

Involvement of Ethylene in Chlorophyll Degradation in Peel of Citrus Fruits¹

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ABSTRACT

The effect of ethylene on chlorophyll degradation in the peel of Robinson tangerine (*X Citrus reticulata* Blanco) and calamondin (*X Citrofortunella mitis* [Blanco] Ingram and Moore) fruits was studied. The chlorophyll degrading system in the peel of these two citrus species was not self-sustaining but required ethylene to function. Chlorophyll degradation ceased immediately when fruit were removed from ethylene and held in ethylene-free air at 0.2 atmospheric pressure. However, at atmospheric pressure, chlorophyll degradation continued for 24 hours in the absence of exogenous ethylene. Although chlorophyllase levels were negatively correlated with chlorophyll content in the peel ($r = -0.981$; $P < 0.01$), the level of chlorophyllase activity did not change when fruit were removed from ethylene, even though chlorophyll degradation had stopped. From these observations, it was concluded that ethylene is necessary for chlorophyll degradation in the two species of citrus studied, but its primary role is not solely for the induction of chlorophyllase activity.

Although ethylene is widely and effectively used to promote Chl degradation in early season citrus fruit, its role in Chl destruction during natural coloring is controversial (2). The mechanism by which ethylene, whether produced endogenously by the fruit or supplied exogenously, degrades Chl also has not been clearly delineated (10). During natural degreening and postharvest degreening with ethylene, the internal structure of the chloroplast breaks down (10, 12, 15). Ethylene also induces an increase in chlorophyllase activity (1, 3, 10, 13), but during natural degreening and regreening of Valencia (late season) orange, chlorophyllase levels parallel Chl content (1). Chlorophyllase levels are inversely related to Chl content during degreening with ethylene (2, 10). Whether the loss of Chl is causally related to the level of chlorophyllase or some other factor is not clearly established. In this paper, we present data which suggest that ethylene is required for Chl degradation and that the level of chlorophyllase is not the regulating factor in Chl degradation.

MATERIALS AND METHODS

Mature green calamondin (*X Citrofortunella mitis* [Blanco] Ingram and Moore) and Robinson tangerine (*X Citrus reticulata* Blanco) fruits were harvested within 2 h prior to treatment. The fruit were washed and air-dried before being placed in chambers containing 7.7 $\mu\text{l/l}$ ethylene or ethylene-free air at 27 C and 95% RH. Ethylene was dispensed continuously into the chambers, as described by Barmore and Wheaton (4), and the concentration

was monitored with a gas chromatograph equipped with a flame ionization detector.

Color change of Robinson tangerine fruit was determined by measuring the a/b ratio, with a Hunter (Hunter Associates Laboratory, Fairfax, VA) Color Difference meter (16), of three marked spots on 10 individual fruit of each treatment at 24-h intervals. This instrument measures reflected light using a L, a , b system. The a value corresponds to a red-green scale on which red values are positive and green values are negative. The b value corresponds to a yellow-blue scale on which yellow is positive. The a/b ratio is negative for green fruit, approximately zero for yellow fruit, and positive for orange fruit. It is presumed that green is due to Chl. Measurements were made within 0.5 h after removal of fruit from the chambers, and the fruit were returned to the chambers immediately after measurements were made.

Chl and chlorophyllase activity of calamondin fruit were extracted and analyzed, as previously described (10).

The amount of ethylene diffusing from calamondin fruit was determined on fruit which had previously been exposed to 7.7 $\mu\text{l/l}$ ethylene for 24 h. Two sets of fruit were used. One set was removed from the ethylene chambers and placed directly into a 0.5-liter glass container. The second set was subjected to 0.2 atm pressure for 0.5 h to remove ethylene which may have accumulated in the fruit. A continual flow of moist air was introduced during evacuation of the ethylene. Ethylene accumulation in the glass containers was then determined during the succeeding 24 h. Each sample consisted of 15 fruit.

All experiments were repeated two to three times with three replications each time. However, data are presented for single experiments when Chl content and chlorophyllase activities of the fruit were different at the beginning of individual experiments. The pattern of treatment effects was the same for all experiments.

RESULTS

Chl degradation in Robinson tangerine peel, determined by an increase in the a/b ratio, increased during continuous exposure to 7.7 $\mu\text{l/l}$ ethylene (Fig. 1). Although there was some color change during the first 24 h of exposure, the greatest change occurred during the succeeding 24 h. Even when the fruit were removed to ethylene-free air for 24 h, they continued to degreen. However, no further degreening occurred if the fruit were kept in ethylene-free air beyond 24 h (Fig. 1). If fruit were returned to 7.7 $\mu\text{l/l}$ ethylene after 24 h in ethylene-free air, degreening continued at a rate near that of fruit maintained continuously in ethylene. The slight degreening occurring in the peel of control fruit could have been caused by a low endogenous ethylene production (8).

Chl degradation in the peel of calamondin fruit was promoted by continuous exposure of the fruit to 7.7 $\mu\text{l/l}$ ethylene (Fig. 2A). The rate of degradation, however, was not constant over the entire exposure period. The maximum rate of Chl degradation occurred between 24 and 48 h. Chl degradation continued for 24 h in the

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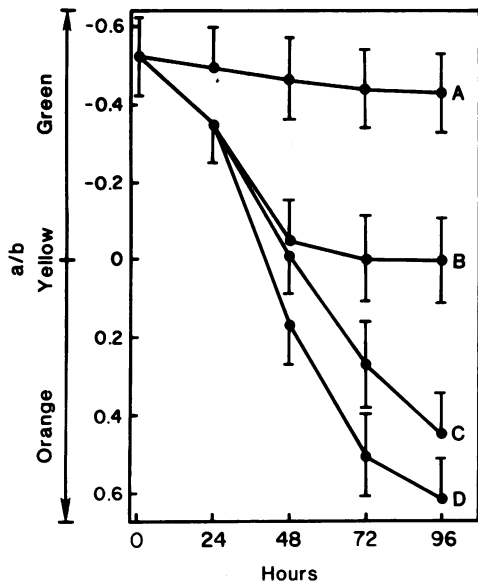


FIG. 1. Change in a/b ratio of peel of Robinson tangerines exposed to continuous or intermittent ethylene at 7.7 $\mu\text{l/l}$. A, Fruit maintained in pure air; B, fruit exposed to 7.7 $\mu\text{l/l}$ ethylene for 24 h and removed to pure air for remainder of experiment; C, fruit exposed to 7.7 $\mu\text{l/l}$ ethylene for 24 h, pure air for 24 h, and 7.7 $\mu\text{l/l}$ ethylene for remainder of experiment; D, fruit exposed to 7.7 $\mu\text{l/l}$ ethylene for entire experiment. Each point represents the mean and SD of 10 fruit.

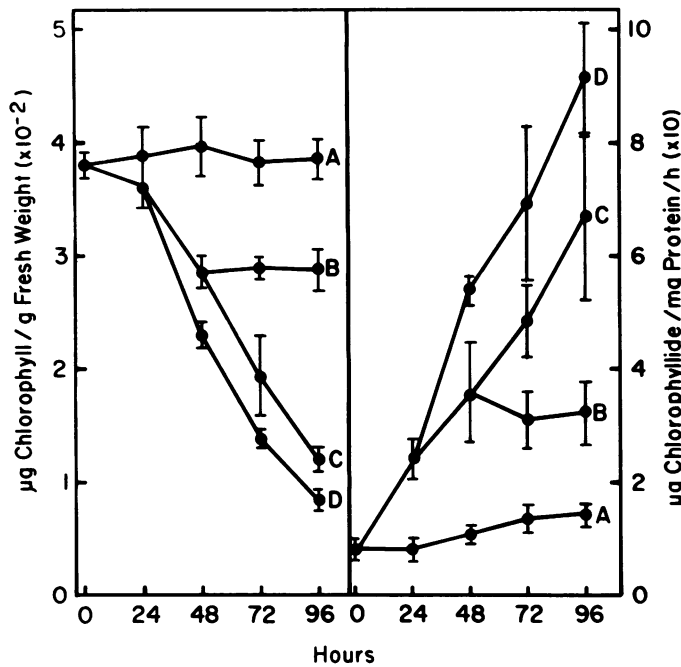


FIG. 2. Change in Chl content (left panel) and chlorophyllase activity (right panel) in the peel of calamondin fruit exposed to continuous or intermittent ethylene at 7.7 $\mu\text{l/l}$. Treatments the same as in Figure 1. Each point represents the mean and SD of three samples of three fruit each.

peel of fruit which had been removed to ethylene-free air following a 24-h exposure to 7.7 $\mu\text{l/l}$ ethylene (Fig. 2A). The rate of degradation was less than the rate in the peel of comparable fruit maintained in ethylene. Within 24 h after the fruit were removed from ethylene, Chl degradation ceased. If, however, the fruit were returned to ethylene after 24 h in ethylene-free air, Chl degradation continued at a rate comparable to that for fruit maintained continuously in ethylene. In contrast to tangerine fruit, no de-

greening occurred in control calamondin fruit in these experiments.

An increase in chlorophyllase activity was also induced by continuous exposure of calamondin fruit to 7.7 $\mu\text{l/l}$ ethylene (Fig. 2B). The largest increase in activity occurred between 24 and 48 h. Chlorophyllase activity continued to increase during the first 24 h after fruit were removed from ethylene and placed in ethylene-free air, but no further increase was observed during the succeeding 24 h. If, however, the fruit were returned to ethylene after 24 h in ethylene-free air, chlorophyllase induction resumed with rates comparable to those for fruit maintained continuously in 7.7 $\mu\text{l/l}$ ethylene (Fig. 2B). The level of chlorophyllase activity was negatively correlated with Chl content of the tissue for all treatments and exposure times ($r = -0.981$; $P < 0.01$).

These data raised the possibility that ethylene in the tissue is an absolute requirement for Chl degradation. Measurements of the ethylene diffusing from calamondin fruit confirmed that ethylene was still present in the fruit for several hours following their removal from ethylene (Fig. 3). However, only trace amounts of ethylene diffused from the fruit 24 h after removal of the fruit from ethylene (Fig. 3). The rate of diffusion of ethylene dropped rapidly after the fruit were removed from ethylene (Fig. 3). Exposing the fruit to 0.2 atm pressure for 0.5 h after removing them from ethylene reduced the total amount of ethylene diffusing from the fruit (Fig. 3). Maintaining the fruit at 0.2 atm pressure following a 24-h exposure to 7.7 $\mu\text{l/l}$ ethylene greatly reduced Chl degradation (Fig. 4A). The induction of chlorophyllase activity also ceased at subatmospheric pressure (Fig. 4B).

DISCUSSION

The natural coloring of citrus fruits consists of two phases, the degradation of Chl and the biosynthesis of carotenoids (16). Although ethylene promotes both phases, it is primarily used to remove the Chl, since carotenoid synthesis (especially β -citaurin) is sensitive to temperature, and carotenoid accumulation is reduced at degreening temperatures (16). The nature of the ethylene-induced Chl degrading system has not been fully described. However, two conclusions can be drawn from the data presented here. First, the Chl degrading system in calamondin and Robinson tangerine fruits is not self-sustaining but requires a continuous supply of ethylene to continue functioning. Second, although chlorophyllase activity may be an integral part of the Chl degrading system, it does not regulate Chl degradation.

Whether ethylene participates directly, *i.e.* interacts with the thylakoid membranes to release Chl molecules, or whether it

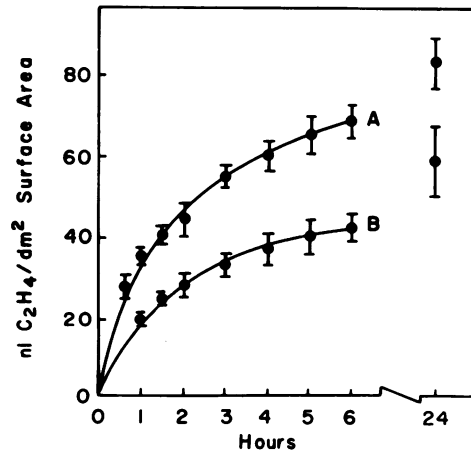


FIG. 3. A, Cumulative ethylene diffusing from calamondin fruit immediately following a 24-h exposure to 7.7 $\mu\text{l/l}$ ethylene; B, fruit exposed to 0.2 atm pressure for 0.5 h before ethylene determinations started. Each point represents the mean and SD of three samples of 15 fruit each.

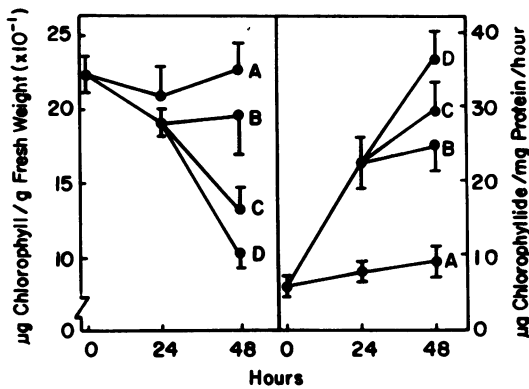


FIG. 4. Left panel, change in Chl content in the peel of calamondin fruit exposed to continuous or intermittent ethylene at $7.7 \mu\text{l/l}$. A, Fruit maintained in pure air; B, fruit exposed to $7.7 \mu\text{l/l}$ ethylene for 24 h, then maintained at 0.2 atm air pressure for remainder of experiment; C, fruit exposed to $7.7 \mu\text{l/l}$ ethylene for 24 h, then maintained at 1.0 atm air pressure for remainder of experiment; D, fruit exposed to $7.7 \mu\text{l/l}$ ethylene for entire experiment. Right panel, change in chlorophyllase activity in acetone powder extracts of calamondin fruit peel during continuous or intermittent exposure to ethylene at $7.7 \mu\text{l/l}$. Each point represents the mean and SD of six samples of three fruit each.

induces another enzyme whose activity precedes chlorophyllase activity in Chl degradation is not certain. If another enzyme is induced by ethylene, its turnover rate is obviously much greater than that of chlorophyllase. Suttle and Kende (14) suggest that the ethylene-induced increase in membrane permeability during senescence is enzyme-mediated, and the same may be true for the release of Chl from thylakoid membranes.

The nonsustaining nature of the Chl degrading system in citrus fruits is typical of processes promoted by ethylene in nonclimacteric fruit (5, 11). Nonclimacteric fruit generally respond to alternate ethylene and air treatments, whereas, in climacteric fruit, processes triggered by ethylene are sustained by the autocatalytic production of ethylene. In addition, processes promoted by ethylene in nonclimacteric fruit respond directly to ethylene concentrations. Within certain limits, Chl degradation in citrus peel has been shown to vary with ethylene concentration (8).

The carryover effect of ethylene for 24 h following the removal of the fruit from ethylene may be due to the ethylene remaining in the fruit or to the autocatalytic production of ethylene by the fruit. Although citrus fruits are nonclimacteric (5, 6, 11), they are capable of producing small quantities of ethylene (6). A carryover effect of ethylene for 1 additional day has been reported for degreening Hamlin oranges and Dancy tangerines (8). Essentially no carryover effect of ethylene was observed for lemons (7).

The absolute requirement for ethylene to sustain Chl degradation after the process has been initiated (this study; Refs. 7 and 8) suggests that ethylene may also be involved in Chl degradation during the natural coloring of citrus fruits. However, this conflicts with the conclusion of Apelbaum *et al.* (2) that endogenous ethylene is not the primary inducer for the natural color change in Shamouti oranges. However, they did not report internal ethylene concentrations of the fruit. In contrast, untreated Hamlin

oranges had color changes equivalent to treatment with 0.03 to $0.05 \mu\text{l/l}$ ethylene, a level of ethylene which was also found internally in untreated fruit (8). In our study, subatmospheric pressure greatly reduced Chl degradation, whereas, in the study of Apelbaum *et al.* (2), subatmospheric pressure did not alter color change. Differences in the sizes of calamondin and Shamouti orange fruit may have affected removal of ethylene with vacuum, or the mechanism of Chl degradation in Shamouti oranges may differ from that in calamondin and Robinson tangerine fruit. Chl degradation continues in Satsuma mandarin peel for 3 to 4 days after being induced with unusually high concentrations (500 – $1,000 \mu\text{l/l}$) of ethylene for about 15 h (9). However, inasmuch as CO_2 buildup inhibited Chl degradation, it is likely that ethylene remaining in the tissue was responsible for Chl degradation (9).

Although ethylene induces chlorophyllase activity in citrus peel and chlorophyllase is necessary for the ultimate degradation of Chl, chlorophyllase apparently is not the regulator of Chl degradation during degreening with ethylene. Chlorophyllase levels increased when ethylene was present, even though the Chl content was low; but, levels changed little when ethylene was removed. On the other hand, Chl degradation seemed to occur when chlorophyllase activity was high or was increasing. Thus, in our study, ethylene in conjunction with chlorophyllase was necessary for Chl degradation in calamondin peel.

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