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INHIBITION OF ETHYLENE PRODUCTION AND ACC OXIDASE ACTIVITY IN AVOCADO BY ACETALDEHYDE VAPOURS

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Abstract

Exogenous application of acetaldehyde (AA) vapour (5000 ppm for 18 h) to peeled avocado fruits prior to storage caused inhibition of fruit ripening. This inhibition was characterized by a delay in fruit softening and reduction in ethylene production. Moreover, addition of 1-aminocyclopropane-l-carboxylic acid (ACC), the precursor of ethylene, to avocado disks *(in situ)* or to avocado extract *(in vitro)* showed that AA reduced ethylene production by inhibiting ACC oxidase activity. CO_2 production and protein levels were the same in the control and treated disks. The levels of total free sulfhydryl (SH) group compounds increased, although the total amino acids level was reduced during ripening of the AA-treated avocado. AA treatment inhibited fruit pulp oxidation, while the control fruits oxidized and became brown.

1. Introduction

Acetaldehyde (AA) and ethanol are two products of anaerobic respiration in the fruit, they accumulate during ripening and contribute to the fruit aroma (Fidler, 1968). These metabolites have been shown to be capable of retarding senescence and inhibiting ethylene production in plants. Ethanol has been reported to inhibit ripening in whole tomato fruits (Kelly and Saltveit, 1988) and to inhibit ethylene production in tomato pericarp disks (Saltveit and Mencarelli, 1988).

Acetaldehyde has been shown to inhibit the fruit softening associated with reduced polygalacturonase activity in peaches and nectarines (Lurie and Pesis, 1992), and in tomato (Pesis and Marinansky, 1993). In halved grapes, AA has been found to inhibit ethylene production, whereas ethanol did not (Pesis and Marinansky, 1992). A short pre-storage anaerobic treatment of mango fruit has been reported to retard ripening, while neither ethanol nor AA vapour treatments were found effective, probably because they failed to penetrate into the tissue (Burdon et al., 1994). In avocado, prestorage treatment for 24 h in a low O_2 atmosphere, which induces endogenous AA and ethanol production during treatment, reduced chilling injury (CI) symptoms at 2C, fruit softening and ethylene production (Pesis et al., 1994). Increased levels of free SH group compounds found in the pulp and peel of the treated fruits might account for CI reduction (Pesis et al., 1994).

AA is a very reactive compound which is capable of binding covalently to amino groups of proteins forming a Schiff base (Mauch et al., 1986; Perata et al., 1992). However, it seems that the AA molecule cannot easily penetrate through the fruit peel. In tomato and grape, the vapour penetration is mainly through the stem (Pesis and Frenkel, 1989; Pesis and Marinansky, 1993). On the other hand, in mango, in which the stem area is hard, AA vapour could not easily penetrate (Burdon et al., 1994).

In the present work the effect of AA on the ripening of whole peeled fruit was studied, thus eliminating the problem of the vapour penetration.

2. Material and methods

Mature avocado *(Persea americana* cv. Fuerte) fruit were harvested from the central area of Israel at the end of the season. The whole fruits were peeled with a commercial peeler (1-2 mm peeled) and treated on the day of harvest. The fruit were enclosed in three 20 1 glass jars (20 fruits in each) and exposed to AA vapour flow in humidified air at a flow rate of 400 ml/min. The applied AA concentration was 5000 ppm for 18 hours at 18C. After application, the jars were ventilated with humid air for another 24 h. Control fruits were peeled on the same day and ventilated with humid air alone for the same period of time as the treated ones. During treatment AA, ethanol, CO_2 and O_2 concentrations in the head space of the jars were checked by gas chromatography (GC). At the end of the treatment, all fruits were transferred to cardboard boxes for further examination at 18C. Fruit firmness was measured on two pared sides of each fruit (10 measurements/treatment) using an electronic penetrometer (Chatillon, N.Y., NY) with a 6.5 mm conical tip.

The ethylene production capacity of the discs was determined with and without the application of 1-aminocyclopropane-1-carboxylic acid (ACC). Each day five new fruits were taken from each treatment for five replicates. From each fruit 10 disks were cut from the inner part of the pulp, five disks for each flask (duplicates for -ACC and +ACC). The disks (1 cm diameter, five disks per gram) were placed in 25 ml conical flasks on filter paper (Whatman No. 1) soaked with 500 µl 0.3 M Manitol, with or without 5 mM ACC. The flasks were seated with serum caps for 1 h for GC measurements of headspace ethylene, CO_2, AA and ethanol (Pesis and Marinansky, 1992).

Total SH content was determined by boiling 0.5 g of outer or inner pulp in 5 ml of 0.02 M phosphate buffer (pH 5.7; five samples/treatment) for 10 min. After filtration (millipore 0.45 HA filter), the water-soluble SH compounds were determined in the mixture, using 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) reagent. The DTNB-reactive compounds were determined spectrophotometrically at 412 nm according to Ellman (1959). Total amino acids content was determined using the ninhydrin reaction (Yemn and Cocking, 1955).

ACC oxidase activity *in vitro* was extracted and determined by the procedure described by Fernandez-Maculet and Yang (1992). Protein concentration was determined using BioRad reagent according to Bradford (1976).

3. Results and discussion

The firmness of untreated fruits decreased linearly during storage at 18C, while firmness was maintained in AA-treated fruits (Fig. 1). Three days after treatment, the firmness of the AA-treated fruits was around 80 (N) while that of the controls was around 50 (N). Inhibition of

softening was found also peaches that were treated with AA for 24 h prior to storage (Lurie and Pesis, 1992).

There was no significant difference between treated and non-treated fruits in the production of CO_2 (Fig. 2), which indicates that AA does not affect mitochondrial activity. In animal tissues (e.g., liver) AA has been shown to inhibit mitochondrial respiration (Cederbaum et al., 1974). In non-climacteric fruits (blueberry, strawberry, citrus, grape) AA can increase CO_2 production but not O_2 uptake, probably by increasing the activity of decarboxylating enzymes (Janes et al., 1978; Pesis and Marinansky, 1992).

Ethylene production in the peeled control fruit reached the climacteric peak on the second day (Fig. 3). This fast increase in ethylene production was due to the fruit peeling; wounding is known to accelerate ripening processes (Starrett and Laties, 1991). On the other hand, peeled fruits that were treated with AA vapour produced little ethylene. After 4 days, ethylene production in the treated fruits began to increase (Fig. 3). Addition of ACC, the immediate ethylene precursor, did not cause a significant increase in the ethylene production of either treated or non-treated fruits (Fig. 3). This indicates that there was enough endogenous ACC in the peeled avocado fruit to maintain ethylene production. Prestorage treatment with AA vapour for 18 h significantly reduced ACC oxidase activity *in situ* (ethylene production of the disks treated with ACC) during 4 days storage at 18C (Fig. 3). Similarly, in halved grapes supplied with ACC, AA had succeeded in reducing ethylene production (Pesis and Marinansky, 1992).

ACC oxidase activity from treated and non-treated fruits was measured during the 5 days of storage. AA vapour treatment inhibited ACC oxidase activity *in vitro* (Fig. 4). The inhibition of the enzyme activity *in vitro* by AA was correlated with the inhibition of the fruit disk ethylene production *in situ.* In various proteins it has been shown that AA can form covalent bonds with NH_2 residue of lysine via a Schiff base (Mauch et al., 1986; Perata et al., 1992). ACC oxidase includes 28 lysine residues out of 314 amino acids (Dong et al. 1992). These free NH_2 residues could probably interact with the AA molecule and affect ACC oxidase activity.

Total free SH group compound (mainly the amino acid, L-cysteine and tripeptide glutathione) contents increased in the AA-treated fruits during ripening, while in the control fruits it remained at a constant level (Fig. 5). It is interesting to point out that in the treated fruit, the total amino acid content decreased, while in the control fruits it remained high (Fig. 6). This probably indicates that protein breakdown was higher in the control fruits thus balancing up the free amino acids. The initial levels of free SH group, in the avocado pulp was quite high (800 nmol/gFW) (Fig. 5), probably because these fruits were harvested at the end of the harvesting season, while in fruit harvested at the beginning of the season the initial levels were almost zero (Pesis et al., 1994). Increase in glutathione levels during maturity has also been found in grape berries (Adams and Liyanage, 1993). In a previous work we showed that in low-O_2- pretreated avocado which exhibited reduced CI symptoms, higher levels of free SH groups were found in the peel and the pulp during storage at k (Pesis et al., 1994). It is possible that in AA-treated avocado the higher levels of SH group compounds than in the control fruits, maintained the tissue in a less oxidized form, owing to the inhibition of the oxidizing enzymes by AA vapour.

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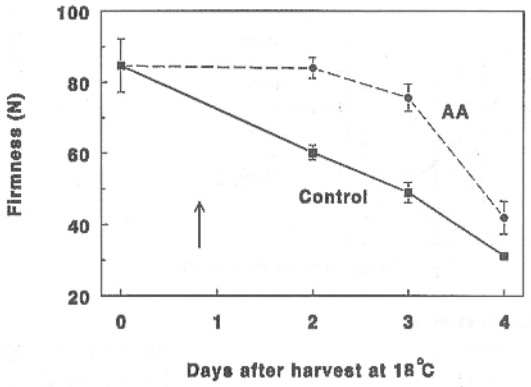

Figure 1 - Effect of acetaldehyde (AA) vapours given for 18 h immediately after harvest on firmness of peeled avocado fruit during storage at 18C. Arrow indicates removal from treatment. Control: ——, AA: -----. Data are means of five measurements ± SE.

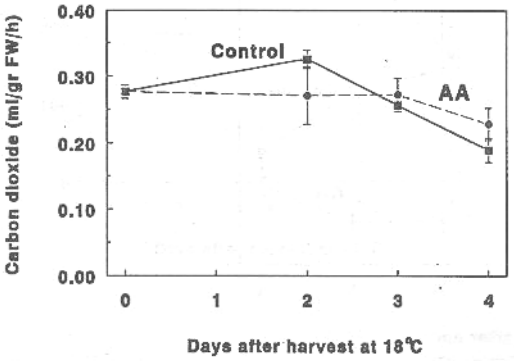

Figure 2 - Effect of acetaldehyde (AA) vapours given for 18 h immediately after harvest on CO_2 production of disks. Every day disks were cut from the inner part of the peeled avocado fruit during storage at 18C. Control: ——, AA: -----.

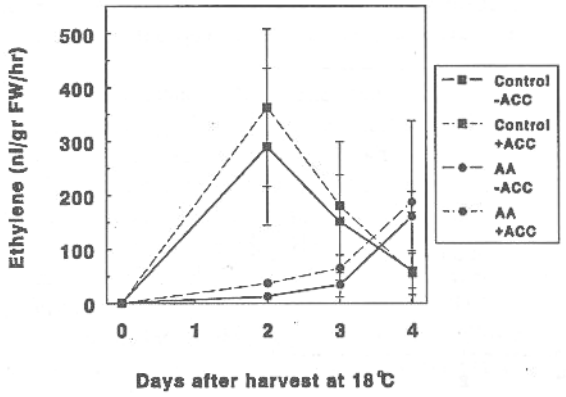

Figure 3 - Effect of acetaldehyde (AA) vapours given for 18 h immediately after harvest on ethylene production of disks with and without addition of ACC. Every day disks were cut from the inner part of the peeled avocado fruit during storage at 18C.

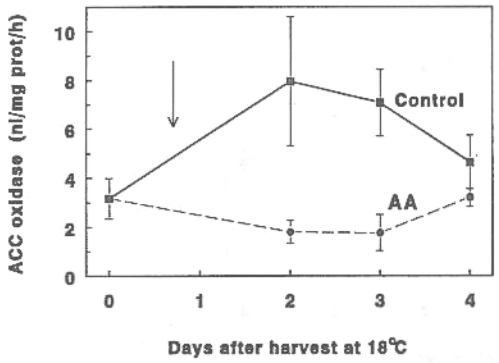

Figure 4 - Effect of acetaldehyde (AA) vapours given for 18 h immediately after harvest on ACC oxidase *in vitro* activity. The enzyme was extracted every day from peeled avocado fruit during storage at 18C. Control: ——, AA: -----.

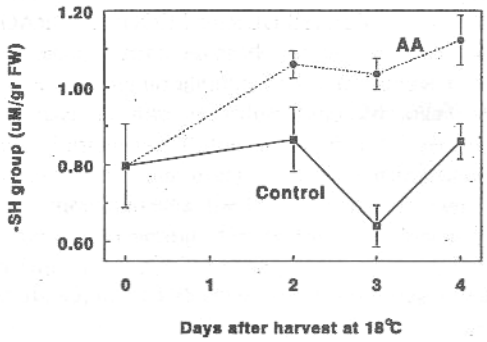

Figure 5 - Effect of acetaldehyde (AA) vapours given immediately after harvest for 18 h on total free SH group compounds from the inner parts of peeled avocado fruit during storage at 18C. Control: ——, AA: -----.

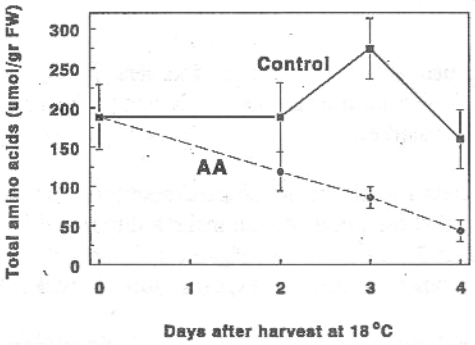

Figure 6 - Effect of acetaldehyde (AA) vapours given immediately after harvest for 18 h on total amino acids content in peeled avocado fruit during storage at 18C. Control: ——, AA: -----.