South African Avocado Growers' Association Yearbook 1989. 12:53-55

Effect of abscisic acid on abscisic acid metabolism, PPO activity, phenolics and quality in ripening avocado (*Persea americana* Mill) fruit

[1]J G M CUTTING, [2]J P BOWER, [3]P J HOFMAN and [3]B N WOLSTENHOLME
[1] Department of Horticulture, University of Stellenbosch, Stellenbosch 7600
[2]Citrus & Subtropical Fruit Research Institute, Nelspruit 1200
[3]Department of Horticultural Science, University of Natal, PO Box 375, Pietermaritzburg 3200

ABSTRACT

The effect of endogenous and applied ABA on ABA metabolism, PPO activity, phenolic levels and postharvest quality was investigated. Results using radioactive ABA showed that the ripening fruit both metabolised and catabolised ABA. High levels of ABA increased PPO activity, caused a decline in phenolic substrate levels and increased mesocarp (flesh) browning. This study has indicated a vital role for ABA and thereby stress, in fruit quality.

UITTREKSEL

Die effek van endogene en toegediende ABA op ABA metabolisme, PPO-aktiwiteit, die vlak van fenole en na-oes kwaliteit is nagegaan. Resultate met radioaktiewe ABA dui aan dat rypwordende vrugte ABA metaboliseer en kataboliseer. Hoe konsentrasies ABA verhoog PPO-aktiwiteit, lei tot 'n afname in fenoliese verbindings en 'n toename in mesokarp (vlees) verbruining. Hierdie studie dui op 'n belangrike rol vir ABA, en dus vir spanning, op vrugkwaliteit.

INTRODUCTION

One of the manifestations of poor fruit quality in export avocados is flesh (mesocarp and endocarp) discolouration. In avocado, Kahn (1975) noted an association between polyphenol oxidase (PPO) activity and susceptibility to flesh browning, while the level of phenolic substrate was also involved in browning of ripening fruit (Golan, Kahn & Sadovski, 1977). Refrigerated storage of fruit for 31 days, especially with treatments such as waxing to prolong shelf-life, increased the incidence of flesh discolouration. This was associated with increased activity of PPO (van Lelyveld & Bower, 1984). It has also been found that a history of preharvest water stress predisposes the fruit to higher postharvest levels of soluble PPO (Bower & van Lelyveld, 1986).

The role of the plant growth regulator abscisic acid (ABA) in fruit ripening is equivocal (Coombe, 1976; Goodwin, 1978; Rhodes, 1981), and it may act indirectly by inducing

ethylene synthesis, or altering the sensitivity of plant tissues to ethylene. A recent study (Cutting & Bower, unpublished data) found that storage of avocado fruit under high humidity conditions for four weeks at 5,5°C significantly improved internal fruit quality.

Using radioimmunoassay, it was shown that free ABA increased in avocado fruits with development to maturity, as the season progressed and as the fruits ripened (Cutting, Lishman, Hofman & Wolstenholme, 1986; Cutting, Hofman, Lishman & Wolstenholme, 1986). A more recent study (Cutting, Bower & Wolstenholme, 1988) showed that high levels of ABA significantly increased PPO activity, reduced internal fruit quality and reduced time to ripeness. In addition, it was found that the greater the quantity of infused ABA prior to ripeness, the less the amount of detected ABA at full softness, indicating some form of accelerated metabolism.

This paper reports upon the metabolism of both applied and endogenous ABA and the effect of ABA on polyphenol oxidase activity, phenolic substrate and internal quality in ripening avocado fruits.

MATERIALS AND METHODS

Fruit used in this study was of the cv Fuerte from a commercial orchard near Nelspruit. The trees were subjected to normal cultural practices and were not under any cultural stress when the fruits were harvested. The fruits were randomly selected from an eight-year-old (in 1988) orchard and had attained legal maturity (80 per cent or lower moisture content).

One hundred mature fruits were harvested with long pedicels in May 1988 and immediately transported to the laboratory where the pedicels were trimmed to leave a 10 mm stub. Fifty of the fruits were passively infused with 270ng.g^{-1} ABA and 50000DPM ^{14}C-ABA (specific activity 25mCu.$mmol^{-1}$) per fruit, using the method of Cutting, Bower & Wolstenholme (1988). Fifty fruits were infused with water and used as a control.

Each day four treated and four control fruits were peeled and the flesh (meso- and endocarp) evaluated for quality. The flesh was then removed, rapidly frozen in liquid nitrogen, lyophilised and stored at -20°C until analysed. The trial was terminated after 10 days, by which time the remaining fruit had reached a firmometer reading of 100 indicating eating ripeness (Swarts, 1981). These fruits were visually assessed for internal quality.

The following conditions were used as standard for the HPLC separations that were used in this study. The separation was achieved on a Spectra Physics HPLC using a Waters semiprep C-18 column. The gradient used was 25 per cent methanol: 75 per cent O, 1N acetic acid to 100 per cent methanol over a 25-minute period, using a flow rate of 2,5 ml per minute. UV monitoring was at 260 nm. One minute fractions were collected for the different studies.

HPLC analysis of the infused ^{14}C-ABA

Five gram samples from each fruit were pooled within treatments which resulted in 20 g

composite samples. The 20 g lyophilised flesh samples were homogenised in 200 ml 80 per cent menthanol with 20 mg BHT.L^{-1} added as an antioxidant. After extraction for 24 hours in the dark at 4°C, the samples were filtered and reduced to dryness under vacuum at 30°C. The sample was then reconstituted in 10 ml methanol and again reduced to dryness in a Savant concentrator. The samples were then dissolved in a 1 ml methanol, filtered through a 0,45 µm disposable filter and 0,5 ml injected into the HPLC. The one minute fractions were collected directly into scintillation vials, followed by the addition of scintillant and the radioactivity determined using a liquid scintillation counter.

ABA immunohistograms

One gram flesh samples were homogenised in 20 mℓ 80 per cent methanol and extracted as above. After filtration the sample was reduced to the aqueous phase in a rotary evaporator to which was then added 10 mℓ O, 1N acetic acid. The sample was then concentrated on a Seppak C-18 cartridge and eluted off the cartridge with 4 ml 100 per cent methanol and reduced to dryness. The sample was then dissolved in 1 ml methanol, filtered and 0,5 ml injected into the HPLC as above. The one minute fractions (2,5 ml) were reduced to dryness in a Savant concentrator, then redissolved in 2 ml methanol, methylated and subjected to radioimmunoassay for ABA (Cutting, Hofman, Lishman & Wolstenholme, 1986).

1 Determination of free ABA

One gram flesh samples were extracted as above. After reduction to the aqueous phase, the sample was added to 10 ml potassium phosphate buffer (0,5M, pH 8,5). After extraction with diethyl ether (2X20 ml) the aqueous phase was retained and acidified to pH 2,5 and further extracted with 2 X 20 ml diethyl ether. The organic phase was retained and reduced to dryness. The sample was then dissolved in 2 ml methanol, methylated and subjected to radioimmunoassay for ABA as above.

Determination of PPO and total phenolic compounds

PPO activity was determined by the method of van Lelyveld & Bower (1984) and total phenols were determined colorimetrically using the method of Torres, Mau-Lastovicka and Rezaaiyan (1987).

RESULTS

^{14}C-ABA metabolism

The proportion of the ^{14}C radio-activity that co-eluted with authentic ABA, declined with time from over 90 per cent of the available radio-activity immediately after infusion, to less than 10 per cent at full ripeness. Four typical radio-activity HPLC histograms corresponding to time zero, 24 hours after treatment, 72 hours (three days) and 144 hours (six days) after treatment are presented in Figure 1.

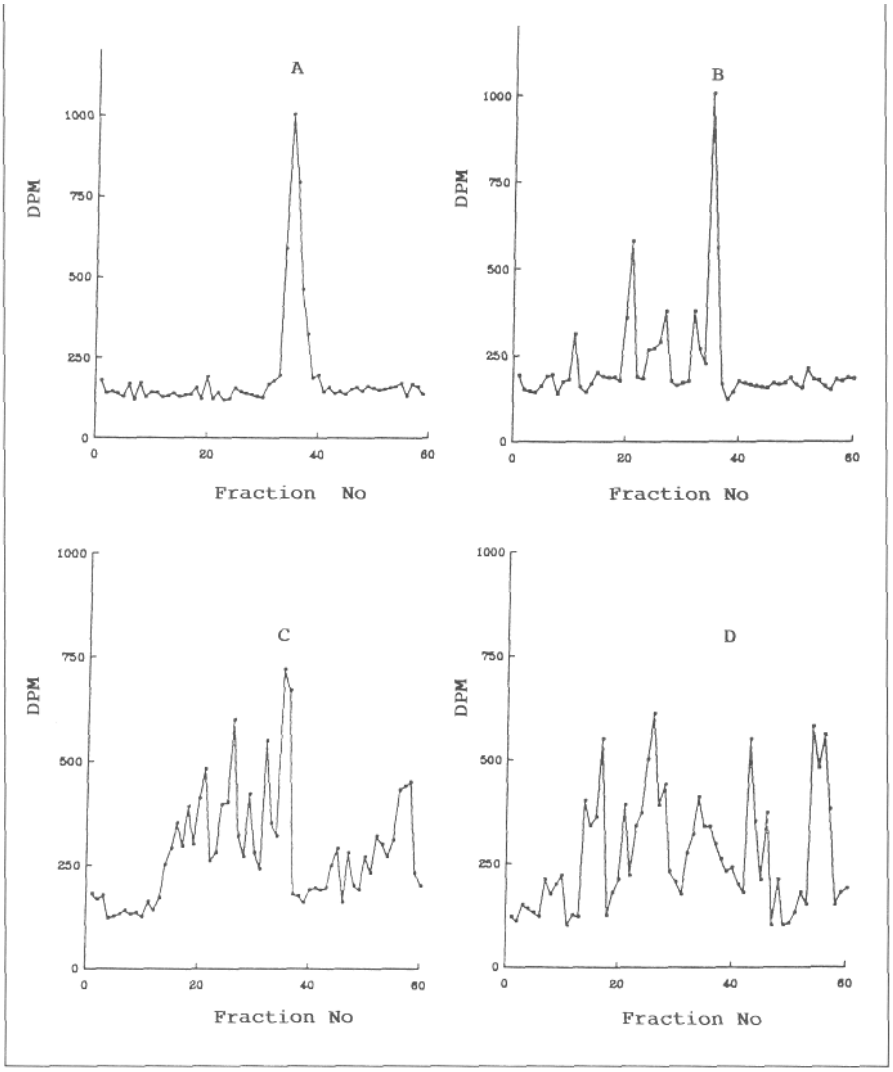

Fig 1 HPLC isolation in 0,5 min fractions of infused ^{14}C-ABA in ripening avocado fruit after one hour (A), one day (B), three days (C) and six days (D). Authentic ABA eluted in fraction 34-37 (17 to 18 minutes).

ABA immunohistograms

There were three zones of immunological activity in the immunohistograms. One band co-eluted with authentic free ABA. Levels of ABA were always higher in the infused treatment than the control. A typical ABA immunohistogram is presented in Figure 2.

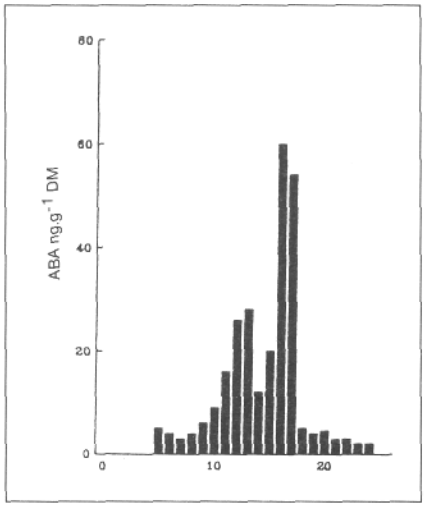

Fig 2 Typical immunohistogram for ABA from an avocado fruit 24 hours after harvest. One minute fractions were collected and assayed.

Free ABA

The free ABA trends are presented in Figure 3. ABA levels declined from the theoretical 270 ng.g^{-1} in the infused fruit to about 70 ng.g^{-1} during the first 48 hours. Thereafter levels stabilised, but there was a marked coinciding with the respiratory during the climacteric about 48 hours before full softness. The level of ABA then declined to about 50 ng.g^{-1} at full softness.

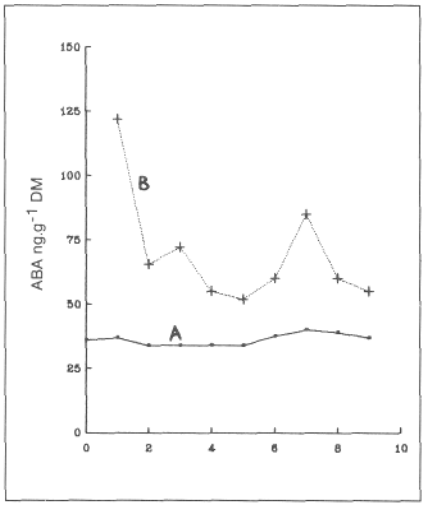

Fig 3 Free ABA in ripening avocado fruit.

A = control fruit

B = ABA-infused fruit.

In the control fruit ABA levels remained consistent at about 35 $ng.g^{-1}$ for the first five days, increasing slightly (but not significantly) to about 40 $ng.g^{-1}$ during the climacteric. At full softness the ABA concentration was about 35 $ng.g^{-1}$.

PPO and total phenolics

Polyphenol oxidase (PPO) activity declined during the first 24 hours after harvest from over five to about two. Thereafter PPO activity slowly declined to about 0,25 for control fruits and 0,35 for ABA infused fruits (Figure 4). The PPO activity in the ABA infused fruit was higher than the control for most of the softening period. This was particularly obvious during the last 72 hours of ripening.

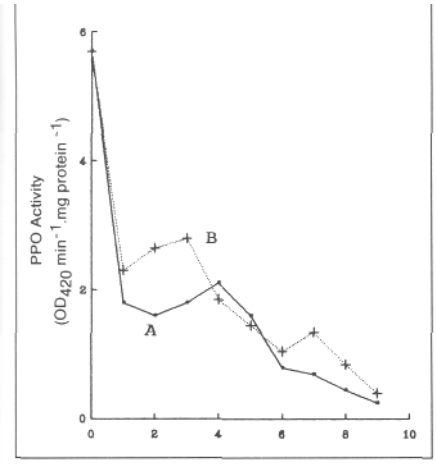

Fig 4 Soluble PPO activity in control (A) and ABA-infused fruit (B) during ripening.

The level of total phenolic compounds was about 200 $mg.g^{-1}$DM at harvest. Levels tended to fluctuate considerably in the control fruit but were still at about 200 at ripeness. In contrast, total phenols declined rapidly after four days softening and remained about 25 per cent lower in the ABA infused fruit from five days after harvest until full softness (Figure 5).

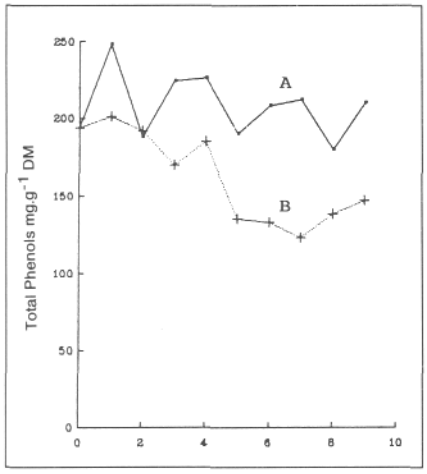

Fig 5 Total phenolic levels in control (A) and ABA-infused fruit during ripening.

Internal fruit quality

The internal fruit quality as measured by flesh discolouration is presented in Table 1. Only two control fruits showed any discolouration. However, in the ABA-treated fruits internal discolouration began 48 hours prior to full softness, and nearly all of the treated fruits had mesocarp discolouration at full softness.

TABLE 1 The effect of infused ABA on internal fruit quality during ripening. Results are expressed as a percentage of the fruits showing mesocarp discolouration and vascular browning

Days after harvest	Control fruit	ABA-treated fruit
	%	%
0	0	0
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	50
8	0	75
9	10	90

DISCUSSION

ABA has been associated with avocado fruit ripening (Adato, Gazit & Blumenfeld, 1976) and was recently shown to influence PPO activity and hence some aspects of fruit quality (Cutting, Bower & Wolstenholme, 1988). The ABA effect on fruit PPO activity did

not appear to be reversible, but rather increased as the fruit became more mature (Cutting & Bower, 1987), possibly due to an increase in oil content and a significant decrease in water content (Pearson, 1975).

The results reported here show that while the infused was indeed metabolised, the fruit had the capacity to produce ABA (whether from bound forms or synthesis is unknown) and to maintain a certain fruit ABA fruit concentration. Therefore the ABA content at harvest determined the ABA content at full softness (ripeness). Of interest was that ABA levels rose during the climateric, a period of known accelerated metabolism (Leopold & Kriedemann, 1975).

ABA also resulted in a higher PPO activity. Results showed that during the last 48 hours (two days) of ripening, PPO activity was almost double that in the control fruit. This coincided with the period when visual browning occurred and overlapped with a period when there was decrease in total fruit phenolic concentration. It would, therefore, appear that browning occurs during the final 48 hours of ripening (at room temperature) and that phenolic substrate is utilised in this reaction. This result is of importance as the level of substrate is reported to be involved in the browning of ripening fruit (Golan, Kahn & Sadovski, 1977). Finally, the amount of browning under optimum ripening conditions appears to be predetermined by the maturity of the fruit and the amount of stress to which the tree (and therefore the fruit) has been subjected.

REFERENCES

ADATO, I, BLUMENFELD, A & GAZIT, S, 1976. Relationship between changes in abscisic acid and ethylene production during ripening of avocado fruit. *Aust J Plant Physiol,* 3, 555 - 558.

BOWER, J P & VAN LELYVELD, L J, 1985. Effect of stress history and container ventilation on avocado fruit polyphenol oxidase activity. *J Hort, Sci,* 60, 545 - 547.

COOMBE, BG, 1976. The development of fleshy fruits. *Ann Rev Plant Physiol, 27,* 507 - 528.

CUTTING, J G M & BOWER, J P, 1987. Effect of harvest date and applied ABA on browning potential of avocado fruit. *SA Avocado Growers' Assoc Yrb,* 10, 130 - 132.

CUTTING, JGM, BOWER, JP & WOLSTENHOLME, B N, 1988. Effect of harvest date and applied ABA on polyphenol oxidase levels in avocado *(Persea Americana Mill)* fruit. *J Hort Sci,* 63, 509 - 51 5.

CUTTING, JGM, HOFMAN, PJ, LISHMAN, AW & WOLSTENHOLME, BN 1986. Abscisic acid, isopentenyl adenine and isopentenyl adenosine concentration in ripening avocado fruit as determined by radio-immunoassay. *Acta Hort,* 179,793 - 800.

CUTTING, JGM, LISHMAN, A W, HOFMAN, P J & WOLSTENHOLME, B N, 1986. Plant growth substance trends in developing avocado fruit as determined by radio-immunoassay. *Acta Hort,* 175, 285 - 289.

GOODWIN, PB, 1981. Phytohormones and fruit growth. In: Phytohormones and related compounds a comprehensive treatise. Vol III. Letham, D S, Goodwin, P B & Higgins, T J (eds). Elsevier North Holland Biomedical Press, Amsterdam.

KAHN, V, 1975. Polyphenol oxidase activity and browning of three avocado varieties. *J*

Sci Food Agric, 26, 1319 - 1324.
LEOPOLD, A C & KRIEDEMANN, P E, 1975. Plant growth and development. 2nd Edition. McGraw-Hill, New York.
MILLBORROW, BV & ROBINSON, D R, 1973. Factors affecting the biosynthesis of abscisic acid. *J Exp Bot,* 24, 537 - 548.
PEARSON, D, 1975. Seasonal English market variation in the composition of South African and Israeli avocados. *J Sci Food Agric,* 26, 207 - 213.
RHODES, M J C, 1981. The maturation and ripening of fruits. In: Senescence in plants, Thimann, KV (ed). CRC Press, Boca Raton.
SWARTS, D H, 1981. Fermometer ondersoeke by avokado's. *SA Avocado Growers' Assoc Yrb,* 4, 42 - 46.
TORRES, AY, MAU-LASTOVICKA, T & REZAAIYAN, R, 1987. Total phenolics and high performance liquid chromatography of phenolic acids of avocado. *J Agric Food Chem,* 35, 921 - 925.
VAN LELYVELD, L J & BOWER, J P, 1984. Enzyme reactions leading to avocado fruit mesocarp discolouration. *J Hort Sci,* 59, 257 - 263.
YOLAN, A, KAHN, V & SADOVSKI, AJ, 1977. Relation between polyphenols and browning in avocado mesocarp. Comparison between the Fuerte and German cultivars. *J Agric Food Chem,* 25, 1253 - 1260.