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Effect of ozone or carbon dioxide pre-treatment during long-term storage of organic table grapes with modified atmosphere packaging

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ABSTRACT

The aim of this study was to maintain the quality of organic table grapes extending its shelf-life during long-term storage by using organic approved methods. The effectiveness of pre-treatments with different concentrations of O_3 (5, 10, 20 μL L^{-1}) or CO_2 (50%, 70%) followed by storage under modified atmosphere packaging (2% O_2—5%CO_2) were evaluated on late-season organic Scarlotta table grapes as alternatives to the usual commercial SO_2 application. The main quality attributes as mass loss, decay incidence, rachis chlorophyll content, antioxidant activity, phenolic compounds and acetaldehyde content, were measured at harvest and after 15, 30, 45 days of cold storage (0 °C) under simulated shipping conditions and one week of shelf-life (15 °C). The O_3 at 20 μL L^{-1} controlled the concentration of acetaldehyde, preserved rachis chlorophyll content and skin colour; in addition, the cumulative decay incidence was reduced compared to untreated samples, however, CO_2 caused organoleptic quality loss with strong stem browning and perceived off-flavours; moreover, it was effective to preserve the initial sensory quality and to control the decay. The results encourage the use of this alternative approach treatment in other cultivars and under commercial conditions.

1. Introduction

Table grapes (*Vitis vinifera* L.) is highly perishable after harvest and exposed to serious quality losses due to water loss with stem drying and browning, berry softening and pathological decay, mainly caused by grey mould (Sanchez-Ballesta et al., 2007).

Grey mould (*Botrytis cinerea*) is the most important postharvest disease because of the damage caused in the harvest season and during storage, it can also develop at low temperature, shortening the storage duration and marketing (Ciccarese, Stellacci, Gentilesco, & Rubino, 2013). Moreover, maturity increases significantly berry's susceptibility to infection and decay symptoms during postharvest handling (Chervin, Aked, & Crisosto, 2012; Teles, Benedetti, Gubler, & Crisosto, 2014).

SO_2 is used to control grapes decay; grapes is fumigated either by repeated application of gas in storage room or by continuous release in generating pads (Chervin et al., 2012). SO_2 is registered as adjuvant in different countries, but several problems arise from its application as bleaching, injuries to rachis and berries, pitting, off-flavours, excessive sulfite residues, corrosion of the equipment, workers and air quality safety issues. 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); in addition, it is not allowed as post-harvest treatment on organic grapes in Europe and USA.

Due to the growing demand for fresh organic products, several efforts focus on developing alternative strategies to control postharvest decay of organic table grapes; these strategies should be safe, effective, economical and compatible with commercial handling procedures (Romanazzi, Lichter, Mlikota Gabler, & Smilanick, 2012). Moreover, the integration of two or more treatments/means can be worthwhile than the use of single treatments (Wilson, 1997).

After O_3 was declared GRAS substance by US FDA, it has been widely investigated into commercial applications in the food industry; it has been extensively tested to control table grapes decay (Mlikota Gabler, Smilanick, Mansour, & Karaca, 2010; Smilanick, Mlikota Gabler, & Margosan, 2010, pp. 85–86). A constant low dose of O_3 (100 ppb day, 300 ppb night cycle) reduced the spread of grey mould and prolonged grapes storage (Smilanick et al., 2010, pp. 85–86). Therefore, O_3 is considered a promising antimicrobial agent to extend the storage period and shelf-life of table grapes.

In addition, postharvest treatment with short-term exposure to high

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CO_2 concentrations is an effective treatment to maintain quality and to control decay development in grapes (Crisosto, Garner, & Crisosto, 2002b; Retamales, Defilippi, Arias, Castillo, & Manriquez, 2003; Sanchez-Ballesta et al., 2007, 2006; Teles et al., 2014). However, low concentration of O_2 (< 1%) induces anaerobic respiration leading to undesirable metabolic reactions, resulting in off-odours and off-flavours (Candir et al., 2012), while, high CO_2 concentration (> =15%) results in stem and berry browning (Crisosto et al., 2002b; Retamales et al., 2003).

Furthermore, modified atmosphere packaging (MAP) is considered as a non-toxic method for keeping the quality of fruit and vegetables and could be a method to control table grapes postharvest decay and to maintain their visual and sensory quality (Artés-Hernández, Aguayo, & Artés, 2004). MAP brings to a respiratory activity reduction, softening and ripening retardation, restraint of pathogens and incidence reduction of various physiological disorders (Caleb, Mahajan, Al-Said, & Opara, 2013). MAP, using a semi-permeable film or using a smart device able to manage the package internal atmosphere (Altieri, Genovese, Matera, Tauriello, & Di Renzo, 2018; Matera, Genovese, Altieri, Tauriello, & Di Renzo, 2017), it has been proven to prolong the storability of perishable commodities (Hagenmaier, 2005).

Several authors consider MAP with 15%O_2 and 10%CO_2 a suitable technique as SO_2 alternative (Artés-Hernández et al., 2004; Crisosto et al., 2002b).

However, few studies have evaluated the effect of these treatments on common quality attributes as phenolic and aromatic compounds, in addition to decay control (Sanchez-Ballesta et al., 2007, 2006; Ustun et al., 2012). Therefore, this study concerns the effects of pre-treatments with O_3 (5, 10 and 20 μL L^{-1}) or CO_2 (50 and 70%) on organic Scarlotta table grapes subsequently stored in MAP (2%O_2—5%CO_2). Decay incidence, sensorial quality, antioxidant activity, total phenolic compounds, total and individual anthocyanins were measured during 45 days cold storage (CS) period (0 ± 0.5 °C) followed by 7 days commercial shelf life (SL) period (15 ± 1.0 °C).

2. Material and methods

2.1. Plant material

Four-year-old organic Scarlotta seedless brand “Sugranineteen” table grapes, with historic and current high incidence of grey mould, was harvested early in the morning, in Gioia del Colle (South-East of Italy)at the end of September 2014.

The harvested clusters were transported to the laboratory and immediately pre-cooled after a selection based on uniform berry size, colour, firmness and lack of evident defects or diseases. The selected clusters met European Union (“EU”) Class 1 and were in agreement with Sun World Quality Specifications. Bunches were randomly distributed into batches and every single bunch was placed inside plastic boxes. Each box constituted a replicate and each sample was composed of five replicates.

2.2. Pre-treatments

Grapes boxes were placed inside different sealed barrels provided with two pipes, the first one for removing the air and the second to introduce the gas for the grapes treatment as follows:

i) O_3 concentration at 5, 10 and 20 μL L^{-1} mixed with air for 30 min at 0 °C;
ii) CO_2 concentration at 50 and 70% mixed with air for 24 h at 0 °C.

All pre-treatments were compared to untreated grapes (control). Except for the non-packed control, all the single grapes boxes were packed separately in film bags, made of polyamide and polyethylene (PA/PE), under MAP (2%O_2—5%CO_2), hermetically sealed. Subsequently, they were stored in a container at 0 ± 0.5 °C and 90–95% relative humidity (RH) for 45 days. At the end of CS, the temperature was raised to 15 ± 1.0 °C for one week to simulate SL.

The main grape berries quality parameters were measured immediately at harvest, during CS at intervals of 15, 30 and 45 days, and at the end of the SL.

2.3. Decay incidence (DI) and mass loss (ML)

DI was measured in naturally infected Scarlotta organic grapes after each sampling time and after the SL period. DI was calculated as the mass of the decayed berries after removal from the entire cluster.

ML percentage was determined according to the following expression: % $WL_t = (M_0\text{-}M_t) \times 100/M_0$, where % WL_t is the percentage ML at time t, M_0 is the initial sample mass and M_t is the sample mass at time t.

2.4. Mechanical and chemical attributes, colour

Mechanical characteristics included: force needed to detach berries from the rachis, maximum force necessary to puncture the skin of berry, force required to compress a berry through a flat cylinder probe.

The soluble solid content (SSC) was determined with a digital refractometer (Atago, Japan), total titratable acidity (TA) was expressed as g L^{-1} of tartaric acid, pH was quantified by a pH-meter (Crison, Spain).

The berry skin colour was measured with a colourimeter (Minolta CR 400 ChromaMeter, Japan). Colour parameters L^*, a^*, b^* were recorded using the CIELAB colour system, and then were calculated:

- Hue angle (h°) [$\tan^{-1}$ b/a];
- Colour index for red grapes (CIRG) as CIRG = [(180-h°)/(L^*+C^*)] (Carreño, Martinez, Almela, & Fernandez-López, 1995).

2.5. Rachis chlorophyll, antioxidant activity (AA), total phenolic content and anthocyanins

The rachis chlorophyll was measured by spectrophotometer at wavelengths of 652.4, 665.2 and 470 nm, its content was expressed as mg kg^{-1} using Lichtenthaler's formula (Lichtenthaler, 1987).

AA in skin and flesh was analyzed with the ABTS [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] assay. The results were expressed as mmol Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalent antioxidant activity (TEAC) for kg^{-1} of skin or flesh according to the method reported by Re, Pellegrini, Pannala, Yang, and Rice-Evans (1999).

Total phenolic content, expressed as gallic acid equivalent (GAE) mg kg^{-1}, was determined on grapes flesh and skin extract by Folin-Ciocalteau method (Singleton, Orthofer, & Lamuela-Raventos, 1999).

Anthocyanins were determined on skin extract using a Waters 600 E HPLC, (Waters Inc.). Tentative identification of anthocyanins was achieved by combining the elution pattern with data reported in literature (De la Cruz et al., 2012; Revilla & Ryan, 2000; Singh Brar, Singh, & Swinny, 2008); the results were expressed as mg kg^{-1} malvidin-3-glucoside equivalent. Total anthocyanins content was determined using skin extract in agreement with Gambacorta et al. (2011).

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

The extraction of volatile compounds was carried out by headspace solid phase micro-extraction using a triphasic fibres DVB/Carboxen/PDMS 50/30 μm. Gas chromatography/mass spectrometry (GC/MS) analysis was performed using a Thermo Scientific ISQ™ QD Single Quadrupole GC/MS.

Chromatographic data identification of acetaldehyde and ethanol components was based on the comparison of their GC retention times