal oxygen respiration rate ($R_{\text{RmaxO}_2}$).

2.4 Film permeability

Different pieces (130×125 mm) of previously mentioned film packaging PAPE (85 µm thickness) were sealed on two sides forming pouches, these pouches were flushed with 4% O$_2$: 15% CO$_2$: 81% N$_2$. During the sampling period, the pouches (three replicates) were stored at 20 °C and 40 % RH. During a storage period of 10 days, the changes in headspace gas composition were measured at interval times by using a CheckMateII O$_2$/CO$_2$ analyser (PBI Dansensor, Ringsted, Denmark). In addition, the O$_2$ and CO$_2$ permeation process through the packaging film, were calculated by derived Fick’s law and the obtained result were expressed in mol m$^{-1}$ s$^{-1}$ Pa$^{-1}$ (Larsen et al., 2000).
2.5 Modelling approach to simulate gas headspace composition

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

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

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

where \( J \) is the gas flux per time unit through the film (mol s\(^{-1}\)); \( Pe \) is the gas permeability coefficient of the film (mol m\(^{-1}\) s\(^{-1}\) Pa\(^{-1}\)); \( S \) is the surface area of film (m\(^2\)); \( e \) is the film thickness (m); and \( \Delta P \) is the gas partial differential pressure between the outside and inside of the package (Pa).

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

\[
RR_{O2} = \frac{RR_{maxO2} \times pO2}{Km_{appO2} \times pO2}
\]

where \( RR_{O2} \) is the oxygen respiration rate (mmol kg\(^{-1}\) h\(^{-1}\)); \( RR_{maxO2} \) is the maximum oxygen respiration rate; \( pO2 \) is the gase partial pressures (kPa); \( Km_{appO2} \) is the apparent constant of Michaëlis-Menten equation (kPa) defined as the amount of substrate providing the reaction rate of \( RR_{maxO2}/2 \).

In addition, the temperature quotient (\( Q_{10} \)) was calculated from the slope of the regression line to obtain the temperature dependence of respiration. \( Q_{10} \) indicates the
increase in the respiration rate caused by a 10 °C increase in temperature. \( Q_{10} \) values were calculated using the following equation, where \( R_2 \) and \( R_1 \) are relative respiration rates at two temperatures, \( T_2 \) and \( T_1 \) (\( T_2 > T_1 \)).

\[
Q_{10} = \left( \frac{R_2}{R_1} \right)^{10/(T_2 - T_1)}
\]

2.6 Decay incidence and weight loss

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

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

2.7 Mechanical attributes

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

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

2.9 Color analysis

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

- Hue angle ($h^\circ$) [$\tan^{-1} b/a$] which is the attribute of a visual sensation according to which an area appears to be similar to one of the perceived colors, red, yellow, green and blue, or a combination of two of them;

- Color index for red grapes (CIRG) as CIRG=$\left\{ \frac{(180-h^\circ)}{(L^*+C^*)} \right\}$ based on the parameters $L^*$, $a^*$, $b^*$, and its characterized by showing a high correlation with the external visual color of the fruit (Carreño et al., 1995).

2.10 Rachis chlorophyll content

Rachis chlorophyll was extracted by immersing 0.5 g of fresh chopped rachides (grinded in cold liquid nitrogen) in 25 mL of pure methanol (99.9%) for 24 h at 4 °C in darkness. The quantification of chlorophyll was carried out by using a spectrophotometer (UV-1800, Shimadzu, Japan), then the samples were exposed to
visible light at a wavelength of (652.4, 665.2 and 470 nm; the content was expressed as 250 mg kg\(^{-1}\) fresh weight using Lichtenthaler’s formula (Lichtenthaler, 1987).

**2.11 Extraction of phenolic compounds**

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

**2.12 Analysis of antioxidant activity**

Antioxidant activity (AA) in skin and flesh was analyzed with the ABTS [2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] assay, this assay is based on free-radical-scavenging activity. The results were expressed as mmol Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) equivalent antioxidant capacity (TEAC) for kg\(^{-1}\) of skin or flesh. For the calibration process, Trolox standard solutions were prepared at a concentration ranging from 10 to 800 μmol L\(^{-1}\). Antioxidant activity was measured in the ABTS assay through the ability of antioxidants to scavenge the ABTS radical cation (ABTS\(^{•+}\), a blue/green chromophore) by inhibiting its absorption at 732 nm. The ABTS antioxidant test was performed according to the method reported by Re et al. (1999) with slight modifications. In order to produce ABTS\(^{•+}\), 7 mmol L\(^{-1}\) ABTS solution was reacted with 2.45 mmol L\(^{-1}\) potassium persulfate aqueous solution for 16 h at room temperature and darkness conditions. The solution of ABTS\(^{•+}\) was then diluted
with ethanol to an absorbance of 0.90±0.03 at 732 nm; after the addition of either 100 μL of skin extract (diluted at 1:20 with ethanol) or undiluted pulp juice to 3.9 mL of diluted ABTS$^{•+}$ solution, the absorbance was measured after 5 min (Ferrara et al., 2015).

### 2.13 Total phenolic content

Total phenols content was determined on grape fleshes and skins extract, the phenolic content was determined by Folin-Ciocalteu method using an UV-visible spectrophotometer (Beckman Coulter, USA). Sample solution of 100 μL of skin extracts (diluted at 1:25 with ethanol/water/HCl solution (70/30/1 v/v)) or undiluted pulp juice was added to 500 μL of H$_2$O and then to 100 μL of Folin-Ciocalteu’s reagent; homogenized and incubated at room temperature for 5 min, after 500 μL of 10% of sodium carbonate was added, the mixture was then incubated for 90 min at room temperature, following this incubation period, the absorbance was measured at 700 nm. Total phenolic content was calculated on the basis of a calibration curve of gallic acid and expressed as mg gallic acid equivalent (GAE) kg$^{-1}$ of fresh weight. The standard calibration curve was performed from 0.08 to 0.002 mg mL$^{-1}$ of gallic acid pure standard (Sigma Aldrich).

### 2.14 Anthocyanins analysis

Anthocyanin composition was determined on grape skins extract, by using a Waters 600 E HPLC, (Waters Inc.), which included a quaternary pump, a PDA and an injection valve with a 20 μL loop. Sample extracts, previously filtered on a 0.45 μm nylon membrane, were injected into a NovaPack C18 (150 × 3.9 mm, 4 μm particle size, Waters Inc.) column maintained at 30 °C and eluted at a flow rate of 1 mL min$^{-1}$ with 10% formic acid (solvent A), and acetonitrile (solvent B). The gradient program for solvent A was 0-1 min 95%, 1-22 min 60%, 22-27 min 30%, 27-35 min 30%.
eluates were monitored at 520 nm, and quantitative analysis was made according to the external standard method with a calibration curve obtained by injection of solutions at different concentration of malvidin-3-glucoside (Sigma Aldrich) ($R^2 = 0.9991$). Tentative identification of anthocyanins was achieved by combining the elution pattern and data reported in literature (Revilla and Ryan, 2000; Singh Brar et al., 2008; Acevado De la Cruz et al., 2012); the results were expressed as mg kg$^{-1}$ malvidin-3-glucoside equivalents.

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

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

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

Frozen grapes were pounded in a mortar until obtaining a homogeneous compound. For each measurement, fibre was exposed to the headspace of a 12 mL screw-capped vial, which contained 2 g of puree with 2 mL of 0.1 mol phosphor-citrate buffer pH 5, 100 μL of pectolytic enzymes for oenological use Endozym (diluted 1000 times in water), and 30 μL of internal standard solution (3-pentanone). The extraction was performed for 30 min at 50±1 °C with an equilibration time of 5 min. All used fibres were conditioned by keeping them in the GC injector following instructions from manufacturer. After the extraction step, fibres were desorbed in a split/splitless injector at 220 °C, for 1.5 min split ratio was 1:20. Gas chromatography/mass spectrometry (GC/MS) analysis was
performed on a Thermo Scientific ISQ™ QD Single Quadrupole GC-MS System. Compounds were separated on a WAX MS capillary column (20 m × 0.1 mm i.d.; 0.1 µm film thickness), by applying the following temperature program: 50 °C for 0.1 min, 50-180 °C at 13 °C min⁻¹, 180 - 220 °C at 18 °C min⁻¹. Mass detector conditions were electronic impact mode at 70 eV, source temperature 250 °C and mass scanning acquisition range: 34 - 200 Da. Carrier gas was helium with a constant flow at 0.4 mL min⁻¹.

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

2.16 Sensory quality

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

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

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

3 Results and discussion

3.1 Film permeability

According to Lamikanra (2002), the commercial used film packaging PAPE showed a low permeability against O$_2$ and CO$_2$ transmission rate ($7.01 \times 10^{-16}$ and $1.98 \times 10^{-15}$ mol m$^{-1}$ s$^{-1}$ Pa$^{-1}$, respectively).

3.2 Respiration rate

Apparent Michaelis-Menten parameters of table grapes were estimated from respiratory activity in the closed system, which allowed the RR$_{O2}$ determination through the oxygen depletion. Then, the RR$_{\text{maxO2}}$ and the Km$_{\text{appO2}}$ were estimated on the Lineweaver plot. The RR$_{\text{maxO2}}$ and the Km$_{\text{appO2}}$ of table grapes were 1.90 mmol h$^{-1}$ kg$^{-1}$ and 34.63 kPa respectively, demonstrating low physiological activity of table grapes. Furthermore, the effect of temperature on respiration in fresh fruits and vegetables is very significant (Cameron et al., 1994). A wide variety of enzymatic reactions are involved in respiration. The rate of all of these reactions increases exponentially with increasing temperature within the physiological temperature range (Exama et al., 1993). The mathematical description of respiration increases ($Q_{10}$) of table grapes was around 2.12 such as main of fruits and vegetables.

3.3 Predicted headspace O$_2$ and CO$_2$ concentration
Fruit and vegetables consume oxygen and produce carbon dioxide while packed, giving rise to a modification of the headspace gas composition (Jayas and Jeyamkondan, 2002). The respiration of the packed product and the gas permeability of the film influence the change in gaseous composition of the environment surrounding the product. As expected during cold storage, MAP was able to increase significantly CO₂ percentage with a tight decrease in O₂ concentrations in the surrounding of grapes bunches. The gas equilibrium was reached after around 16 days for CO₂ and 3 days for O₂ (15% and 1.8%, respectively) at 0 °C of storage (Figure 1). However, during simulated commercial shipping at 15 °C for one week, a fast increase in CO₂ and O₂ concentrations were recorded after one day reaching 15.8% and 2%, respectively, due to the suddenly increase of temperature. The registered increase was followed by a severe decrease in CO₂ concentration reaching 6.4% at the end of storage, while, O₂ concentration returned to the initial equilibrium (1.8%). Thus suggesting that headspace gas condition in the package was affected by the increasing temperature, which had an impact on grapes respiration rate and film barrier properties.
3.4 Weight loss

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight loss (%)</th>
<th>Decay incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15d</td>
<td>30d</td>
</tr>
<tr>
<td>Control-MAP</td>
<td>0.68a</td>
<td>0.65a</td>
</tr>
<tr>
<td>O₃-5 µL L⁻¹</td>
<td>0.47bc</td>
<td>0.54a</td>
</tr>
<tr>
<td>O₃-10 µL L⁻¹</td>
<td>0.51bc</td>
<td>0.56a</td>
</tr>
<tr>
<td>O₃-20 µL L⁻¹</td>
<td>0.45c</td>
<td>0.60a</td>
</tr>
<tr>
<td>CO₂-50%</td>
<td>0.58b</td>
<td>0.63a</td>
</tr>
<tr>
<td>CO₂-70%</td>
<td>0.53bc</td>
<td>0.63a</td>
</tr>
</tbody>
</table>

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.

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.

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Physical qualities</th>
<th>Chemical qualities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Berry detachment force 2.82 N</td>
<td>Berry firmness 11.83 N</td>
</tr>
<tr>
<td>Treatment</td>
<td>45d</td>
<td>55d+SL</td>
</tr>
<tr>
<td>Control-MAP</td>
<td>2.37a</td>
<td>2.31a</td>
</tr>
<tr>
<td>O₃-5 µL L⁻¹</td>
<td>2.35a</td>
<td>2.15a</td>
</tr>
<tr>
<td>O₃-10 µL L⁻¹</td>
<td>2.46a</td>
<td>1.99a</td>
</tr>
<tr>
<td>O₃-20 µL L⁻¹</td>
<td>2.18a</td>
<td>2.23a</td>
</tr>
<tr>
<td>CO₂-50%</td>
<td>2.31a</td>
<td>1.99a</td>
</tr>
<tr>
<td>CO₂-70%</td>
<td>2.49a</td>
<td>2.21a</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>L*</th>
<th>H°</th>
<th>CIRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>45d</td>
<td>55d+SL</td>
<td>45d</td>
</tr>
<tr>
<td>Control-MAP</td>
<td>38.71</td>
<td>1.98</td>
<td>4.24</td>
</tr>
<tr>
<td>O₃-5 µL L⁻¹</td>
<td>40.10a</td>
<td>38.65b</td>
<td>11.83a</td>
</tr>
<tr>
<td>O₃-10 µL L⁻¹</td>
<td>39.25a</td>
<td>38.90ab</td>
<td>-0.75ac</td>
</tr>
<tr>
<td>O₃-20 µL L⁻¹</td>
<td>38.88a</td>
<td>36.80c</td>
<td>-12.24c</td>
</tr>
<tr>
<td>CO₂-50%</td>
<td>40.44a</td>
<td>38.89ab</td>
<td>4.48ab</td>
</tr>
<tr>
<td>CO₂-70%</td>
<td>41.51a</td>
<td>39.89ab</td>
<td>5.27ac</td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Antioxidant activity (mmol kg⁻¹)</th>
<th>Total phenolic content (mg GAE kg⁻¹)</th>
<th>Total anthocyanins (mg Malvidin-3-glucoside kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skin 78.32</td>
<td>Flesh 14.05</td>
<td>110.89</td>
</tr>
<tr>
<td></td>
<td>14.05</td>
<td>451.45</td>
<td>114.90</td>
</tr>
<tr>
<td>Treatment</td>
<td>15d</td>
<td>30d</td>
<td>45d</td>
</tr>
<tr>
<td>Control-MAP</td>
<td>75.44a</td>
<td>79.63a</td>
<td>78.18a</td>
</tr>
<tr>
<td>O₃-5 µL L⁻¹</td>
<td>53.60b</td>
<td>72.57ab</td>
<td>80.69a</td>
</tr>
<tr>
<td>O₃-10 µL L⁻¹</td>
<td>69.03ab</td>
<td>75.28a</td>
<td>77.41a</td>
</tr>
<tr>
<td>O₃-20 µL L⁻¹</td>
<td>73.87a</td>
<td>84.2a</td>
<td>77.01a</td>
</tr>
<tr>
<td>CO₂-50%</td>
<td>79.72a</td>
<td>86.24a</td>
<td>60.34b</td>
</tr>
<tr>
<td>CO₂-70%</td>
<td>56.16b</td>
<td>57.64b</td>
<td>82.11a</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Dp 0.96 mg kg⁻¹</th>
<th>Cy 9.87 mg kg⁻¹</th>
<th>Pt 1.56 mg kg⁻¹</th>
<th>Pn 24.66 mg kg⁻¹</th>
<th>Mv 11.62 mg kg⁻¹</th>
<th>Ac-A 17.12 mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>15d</td>
<td>30d</td>
<td>45d</td>
<td>15d</td>
<td>30d</td>
<td>45d</td>
</tr>
<tr>
<td>Control-MAP</td>
<td>1.15</td>
<td>1.34</td>
<td>0.64</td>
<td>7.85a</td>
<td>5.83ac</td>
<td>10.44a</td>
</tr>
<tr>
<td>O₃ 5 µL L⁻¹</td>
<td>0.88a</td>
<td>0.80ab</td>
<td>0.65a</td>
<td>10.57ab</td>
<td>12.87b</td>
<td>6.93ab</td>
</tr>
<tr>
<td>O₃ 10 µL L⁻¹</td>
<td>0.80a</td>
<td>0.63ab</td>
<td>0.43a</td>
<td>9.68ab</td>
<td>9.50ab</td>
<td>7.72ab</td>
</tr>
<tr>
<td>O₃ 20 µL L⁻¹</td>
<td>0.70a</td>
<td>1.23ab</td>
<td>1.05a</td>
<td>10.69a</td>
<td>4.88ad</td>
<td>8.44ab</td>
</tr>
<tr>
<td>CO₂-50%</td>
<td>0.64a</td>
<td>0.46ab</td>
<td>0.47a</td>
<td>4.74bc</td>
<td>4.38cd</td>
<td>6.08ab</td>
</tr>
<tr>
<td>CO₂-70%</td>
<td>0.47a</td>
<td>0.54ab</td>
<td>0.25b</td>
<td>1.08c</td>
<td>0.98d</td>
<td>4.44b</td>
</tr>
</tbody>
</table>

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.
3.5 Decay incidence

The effects of pretreatments and storage time were investigated in naturally infected clusters of organic table grapes cv. Scarlotta® (Table 1), where natural incidence of decay was mostly caused by *B. cinerea*. The relative observations did not show any significant differences between packed untreated and pretreated samples until 15 days of cold storage with decay incidence around 0 to 0.55%, at the opposite in non-packed control the decay attained 27% (data not shown). Consequently, the non-packed control samples were excluded from the trial after the first sampling time (15 days of CS). Moreover, the situation was worsening after 30 days of CS, where the packed control grapes reached 2.04% of decay; however, the remaining pretreated grapes showed less than 1% decay. After 45 days of CS, pretreated samples with O$_3$ at 20 µL L$^{-1}$ and CO$_2$ at 70% maintained significant high control of decay (less than 1% decay) compared to packed control (2.51%) and a remaining pretreatments. Furthermore, after 55 days of CS + SL, trend of decay incidence decreased in samples pretreated with O$_3$ at 20 µL L$^{-1}$ and CO$_2$ reaching values around 0% decay compared to packed control samples with 1.2% of decay. Cumulative decay incidence confirmed also the efficiency of these pretreatments (Figure 2); to control the decay after 45 days of CS and 55 days CS + SL by reducing it 5 to 6 fold compared to packed control.

The effect of pretreatment with O$_3$ or CO$_2$ at high concentration to control berries decay, was confirmed in previous work in which the decay decreased through direct action against *B. cinerea*, including partial inhibition of conidia germination (Mlikota Gabler et al., 2010, Karaca et al., 2012, Teles et al., 2014). The efficiency of these gases in controlling decay could be due to the internal increase of ethanol.
and acetaldehyde to fungal-toxic concentrations (Pesis, 2005) or the formation of reactive oxygen species associated with stilbene synthase gene expression and resveratrol accumulation (Sanchez-Ballesta et al., 2006; Romero et al., 2008; Minas et al., 2010).

According to current E.U. marketing regulations EC N° 543/2011, the maximum decay rate accepted is 1% by weight of table grapes bunches at the receiving point for Class I EU grapes. During all storage period (CS and SL), all samples packed under MAP, controlled decay incidence below this accepted maximum rate (1%); moreover, samples pretreated with O$_3$ at 20 µL L$^{-1}$ and CO$_2$ at 50%, yielded cumulative decay incidence within this limit after CS and SL, by reaching the minimal quality standards for commercial table grapes.

**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.

### 3.6 Physical and chemical qualities

During the 45 days of cold storage, no significant differences of physical and chemical quality parameters were obtained between pretreated samples and control on organic table grapes cv. Scarlotta® at different sampling time. However, after simulated commercial shelf life (Table 2), some significant differences were observed, such the decreases of skin firmness, in samples pretreated with CO$_2$ at 70%
(0.11 N) and O$_3$ at 5 µL L$^{-1}$ (0.12 N) compared to control (0.16 N). Likewise, pH and SSC in samples pretreated with O$_3$ at 5 - 10 µL L$^{-1}$ (pH: 3.17 and 3.18, respectively) and CO$_2$ at 70% (pH: 3.22 and SSC: 3.14%) compared to control (pH: 3.29 and SSC: 14.75). These registered variations remained very tiny and no relevant. Similar results for changes in physical and chemical attributes were reported in “Red globe” grapes stored at 0 °C under several controlled atmospheres for up to 3 months weeks (Crisosto et al., 2002a), in “Superior seedless” grapes stored under different MAP and after 7 days CS + SL (Artés-Hernández et al., 2006), and in “Flame Seedless” grapes pretreated with 40% CO$_2$ and conserved under controlled atmosphere (Teles et al., 2014).

### 3.7 Berry color

The obtained results showed that most pretreatments retained the $L^*$ and $h^\circ$ of berries over all sampling times with no relevant changes; however, after SL, $L^*$ decreased significantly regardless of pretreatments and the lower values were noted in samples pretreated with O$_3$ at 5 and 20 µL L$^{-1}$ (36.49 and 36.8, respectively) compared to control (38.65) (Table 3). In addition, $h^\circ$ increased significantly in almost all samples except samples pretreated with O$_3$ at 20 µL L$^{-1}$ and packed control (12.79 and 17.72) compared to value at harvest (1.98). These similar results were already mentioned in unwrapped “Flame seedless” grapes after 18 days of CS (Martínez-Romero et al., 2003), in organic “Crimson” grapes after 30 days of CS immersed in hot ethanol (Mlikota Gabler et al., 2005), in “Red globe” grapes packed in MAP bags during three months of CS (Candir et al., 2012). According to Mlikota Gabler et al. (2005), the increases in $h^\circ$ and decrease in $L^*$ values indicate a
progression in berry color toward brown, but the darker or deeper red color that they reported are not visible to the naked eye.

In addition, CIRG permitted the objective definition of the external color in all samples, based on this index, after SL only samples pretreated with \( \text{O}_3 \) at 20 \( \mu \text{L L}^{-1} \) (4.05) maintained red skin color compared to the harvest time (4.24). Furthermore, following the criterion of Carreno et al. (1996), the remaining samples presented pink skin color with values ranging between 3.22 and 3.69.

### 3.8 Rachis chlorophyll content

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

Compared to harvest values (188.09 mg kg\(^{-1}\)), trend of rachis chlorophyll content decreased significantly during storage period, principally after 45 days of cold storage where samples pretreated with \( \text{CO}_2 \) at 50 and 70\% presented very low concentration of chlorophyll (59.45 and 80.48 mg kg\(^{-1}\), respectively) (Figure 3), while, rachis green color was maintained in samples pretreated with \( \text{O}_3 \) at 20 \( \mu \text{L L}^{-1} \) (139.89 mg kg\(^{-1}\)) followed by packed untreated samples (119.08 mg kg\(^{-1}\)) . However, only samples pretreated with \( \text{O}_3 \) at 20 \( \mu \text{L L}^{-1} \) maintained chlorophyll content during the whole period of CS. The obtained results concerning rachis chlorophyll content confirmed the subjective results obtained by the panelist for rachis browning after 45 days of CS.

The green color loss or rachis browning affects overall cluster quality and has been associated mainly to water loss (Valverde et al., 2005; Lichter et al., 2011) and
oxidation processes (Carvajal-Millán et al., 2001). However, several studies suggested that other factors could be involved in rachis green color loss due to green pigments degradation during the chlorophyll breakdown pathway and the consequent formation of pheophytin-a by the putative enzyme Metal Chelating Substance (MCS) (Shioi et al., 1996; Suzuki et al., 2005). Moreover, Hörtensteiner and Kräutler (2011) reported that a non-enzymatic or a species-specific reaction generates a series of non-colored catabolites that accumulates inside vacuole. Alternatively, results of the work carried out by Carvajal-Millán et al. (2001) showed that clusters with severe rachis browning symptoms had higher polyphenol oxidase (PPO) activity than clusters with less rachis browning symptoms in “Flame Seedless” grapes. Additionally, Balic et al. (2012) described a list of 30 senescence associative genes (SAGs). Suggesting that in rachis of “Red Globe” grapes stored at 0 °C for 90 days, 10 genes increased the level of transcript abundance, while another 7 did not show significant differences as compared with harvest. Additionally, Silva-Sanzana et al. (2016) observed through histological analysis of rachis that MAP storage increases rachis postharvest quality of “Red Globe” grapes by reducing green color loss reported due to a combination of processes involving a delay of green pigments degradation and a less accumulation of brown compounds at the periderm and cortex tissues, thus preventing green pigments masking.
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 $\text{FWER} \leq 0.05$.

3.9 Antioxidant activity and phenolic compounds

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

Samples pretreated and untreated did not show the same trend for AA, total phenolic content and total anthocyanins. During CS, AA in flesh berries increased in all samples untreated and pretreated with $\text{O}_3$; this activity in skin berries was almost maintained in all samples; while, in samples pretreated with $\text{CO}_2$ at 50%, AA increased significantly in flesh and skin berries reaching high values after 15 and 30 days respectively, and decreased drastically at the end of storage. Regarding samples pretreated with $\text{CO}_2$ at 70%, AA decreased significantly after 15 and 30 days of cold storage and remained similar to those achieved in freshly harvested grapes. Sanchez-Ballestra et al. (2007) and Romero et al. (2008) reported the same results in “Cardinal” grapes treated with $\text{CO}_2$. The same observation was noted also regarding the total phenol content, where the trend in flesh berries increased significantly in all conserved samples, while in skin berries its concentration was maintained until the end of storage in almost all samples, exception of those pretreated with $\text{CO}_2$. In
previous work it was demonstrated that the antioxidant capacity is dependent on the level and type of phenolic compounds (Lutz et al., 2011).

Concerning the development of total anthocyanins in skin berries, the results showed two distinguished trends. In samples pretreated with $O_3$ the concentration of anthocyanins increased significantly, which was paralleled by an increase in antioxidant activity during cold storage (Table 4), this correlation was also observed in “Cardinal” grapes after 12 days of cold storage (Sanchez-Ballesta et al., 2007). Which could be explained by the fact that $O_3$ reacted as elicitors for the biosynthesis of phenolic compounds (Vincente et al., 2014).

However, total anthocyanin decreased significantly in samples pretreated with $CO_2$ during storage period to reach values of 100.82 and 62.57 mg Malvidin-3-glucoside kg$^{-1}$ in samples pretreated with $CO_2$ at 70% and 50%, respectively. The results obtained are in agreement with those of Artés-Hernandez et al. (2003) which observed a decrease in total anthocyanin content in “Napoleon” grapes when using an atmosphere of 15% $CO_2$ and 5% $O_2$. Even if, it is already known that anthocyanin synthesis continues after harvest and also during long-term cold storage by activating phenylpropanoid gene expression, total anthocyanin accumulation and antioxidant activity, whereas the application of $CO_2$ treatment reduces/inhibits these responses (Sanchez-Ballesta et al., 2007, Romero et al., 2008).

### 3.10 Anthocyanins content

The anthocyanin profile consisted of twelve compounds (seven of them acylated), with the prevalence of peonidin (Pn) forms (Figure 4) in agreement with what was reported by Cantos et al. (2002); Sanchez-Ballesta et al. (2007) and Ferrara et al. (2015). The most abundant anthocyanin present was peonidin-3-glucoside
(37%), followed by malvidin-3-glucoside (18%), cyanidin-3-glucoside (15%), and in lower quantities than other anthocyanins: petunidin-3-glucoside (2%) and delphinidin-3-glucoside (1%) (Table 5). According to Liang et al. (2011), the most abundant anthocyanin present in pink and red-colored cultivars was Pn forms, whereas cyanidin, malvidin and petunidin forms were abundant in red-black cultivars. This suggests that different proportions of individual anthocyanin compounds, in addition to their absolute amount, can affect the skin color of grapes (Ferrara et al., 2015). This result was confirmed at the end of cold storage where pretreatment with O\(_3\) at 20 µL L\(^{-1}\) presented the highest concentration of Pn (38.66 mg kg\(^{-1}\)) and CIRG (4.43).

The individual anthocyanins in almost all samples showed not significant variation during storage period, and their values remained similar to those achieved in freshly harvested grapes. Except for cyanidin and delphinidin, where their concentrations decreased significantly in samples pretreated with CO\(_2\) at 70%. Moreover, peonidin and acylated anthocyanins showed a similar trend with total anthocyanin content in samples pretreated with O\(_3\) at 20 µL L\(^{-1}\).

**Figure 4.** Anthocyanin profile of a skin extract of berries at harvest from organic table grapes cv. Scarlotta\(^\circ\) berries [peaks: 1 = delphinidin-3-glucoside; 2 = cyanidin-3-glucoside, 3 = petunidin-3-glucoside, 4 = peonidin-3-glucoside; 5 = malvidin-3-glucoside; 6–12 = acylated anthocyanins].
3.11 Acetaldehyde and ethanol

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

Compared to the acetaldehyde and ethanol values at harvest (0 µg kg\(^{-1}\) and 0.08 mg kg\(^{-1}\), respectively), trend of both components increased significantly during storage period in all samples (Figure 5). Several not significant variation of acetaldehyde and ethanol compounds were observed during conservation between samples at different sampling time (P<0.01). Except at the end of cold storage, where samples pretreated with O\(_3\) at 20 and 10 µL L\(^{-1}\) present significant low values of acetaldehyde (24.59 and 32.51 µg kg\(^{-1}\)) compared to packed untreated samples (41.16 µg kg\(^{-1}\)). In addition, samples pretreated with O\(_3\) at 20 µL L\(^{-1}\) presented perceived low trend of acetaldehyde paralleled with trend of remaining samples. Samples pretreated with CO\(_2\) and packed untreated presented high concentration of acetaldehyde during period of storage, similar result was also obtained by Teles et al. (2014) on organic “Flame Seedless” grapes pre-stored with CO\(_2\) at 40% for 24 - 48 h and conserved for four weeks. According to Candir et al. (2012), a low concentration of O\(_2\) induces anaerobic respiration, which leads to undesirable metabolic reactions, such as tissue breakdown and accumulation of acetaldehyde and ethanol in the tissue, resulting in off-odors and off-flavors. The results concerning acetaldehyde and
ethanol content in berries at the end of cold storage confirmed the subjective results obtained by the panelist for the perceiving off-flavor after 45 days of CS.

Figure 5. Effects of pretreatments and MAP on acetaldehyde (A) and ethanol (B) content on 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≤0.05

3.12 Sensory quality

After 45 days of cold storage, clusters pretreated with O₃ maintained good visual appearance compared to the control and good aroma was mainly conserved by untreated and treated samples with O₃ at 20 µL L⁻¹, while it decreased drastically in clusters pretreated with CO₂ at 50 and 70% (Figure 6). The same results were obtained after SL, except for good aroma, where also, samples pretreated with O₃ at 5 and 10 µL L⁻¹ presented fair score. Furthermore, as described after 45 days of CS regarding juiciness and firmness of berries, also after SL almost all samples preserved juiciness of berries, for firmness untreated paked samples and pretreated with O₃ at 20 µL L⁻¹ were at limit of marketability while in remaining samples, it was
judged as fair. Concerning sweetness after SL; packed control and pretreated samples with O$_3$ at 20 and 10 µL L$^{-1}$ obtained limit of marketability score, while in remaining samples was refereed as very fair (Figure 6).

Equally, samples at both sampling time presented sourness in the taste, which was considered as fair in almost all samples after 45 days of CS and fair in packed control and pretreated samples with O$_3$ at 20 µL L$^{-1}$. Although after SL, in practically all samples the score increased to reach limit marketable score and was perceived as severe in samples pretreated with O$_3$ at 5 µL L$^{-1}$ and CO$_2$ at 70%. Moreover after SL, the panelist detected severe off-flavor in berries pretreated with O$_3$ at 5 µL L$^{-1}$ and CO$_2$ at 70%, limit of marketability in samples pretreated with O$_3$ at 10 µL L$^{-1}$ and CO$_2$ at 50%, and fair in packed control and pretreated samples with O$_3$ at 20 µL L$^{-1}$.

The observed off-flavors could be due to an accumulation of fermentative volatiles compounds in berries like ethanol and acetaldehyde (Candir et al., 2012; Teles et al., 2014). Consequently, gas composition inside package did not influence grapes flavor; our data confirm results obtained by Artés-Hernández et al., (2006) in “Superior Seedless” grapes stored under MAP. Regarding rachis browning attribute, SL increased the perceptibility of rachis browning to limit of marketability especially in packed control and pretreated samples with O$_3$ at 10 and 20 µL L$^{-1}$, comparing to samples stored for 45 days. This stem browning could be correlated to the condensation of CO$_2$ inside package (Crisosto et al., 2002a, Chen et al., 2011, Candir et al., 2012, Teles et al., 2014), produced by the accelerated respiration of the products generated mainly by the stress of high temperature during SL.
Conclusion

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

Figure 6. Effects of pretreatments and MAP on organic table grapes cv. Scarlotta® sensory quality after CS (A) and SL (B).
during storage, transportation or marketing could be a commercially practical alternative for postharvest handling of organic grapes. Therefore, further trials with other cultivars should be worth to validate the applied protocol.

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