RELATIONSHIP BETWEEN PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS IN AVOCADO FRUITS STORED AT CHILLING TEMPERATURE

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Abstract

The effects of chilling on avocado fruit physiology and biochemistry have been examined following the evolution in the respiration and ethylene production rates, texture, and electrophoretic profiles of total protein extracted from the mesocarp of fruits stored at 3 °C, as compared to fruits at 20 °C. The respiration and ethylene production rates were drastically reduced at 3 °C, showing no climacteric peaks, and fruit softening was almost absent. External chilling injury symptoms (pitting and browning of the skin) were observed after three weeks of cold storage. The pattern of electrophoretically resolved polypeptides indicated that the changes observed during avocado ripening at 20 °C (increases in 15.8, 21, 23.5, and 25.8 kD polypeptides; decreases in 18.2, 23, and 24 kD polypeptides) were not present during storage at 3 °C for two months. It is proposed that differential synthesis and/or degradation of some of the mentioned polypeptides is required for avocado fruit ripening. No chilling-induced polypeptide was found using this approach.

1. Introduction

Low temperature storage is probably the most extensive postharvest technology for preservation of fruit and vegetables. However, some problems arise when this technology is applied to chilling-sensitive crops such as fruits of tropical or subtropical origin. During low temperature storage, these commodities may develop some physiological and biochemical disturbances, frequently occurring with visual symptoms, named chilling injury (CI). The appearance of CI is mainly dependent on the species, physiological stage, and temperature and duration of storage. For commercial purposes, it is defined as a temperature threshold for prolonged cold storage of a given crop below which it is expected to find a percentage of CI symptoms commercially unacceptable. In avocado (*Persea americana* Mill) cv. 'Hass' fruit, 5 °C is considered the lowest safe temperature for prolonged cold storage. The CI symptoms in avocado fruit are scalding, pitting of the skin, discoloration of the mesocarp, failure of the fruit to soften properly after removal to nonchilling temperature, atypical respiratory rate patterns and reduced ethylene production.

Since plants respond to a wide variety of environmental stresses (high-temperature, anaerobic and water stress, heavy metals, etc) by alteration of gene expression (Sachs and Ho, 1986), a similar response during low

temperature exposure should be expected. Thus, some specific polypeptides and several cDNA clones have been related to the acclimation process (in which exposure of a plant to cold results in a lowering of the temperature at which the plant is damaged or killed by chilling or freezing temperatures). In tomato fruit exposed to 4 °C, transcriptional activation of a thiol protease gene was found by Schaffer and Fischer (1990), while Watkins et al. (1990) reported a three-fold increase in a mRNA homologous to a cDNA clone (pTOM13) corresponding to the EFE gene. Wilson et al. (1990) showed that low temperature storage of pears was accompanied by altered gene expression.

It is also clear that fruit ripening involves a series of biochemical events that are brought about by the coordinated expression of specific genes, and this matter has been well documented by different studies on avocado fruit ripening. Several of the changes observed in the mRNA population during avocado ripening are modified by low oxygen condition (Kanellis et al., 1991) and during low temperature storage (Dopico et al., 1993). Therefore, it was of interest to analyze whether these changes are also reflected in the polypeptide pattern, attempting to relate them to the timing of occurrence of CI during storage of 'Hass' fruit at 3 °C.

2. Material and methods

Mature 'Hass' fruits were harvested from a commercial orchard, selected for healthy appearance and stored at 20 ° and 3 °C in cold-store rooms. The concentrations of CO₂ (respiration rate) and C₂H₄ (ethylene production) evolved in the storage atmosphere by 25 fruits placed in respiration chambers at each temperature were measured by gas chromatography in an 1-mL gas sample taken from the outlet of the respiration chambers. Fruit softening was measured in three peeled areas (approximately 1 cm in diameter) of the flesh of five fruits using an Instron 1140 texturometer. Periodically, three fruits were peeled and one longitudinal half of each fruit was cut into pieces, frozen in liquid N₂ and stored at -80 °C until used for protein extraction.

Proteins were extracted using the method of Kanellis et al. (1991) and fractionated by SDS-PAGE using 10-15 % gradient polyacrylamide gels. After electrophoresis the gels were silver stained and scanned using the Biolume system (Millipore) for assignment of molecular weights.

3. Results

The avocado fruits were soft and ripe on day 9 at 20 °C. The SDS-PAGE profile confirmed that avocado fruit ripening involves protein synthesis and degradation. Thus, though most polypeptides were present in all the samples, some variations were observed during ripening. The most striking changes were found after the climacteric peaks in respiration and ethylene production rates on day 7, coincident with the initiation of the largest drop in fruit firmness (Figures 1-2). Among these changes, an increase in a 15.8 kD polypeptide was the clearest one. Other increases were found in polypeptides with an

Figure 1. Respiration and ethylene production rates of avocado fruit stored at 20 °C and 3 °C

(.....) ethylene production
(—) respiration

approximate molecular weight of 21, 23.5 and 25.8 kD, while 18.2, 23 and 24 kD polypeptides showed decreases in their levels.

Fruits at 3 °C showed few modifications in the respiration and ethylene production rates, with no clear climacteric peaks (Figure 1), and did not ripen during storage, as shown by fruit firmness (Figure 2). Accordingly, no change in the polypeptide pattern was found during 3 °C storage, and all the samples obtained during storage showed a polypeptide pattern similar to that of prestored (day 0) fruit. Therefore, no new polypeptide could be related to the appearance of chilling injury symptoms, which were observed during 3 °C storage as pitting and scalding of the skin, and upon rewarming of fruits to 20 °C as abnormal patterns in their respiration and ethylene production rates (data not shown).

Figure 2. Flesh firmness (mean $\pm$ SD) of avocado fruit during storage at 20 °C (♦) and 3 °C (□)

4. Discussion

Our data confirm previous results pointing out that synthesis and/or degradation of specific polypeptides are involved in avocado fruit ripening (Christoffersen et al., 1982; Kanellis et al., 1991). It is proposed that modifications in the level of specific proteins are needed for avocado fruit to ripen, since the absence of changes in the polypeptide pattern obtained by SDS-PAGE was accompanied by the lack of ripening during storage at 3 °C. This suggestion is supported by our observations that the delay in ripening at 7 °C is also accompanied by a delay in the above mentioned changes in the polypeptide pattern observed during ripening at 20 °C (Zamorano y Merodio, 1993). Furthermore, Dopico et al. (1993) found that the expression of several mRNAs homologous to ripening-related cDNA clones was either undetectable or at a very low level during storage of avocado fruit at 3 °C. Similar results showing that low oxygen suppresses the expression of cellulase and polygalacturonase in avocado fruit (Kanellis et al., 1991) are consistent with the view of fruit responding to storage conditions by alteration of gene expression.

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