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Influence of Storage Atmospheres and Procedures on 1-Aminocyclopropane-1-Carboxylic Acid Concentration in Relation to Flesh Firmness in 'Golden Delicious' Apple

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Abstract. **Firmness loss, increase in 1-aminocyclopropane-1-carboxylic acid (ACC) concentrations, and increase in internal ethylene concentrations were greatest in air-stored fruit of 'Golden Delicious' apple (*Malus domestica* Borkh.) and lowest in controlled-atmosphere (CA)-stored fruit receiving a "rapid CA" or a "prestorage high carbon dioxide" storage procedure. Changes in apples kept in "slow CA" were intermediate. The accumulation of ACC in fruit was related closely to the subsequent flesh softening and increase in internal C_2H_4 concentration, and these processes were suppressed to different degrees in CA-stored fruit, depending on the storage procedures.**

'Golden Delicious' apples soften at a relatively high rate in air storage or in conventional CA storage ("slow CA") in which the required atmosphere is established slowly by fruit respiration. Excellent firmness retention in this cultivar was achieved, however, when a "prestorage high CO_2 treatment" (5) or a "rapid CA" storage procedure (8) was used; both were equally effective (9).

The role of ethylene in initiating fruit ripening and softening is well-known (1). In postclimacteric apples, C_2H_4 is synthesized by the sequence: methionine → S-adenosylmethionine (SAM) → 1-aminocyclopropane-1-carboxylic acid (ACC) → C_2H_4 (3). During ripening of various climacteric fruits, increased C_2H_4 synthesis is accompanied by increased ACC concentration in the tissues (6). However, both the conversion of SAM to ACC and the conversion of ACC to C_2H_4 are restricted in preclimacteric fruit tissues (2, 6). Ethylene production and flesh softening of apples during ripening in air at 15° to 20°C also are suppressed by treatments of preclimacteric apples with 1–3% O_2 or 15–20% O_2 + 20–30% CO_2 (10). ACC synthesis in these tissues is delayed initially, but not prevented, and eventually ACC is accumulated in large quantities (10).

This paper describes the changes of ACC levels in the fruit tissue in relation to the rates of flesh softening in 'Golden Delicious' apples stored in air and CA, preceded by a "prestorage high CO_2", "rapid CA", or "slow CA" storage procedure.

Samples of 'Golden Delicious' apples (5 one-fruit replicates per sample for each storage treatment and examination date) were obtained from 5 trees (one apple per tree) in a commercial orchard at Summerland, B.C. on 23 Sept. 1982. They were cooled in 0°C air for 48 hr and then sealed in 3, gas-tight cabinets (0.6 × 1.2 × 2.4 m) placed at 0° on 25 Sept. The storage atmosphere of 2.5% O_2 + 1.5% CO_2 in the "rapid CA" cabinet was established within 24 hr of sealing by use of N_2 and CO_2 gases; it was established in 25 days through fruit respiration in the "slow CA" cabinet. The "17% CO_2 + rapid CA" samples were exposed to an atmosphere of 5% O_2 + 17% CO_2 during the first 10 days followed by storage in 2.5% O_2 + 1.5% CO_2. All CA samples were transferred to 1 cabinet on 12 Oct. and the storage atmosphere was maintained at 2.5% O_2 + 1.5% CO_2 throughout the remainder of the storage period. The air storage samples were kept continuously in a 0° cold storage room (12 × 8.5 × 3.7 m). The concentrations of C_2H_4 inside the storage cabinets were neither controlled nor monitored.

Two samples of fruit for each storage treatment and removal date were removed after 0, 28, 59, 88, and 118 days of storage; and internal C_2H_4 concentration, severity of CO_2 injury, flesh firmness, and ACC concentration were determined on each fruit of the 1st and 2nd sample (5 one-fruit replicates) immediately upon removal from storage and after 1 day in 20°C air, respectively.

A 1-ml gas sample was withdrawn with a syringe from the seed cavities of each apple submerged under water, and C_2H_4 concentration was determined by gas chomatography. Internal C_2H_4 concentrations are given only for those fruit which were kept for 1 day in open air at 20°C after removal from storage, because they would be correlated directly to the C_2H_4 production rates (4). Internal C_2H_4 measurements were not made on fruit immediately after removal from the CA cabinets, as they would represent the sum of the rate of C_2H_4 production and concentration inside the storage cabinet.

The skin surface areas afflicted with CO_2 injury were determined for each fruit. Flesh firmness was determined on each fruit with a Magness-Taylor penetrometer (2 measurements made on opposite pared sides, 11.1-mm tip). For ACC determination, 2 opposite sectors of cortical tissue from each fruit (about a quarter of an apple weighing 50–60 g) were diced and blended with a Waring blender, in 3 ml of 2% HCl (w/v) per g of fruit tissue. The homogenate then was filtered through Whatman No. 4 filter paper under suction from a water aspirator. The ACC concentration in the extract was assayed according to the method of Lizada and Yang (11) after neutralizing with NaOH.

The low levels of ACC (0.3 nmol/g) and the absence of measurable C_2H_4 in 'Golden Delicious' apples picked at the preclimacteric stage on 23 Sept. 1982 (Fig. 1) suggest that synthesis/availability of ACC is a limiting factor regulating C_2H_4 production. It has been shown that preclimacteric fruit tissue lacks capability not only for ACC synthesis but also for the conversion of ACC to C_2H_4 (2, 6). In this study, the ACC level in fruit stored in 0°C air increased markedly (to 39 nmol/g after 27 days) during the 1st month of air storage, then declined sharply (to 7.5 nmol/g after 59 days) during the 2nd month of storage and remained at a low and constant level (3.7 and 4.9 nmol/g after 88 and 118 days, respectively) during the 3rd and 4th month of storage (Fig. 1). The results suggest that the enzyme responsible for ACC synthesis was synthesized at maximum rate during the 1st month of storage in 0° air. The rapid and steady conversion of ACC to C_2H_4 also could be a cause of reduced ACC levels in later periods of storage. Whether the induction of ACC synthase, which catalyzes the formation of ACC from SAM (12), during the early part of air storage at 0° is triggered by the removal of fruit from the tree, by chilling of fruit in the cold storage, and/or by other factors is yet to be determined.

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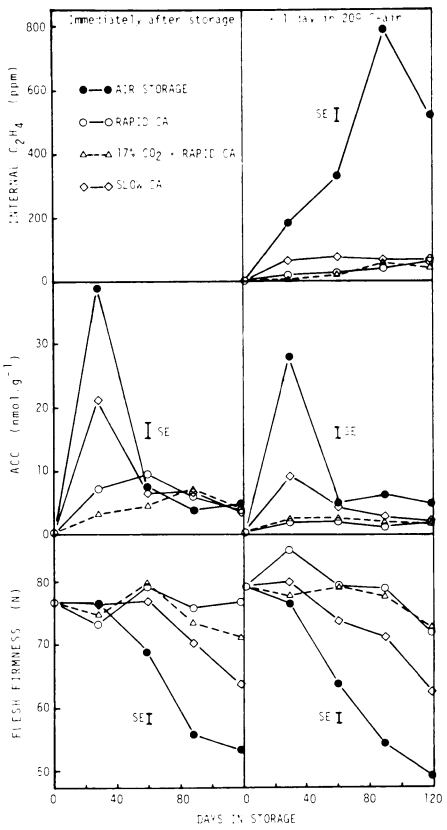

Fig. 1. Changes in flesh firmness and 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene (C_2H_4) concentrations in stored 'Golden Delicious' apples in relation to the storage atmospheres (air vs. CA), storage procedures ("rapid CA" vs. "slow CA" vs. "pre-storage high CO_2"), and storage duration.

The internal C_2H_4 concentration in fruit stored in 0°C air for 2 and 3 months was much higher than that in fruit stored for only 1 month. A decline in ACC level during the 2nd and 3rd month suggested that the ethylene-forming enzyme, which converts ACC to C_2H_4, was not fully developed until the 3rd month of air storage.

The firmness data (Fig. 1) confirm previous reports (8, 9) that the softening rate in 'Golden Delicious' apples is related to the storage atmospheres and the rapidity of establishing the CA conditions. The effectiveness of "rapid CA", "high CO_2", "slow CA", or air storage in maintaining flesh firmness appeared to be related closely to their inhibitory effects on the synthesis/accumulation of ACC and on the subsequent conversion of ACC to C_2H_4 (Fig. 1).

Both "rapid CA" and "high CO_2 prestorage treatment" were more effective than "slow CA" in suppressing the rise of ACC level which occurred during the 1st month of CA storage (Fig. 1). This is expected because it took 25 days to establish the required low-O_2 CA conditions under the "slow CA" storage procedure, during which time an active synthesis of ACC must have occurred. Only small and fairly comparable amounts of ACC and C_2H_4 were detected in fruit subjected to a "rapid CA" or a "prestorage high CO_2" storage procedure (Fig. 1). This could explain, at least in part, why the rates of flesh softening in these fruit samples were lowest and comparable to one another (Fig. 1) (9). Fruit samples from "slow CA", which were firmer than those from air storage but less firm than those from the "rapid CA" or the "prestorage high CO_2" storage procedure, accumulated ACC at an intermediate rate (Fig. 1).

The low levels of ACC and C_2H_4 in the fruit stored with a "rapid CA" or "prestorage high CO_2" storage procedure indicate that the onset of ripening was delayed and the ripening processes were suppressed throughout the storage period, thereby restricting the synthesis of ACC, the formation of C_2H_4, and the softening process. Continuous exposure of preclimacteric apples to elevated CO_2 or low O_2 atmospheres delays ACC synthesis initially, but eventually results in an accumulation of ACC (10), due possibly to an inhibitory effect of high CO_2 on the formation of ACC (10) and the inhibitory effects of low O_2 (3, 10) and high CO_2 (10) on the conversion of ACC to C_2H_4.

In conclusion, the rapid accumulation of ACC and the subsequent, high rates of C_2H_4 production are related closely to the rapid flesh softening observed in fruit stored in air; these processes can be suppressed greatly by storage of fruit in CA, provided the fruit were exposed to low O_2 and/or high CO_2 regimes immediately following harvest. CO_2 injury has been a problem associated with the "prestorage high CO_2" storage procedure (7), and in this study about 70% of the CO_2-treated fruit (9% of the total skin surfaces) exhibited CO_2 injury. Consequently, 'Golden Delicious' apples designated for late marketing should be stored in CA using the simple and safe "rapid CA" storage procedure (8, 9), in order to suppress the synthesis of ACC and C_2H_4, to reduce the loss of flesh firmness, and to maximize the storage life of the fruit.

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