Postharvest Biology and Technology 147 (2019) 214–221

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Effects of chitosan treatment on avocado postharvest diseases and expression of phenylalanine ammonia-lyase, chitinase and lipoxygenase genes

Chinelo Obianom[a], Gianfranco Romanazzi[b], Dharini Sivakumar[a,*]

[a] Postharvest Technology Group, Department of Crop Sciences, Tshwane University of Technology, Pretoria 0001, Pretoria, South Africa
[b] Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Ancona, Italy

ARTICLE INFO

Keywords:
Biofungicide
Colletotrichum gloeosporioides
Epicatechin
Fruit quality
Induced defence
Lasiodiplodia theobromae

ABSTRACT

Stem-end rot (*Lasiodiplodia theobromae*) and anthracnose (*Colletotrichum gloeosporioides*) are major postharvest diseases in avocado that cause severe postharvest losses throughout the supply chain. One of the strategies to reduce development of such decay agents resides in the application of resistance inducers (e.g. chitosan), capable of reducing fungal growth and inducing resistance in fruit tissues.

The influence of chitosan treatment (1% or 1.5% w/v) was investigated on decay incidence, gene expression of phenylalanine ammonia-lyase (*PAL*), chitinase (*CHI*) and lipoxygenase (*LOX*) and antioxidant enzyme activity [superoxide dismutase (SOD) and catalase (CAT)] in i) drop-inoculated (without wounding) or ii) artificially infected (with wounding) avocado (cv. 'Hass') with *L. theobromae* or *C. gloeosporioides* pathogens and also in iii) naturally infected (natural inoculum) avocado. Fruit were dipped in 1% or 1.5% w/v chitosan dissolved in water for 3 min, followed by storage for 14 d and 28 d respectively at 7.5 °C, and thereafter for 5 d at 18 °C to simulate market shelf conditions.

Chitosan at 1.5% significantly reduced the incidence of stem-end rot and anthracnose in both inoculated and naturally infected avocados. The up-regulation of *PAL* and down-regulation of *LOX* genes moderately allowed higher epicatechin contents (90 mg kg^{-1} FW) in the exocarp, which could have contributed to improved anthracnose control. The up-regulation of *CHI* genes and higher SOD activity could have contributed to control of stem-end rot. Chitosan solution (1.5%) retained moderate levels of C7 sugars and firmness up to 5 d shelf life. The control of stem-end rot and anthracnose of avocados obtained with 1.5% chitosan can be ascribed to a combination of its antifungal and eliciting properties.

1. Introduction

'Hass' avocado is the most popular cultivar globally retailed and consumed, due to their characteristic nutty flavour and high nutritional value (Dreher and Davenport, 2013). Avocado fruit is mainly susceptible to postharvest fungal pathogens that gain entry via natural openings, wounded surfaces and by direct penetration into the host cell tissue (Zeng et al., 2010; Prusky et al., 2014). Considerable postharvest losses, limiting storage and marketability of the fruit, are caused mainly by anthracnose (*Colletotrichum gloeosporioides* (Penz. & Sacc.) and stem-end rot (including *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.) (Zeng et al., 2010; Srivastava and Ahmad, 2014). In avocado fruit, once the spores infect the host through the pericarp, the fungi remain quiescent and start colonizing with initation of fruit ripening (Prusky et al., 2009).

South Africa and other avocado exporting countries apply imidazole (a.i. prochloraz) as commercial fungicide in the packhouse as a spray or dip to control anthracnose and stem-end rot during storage and transportation. The allowable maximum residue level (MRL) for South African avocados was set at 2 mg kg^{-1}, however some importing countries in Europe prefer fruit with lower MRL due to the negative impact of fungicide residues on human health. Acid prochloraz treatments were reported to reduce the concentration of prochloraz and improve the fruit quality by controlling postharvest decay (Feygenberg et al., 2014). However, due to the corrosive nature of HCl, this application was not commercially adopted by the avocado industry (Mavuso and Van Niekerk, 2013). Despite the efficiency in managing disease occurrence in fruit with synthetic fungicides, limitations on the application of prochloraz have necessitated the search for alternatives, principally natural treatments that are environmentally friendly and

* Corresponding author.
E-mail address: sivakumard@tut.ac.za (D. Sivakumar).

https://doi.org/10.1016/j.postharvbio.2018.10.004
Received 16 July 2018; Received in revised form 26 September 2018; Accepted 4 October 2018

safe with the capability of replacing the use of synthetic fungicides. Recently, exposure of infected avocado to thyme oil vapours were shown to reduce anthracnose (Bill et al., 2016), and the combination of thyme oil and half-strength prochloraz (500 mg L^{-1}) in aqueous solution effectively reduced stem-end rot incidences in our previous studies. Hence, it is beneficial to develop an alternative treatment that can be easily implemented in the packline to control both postharvest diseases for the avocado industry. Recently, intensive research has been conducted on the retention of postharvest quality of avocados via application of edible coatings such as methylcellulose and chitosan (Mura et al., 2011; Tesfay et al., 2017). Chitosan is an *N*-deacetylated derivative of chitin, a natural polymer with polycationic characteristics [poly (*β*-(1-4)-*N*-acetyl-D-glucosamine)], synthesized by a number of living organisms including crustaceans (Romanazzi et al., 2017). Chitosan coatings exhibit film-forming properties (Sivakumar et al., 2005), creating a modified atmosphere around the product which improves its shelf life, overall appearance and concurrently reduces fruit weight loss during storage and/or transit (Ali et al., 2011; Romanazzi et al., 2017). Chitosan has shown a direct effect on the morphology of pathogens through its fungistatic and fungicidal capabilities in the host against *Botrytis* cinerea (El Ghaouth et al., 1992), where the chitosan coating on intact strawberries did not stimulate synthesis of chitinase and β-1,3-glucanase activities. Chitosan coatings are prepared for laboratory studies dissolving the biopolymer in glacial acetic acid, a process which is not economically viable to the avocado industry. Recently, a water-soluble chitosan formulation was shown to improve the postharvest quality of fruit crops such as table grapes, sweet cherries and strawberries (Feliziani et al., 2013; Romanazzi et al., 2016) through reduction of decay. Landi et al. (2014) demonstrated that this formulation induced defence responses of phenylalanine ammonia lyase (*PAL*), chitinase (*CHI*) and β-1, 3-glucans in strawberries stored up to 6, 24 and 48 h post-treatment. However, the information on chitosan's effect on gene expression of (*PAL*), chitinase (*CHI*) and β-1, 3-glucans, and the antioxidant enzyme activities are limited on sub-tropical fruits. It is important to demonstrate the long-term effect of the water-soluble chitosan formulation on induced defence response until the fruit reach the shelf, to provide protection against invading postharvest pathogens.

The objectives of this study were to investigate the effects of chitosan on the (i) control of incidence of artificially inoculated and naturally occurring anthracnose and stem-end rot in avocado 5 d after cold storage; (ii) expression of the defence-related genes *PAL*, *CHI* and *LOX*; (iii) activities of antioxidant enzymes SOD and CAT in inoculated and naturally infected avocados; (iv) epicatechin content and C7 sugars in naturally infected fruit ; (v) fruit firmness of 'Hass' avocado in naturally infected fruit; (vi) sensory properties of the fruit.

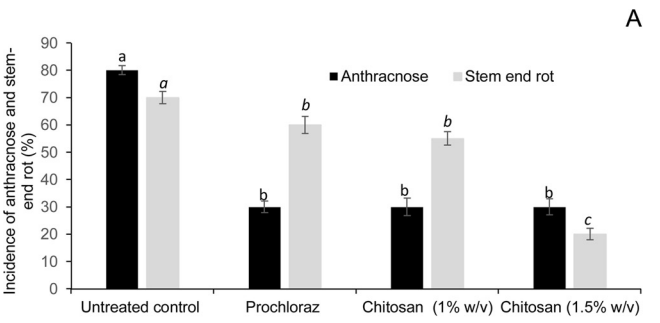
2. Materials and methods

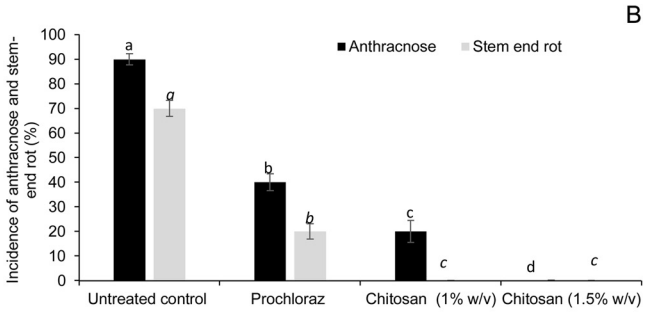
2.1. Fungal isolates

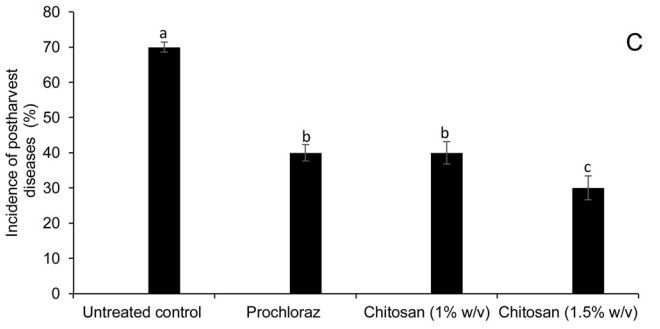
C. gloeosporioides and *L. theobromae* were isolated from avocado fruit [Fruit and Vegetable Technology Laboratories (Tshwane University of Technology) and Westfalia Fruit Laboratories (Tzaneen, South Africa)]. The fungal isolates were cultured and maintained on Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) (Sigma-Aldrich, Johannesburg, South Africa) at 25–30 °C respectively. For the fruit inoculation experiments, a spore suspension (10^6 spores mL^{-1}) was prepared according to Bill et al. (2017) from a viable culture.

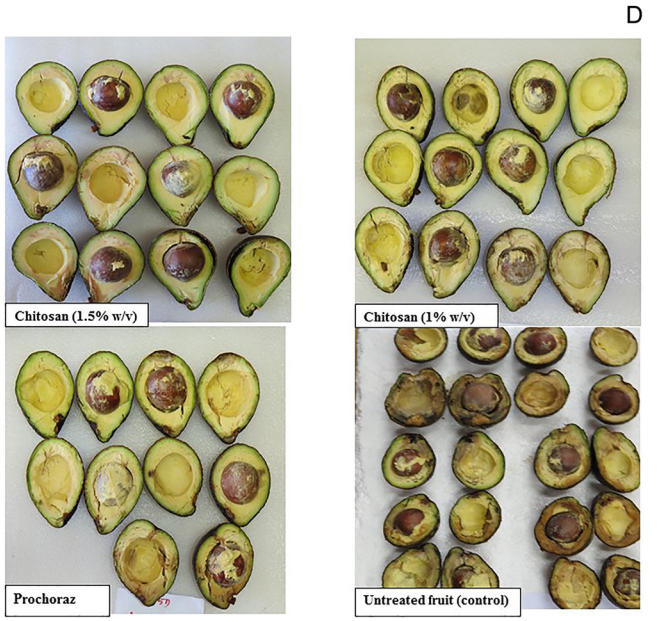
2.2. Postharvest treatments

The chemicals used for postharvest treatments in this study include a chitosan-based product (Chito Plant, ChiPro GmbH, Bremen, Germany) dissolved in water at two different concentration 1.0% or 1.5% and currently adopted prochloraz (450 g L − 1; imidazloe) (Adama SA (Pty) Ltd, Cape Town, South Africa) treatment. Fruits dipped in









(caption on next page)