Antioxidants in ‘Hass’ avocado

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ABSTRACT

The potential involvement of C7 sugars as antioxidants in various avocado tissues was tested. Total antioxidant activity was found to be similar in leaf, exocarp and seed tissue; however, the concentration of total antioxidants in mesocarp tissue was lower than in other tissues at all three sampling dates. The various tissues examined had different predominant antioxidant systems; while leaf tissue contained high concentrations of anthocyanins, ascorbic acid was found to be the main antioxidant in the seed as well as the rind tissue. In mesocarp tissue, however, C7 sugars, and particularly mannoheptulose, played the major antioxidant role. As levels of both C7 sugars declined significantly as harvest maturity was approached, the reduction in C7 sugars could be related to deterioration in post-harvest quality. Therefore, management practises should be established to increase the concentration of these sugars in the mesocarp prior to harvesting.

INTRODUCTION

The quest for the role of the abundant C7 sugars mannoheptulose and perseitol in avocado is ongoing. Previous research by Liu *et al*. (2002) indicated that these carbohydrates might play a role as ripening inhibitors. Our previous findings (Bertling and Bower, 2005) suggest importance of these carbohydrates in sink strength of fruit and later on in fruit ripening. Cowan (2004) proposed that these C7 sugars have various important functions, amongst them protection of certain key enzymes essential for fruit growth and development, from damage by reactive oxygen species (ROS). Therefore, C7 sugars could form part of a pool of antioxidant systems which not only protect the fruit against damage but would also carry health benefits for the consumer of the fruit and hence could become an important marketing issue.

Antioxidant (AO) systems are present in cells to guard cellular structures against naturally occurring, extremely reactive compounds (particularly ROS) such as the oxygen ions, the hydroxyl radical and other free radicals as well as hydrogen peroxide. Such compounds, which form as natural by-products of the normal oxygen metabolism, could, due to their high chemical reactivity, damage proteins, DNA and lipids – a damage which would eventually lead to cell death. The removal of such compounds from cells is afforded either by ROS scavenging enzymes (e.g. superoxide dismutase, ascorbate peroxidase and catalase) which will inactivate the ROS or by small scavenging molecules (e.g. ascorbic acid, glutathione, polyphenols) which combine with the ROS to form non-toxic compounds.

ROS commonly occurring in plant tissue are the superoxide anion, which is scavenged by superoxide dismutase (SOD), as well as hydrogen peroxide, which is scavenged by catalase and ascorbate peroxidase, and using the ascorbate-glutathione cycle. Hydroxyl radicals and singlet oxygen are scavenged non-enzymatically by compounds such as carotenoids, ascorbate and α-tocopherol.

Under optimal growing conditions the production of antioxidants in cells is low and ROS occurring in cells are scavenged by the AOs present. During normal cell metabolism, ROS and AOs exist therefore in equilibrium. Stress (drought, chilling, heat shock, UV, pathogens, light) can, however, increase the production of ROS dramatically (Mittler, 2002) which then would need to be counteracted by an increase in AOs. However, when exposed to stressful conditions, the present pool of antioxidants cannot scavenge all ROS produced, which will result in non-healthy, damaged fruit.

Certain information on antioxidant activity of avocado is available. Vinokur and Rodov (2006) reported on the lipophylic versus hydrophilic radical scavenging activity of avocado, while Song and Barlow (2004) published that the antioxidant activity in avocado seeds is – like in most fruit – higher than in the edible portion. Furthermore, Ekor *et al*. (2006) reported that the administration of a methanolic extract of avocado leaves to rats increased the activity of the antioxidant enzymes SOD and catalase in these mammals.

Certain C6 sugar alcohols (sorbitol and mannitol) have been found to act as antioxidants when added to produce (Faraji and Lindsay, 2004). Similarly, C7 sugar alcohols could form an important part of the pool of antioxidants in avocado, protecting the developing fruit from oxidative stress and, therefore, playing an important role in the development of healthy fruit. Therefore, we investigated if perseitol and mannoheptulose form part of the total AOs in avocado tissue, which AOs are present in various ‘Hass’ avocado tissues and if the pool of antioxidant systems and its main fractions changes during fruit development.

MATERIALS AND METHODS

Leaves as well as fruit were sampled from approximately eight-year-old ‘Hass’ avocado trees in the KZN Midlands during November 2005 (early fruitset), March 2006 and June 2006 (close to harvest date). Samples were transported to the laboratory and 1 to 5 g leaf or fruit tissues – the latter separated into rind, flesh and seed – shock-frozen and subsequently freeze-dried.

Total antioxidant capacity was determined according to Benzie and Strain (1996) using the FRAP assay, while ascorbic acid was analysed using the colour reaction with 2,4-dinitrophenylhydrazine (DNPH) according to Böhm *et al*. (2006). Anthocyanins were determined spectrophotometrically (Steyn *et al*., 2006) and sugars were determined via HPLC according to Liu *et al*. (2002).

Statistical evaluations were carried out using GenStat® Version 7.1.

RESULTS AND DISCUSSION

With the maturation of new leaves from November’05 to March’06 the levels of total antioxidants in these tissues increased slightly while exocarp and seed showed no alterations in total antioxidants. However, mesocarp tissue was consistently found to have the lowest total antioxidant concentration (**Figure 1**). This might be an indication why particularly the mesocarp is sensitive to various disorders like grey pulp, seed cavity browning or vascular browning, as the capacity of flesh tissue to counteract oxidative stress is significantly lower than that of other tissues.

Of the three components of antioxidants analysed, ascorbic acid was found to be the highest in seed tissue; however, variations between samples were too great to distinguish significant differences between tissues. Nonetheless, at all three sampling dates the mesocarp showed significantly lower ascorbic acid concentration than the seed (**Figure 2**).

Throughout fruit development only very low concentrations of anthocyanins were detected (**Figure 3**). Young leaves, which are known to contain high concentrations of anthocyanin (Close and Beadle, 2003), had higher concentrations of this antioxidant pigment in November' 05 than the exocarp, seed and mesocarp. No anthocyanins could be detected in the latter tissue at any of the three sampling dates.

Furthermore, ascorbic acid accounted for around half the antioxidant activity in the seed and mannoheptulose for up to half of the antioxidant activity in mesocarp, and up to one fifth of the total antioxidants in other tissues (**Table 1**).

The opposite trend to ascorbic acid and anthocyanin concentrations in the various tissues was established for C7 sugars; the tissue lowest in anthocyanins and ascorbic acid had the highest mannoheptulose and perseitol concentration. Particularly in younger fruit higher mannoheptulose – and to a lesser degree perseitol – concentrations were determined in the mesocarp compared to the seed and exocarp. The decline in mannoheptulose as fruit approached harvesting maturity underlines our previous assumptions (Bertling and Bower, 2006) of the importance of C7 sugars in fruit quality. Of the C7 sugars D-mannoheptulose was found in approximately four times the concentration as perseitol (**Figure 4** and **5**). Perseitol declined in a similar manner as mannoheptulose, showing higher concentration in the mesocarp than in other tissues, and perseitol was, similar to mannoheptulose, declining as fruit approached harvest maturity.

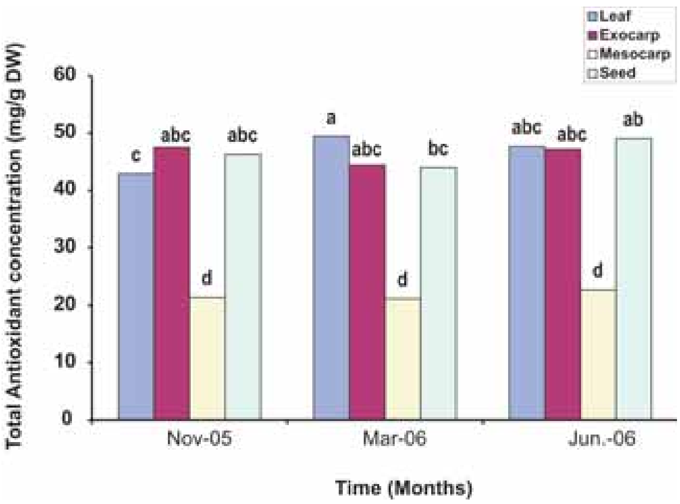

Figure 1: Total antioxidant concentration of 'Hass' avocado tissue during different times of plant development. LSD (5%) = 5.2 (n=12).

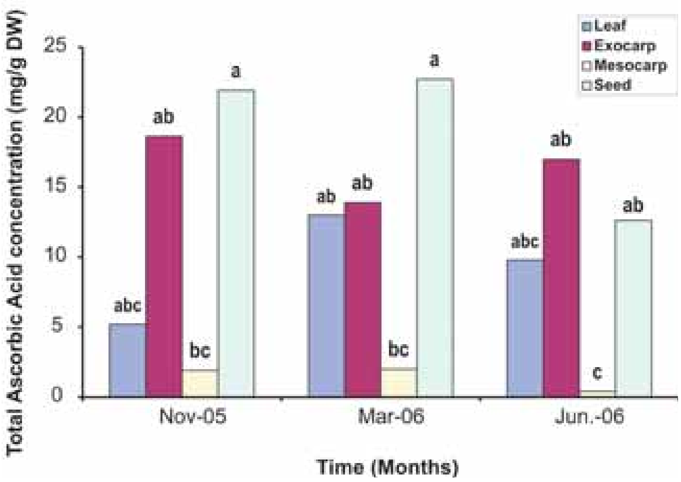

Figure 2: Total ascorbic acid concentration of 'Hass' avocado during different times of plant development, LSD (5%) = 18.6 (n=12).

Table 1: Distribution of antioxidants, their fractions and % of total antioxidants of these fractions in certain plant tissues (in brackets) of 'Hass' avocado.

		Plant tissue							
Compound	**Sampling date**	**Leaf**		**Exocarp**		**Mesocarp**		**Seed**	
Total Antioxidants (AOs) (mg/g DW)	Nov. 05	42.88		47.53		21.41		46.28	
	Mar. 06	49.43		44.41		21.21		44.01	
	Jun. 06	47.71		47.17		22.76		49.03	
Ascorbic Acid (mg/g DW) (% total AO fraction)	Nov. 05	5.2	(12.1)	18.6	(39.13)	1.9	(8.87)	21.9	(47.32)
	Mar .06	13.0	(26.30)	13.9	(31.30)	2.0	(9.43)	22.7	(51.58)
	Jun. 06	9.8	(20.54)	17	(36.04)	0.4	(1.76)	12.6	(25.70)
Anthocyanin (mg/g DW)	Nov. 05	0.011	(0.03)	0.003	(0.006)	n.d.		0.002	(0.004)
	Mar. 06	0.004	(0.08)	0.002	(0.005)	n.d.		0.002	(0.005)
	Jun. 06	0.009	(0.02)	0.009	(00.19)	n.d.		0.002	(0.004)
Mannoheptulose (mg/g DW)	Nov. 05	6.03	(14.06)	9.42	(19.82)	10.7	(50.2)	7.95	(17.18)
	Mar. 06	5.58	(11.29)	7.05	(15.87)	10.4	(49.2)	6.69	(15.20)
	Jun. 06	6.24	(13.08)	8.44	(17.89)	7.21	(31.7)	5.34	(10.89)
Perseitol (mg/g DW)	Nov. 05	0.25	(0.58)	1.34	(2.82)	2.06	(9.6)	1.62	(3.50)
	Mar. 06	0.44	(0.89)	0.37	(0.83)	2.73	(12.9)	0.43	(0.98)
	Jun. 06	0.11	(0.23)	0.36	(0.76)	0.25	(1.1)	0.89	(1.82)

n.d. = not detectable

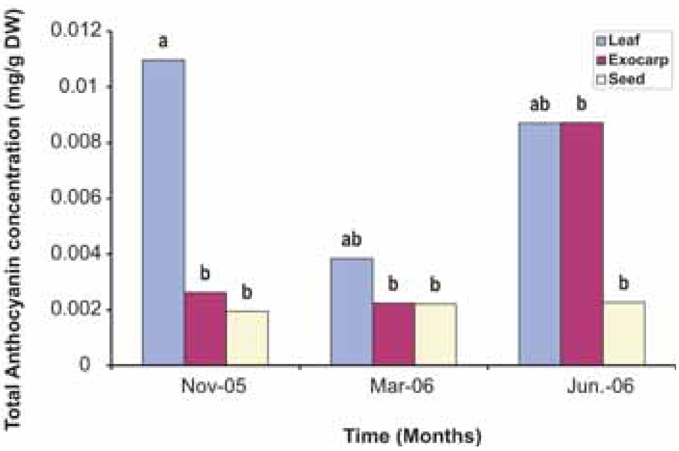

Figure 3: Total anthocyanin concentration of 'Hass' avocado during different times of plant development, LSD (5%) = 7.5 (n=12).

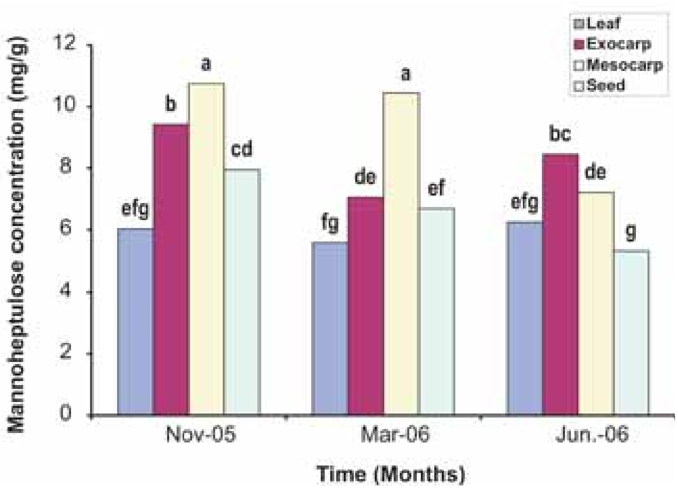

Figure 4: Mannoheptulose concentration of 'Hass' avocado during different times of plant development, LSD (5%) = 1.9 (n=12).

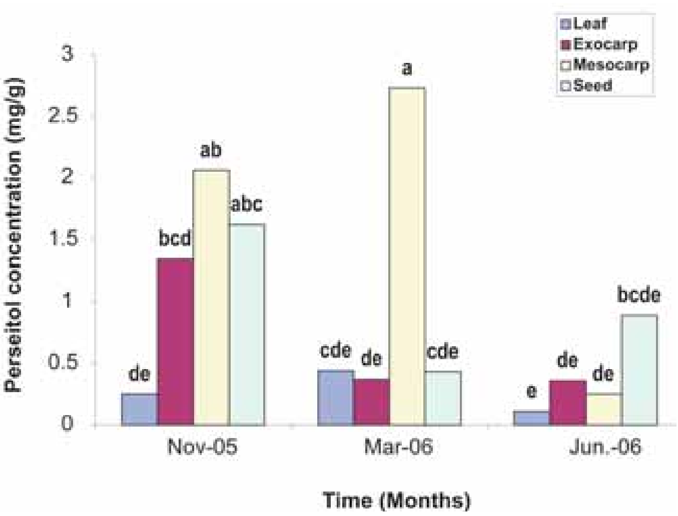

Figure 5: Perseitol concentration of 'Hass' avocado during different times of plant development, LSD (5%) = 1.2 (n=12).

Our results demonstrate that different antioxidant systems are present in the various tissues of avocado. Our current data also provide evidence that the deterioration of post-harvest quality in 'Hass' avocado might be due to the lack of C7 sugar antioxidants in the mesocarp which could counteract the oxidative stress and subsequently browning of flesh tissue. This lack of antioxidants in the mesocarp tissue would lead to the occurrence of oxidation products which would become visible as various browning disorders. Therefore, as levels of C7 sugars are declining towards maturity, the fruit's ability to withstand stress is reduced and subsequently fruit quality declines.

CONCLUSION

The data presented provide evidence that C7 sugars are not only important as storage compounds but could also play an important role as antioxidants, particular in mesocarp tissue. It is therefore essential to find means of increasing C7 sugar levels in the mesocarp in order to improve post-harvest quality.

LITERATURE CITED

BENZIE, I.F.F. and STRAIN, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power". *Analytical Biochemistry* 239(1): 70-76.

BERTLING, I. and BOWER, J.P. 2005. Sugars as energy sources – is there a link to avocado fruit quality? *South African Avocado Growers' Association Yearbook* 28: 24-27.

BERTLING, I. and BOWER, J.P. 2006. Avocado sugars during early fruit development. *South African Avocado Growers' Association Yearbook* 29: 38-39.

BÖHM, V., KÜHNERT, S., ROHM, H. and SCHOLZE, G. 2006. Improving the Nutritional Quality of Microwave-vacuum Dried Strawberries: A Preliminary Study. *Food Science Technology International* 12(1): 67-75.

CLOSE, D.C. and BEADLE, C.L. 2003. The Ecophysiology of Foliar Anthocyanin. *The Botanical Review* 69(2): 149-161.

COWAN, A.K. 2004. Metabolic control of avocado fruit growth: 3-hydroxy-3-methylglutaryl coenzyme a reductase, active oxygen species and the role of C7 sugars. *South African Journal of Botany* 70: 75-82.

EKOR, M., ADEPOJU, G.K.A. and EPOYUN, A.A. 2006. Protective effect of the methanolic leaf extract of *Persea americana* (avocado) against paracetamol-induced acute hepatotoxicity in rats. *International Journal of Pharmacology* 2(4): 416-420.

FARAJI, H. and LINDSAY, R.C. 2004. Characterisation of the antioxidant activity of sugars and polyhydric alcohols in fish oil emulsions. *Journal of Agricultural Food Chemistry* 52: 7164-7171.

LIU, X., SIEVERT, J., ARPAIA, M.L. and 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.

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

SONG, Y.Y. and BARLOW, P.J. 2004. Antioxidant activity and phenolic content of selected fruit seeds. *Food Chemistry* 88(3): 411-417.

STEYN, W.J., HOLCROFT, D.M., WAND, S.J.E. and JACOBS, G. 2004. Anthocyanin degradation in detached pome fruit with reference to pre-harvest red color loss and pigmentation patterns of blushed and fully red pears. *Journal of the American Society for Horticultural Science* 129(1): 13-19.

VINOKUR, Y. and RODOV, V. 2006. Method for determining total (hydrophilic and lipophylic) radical-scavenging activity in the sample of fresh produce. *Acta Horticulturae* 709: 53-60.