The combined effect of edible coating and ozonated cold storage in avocado (*Persea americana* Mill.) fruit quality

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ABSTRACT

Postharvest avocado fruit quality deterioration as well as uneven fruit ripening during cold storage time and shelf life are some of the major challenges that require special attention by fruit industries. The aim of this work was to investigate the effect of ozone treatment in relation to edible coating in enhancing avocado fruit quality. Fruit were treated with and without edible coating of moringa with carboxymethyl cellulose (CMC) and then exposed to intermittent ozone of 0.25 ppm for 12 h during cold storage. The ozone exposure frequency was designed on a weekly basis, where fruit with and without coatings were exposed to ozone at a frequency of once, twice, three or four times during the cold storage period. Fruit physical quality attributes and chemical analysis were done and the data were analyzed using Genstat 18 version. 'Gem' fruit treated with moringa CMC (1%) that were exposed to ozone of two times at 7 d, 14 d, 7 d, 21 d, 14 d, 21 d and three times at 0 d, 7 d, 14 d, 0 d, 7 d, 21 d, 7 d, 14 d and 21 d had significantly lower mass loss (3.25±1.2%), electrical conductivity (3.5±0.9 m.mohs/cm) and respiration rate (160±36 mg/kg/h), ethylene accumulation (3.5±1.2 mg/kg/h) compared to the untreated control with respective values of (7.8±1.4%), (11.3±1.3 m.mohs/cm), (220±29 mg/kg/h), (11.8±1.8 mg/kg/h). The same treatment had higher values for firmness (80.0±6.25 N) and phytochemical characteristics, mainly D-mannoheptulose (5.67±0.6 g/kg against 1.206 g/kg) and the volatile hexanal (834 \pm 95 µg/l against 672 \pm 63 µg/l). In conclusion, intermittent ozone treatment together with edible coating could enhance postharvest fruit quality and reduce uneven ripening in 'Gem' fruit during storage, if ozone is applied at optimum dose and storage conditions are adequate: (low temperature (5 °C) and high relative humidity (90-95%).

Keywords: Mass loss, ethylene, respiration, mannoheptulose, hexanal

INTRODUCTION

Avocado (*Persea americana*, Mill.) is an extremely perishable fruit with very high metabolic rate, resultant in a short postharvest life of about three to five weeks when stored under optimum conditions (Yahia & Gonzalez-Aguilar, 1998). The postharvest storage life of avocado fruit is limited by its climacteric-ripening pattern which exhibits high ethylene accumulation, stimulating faster ripening as result of the high rate of respiration (Blakey *et al.*, 2012). Avocado fruit are also considered to have a high postharvest mass loss and this is mostly due to moisture loss through transpiration, which has been shown to contribute about 90% of the total fruit mass loss (Cutting & Wolstenholme, 1992).

Fruit and vegetables after harvest are still alive, in

which transpiration, respiration and other metabolic processes continue during the postharvest period. All these biological processes can lead to quality losses, due to external and internal factors. During the postharvest period, fruit exposure to ethylene may occur inadvertently in storage or transit from atmosphere pollution as a by-product of human industrial activities (Chang & Bleecker, 2004) or from ethylene produced by adjacent plant products.

In fruit and vegetables, a strong correlation between storage life and ethylene atmospheric concentration has been found, in which ethylene levels higher than 0.10 μ II–1 would cause important quality loss (Wills & Warton, 2000), leading to shortening of the shelf life by acceleration of the ripening and senescence processes. These accelerated metabolic



beskerming van die grond af op

aktief in die grond en plant

genees bestaande infeksie

word vinnig opgeneem met sterk

bevorder wortelgesondheid, stand en lewenskragtigheid

Met 'n gesonde wortelstelsel word water en nutriënte optimaal opgeneem vir optimale groei en opbrengs





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processes depend on a number of variables, the most important being fruit sensitivity to ethylene, duration of exposure, ethylene concentration, atmospheric composition and temperature (Saltveit, 1999).

To reduce adverse effects of ethylene on vegetable and fruit quality, the level of ethylene concentrations should be kept below the threshold, depending on tissue sensitivity in storage areas (Wills & Warton, 2000). Furthermore, there are postharvest treatments and technologies in practice used for delaying and/ or inhibiting ethylene production, as well as removing ethylene under controlled atmospheric condition. Globally, in avocado fruit industries there have been challenges of fruit softening on arrival to long distance markets, such as EU countries. As a result this has posed a desperate need to find a solution that would remove ethylene accumulation during cold storage.

Previously, different methods have been used by industries in order to increase fruit shelf life. 1-Methylcyclopropene (1MCP), potassium permanganate have been used to reduce ethylene accumulation. However, due to safety concerns by EU countries from using chemical based treatments, the avocado industry has gradually begun to abandon these chemicals to avoid rejections from export to EU countries due to Maximum Residual Limits (MRLs).

Ozone is a naturally produced three oxygen atomic molecules used in postharvest fruit treatments. The use of ozone for ethylene degradation in air has been well-documented (Dickson *et al.*, 1992). The physicochemical characteristics of O_3 in terms of solubility in water and reactivity make it useful in the food industry for food preservation and equipment sterilization (disinfectant and sanitizer) and promoting shelf life extension (Suslow, 2004). Ozone has been listed as a GRAS (generally recognize as safe) material by the Food and Drug Administration (FDA) (2001) and approved for use during food processing (raw and processed fruit and vegetables), and for treatment and storage, both in gas or aqueous phases.

Edible coatings are ecologically friendly substitutes applied on fresh produce to reduce water transfer, gaseous exchange and oxidation processes (Dhall, 2013). Among edible coatings, CMC was reported on avocado fruit (Maftoonazad & Ramaswamy, 2005). Tesfay & Magwaza (2017) reported 'Fuerte' and 'Hass' avocado fruit coated with moringa +1% CMC had lower rates of respiration and higher values of firmness, compared with the uncoated control.

This experiment investigated the efficacy of intermittent application of ozones in reducing the fruit sensitivity towards ethylene by slowing down its accumulation greater than thresh-hold amount, as well as ozone compatibility with edible coatings in maintaining fruit quality.

MATERIALS AND METHODS

Fruits were collected from Westfalia Fruit, Merensky packhouse in Howick. Avocado cultivar 'Gem' was used for the experiment, 840 fruit were assigned for two levels of coating (control, CMC 1% + Moringa leaf) treatments. The fruit were equally divided, each group

had 420 fruit and the two groups were then exposed to ozone treatments during cold storage. Ozone application times (0.25 ppm) was for 12 h (0 d, 7 d, 14 d, 21 d) and its matrix. Each treatment had 60 fruit with 3 replications and 20 fruit per replication. The storage room was set at 5.5 °C for 21 days and afterwards fruit were moved to ambient condition for ripening and the fruit shelf life to each treatment evaluated.

Fruit physical and biochemical parameters were measured:

Fruit ethylene production measurement

Fruit ethylene production was measured with an F-950 handheld ethylene analyser (Felix Instruments, QC Applied Food Sciences) using fixed volume mode which samples 15 ml from the headspace. Sampling was done every seven days (Blakey *et al.*, 2012). Each fruit was sealed in a 1 L jar for 15 min and the readings recorded as a rate of ethylene in ppm.

Fruit CO, production measurement

Fruit CO₂ production was measured with an environmental gas monitor (EGM-1, PP Systems, Hitchin, UK) every seven days (Blakey *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.

Fruit firmness measurement

Fruit firmness was determined every seven days during cold storage and during shelf life 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. In the firmness readings, 100 represented hard, unripe fruit, and 60 soft, ripe fruit (Standard ISO 7619, International Organization for Standardization).

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 3 h 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:

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

where EC = electrical conductivity; ECi = 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 homogenized 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 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 EZ2.3, GenVac LTD, Ipswich, England). Dried samples were re-suspended in 2 ml ultra-pure water, filtered through a 0.45 µm nylon filter into HPLC vial, and sugars were analyzed 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.

Hexanal and Pentanal determination using Gas mass spectrophotometry (GC/MS)

Fruit volatiles were determined according to Obenland et al. (2012) with slight modification. Briefly, mesocarp tissue (20 g) was cut and homogenised with 40 mL saturated NaCl for 1 min. The addition of NaCl was to limit the formation of volatiles in order to figure out the actual volatiles during the time of sampling. Volatile compounds were analyzed using coupled Varian 3800 gas chromatography (Varian Palo Alto, California, USA) and Varian 1200 mass spectrometry (GC-MS). The GC was equipped with an Alltech EC-WAX column of 30 m x 0.25 mm internal diameter x 0.25 µm film thickness (Alltech Associates Inc., Deerfield, Illinois, USA). Helium was used as the carrier gas at a flow rate of 1 mL/min. Compound identification was carried out using the NIST05 mass spectral library and comparisons with retention times of chemical standards, as well as comparisons between calculated Kovats retention indices and those published in the literature. Clean chromatoprobe traps were run in GC-MS as control to identify background contamination.

Data analysis

The data collected were analysed using statistical software using Gen-Stat 18.1. Standard error values were calculated where a significant standard deviation was found at $P \le 0.05$ between individual values.

RESULTS AND DISCUSSION Fruit respiration

The fruit were respiring at a slower rate for the first 14 d during cold storage and started to increase at a faster rate towards the end of the cold storage (Fig. 1). In the current study, a gradual increase in the rate of respiration was observed during postharvest storage, indicating that the rate of avocado fruit respiration increases as the fruit ripens or senesces. For instance, in 'Gem', combined effects of coating and ozone exposure treatments had a significant effect on fruit respiration with fruit displaying the slower rate (170±55 mg/kg/h) compared to untreated fruit (220±60 mg/kg/h). Coated fruit with two times (14 d, 21 d) and three times (7 d,14 d, 21 d) ozone exposure, significantly reduced fruit respiration rate to $(160.0\pm40.8 \text{ mg/kg/h})$ and $(130.0\pm20.3 \text{ mg/kg/h})$, respectively. There were differences in fruit oxidation during the storage period. In avocado, typical a climacteric fruit, the increase in respiration rate, which is triggered by ethylene accumulation, is accompanied by a complex of biochemical changes resulting in fruit softening. The results observed herein are similar to those reported in other studies on avocado fruit, where respiration have been shown to increase with time and the rate reduced by coating treatments (Jeong, Huber, & Sargent, 2002; Jeong, Huber, & Sargent, 2003).

Minas *et al.* (2014) reported ozone delays ripening and inhibits ethylene production and respiration rate of Kiwi fruit. They further stated that upon transfer to 20 °C after 2 months, control fruit exhibited a typical climacteric rise in ethylene production, as opposed to ozone-treated fruit, which consistently showed basal levels of ethylene production.

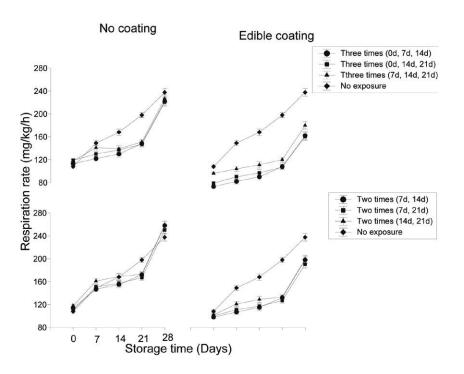


Figure 1: Effect of ozone on coated as well as uncoated avocado fruit CO_2 production during postharvest storage time. Vertical bars represent standard error of the mean value (n = 5). The SE refers to the standard deviation of each measured value from the mean value.



Fruit ethylene production

In 'Gem' avocado, the ethylene production rate was significantly reduced (p<0.05) in fruit coated with CMC and ozone treatments (Fig. 2). Between the two treatments of ozone and control, the treatment consisting of edible coating and ozone had the lowest ethylene levels throughout cold storage and shelf life. At shelf life, the ethylene production rate reached 11.8 mg kg⁻¹ fruit for the control treatment, while it was 3.5 mg kg⁻¹ fruit for the edible coating and ozone.

The increasing pattern of ethylene production observed at shelf life was due to the positive correlation between temperature and the rate of metabolic reactions in fruit tissues (Tesfay & Magwaza, 2017). However, in the current study, fruit treated with edible coating and ozone did not have a similar rise in ethylene production at shelf life, indicating that these treatments suppressed metabolic reactions and delayed tissue sensitiveness towards ethylene concentration in the storage room.

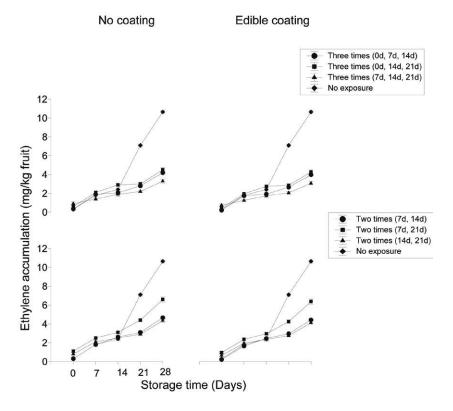


Figure 2: Effect of ozone on ethylene production of coated as well as uncoated fruit. Vertical bars represent standard error of the mean value (n = 5). The SE refers to the standard deviation of each measured value from the mean value.

Fruit mass loss

Coating treatments had a significant influence on mass loss of avocado fruit during postharvest storage. Similarly the uncoated fruit, but exposed to ozone, had also significant differences with untreated control fruit. In this case, fruit mass loss may be reduced due to slowing down of the fruit biological process, possibly occurred due to chemical reaction between ozone and ethylene. Higher fruit mass loss was observed in untreated control compared to treated fruit (p<0.01) (Fig. 3). Further, and perhaps very important, was the rate of mass loss over the storage period. The greatest mass loss was observed during the first ten days of postharvest storage. In fact, most moisture loss and thus mass loss could be expected to occur during the initial cooling of the fruit (Wills, McGlasson, Graham, & Joyce, 1998; Bower & Magwaza, 2004). Fruit moisture loss was consistently declining for all treatments during cold storage as well as during ripening stage at ambient conditions. The most efficient mass loss control was in the fruit coated with a combination of

coated and ozone exposure times. Untreated control fruit continued losing water in the cold room atmosphere. This is due to the cold room atmosphere continually being dried as it passes through the cooling coil, and the resultant deficiency of water being replaced down the concentration gradient from the fruit to the atmosphere (Bower & Magwaza, 2004). Observed moisture loss was mostly due to moisture loss by transpiration, which accounts for about 90% of total mass loss in fruit (Magwaza et al., 2013a). This is in accordance with the literature stating that moisture loss from the fruit results from vapour pressure deficit between the less saturated atmosphere and the fruit, which is close to saturation with water (Cutting & Wolstenholme, 1992; Magwaza et al., 2013a). The differences between control and coating treatments recorded in the current study could be due to the nature of edible coating properties, which is hydrophilic, and it displays less resistance to water loss than the lipophilic coatings (Kester & Fennema, 1986). This observation has been anticipated in avocado fruit tissues which accumulate high oil content that has a lipophilic functional property which exhibits resistance to water loss (Kruger, 2013). The results observed in the current study are also in agreement with Maftoonazad & Ramaswamy (2005), who showed that methyl cellulose retards moisture loss in avocado fruit. Higher postharvest mass loss observed in the untreated control fruit could also be explained by the removal and re-organisation of natural wax on the fruit surface, which is brushed down and reorganised by standard commercial brushing and washing (Magwaza et al., 2013b).

Mesocarp electrical conductivity

The cultivar had low EC during the first 14 d of cold storage (Fig. 4). The fruit membrane only started to release more electrolytes after 14 d and increased EC exponentially during the shelf life storage at ambient condition.





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commences (ca. 2 g stage)

to increase fruit size.

Avocado

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Mango Spray during flowering to increase fruit retention.

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- ✓ When certain foliar applications are tested and proved to result in measurable and positive plant parameter responses.
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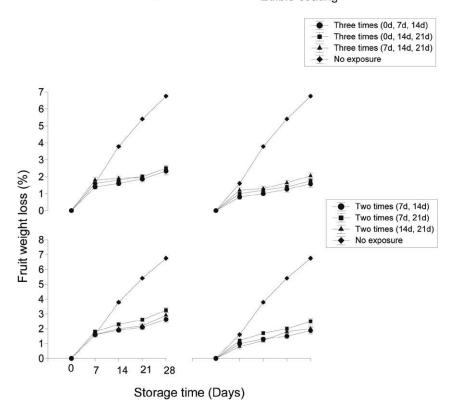


Figure 3: Effect of ozone in coated and uncoated avocado fruit mass loss during postharvest storage time. Vertical bars represent standard error (SE) of the mean value (n = 5). The SE refers to the standard deviation of each measured value from the mean value.

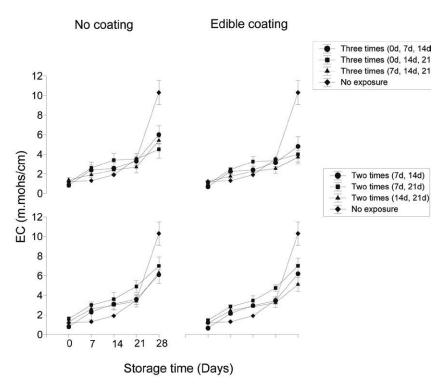


Figure 4: Effect of ozone on avocado mesocarp electrolyte leakage for coated as well as uncoated fruit during storage time. Vertical bars represent standard error of the mean value (n = 5). The SE refers to the standard deviation of each measured value from the mean value.

132

These observations suggest that EC of avocado fruit increases with postharvest storage time. The treatments had a significant effect on mesocarp EC and this was consistent over storage time. Fruit with coating as well as without coating, both exposed to ozone, had respective EC of (3.5± 0.2 m.mohs/cm) and (5.1±0.4 m.mohs/cm) which were significantly lower than that of the control fruit (11.2±0.8 m.mohs/cm). The fruit with any treatments retained fruit firmness longer than other control treatments. The observed retention of firmness may suggest that these treatments resulted in better membrane integrity which improved fruit quality and shelf life. Similar results were reported by Azad (2006), fruit treated with any coating material retains firmness and releases less EC. The observed disrupted ion balance and electrolyte leakage is hypothesized to have resulted from ultra-structural changes in the membranes (Lyons, 1973). In addition, the relative EC of the mesocarp tissue in the current study was gradually increasing during postharvest storage, suggesting a continuous and a gradual loss of

Fruit firmness

cell membrane integrity.

Fruit firmness decreased significantly (p<0.05) with storage period and these trends were similar for both treated and control fruit, although the rate of decrease was not the same (Fig. 5). In 'Gem', the highest value of firmness was maintained by the uncoated fruit and ozone exposure (63±4.25 N) and the coated fruit with ozone exposure (78.0±3.75 N). Upon removal from cold storage, the control fruit had the lowest values of firmness (51±3.0 N) (Fig. 5). The softness of fruit is typical of avocado fruit, resulting from the weakening of the cell wall structure, loss of membrane integrity, hydrolysis of cellulose and hemicellulose as well as depolymerisation of pectin and starch (Seymour, Taylor, & Tucker, 1993). The hydrolysis and depolymerisation of these components are catalysed by the action of several

enzymes, including polygalacturonase, pectin methylesterase, pectatelyase, Rhamnogalacturonase and b-galactosidase (Yaman & Bayoindirli, 2002; Payasi, Mishra, Chaves, & Singh, 2009). Structural changes in the integrity of cell wall are likely to result from the activities of cellulase (Pesis, Fuchs, & Zauberman, 1978; Tucker & Laties, 1984) which decreases cellular cohesion by cell disarrangement and degradation of pectin (Awad & Young, 1979). The gaseous composition of lower O, and higher CO, percentage in coated fruit and ozones is likely to limit the activities of oxidising enzymes, allowing the retention of firmness during postharvest storage (Salunkhe, Boun, & Reddy, 1991). Our findings are therefore in agreement with this argument and that of Jeong, Huber, and Sargent (2000) who stated that firmness of untreated avocado fruit decreased faster whereas fruit treated with both 1-MCP and wax retained firmness for a longer period. Loss of firmness during marketing negatively affect fruit quality, hence the ability of these coating treatments together with ozone to reduce the rate of softening is advantageous in extending shelf life and reducing postharvest losses.

Mesocarp soluble sugars

A significant (p<0.05) differences was observed in concentrations of soluble sugars among the treatments (Figs. 6 and 7). For instance, D-mannoheptulose, which is a dominant soluble sugar in avocado fruit, significantly decreased with storage period from an average of 15 g/kg at the beginning of the experiment to less than 6-7 g/ kg upon ripening. At the end of the storage period, control fruit had the lowest concentration of D-mannoheptulose. Avocado fruit is known to produce the two dominant C7 sugars, Dmannoheptulose and perseitol, and they also produce hexoses as the relatively smaller amount. It is also reported that D-mannoheptulose is known to delay the onset of ripening or else improves fruit shelf life. The treated

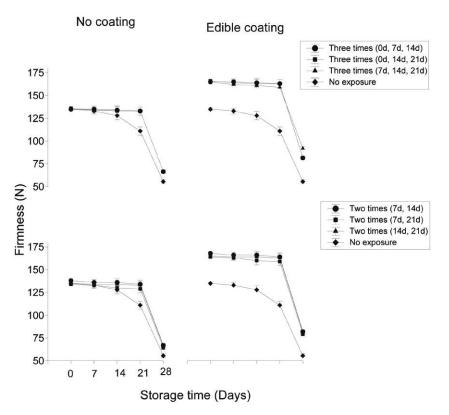


Figure 5: Effect of ozone on avocado fruit softness for coated as well as uncoated fruit during storage time. Vertical bars represent standard error of the mean value (n = 5). The SE refers to the standard deviation of each measured value from the mean value.

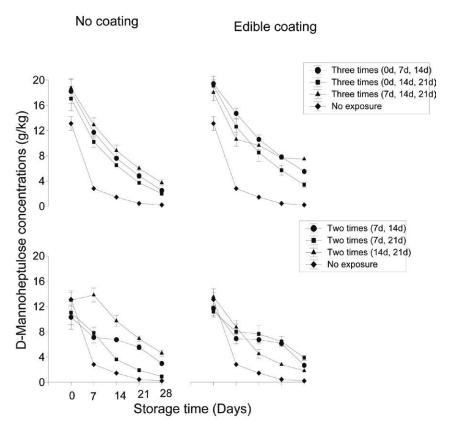


Figure 6: Effect of ozone on mesocarp D-mannoheptulose for coated as well as uncoated fruit during storage time. Vertical bars represent standard error of the mean value (n = 5). The SE refers to the standard deviation of each measured value from the mean value.



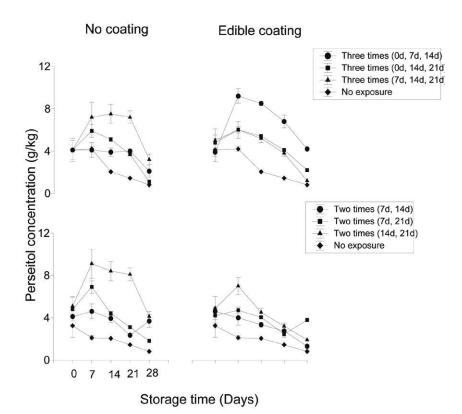


Figure 7: Effect of ozone on mesocarp perseitol for coated as well as uncoated fruit during storage time. Vertical bars represent standard error of the mean value (n = 5). The SE refers to the standard deviation of each measured value from the mean value.

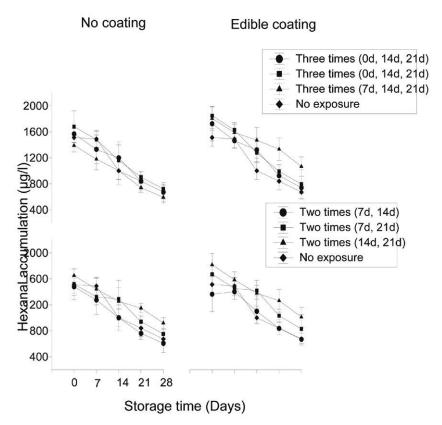


Figure 8: Effect of ozone on mesocarp hexanal for coated as well as uncoated fruit during storage time. Vertical bars represent standard error of the mean value (n = 5). The SE refers to the standard deviation of each measured value from the mean value.



fruit retained these C7 sugars as compared to control. Tesfay and Magwaza (2017), who observed a decrease in the content of C7 sugar in avocado fruit during ripening, reported a similar trend.

Fruit volatiles

Two volatiles were analysed; the concentrations significantly changed during postharvest. The hexanal displayed a declining pattern whereas the pentanal was increasing over the storage time, but its concentration was significantly higher in fruit coated with ozone treatments than control fruit (Figs. 8 and 9). There is a clear correlation observed with fruit maturity and ripening, that hexanal had high concentration at early stages and the amount decreases as the fruit ripens and over-matures. This finding can also give a good insight of hexanal volatiles, an aldehyde that could play a vital role if it can be applied exogenously to delay ripening. This has left a room to further investigate how this volatile affects fruit quality. On the contrary, the pentanal displayed an increasing trend with fruit ripening and maturity. Obenland et al. (2012) also reported similar results that pentanal increases during avocado fruit maturity and the concentration can have an influence in fruit flavour.

Aldehydes and alcohols, such as hexanal, trans-2-hexenal and cis-3-hexen-1-ol, are important for the green, unripe notes in strawberry aroma. Their concentrations are also cultivar and ripeness dependent (Jetti et al., 2007). Our findings are also in agreement with El-Mageed (2007) that ethanol, (Z)-3-hexanol and (E)-2-hexenal, the most abundant volatiles in whole green and ripe 'Fuerte' avocados (Persea Americana Mill), declined with ripening, while overripe fruit had the highest ester concentration (El-Mageed, 2007). Pereira (2010) reported that the most abundant volatiles present in the headspace of unripe, diced 'Simmonds' avocado were sesquiterpenes and hexanal, whereas the amount of these compounds was greatly reduced by ripening.

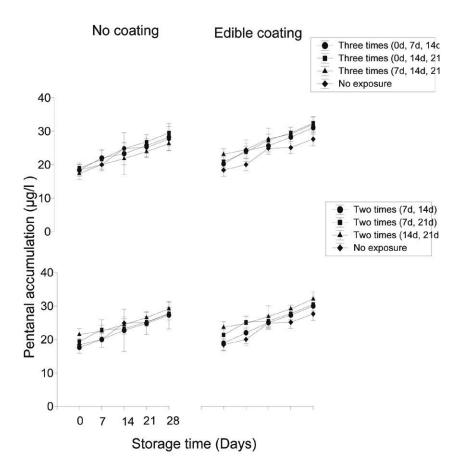


Figure 9: Effect of ozone on mesocarp pentanal for coated as well as uncoated fruit during storage time. Vertical bars represent standard error of the mean value (n = 5). The SE refers to the standard deviation of each measured value from the mean value.

During maturation time of avocados cv. Hass, the concentration of hexanal, (E)-2-hexenal and 2,4-hexadienal greatly declined in amount, while acetaldehyde, methyl acetate, pentanal, and β -myrcene were at higher concentrations in mature fruit and may also have contributed to the overall flavor (Obenland *et al.*, 2012).

CONCLUSION

The combined treatments, edible coating and ozone, enhance fruit quality. The treatments improved the physical appearance and membrane structures, reduced moisture loss, reduced respiration and ethylene accumulation compared to control fruit. Since the treatments are natural and leaves no residues, introduction of edible products and ozone can be very invaluable material for postharvest fruit treatments by fruit industries. It needs to be noted that although consumption of fruit is regarded as nutritional and has health benefits, nowadays consumers are raising a concern on safety issues towards what they consume. Accordingly, using chemicals by fruit industries impose pressure to comply with safety regulations, our research therefore reports on potential use of edible coating and ozone gas that are safe and long lasting. This perhaps boosts our fruit industries' global competitiveness in supplying quality fruit.

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