Effects of smoke, hexanal, and calcium chloride on post-harvest quality of oranges [*Citrus x sinensis* (L.) Osbeck] cvs Msasa and Jaffa under different storage durations and conditions in Tanzania

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Experiments were conducted to assess the effects of hexanal, calcium chloride, and smoke on the post-harvest quality of oranges under ambient (room) temperature (28±2^{0}C) and reduced temperature storage (18±2^{0}C) conditions on two varieties of sweet orange (*Citrus x sinensis* (L.) Osbeck) cvs 'Msasa' and 'Jaffa'. Fruit were dipped in enhanced freshness formulation (EFF) containing hexanal as the key ingredient at 0.01%, 0.02%, and 0.04% (volume/volume), or calcium chloride solution at 1%, 2%, and 4% (weight /volume) for five minutes each, or subjected to a smoking regime, simulating a popular traditional practice, by burning 0.5 kg, 1.0 kg, and 1.5 kg of dried banana leaves, or left untreated (control). Various parameters including physiological weight loss, fruit firmness, total soluble solids (TSS), titratable acidity (TA), and the TSS/TA ratio were assessed to determine effects on post-harvest quality of fruit. Results indicate that hexanal and calcium chloride treatments significantly (p < 0.001) reduced physiological weight loss, maintained fruit firmness and significantly higher TSS in both varieties compared to smoke treatment and untreated controls. Reduced temperature storage also significantly (p < 0.001) lowered physiological weight loss of hexanal- and calcium chloride-treated oranges. Based on the results of this study, post-harvest dip treatments with hexanal solution at 0.02% or calcium chloride solution at 2% coupled with reduced temperature storage at 18°C are recommended to maintain the quality of fresh oranges in Tanzania. On the contrary, the application of smoke is highly discouraged as it reduces the quality of oranges.

Keywords: Post-harvest treatments, post-harvest loss, shelf life, physiological weight loss, fruit firmness, total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio

Post-harvest loss is a general term used to describe the fraction of produce that is rendered unfit for a particular use, whereas the term quality implies the degree of excellence or suitability of a product for a particular use (Abbott 1999). Post-harvest loss is a major constraint in the tropical fresh fruit industry (Kusumaningrum et al. 2015). About 40% to 50% of fresh horticultural produce in developing countries is lost after harvest (Ahmed and Siddiqui 2016; Ugoh et al. 2015). Post-harvest losses are commonly characterized by fruit shrivelling, weight loss, softening, decay, and changes in sugar content and acidity levels (Martin-Diana et al. 2007; Ahmed and Siddiqui 2016), which are major determinants of consumers' choices of fresh fruits. Post-harvest losses are thus barriers to both local and international trade of tropical fresh fruits (Kusumaningrum et al. 2015). The major causes of post-harvest losses in developing countries include improper post-harvest practices, poor post-harvest management systems and lack of supporting infrastructure for quality maintenance (Ahmed and Siddiqui 2016). Moreover, harvesting fruit at stages other than horticultural maturity, storage under inappropriate conditions, especially temperature (Ahmed and Siddiqui 2016), and use of improper post-harvest treatments results in significant post-harvest losses (Heather and Hallman 2008).

Several practices are used by farmers to reduce post-harvest losses and to maintain the quality of fresh fruits. Smoke treatment is a common post-harvest practice used by farmers in Tanzania to hasten fruit ripening for bulk purchase and prior to transportation (Saltveit 1999). However, this technique results in increased fruit softening and reduced fruit shelf life (Watada 1986). Storage temperature is an important factor that affects respiration, transpiration, senescence, and other physiological processes in the fruit (Wani et al. 2014). Storage at low temperatures and high relative humidity is beneficial for maintenance of the natural resistance of the peel to infection (Wardowski et al. 2006). Storage of horticultural products in reduced temperatures has been reported to be effective in delaying the physico-chemical changes related to quality loss in fruits (Wani et al. 2014).

Chlorine, sourced from chloride salts, is commonly used in small-scale, post-harvest treatments of fruits (Bertzer et al. 2002) to extend their shelf life and to reduce incidences of decay (Singh et al. 1993). Calcium chloride has been widely used as a preservative and firming agent in the fruit and vegetable industry for whole and fresh-cut commodities (Martin-Diana et al. 2007). For instance, calcium chloride maintained the quality of apples [*Malus domestica,* L.] (Chardonnet et al. 2003), increased the total soluble solids of fig [*Ficus carica*, L.] fruits (Irfan et al. 2013), reduced weight loss of loquart [*Eriobotrya japonica,* L.] (Akhtar et al. 2010), and also delayed ripening and senescence of strawberries [*Fragaria ananassa,* L.], blueberries [*Vaccinium corymbosum,* L.], apples (Martin-Diana et al. 2007; Mishra 2002), and fig fruits (Irfan et al. 2013). Fruit treatment with a high concentration of calcium chloride, however, has been shown to lower fruit quality and increase susceptibility to fungal diseases (Chardonnet et al. 2003).

Hexanal treatment is an emerging technique for reducing post-harvest losses of fresh horticultural produce (Paliyath et al. 2008; Paliyath 2011) as it inhibits the activities of phospholipase D enzymes, which are responsible for membrane degradation (Paliyath 2011). Hexanal has been used to reduce post-harvest losses in sweet cherry (*Prunus avium* L.) (Sharma et al. 2010) and tomato (*Solanum lycopersicum* L.) (Tiwari and Paliyath 2011). Hexanal was reported to reduce weight losses in mango (*Mangifera indica* L.), extend the shelf life of sweet cherries (Sharma et al. 2010), delay ripening of mango, and increase firmness and maintain total soluble solids in blueberry (Song et al. 2010). However, information is lacking on the effects of hexanal on post-harvest losses and quality maintenance of fresh citrus fruits. The objective of this study was to assess the effect of different post-harvest techniques on the reduction of post-harvest losses and quality maintenance of fresh oranges.

Materials and methods

Fruits of the two most popular sweet orange varieties, ‘Msasa’ and ‘Jaffa’, were harvested from Bwembera and Semngano villages in the Muheza district, Tanga Region in Tanzania. Both varieties were harvested at standard commercial horticultural maturity. Three post-harvest techniques were applied: (i) dipping of fruits in enhanced freshness formulation (EFF), (ii) dipping of fruits in calcium chloride solution, and (iii) smoke treatment. Control groups consisted of untreated oranges of both varieties.

Fruits were completely immersed in EFF solution at 0.01%, 0.02%, and 0.04% (volume/volume) for five minutes or in calcium chloride solutions of 1%, 2%, and 4% (weight /volume) for five minutes each, or subjected to smoke treatment generated by burning dried banana leaves, or were left untreated (control). For the smoke treatment, fruits in three chambers (each with a volume of 12 m^3) were treated with different smoke concentrations obtained from burning 0.5 kg, 1.0 kg, and 1.5 kg of dried banana leaves. The smoke treatment was done three times at intervals of 12 hours. The chambers were ventilated for 30 minutes by opening between smoke treatments. The chambers were located six metres from the source of smoke to reduce heat transfer to the oranges.

There were two storage conditions: ambient storage (28°C ± 2°C) and reduced temperature storage (18°C ± 2°C). Data were collected on the 4th, 8th, and 12th day from the date of fruit harvest (DAH). Thirty fruits of each variety were used for each treatment, each of which was replicated six times making a total of 360 fruits per treatment.

Data collection was stopped on the 12th day from the date of harvest due to the onset of post-harvest deterioration in some treatments.

Data were collected on fruit weight, firmness, total soluble solids (TSS), and titratable acidity (TA). Physiological weight loss and the total soluble solids/titratable acidity (TSS/TA) ratio were calculated. The physiological weight loss was determined by randomly selecting five fruits from each treatment. The selected fruits were numbered and used for measurement of initial and final weights using an electronic balance (BX 4200H, Shimadzu, Japan) and percentage fruit weight loss was calculated. A small peel disc (approx. 2 mm^2) on the opposite side of the fruit cheek was removed, and then fruit firmness was measured using a penetrometer (Wagner fruit test, FT 20 Model, Wagner Instruments, Italy). Total soluble solids content (^{o}Brix) was measured using a hand-held digital refractometer (ATAGO, Japan) according to Nielsen (2010). Titratable acidity of the juice was determined by titration with 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator. The titratable acidity (%) was estimated as per Ranganna (1999) and expressed as per cent anhydrous citric acid. The TSS/TA ratio was computed by dividing the TSS by the TA (%). The data were analysed using R software and, where significant differences existed, using the *F*-statistic, and means were separated using Tukey's Honestly Significant Differences (HSD) ($p \leq 0.05$).

Results

Effects of post-harvest treatments, storage duration and storage conditions on physiological weight loss

Physiological weight loss of fruits in both varieties tested was significantly lower in hexanal treatment in ambient conditions on all three days of observation. However, no significant difference was observed when the fruits were kept in reduced temperature conditions (Fig 1). Overall, the physiological weight loss of oranges was lower in hexanal and calcium chloride-treated fruits compared to both smoked and untreated fruits of both varieties.

Effects of post-harvest treatments, storage duration and storage conditions on firmness

Our results showed that fruits remained firm after hexanal and calcium chloride treatments compared to smoke-treated and control fruits of both cultivars. The firmness decreased, as expected, throughout the study period (Fig 2). The highest firmness was 17.40 N/mm^2 for 'Jaffa' and 13.39 N/mm^2 for 'Msasa' when treated with 0.02% hexanal under ambient storage conditions at 4DAH.

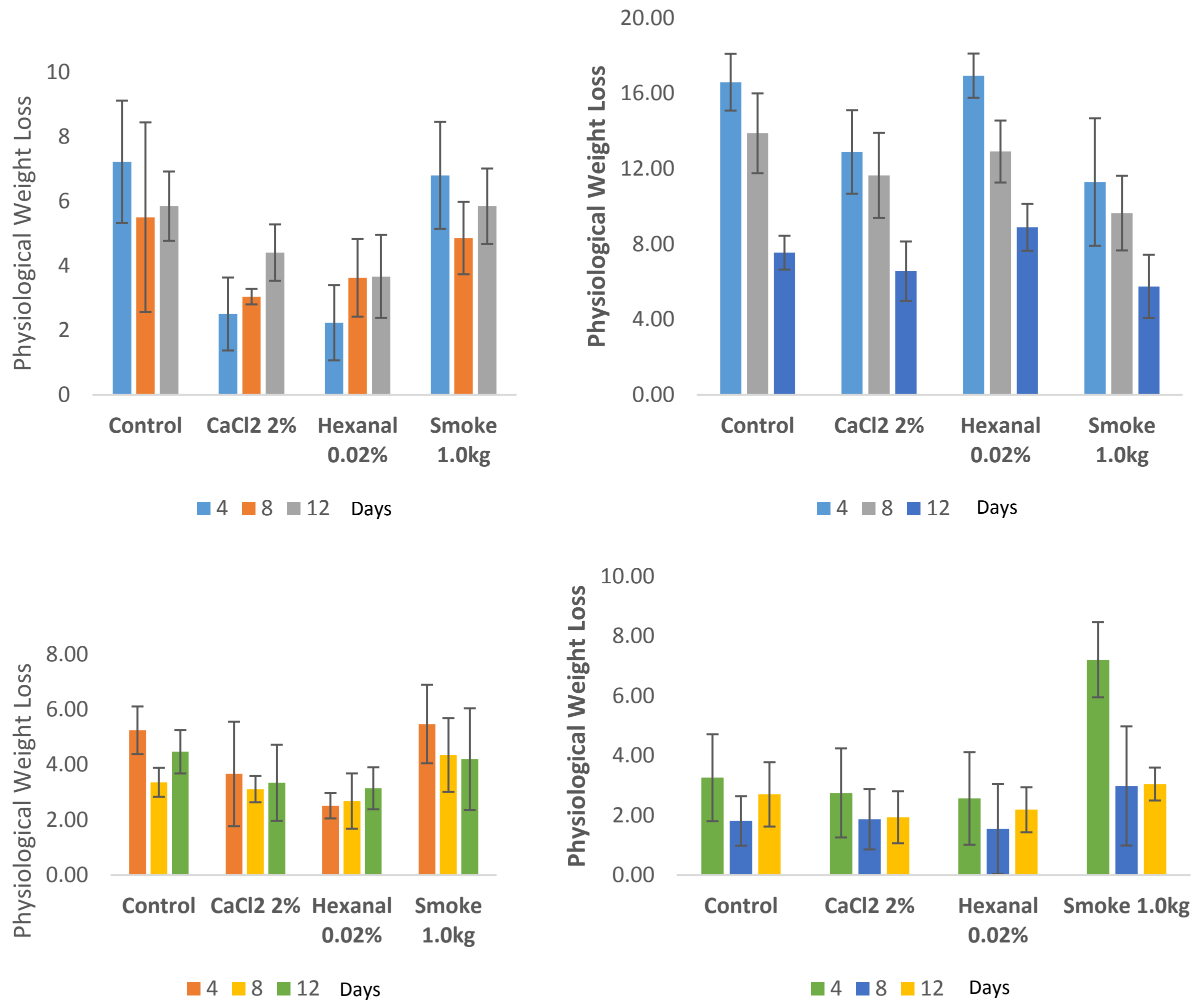

Figure 1: Physiological weight loss in sweet orange in ambient (Left; 28±2°C) and cold (Right; 18±2°C) conditions. Top panel depicts cv ‘Jaffa’ and the bottom panel depicts cv ‘Msasa’. Values are mean ± Standard deviation.

Effects of post-harvest treatments, storage duration and storage conditions on total soluble solids

Total soluble solids increased with duration of treatment in all treatments, conditions and days after harvest, which was not surprising. There were no significant differences in any of the factors tested in both varieties (Fig 3). Since hexanal only delays membrane deterioration, this result is not surprising and follows the anticipated trend. Fruits treated with hexanal at 0.02% had the lowest value for total soluble solids in both varieties.

Effects of post-harvest treatments, storage duration and storage conditions on titratable acidity

Titratable acidity decreased steadily over time in both varieties tested. The decrease was more pronounced in ‘Msasa’ than ‘Jaffa’ fruits stored at room temperature. For fruit stored under reduced temperature conditions the decrease was not as pronounced as in ambient conditions, although there were some anomalies in ‘Msasa’ fruit, at 12DAH, from hexanal and calcium chloride dip treatments (Fig 4).

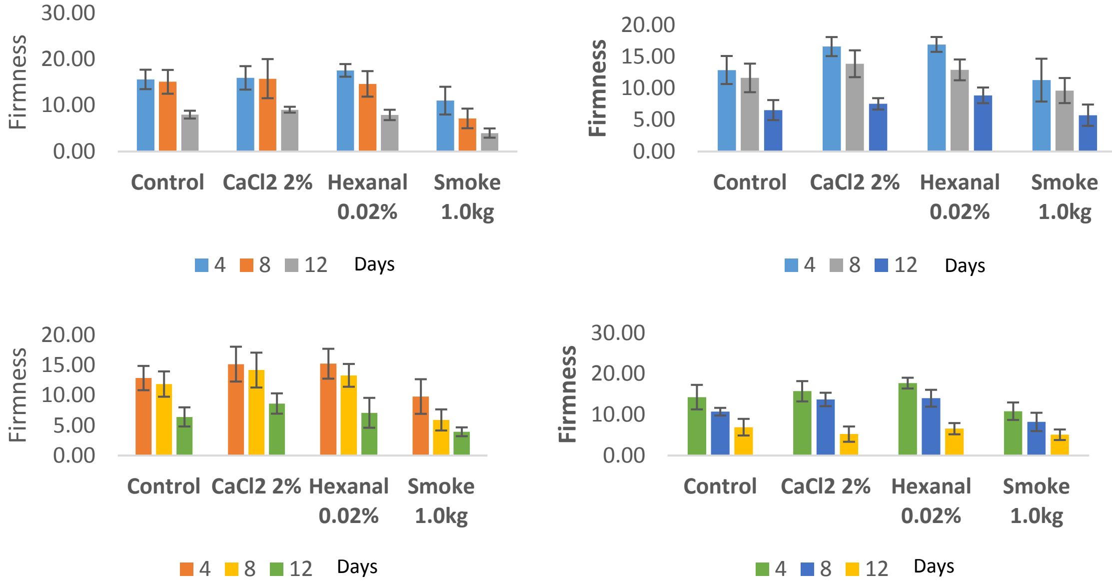

Figure 2: Fruit firmness in sweet orange in ambient (Left; 28±2°C) and cold (Right; 18±2°C) conditions.
Top panel depicts cv 'Jaffa' and the bottom panel depicts cv 'Msasa'. Values are mean ± Standard deviation.

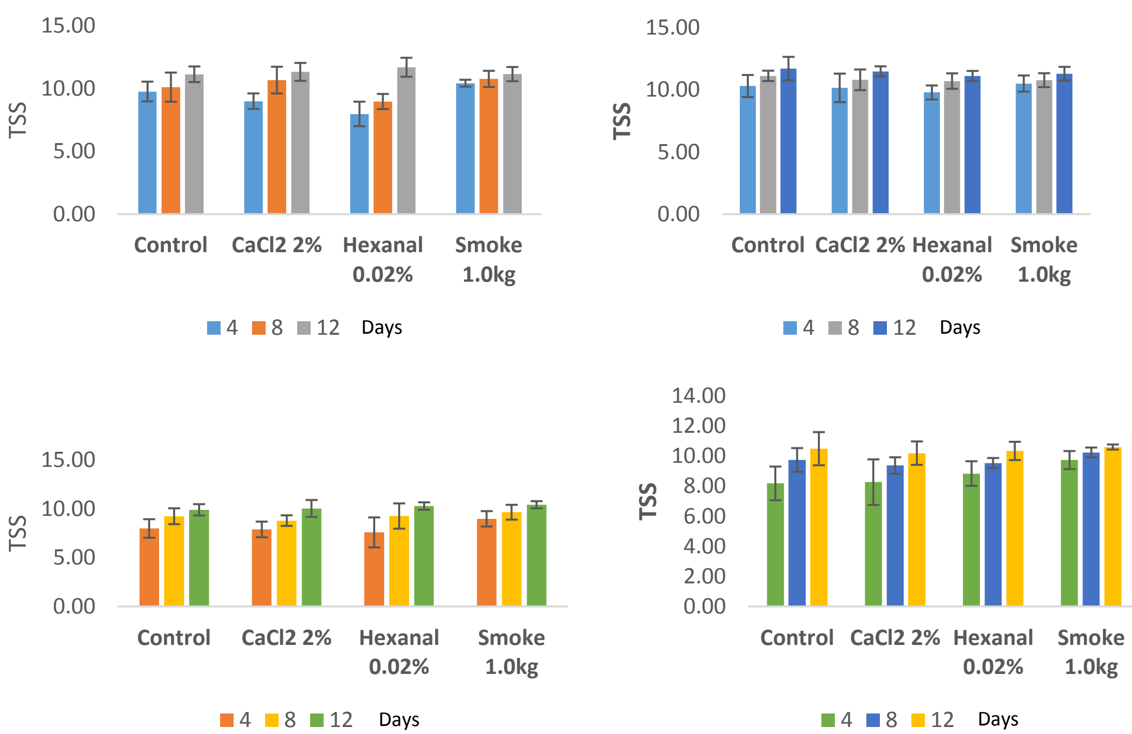

Figure 3: Total soluble solids (°bx) in sweet orange in ambient (Left; 28±2°C) and cold (Right; 18±2°C) conditions.
Top panel depicts cv 'Jaffa' and the bottom panel depicts cv 'Msasa'. Values are mean ± Standard deviation.

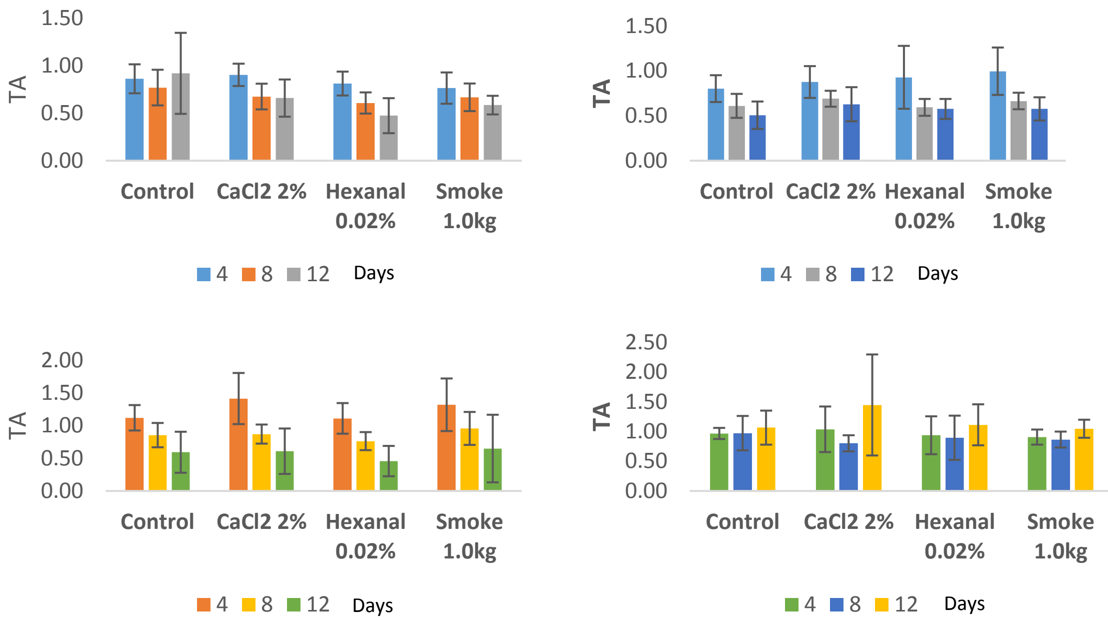

Figure 4: Titratable acidity in sweet orange in ambient (Left; 28±2°C) and cold (Right; 18±2°C) conditions.
Top panel depicts cv ‘Jaffa’ and the bottom panel depicts cv ‘Msasa’. Values are mean ± Standard deviation.

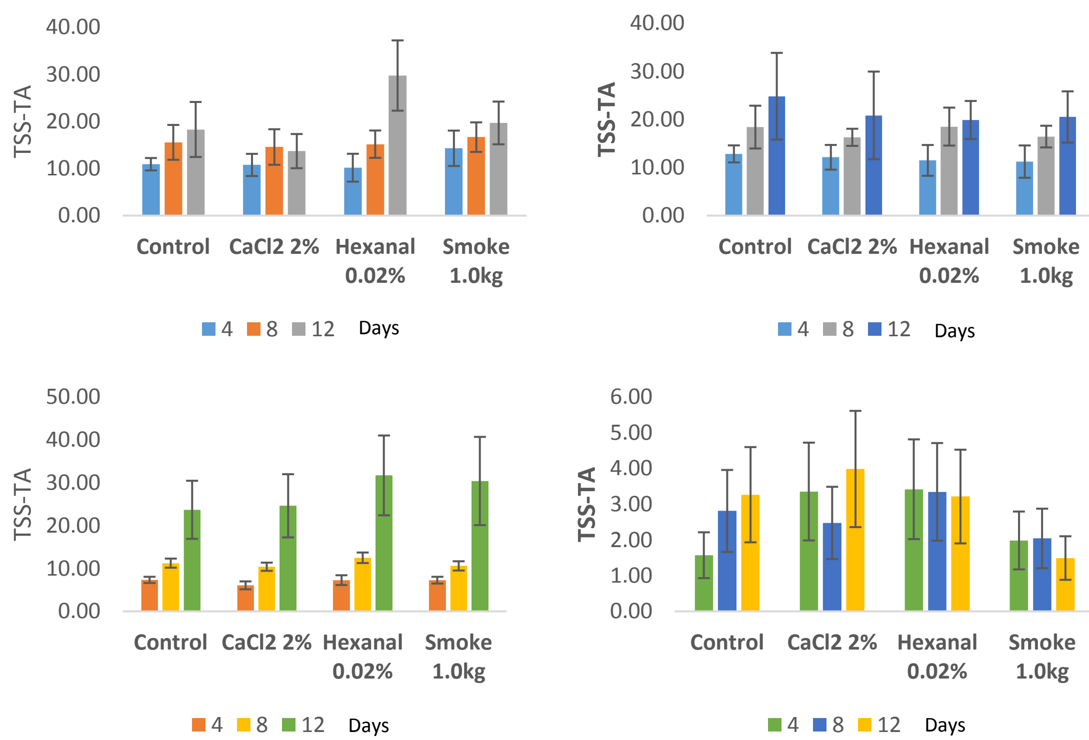

Figure 5: TSS-TA ratio in sweet orange in ambient (Left; 28±2°C) and reduced temperature (Right; 18±2°C) conditions.
Top panel depicts cv ‘Jaffa’ and the bottom panel depicts cv ‘Msasa’. Values are mean ± Standard deviation.

Effects of post-harvest treatments, storage duration and storage conditions on the total soluble solids/titratable acidity ratio

Results showed that TSS/TA increased significantly with storage duration in fruit of both varieties. However, post-harvest factor and storage condition did not alter the TSS/TA ratio significantly in ei ther cultivar (Fig 5). This is not surprising since we noticed opposite trends with TSS and TA as explained earlier.

Overall, our results consistently showed that, treating the fruits with hexanal did slow down the post-harvest damages in physiological factors, thus helping oranges to keep longer in very ordinary conditions that are prevalent in Tanzania. Smoke treatment of oranges, a common practice in Africa, is not beneficial as this research has confirmed. This is the first report of the effects of hexanal in the citrus family, one of the top five fruit crops of the world.

Discussion

Fruit physiological weight loss

Results showed that post-harvest treatments affected physiological weight loss of oranges. Further, the study found that smoke-treated oranges had the highest physiological weight loss. Fruits treated with smoke, shrivelled and lost freshness earlier compared to fruits from the other treatments and the control. Higher smoke concentration aggravated the loss of fruit freshness compared to the other treatments and the control. Smoke treatment results in higher ethylene concentration in the storage room, which accelerates physiological weight loss, ripening and fruit aging (Karthika et al. 2015). Weight loss is an important factor in citrus deterioration during storage, and it is essentially due to water loss by transpiration (Wardowski et al. 2006). A previous study associated the loss of fruit freshness with the rise in transpiration rate and an increase in wilting and shrivelling of stored fruits (Paul and Pandey 2016). Wardowski et al. (2006) have shown that respiration and transpiration rates are high immediately after fruit picking and decline during storage due to shrivelling and drying of peel.

Hexanal-treated oranges had low physiological weight loss regardless of the concentration. El Kayal et al. (2017) reported low weight loss in hexanal-treated raspberry compared to untreated controls. They found that there was delayed wilting in the fruit treated with calcium chloride and the fruit remained fresh for a longer time compared to untreated control fruit. Calcium chloride has been reported to stabilize cellular membranes and consequently delay senescence in horticultural produce Bertzer et al. (2002) and Akhtar et al. (2010) reported delayed senescence and reduced rates of respiration and transpiration in loquat (*Eriobotrya japonica*) treated with calcium chloride. The low weight loss in fresh, hexanal-and calcium chloride-treated oranges translates into maintained quality, improved shelf life and, therefore, potentially expanded market window.

Physiological weight loss increased during the storage period regardless of storage conditions and post-harvest treatment. Those under ambient (room) storage conditions experienced higher physiological weight loss than those under reduced temperature storage conditions. Singh and Reddy (2006) found that the percent cumulative weight loss in oranges during storage under ambient and refrigerated conditions for 17 days duration, increased with increasing storage period under both storage conditions. High temperatures are well known to result in increased rates of respiration, deterioration, and water loss in fresh produce, leading to reduced market, food, and nutritional values (Hailu and Derbew 2015).

Fruit pulp firmness

Results showed that regardless of the concentration of post-harvest treatment used, and the storage time at assessment, the firmest fruits were those treated with hexanal followed by those treated with calcium chloride. Similar results were reported by Sharma et al. (2010) in sweet cherries. Paliyath (2008) also reported high firmness in fruits treated with hexanal formulations after harvest. Hexanal acts as a strong inhibitor of phospholipase D action, and thus slows down ethylene stimulation of fruit ripening and softening processes (Karthika et al. 2015). Calcium chloride-treated fruits had higher firmness than untreated controls. Calcium chloride has been used as a firming agent for many fruits and vegetables (Mishra 2002). Calcium chloride accumulates in the cell walls leading to facilitation of the cross linking of the pectic polymers, which increases cell wall strength and cell cohesion (Akhtar et al. 2010). The greater firmness in hexanal- and calcium chloride-treated fruits may also be associated with fruit cells' turgidity, which maintains fruit freshness and increases fruit shelf life. Results demonstrated that smoke treatment leads to increased fruit softness and senescence. Burning one kilogram of wood produces 2.245 g of ethylene (Todd 2003), which means that the effects of smoke are expected to be similar to those of ethylene (Porat et al. 1999). Fruit firmness is one of the most important fruit quality parameters and thus the degree of fruit firmness has been used as an indicator of fruit quality. Further, firmness is one of the final indices that buyers use to make decisions as to whether to buy fruits or not (Batu 2003). It is therefore important to extend fruit firmness so that shelf life and produce acceptance by buyers are improved, which will also result in reduced produce loss.

The reduced temperature storage conditions led to firmer fruits than the ambient storage conditions. This could be due to reduced metabolic reactions in fruits stored at the reduced temperature. Wardowski et al. (2006) reported that citrus firmness undergoes changes during storage depending on storage conditions, especially humidity. The firmness of most fruits and vegetables showed decreased firmness with increasing temperatures (Bourne 1982). The fruits stored under reduced temperature conditions were firmer than the fruits stored in ambient (room) storage, regardless of the variety.

Fruit juice total soluble solids

Hexanal- and calcium chloride-treated fruits had lower values of total soluble solids than smoke-treated oranges at eight days after harvest. This might be due to delayed fruit ripening in the hexanal- and calcium chloride-treated fruits. It has been reported that hexanal slows down ethylene-induced fruit ripening and softening processes (Karthika et al. 2015). The higher total soluble solids content in smoke-treated fruits might be due to the accelerated breakdown of starch, and ripening of the fruits caused by smoke. Goldschmidt (1997) reported that citrus fruits reveal ripening-related symptoms in response to exogenous ethylene treatment. On the contrary, Mayuoni et al. (2011) reported that ethylene had no effects on total soluble solids and acidic contents of citrus fruit juice. Like this study, Akhtar et al. (2010) also reported higher values of total soluble solids content in calcium chloride-treated fruits and the lowest values in the control group.

Fruit juice titratable acidity

The quality of orange juice is influenced by physico-chemical parameters such as pH, total soluble solid content, and total titratable acidity. Citric acid is the dominant organic acid in citrus fruits (Etienne et al. 2013) and it determines the fruits' organoleptic quality. In our study, the oranges' titratable acidity decreased significantly with storage duration, but it was not affected by post-harvest treatments used. The titratable acidity was high at harvest and decreased with storage time, with higher values seen in ambient storage conditions than in reduced temperature storage conditions. This might be due to the ripening of the fruits, when starch is converted into sugar, which contributes to the sweetness of the fruits. The ripening of fruits is associated with softening, sweetening (or decreased bitterness) and colour change. The reduction in acidity may also be due to the conversion of the acids into sugars and their further utilization in the metabolic processes of the fruits. Faasema et al. (2011) reported a similar result of decreased acidity in citrus fruits during storage.

Fruit juice total soluble solids/titratable acidity ratio

The sugar to acid ratio (TSS/TA ratio) was influenced by storage conditions, and there was a higher TSS/TA ratio in ambient storage conditions than in reduced temperature storage conditions at the fourth day after fruit harvest. This could be due to decreased fruit acidity during ripening, and the reduced fruit metabolic activities in reduced temperature storage. The TSS/TA ratio is a key characteristic determining the taste and texture of fruit, and it contributes to characteristic fruit flavours (Wardowski et al. 2006). The TSS/TA ratio is a more reliable index of maturity than rind colour in sweet oranges especially in the humid, tropical regions (Ladaniya 2008). Although the respiratory rates of mature citrus fruits are relatively low, extended post-harvest storage can result in internal quality changes (Echeverria and Ismail 1987). It can be inferred that longer storage duration positively influenced the TSS/TA ratio of fruits in the current study.

Conclusion and recommendations

The current study shows that 0.02% hexanal treatment reduces physiological weight loss, and improves total soluble solids content and firmness in both *Msasa* and *Jaffa* oranges up to eight days of storage. Similarly, 2% calcium chloride treatment reduces physiological weight loss, increases total soluble solids content, and increases firmness of oranges. Conversely, smoke treatment increases physiological weight loss and reduces fruit firmness under both ambient (room) and reduced temperature storage conditions with the latter giving better results. Based on the results of this study, treatments with either 0.02% hexanal formulation or 2% calcium chloride coupled with reduced temperature storage at 18°C (± 2°C) are recommended for the maintenance of quality of fresh oranges in Tanzania. On the contrary, smoke treatment is highly discouraged as it reduces the quality of oranges. Further studies are required on the cost-benefit analysis of hexanal and calcium chloride treatment of oranges. Research is also needed to evaluate the effect of hexanal and calcium chloride treatments on the quality maintenance of other tropical fruits in Tanzania.

Acknowledgement

This research was supported by the Canadian International Food Security Research Fund (CIFSRF)-Canada, Global Affairs Canada, and the International Development and Research Centre (IDRC), Canada.

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