The physiological effects of ultra-low temperature shipping and cold chain break on 'Hass' avocados

RD Kok, JP Bower and I Bertling

Horticultural Science, School of Agricultural Science and Agribusiness
University of KwaZulu-Natal, Pietermaritzburg, South Africa
Private Bag X01, Scottsville 3209, South Africa
E-mail: richardkok87@gmail.com

ABSTRACT
Avocados are renowned as a 'healthy food' due to their nutritional value (Bergh, 1992), as well as containing relatively high concentrations of anti-oxidants, however the fruit also contain high amounts of C7 sugars which can act as anti-oxidants. Additionally, C7 sugars and other anti-oxidants play important roles in fruit quality (Liu *et al*., 1999; Tesfay, 2009). Therefore it is important to understand how varying storage conditions and treatments affect the levels of these physiological parameters. Treatments of cold chain break/delay included a deliberate 8 hour break at day 14 of cold storage where fruit were removed from cold storage, a 24 hour delay before cold storage and a control of 28 days where no break was involved. A 56 day extended storage period was also used. Early, mid and late season 'Hass' avocados were stored at 1°C or 5.5°C for 28 days. Additional treatments included fruit treated and not treated with 1-MCP as well as waxed and unwaxed fruit. The use of 1-MCP maintained higher levels of anti-oxidants, ascorbic acid and C7 sugars for both the 28 day and the 56 day storage periods. The 24 hour delay had a tendency to increase consumption of physiological reserves. The use of 1°C resulted in anti-oxidant and ascorbic acid levels decreasing, while maintaining higher sugar levels. Overall, high stress on fruit decreased reserves resulting in poor quality fruit.

INTRODUCTION
Avocados are well known for their nutritional value (Bergh, 1992) and considered as a health food. The avocado fruit have important and unique combinations of characteristics, such as significant amounts of B-complex vitamins, a high ratio of unsaturated to saturated fatty acids (Slater *et al*., 1975), a notable amount of potassium and iron (Wolstenholme, 1990), as well as a large amount of anti-oxidant substances, such as C7 sugars (Tesfay, 2009), β-carotene (Human, 1987) and α-tocopherol (Terasawa *et al*., 2006). These anti-oxidant compounds have important functions in ripening and quality (Tesfay *et al*., 2011). Anti-oxidant systems are present in cells to guard cellular structures against naturally occurring reactive oxygen species (ROS) such as oxygen ions, hydroxyl radical, other free radicals, as well as hydrogen peroxide. These compounds form as natural by-products of the metabolism associated with avocado fruit post-harvest and can cause damage to proteins, DNA and lipids, eventually leading to cell death. With the implementation of a stress, such as chilling, heat shock, water stress or pathogens, the production of ROS can increase significantly (Mittler, 2002), which then would need to be counteracted by the anti-oxidants present. However, if the pool of available anti-oxidants cannot scavenge all ROS produced, it will result in poor quality, damaged fruit.
Avocado fruit contain large amounts of C7 sugars, most notably mannoheptulose and perseitol, whose functions were largely unknown. Liu *et al*. (1999) reported that these C7 sugars were the major non-structural carbohydrates present in avocado fruit tissues. Further research by Tesfay (2009) indicated that mannoheptulose is of paramount importance as a transport sugar. Perseitol in avocados, on the other hand, seems to act as the storage product of mannoheptulose, which can be easily converted into mannoheptulose (Tesfay *et al*., 2011). While the roles of sugars found within avocado fruit is still uncertain, it is probable that the collection of C7 sugars in the mesocarp tissue of avocados plays an important role in the post-harvest quality (Tesfay, 2009). Altering the storage temperature and duration may lead to changes in carbohydrate storage and usage and ultimately affect final fruit quality of the avocado fruit (Eaks, 1990; Spalding, 1976).

The aim of this study was to ascertain the influence of varying storage conditions of ultra-low temperature, 1-MCP, cold chain breaks/delay and extended storage on anti-oxidants. Understanding the changes that occur in the pool of anti-oxidants in avocado

fruit due to manipulation of storage conditions, may help maintain quality and help to enhance the image of the avocado in terms of being a healthy fruit.

MATERIALS AND METHODS

Fruit

'Hass' avocado fruit were obtained from Cooling Estate near Wartburg, KwaZulu-Natal (29°27'S, 30°40'E). The fruit were harvested at three stages during the season at significantly different maturity levels from one orchard block. Early-season fruit were harvested on 28 July 2010 with a moisture content of 72% (thus equivalent dry matter (DM) of 28%). The mid season fruit were harvested on 2 September 2010 with a moisture content of 66% (DM 34%), and the late season fruit were harvested on 16 September 2010 with a moisture content of 60% (DM 40%). Post-harvest operations of waxing, 1-MCP, forced air cooling, grading, sizing and packing to 'count 20' were conducted at the pack house. Half the fruit samples were collected off the pack line before waxing, while the other half remained on the pack line to be waxed. Fruit treated with 1-MCP were to standard export protocols of 300 ppb 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. Fruit which were subject to the 24 hour delay before entering cold storage were sorted on the pack line and left overnight within the pack house and then subject to temperature and 1-MCP treatments after 24 hours along with the rest of the treatment structures. After the initial pack house treatments, all fruit were transported to the Horticultural Science Laboratories at the University of KwaZulu-Natal, and immediately prepared for simulated shipping for a period of 28 days and 56 days. The cold chain breaks/delay applied were a 24 hour delay before cold storage (implemented at pack house), as well as a deliberate 8 hour break out of cold storage at day 14 of cold storage. These cold chain breaks and delay treatments were compared against fruit under a maintained cold chain with no breaks for 28 days. Half the fruit were stored at 1°C (±0.5°C) and the other half at 5.5°C (±0.5°C). 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.

Sampling

Core of mesocarp tissue as well as strips of exocarp tissue (approximately 2.5 g fresh mass respectively) were taken (Blakey *et al*., 2010) from three randomly selected fruit within each treatment combination immediately after the allocated 28 days or 56 day storage period. Tissue sample were flash-frozen in liquid nitrogen, lyophilised, ground and stored at -20°C until further physiological analysis.

Measurement of total anti-oxidant capacity

Anti-oxidant levels were first determined as 'total anti-oxidant capacity' (TAOC) using the FRAP (ferric reducing ability of plasma) assay (Benzie & Strain, 1996) and expressed as μmol $FeSO_4 7H_2O$ g^{-1} DW equivalents. This method involves the reduction of a ferric-tripyridyltriazine complex to its ferrous form, which is visible in the blue colour change in the presence of anti-oxidants. This colour change enables the quantification of the combined anti-oxidant capacity of the anti-oxidant molecules present in the exocarp tissue. Prior to measurement, a fresh FRAP reagent solution (300 mM/L sodium acetate buffer pH 3.6, 10 mM/L TPTZ in 40 mM/L HCl and 20 mM/L $FeCl_3.6H_2O$) was prepared in a ratio of 10:1:1. A 30 μL aliquot of the sample was mixed with 900 μL of the FRAP reagent solution and absorbance readings taken at 593 nm after 10 minutes. Results were expressed as mg $FeSO_4.7H_2O$ g^{-1} DW equivalents.

Determination of ascorbic acid concentration

The ascorbic acid (AsA) concentration was determined according to Böhm *et al*. (2006) with slight modifications. Briefly, 100 mg of exocarp sample was mixed with 5 mL 0.56 M metaphosphoric acid, vigorously shaken, centrifuged at 2988 x g for 5 minutes and the supernatant transferred into a volumetric flask. This procedure was repeated twice and the combined extracts made up to 20 mL using 0.56 M meta-phosphoric acid. Subsequently, 200 μL of this extract were mixed with 300 μL 0.3M trichloroacetic acid, centrifuged at 17212 x g for 10 min. Sub-samples of the supernatant (300 μL aliquots) were mixed with 100 μL 2, 4-dinitrophenylhydrazine reagent (0.013 M in 30% perchloric acid) and heated to 60°C for 1 hour and subsequently cooled in an ice bath for 5 minutes. Thereafter, 400 mL 15.75 M sulphuric acid was added to the sample and the absorbance read at 520 nm after 20 minutes. The ascorbic acid concentration was calculated by comparison of the values obtained with an L-ascorbic acid standard curve and expressed in mg AsA g^{-1} DW.

Determination of sugar concentrations

Sugars were analysed according to Tesfay *et al*. (2010) with slight modifications. A 10 mL aliquot of 80% (v v^{-1}) ethanol was added to 100 mg of mesocarp, heated in an 80°C water bath for 60 minutes and then incubated at 4°C for 24 hours. Samples were then filtered through glass wool and dried in a rotary evaporator (SpeedVac Concentrator, Savant, Farmingdale, NY). Dried samples were re-suspended in 2.0 mL ultra pure water, placed on a Vortex® for 1 minute, and filtered through a 0.45 μm nylon syringe filter. The analysis was performed using an isocratic HPLC system (LC – 20AT, Shimadzu Corporation, Kyoto, Japan) equipped with a refractive index detector (RID-10A, Shimadzu Corporation, Kyoto, Japan) and a Rezex RCM – Monosaccharide column (300 mm x 7.8 mm) (8 micron pore size; Phenomenex®, Torrance, CA, USA). Individual sugars were identified and quantified by comparing retention times of mannoheptulose and perseitol sugar standards (Glycoteam GmbH, Hamburg, Germany).

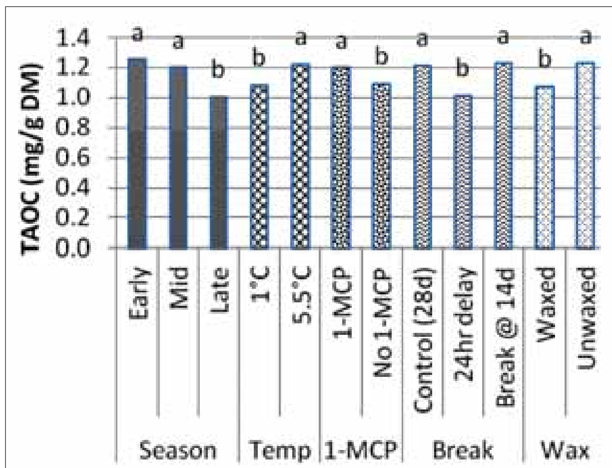

Figure 1. Effects of season maturity variances, storage temperature, 1-MCP, cold chain breaks and waxing on total anti-oxidant capacity after 28 days cold storage. Season and cold chain break $LSD_{0.05}$ = 0.116; Temperature, 1-MCP and waxing $LSD_{0.05}$ = 0.095.

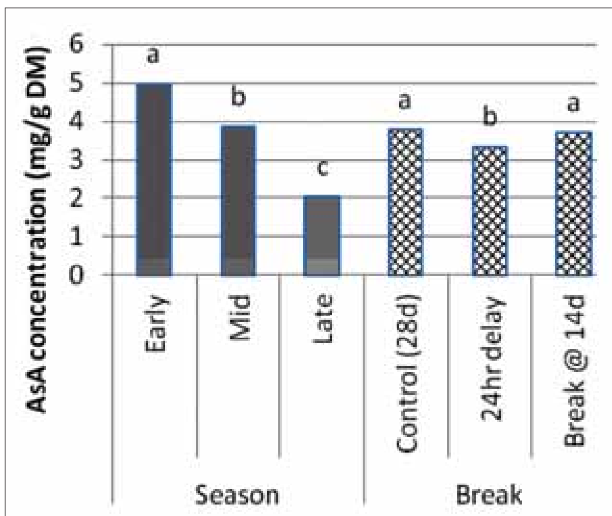

Figure 2. Effects of season maturity variances and cold chain breaks on ascorbic acid concentration after 28 days cold storage. $LSD_{0.05}$ = 0.271.

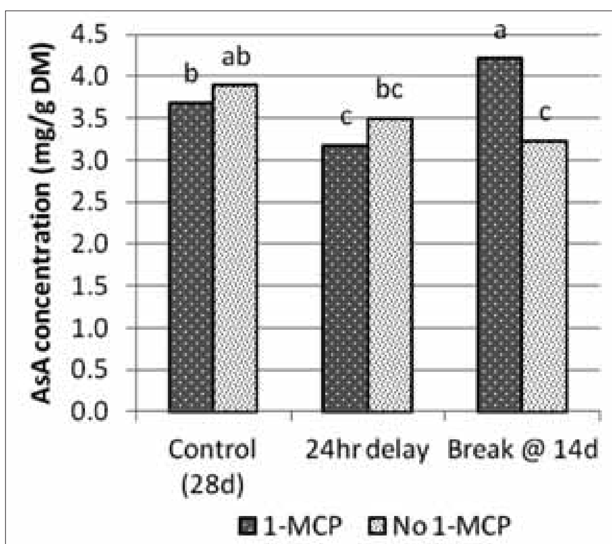

Figure 3. Effect of cold chain breaks and 1-MCP treatments on ascorbic acid concentration after 28 days cold storage. $LSD_{0.05}$ = 0.383.

Statistical analysis

A general analysis of variance between the treatment combinations of storage temperature, 1-MCP treatments, waxing and cold chain breaks was run using Genstat®14 (VSN International, Hemel Hempstead, UK), where the ANOVA, table of means, and LSD at $P < 0.05$ was computed to identify significantly different treatment combinations. Each treatment combination consisted of three samples, each constituting a single replication.

RESULTS AND DISCUSSION

Anti-oxidants in the exocarp

The TAOC and AsA concentration in the exocarp showed similar trends through the season, supporting the correlation between these two measurements in the seed and exocarp found by Tesfay *et al*. (2010). All main effects had a significant effect on TAOC of fruit stored for 28 days (Figure 1). Season maturity and cold chain breaks were significant for AsA (Figure 2). As found by Tesfay (2009) and Landahl *et al*. (2009), early season fruit maintained the highest anti-oxidant concentration, and as the season progresses the concentration decreased. The reason for this decline may be attributed to more than just storage conditions, but pre-harvest conditions as well. The anti-oxidant levels in the fruit tissues might have declined due to high levels of reactive oxygen species (ROS). Either fruit may have been exposed to stressful conditions (temperature, water and light stress) (Mittler, 2002), or the post-harvest stress may have resulted in an increased usage of anti-oxidants scavenging the abundant ROS. Once there are ROS present, anti-oxidants will be used in order to counteract the highly reactive nature and destructive action of the ROS.

The ultra-low storage temperature of 1°C resulted in a significant decrease in TAOC compared with 5.5°C storage. This is attributed to the influence of chilling stress caused by the ultra-low temperature. If ROS were not scavenged by the anti-oxidants present, significant chilling injury would have resulted. This result links to the chilling injury levels associated with the 1°C treatment which resulted in a higher level of chilling injury.

The use of 1-MCP significantly reduced the decline in the TAOC during storage. It is suggested that 1-MCP acted by reducing the metabolic activity during storage through inhibiting the ripening process (Lemmer & Kruger, 2003) and, thus, production of free radicals.

The influence of cold chain breaks/delay showed that a break within storage (*i.e*. break at day 14) did not influence the TAOC or AsA significantly, but the 24 hour delay significantly reduced the TAOC and AsA. It is possible that such fruit were exposed to a high amount of water loss which could have resulted in a stress induced increase in ROS in the exocarp, subsequently reducing exocarp anti-oxidant levels due to use as a protectant. The interaction between cold chain breaks and 1-MCP treatments was found

to be significant of AsA in the exocarp and indicated that for the break at day 14 there was a significant difference experienced between the two 1-MCP treatments (Figure 3). It appears that the use of 1-MCP is negating the effect of a cold chain break/delay with respect to the consumption of AsA. The trend does not correlate directly to the results found for the physical data (Kok *et al*., 2011) in terms of influencing days to ripening or fruit softening percentage, but indicates that there is an influence on a physiological level when a break occurs within cold storage and 1-MCP is not used. No previous work has investigated this issue, therefore it is suggested that with no 1-MCP the effects of partial fruit ripening on the production of free radicals, which occurred during fruit warming period of the cold chain break, resulted in the ascorbic acid reserves being used as metabolic activity is increased briefly, which is not the case if some fruit ripening does not occur, as may be the case with the use of 1-MCP.

Waxed fruit incurred a higher chilling injury than the unwaxed fruit and therefore it is suggested that the wax creates some form of stress which results in anti-oxidants being used and thus the lower level of TAOC post-storage.

Similar results to the 28 days storage were found after 56 day storage with respect to seasonal maturity and waxing treatments for TAOC (Figure 4) as well as AsA (Figure 5). The 1-MCP treatment did not have significantly different anti-oxidant levels, but the trend was similar to 28 day storage, confirming the breakdown of mannoheptulose (Blakey *et al*., In press) in storage as well as reducing levels of the storage sugar perseitol.

There was a significant interaction between seasonal maturity and storage temperature (Figure 6). As the season progressed, TAOC of the exocarp decreased significantly in the 5.5°C storage, whereas the 1°C storage showed that early season fruit had a reduced TAOC compared with the mid as well as the late season fruit, where the latter had the highest value for the 1°C storage, yet not significantly higher. These results correlate with chilling susceptibility where, as the season progressed, the chilling injury increased in 5.5°C storage, while in 1°C storage early season fruit experienced the highest chilling injury, which decreased as the season progressed. This again indicates that the severity of chilling injury is correlated to the TAOC of the exocarp. A further possible reason for the TAOC decreasing in the exocarp of fruit in 5.5°C storage as the season progresses, is the higher metabolic activity associated with the 5.5°C treatment and more mature fruit, as shown by the degree to which it softened in storage as well as the rate of ripening after storage.

Sugar analysis – mannoheptulose and perseitol

The results for perseitol are similar to those found for mannoheptulose. Perseitol acts as the storage product of mannoheptulose, and can be easily converted to mannoheptulose (Tesfay, 2009). Only the use of 1-MCP had a significant influence on fruit C7 sugar

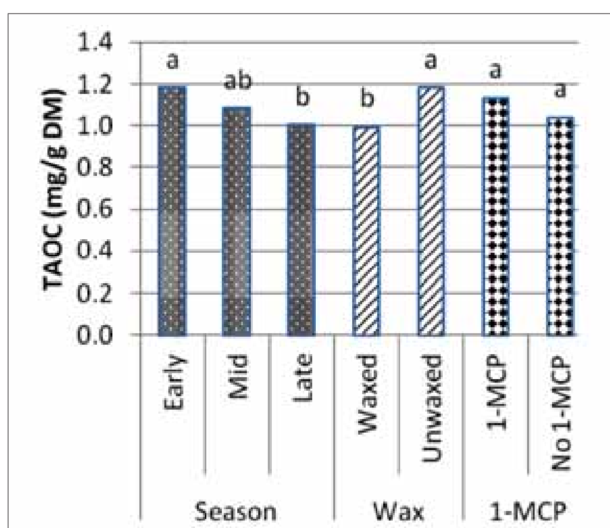

Figure 4. Effects of season maturity variances, waxing and 1-MCP on total anti-oxidant capacity after 56 day extended cold storage. Season $LSD_{0.05}$ = 0.142; 1-MCP and waxing $LSD_{0.05}$ = 0.116.

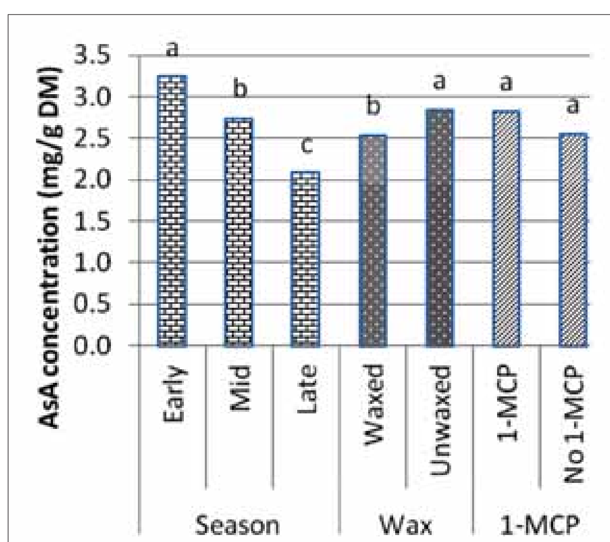

Figure 5. Effects of season maturity variances, waxing and 1-MCP treatments on ascorbic acid concentration after 56 days extended cold storage. Season $LSD_{0.05}$ = 0.365; Wax and 1-MCP $LSD_{0.05}$ = 0.298.

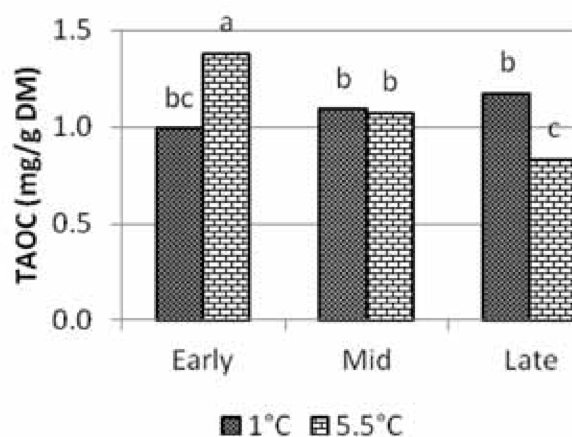

Figure 6. Effect of season maturity variances and storage temperature on total anti-oxidant capacity after 56 day extended cold storage. $LSD_{0.05}$ = 0.201.

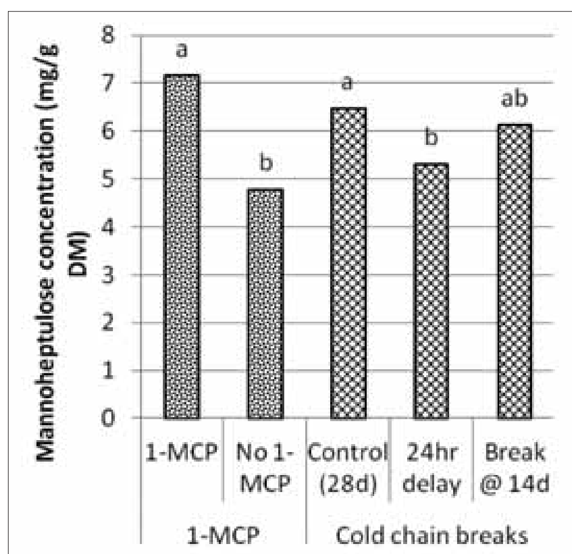

Figure 7. Effects of 1-MCP treatments and cold chain breaks on mannoheptulose concentration after 28 days cold storage. 1-MCP $LSD_{0.05}$ = 0.834; Cold chain breaks $LSD_{0.05}$ = 1.021.

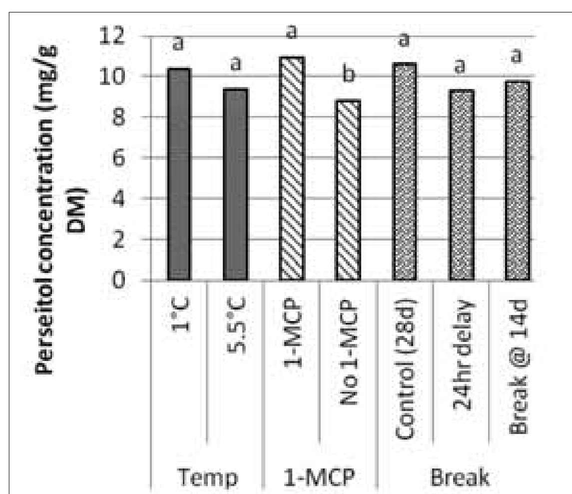

Figure 8. Effects of storage temperature, 1-MCP treatments and cold chain breaks on perseitol concentration after 28 days cold storage. 1-MCP and temperature $LSD_{0.05}$ = 1.154; Cold chain breaks $LSD_{0.05}$ = 1.413.

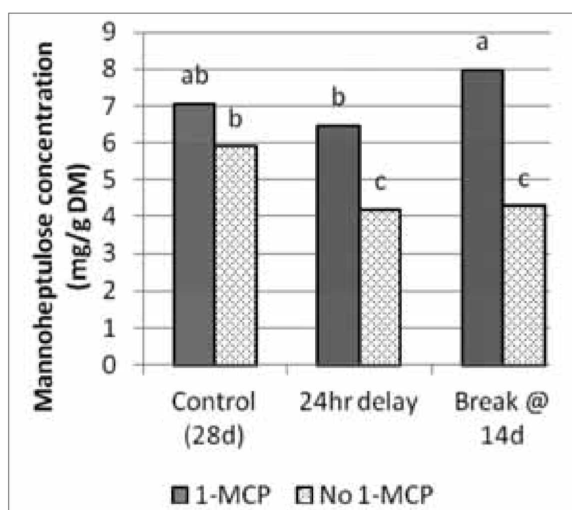

Figure 9. Effect of cold chain breaks and 1-MCP treatments on mannoheptulose concentration after 28 days cold storage. $LSD_{0.05}$ = 1.444.

levels (Figures 7 and 8). Application of 1-MCP reduced the consumption of mannoheptulose and perseitol, as expected, seemingly due to the reduced effect of ethylene and the subsequent reduction in the rate of metabolic activity resulting from the suppression of ripening. Although Blakey *et al*. (2010) found that storage at 1°C significantly reduced the consumption of C7 sugars, this could not be demonstrated.

The effect of cold chain breaks/delay on C7 sugars was not found to be significant, however, the trend shown (Figure 7 and Figure 8) indicated that the 24 hour delay before cold storage leads to an increased consumption of C7 sugars, as one would expect due to the greater time at higher temperature with therefore higher respiration. It was also observed that the break at day 14 resulted in a decreased level of mannoheptulose when compared with the control treatment. A cold chain break triggers metabolic activity of fruit as it warms up, hence leading to the consumption of sugar reserves. Blakey and Bower (2009) found that a break in the cold chain resulted in a significant amount of softening of the fruit, which would in turn result in the increased consumption of sugars.

1-MCP may negate the effect of cold chain breaks with respect to mannoheptulose sugar consumption (Figure 9). Without the use of 1-MCP the increase in metabolic activity, due to the stresses and triggers involved with cold chain breaks, probably results in a decrease in mannoheptulose reserves.

For the extended storage it was found that only 1-MCP effected mesocarp mannoheptulose levels. A similar trend was shown for perseitol, however not significant. The use of 1-MCP appears to be a useful treatment in terms of maintaining mannoheptulose levels. The non-significant trend for waxing may be linked to waxed fruit incurring a higher chilling injury, and as a measure to reduce the damage, sugar reserves are consumed.

CONCLUSION

Maintaining high levels of anti-oxidants in the exocarp plays a vital role in countering cold stress, particularly due to the likely stress involved in ultra-low temperature storage and the prevention of chilling injury under these conditions. The overall occurrence of external and internal quality defects was low in this experiment. In general, however, the more stressful the storage, whether due to temperature or breaks, the higher the consumption of anti-oxidants, which will likely compromise potential fruit quality. However, 1-MCP does help to mitigate the effects. While for 28 days storage, a protocol of 1°C may be best even if cold chain breaks occur, this will not be sufficient to ensure quality if storage of 56 days is considered. In this case, a combination of 1°C and 1-MCP is needed. If this is done, then 56 days storage is potentially achievable in a commercial context. Further research into the effects of cold chain breaks, 1-MCP applications and temperatures on anti-oxidant levels in both the exocarp and mesocarp is required.

Particular focus on the continuous measurement of AsA and C7 sugars throughout the storage as well as the ripening period is required to elucidate how these anti-oxidants are used by the fruit to counter ROS following stressful cold storage.

REFERENCES

BENZIE, I.F.F. & STRAIN, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power'. *Analytical Biochemistry,* 239: 70-76.

BERGH, B.O. 1992. Avocado and human nutrition. I. Some human health aspect of the avocado. *Proceedings of the Second World Avocado Congress* 1: 25-35. University of California, Riverside, CA, USA.

BLAKEY, R.J. & BOWER, J.P. 2009. The importance of maintaining the cold chain for avocado ripening quality. *South African Avocado Growers' Association Yearbook,* 32: 48-52.

BLAKEY, R.J., BOWER, J.P. & BERTLING, I. 2010. Post-harvest avocado physiology. *South African Avocado Growers' Association Yearbook,* 33: 56-60.

BLAKEY, R.J., TESFAY, S.Z., MATHABA, N., BERTLING, I. & BOWER, J.P. In press. Some initial changes in avocado physiology due to ethephon. *International journal of postharvest technology and innovation.*

BÖHM, V., KÜHNERT, S., ROHM, H. & SCHOLZE, G. 2006. Improving the nutritional quality of microwave-vacuum dried strawberries: A preliminary study. *Food Science Technology International,* 12: 67-75.

EAKS, I.L. 1990. Change in the fatty acid composition of avocado fruit during ontogeny, cold storage and ripening. *Acta Horticulturae,* 269: 141-152.

HUMAN, T.P. 1987. Oil as a by-product of the avocado. *South African Avocado Growers' Association Yearbook,* 10: 159-162.

KOK, R.D., BOWER, J.P. & BERTLING, I. 2011. Ultra-low temperature shipping and cold chain management of 'Hass' avocados: Investigation into reducing shipping costs. *South African Avocado Growers' Association Yearbook,* 34: 13-19.

LANDAHL, S., MEYER, M.D. & TERRY, L.A. 2009. Spatial and Temporal Analysis of Textural and Biochemical Changes of Imported Avocado cv. Hass during Fruit Ripening. *Journal of Agricultural Food Chemistry,* 57: 7039-7047.

LIU, X., ROBINSON, P.W., MADORE, M.A., WITNEY, G.W. & ARPAIA, M.L. 1999. 'Hass' avocado carbohydrate fluctuations. II. Fruit growth and ripening. *Journal of the American Society for Horticultural Science,* 124: 676-681.

LEMMER, D. & KRUGER, F.J. 2003. Laboratory based evaluation of 1-Methyl cyclopropene (1-MCP): With five South African commercial export avocado cultivars. Proceedings V World Avocado Congress. Pp. 611-616.

MITTLER, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science,* 7 (9): 405-410.

RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M. & RICE-VANS, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine,* 26:1231-1237.

SLATER, G.C., SHANKMAN, S., SHEPHERD, J.S. & ALFIN-SLATER, R.B. 1975. Seasonal variation in the composition of California avocados. *Journal of Agricultural and Food Chemistry,* 23: 468-474.

SPALDING, D.H. 1976. Storage of avocados, Pp. 109-113. In: J.W. Sauls, R.L. Phillip and L.K. Jackson (eds.). Proc. 1st Intl. tropical fruit short course: The avocado. University of Florida. Press, Gainesville.

TERASAWA, N., SAKAKIBARA, M. & MASATSUNE, M. 2006. Antioxidative activity of avocado epicarp hot water extract. *Food Science and Technology Research,* 12: 55-58.

TESFAY, S.Z. 2009. Special carbohydrates of avocado – their function as 'sources of energy' and 'antioxidants'. Horticultural Science. School of Agriculture Sciences and Agribusiness. Pietermaritzburg, University of KwaZulu-Natal, PhD.

TESFAY, S.Z., BERTLING, I. & BOWER, J.P. 2010. Anti-oxidant levels in various tissues during the maturation of 'Hass' avocado (*Persea americana* Mill.). *Journal of Horticultural Science & Biotechnology,* 85: 106-112.

TESFAY, S.Z., BERTLING, I. & BOWER, J.P. 2011. *D*-mannoheptulose and perseitol in 'Hass' avocados: Metabolism in seed and mesocarp tissue. *South African Journal of Botany*. doi: 10.1016/j.sajb.2011.10.006.

WOLSTENHOLME, B.N. 1990. Avocados and health issues. *South African Avocado Growers' Association Yearbook,* 10: 1-2.