THE EFFECT OF ETHYLENE REMOVAL DURING CONTROLLED ATMOSPHERE STORAGE OF RED DELICIOUS, EMPIRE, JONATHAN, IDARED, AND LAW ROME APPLES

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Development and implementation of controlled atmosphere storage technology for apples during the last decade has been increasingly directed at attenuating the synthesis and action of ethylene (19). Ethylene is the fruit ripening hormone (3,4) and is responsible for initiation of ripening capacity of apples during development on the tree or during storage. Oxygen is required for the synthesis and action of ethylene (3,9,10,15) so it is important to quickly lower the O_2 level in storage to the safest minimum level. Carbon dioxide is a natural occurring competitive inhibitor of ethylene action (4) so CO_2 level in storage is kept at the highest safe concentration. Ethylene synthesis and action is temperature dependent (5) so it is important to quickly cool and maintain the fruits at the lowest safe storage temperature. Ethylene initiates autocatalytic ethylene production (20) and the respiratory climacteric in apples so it is important to keep the ethylene level in the CA storage atmosphere below 1 ppm (1,14,16) to prevent induction of rapid ethylene production. And, best results for long-term storage can be realized when appropriate CA technology is applied to apples that are mature but preclimacteric with respect to ethylene so maturity and ripening development at harvest/storage is of utmost importance (7,8,16).

The varied results of CA storage investigations with apples from around the world (19) reflect inherent complexities when variables such as cultivar, climate, nutrition and maturity, are interacted with storage variables of handling, temperature, O_2, CO_2, ethylene, humidity and time. The investigations reported here evaluated the application of low ethylene CA storage to the Empire, Jonathan, Red Delicious, Idared, and Law Rome cultivars grown under Michigan conditions. The fruits were cooled within one day of harvest and brought to CA storage conditions in 7 or 14 days to examine the latitude of effectiveness for commercial feasibility. The objective was to determine the influence of 1.5 or 3% O_2 with 3 or 5% CO_2 with high or low ethylene levels during storage at 0^oC for up to 8 months.

Materials and Methods

Fruits of the various cultivars were harvested from daminozide-treated trees in commercial orchards in the Sparta, Michigan area. Maturity of fruit at harvest was assessed by determining the development of the autogenous ethylene climacteric. This was done by determining the onset of the induced ethylene climacteric (6) for three 10-fruit samples at intervals during the harvest season and by measuring the level of ethylene in the internal atmosphere of the fruit. Empire and Jonathan (Jonared) fruits were harvested on Oct. 2 and Oct. 9 and Red Delicious (Red Chief), Idared and Law Rome were harvested on Oct. 9. The fruits of each variety were randomized into treatment replicates of 60 to 90 fruits each placed in 20 l plastic containers and stored in air at 0^oC within 24 hours of harvest. After 7 or 14 days the containers were sealed with covers fitted with inlet and outlet tubulation and ventilated with gas mixtures prepared from liquified N_2, CO_2 and air to provide 3 or 5% CO_2 with 1.5 or 3% O_2. The gas mixtures were humidified, brought to the dew point at the storage temperature of 0^oC and distributed to the containers via a calibrated capillary flow rate control system. Two ethylene levels were established by ventilating the containers at 30 ml/min (high ethylene level) or 300 ml/min (low ethylene level). The 30 and 300 ml/min flow rates provided about 0.2 and 2 atmosphere changes per hour. The combination of 2 storage delays, 4 CA gas mixtures, 2 ethylene levels and 3 replications comprised a total of 48 containers per harvest for each variety. Additional fruits were maintained in air at 0^oC. Fruits were examined before storage and after 2,4,6 and 8 months.

Fruit Analyses

Internal ethylene was determined on a 10-fruit sample with individual fruits employing gas chromatography. Flesh firmness was determined with a Effegi penetrometer with an 11 mm diameter tip and the same fruits were used for determination of the starch index (18). The time of the induced ethylene climacteric was determined with three to five 10-fruit samples in the usual manner with dry lime and sealed containers (6). Acetone and freeze-dried samples were prepared at each sampling interval for measurement of ACC (1-amino-cyclopropane-1-carboxylic acid) content (17), ACC synthase (2), protein content, malic enzyme activity, and water soluble polyuronide content (11).

Results and Discussion

Maturity at Harvest

Empire fruits harvested Oct. 2 and Oct. 9 were judged to be preclimacteric with respect to ethylene based on internal ethylene and development of the ethylene climacteric. The date of the autogenous ethylene climacteric was determined to be Oct. 20. Jonathan fruits were at the onset of the ethylene climacteric at the harvest of Oct. 2 and well into the ethylene climacteric by Oct. 9. The onset of the autogenous ethylene climacteric of Red Delicious was determined to be Oct. 6 to 9 and internal levels confirmed this on Oct. 9. The Idared were well into the ethylene climacteric by the harvest of Oct. 9 and were judged to be too far advanced in ripening for good results from long-term storage. The Law Rome were preclimacteric at the harvest of Oct. 9 and the autogenous ethylene climacteric was determined to be Oct. 12-17. They were judged to be ideal for long-term CA storage at harvest on Oct. 9.

Storage Studies

Empire: The ventilation rates of 30 and 300 ml/min were employed to provide high and low ethylene levels, respectively, in the storage containers. However, even the low ventilation rate kept the ethylene level below 1 ppm in the storage chambers for 8 months. Ethylene level increased earliest for fruits of the Oct. 9 harvest and delayed for 14 days before applying an atmosphere of 3% CO_2 + 3% O_2. The ethylene levels in the storage chambers of fruits kept in the other atmospheres for 8 months were mostly below 0.1 ppm.

Flesh firmness (Fig. 1) of Empire apples kept for 8 months in all CA mixtures after a 7-day delay was maintained at nearly the initial value for the harvest of Oct. 2 and for 4 months for the fruits harvested Oct. 9. The 1.5% O_2 with either 3% or 5% CO_2 gave the best firmness retention while the 3% O_2 + 5% CO_2 was consistently least effective in retaining flesh firmness. The incidence of a flesh browning disorder was most prevalent at 5% CO_2 + 1.5% O_2 with fruits from the second harvest and to a lesser extent with the 3% CO_2 + 1.5% O_2. This was occasionally observed in fruits from the 5% CO_2 + 3% O_2 but not with the 3% CO_2 + 3% O_2 mixture. Fruits from the first harvest were essentially free of this CO_2-linked disorder associated with low O_2. This is not a senescence disorder associated with ripening because the flesh was still firm. The disorder was not seen in Empire stored in air at 0^oC nor when stored in 3% CO_2 + 2% O_2 at 3.3^oC. The disorder may be a chilling disorder aggravated by CO_2 at low O_2 levels.

A delay of 14 days before establishing the CA condition resulted in greater accumulation of ACC in Empire apples and the level remains higher throughout 8 month's storage than in fruits with CA established in 7 days (Fig. 2). Moreover, ACC levels remain at a low and constant level in these fruits at all CA mixtures. Fruit stored in air at 0^oC accumulated ACC in a linear manner from the beginning of the storage period.

Jonathan: Jonathan fruits of the first harvest responded similar to Empire in flesh softening with respect to delay in establishing CA (Fig. 3). Fruits of the second harvest softened most quickly if delayed 14 days before CA. Little differences in flesh firmness were noted among fruits regardless of the CA mixture but generally the firmest fruits were observed from the 1.5% O_2 with 3 or 5% CO_2. The 3% CO_2 + 3% O_2 was the least effective of all gas atmospheres in delaying the initiation of ethylene production by the fruits during storage. Even fruits from the harvest of Oct. 9 which were already well into their ethylene climacteric at harvest were kept at low ethylene levels during 8 months of storage providing an atmosphere of 1.5% O_2 with 3 or 5% CO_2 or 3% O_2 with 5% CO_2 was established within 7 days. The maintenance of the low ethylene level in the storage chamber may explain the good retention of flesh firmness.

The level of ACC in Jonathan fruit remained low and even declined over the course of 4 to 8 months of storage regardless of the CA mixture but increased quickly in fruits held in air at 0^oC (Fig. 4). The ACC content of fruits from CA differed little and this was consistent with little differences observed in flesh softening.

Red Delicious: Flesh softening of Red Chief Red Delicious progressed very slowly over the 8-month storage period and occurred most slowly in 1.5% O_2 with 3 or 5% CO_2 (Fig. 5). The 3% O_2 with 3% CO_2 was the least effective atmosphere to retain flesh firmness. It is important to note that even in the 3% O_2 and 3% CO_2 with the low ventilation rate the ethylene level in the storage chambers was maintained below 1 ppm. And this was also seen for fruit delayed for 14 days before applying CA. It is likely that the low ethylene levels maintained in storage chambers account for the delay in flesh softening. The ethylene level in the storage chambers was directly related to O_2 level, inversely related to CO_2 and directly related to the duration of time before CA was applied.

Idared: Flesh softening of Idared progressed in about a linear fashion regardless of CA storage atmosphere or whether CA was established in 7 or 14 days (Fig. 6). The 5% CO_2 + 3% O_2 CA was the least effective atmosphere to retard flesh softening. The fruits in all CA mixtures continued to develop ethylene production capacity gained prior to when they were harvested and even 1.5% O_2 with 5% CO_2 applied within 7 days was only marginally effective in delaying the development of flesh softening beyond 4 months of storage. And ethylene scrubbing by ventilation was not effective in delaying ripening. The level of ethylene within the fruits was too high and fruit ripening had progressed too far by the time the fruits were placed under controlled atmosphere conditions to effectively attenuate ethylene action by removing it from the storage atmosphere.

Law Rome: Law Rome fruits were judged to be of ideal maturity for long term Ca storage at harvest Oct. 9. The fruits were preclimacteric with respect to ethylene and the date of the autogenous ethylene climacteric was determined to be Oct. 12-17. Flesh firmness at harvest was 21.0 lb and no softening was evident after 14 days at 20^{o}C. Delaying the application of CA for 7 or 14 days allowed development of capacity of the fruits to produce ethylene at about the same rate after 2 months whether or not ethylene was removed from the storage atmospheres. Little loss of flesh firmness occurred in fruits kept at 1.5% O_2 with 3 or 5% CO_2 provided the atmosphere was established within 7 days of harvest (Fig. 7). However, at 3% O_2 with either 3 or 5% CO_2 flesh firmness of fruits for which CA was applied within 7 days declined in a linear manner to 15 to 16 lbs after 8 months of CA storage. A similar linear decline in flesh firmness was seen for all CA conditions for fruits when a 14 day delay was imposed before establishing CA. In this case the flesh firmness change essentially paralleled that of fruits kept in air. And this is consistent with the interpretation that CA, to be effective in delaying ripening during storage, must be applied before fruits have gained capacity to produce ethylene. CA storage at 1.5% O_2 with 3 or 5% CO_2 yielded only marginally firmer fruits (16 lbs) after 8 months than observed for fruits kept at 3% O_2 with 3 or 5% CO_2 (15 lbs) or in air (14 lbs) at the same temperature.

Retention of flesh firmness is the single most important parameter to employ to assess the effectiveness of application of CA technology for apples. This is fortunate because the determination is simple and objective. If fruits lose 10% or less in flesh firmness during prolonged CA storage (8 months), it is obvious that ripening has been effectively attenuated. If flesh firmness decrease of fruits in CA parallels the track of fruits stored in air at the same temperature it should likewise be obvious that CA technology has not been effective. Ethylene action is the root cause of flesh softening in apples because it initiates the biochemical capacity for cell wall degradation. The manifold other processes attending ripening are likewise initiated by ethylene action. Recognition of the critical role ethylene plays in fruit ripening and the requirements of ethylene action should continue to foster development and application of successful controlled atmosphere technology.

In summary, the results of our investigation indicate that markedly beneficial results may be expected in terms of delaying ripening of apples by reducing the ethylene level in CA storage rooms. Ripening delay may be realized if: the apples are preclimacteric with respect to ethylene at harvest and at the time the CA is applied; CA conditions of 1.5 to 3% O_2 with 3 to 5% CO_2 are applied within 7 days of harvest and cooling is effected within 1 day of harvest; and the ethylene level within the fruit and the CA storage is kept below 1 ppm during the first few months of the storage period. These conditions can be met by applying currently available technology. A CO_2 level at or below that of O_2 should be employed for Empire and Law Rome when storing at O_2 levels below 3% as a precaution to prevent an apparent CO_2-linked low O_2 disorder at 0^oC.

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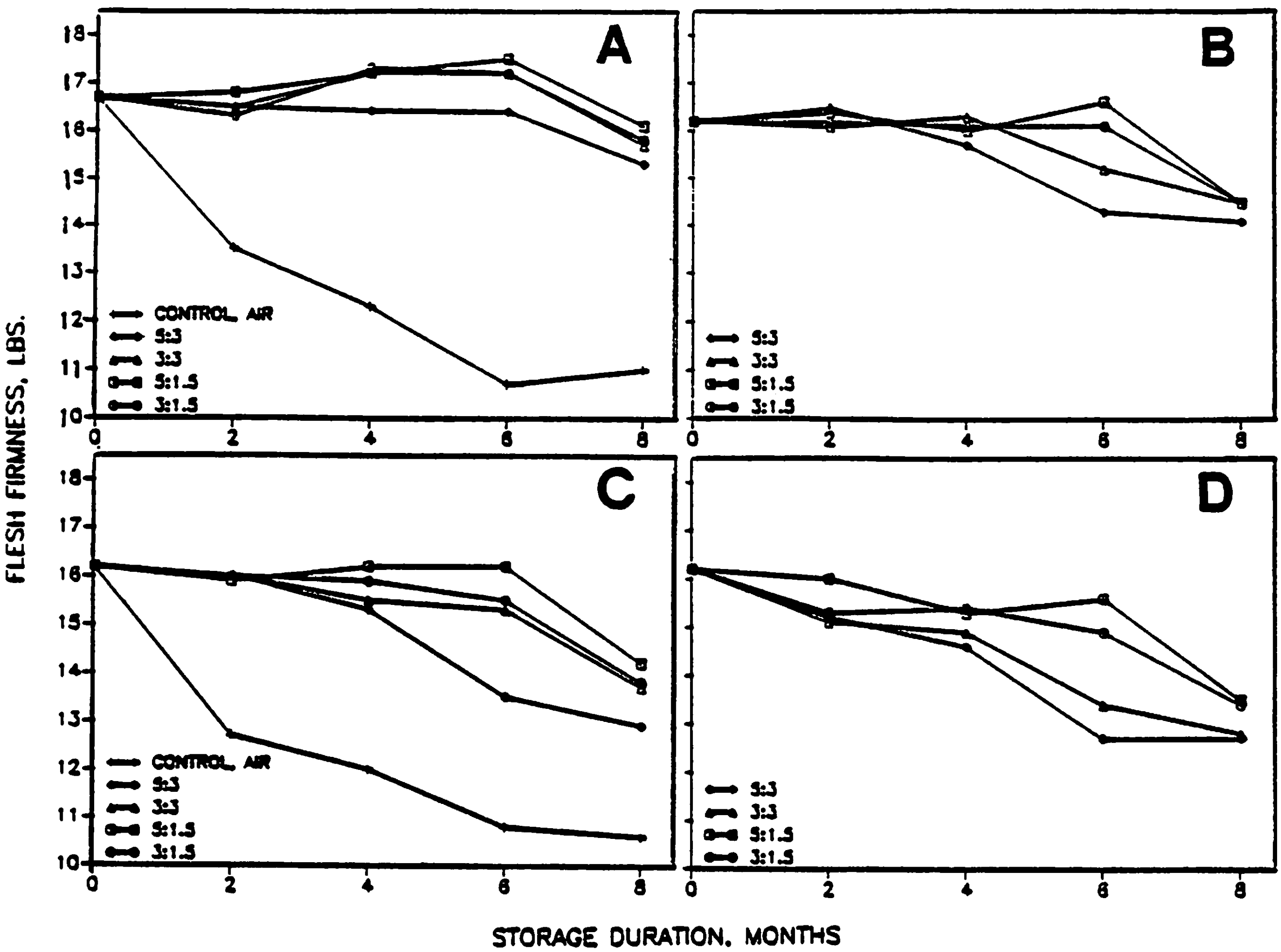

Fig. 1. Changes in flesh firmness of 'Empire' apples in relation to harvest date, time of imposition at CA, and storage atmospheres. (A) 7 day and (B) 14 day delay of CA, first harvest, (C) and (D), second harvest, respectively.

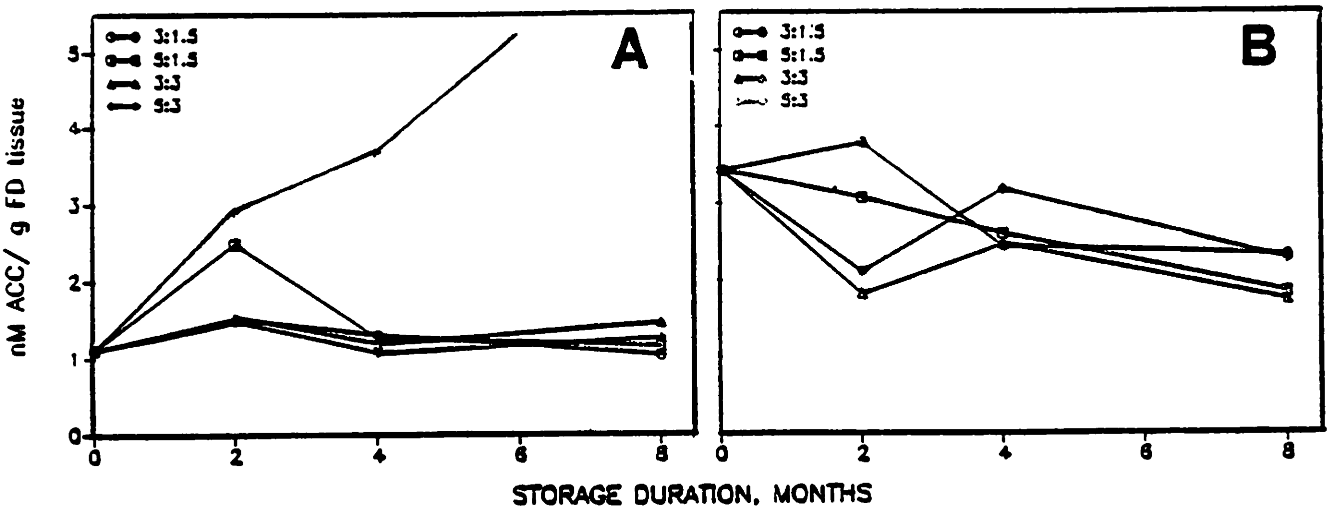

Fig. 2. Changes in 1-amino-cyclopropane-1-carboxylic acid (ACC) content of 'Empire' apples in relation to time of imposition at CA and storage atmospheres . (A) 7 day and (B) 14 day delay of CA first harvest .

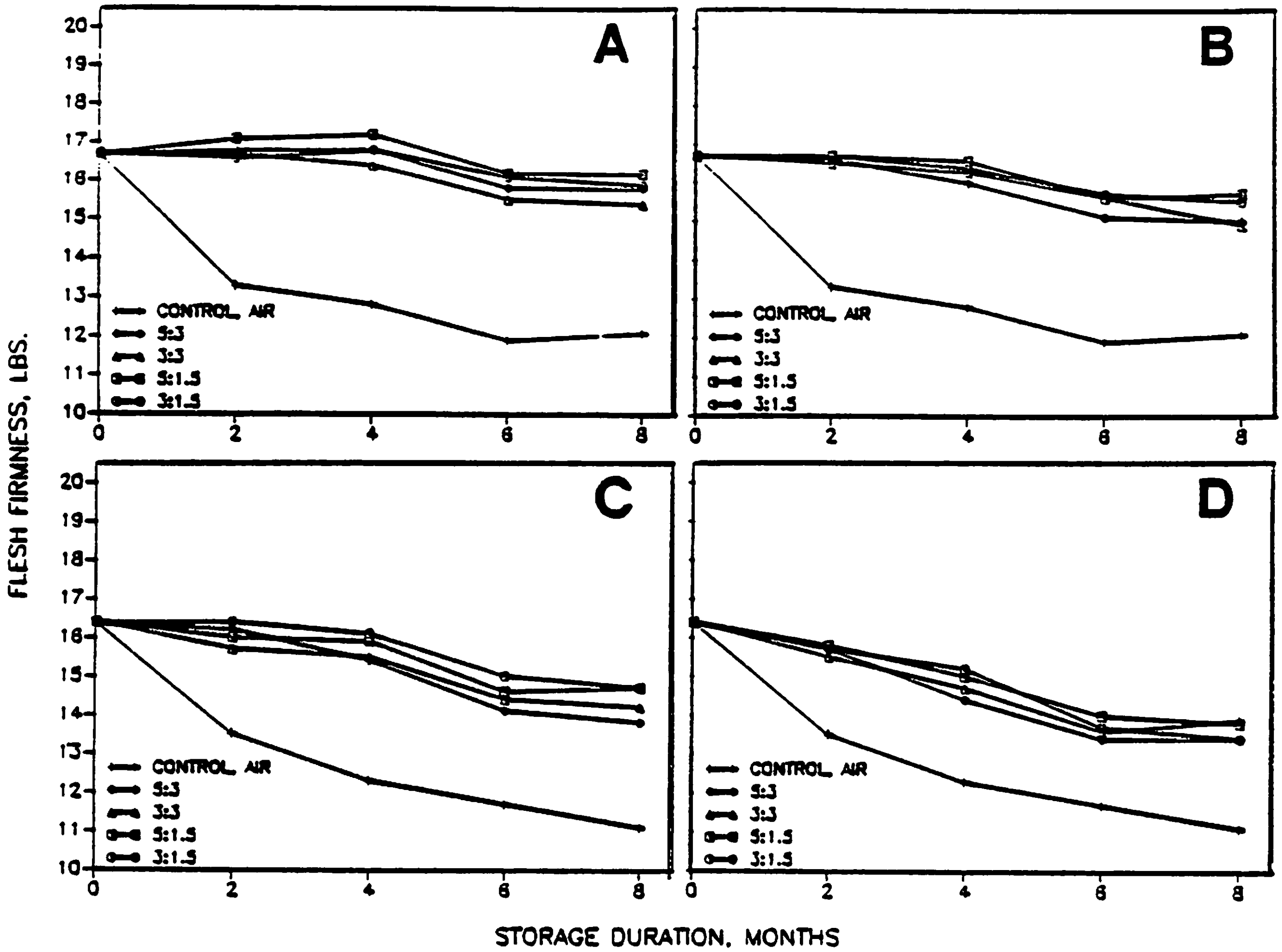

Fig. 3. Changes in flesh firmness of 'Jonathan' apples in relation to harvest date, time of imposition at CA, and storage atmospheres. (A) 7 day and (B) 14 day delay of CA, first harvest, (C) and (D), second harvest, respectively.

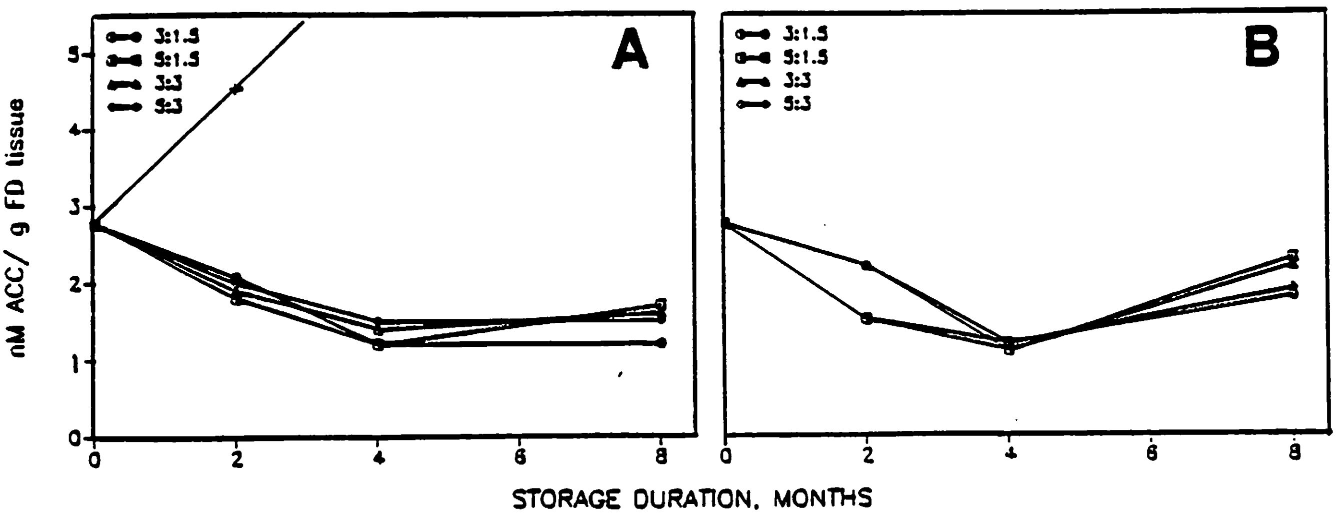

Fig. 4. Changes in 1-amino-cyclopropane-1-carboxylic acid (ACC) content of 'Jonathan' apples in relation to time of imposition at CA and storage atmospheres . (A) 7 day and (B) 14 day delay of CA first harvest .

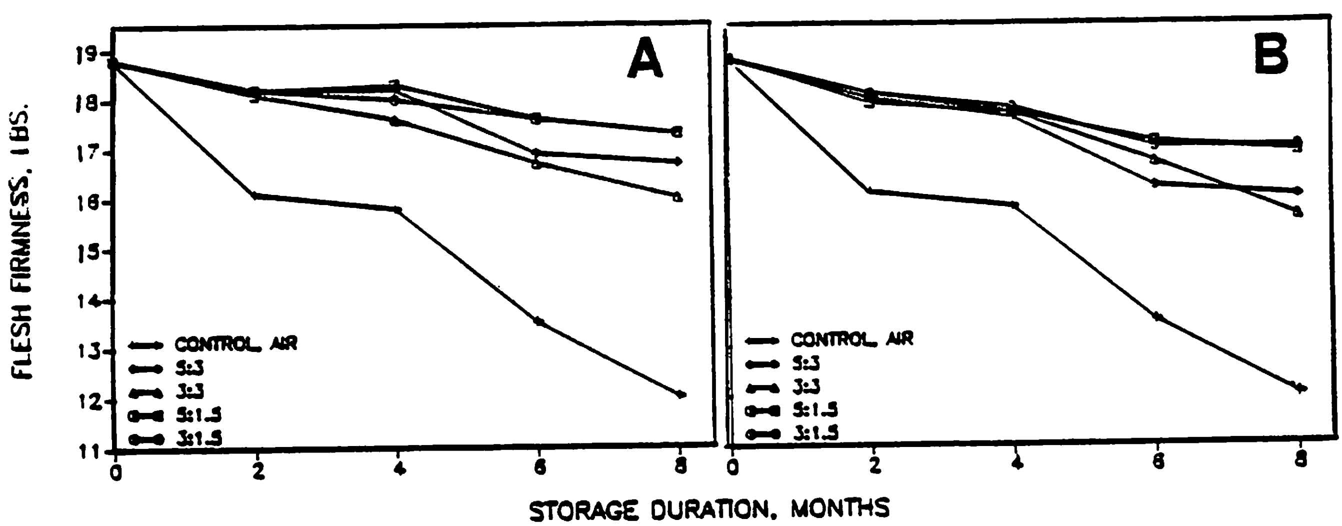

Fig. 5. Changes in flesh firmness of 'Red Chief' apples in relation to time of imposition at CA and storage atmospheres .
(A) 7 day and (B) 14 day delay of CA .

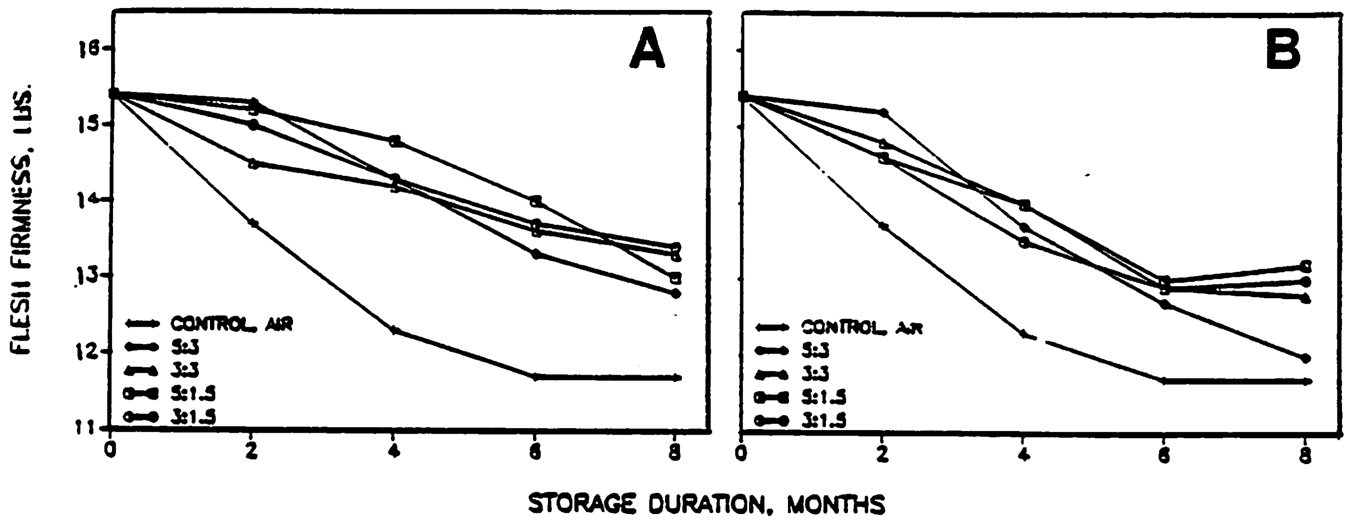

Fig. 6. Changes in flesh firmness of 'Idared' apples in relation to time of imposition at CA and storage atmospheres . (A) 7 day and (B) 14 day delay of CA .

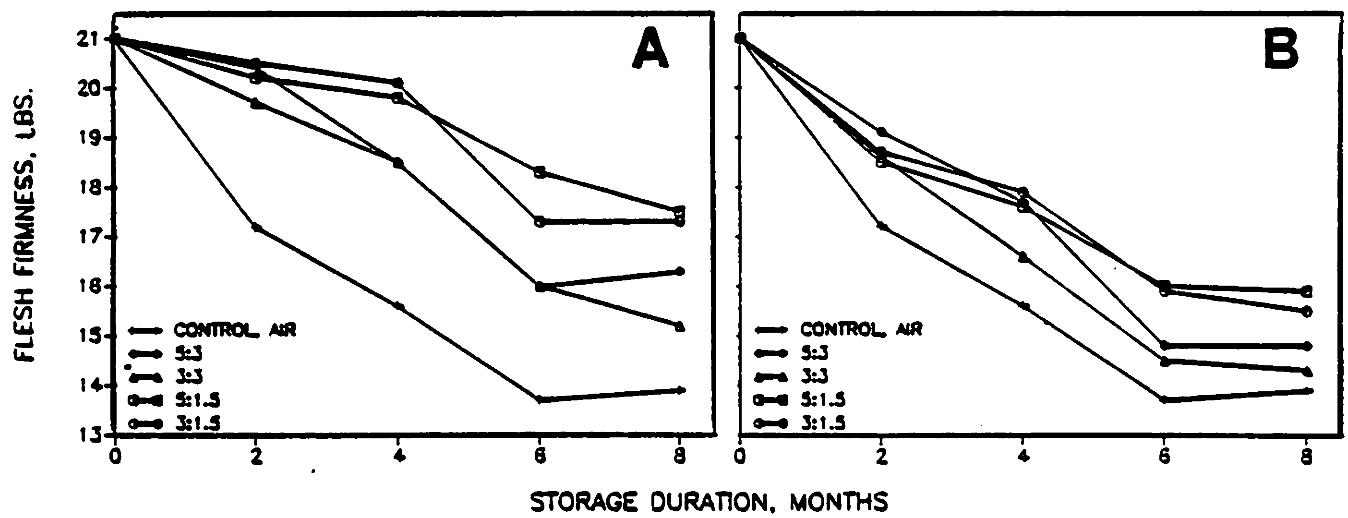

Fig 7. Changes in flesh firmness of 'Law Rome' apples in relation to time of imposition at CA and storage atmospheres . (A) 7 day and (B) 14 day delay of CA .