



Antifungal protection and antioxidant enhancement of table grapes treated with emulsions, vapors, and coatings of cinnamon leaf oil



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ARTICLE INFO

Article history:

Received 26 January 2012

Accepted 15 July 2013

Keywords:

Table grapes

Fungal decay

Antioxidant status

Cinnamon leaf oil

ABSTRACT

Table grapes have high market value in international markets due to their attractive taste and high antioxidant content. However, their market potential is limited by losses due to *Botrytis cinerea* Pers. Fr. Cinnamon leaf oil (CLO) is a natural fungicidal and antioxidant agent that can be used to avoid postharvest losses due to *B. cinerea* Pers. Fr. and to increase the antioxidant levels of this produce. CLO was applied to grapes as water emulsions (0, 0.5, 2.5, and 5 g L⁻¹), as vapors (0, 0.196, 0.392, and 0.588 g L⁻¹), or as a chemical incorporated into pectin coatings (0 and 36.1 g L⁻¹). Afterwards, berries were stored at 10 °C for 15 d and were evaluated periodically for the fungal decay index, the total phenolic and flavonoid contents and the antioxidant activity using the Trolox equivalent antioxidant capacity and DPPH• radical inhibition methods. The odor acceptability of the treated berries was evaluated after 10 d of storage. The CLO emulsion (5 g L⁻¹) significantly reduced the fungal decay without affecting the antioxidant properties of the berries. The application of CLO as a vapor was more effective according to the evaluated parameters than the emulsions; all tested concentrations inhibited fungal decay and increased the flavonoid content and antioxidant activity. When CLO was incorporated into the pectin, no fungal decay appeared, and the highest antioxidant activity was observed after 15 d of storage. Additionally, all treatments, except the emulsion treatment, increased the odor acceptability of the treated berries compared to the control berries. From this study, it can be concluded that CLO as vapors or coatings can be used to control decay and increase the antioxidant health benefits of grapes due to CLO's antifungal and antioxidant properties.

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1. Introduction

Grapes have been studied because of their antioxidant properties and are considered important sources of phenolic compounds, which are mainly responsible for the antioxidant properties of grapes (Baiano and Terracone, 2011). Anthocyanins, tannins, and flavonoides contribute to the characteristic colors and flavors of grapes and, moreover, are powerful antioxidants (Lutz et al., 2011). Nevertheless, table grapes are very susceptible to postharvest rot caused by fungi, *Botrytis cinerea* being among the most prevalent (Chervin et al., 2009). The major symptom of *B. cinerea* infestation is brown discoloration, after which the skin of the fruit begins to loosen, followed by a surge of white hyphae filaments and, finally, masses of gray-colored spores (Elmer and Reglinski, 2006). The most utilized commercial treatment to control *Botrytis* infestation around the world is fumigation using sulfur dioxide (SO₂)

and other synthetic gases (Rivero and Quiroga, 2008). However, this treatment can be dangerous to consumers' health and to the environment (Lichter et al., 2002); thus, alternative natural technologies may help maintain berry quality without the use of these synthetic products.

The essential oil of cinnamon leaves (CLO) is recognized for its aroma and medicinal properties (Ayala-Zavala et al., 2008a; Singh et al., 2007) and has been identified as a GRAS (Generally Recognized As Safe) product by the USFDA (US Food and Drug Administration, 21CFR582.20). The antifungal and antioxidant properties of CLO are due to volatile components such as eugenol and cinnamaldehyde (Combrinck et al., 2011). These volatile phenolic compounds are able to damage the fungal cells, thus slowing the development of *B. cinerea* (Ayala-Zavala et al., 2008a; Combrinck et al., 2011), and are highly capable of neutralizing free radicals (Singh et al., 2007). Despite its beneficial properties, CLO's application to fruits and vegetables postharvest remains a challenge due to the high volatility and high reactivity of such bioactive compounds and its strong aroma, which could affect the sensorial attributes of the fruit (Ayala-Zavala et al., 2008b).

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Table 1

Physicochemical characterization of grapes used in the experiments for replication purposes. The selection of the lots of berries for the replication of experiments (3 times) was performed, verifying that the grapes displayed values similar to those shown in this table.

Parameter	Mean value \pm SD
pH	4.44 \pm 0.03
TA (%)	0.34 \pm 0.01
TSS (%)	19.9 \pm 0.12
Color	
L	29.6 \pm 3.26
C	20.9 \pm 2.35
$^{\circ}$ Hue	52 \pm 9.10
Firmness (N)	2.58 \pm 0.48

Immersion into solutions, exposure to vapors, and coatings are among the alternative means of incorporating essential oils into the postharvest treatments of fruits and vegetables (Kader, 2002). Immersion into solutions is one of the most practical and developed techniques that is used to sanitize fruits and vegetables (Kader, 2002). Treatments involving gas vaporization have also been contemplated for use in the preservation of the quality of fruit postharvest. In the procedure for using this protective treatment, the essential oil is volatilized in the atmosphere within the package of the relevant fresh produce (Kader, 2002). In coatings, the active agent can be incorporated onto the surface of the fruit and will thus exert a protective function (Embuscado and Huber, 2009). Based on this information, the present work aims to evaluate the use of CLO as an antifungal and antioxidant agent when applied as emulsions, vapors, and coatings to table grapes postharvest.

2. Materials and methods

2.1. Plant materials

The grapes (*Vitis vinifera* L., Cv 'Redglobe') used in the present study were obtained from commercial vineyards in the region of Pesqueira, Sonora, Mexico. Berries from the cluster, harvested at commercial maturity, were randomly selected according to their size uniformity, color, and the absence of diseases or damage.

2.2. Physicochemical quality parameters

The berries were initially characterized using physicochemical parameters (Table 1) to select the grapes for use in the replication studies, which were repeated three times.

2.2.1. Soluble solids, pH, and titratable acidity

Twenty berries from 5 different clusters were placed in cheese-cloth and squeezed manually, and the juice was analyzed for the total soluble solids (TSS), pH, and titratable acidity (TA). The TSS were determined at 20 °C using a refractometer (Atago DBX-55, Atago Co., Ltd., Tokyo, Japan) and expressed as a percent (%). The TA, expressed as a percent (%) of tartaric acid, was determined by diluting a 5 mL aliquot of berry juice with 95 mL distilled water and then titrating it to a pH of 8.2 using 0.1 mol L⁻¹ NaOH. The TA and pH were measured using an automatic titrator (Mettler-Toledo, Columbus, OH, USA). Samples were evaluated in triplicate.

2.2.2. Surface color measurement

Fruit surface color was measured on 20 fruits using a colorimeter (CR 200, Minolta, Ramsey, NJ, USA), which provided CIE L*, a*, and b* values. Negative a* values indicate green skin color, and higher positive a* values indicate red skin color. Higher positive b* values indicate yellow skin color. These values were then used to calculate the hue degree ($h^{\circ} = \arctangent(b^*a^{*-1})$), where

0° = red-purple; 90° = yellow; 180° = bluish green; and 270° = blue, as well as the chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$), which indicates the intensity or color saturation.

2.2.3. Firmness

Fifteen berries were evaluated by compression (1 mm) with a flat disk on an automatic texture analyzer (Firm Tech 2, Bioworks Inc., KS, USA). The results were expressed in Newtons (N).

2.3. CLO treatments

Berries were detached from the clusters and subjected to CLO emulsion immersion, vapors, and pectin coatings. The effect of the CLO (*Cinnamomum zeylanicum*, Ceylon origin, purchased from Sigma-Aldrich, Toluca, Mexico) as an antifungal and an antioxidant agent for grapes was evaluated during grape storage. The volatile constitution of this oil has been previously reported (Ayala-Zavala et al., 2008b). Eugenol accounts for approximately 78% (w w⁻¹) of the total volatiles, and other minor CLO constituents detected included cinnamaldehyde, copaene, and β -caryophyllene.

2.3.1. Emulsion treatment

Berries were treated with different concentrations of CLO emulsions (0, 0.5, 2.5, 5 g L⁻¹). The emulsification process was performed mechanically using a homogenizer at 50 s⁻¹ for 5 min. The emulsion was stirred until the treatment was performed on the berry samples to assure the complete homogenization of the CLO. The berries were submerged in the essential oil emulsions for 2 min, air-dried and packed in polypropylene trays. Twenty trays containing 6 berries from each type of treatment were stored at 10 °C, this arrangement was repeated for the vapor and the edible coating experiments.

2.3.2. Vapor treatment

The table grape berries were exposed to different headspace concentrations of the CLO vapors (0, 0.196, 0.392, 0.588 g L⁻¹). Fruit were placed in 0.62 L polypropylene trays, and 0, 0.122 g, 0.243 g, and 0.365 g of CLO were added into individual small glass containers, which were subsequently placed inside the plastic trays before the lids were used to cover the trays to achieve the desired concentrations. The volatile CLO compounds were vaporized inside the containers spontaneously for 30 min at 25 °C.

2.3.3. Pectin + CLO coatings

CLO enriched coatings were prepared with 15 g of food grade pectin (methoxy = 8.1%) dissolved in 0.5 L of distilled water and homogenized for 15 min. Then, 4.95 mL of glycerol was added, and the mixture was homogenized for 15 min. CLO (18.05 g) was added to achieve a final oil in pectin solution concentration of 36.1 g L⁻¹. The berries were submerged in the coating solution for 2 min and allowed to dry at room temperature (25 °C) for 15 min. Two control treatments were also evaluated: fruit without coating and fruit with a pectin-only coating. Six berries (\approx 100 g) for each type of treatment were packed in twenty polypropylene trays, and the twenty trays were stored at 10 °C. At 5 d intervals, samples were taken to evaluate the fungal decay, total phenolic content, total flavonoid content, and antioxidant capacities.

2.4. Fungal decay index caused by *B. cinerea*

The inoculation method was used to evaluate the fungicide effect of the CLO treatments in controlling the postharvest decay of grapes. The fungal pathogen, *B. cinerea* Pers. Fr., was isolated from infected grapes that showed a typical gray mold infestation symptom. The isolate was purified using a single spore isolation

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