

ENZYME ACTIVITIES AND THE APPEARANCE OF PULPSPOT IN AVOCADO FRUIT

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OPSOMMING

Peroksidase en polifenoloksidase is laer in die proksimale helfte van die avokadovrug in vergelyking met die hele vrug. Die hoër aktiwiteit in die distale kant mag gekoppel wees aan verbruining in daardie gedeelte. Die hoër peroksidase aktiwiteit mag verband hou met die hoër etileenvlakke en die patroon van vrugrypwording. Feniëlanalies ammoniumliase-aktiwiteit was hoër in vrugte met pulpvlak teenoor vrugte sonder pulpvlak en dit mag verhoogde substraatsintese tot gevolg he.

SUMMARY

Peroxidase and Polyphenol oxidase activities are lower in the proximal half of avocado fruit compared with the whole fruit: The higher activity in the distal part may be associated with browning disorders in this region, and the higher peroxidase activity may be connected with higher ethylene levels and the fruit ripening pattern. Phenylalanine ammonia-lyase activity was higher in pulpspot-affected than in healthy fruit, and this may result in increased substrate synthesis.

INTRODUCTION

Browning disorders such as pulpspot, vascular browning and "lead discolouration" of avocado mesocarp are major limiting factors in the marketing of fresh or low temperature stored avocado fruit. After storage at 5,5°C for approximately 30 days, cut fruit may develop pulpspot after ripening; this appears as a spreading discolouration around the cut vascular bundles. The exact nature of the factors involved in "triggering" the appearance of pulpspot is unknown.

Kahn (1975) has shown that the activity of the browning enzyme Polyphenol oxidase (PPO) can differ in different avocado cultivars according to the rate of browning of their fruit mesocarp. The concentration of the natural phenolic substrates present may, however, also play a part in the rate of browning (Kahn, 1977a) and this in turn may be influenced by the rate of synthesis of substrates via the enzyme

phenylalanine ammonia-lyase (PAL).

Adato and Gazit (1977) established that the rate of ethylene evolution in the avocado fruit follows the pattern of ripening (softening) of the fruit. These results suggest that the associated enzyme systems, such as peroxidase (PO), may also follow a similar pattern.

It was the intention of the present investigation to study the changes in these enzyme activities within the avocado fruit with particular reference to the pulpspot disorder.

MATERIALS AND METHODS

Fully mature Fuerte avocado fruit, as determined by the oil content, were picked at the Burgershall Substation of the Citrus and Subtropical Fruit Research Institute and ripened at 22°C. Fruit affected with pulpspot were obtained from fruit stored at 5,5°C for 30 days before being placed at 22°C for ripening. The eating ripe stage (softness) was determined with the firmometer test (Swarts, 1981).

Ripe fruit were cut in half to analyze the distal and proximal halves separately: other fruits were pulped whole to provide a comparison with the halves. The mesocarp was mashed, well mixed and stored at — 20°C until required for analysis of enzyme activities.

Crude enzyme extraction for PPO and PO was made by grinding 5 g of fruit tissue with 10 ml of 10 mM acetate buffer, pH 5.0, in a mortar and pestle at room temperature, 22°C ± 2°C (Kahn, 1977b). The homogenate was squeezed through a gauze cloth and centrifuged in a Sorval Superspeed RC-2 centrifuge at 18,000 x g for 45 min. at 0 to 4°C. The supernatant was used as the crude enzyme extract for PO and PPO assay. Enzyme activities were determined as described earlier (Van Lelyveld and Pretorius, 1973; Van Lelyveld and Bozalek, 1979; and Van Lelyveld, Alcock and Nel, 1981).

For extraction and assay of phenylalanine ammonia-lyase (PAL), 5 g of fruit tissue was homogenized in ice-cold 20 mM Tris HCl buffer, pH 8.5, containing 1.4 mM B-mercaptoethanol. Insoluble polyvinylpyrrolidone (Plyclar AT) was added during the homogenization by mortar and pestle. Homogenates were squeezed through gauze cloth and centrifuged at 18,000 x g for 45 min. at 0 to 4°C in a Sorval Superspeed RC-2 centrifuge. The supernatant was used for PAL assay.

PAL activity was determined spectrophotometrically in a Pye Unicam SP8-200 Spectrophotometer by the method of Lamb et al. (1979). Enzyme activity is expressed in S.I. units of specific activity Cu katals. kg protein⁻¹). The protein content of the extracts was determined, following precipitation of protein by 5% trichloroacetic acid, by the method of Lowry et al. (1951) as modified by Leggett-Bailey (1962).

Enzyme activity for PO is expressed as volume activity (μ^{-1}) with the extinction coefficient of 7.86 cm² per mol at 420 nm and 25°C by 0,5 ml enzyme extract corresponding 0.5 g fresh weight material. PPO activity represents the OD change at 480 nm and 25°C over one minute brought about by 0.1 ml enzyme corresponding to 0.1 g fresh weight material.

TABLE 1. Activities of PO, PPO and PAL in whole avocado fruit, proximal half and distal half. The figures in brackets after the enzyme activities are the number of samples taken.

Fruit sample	PO Volume activity (μr^{-1})	PPO OD change at 480 nm	PAL $\mu\text{ kat. kg. protein}^{-1}$
Whole	0.269 (4)	0.048 (4)	1.545 (4)
Proximal	0.225 (4)	0.033 (4)	2.320 (4)
Distal	0.264 (4)	0.043 (4)	1.458 (4)
L.S.D.	0.05 = 0.028 0.01 = 0.040	0.05 = 0.012	N.S.

RESULTS

Enzyme activities were determined for PO, PPO, and PAL in the whole fruit and in the proximal and distal halves separately (Table 1).

Peroxidase activity in the proximal half of the fruit was significantly lower compared with the distal half and the whole fruit. Polyphenol oxidase activity in the proximal half was lower than in the whole fruit. Phenylalanine ammonia-lyase activity showed no significant difference in any part of the fruit.

In a separate experiment, PAL activity in pulpspot fruit was shown to be $4.85 \mu\text{ kat. kg protein}^{-1}$ as compared with a value in healthy fruit of $1.19 \mu\text{ kat. kg protein}^{-1}$, thus indicating a significantly (L.S.D. = 1,762) higher activity of the enzyme in pulpspot fruit.

DISCUSSION

The lower activities of PO and PPO in the proximal half of the fruit may account for the fact that some of the browning disorders appear in the distal half of the fruit: pulpspot, however, appears in the whole fruit when present.

Adato and Gazit (1977) established that in mature fruit ethylene production starts first in the distal part which is also first to ripen. There may, therefore, be an association between this higher ethylene evolution, ripening and higher PO activity in the distal end of the fruit. Shannon, Uritani and Imaseki (1971) found that PO activity was increased in sweet potato slices in the presence of ethylene.

Although PAL activity was not significantly different in the proximal and distal parts of the fruit, it was higher in pulpspot-affected than in healthy fruit. PAL is a key enzyme in the synthesis of phenolic substances (Camm and Towers, 1973). It is likely, therefore, that the higher PAL activity results in increased synthesis of phenolic substrates involved in the browning by the PPO enzyme. Kahn (1977a) concluded that the different rates of browning of avocado cultivars may be directly correlated to the amount of PPO activity and/or the concentrations of the natural substrates present. An investigation of the factors that may stimulate PAL activity in the avocado

fruit after harvesting and during or after storage may therefore help to elucidate the nature of the "trigger" that leads to the development of the pulpspot disorder.

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