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Carotenoids in Citrus: Their Accumulation Induced by Ethylene

Application of ethylene to citrus fruit after harvest enhanced external orange and red color by inducing accumulation of specific carotenoid pigments. Cryptoxanthin, β-citraurin, and to a lesser extent, violaxanthin, accumulated in the flavedo of Robinson and other citrus cultivars following treatment with ethylene or 2-chloroethylphosphonic acid (ethephon).

Ethylene has long been used to reduce the chlorophyll content of citrus peel following harvest. More recently, preharvest application of ethylene supplying materials to citrus resulted in hastening degreening on the tree (Young *et al.*, 1970). Although some early work by Baier (1932) indicated that ethylene could also improve orange pigmentation of the fruit, the work of Miller *et al.* (1940) generally has been accepted and often quoted, "Carotenoids in the peel showed no significant changes as a result of ethylene treatment."

We found, however, that postharvest ethylene application to certain citrus cultivars resulted in substantial increase of carotenoid pigments and consequently improved fruit color. In the cultivars studied, the pigments that increased most were those that contributed most to the orange and red colors, *i.e.*, cryptoxanthin (orange) and β-citraurin (reddish orange) (Figure 1). *Trans-* and *cis*-violaxanthin (yellow) increased to a lesser extent.

Subsequent to the initial preparation of this manuscript, Daito and Hirose (1970) reported enhanced carotenoid pigmentation following ethephon treatment. Our results were qualitatively similar to theirs. However, we have provided in more detail the changes in individual pigments resulting from ethylene treatment and have related them to external fruit appearance.

EXPERIMENTAL

Ethylene was applied to fruit of several cultivars, including Robinson, Orlando, Temple, Pineapple, and Hamlin. Although responses varied, ethylene application caused increased carotenoid accumulation in all tested cultivars. Since the Robinson gave the most marked response and was studied in greatest detail, most of the results reported here will be based on work with Robinson fruit.

Fruit were placed in 20-l. jars at room temperature and ethylene was added to give a concentration of 10 ppm. Ethylene was added on three consecutive days. The jars were flushed with air prior to each ethylene addition and then closed for 24 hr. After the third day, the jars were maintained at room temperature with a loose plastic cover to reduce moisture loss.

External fruit color was measured with a Hunter Color and Color Difference Meter. With this reflectance instrument, "a" values are on a green to red scale and "b" values on a blue to yellow scale. The a/b ratio increased with increasing orange or red color and was well correlated with visual differences among the fruit and with the USDA citrus fruit color standards (Harding and Sunday, 1953). This ratio was used in quantitative color evaluation.

Carotenoid pigments in the peel samples were extracted, saponified, and then tentatively identified and quantitatively determined using high pressure liquid chromatography (Stewart and Wheaton, 1971). The identity of the pigments was confirmed by studies of crystalline carotenoids obtained from the peel of several hundred kilograms of Robinson incubated with 10 ppm of ethylene for 3 days and then held at room temperature until a red color developed. Cryptoxanthin was identified by cochromatography with crystalline material from Dancy tangerines, egg yolk, yellow papaya, and balsam apple using the tlc method of Hager and Meyer-Bertenrath (1966), zinc carbonate tlc, and liquid chromatography (Stewart and Wheaton, 1971). Visible absorption peaks in hexane were at 477, 449, and 426 (sh). The mass spectrum showed the parent peak at *m/e* 552.4315; calculated for $C_{40}H_{56}O$ 552.4330. β-Citraurin was identified by cochromatography using the methods given above with a crystalline sample from Dancy tangerine. Visible absorption maxima in hexane were at 480, 453, and 428 (sh). There were no well-defined peaks in ethanol. A strong peak in the infrared spectrum was observed at 1665 (conjugated carbonyl). The mass spectrum showed the parent peak at *m/e* 432.3067; calculated for $C_{30}H_{40}O_2$ 432.3027.

RESULTS AND DISCUSSION

Robinsons harvested in October and placed in 10 ppm of ethylene for 3 days degreened in 1 week. During the following 2 weeks, the fruit changed from yellow to orange and then red. Control fruit which received no ethylene degreened

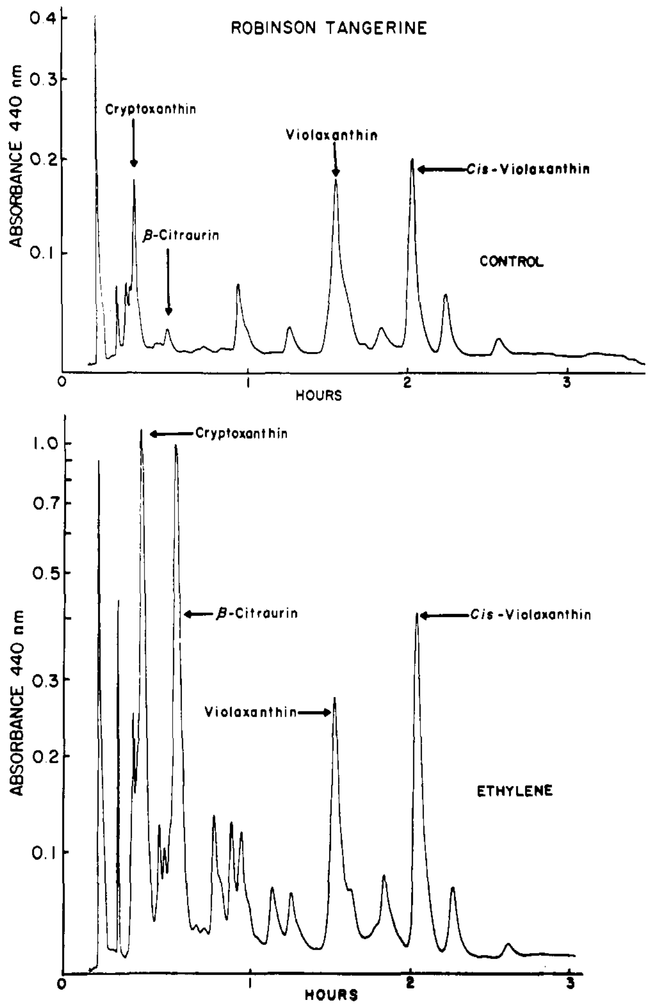

Figure 1. Liquid chromatographic separation of carotenoid pigments from the flavedo of control and ethylene-treated Robinson tangerine fruit

slowly and then remained yellow until decay commenced. Analysis of the carotenoids from these fruit revealed that the levels of cryptoxanthin and β-citraurin increased rapidly in the ethylene-treated fruit (Figure 2). These two pigments undoubtedly contributed to the orange and red color of this fruit. Violaxanthins were predominant pigments in the yellow control fruit. The results obtained by dipping fruit in 250 to 1000 ppm of ethephon were similar to those obtained with ethylene gas.

Orlando tangelos, Temples, and oranges harvested after natural degreening had occurred on the tree also responded to ethylene treatments with improved color and higher levels of carotenoids. Orlando tangelos exposed to ethylene for 5 days had an a/b ratio of 1.29 ± 0.05, as compared with 0.70 ± 0.07 for controls (mean and standard error of the mean). Temples, after 4 day's exposure to ethylene, had an a/b ratio of 0.83 ± 0.02, compared with 0.40 ± 0.03 for the controls. Pineapple and Hamlin oranges both showed an increase in color when exposed to ethylene.

We also found that ethylene-induced carotenoid accumulation is quite temperature sensitive and is inhibited at 30 °C and above. This finding is supported by earlier observations of Baier (1932) that, although ethylene degreening occurs most

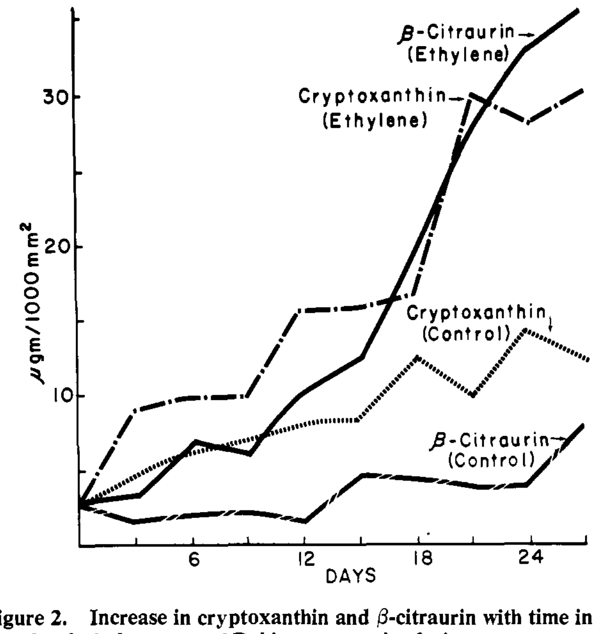

Figure 2. Increase in cryptoxanthin and β-citraurin with time in the flavedo of ethylene-treated Robinson tangerine fruit

rapidly at relatively high temperatures, optimum fruit color is achieved at lower degreening temperatures. Perhaps the importance of ethylene in enhancing citrus peel carotenoid levels generally has not been recognized because of the relatively high temperatures frequently used for ethylene degreening.

Recently, compounds other than ethylene have been found to have an effect on citrus carotenoids. Cooper *et al.* (1968) reported an increase in carotenoids from preharvest application of abscisic acid, but found no ethylene being evolved from the treatment. The pigments involved were not identified. Coggins *et al.* (1970) discovered that an application of 2-(4-chlorophenylthio)triethylamine HCl to harvested fruit caused a dramatic increase in lycopene content, although this is not a major peel carotenoid pigment in most citrus varieties. Our work demonstrates that the accumulation of specific normally occurring citrus carotenoid pigments can be greatly enhanced and fruit color improved by postharvest ethylene application.

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