Enhancement of 'Hass' avocado shelf life using ultra-low temperature shipping or 1-MCP treatment and cold chain management

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ABSTRACT

Long distance shipping requires extended storage which may result in fruit partially soft on arrival or quality defects. Solutions may be the use of lower shipping temperatures or 1-MCP, although the latter may result in post shipping ripening problems. 'Hass' avocado fruit were harvested at early, mid and late season and stored at 1°C or 5.5°C for 28 and 56 days. Additional treatments were 1-MCP, waxing and cold chain breaks (24 hour delay, break at day 14). Storage at 1°C was comparable to the use of 1-MCP. Cold chain breaks reduced the quality and ripening period, with a 24 hour delay before storage being highly detrimental. The 1°C treatment suppressed the effects of cold chain breaks to some extent. Storage at 5.5°C resulted in partial in-transit ripening if 1-MCP was not used. Mid season fruit were the most sound in terms of quality. After 56 days the 1°C treatment caused significant external chilling injury, but internal quality was unaffected. Waxing was advantageous in decreasing external chilling damage. It is suggested that 1°C can be used as an alternative to 1-MCP for long distance shipping up to 28 days but not 56 days.

Mejora en la vida util del avocado 'hass' usando Ultra-baja temperatura durante el translado o tratamiento con 1-MCP y manejo de la cadena de frio

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Los traslados de larga distancia requieren de almacenados prolongados, lo que puede r sultar en frutos parcialmente ablandados al arribo o con defectos en la calidad. Una solución podría ser el uso de menores temperaturas durante el traslado o del 1-MCP, aunque este ultimo puede resultar en problemas de maduración posteriores al traslado. Avocados 'Hass' fueron cosechados temprano, en el medio y al final de la temporada y almacenados a 1ºC o 5.5ºC por 28 y 56 días. Tratamientos adicionales fueron el agregado de 1-MCP, encerado y cortes en la cadena de frío (atraso de 24 horas, y corte al día 14). El uso de almacenamiento a 1ºC produjo resultados similares al uso de 1-MCP. Cortes en la cadena de frío reduj ron la calidad y el periodo de maduración, con las 24 horas de atraso previas al almacenamiento siendo el tratamiento más nocivo. El tratamiento de 1ºC suprimió los efectos nocivos de los cortes en la cadena de frío, hasta un determinado punto. El almacenamiento a 5.5ºC result en una maduración parcial en transito si esto no esta combinado con el uso de 1-MCP. Los frutos de media estación produjeron los mejores resultados en calidad. Después de 56 días, el uso de 1ºC causo una significativa injuria externa por enfriado, pero la calidad interna permaneció intacta. El encerado fue beneficioso en disminuir el daño externo por enfriado. Es sugerido que el uso de 1ºC puede ser una alternativa al uso de 1-MCP dur ante traslados de larga distancia hasta 28 días, pero no para traslados de 56 días.

Key words: Ultra-low temperature shipping, 1-MCP, cold chain breaks, extended shipping

1. INTRODUCTION

The South African avocado industry has unique challenges that are present with respect to the logistical effort required to export avocados to the European market (Dodd *et al*., 2007), and where fruit quality is of major importance for the export markets the industry should take all possible measures in order to ensure the fruit that is exported will arrive at its market with the desired quality. For fruit to reach the European markets it can entail a transport period of up to 30 days, and with such an extensive time frame there is an obvious need to slow the natural ripening processes of the avocado fruit. The current technologies used are controlled- and modified atmosphere (CA and MA) or the use of an ethylene receptor blocker 1-methylcyclopropene (1-MCP), but it has been found that problems are still associated with the use of these protocols. Examples of such problems include 1-MCP fruit having uneven ripening (Mare *et al*., 2002), extreme CA conditions with high CO_2 levels (>5%) leads to carbon dioxide poisoning, inhibiting ripening, body rots, hypoxia and anoxia (Kader, 2003; Arpaia *et al*., 1990; Burdon *et al*., 2008), and not least of which are the costs involved to implement these technologies (approximately US$ 800-1500). With this an opportunity has arisen in trying to achieve the same, or even better, export standards without the use of current protocols (ie. 1-MCP and 5.5°C) by instead using ultra-lower temperatures such as 1°C for 'Hass'. If it is found that shipping at lower temperatures is comparable to the use of CA or 1-MCP, then costs would be reduced drastically. There has also been indication that cold chain breaks associated with the logistics have severe effects of the final quality of the fruit (Undurraga *et al*., 2007). There has been very little work done on this issue and the actual losses that are involved due to this problem are unknown. Work conducted by Blakey and Bower (2009) as well as Kok *et al.* (2010) illustrated that the use of 1°C significantly improved the quality of the fruit where as fruit at 5.5°C which incurred the same cold chain break treatment had a figure of less than 30% being of acceptable quality. To date there has been no physiological work done with respect the effect cold chain breaks have on the fruit with respect to enzyme activity. It is clear therefore that this could be a big opportunity to determine what actually is involved within the fruit on a physiological level when a cold chain break occurs, and link these results to the final quality of the fruit and the expected shelf life. If it could be understood on what happens to the fruit, it may be possible to find a solution to the problem which could be hugely beneficial to the industry.

Enquiries with respect to the possibility of lengthening the storage period time influenced the addition of an extra treatment being a storage time of 56 days. The reason for the interest in this is due to estimating the limits of ultra-low temperature shipping. It would also have its practical implications of providing an estimated result if a container of fruit were subject to a delay in the port or market whilst in cold storage. There also may be an opportunity in that fruit could be strategically held back from the market in order to receive higher prices.

The study aimed to ascertain whether storage at 1°C as opposed to current industry protocol 5.5°C is comparable to the use of 1-MCP during simulated shipping, as well as to ascertain the effects of cold chain breaks on the final fruit quality for 'Hass'. Furthermore an investigation into an extended storage time of 56 days was conducted to ascertain whether it can be a credible option for export.

2. MATERIALS AND METHODS

'Hass' avocado fruit were obtained from a designated packhouse near Wartburg, KZN (29°27'S, 30°40'E). The fruit were harvested throughout the season at significantly different maturity levels from one block in the orchard. Early season fruit were harvested on 28 July 2010 with a moisture content of 72%. The mid season fruit were harvested on 2 September 2010 with a moisture content of 66%, and the late season fruit were harvested on 16 September 2010 with a moisture content of 60%. Post harvest operations of waxing, 1-MCP, forced air cooling, grading, sizing and packing to 'count 20' were all conducted at the packhouse. Half the fruit samples were collected off the packline before waxing, while the other half remained on the packline to be waxed. The fruit treated with 1-MCP were to standard export protocols for sixteen hours at a temperature of 5.5°C, whilst the untreated fruit were stored under the same temperature for the same period but without 1-MCP. After the initial packhouse treatment, all fruit were transported to the laboratories of the Horticultural Science Department at the University of KwaZulu-Natal, and immediately prepared for simulated shipping for a period of 28 days. Half the fruit were stored at 1°C (±0.5°C) and the other half at 5.5°C (±0.5°C). Cold chain break treatments were applied throughout the cold storage period where there was a control (no break for 28 days and 56 days), 24 hour delay before cold storage, and a break at day 14 where the break would be regulated to 8 hours. To monitor the internal temperature and relative humidity of the storage containers, HOBO® H8 data loggers were used. Each of the treatment combinations consisted of ten fruit replicates. Before storage, fruit mass, ethylene evolution and fruit softness were also measured.

Fruit were visually assessed after storage after warming up to room temperature for external damage, including chilling injury and lenticel damage. After ripening at room temperature (20-25ºC), fruit were cut and assessed for anthracnose, stem-end rot, vascular browning and mesocarp discolouration. Ethylene evolution was also determined during the ripening period on a daily basis. The data was analysed in the form of a factorial design, where each treatment combination consisted of ten fruit, each constituting a single replication. A general analysis of variance was run using Genstat12, where the ANOVA, table of means, and LSD was computed to identify significantly different treatment combinations.

3. RESULTS

<u>3.1 Fruit Softening</u>

The interaction of the seasonal maturity variances and temperature showed that the 1^{o}C treatment significantly suppressed ($P < 0.001$) the amount of softening occurring within cold storage in comparison to the 5.5^{o}C treatment (**Figure 1**), however the mid season fruit experienced the highest percentage softening when one may expect the late season fruit to incur the highest softening. The interaction of 1-MCP with the seasonal maturity variances showed a similar trend ($P < 0.001$) (**Figure 2**). The cold chain break interaction showed that the early season fruit were least affected by the cold chain breaks whereas the mid and late season fruit followed the expected trend where the 24 hour delay causes the highest amount of softening followed by the break within storage at day 14 and then the least softening resulting from no break occurring (**Figure 3**). The most important finding was that the fruit stored at 1^{o}C, regardless of whether 1-MCP treatment was used, had substantially less softening than fruit at 5.5^{o}C (**Figure 4**). It highlighted that even fruit treated with 1-MCP at 5.5^{o}C still experience significantly higher softening throughout storage as compared to fruit at 1^{o}C without the use of 1-MCP ($P < 0.001$).

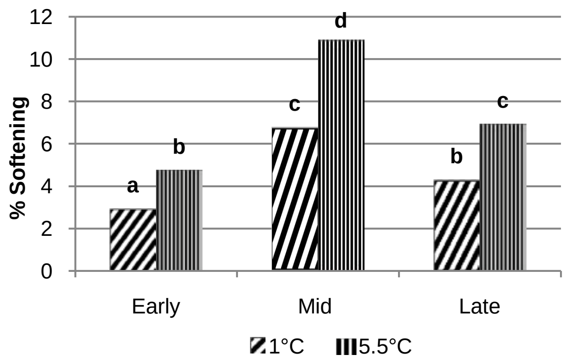

Figure 1. Percentage softening after storage period of 28 days as influenced by maturity stages and temperature treatments.

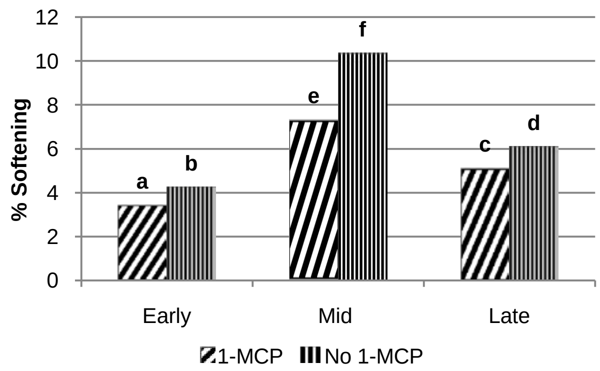

Figure 2. Percentage softening after storage period of 28 days as influenced by maturity stages and 1-MCP treatments.

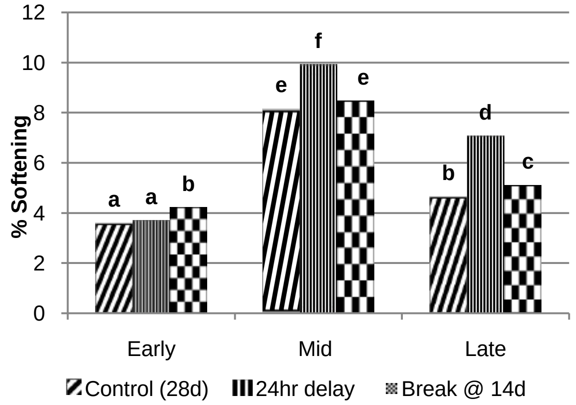

Figure 3. Percentage softening after storage period of 28 days as influenced by maturity stages and cold chain break treatments.

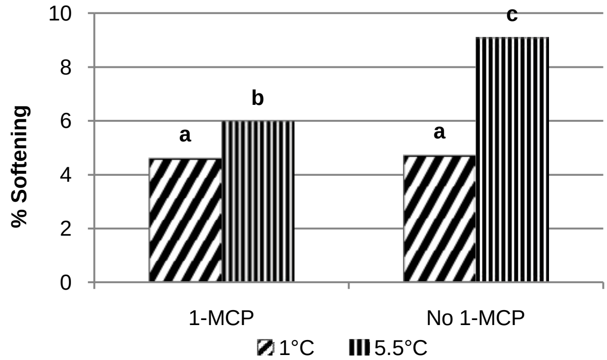

Figure 4. Percentage softening after storage period of 28 days as influenced by treatment with 1-MCP and temperature.

3.2 Mass Loss

The mass loss, measured as a percentage, can be assumed to be predominantly due to the loss of water. The main effects of temperature, waxing and cold chain breaks were significant ($P < 0.001$) in differences experienced amongst their respective treatments. In **Figure 5** it can be seen that storage at 5.5^{o}C resulted in a higher fruit mass loss than 1^{o}C, waxed fruit experienced less mass loss than unwaxed fruit, and the 24hr delay treatment had a significantly higher mass loss when compared to the control fruit.

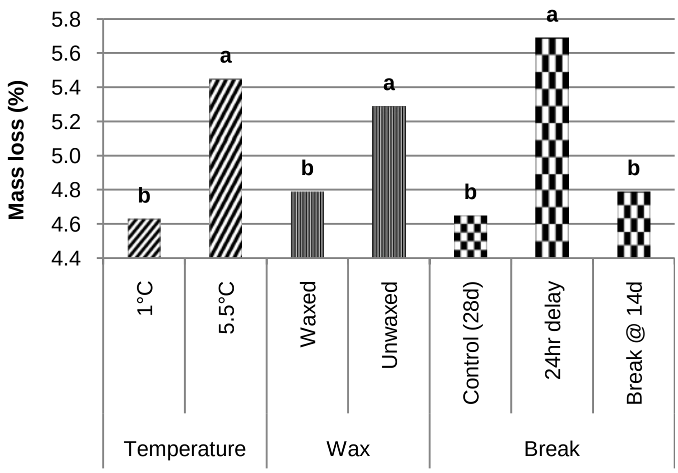

Figure 5. Main effects of temperature, waxing and cold chain breaks on the percentage mass loss of fruit after 28 days cold storage.

3.3 Days to ripening

The season maturity variance treatment showed the early season fruit had the longest days to ripening at an average of approximately 8, followed by the mid season fruit at 7.5 days and the late season fruit at 7 days ($P < 0.001$). The interaction of season maturity variance and temperature ($P < 0.001$) showed that the 1^{o}C fruit maintained consistency in the number of days taken to ripen throughout the season, whereas the 5.5^{o}C was not only significantly worse in comparison to the 1^{o}C but also seemed to significantly decline in performance as the season progressed (**Figure 6**). A similar effect was shown for the 1-MCP treatments applied throughout the season yet the 1-MCP treated fruit did significantly decline ($P < 0.001$) in the late season (**Figure 7**).

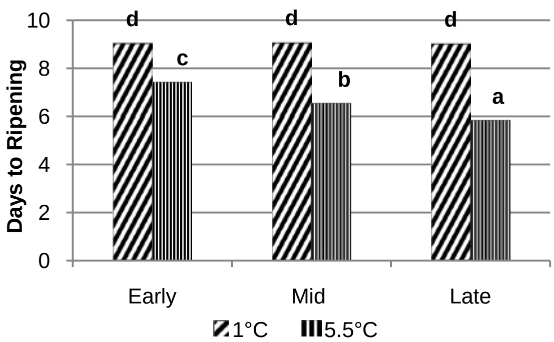

Figure 6. Effect of temperature and seasonal maturity variances on the days to ripening after 28 days storage.

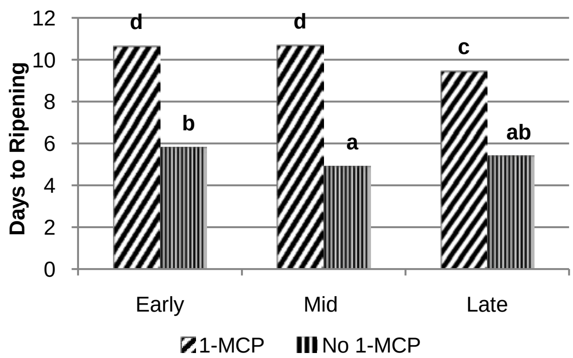

Figure 7. Effect of 1-MCP and seasonal maturity variances on the days to ripening after 28 days storage.

3.4 External chilling injury

The 1^{o}C early season fruit experienced significantly higher ($P < 0.001$) chilling injury when compared to the higher temperature of 5.5^{o}C. There was however an interesting trend shown in that the 1^{o}C improved as the season progressed and fruit matured where as the 5.5^{o}C got progressively worse in performance as the season progressed (**Figure 8**). It can be noted that by the late season there is no significant difference between the two temperature treatments with respect to chilling injury.

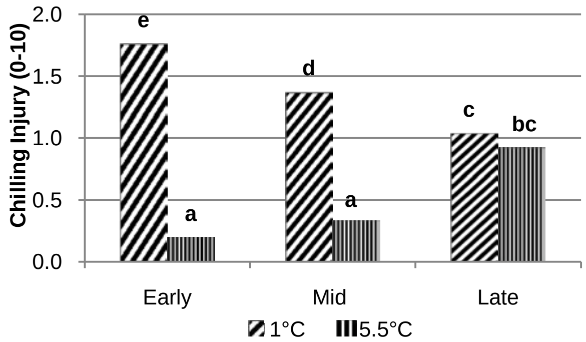

Figure 8. Effect of storage temperature and season maturity variances on external chilling injury at 28 days (scale of 0-10).

3.5 Internal quality

The important parameters with respect to the internal quality include mesocarp discolouration, vascular browning, anthracnose and stem-end rot.

3.5.1 Vascular browning

Only 13 of 720 fruit within the trial experienced a case of vascular browning, hence no significant differences were found.

3.5.2 Mesocarp discolouration

The main effects of temperature as well as waxing were found to show significant differences ($P < 0.013$). The 5.5^{o}C treatment induced a significantly higher incidence of mesocarp discolouration compared to the 1^{o}C treatment. The wax treatment induced a higher amount of mesocarp discolouration compared to the non wax treatment.

3.5.3 Anthracnose and stem end rot

Both anthracnose as well as stem end rot indicated that the 1-MCP treated fruit experienced a significantly ($P > 0.001$) higher incidence compared to the untreated 1-MCP fruit.

3.6 56 day storage

3.6.1 Days to ripening

There was a substantial difference ($P > 0.002$) shown between the two temperature treatments throughout the season in terms of the days to ripening for the 56 day storage trial, where in the early season there was up to a 6.5 day ripening difference. For the 5.5^{o}C treatment it was found that the shelf life of the fruit would be minimal at best and in fact majority of the fruit at the 5.5^{o}C temperature had nearly fully ripened within storage, not even the use of 1-MCP could significantly extend the ripening period at this temperature. However the 1^{o}C treatment proved to significantly increase the ripening period to such an extent it was comparable to the shelf life experienced by fruit under the 28 days storage trial. There was the trend shown that as the season progresses the 1^{o}C significantly declines but still allows for an acceptable shelf life even in the late season (**Figure 9**).

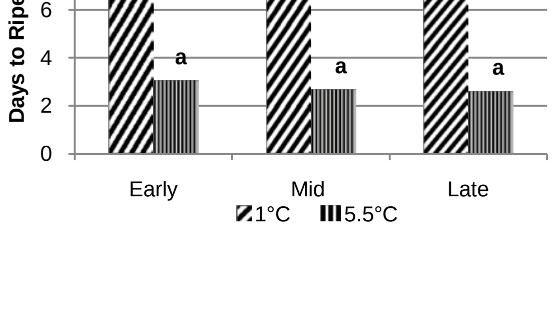

Figure 9. Effect of temperature and seasonal maturity variances on the days to ripening after 56 days storage.

3.6.2 External quality

It was found that the early season fruit incurred the highest amount of chilling injury at approximately 3.7 on a scale of 10, where as the 5.5°C was well below 0.5 (**Figure 10**). The mid and late season fruit did have significantly less chilling injury however which does shed some positive aspects (P > 0.001).

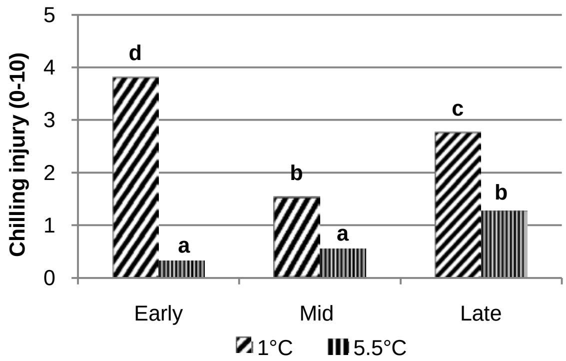

Figure 10. Effect of storage temperature and season maturity variances on external chilling injury at 56 days (scale of 0-10).

3.6.3 Internal quality

Approximately 12% of the fruit within the 56 day storage trial experienced some form of internal disorder. The main findings were those of mesocarp discolouration as well as vascular browning (**Figure 11**). The results indicated that the use of 1-MCP significantly suppressed the occurrence and severity of these disorders (P > 0.042). The 1°C treatment also managed to suppress the occurrence of mesocarp discolouration to a certain extent (P > 0.017) due to the same effect in reducing the softening percentage within storage (**Figure 12**).

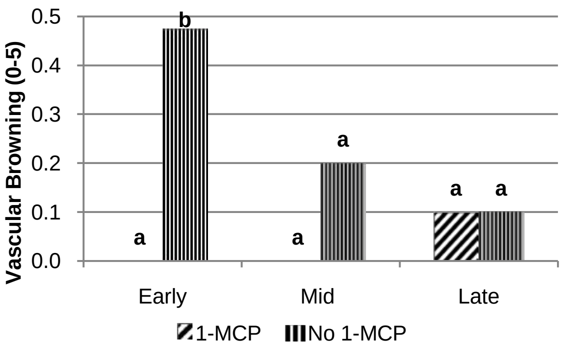

Figure 11. Effect of 1-MCP treatments and season maturity variances on vascular browning at 56 days (scale of 0-5).

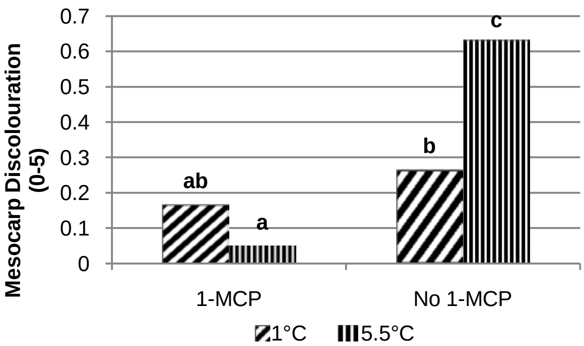

Figure 12. Effect of 1-MCP treatments and storage temperatures on mesocarp discolouration at 56 days (scale of 0-5).

4. DISCUSSION

One of the important parameters which were measured was that of fruit softening, and one of the notable results was that of the temperature and 1-MCP treatment interaction. These results concurred with those found in the preliminary study conducted in the 2009 season (Kok *et al*., 2010). This is positive in that it indicates that the use of an ultra low temperature, namely 1°C, could be a viable alternative to the use of the 1-MCP chemical in order to reduce softening of the fruit whilst in cold storage. Unfortunately it was found that the treatment interactions of 1-MCP as well as 1°C were found to be not significant in terms of negating cold chain breaks. It was indicated however that if a cold chain break occurs within storage (ie. break at day 14) then both the implied treatments successfully negate the effect of cold chain breaks in terms of fruit softening which correlate to findings by Kok *et al.* (2010) as well as the findings by Blakey and Bower (2009). It was the 24 hour delay which was unable to be rectified, which is to be expected to a certain extent due to this occurring before any of the treatments being implemented and the subsequent water loss involved with the break is irreversible.

In terms of the mass loss involved during the storage period there were no unexpected trends and again the results were similar to those in the preliminary study (Kok *et al.*, 2010) as well as work done by Blakey and Bower (2009). The waxing treatment indicated that the unwaxed fruit lost a significantly higher amount of water when compared to the waxed fruit, as found by Bower and Jackson (2003), however this did not translate to any subsequent softening as there was no significant difference found for the waxing treatments in terms of softening. The results for the cold chain breaks illustrated that the 24 hour delay causes significant mass loss which is to be expected due to the vapour pressure deficit (VPD) between the fruit and the surrounding atmosphere being higher for warm fruit compared to cold fruit. This clearly illustrates the importance of cooling the fruit as fast and soon as possible after being harvested in order to reduce the VPD between the fruit and atmosphere, and therefore the subsequent water loss (Mitchell, 1992). There was a difference between the fruit which experienced no break and the ones which experienced an 8 hour cold chain break at day 14 of cold storage in that the break at day 14 increased the amount of water loss but not significantly.

Another important factor is that of the overall shelf life of the fruit once they have been removed from storage. The results for the days to ripening showed that even though the mid season fruit experienced the highest softening, the days to ripening trend was not influenced. One may normally expect a correlation between fruit softening in storage and the days to ripen. Overall it is evident that 1^{o}C extended the ripening of the fruit, which is to be expected as there is a lowering of enzyme activity, respiration and ethylene production (Brady, 1987). The importance of the 1^{o}C being able to maintain a consistency in the number of days to ripen throughout the season should not be overlooked.

The external chilling injury involved in this study is of vital importance due to the temperatures involved. It is often debated whether the fruit will be able to withstand a temperature such as 1^{o}C without incurring significant damage. As seen in the results there was a higher external chilling injury rating for the 1^{o}C fruit however the results also need to be place in perspective. On a scale of 0-10 the early season fruit average at approximately 1.7 and by the late season this is just under 1. There are naturally individual fruit which do get extensive chilling injury but overall majority of the fruit within this trial were of good external quality at 1^{o}C. Chilling injury may not be a significant factor for the ‘Hass’ cultivar as it does change colour when ripened, so the external chilling injury is easily masked. A semi-commercial trial conducted on ‘Hass’ at 1^{o}C showed that minimal severe incidences of chilling injury were found (van Rooyen, 2009). In terms of waxing it was found that the waxed fruit experienced significantly higher chilling injury, where the same result was found by Kok *et al*. (2010). This is still an unexpected trend due to previous findings by Bower & Jackson (2003) as well as Bower & Magwaza (2004) indicating that waxing reduced the incidence of chilling injury.

With respect to the internal quality of the fruit the results showed that the use of 1^{o}C may be beneficial. The 5.5^{o}C fruit incurred higher incidences of mesocarp discolouration which is expected due to the higher softening involved with the increased temperature leading to the decrease of cell wall and membrane integrity, which allows for an increased probability of rupturing and solute leakage. Once this occurs in either the mesocarp or vascular bundles, PPO is released from the thylakoids, where it is latently held, and spills into the cytoplasm where browning occurs (Bower and Cutting, 1988). The study conducted by van Rooyen (2009) confirms that the internal quality is excellent at 1^{o}C for ‘Hass’. With respect to the waxing treatment it was found that the waxed fruit had a significantly higher incidence of mesocarp discolouration. These results correlate to those of Bower and Jackson (2003).

The 56 day storage trial produced some interesting and useful information. With the issue of the ripening period it was shown that if 1^{o}C is used as the storage temperature, a storage time of 56 days may be a credible option in terms of the shelf life of the fruit. The use of 5.5^{o}C, even with 1-MCP, produced a poor shelf life. The big issue of this trial was the external chilling factor as it would ultimately determine the outcome of the 56 days storage length in terms of rendering it a credible option overall or not. As expected there was substantial chilling injury in some cases and overall the use of this temperature for early season fruit is not advisable however for the mid and late season fruit it may be a credible option. The internal quality showed that the use of 1-MCP significantly suppressed the occurrence and severity of these disorders. The reason for this could be linked to the ability of 1-MCP to reduce the amount of softening within storage, hence maintaining the cell wall and membrane integrity over the extended time period.

5. CONCLUSION

It was found that shipping at 1^{o}C in the absence of 1-MCP was comparable, and in most cases even better, than the use of the protocol temperature of 5.5^{o}C in conjunction with the use of 1-MCP. There is a strong possibility that the use of 1°C could be a credible alternative to the use of 1-MCP as this study showed positive results towards this outcome as well as previous studies in recent years. The positive aspects not only include the elimination of significant costs involved with 1-MCP but there is the advantage of significantly reducing softening leading to enhanced shelf life and internal quality as well as negating the detrimental effects of in-storage cold chain breaks. This is probably due to the core temperature of the fruit not being able to rise sufficiently within an 8 hour break to reactivate the enzymes involved with softening and break down of cell walls. The one area that was of concern was if a delay of 24 hours occurs before the fruit are placed into cold storage. It was found that this delay causes a significant amount of water loss from the fruit, which makes them more susceptible to chilling injury as well as ripening after storage occurring at a much faster rate.

In terms of the fruit maturity, it was found that the early season fruit had the longest days to ripen as well as being the least affected by cold chain breaks, however the relative immaturity of the fruit results in internal disorders as well as uneven ripening. It was indicated that the mid season fruit were most sound in terms of ripening and quality aspects.

Shipping at 1°C may cause concern of substantial chilling injury. However, it has been shown in this study that there is minimal chilling injury present. The early season fruit resulted in a higher rating of chilling injury as one would expect, yet this level is still not drastic and may be even acceptable when one considers that the 'Hass' cultivar does have the advantage of a colour change which will mask the trace amounts of chilling injury. The mid and late season fruit proved to be perfectly acceptable in terms of the chilling injury shown.

The 56 day storage trial produced data which may be useful to growers and suppliers wishing to hold back fruit from the market to receive a higher price. It was shown that the 1°C is needed for this storage time period in order to acquire a decent shelf life. The use of 1-MCP is also essential for this extended storage period, not only to help extend the shelf life but also to maintain the internal quality and integrity of the fruit. If 1-MCP is not used there are issues of excessive softening and subsequent cell rupture leading to browning and discolouration of the mesocarp. Unfortunately the use of 1°C can inflict significant external chilling injury. It was found that this storage temperature would not be a viable option for early season fruit as there is excessive chilling injury involved. However the mid and late season fruit are affected significantly less. The best option for this storage period would be to place the fruit in a "Ready ripe" programme after storage so that the chilling injury would be masked in the mid and late season fruit.

Overall, the data resulting from this study implies that 1°C can be used as an alternative method to extend shelf life, improve internal quality and negate in-storage cold chain breaks of the 'Hass' avocado fruit.

Therefore, it appears that low temperature shipping may be the future of the South African avocado industry by phasing out other technologies used to export avocado fruit.

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