Effects of low temperature storage and cold chain breaks on anti-oxidants and C7 sugars in 'Fuerte' avocados from South Africa

A Lütge, I Bertling and JP Bower

Horticultural Science, School of Agricultural Science and Agribusiness
University of KwaZulu-Natal, Pietermaritzburg, South Africa
Private Bag X01, Scottsville 3209, South Africa
E-mail: andrel@westfaliam.co.za

ABSTRACT

To ensure hard fruit on arrival at European markets, South African exporters may make use of an average storage temperature of 5.5°C during the season, as well as other expensive technologies, such as controlled atmosphere or 1-MCP. Previous work has shown that various post-harvest quality issues can be resolved by lowering the storage temperature, using waxes and other water loss preventing treatments, and by avoiding cold chain breaks (Lütge *et al*., 2010). This study investigates the effect these treatments have on anti-oxidant and C7 sugar levels in 'Fuerte' avocados. 'Fuerte' avocados were harvested at three dates during the season, with moisture contents of 74% (early), 68% (mid) and 63% (late). Fruit were subjected to treatments of temperature (2°C and 5.5°C), 1-MCP (treated and untreated), waxing (waxed and non-waxed) and cold chain breaks (no break, 24 hour delay and break at 14 days) for 28 days as well as 56 days. Anti-oxidant concentrations in the exocarp, as well as mannoheptulose and perseitol concentrations in the mesocarp, were highest in early-season fruit and declined significantly as the season progressed. Results suggest a link between anti-oxidant concentrations in the exocarp and the rate of metabolic activity during cold storage. Although trends were visible for different treatments, the level of C7 sugars found in the mesocarp did not conclusively indicate differences between storage treatments, storage duration or breaks in the cold chain. Results indicate that post-harvest stress conditions, such as lengthy cold storage times, high fruit moisture loss, cold chain breaks and ripening during cold storage, generally cause a reduction in anti-oxidants of the fruit. Thus, treatments which reduce these stresses and prevent ripening during cold storage, may maintain the levels of anti-oxidants and C7 sugars in the fruit more effectively than a higher storage temperature and possibly maintain a higher final fruit quality.

Key words: low temperature shipping, 1-MCP, wax, total anti-oxidant capacity, mannoheptulose, perseitol, ascorbic acid

INTRODUCTION

Numerous anti-oxidant systems exist in avocado fruit and in varying levels, depending on the stage of fruit development (Tesfay *et al*., 2010a). Anti-oxidants are produced in cells to protect cellular structures against reactive and damaging compounds, particularly reactive oxygen species (ROS), which are formed during oxidative stress (Fang *et al.*, 2002). Anti-oxidants function to scavenge ROS, and maintain a balance between anti-oxidants and ROS so that normal cell metabolism can proceed; however, under stress conditions, this balance can be easily disturbed and result in an accumulation of ROS, causing membrane damage and ultimately visible symptoms of chilling injury.

Ascorbic acid (AsA) is an important 'broad spectrum' anti-oxidant found in avocados. It helps regenerate tocopherol, another important anti-oxidant compound that limits membrane damage (Tesfay *et al*., 2010a). Tesfay *et al*. (2010a) reported that the total anti-oxidant capacity (TAOC) and AsA concentration was highest in the exocarp and seed of 'Hass' avocados and lowest in the mesocarp, thus these parameters were chosen as measurements to depict the anti-oxidant levels in the exocarp. In post-harvest avocado physiology, C7 sugars are believed to perform various important roles. Mannoheptulose has been proposed as a possible source of energy (Liu *et al*., 1999), the main anti-oxidant in the mesocarp (Tesfay *et al*., 2010a) and a major source of carbon (Blakey *et al*., 2010). Perseitol functions as a storage carbohydrate and as an important anti-oxidant in the mesocarp of avocados, albeit with a significantly lower ability to scavenge ROS than man-

noheptulose (Tesfay *et al*., 2010a). Although the exact roles of these sugars have not been elucidated, there are indications that the pool of C7 sugars in the mesocarp of avocados plays an important role in the post-harvest quality of avocados (Bertling *et al*., 2007; Liu *et al*., 1999; Tesfay *et al*., 2010a).

The altering of storage temperature and duration may lead to changes in carbohydrate storage and usage and, ultimately, affect final fruit quality. However, information on the effect of different storage treatments and temperatures as well as cold chain breaks and extended storage periods on the anti-oxidant levels in the exocarp of avocados is limited, particularly for 'Fuerte' avocados. Thus the objective of the study was to determine the effects of different storage treatments, cold chain breaks and storage period on the anti-oxidant concentrations in the exocarp and of C7 sugars in the mesocarp.

MATERIALS AND METHODS

Fruit material

Export grade 'Fuerte' avocado fruit were obtained from Wartburg (29°27'S, 30°40'E) in KwaZulu-Natal. The fruit were collected on 25/06/2010 (early season, 74% moisture), 12/08/2010 (mid season, 68% moisture) and 16/09/2010 (late season, 63% moisture). The fruit were harvested from the same block and were 'count 16' in size. The 1-MCP treatment was applied at the registered rate (500 ppb) for 16 hours in cold storage at 5.5°C, whilst the untreated fruit were stored under regular atmosphere (RA) at the same temperature for the same period. Fruit were stored under RA for 28 and 56 days, at air delivery temperatures of either 2°C (±1°C) or 5.5°C (±1°C).

Treatments

Treatments of temperature (2°C or 5.5°C), 1-MCP (treated or untreated) and waxing (waxed or non-waxed) over three harvest dates were applied, with 10 fruit for each treatment combination, each fruit constituting a single replication. Cold chain breaks included a 24 hour delay in cooling and a break after 14 days of cold storage, where fruit were placed in the laboratory for eight hours at 20±2°C and then returned to cold storage.

Post-storage sampling from fruit tissue

Upon removal from storage, a core sample of the mesocarp and an exocarp sample of three randomly

Table 1. Seasonal changes in total anti-oxidant capacity (TAOC) and ascorbic acid (AsA) concentration in the exocarp, and mannoheptulose and perseitol concentrations in the mesocarp of 'Fuerte' avocado fruit upon removal from cold storage. Fruit were stored for 28 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. *

Treatment				TAOC (mg $FeSO_4.7H_2O$ g^{-1} DW)	AsA (mg g^{-1} DW)	Mannoheptulose (mg g^{-1} DW)	Perseitol (mg g^{-1} DW)
Early	2°C	1-MCP	waxed	1.38 bc	2.86 bcde	8.38 a	6.03 cde
			Non-waxed	1.73 abc	3.07 bcde	7.57 a	5.28 cde
		No 1-MCP	waxed	1.61 abc	3.57 ab	5.30 a	4.54 de
			Non-waxed	1.78 ab	3.83 a	5.13 a	13.75 ab
	5.5°C	1-MCP	waxed	1.89 a	3.16 abcd	7.30 a	5.34 cde
			Non-waxed	1.31 c	3.19 abcd	5.48 a	5.57 cde
		No 1-MCP	waxed	1.35 bc	3.30 abc	7.00 a	7.01 bcde
			Non-waxed	1.51 abc	2.54 defg	4.49 a	8.66 abcde
Mid	2°C	1-MCP	waxed	0.71 d	1.57 ijk	6.16 a	5.31 cde
			Non-waxed	0.53 de	2.38 efgh	5.42 a	2.11 e
		No 1-MCP	waxed	0.48 de	1.85 ghijk	5.26 a	4.64 de
			Non-waxed	0.72 d	1.36 ijkl	7.51 a	9.75 abcd
	5.5°C	1-MCP	waxed	0.74 d	1.88 ghij	4.52 a	6.17 cde
			Non-waxed	0.67 d	2.70 cdef	4.92 a	4.92 cde
		No 1-MCP	waxed	0.72 d	1.76 hijk	6.63 a	5.12 cde
			Non-waxed	0.28 de	1.10 kl	4.33 a	12.10 abc
Late	2°C	1-MCP	waxed	0.60 d	1.41 ijkl	5.63 a	5.48 cde
			Non-waxed	0.46 de	1.21 ijkl	4.84 a	9.70 abcd
		No 1-MCP	waxed	0.54 de	1.20 ijkl	5.76 a	7.44 abcde
			Non-waxed	0.50 de	1.29 ijkl	4.55 a	9.21 abcde
	5.5°C	1-MCP	waxed	0.52 de	1.12 jkl	6.66 a	14.47 a
			Non-waxed	0.68 d	1.96 fghi	4.77 a	6.24 cde
		No 1-MCP	waxed	0.38 de	1.16 jkl	4.59 a	6.49 cde
			Non-waxed	0.12 e	0.76 l	4.03 a	10.60 abcd
LSD				0.4638	0.7569	4.583	7.222

* Each point represents the mean of 3 fruit.
Different letters indicate significant differences within each column.

selected fruit for each treatment combination were collected according to Blakey *et al*. (2010). The samples were flash-frozen in liquid nitrogen, freeze-dried, ground and subsequently stored at -20°C until further physiological analysis.

Anti-oxidant analysis

Anti-oxidant levels were determined as 'total anti-oxidant capacity' (TAOC) using the FRAP assay as described by Benzie and Strain (1996) with slight modifications according to Tesfay *et al*. (2011). Results were expressed as mg $FeSO_4.7H_2O$ g^{-1} DW equivalents.

AsA analysis

Ascorbic acid concentrations were determined according to Böhm *et al.* (2006), using the colour reaction with 2,4-dinitrophenylhydrazine (DNPH). Absorbance readings from tissue extracts at 520 nm were compared with values obtained from an L-ascorbic acid standard curve, and expressed in mg AsA/g DW.

Sugar analysis

Sugar concentrations were measured according to Liu *et al*. (2002). An isocratic HPLC system was used and individual sugar concentrations were determined by comparison with mannoheptulose and perseitol sugar standards.

Statistical analysis

Statistical analyses were conducted using GenStat® version 12.1 and analysed in the form of a factorial design. For the physiological analyses, each treatment combination consisted of three samples, each constituting a single replication.

RESULTS AND DISCUSSION

28 day Storage

Anti-oxidants in the exocarp

Anti-oxidants were found to be highest early in the season, when fruit are most susceptible to ECI, and decreased as the season progressed (Table 1). Results suggest that the TAOC of the exocarp increases as the post-harvest stress is increased, to counter the dramatic increase in ROS production under stress conditions (Mittler, 2002). Storage at 5.5°C had a tendency to maintain TAOC and AsA concentrations less effectively than 2°C throughout the storage period, particularly when fruit were not treated with 1-MCP (Figure 1a and 2b). Treatment of fruit with 1-MCP resulted in a significantly higher concentration of AsA, particularly in the mid and late season fruit (Figure 2a). Waxing had a tendency to maintain higher anti-oxidant levels through the storage period as early season, non-waxed fruit stored at 2°C resulted in a slightly higher TAOC and higher levels of AsA than waxed fruit at this temperature (Table 1), suggesting that the increased water loss associated with non-waxed fruit resulted in an increased stress level, therefore the enhanced production of anti-oxidants. Non-waxed fruit stored at 5.5°C and not treated with 1-MCP (P = 0.001) had the lowest concentrations of AsA in all seasons (Table 1).

It would be expected that higher levels of stress (*i.e*., lower storage temperature) result in increased production of ROS, therefore a greater usage of anti-oxidants to counteract these species. However, it appears that the anti-oxidant concentrations did not decline to the same degree in the lower as high temperature storage. This suggests that the lower storage temperature did not result in an increase in ROS levels.

A further explanation could be that the usage of anti-oxidants is reduced at this lower temperature, resulting in a significantly higher TAOC (Figure 1a) and AsA concentration (Figure 2b) than at a storage temperature of 5.5°C without the use of 1-MCP and a storage temperature of 5.5°C, respectively. This could be explained by the reduced metabolic activity of the fruit under these treatments, which possibly reduces the use of anti-oxidants and enzyme activity in cold storage. Such treatment combinations favoured fruit softening during storage and include a higher storage temperature without the use of 1-MCP (Figure 1a and 2b), mid and late season fruit not treated with 1-MCP (Figure 2a) and the cold chain breaks when fruit were stored at 5.5°C (Figure 1b and 2d). Conversely, fruit which received treatments that minimised metabolic

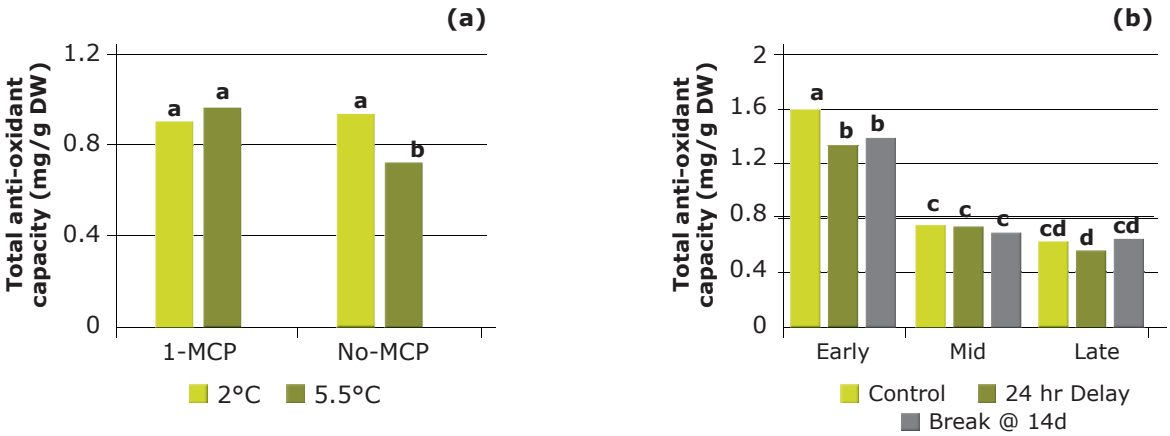

Figure 1a and b. Treatment effects on mean TAOC (mg $FeSO_4.7H_2O$ /g DW) in the exocarp of 'Fuerte' avocados following 28 days of cold storage. Different letters indicate significant differences between treatments (P ≤ 0.05). LSD = 0.1893 (A) and 0.1423 (B).

activity during storage, tended to maintain higher levels of TAOC and AsA, however, upon removal from storage and subsequent increase in metabolic activity of the fruit, these anti-oxidants possibly decrease in concentration as they attempt to scavenge the harmful compounds caused during stress.

Sugars in the mesocarp

Mannoheptulose (Figure 3) and perseitol concentrations after 28 days of cold storage had a tendency to decrease in the mesocarp tissue as the season progressed, as reported by Liu *et al*. (1999) in 'Hass' avocados. Results suggest that reducing the storage temperature from 5.5°C to 2°C allowed for better preservation of the limited pool of C7 sugars found in the mesocarp, and that treatments which delayed ripening during storage more effectively, tended to reduce the consumption of C7 sugars during storage (Figure 3). This preservation of energy reserves and anti-oxidant capacity of the mesocarp may be one of the primary reasons for the increase in shelf-life noted under these treatments in previous studies (Lütge *et al*., 2010).

Liu *et al*. (1999) as well as Landahl *et al.* (2009) also reported that mannoheptulose concentrations decreased during cold storage. Liu *et al.* (1999) proposed that this vital sugar is used as a carbon energy source for respiration during cold storage. Tesfay *et al*. (2010b) proposed that perseitol is also the main storage carbohydrate which is easily converted to mannoheptulose, a compound identified as an energy source in the mesocarp, and importantly, the

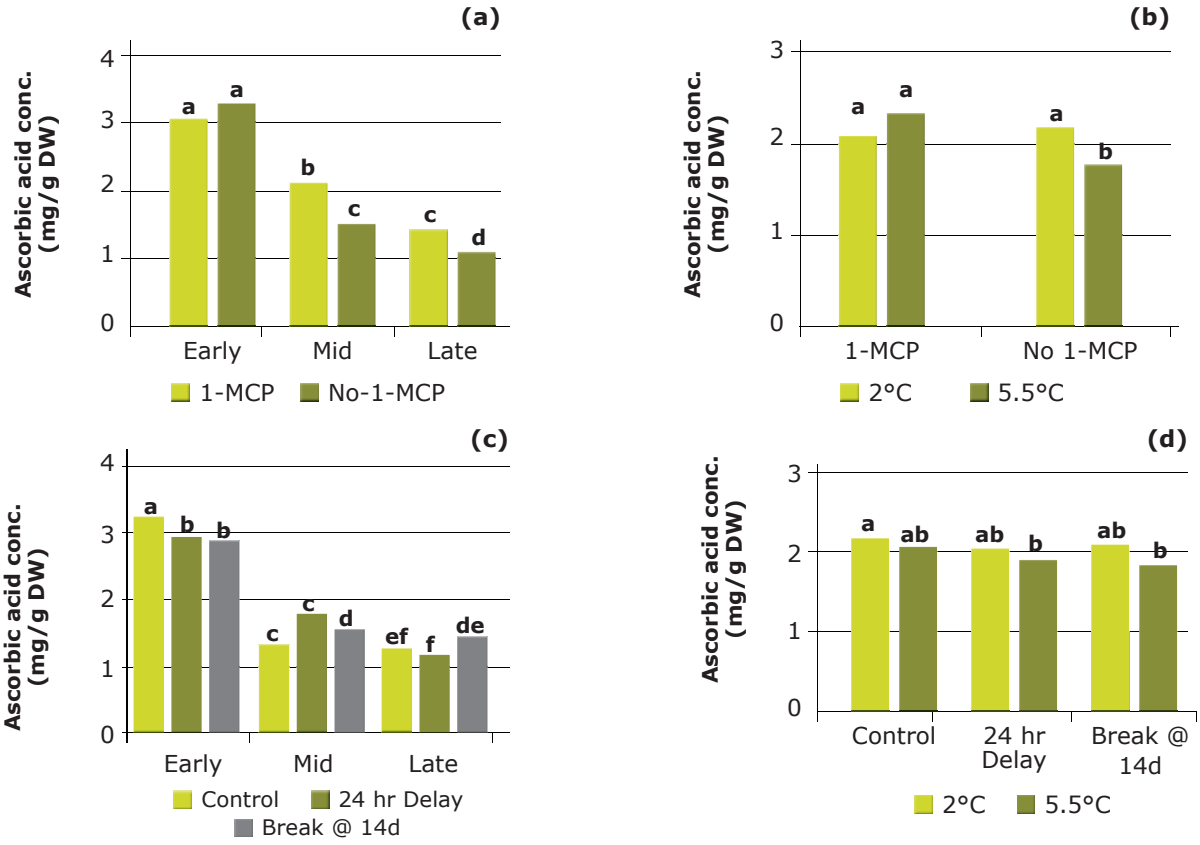

Figure 2a-d. Treatment effects on mean ascorbic acid concentration (mg/g DW) in the exocarp of 'Fuerte' avocados following 28 days cold storage. Different letters indicate significant differences between treatments (P ≤ 0.05). LSD = 0.3784 (a), 0.309 (b), 0.2924 (c) and 0.2387 (d).

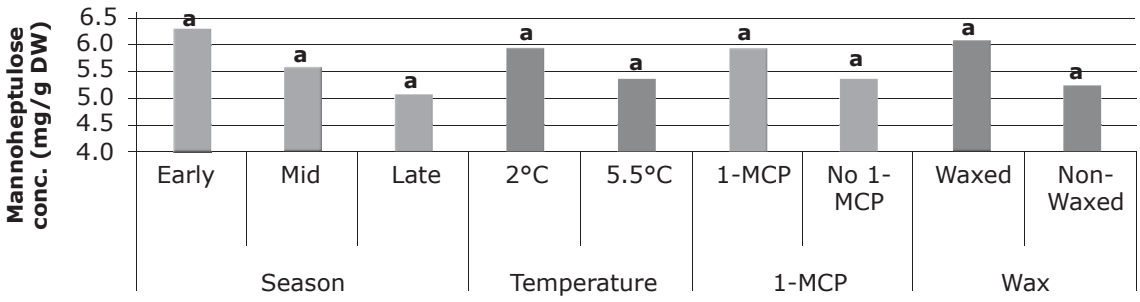

Figure 3. Mannoheptulose conc. (mg/g DW) of 'Fuerte' avocado mesocarp following 28 days cold storage. Different letters indicate significant differences for each treatment (P ≤ 0.05). LSD = 1.62 (season), 1.323 (temperature), 1. 323 (1-MCP) and 1. 323 (wax).

main transport sugar (Tesfay *et al.,* 2010b). Tesfay *et al.* (2010a) reported that the concentration of mannoheptulose in the mesocarp of 'Hass' avocados was higher than perseitol at the time of harvest, as was found in this study after one day of cold storage at 5.5°C (data not shown). The mannoheptulose concentration detected after removal from cold storage was similar, and in some cases lower than that of perseitol, suggesting that mannoheptulose, being the main transport sugar, may be utilised more easily than perseitol during cold storage. This is supported by the findings of Liu *et al*. (1999), who reported that perseitol concentrations did not decrease during cold storage but did decrease significantly during ripening, thus, as more energy is required during ripening, it appears that mannoheptulose is used up during storage and then perseitol is utilised when mannoheptulose has been depleted. Thus, by the time measurements were taken, mannoheptulose concentrations were low and had a minimal effect on fruit quality.

Cold chain breaks and delays

Anti-oxidants in the exocarp

The two cold chain disruptions significant reduced the levels TAOC (Figure 1b) and the concentration of AsA (Figure 2c) in the exocarp, compared with the control, but only early in the season. Treatment effects on the delay in cooling and cold chain break were inconsistent through the season and, due to the complexity of the treatment structure and the variation in TAOC between individual fruit, no significant treatment differences could be identified (data not shown). However, lowering the storage temperature had a tendency to minimise the reduction in AsA concentration following breaks in the cold chain, compared with a storage temperature of 5.5°C (Figure 2d), confirming the effect of a lower storage temperature on minimising the softening effects of a cold chain break (Lütge *et al*., 2010). The amount of AsA decline following a break during storage, was significantly reduced by waxing in early and mid season-fruit (data not shown).

Sugars in the mesocarp

The cold chain break during storage resulted in a significant reduction of mannoheptulose concentration; however, no treatments had a significant effect on either C7 sugars after such a break (data not shown).

56 day storage (data not shown)

Anti-oxidants in the exocarp

The TAOC after 56 days of cold storage was generally lower than after 28 days, shown in the decreased TAOC from 0.884 mg $FeSO_4.7H_2O$ g^{-1} DW equivalents to 0.835 mg $FeSO_4.7H_2O$ g^{-1} DW equivalents after 56 day storage.

Similar to 28 day cold storage, early season fruit had a significantly higher TAOC than mid and late season fruit after 56 days of cold storage. Lowering the storage temperature, as well as waxing, had a tendency to lower the TAOC compared with higher temperature storage and non-waxed fruit. The use of 1-MCP resulted in a significantly lower TAOC early in the season, particularly of non-waxed fruit.

The concentration of AsA remaining in the exocarp of fruit after 56 days of cold storage was generally lower than after 28 days, shown in the significant decrease in the grand mean AsA concentration from 2.093 mg/ml after 28 days to 1.762 mg/ml after 56 days. As found following 28 day storage, early season fruit had a significantly higher concentration of AsA than mid and late season fruit, at either storage temperature. Early season fruit stored at 5.5°C had a significantly lower rind concentration of AsA than those stored at 2°C.

Sugars in the mesocarp

Similar trends to those found after 28 days of cold storage were noticed for mannoheptulose and perseitol concentrations after 56 days, although levels of these sugars were generally lower after 56 than 28 days of storage.

CONCLUSION

Maintaining high levels of anti-oxidants in the exocarp will be vital in countering cold stress, particularly due to the likely stress involved in cold disinfestation treatments and the prevention of ECI under these conditions. Reducing storage temperatures, 1-MCP use and waxing, all tended to maintain higher levels of anti-oxidants in the exocarp. The overall occurrence of external damage and internal quality defects were fairly low during this experiment, thus no conclusive results were obtained with respect to the ability of anti-oxidants in the exocarp and sugars in the mesocarp to reduce or prevent chilling damage and internal disorders, respectively. The sugar results were not as conclusive as anticipated, and results do not indicate that sugars in the mesocarp after storage correlate to treatment effects on fruit quality. Further research into the effects of different storage treatments and temperatures on anti-oxidant levels in both, the exocarp and mesocarp, are 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 the damaging compounds following cold exposure.

ACKNOWLEDGEMENTS

The authors would like to thank SAAGA and The Hans Merensky Foundation for financial assistance.

REFERENCES

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

BERTLING, I., TESFAY, S.Z. & BOWER, J.P. 2007. Antioxidants in 'Hass' avocado. *South African Avocado Growers' Association Yearbook,* 30: 17-19.

BÖHM, V., KHUNERT, 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.
BLAKEY, R.J., BOWER, J.P. & BERTLING, I. 2010. Post-harvest avocado physiology. *South African Avocado Growers' Association Yearbook*, 33: 56-60.
FANG, Y.Z., YANG, S. & WU, G.Y. 2002. Free radicals, antioxidants and nutrition. *Nutrition*, 18: 872-879.
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.
LIU, X., SIEVERT, J., ARPAIA, M.L. & MADORE, M.A. 2002. Postulated physiological role of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. *Journal of the American Society for Horticultural Science*, 127: 108-114.
LÜTGE, A., BOWER, J.P. & BERTLING, I. 2010. Low Temperature Shipping and Cold Chain Management of 'Fuerte' Avocados: An opportunity to reduce shipping costs. *South African Avocado Growers' Association Yearbook*, 33: 39-43.
MITTLER, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7(9): 405-410.
TESFAY, S.Z., BERTLING, I. & BOWER, J.P. 2010a. 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. 2010b. D-mannoheptulose and perseitol in 'Hass' avocado: metabolism in seed and mesocarp tissue. *South African Journal of Botany*, (2011). Doi: 10.1016/j.sajb.2011.10.006.