

Fig. 3. Relative mobility (RM) of ABA as a function of tea crabapple leaf age. RM defined as the amount of ABA present in the diffusate divided by the amount of residual ABA in leaves following exudation. (A = apical, M = medial, B = basal.)

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## Effect of Washing 'Hamlin' Orange on Chlorophyll and Carotenoid Changes During Degreening<sup>1</sup>

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**Abstract.** 'Hamlin' oranges were washed by hand or with various mechanical-washer procedures. The latter included variations in washing and brushing time and brush-bristle size. Washing procedures had little effect on subsequent chlorophyll loss during degreening. Carotenoid synthesis was significantly reduced by washing before degreening. These reductions in carotenoid levels were not appreciably affected by changes in washing time or brush-bristle size. Hand-washing oranges with a sponge reduced carotenoid synthesis less than mechanical washing. Carotenoid synthesis was reduced by less brushing than was required to clean the fruit, but "over" brushing had little further effect.

Early-maturing cultivars of citrus are normally degreened to improve their color before packing and marketing. Degreened fruit are usually not washed until after they are colored and ready for packing. Processes which follow washing, however, such as grading or color sorting (4), would be more effective if done before degreening. Washing also may contribute substantially to decay control (3, 5, 6) where *Collectotrichum gloeosporioides* Penz., or possibly *Diplodia natalensis* Pole-Evans, is a problem, and particularly when followed by a fungicide application (5, 6). Previous work has indicated that washing retards degreening (2); although in our work, washing had little or no effect on the rate of chlorophyll loss in several cultivars (3, 4). Some reduction in carotenoid synthesis was noted in tests on 'Hamlin' orange, however (3). Since there would be advantages to washing before degreening, work on 'Hamlin' orange was continued to determine if other washing procedures might minimize the color-development problem.

#### Materials and Methods

Fruits of 'Hamlin' orange [*Citrus sinensis* (L.) Osb.] grown on rough-lemon [*C. limon* (L.) Burm. f.] rootstock were harvested and prepared for degreening within 6 hr (except where delay was a treatment). Oranges were washed with a commercial washer with 6 transverse brushes rotating at ca 150 rpm. Single samples of 20 fruits each were washed with city tap water and a nonfugicidal cleaner. A brusher with 10 brushes rotating at ca 200 rpm followed the washer. A water rinse was provided between the washer and brusher. Fruits were dried in a warm-air (63° C) roller-drier. The degreening room was held at 29° C with 90 to 95% relative humidity and 5 to 10 ppm ethylene. Oranges were left in ethylene to complete degreening, then transferred to 21°, except in 1973. In the 1973 tests, oranges were left in ethylene at the treatment temp continuously to avoid effects of changing conditions on pigment responses. Changes in chlorophyll were determined by a light-transmittance difference meter (1) with an integrating sphere sample system and filters for measurements which were recorded as  $\Delta OD$  695-740 nm. Changes in carotenoids were measured by using a reflectance attachment to the difference meter

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and data recorded as  $\Delta R$  540-650 nm. Fruit were oriented so that repeated measurement were comparable.

The treatment combinations varied among the 7 tests (Table 1). All brushes were made of synthetic fiber. The brushes with 8- and 12-mil (.203 and .305 mm) diameter bristles were of uniform 2.5 cm length while the 14-mil (.356 mm) diameter bristles were of a scalloped "tumbler" design. The brusher unit was equipped with 12-mil brushes in all tests. Fruits were moved continuously and at a nearly uniform rate through the units. Variations in brush contact time were provided by repeating the washing and brushing cycles. In the 1973 tests, visual estimates of the cleaning efficiency of the various treatments were recorded, based on estimated percentage of the rind surface cleaned.

Chlorophyll and carotenoid data from the last 4 tests were analyzed statistically using individual fruit measurements. Numerous significant differences were found, but many of these were inconsistent from test to test or involved treatment differences of less than .01. The latter approach the level of accuracy with the difference meter and are well below the levels considered minimum for practical treatment differences (ca .10  $\Delta OD$  for chlorophyll and ca .07  $\Delta R$  for carotenoids). For this reason, emphasis will be placed on the more important and consistent responses.

### Results and Discussion

No effect of washing on chlorophyll loss was evident in 1970 and 1971 tests (data not shown). Significant effects of washing on chlorophyll changes were found in 3 of the last 4 tests. These were inconsistent between tests, however, and as shown for the November 5, 1973 test, many of the differences were small (Fig. 1A). Some significant effects in this test were also due to initial sample variations rather than treatment responses.

Of the other factors included in these studies, drying of fruit after washing and the humidity level during degreening had no effect on chlorophyll changes. Brushing significantly lowered the chlorophyll level in the November 5, 1973 tests (data not shown), but differences were small and did not occur in other tests. Number of washing cycles and brushbristle size had small, inconsistent, and usually nonsignificant effects on chlorophyll changes. These results indicate that washing effects on chlorophyll losses were too small to be of practical importance, supporting previous conclusions (3).

Washing had a greater effect on carotenoid changes than on

Table 1. Harvest date, treatment description, number of samples, and pigment measurements made in tests on 'Hamlin' oranges.

Harvest date	Treatment description <sup>z</sup>	No. samples treated	Pigment measured
10-14-70	MW, B, D applied separately or in combinations SW, NW.	7	Chlor.
11-17-70	MW, B, D applied separately or in combinations SW, NW, + low and high humidity.	14	Chlor. (Car.) <sup>y</sup>
11-30-71	1, 2, 3 MW + 1 B, 1 MW + 1, 2, 3 B, SW, NW.	8	Chlor. (Car.)
11-13-72	1, 3 MW + 8, 12, 14-m + 0, 1, 2, 3 B, SW, SWC, NW.	27	Chlor. (Car.)
12-4 & 5-72	1, 3 MW + 8, 12, 14-m + 0, 1 B, SW, SWC, NW, + 0 or 1-day delay before washing.	30	Car.
11-5-73	1, 3 MW + 8, 12, 14-m + 0, 1, B, SW, SWC, NW.	15	Chlor. Car.
11-12-73	1, 3 MW + 8, 12, 14-m + 0, 1 B, SW, SWC, NW + degreened at 27 or 29°C.	30	Chlor.

<sup>z</sup> Treatment abbreviations are: MW, mechanical-washer; B, brusher; D, drier; SW, sponge-washed by hand; SWC, sponge-washed with fruit cleaner; NW, not washed; 0, 1, 2, 3 range in number of cycles tested; 8, 12, 14-m bristle fiber diameter, mils.

<sup>y</sup> Carotenoid measurements enclosed in parentheses were made only at the end of the test.

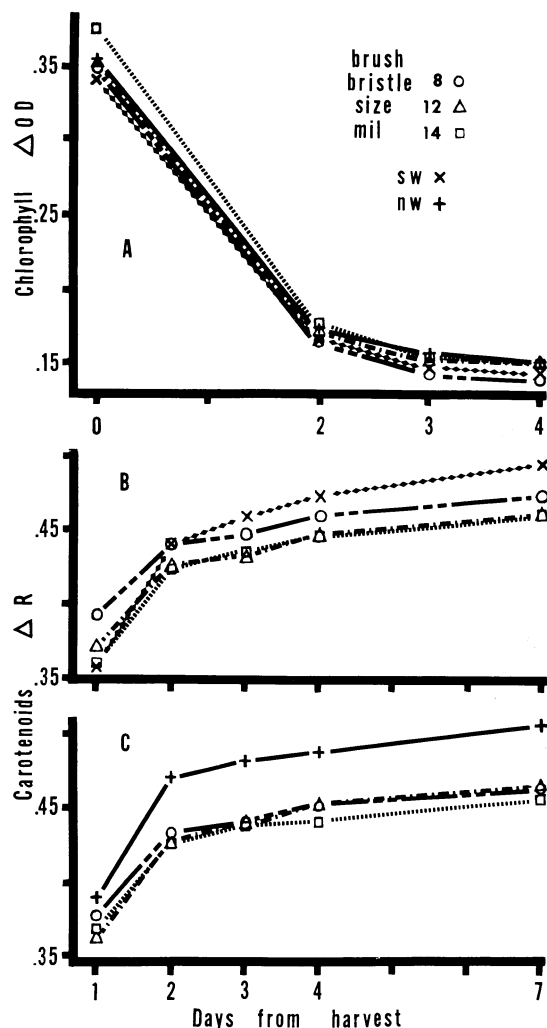


Fig. 1. Pigment changes in 'Hamlin' orange during degreening in relation to washing treatments. A. Chlorophyll measurements ( $\Delta OD$  695-740 nm) in fruit washed mechanically for 3 cycles. B, C. Carotenoid measurements ( $\Delta R$  540-650 nm) in fruit washed mechanically 1 time (B) or 3 times (C). All fruit brushed 1 cycle. SW, sponge washed by hand; NW, not washed. Fruit harvested November 5, 1973.

chlorophyll changes in 'Hamlin' orange (Fig. 1B and C). Significantly higher carotenoid levels developed in unwashed than in mechanically washed fruit. Levels in sponge-washed fruits were intermediate and on day 7 significantly better than in mechanically washed fruit. The small differences present at the start of the test (Fig. 1), were mainly due to differences in chlorophyll levels to which this measurement was also sensitive, and were not necessarily related to later treatment effects. No consistent effects of brush-bristle size occurred, and there was no effect of number of washer or brusher cycles.

Washing significantly reduced the rate of carotenoid development in the December 4, 1972 test (Fig. 2), and this effect was greater in mechanically washed than in sponge-washed fruit. Differences shown between dates of harvest were largely due to initial sample differences. Significantly lower carotenoid levels developed in fruit washed 3 times compared to those washed once. No effect of bristle size or number of brusher cycles was found.

Consistently higher carotenoid levels were also found in unwashed oranges in other tests where measurements were made. Levels were intermediate in sponge-washed fruit and lowest in those mechanically washed. An increased number of washing cycles significantly reduced carotenoid levels of oranges in the November 13, 1973 test, suggesting that the duration of brush contact had some effect.

Washing 'Hamlin' oranges before degreening primarily reduced carotenoids, rather than a general delay in color development, as has

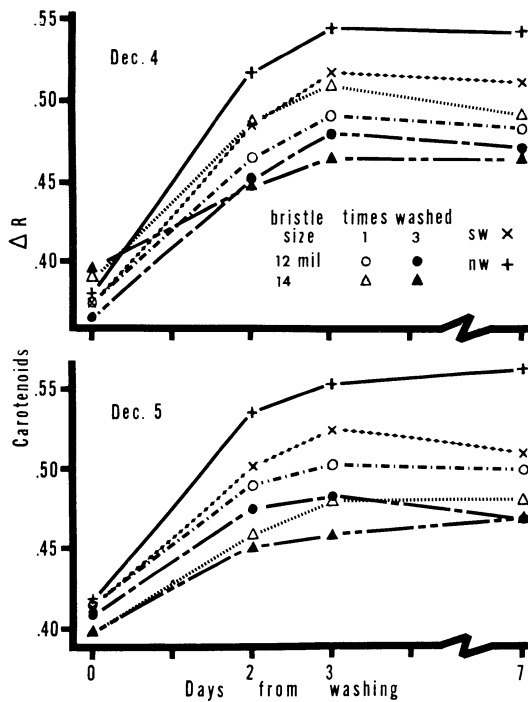


Fig. 2. Carotenoid changes in 'Hamlin' oranges during degreening in relation to washing treatments. Measurements of light reflectance  $\Delta R$  540-650 nm of fruit harvested on December 4 and 5, 1972, washed on December 5, and degreened 3 days. Mechanically washed treatments shown were brushed for 1 cycle.

been reported on the basis of visual observations (2, 5, 6). Low carotenoid levels, like visible chlorophyll, may give an impression of incomplete degreening. Also, low chlorophyll levels in the rind may be masked if carotenoid synthesis has been adequate.

The reduced carotenoid levels in washed fruit were presumed to be due to reduced synthesis caused by brushing. This inhibition could have resulted either directly from physical injury, or indirectly by reduced gas exchange caused by the plugging of stomata. Available information from these studies and previous work (3) does not identify a mechanism for reduced carotenoid synthesis. However, the occurrence of slight injury to the flavedo would be expected to cause an increase in the respiration rate. This in turn could inhibit carotenoid and other synthetic processes, while the breakdown of chlorophyll was unaffected.

Washing treatments, especially the number of cycles, affected

cleaning more than carotenoid changes. This suggested that most of the effect on pigment changes occurred on initial contact of fruit with the brushes and before the fruits were completely cleaned. However, cleaning efficiency and inhibition of carotenoid changes were only indirectly related. Unless fruit are kept moving through a mechanical washer, they tend to maintain a relatively stable orientation on the brushes. Some areas on the fruit, therefore, may have excessive brush contact before movement through the washer causes the remainder of the fruit to be washed. Such fruit orientation problems were minimal in our tests, because fruit were kept moving through the washer. The scalloped "tumbler" brushes were expected to provide better fruit movement and cleaning, but cleaning data (not shown) indicated that results were only slightly better than those for other brushes. Sponge-hand washed fruit were more uniformly treated, with less chance of over-cleaning. This may explain the improved carotenoid development in these fruit compared to mechanical washing.

No practical means of maintaining carotenoid levels in washed 'Hamlin' oranges was evident in these tests. Caution, therefore, should be exercised in washing oranges prior to degreening. Reductions in color caused by washing often are too small to be detected by casual visual observation (5), but where color is marginal as in early 'Hamlin' oranges, these changes can be important. The advantages of washing before degreening should be considered, however, where fruit has good carotenoid levels at harvest, or where conditions indicate a potential for serious *Collectotrichum* decay as occurs in 'Robinson' tangerines (3, 5, 6).

'Hamlin' orange appears to be more sensitive to washing effects than other cultivars. Little or no carotenoid loss has been noted in washed 'Robinson' and 'Dancy' tangerines (3, 6). Reduced carotenoid levels in 'Marsh' grapefruit (2, 5) are of little concern, since there is a preference for lower carotenoid levels in that fruit than in the orange.

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