The efficacy of combined application of edible coating and moringa extract in enhancing fruit quality in avocado (Persea americana Mill)

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ABSTRACT

South African (SA) fruit industries have a huge concern of fruit losses during postharvest storage time, as fruit perishes rapidly due to high rate biological processes as well as fruit disease occurrences. Commercially, avo-shine has been used to enhance fruit quality, improving glossiness and reducing water loss of avocado fruit during postharvest storage. However, due to increasing public health awareness, an edible coating is to replace the synthetic wax. In addition to enhancing fruit quality by reducing water loss, an edible coating material also contains antifungal properties. This experiment was designed to investigate a novel moringa leaf extract as one of edible coatings, together with other commercially available edible coatings such as chitosan and carboxymethyl cellulose (CMC), to improve fruit quality in avocado cultivars, Fuerte and Hass. A level of moringa extract, two levels of chitosan (0.5, 1%), CMC (0.5, 1%) were used to treat avocado fruit for postharvest storage. Fruit quality attributes were measured and the moringa 2% with emulsifier and moringa containing edible coatings had positive results in improving the fruit quality for both cultivars. For 'Fuerte', the treatment CMC (1%) and moringa 2% significantly improved fruit quality, reducing fruit weight loss and slowing down the respiration process. The same treatment also increased the fruit phytochemical characteristics, such as carbohydrates, fatty acids and decreased polyphenol oxidase (PPO) and lipid peroxidation. The result also showed moringa containing treatments improved fruit quality. For 'Hass' fruit, similar results were also found for the same treatments. In conclusion, the investigated edible coatings containing moringa leaf extracts improved fruit quality and shelf life. It could therefore potentially be commercialised as new edible coating for future industry usage with further investigation.

INTRODUCTION

Avocado (*Persea americana*, Mill) is a climacteric fruit, ripening fruit exhibits high ethylene accumulation stimulating onset of ripening and CO₂ accumulation as result of high rate of fruit respiration (Blakey *et al*., 2012). Huge magnitude of postharvest fruit loss is reported due to high rate of biological process as well as fruit diseases. Various types of step wise postharvest fruit treatments have been used to reduce these losses and instead improve fruit quality and shelf life. During postharvest fruit handlings, picked fruit needs to be sorted, graded, washed, treated and coated as to maintain their quality during pre-storage.

Fruit coating is commonly used to reduce fruit water loss. The SA avocado industry has previously been using a synthetic wax commercially known as 'avo-shine' to treat fruit during postharvest storage (Kremer-Kohne & Duvenhage, 1997). Avocado fruit coated with avo-shine is expected to reduce fruit moisture loss by a larger amount compared to uncoated fruit. However, avocado fruit treated with avoshine is prohibited by EU markets. Instead, fruit are supplied to EU markets unwaxed. Although the fruit shelf life can be improved with coating material, consumers' preference to untreated ones also coincided with the recent rising of a need of organic produce. It is also evident that the search for naturally produced coating material to treat this fruit can significantly enhance the fruit quality and further lift up consumers' acceptability in EU markets.

Edible coatings are an environmentally friend-

ly technology that is applied on many products to control moisture transfer, gas exchange or oxidation processes (Dhall, 2013). An advantage of edible coating is the reduction of synthetic packaging waste, because these coatings are composed of biodegradable raw material. Components of edible films and coatings can be divided into three categories: hydrocolloids, lipids and composites (Dhall, 2013). Another distinguished characterstic of edible coatings is the high potential to carry active ingredients such as antibrowning agents, colourants, flavours, nutrients, spices and antimicrobial compounds that can extend product shelf life and reduce the risk of pathogen growth on food surface (Pranoto *et al*., 2005).

Moringa oleifera Lam. is a tree that grows widely in many tropical and subtropical countries. Moringa oleifera's leaves, seeds, bark, roots, sap and flowers are widely used in traditional medicine and the leaves and immature seed pods are used as food products in human nutrition (Tesfay *et al*., 2011, 2016). Leaf extracts exhibit the greatest antioxidant activity. This study, therefore, investigated the efficacy of commercially known hydrophilic polysaccharide based edible coatings, carboxymethyl cellulose (CMC) and chitosan which incorporates moringa leaf extracts as novel approach in enhancing fruit quality and shelf life.

MATERIALS AND METHODS

Fruits were collected from Westfalia Fruit's Merensky pack house in Howick. Two avocado cultivars, Hass and Fuerte, were used for the experiment and 360 fruit were assigned for six treatments: Avo-shine®, Moringa 2% (M), M+Chitosan 0.5% (CN 0.5%), M+CN 1%, M+Carboxylmethyl Cellulose (CMC) 0.5%, M+CMC 1%. Each treatment had 60 fruit for three replications and there were 20 fruit per replication.

The fruit were dipped in treatment solutions for 1 min and left on bench top to drain out. The fruit were transferred to a cold room which were set at 5.5ºC for 21 days and were afterwards moved to ambient condition for ripening. Fruit in each treatment were evaluated for shelf life.

Over the course of the experiment, various physical and chemical quality attributes were measured:

Fruit firmness

Fruit firmness was determined every seven days during cold storage using a hand-held firmness tester (Bareiss, Germany). 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. Firmness readings with 100 representing hard, unripe fruit and 60 soft, ripe fruit (Standard ISO 7619, International Organisation for Standardisation).

Fruit CO₂ production

Fruit CO₂ production was measured with an environmental gas monitor (EGM-1, PP Systems, Hitchin, UK) every seven days (Tesfay *et al*., 2012). Each fruit was sealed in a 1 L jar for 10 min, after which the headspace CO₂ concentration was determined and

the results calculated as a rate of $CO₂$ production (mg kg−1 FW h−1), taking into account fruit mass, headspace and ambient room CO₂ concentration.

Electrical conductivity (EC)

The electrolytes conductivity from mesocarp tissue leakage was determined using a multi-range conductivity meter (HI 9033, Hanna Instruments, Johannesburg, South Africa) (Venkatarayappa *et al*., 1984), with slight modification. Briefly, a mesocarp plug was taken from the cut-half of each fruit at the distal end, between the seed and the mesocarp. A disc of 11 mm thickness (2.0 - 2.5 g) was cut from this plug, rinsed three times in distilled water and placed in a boiling tube containing 25 ml distilled water. The tubes were then shaken for three hours and the solution was ready for the EC analysis. The EC of each tube was recorded before and after boiling, the electrolyte leakage calculated as the EC according to the following formula:

$$
EC = \frac{ECf\text{-}ECi}{n}
$$

where $EC =$ electrical conductivity; $EC =$ initial reading; $ECf = final reading; n = number of samples.$

Carbohydrate determination

Freeze-dried mesocarp powder (0.10 g) was mixed with 10 ml 80% (v/v) ethanol and homogenised for 1 min. Thereafter, the mixture was incubated in an 80°C in a water bath for 60 min to extract the soluble sugars. Subsequently, the mixture was stored at 4°C overnight to facilitate the release of soluble sugars. The mixture then centrifuged at 12000 g for 15 min at 4° C, the supernatant was filtered through glass wool and the filtrate was taken to dryness in a GenVac (model). Dried samples were re-suspended in 2 ml ultra-pure water, filtered through a $0.45 \mu m$ nylon filter into HPLC vial, and sugars were analysed according to Liu *et al*. (1999), using an isocratic HPLC system equipped with a refractive index detector on a Phenomenex® column (Rezex RCM– Monosaccharide). The concentration of individual sugars was determined by comparison with authentic sugar standards.

Crude fat determination

The Soxlet method was used for the determination of crude fat plant tissue samples (AOAC Official Method 920.39). The fat is extracted by the ether, which drops back into the flask. The ether is evaporated from the flask, leaving the fat behind.

Total protein quantification

The protein concentration of the samples was quantified by the Bradford microassay (Bradford, 1976). Bradford dye reagent was prepared by diluting the dye concentrate with distilled water at a ratio of 1:4. The diluted dye (1 ml) was added to a plastic cuvette and mixed with protein extract. Subsequently,

samples were incubated at room temperature for 5 min and read spectrophotometrically at A595 nm. The protein concentration was determined by comparing results with a standard curve constructed using bovine serum albumin (BSA).

Polyphenol oxidase (PPO) activity determination

Total polyphenol oxidase (PPO; EC 1.14.18.1) activity was assayed according to Van Lelyveld *et al*. (1984) with slight modification. One hundred microliter of protein extract (100 μL) was added to a mixture of 1.450 ml 10 mM acetate buffer (pH 5.0) and 1.450 ml 20 mM 4-methyl-catechol. PPO activity was expressed as the change in optical density (OD) at 420 nm min $^{-1}$ mg protein $^{-1}$.

Lipid peroxidation determination

Lipid peroxidation was measured using the amount of reaction mixture of malondialdehyde (MDA) with thiobarbituric acid (TBA) and forms a TBA– MDA complex as end product (Chong *et al*., 2005). Briefly, 1 ml crude protein extract was added to a test tube containing 1 ml 20% (w/v) TCA, 0.01% (w/v) BHT and 0.65% (w/v) TBA. Samples were mixed vigorously, incubated at 95ºC for 30 min, cooled on ice and centrifuged at 3000×g for 10 min. Absorbance was read at 532 and 600 nm with a UVvisible spectrophotometer. Total MDA equivalents (nmol g-1 DW) were calculated according to Heath and Packer (1968) as:

Total MDA = Extraction buffer(ml)
\n× protein supernatant
$$
\left[\frac{(A532nm \cdot A600nm)}{155} \right]
$$

\n× 10³/tissue(g)

Scanning electron microscopic (SEM) fruit membrane image analysis

Samples were sputter coated with gold using an EIKO IB3 gold coater and viewed using the SEM (Zeiss EVO LS15).

Data analysis

The data collected was analysed with statistical software using GenStat 17.1. Standard error values were calculated where a significant standard deviation was found at $P \le 0.05$ between individual values.

RESULTS AND DISCUSSION

Moisture loss in the control fruit was higher than coated ones ($P \le 0.01$). Fruit moisture loss was consistently declining for all treatments almost in straight line pattern during cold storage, as well as ambient conditions. The treatments' effects did not have huge impact on fruit weight loss (Fig. 1), it could also be due to the nature of edible coating properties, which is hydrophilic, and it displays less resistance to water loss (Kester & Fennema, 1986). This observation has been anticipated in

Figure 1. Effect of postharvest edible coatings in avocado fruit weight loss during storage time. Vertical bars represent standard error of the mean value ($n = 5$).

Figure 2. Effect of postharvest edible coatings in avocado mesocarp electrolyte leakage during storage time. Vertical bars represent standard error of the mean value $(n = 5)$.

avocado fruit tissues which accumulates high oil content that has lipophilic functional property which exhibits resistance to water loss (Kruger, 2013). Our result also, in agreement with Maftoonazad and Ramaswamy (2005), methyl cellulose retards moisture loss in avocado fruit.

The treatments had a significant effect on mesocarp electrolyte conductivity (EC) over storage time. There were less mesocarp EC of the two cultivars during cold storages for 14 days (Fig. 2). The fruits' membrane only started to release more electrolytes after 14 days and increased EC exponentially during the shelf life storage at ambient condition. Fruit coated with CMC and chitosan containing moringa leaf extract had less EC during storage than the control ($P \le 0.05$). The two CMC at 0.5% and 1% containing moringa leaf extract and the moringa 2% with emulisifier retained the firmness and resulted in better membrane integrity, which eventually improves fruit quality and shelf life. Similar results were reported by Azad (2006), fruit treated by any coating material retains firmness and releases less EC. Electrolyte leakage and disrupted ion balance resulting from ultra-structural changes in the membranes (Lyon, 1973). The relative EC of the fruit tissue continually increased after harvest, suggesting a gradual loss of cell membrane integrity.

There were differences in fruit oxidation during the storage times. Both Hass and Fuerte cultivars displayed similar fruit respiration patterns (Fig. 3). The fruit respired at a slower rate for 14 days during cold storage and started to increase at a significant rate towards the end of the cold storage. The treatments had a strong effect on fruit respiration. The fruit respiration had a slower rate in treated fruit than the control in the storage period. The moringa 2% and the CMC with moringa significantly delayed fruit respiration rate.

In avocado, a climacteric fruit, the increase in respiration rate, which is triggered by ethylene accumulation, is accompanied by a complex of bio-

Figure 3. Effect of postharvest edible coatings in avocado fruit CO₂ production during storage time. Vertical bars represent standard error of the mean value (*n* = 5).

Figure 4. Effect of postharvest edible coatings in avocado fruit softness during storage time. Vertical bars represent standard error of the mean value ($n = 5$).

chemical changes resulting in fruit softening. These results are complementary to those of other studies on avocado (Jeong *et al*., 2002, 2003).

Firmness of fruit significantly decreased ($P \le 0.05$) with storage period for both treated and control fruit. At the end of storage, control fruit clearly showed the lowest firmness (Fig. 4). The maximum firmness was maintained by the moringa 2% and CMC 1% containing moringa. Softness of fruit is due to deterioration in the cell structure, loss of membrane integrity (Seymour *et al*., 1993) and is a biochemical process of pectin and starch catalysis by enzymes. During the fruit ripening process, depolymerisation pectins occur with an increase in pectinesterase and polygalacturonase activities (Yaman & Bayoindirli, 2002). These cell wall structural changes are due to the activities of degrading cellulase enzymes in the cell wall (Pesis *et al.*, 1978; Tucker & Laties, 1984) that result in decreased tissue cohesiveness resulting from the degradation of pectin and cell disarrangement (Awad & Young, 1979). Gaseous compositions of lower percentage of $O₂$ and high percentage of $CO₂$ limit the activities of oxidising enzymes and allow retention of the firmness during storage (Salunkhe et al., 1991). Our findings were in agreement with Jeong *et al.* (2000), firmness of untreated avocado fruit decreased whereas fruit treated with both 1-MCP and wax retained firmness for a longer period. Loss of firmness affects the fruit quality during marketing. Chitosan coating was reported to retain fruit firmness by inhibiting the macerating enzyme activity, such as polygalacturonase, pectate lyase, and cellulase in tomatoes (Reddy *et al*., 2000) and peaches (Atkinson *et al*., 2012).

Polyphenol oxidase (PPO) activity of mesocarp tissue of both fruit cultivars significantly increased in the storage period for all treatments. Starting at 7 days up to the end of the storage period, PPO activity for control fruit increased linearly, dominating over the treated fruit (Fig. 5). The moringa 2% and CMC 1%

Figure 5. Effect of postharvest edible coatings in avocado mesocarp polyphenol oxidase (PPO) activity during storage time. Vertical bars represent standard error of the mean value $(n = 5)$.

Figure 6. Effect of postharvest edible coatings in avocado fruit mesocarp lipid peroxidation during storage time. Vertical bars represent standard error of the mean value $(n = 5)$.

with moringa had the least PPO activity amongst the treatments. Browning, as a symptom of CI in persimmon fruit, involves oxidation of phenolic substrates mediated by PPO (Tomas-Barberan & Espin, 2001). We speculate that the maintenance of membrane integrity in treated fruit contributed to the reduction in skin and flesh browning by preserving cell membrane structure and membrane integrity.

Lipid peroxidation of fruit significantly increased with the storage period. There were significant (P \lt 0.05) differences among treatments. The control fruit scored the highest lipid peroxidation (Fig. 6). The moringa 2% and CMC 1% with moringa leaf extracts had the least fruit lipid peroxidation. The oxidation of polyunsaturated fatty acids results in oxidants, such as peroxide ions and malondialdehyde (MDA). The lipid peroxidation activation increases with stresses and depends on the degree of cold stress and fruit prone to chilling injury (CI). High fruit lipid peroxidation, accumulation of MDA is often taken as an indicator of CI (Wongsheree *et al*., 2009), further affects the fruit quality. The pattern of MDA content of avocado during cold storage, followed by shelf life at ambient condition, tended to increase in the control fruit.

Figure 7. Effect of postharvest edible coatings in avocado fruit carbohydrate accumulation during storage time. Vertical bars represent standard error of the mean value $(n = 5)$.

Figure 8. Effect of postharvest edible coatings in avocado fruit carbohydrate accumulation during storage time. Vertical bars represent standard error of the mean value $(n = 5)$.

Plate 1. Scanning Electronic Microscopic (SEM) image analysis of avocado membrane structure of two treatments: Control; Caroboxymethyl cellulose (CMC) plus Moringa (M), during postharvest storage at ambient condition.

Figure 9. Effect of postharvest edible coatings in avocado ('Fuerte') fruit fatty acid accumulation during storage time.

There were significant differences ($P \leq 0.05$) in carbohydrates concentration among the treatments. Fruit carbohydrates significantly decreased in the storage period. At the end of the storage period, fruit retained carbohydrates at different concentrations for the treatments. The treatments had a stronger effect on the dominantly produced C7 sugars than the hexoses (Figs. 7, 8). Avocado fruit is known to produce the two dominant C7 sugars,

D-mannoheptulose and perseitol, and they also produce hexoses as relatively smaller amounts. It is also reported that D-mannoheptulose is known to delay the onset of ripening and improves fruit shelf life. The fruit treated with CMC as well as chitosan containing moringa extracts retained these C7 sugars as compared to control. A decrease in the C7 sugar content of avocados during ripening has been reported by Bertling and Bower (2005), C7 sugars were reported to decrease with fruit maturity.

The crude fat content of the fruit were not significantly different at 'ready-to-eat' stage (Fig. 1). The moringa 2% and the chitosan 1% with moringa leaf extract increased the fatty acid content, compared to untreated fruit.

The fruit membrane image of the control and fruit treated with CMC 1% with moringa extract were also viewed under SEM over the shelf life period. The result showed that the treated fruit retained membrane integrity as compared to untreated fruit (Plate 1). The onset of ripen-

Where $D6 = Day 6$; CN = Chitosan; CMC = Carboxymethyl cellulose

Plate 2. $A =$ Control; $B =$ Moringa (M) ; $C = M + CN (0.5\%)$; D = M+CN (1%); $E = M + CMC$ 0.5%; $F = M + CMC$ 1%.

ing was delayed, fruit shelf life improved beyond six days, while the untreated fruit reached 'to ready-to eat' softness in six days. This SEM result further supported our findings that fruit treated with coating material and moringa leaf extracts had less respiration and moisture loss and high firmness.

Similarly fruits were also evaluated for their rate of fruit ripening and internal quality. Out of 20 fruits the untreated fruit had shorter shelf life as compared to treated ones (Table 1, Plates 2, 3). The majority of untreated fruit (85%) were ready to eat within six days, while the treated fruit had only 25%. The treated fruit extended their shelf life for more than nine days (Table 1, Plate 1).

Overall, our findings were found in agreement with Azad (2006). Coated avocados with methyl cellulose demonstrated lower respiration rates, greener colour and higher firmness during the entire storage period, compared to the uncoated control.

In conclusion, the study reported the potential of this novel edible coating containing moringa leaf extracts, improving avocado fruit quality during storage period. Fruit firmness was retained, while respiration, EC, PPO and lipid peroxidation were minimal.

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