



Carboxyl methylcellulose (CMC) containing moringa plant extracts as new postharvest organic edible coating for Avocado (*Persea americana* Mill.) fruit



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ABSTRACT

This study investigated the potential of edible carboxyl methylcellulose (CMC) containing moringa leaf and seed extracts as a novel postharvest treatment for maintaining storage quality and controlling diseases in 'Hass' and 'Gem' avocado fruit. The study also investigated the antifungal activity of methanolic and ethanolic moringa plant extracts. Briefly, 1% CMC was blended with 2% of moringa leaf (MLE) or seed extract (MSE). After the fruit was dipped in either CMC + MLE or CMC + MSE, it was stored at 5.5 °C (95% RH) for 21 days. After cold storage, fruit were stored at ambient conditions (21 ± 1 °C) and 60% RH to simulate retail conditions. Postharvest quality attributes such as ethylene production, respiration rate and fruit firmness were measured. Both coatings were also tested against postharvest fungi in reference to potato dextrose agarose (PDA). Coated fruit had lower mass loss, ethylene production and respiration rate compared to the uncoated fruit. Ethanolic leaf extract had an inhibition of 43.6% and 42.9% against *C. gloeosporioides* and *A. alternata*, respectively. Scanning electron microscopy analysis revealed damaged hyphal structures for all pathogens exposed to coatings while such structures remained intact in uncoated fruit. Ethanolic moringa leaf extracts proved to have higher antimicrobial activity compared to methanolic extracts. The findings reported in this study demonstrated that CMC containing moringa extract suppresses diseases, prolongs the shelf-life and maintain the overall quality avocados during postharvest supply chain. The CMC blended with moringa extracts could potentially be commercialized as a new organic edible coating for avocado fruit industry.

1. Introduction

Avocado (*Persea americana*, Mill) is a climacteric fruit characterized with high ethylene production and respiration rate during postharvest storage. The high rate of biological activities as well as postharvest fungal diseases is a major cause of avocado postharvest losses. Anthracnose caused by *Colletotrichum gloeosporioides* and stem-end rot caused by different fungal species of *Colletotrichum*, *Botryodiplodia*, *Dothiorella*, *Phomopsis* and *Lasiodiplodia* genera (Perez-Jimenez, 2008) are the most common fungal diseases in the avocado industry. A survey conducted by Sanders and Korsten (2000) showed a disease incidence of up to 80% in South Africa. Various postharvest fruit treatments have been used to maintain quality and reduce these postharvest losses. For instance, synthetic fungicides such as copper oxychloride and copper

hydroxide are common preharvest treatment used to control anthracnose and stem-end rot whilst prochloraz is used as a postharvest treatment (Perez-Jimenez, 2008). However, health concerns regarding chemical residues on treated fruit and their undesirable effect of on environment, chemical treatments have become unfavourable. Additionally, avocado fruit destined for the European Union should not be treated with synthetic waxes. This has detrimental effect on fruit quality as unwaxed fruit are prone to shriveling.

Consumer awareness for health and environmental benefits of using edible, natural and food safe coatings has warranted more postharvest research. By definition, edible coatings are natural food safe and ecologically friendly substitutes applied to reduce water transfer, gaseous exchange and oxidation of fresh produce (Dhall, 2013). Studies by Bill et al. (2014), Sivakumar and Bautista-Baños (2014), Sellamuthu et al.

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(2013) have demonstrated the effectiveness of edible coatings and essential oils in reducing postharvest diseases and prolonging shelf-life of avocado fruit. Carboxymethyl cellulose (CMC) is one of the edible coatings that has dominated the food industry and has been reported to enhance quality and extend shelf life of avocado (Maftoonazad and Ramaswamy, 2005). Commercially, prochloraz is another common treatment used to control postharvest fruit diseases with regulatory conditions linked to different maximum residual limits (MRLs) in order to ship the fruit to international export markets (Perez-Jiminez, 2008). How, its application still imposes pressure to comply with safety regulations. There is therefore a need to develop alternative postharvest treatments to comply with this regulation while effectively reducing postharvest losses. As a result, various attempts have been made on developing other alternative control methods that are safe and long-lasting (Busani et al., 2012).

Moringa (*Moringa oleifera* Lam.) is a tree that grows in many tropical and subtropical countries. Studies have shown that leaf extracts of moringa have high antioxidant activity and exhibit antibacterial potential against several organisms (John et al., 2013). The antimicrobial properties of moringa leaf extracts are as a result of its active phytochemicals which include, Sitosterol, Niazin A, Stigma sterol, Kaempferol and Quercetin (Rao et al., 2001). Yousef et al. (2015) reported the use of moringa products on storability and fruit quality properties of 'Fuerte' avocado. A recent study by Tesfay and Magwaza (2017) showed improved fruit quality and longer shelf-life of 'Hass' and 'Fuerte' avocado fruit treated with 1% CMC (w/v) containing moringa leaf extract (MLE). However, the efficacy of moringa leaf and seed extracts on 'Gem', a newly-developed late-season cultivar, has not been evaluated. Moreover, to the best of our knowledge, the most appropriate and effective extraction method for moringa has not yet been determined. This is of importance for industry recommendations as avocado cultivars differ in a variety of characteristics, ranging from anatomical features to those related to fruit quality. Furthermore, there is currently no literature evidence reporting the use of moringa extracts against avocado postharvest diseases. Therefore, this study investigated the efficacy of commercially known hydrophilic polysaccharide based edible coating CMC combined with moringa leaf and seed extracts as a novel approach for enhancing quality and prolonging the shelf-life of 'Hass' and 'Gem' avocado fruit. The study also evaluated *in vitro* and *in vivo* antifungal activity of moringa extracts against postharvest diseases of avocado.

2. Materials and methods

2.1. Fruit sampling

Two avocado cultivars 'Hass' and 'Gem' used in this study were harvested from Everdon Estates, a commercial avocado farm of Westfalia Fruits™ a division of Hans Merensky Holdings (Pty) LTD. The experimental farm is situated in Howick, a cool subtropical area of KwaZulu-Natal Province, South Africa (Latitude: 29°45'0S, Longitude: 30°25'0E). A total of 360 (180 'Hass' and 180 'Gem') count 18 fruit with an average mass ranging of 211–235 g, were harvested and packed in open-top display boxes. Harvested fruit was immediately transported in a well-ventilated vehicle to Postharvest Laboratory of the University of KwaZulu-Natal.

2.2. Postharvest treatments and storage

Upon arrival at the Postharvest Laboratory, fruit was either assigned to three postharvest coating treatments, including control, moringa leaf extract (MLE) + 1% CMC, moringa seed extract (MSE) + 1% CMC. Each treatment was replicated 3 times with each replication consisting of 20 individual fruit. Fruit was dipped in the treatment solutions for 1 min and left to dry on the laboratory bench at room temperature (21 ± 1 °C). The fruit was thereafter transferred to cold room with the

delivery air set at 5.5 °C and RH of 95% ± 2% for 21 days, simulating export conditions. After cold storage, fruit was allowed to ripen at ambient conditions (21 ± 1 °C) and 60% RH for 7 days, simulating retail conditions.

2.3. Plant tissue extraction process

Moringa leaf and seed were extracted as described by Mendoza et al. (2013) with some modifications as follows:

2.3.1. a. Methanol extraction

100 g of moringa plant tissues were extracted with 1 L of methanol/HCl 1% (v/v) for 2 h with constant agitation at 4 °C. Extracts were concentrated in a rotary evaporator and 20 mL of distilled water was added. Finally, crude extract was subjected to sequential liquid-liquid extraction with hexane, chloroform and finally ethyl acetate.

2.3.2. b. Ethanol extraction, method A

100 g of moringa plant tissues were extracted with 1 L of ethanol 70% (v/v) for 2 h with constant agitation at 4 °C. Extracts were concentrated in a rotary evaporator to get crude extracts which were subjected to sequential liquid-liquid extraction with hexane, chloroform and finally ethyl acetate.

2.3.3. c. Ethanol extraction, method B

100 g of moringa plant tissues were extracted with 1 L of ethanol 70% (v/v) for 2 h with constant agitation at 4 °C. Extracts were concentrated in a rotary evaporator and 20 mL of distilled water was added. Finally, crude extract was subjected to sequential liquid-liquid extraction with hexane, chloroform and finally ethyl acetate.

The extracts were thereafter kept in cold storage for amending mycelium growing media for the *in-vitro* determination of antifungal properties.

2.4. Postharvest fruit quality evaluation

During postharvest cold storage and shelf life simulation at room temperature, the fruit was evaluated for various physical and chemical properties, including ethylene production, respiration rate, fruit firmness and mass loss.

2.4.1. Fruit ethylene production and respiration rate

Fruit ethylene production was measured with a handheld ethylene analyzer (F-950 three gas analyzer, Felix instruments, Camas, WA98607, USA) using fixed volume mode which samples 15 mL from the headspace. Each fruit was sealed in a 1 L jar for 15 min, the ethylene content was calculated and expressed as $\text{kg}^{-1} \text{h}^{-1}$. To determine respiration rate, fruit CO_2 production was measured using environmental gas monitor (EGM-1, PP Systems, Hitchin, United Kingdom) at seven days interval using a method previously defined by Blakey et al. (2012). Each fruit was sealed in a 1 L jar for 10 min, after which the headspace CO_2 concentration was determined and the results were expressed as $\text{mg kg}^{-1} \text{h}^{-1}$, taking into account fruit mass, headspace and ambient room CO_2 concentration (Tefsay and Magwaza, 2017).

2.4.2. Fruit firmness and mass loss

Fruit firmness was determined every seven days during cold storage using a hand-held firmness tester (Bareiss, Germany) based on a method described by Tesfay and Magwaza (2017). Two readings, on a scale of 100 (hard, unripe) to < 60 (ready to eat), were taken at the equatorial region of the fruit on opposite sides (Magwaza and Tesfay, 2015; Tesfay and Magwaza, 2017). The avocado fruit mass was measured using a Mettler Toledo digital balance (± 0.01 g).

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