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Postharvest grapefruit seed extract and chitosan treatments of table grapes to control *Botrytis cinerea*

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Abstract

'Redglobe' table grapes (*Vitis vinifera* cv. Redglobe), undergoing deterioration were selected as model fruit with, *Botrytis cinerea*, to test the antifungal activity of grapefruit seed extract (GSE) in vitro and in vivo. The results of inhibition of spore germination and radial growth of *B. cinerea* in vitro indicated that GSE could efficiently inhibit the growth of the tested fungi.

The effectiveness of GSE and chitosan to control postharvest decay and quality of 'Redglobe' grape berries stored at 0–1 °C was also investigated. Chitosan and GSE treatments, alone or combined, significantly reduced postharvest fungal rot of the fruit compared with controls challenged with *B. cinerea*. Differences in weight loss, color change, ripening, sensory quality and microorganism index between grapes treated with GSE and control fruit suggested that GSE had both antifungal and antioxidative activity. Moreover, the sensory analyses revealed beneficial effects in terms of delaying rachis browning and dehydration and maintenance of the visual aspect of the berry without detrimental effects on taste, or flavour. GSE and chitosan might have a synergistic effect in reducing postharvest fungal rot and maintaining the keeping quality of 'Redglobe' grapes.

Keywords: Grapefruit seed extract; Chitosan; *Botrytis cinerea*; Table grape; Quality attributes

1. Introduction

Gray mold, caused by *Botrytis cinerea* Pers., is the most economically important postharvest disease of table grapes (Cappellini et al., 1986). It is responsible for significant losses of table grape berries both before and after harvest, and is a major obstacle to long-distance transport and storage. Control of the disease is especially important in storage because it develops at low temperatures (−0.5 °C) and spreads quickly among berries (Karabulut et al., 2004).

During the last 50 years, management of *B. cinerea* has relied heavily on the use of chemicals such as sulfur dioxide (SO_2) (Rosslenbroich and Stuebler, 2000). This approach, however, is not regarded as sustainable, because of the relative ease with which fungicide-resistant strains of *B. cinerea* emerge within vineyard populations (Latorre et al., 1997) and because of increasing public concern about chemical residues and environmental health (Janisiewicz and Korsten, 2002). In addition, SO_2 is not allowed for use in organic produce as a postharvest treatment (Mlikota and Smilanick, 2001). Thus alternative approaches are necessary to maintain the marketable quality of table grapes.

Grapefruit seed extract (GSE) is a commercial product derived from the seeds and pulp of grapefruit (*Citrus paradisi Macf.* Rutaceae). GSE is an effective broad-spectrum bactericide (Coombe, 1989; Reagor et al., 2002; Lee et al., 2005), fungicide (Heggers et al., 2002), and antiviral and antiparasitic (Ionescu et al., 1990; Tirillini, 2000) natural extract. And GSE is also environmentally safe without toxicity to humans or animals at effective concentrations. Ionescu et al. (1990) demonstrated that GSE performed as well as other antimicrobial agents tested on 770 strains of bacteria, and 93 strains of fungus. Inhibitory activity of GSE on yeast and some yeast-like fungi showed different efficacy depending on the strains and was generally weaker than that on bacteria and fungi (Ionescu et al., 1990). The safety of

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GSE has been tested in several areas, with Heggers et al. (2002) showing that GSE was not detrimental to human fibroblast skin cells in vitro, while still retaining high antimicrobial activity.

Chitosan, a mostly deacetylated β-1,4-linked D-glucosamine polymer, is a structural component of fungal cell walls. Chitosan has been reported to enhance disease resistance against many fungal diseases, when applied as either a pre- or postharvest treatment (El Ghaouth et al., 2000; Reglinski et al., 2005). In addition, chitosan can be directly antimicrobial and has been shown to interfere with germination and growth of several phytopathogenic fungi, including *B. cinerea* (Ben-Shalom et al., 2003). Furthermore, various investigators have demonstrated that a chitosan coating has the potential to inhibit decay and hence prolong the storage life of a variety of produce including strawberries (Han et al., 2004) and table grapes (Romanazzi et al., 2002), especially when enriched with other antimicrobial agents (Sivakumar et al., 2005; Zivanovic et al., 2005; Romanazzi et al., 2006).

The purpose of this study was to evaluate the antifungal efficacy of GSE and chitosan, alone or combined, compared with thiabendazole, to control *B. cinerea* in vitro and test the effects on decay and keeping quality of 'Redglobe' grapes in vivo.

2. Materials and methods

2.1. Table grapes

'Redglobe' grapes (*Vitis vinifera* cv. Redglobe, 19% soluble solids and 0.4% tartaric acid) were harvested from a commercial planting located in the Daxing area (Beijing, China). Only fruit of uniform size, color and shape were selected to obtain homogeneous batches, and all were chosen to have been absence of injuries.

2.2. Fungi and chemicals

B. cinerea was isolated from infected grape berries, stored at −80 °C on silica gel and cultured on potato dextrose agar (PDA) for 2 weeks at 25 ± 1 °C. A spore suspension was created by flooding plates with a small volume of sterile distilled water containing 0.05% (v/v) Tween-80 (Sigma Chemical Co., St. Louis, U.S.A.), and spores were removed by gently scraping with a glass rod. The resulting spore suspension was filtered through four layers of cheesecloth to remove mycelial fragments and diluted with sterile water to obtain an absorbance of 0.25 at 425 nm as determined by a BioMate5 Union ultraviolet spectrophotometer (UNI, Hamburg, Germany). The density was about 1.2×10^6 spores/mL and was diluted with sterile water to obtain the desired spore concentrations. A volume of 50 mL of inoculum per 1200 berries was applied with an air-brush sprayer.

GSE (Citricidal™, 60% grapefruit extract and 40% vegetable glycerine) was purchased from Bio/chem. Research (CA, U.S.A.). GSE was dissolved in distilled water with 0.05% (v/v) Tween-80 as a surfactant to make a 1% (v/v) stock solution. Crab-shell chitosan (Sigma Chemical Co., St. Louis, U.S.A.) was ground to a fine powder in a mortar, washed three times with distilled water, pelleted by low-speed centrifugation, and air-dried at room temperature. Sheets of chitosan were solubilized by stirring in 0.25N HCl, centrifuging to remove insoluble material, and precipitating by neutralization with 1N NaOH. The chitosan pellets were recovered by centrifugation, washed with deionized water to remove salts, and freeze-dried (Benhamou et al., 1994). For experimental use, the 1% (w/v) solution of chitosan was prepared by dissolving, under continuous stirring, the purified chitosan in 0.5% (v/v) acetic acid (Romanazzi et al., 2002). When dissolved, the pH of the chitosan solution was adjusted to 5.6 using 1N NaOH, and 0.05% (v/v) surfactant Tween-80 was added to improve the wetting properties of the solution. GSE was added to chitosan (1%, w/v) to make the combination of 1% chitosan with 0.1% GSE. For comparison, 0.1% of thiabendazole (TBZ Deco Chemicals, China), a commonly used fungicide with the $EC_{50} < 2$ μg/mL for *B. cinerea* (Lima et al., 2006), was also prepared. All stock solutions were filter-sterilized through a 0.22 μm pore size filter.

2.3. Antifungal assay

Two methods were used to determine the effect of 0.5% GSE, 1.0% chitosan, 1.0% chitosan containing 0.1% GSE, and 0.1% TBZ on the germination of spores of *B. cinerea*. In the first method, spores (12,500 spores/mL) were mixed with various antifungal agents at room temperature (22–24 °C) in a final volume of 2 mL. After 60 s, the spore suspensions were diluted 100-fold in sterile water and 10 μL was plated on PDA. After 48 h incubation at 24 °C, the number of colonies per plate was counted. Data were expressed as the percentage of germinated spores.

To further characterize the antifungal activity, we carried out a parallel study using PDA plates containing antifungal agents at a 1% final concentration as mentioned above. Antifungal agents were added separately to PDA at 60 °C, mixed rapidly and poured into Petri dishes. After the agar had cooled, a small amount of mycelium of the *B. cinerea* was added to each plate. After incubation at 24 °C for 72 h the control had fungal growth to the edge of plates, and the diameter of the mycelial colony was measured (Ait Barka et al., 2004). In addition, after 5 days of incubation at 24 °C, a thin layer of mycelium was aseptically removed, placed in a drop of lactophenol-cotton blue (0.1%, w/v) on a microscope glass slide, stained for 2 h and observed under a microscope (BX51T, Olympus, Japan). For each treatment, six replicate plates were used.

2.4. Treatment of single detached berries

The effectiveness of treatments of antifungal agents on gray mold incidence of 'Redglobe' grape berries sprayed with a spore suspension of *B. cinerea* was determined. Berries were either cut from the rachis with pedicel intact, or pulled from the rachis (pedicel detached), which exposed the berry flesh and enabled wound inoculation to occur. The berries were then sprayed with the spore suspension at about 1.0×10^5 spores/mL as described previously (Mlikota et al., 2005). Inoculated berries were kept at 15 °C in a covered plastic box for 2, 12, 24, or 48 h prior to