INFLUENCE OF ETHYLENE REMOVAL ON THE PROTEIN PATTERN OF AVOCADO FRUITS AT LOW TEMPERATURE STORAGE.

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Low temperature storage is a widespread technology to prolong the shelf life of fruits. The removal of C_2H_4 from the surrounding atmosphere during cold storage of avocados has been proposed by Zauberman and Fuchs (1973) to prevent chilling injury (CI). Moreover, CI occurrence increases when avocados cv. Fuerte stored at low temperatures are treated with 100 ppm C_2H_4 (Lee and Young, 1984).

On the biochemical aspect, C_2H_4 is related in avocado with the synthesis of several cell wall degrading enzymes involved in fruit softening and with enzyme activities of the C_2H_4 synthesis pathway. In general, the protein pattern of Hass fruits is correlated with the C_2H_4 evolution during the postharvest period (Christoffersen et al., 1982), and the levels of several mRNAs that change during ripening of avocados was clearly affected by pulses of propylene, an C_2H_4 -like factor (Starret and Laties, 1991).

Since C_2H_4 appears to affect the synthesis of enzymes related with the CI symptoms of avocado (atypical respiration and C_2H_4 production rates, and failure to soften properly upon warming) we have studied the CO_2 and C_2H_4 production, flesh firmness and protein profile of Hass fruits stored at 7°C with or without C_2H_4 removal.

Fruits were stored in cold store rooms at 90% RH. External CO_2 and C_2H_4 production was measured by gas chromatography on avocados placed in respiratory chambers. In a batch of fruit, C_2H_4 was removed from the chamber by chemisorption. One sachet (10 g of Green Keeper, manufactured by Pro-market SA, Spain) per kg of fruit was placed into the correspondent respiration chamber. Flesh firmness was measured using an Instron 1140 Testing machine, fitted with a double plateprobe.

In order to avoid coring effects, samples were taken by cutting three peeled fruit halves into pieces. The pieces were immediately frozen in liquid N_2 , randomized and ground to a fine powder with a coffee mill. Water soluble proteins were extracted according to Kanellis et al. (1989) with minor modifications, and subjected to SDS-PAGE using 10 to 15% gradient polyacrilamide gels. Silver stained gels were scanned with the BioImage system (Millipore Corporation).

The rates of CO_2 and C_2H_4 production were clearly reduced by low temperature. The respiration pattern was similar between both conditions, showing an irregular behavior during the first 25 days of storage. After this breaking point a slow increase was observed. Ethelene production was more affected by the temperature, being always below $1.5 \mu\text{l/kg/hr}$. The treatment with C_2H_4 removal reduced these levels and the peaks on C_2H_4 evolution were smoothed.

Softening of fruit during storage shows that delay of ripening was not affected by the treatment with C_2H_4 removal. This suggests that induction of the synthesis of softening related enzymes should not solely be attributed to

high C_2H_4 levels. Instead, the hypothesis of a genetic control should be considered. Thus, some changes in gene expression during avocado ripening have been shown to be suppressed by storage conditions retarding ripening (Kanellis et al., 1989).

With the approach used in this study, no relevant differences have been found at low temperature with respect to normal ripening. However, accumulation of some polypeptides (i.e.: 71, 55, 45, 30, 29, 24, 15 kDa) was delayed. The delay was more evident in samples of fruits treated with C_2H_4 removal, notably for proteins of 15 and 24 kDa. Polypeptides disappearing during ripening at 20°C (42 and 26 kDa) decreased their levels more slowly at 7°C. Proteins of 20 and 15.5 kDa disappeared later in avocados stored with C_2H_4 removal.

More accurate analyses are needed, but these results suggest that the delay of the ripening at low temperature could be related to a delay on the evolution of the protein profile.

REFERENCES

- Christoffersen, R.E., E. Warm and G.G. Laties. 1982. Gene expression during fruit ripening in avocado. *Planta*, 155:52-57.
- Kanellis, A.K., T. Solomos and A.K. Mattoo. 1989. Hydrolytic enzyme activities and protein pattern of avocado fruit ripened in air and in low oxygen, with and without C_2H_4 . *Plant. Physiol.* 90:257-266.
- Lee, S.K. and R.E. Young. 1984. Temperature sensitivity of avocado fruit in relation to C_2H_4 treatment. *J. Am. Soc. Hort. Sci.* 109:689-692.
- Starret, D.A. and G.G. Laties. 1991. The effect of C_2H_4 and propylene pulses on respiration, ripening advancement, ethylene-forming enzyme, and 1-aminocyclopropane-1-carboxylic acid synthase activity in avocado fruit. *Plant Physiol.* 95:921-927.
- Zauberman, G. and Y. Fuchs. 1973. Ripening processes in avocados stored in C_2H_4 atmosphere in cold storage. *J. Am. Soc. Hort. Sci.* 98:477-480.