



Exposure to volatiles of essential oils alone or under hypobaric treatment to control postharvest gray mold of table grapes



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ABSTRACT

After harvest, table grapes can easily undergo fungal spoilage, which is mainly caused by *Botrytis cinerea*, the causal agent of gray mold. To reduce such losses, table grapes are usually treated with conventional fungicides during the season, and cold stored in the presence of sulfur dioxide. However, these applications are not permitted in organic agriculture, and at the same time, there is a growing demand from consumers for fresh fruit free from pesticide residues. The application of essential oils and hypobaric treatments are promising alternatives to sulfur dioxide with minimal environmental impacts and limited concerns about human health risks. The aim of this study was to determine the effectiveness for control of postharvest gray mold of table grapes of 24-h exposure to volatiles of essential oils of *Rosmarinus officinalis* (rosemary), *Mentha piperita* (peppermint), and *Thymus vulgaris* (thyme) individually and in combinations with hypobaric treatment at 50 kPa (0.5 atm). Exposure to volatiles of rosemary essential oils under atmospheric pressure and hypobaric conditions reduced by around 65% the incidence and McKinney's Index of gray mold for table grapes that were then stored at room temperature for 9 d and 5 d, respectively, or that were stored at 4 °C for 7 d and followed by 3 d shelf life at 20 °C. Peppermint essential oils similarly controlled gray mold for grapes stored at room temperature and under hypobaric conditions for 24 h. Panel tasting revealed perception of the essential oils soon after the treatments and 24 h later for grape berries exposed to vapors of rosemary, peppermint, and *Lavandula × ibrida* (lavender). Then 48 h after treatment, the rosemary and peppermint essential oils were no longer perceived on grapes stored at 4 °C and at 20 °C. Exposure to volatiles of the rosemary and peppermint essential oils alone or in combination with hypobaric treatment might represent an innovative method to control postharvest gray mold of table grapes, although at least 48 h were needed between exposure to volatiles of essential oils and presentation to consumers.

1. Introduction

After harvest, table grapes are perishable, as they are particularly susceptible to drying, mechanical injury, decay, and physiological disorders. The most economically important postharvest disease of table grapes is gray mold, which is caused by *Botrytis cinerea* (Lichter and Romanazzi, 2017). Gray mold is a major obstacle to long-distance transport and storage of grapes, because it can develop at low temperatures (-0.5 °C), and once present, it spreads quickly among the grape berries.

Over the last 50 years, the use of fungicides or chemicals such as sulfur dioxide (SO₂) have been the main means for management of *B. cinerea* (Rosslenbroich and Stuebler, 2000; Romanazzi et al., 2016b). However, SO₂ can cause bleaching of table grape berries, and it is responsible for decline in the flavor quality and sulfite can induce adverse reactions in some consumers (Montaño García, 1989). In addition, SO₂

and fungicides are not permitted as postharvest treatments under organic production rules (Mlikota Gabler and Smilanick, 2001). For these reasons, alternatives to SO₂ are needed for the control of postharvest decay of table grapes (Romanazzi et al., 2012).

Essential oils are biologically active and they represent rich potential sources of alternative and environmentally acceptable compounds for disease management. The antifungal activities of essential oils have been well documented over recent decades (Deans and Ritchie, 1987; Stavropoulou et al., 2014), and there have been several more recent studies describing the effectiveness of essential oils on the control of postharvest decay of fruit (Cindi et al., 2016; Mari et al., 2016). Such essential oils couple their antimicrobial activities with elicitation of host defenses (Romanazzi et al., 2016a). An added advantage of essential oils is their activity as vapors, a characteristic that makes them attractive as possible fumigants for the protection of stored produce, allowing further fruit manipulation to be avoided (Tripathi et al.,

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2008). On the other hand, treatment of fruit with essential oils needs to be carefully considered, as these might influence the organoleptic characteristics of the fruit that could compromise the acceptability for consumers (Guillén et al., 2007).

Storage of fruit under hypobaric conditions can also be considered as an alternative to conventional fungicides to control postharvest decay (Apelbaum and Barkai-Golan, 1977; Romanazzi et al., 2001; Hashmi et al., 2013). Furthermore, under hypobaric conditions, the vaporization and distribution over the fruit surface of the essential oils can be improved.

The aim of this study was to determine the effectiveness of exposure to volatiles of essential oils of *Rosmarinus officinalis* (rosemary), *Mentha piperita* (peppermint), and *Thymus vulgaris* (thyme) to control postharvest decay of table grapes at atmospheric pressure or under hypobaric conditions (50 kPa), with further storage at room temperature (20 °C) or cold storage (4 °C), followed by exposure to shelf life conditions. In addition, a consumer panel tasted the table grapes after these treatments at different times and temperatures to determine whether differences can be perceived among grape berries exposed to rosemary, peppermint, or *Lavandula × ibrida* (lavender) essential oil vapors, where this last is particularly perceivable by consumers.

2. Materials and methods

2.1. Fruit

The experimental trials were carried out on 'Italia' table grapes (*Vitis vinifera*) grown organically in Apulia Region, South-eastern Italy, according to the standard practice of the area, and harvested in commercial orchards. Fresh table grapes were transferred to the laboratory, where portions of bunches consisting of 10 berries each were selected according to homogeneous size, shape, color, and weight, and absence of injuries to the berries.

2.2. Treatments

The bunch portions consisting of 10 berries each were placed in small plastic trays that were then placed in airtight boxes for 24 h. Within each airtight box, there were seven replicates of the plastic trays, each of which contained four bunch portions of the table grapes. In total within each airtight box, there were 280 berries. In addition, the airtight boxes contained flasks with 5 mL water or the essential oils of rosemary, peppermint or thyme. The chemical compositions of the essential oils used in the experimental trials (including those of lavender) were analyzed by gas chromatography (Perkin Elmer Clarus 500 GC/FID/MS) (kindly provided by Flora Srl, Pisa, Italy) (Table 1).

In the first trials, these airtight boxes were kept at room pressure, while in the following trials, the hypobaric treatment of 50 kPa (0.5 atm) was created within the airtight boxes using a vacuum pump. After 24 h, the plastic trays containing the table grapes were moved out of the airtight boxes and placed into large covered plastic boxes. In addition, the trials carried out with the hypobaric treatment were repeated with the table grapes stored in large covered plastic boxes for 7 d at 4 °C, and then exposed to shelf life at room temperature (20 ± 2 °C). During the storage at room temperature and during the shelf life, the postharvest decay evaluations were carried out as reported below.

2.3. Postharvest decay evaluation

Postharvest decay of the table grapes was recorded daily. Disease incidence was expressed as percentages of infected berries. Disease severity was also recorded according to an empirical scale with six degrees: 0, healthy fruit; 1, 1% to 20% fruit surface infected; 2, 21% to 40% fruit surface infected; 3, 41% to 60% fruit surface infected; 4, 61% to 80% fruit surface infected; 5, > 81% of fruit surface infected and showing sporulation (Romanazzi et al., 2001). This empirical scale

allowed the calculation of the McKinney's Index, which is expressed as the weighted average of disease as a percentage of the maximum possible level (McKinney, 1923). Specifically, this was calculated according to Eq. (1):

$$I = [\Sigma(d \times f)/(N \times D)] \times 100, \quad (1)$$

where d is the category of rot intensity scored on the berry, f is its frequency, N is the total number of berries examined (i.e., healthy and rotted), and D is the highest category of disease intensity that can occur on the empirical scale (Romanazzi et al., 2001).

2.4. Sensory analysis

The technique of triangle discrimination tests (Meilgaard et al., 1999; Lawless and Hildegarde, 2010; Feliziani et al., 2014) was used to determine whether there were any detectable differences in the flavors of the table grapes exposed to the essential oils vapors, as compared to table grapes stored with water. Here the table grapes underwent to exposure to volatiles of rosemary, peppermint or lavender essential oils under hypobaric conditions described above. Once the table grapes were moved from the airtight boxes, they were submitted to tasting trials by untrained panelists: (i) soon after the treatments; (ii) after 24 h at room temperature; (iii) after 48 h at room temperature; and (iv) after 48 h storage at 4 °C.

At the time of the analysis, each panelist was presented with three sets of three berries each: the first set had been exposed to vapors of water or rosemary essential oils, the second to vapors of water or peppermint essential oils, and the third to vapors of water or lavender essential oils. In each set, two of the three berries had the same treatment, while one was different. Samples were presented in a random order and assigned three-digit codes to reduce any influence over the decisions of the panelists. For each set, the panelists were asked to taste the berries and circle the sample number that they believed was the different berry. The analysis was performed under controlled temperature and lighting conditions, in individual booths. The three tests were carried out on three different days, each day at 10:00 h.

2.5. Statistical analysis

The data were analyzed by one-way ANOVA, followed by Tukey's honestly significant difference (HSD) test, to $P \leq 0.05$ (Stat-soft, Tulsa, OK, USA). When the range of the percentages was > 40%, the percentage data were arcsine transformed before analysis, to improve the homogeneity of the variance. The actual values are shown. The experimental trials were repeated at least twice. Data from two or more trials were pooled, and the statistical analysis to determine the homogeneity of the variance was tested using Levene's tests.

To analyze the data obtained from the sensory evaluation panelists, the correct answers of the triangle discrimination tests were compared to the tabulated critical values (Lawless and Hildegarde, 2010).

3. Results

3.1. Postharvest decay following treatments with essential oils

The main postharvest decay observed among the table grapes was gray mold. Other rots caused by *Penicillium* spp., *Rhizopus stolonifer*, and *Aspergillus* spp. occurred rarely. In the trials where the table grapes were exposed to the essential oils at atmospheric pressure and then stored at room temperature, only the vapors of the rosemary essential oils significantly reduced the incidence and McKinney's Index of the gray mold, by 63% and 69%, respectively (Table 2). The treatments with the peppermint essential oils showed a trend to a reduction of both of the measured parameters, while the thyme essential oils were not effective. The severity of the gray mold also showed a tendency to a reduction

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