Seasonal Development of External Color and Carotenoid Content in the Peel of Ripening 'Shamouti' Oranges[1,2]

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Abstract. External peel color and the content of chlorophyll, total carotenoids, carotenes and xanthophylls in the flavedo of 'Shamouti' orange fruits were studied during their development from the young to the overripe fruit stages.

In the early stage, the main change consisted of a decrease in chlorophyll content, resulting in the initial yellowing of the peel. Later, concomitant with the appearance of the typical orange color (color break), total carotenoid content of flavedo, as well as its carotene/xanthophyll ratio increased rapidly. Such increase, however, continued after full color had been attained and until the fruit was decidedly overripe. The advantage of relating the pigment content to the flavedo layer rather than to the whole peel and the suitability of expressing the pigment concentration on a per cm^2 basis are discussed briefly.

Introduction

The typical color changes occurring in the orange peel in the fall, when soil and air temperatures decrease under subtropical conditions are the consequence of simultaneous chlorophyll degradation and carotenoid buildup (12). This has been common knowledge since the early classical studies of Miller, Winston and Shomer (11). It now seems desirable to confirm these trends with more modern equipment and methods. Moreover, a quantitative expression of the external changes in color and their relationship to the pigment composition of peel is needed, since it may throw additional light on the rather sudden visible changes in color occurring at color break. Equipment for assessing external (i.e. reflected) color has become available and has been used for apples (4), tomatoes (1) and pulp from citrus fruits (9).

The present work attempts to bring together data on the seasonal changes of pigment composition of orange peel (including chlorophyll, total carotenoids and carotene/xanthophyll ratio) and concomitant changes in external color from a young fruitlet stage to overripe conditions.

The 'Shamouti' orange used in this study is an early to mid-season eating variety of high export grade. At the beginning of the picking season, fruit, although internally ripe, does not attain satisfactory external color. It is believed that a study of pigment changes is an essential prerequisite of any endeavour to improve peel color at an early date.

Materials and Methods

Samples of 40 fruits were randomly picked (at a height ranging from 1 to 2 m) from all quadrants of 4 adjacent 'Shamouti' orange trees. These trees were about 35 years old, budded on sweet lime stock, and growing in light sandy loam at Rehovot, on the coastal plain of Israel.

Determination of external color. Fruits were wiped, individually measured and weighed, and their external color was assessed with a Hunter Color and Color Difference Meter (Gardner Laboratory Inc.) using the smaller window (25 mm across). The parameters "L" (lightness), "a" (greenness to redness), "b" (blueness to yellowness) were measured on the L scale. Calibration was done with a yellow green ceramic panel ("L" = 51.9; "a" = −3.6; "b" = 30.4), selected because it resembles the hue of fruits during most of the season. Each fruit was measured at 5 different spots along the equator, and fruit averages were calculated. Twenty fruits evincing values closest to the group average were selected for extraction.

Flavedo extraction and pigment determination. All solvents used (petroleum ether, acetone and ethyl ether) were distilled over KOH pellets and zinc powder. All manipulations were carried out under dim light at room temperature.

Fruits were carefully peeled, and discs were removed from the peel along the equator with a stainless-steel cork borer, 10 mm in diameter. Albedo was carefully removed with a sharp scalpel, leaving only flavedo discs. Four replicate samples of about 40 to 80 discs (according to pigment content) were immediately blended in 20 ml acetone containing 0.2 g $CaCO_3$ and 2 ml of a 0.1% acetone solution of the antioxidant 2,6-di-*tert*-butyl-4-methylphenol (Butylated Hydroxytoluene, BHT), using an "Ultra Turrax" homogenizer (Janke & Kunkel, KG) under constant cooling. It was found that this amount of BHT, when carried through the entire determination procedure, contributed no significant color to the final extract, even at the lowest final volume used (10 ml, corresponding to 200 μg BHT per ml). The slurry was transferred to a mortar and ground thoroughly with successive portions of acetone until the residue became colorless. The colored supernatant portions were decanted onto the top of a short Hyflo Super-Cel column for filtration. The acetone extract was measured, an aliquot was put aside for chlorophyll determination, and the rest was evaporated under reduced pressure at 40°C to a volume of 2 ml, a 10-fold amount of 6% KOH in ethanol as well as 0.5 g of hydroquinone antioxidant were added and the flask was blanketed with N and held for one hour at 40°C. The saponified solution was diluted with 2 volumes of water and extracted with ethyl ether several times. The combined ether extracts were washed with saturated NaCl solution until neutral, dried over anhydrous granular sodium sulfate for 30 min, and evaporated to dryness under reduced pressure and constant N flow. The residue was dissolved in a suitable volume of petroleum ether, b.p. 60° – 80°C, and the absorption spectrum was recorded with a DB Beckman spectrophotometer. This spectrum was used to determine the total carotenoid content.

Total carotenoid pigments were fractionated into carotenes, which are hydrocarbons, and xanthophylls, or oxygen-containing carotenoids. For their separation, the unsaponifiable fraction in petroleum ether solution was

[1]Received for publication September 25, 1968. This work was partially supported by a grant from the Israeli Ministry of Agriculture. It is part of the Ph.D. thesis of the first author.

[2]The skillful technical help of D. Raber is gratefully acknowledged.

placed on a column (1.2 × 25 cm) of Fisher alumina (Brockman activity I), mixed with an equal amount of anhydrous powdered sodium sulfate, and the carotene fraction was washed down quantitatively with 3.5–4% acetone in petroleum ether. The residual xanthophylls were eluted with 50% acetone in petroleum ether. The absorption spectra of both fractions were recorded on a DB Beckman spectrophotometer.

Calculation of total carotenoids as well as carotenes and xanthophylls was made from the main absorption peak. This method is obviously an approximation since there are many different carotenoid components (2, 3) and the absorption maxima shift during ripening (see also the following results reported under Seasonal changes), but it is satisfactory for showing the seasonal trends of accumulation. The absorption coefficient at the λ_{max} was assumed to be 2500 (6).

Chlorophyll determinations were carried out on an aliquot of the original acetone extract, as explained under Flavedo extraction, by measuring the absorbance at 663 and 645 nm in a Spectronic-20 Bausch & Lomb spectrophotometer. Total chlorophyll was calculated according to Mackinney (10). All pigment determinations were expressed on a per surface basis.

The results reported in the present study cover mainly the 1966/67 season, with some additional data reported also for 1967/68. Preliminary results for 1965/66, not reported here, were essentially similar.

Results and Discussion

Relative importance of different peel layers. The citrus peel is composed of 2 clearly defined layers. The outer one—the flavedo—contains all the visible carotenoids, whereas the internal layer—the albedo—is almost devoid of visible carotenoids. In the 'Shamouti' orange, a rather thick-peeled variety, the ratio of thickness of albedo to flavedo is at least 6:1 at full ripeness. In one particular case (March 9, 1967) flavedo carotenoid content based on fresh weight was 353 μg/g while albedo content on the same basis was only 8 μg/g.

Data on peel pigments found in the literature are generally expressed per fresh weight of whole peel, and thus unduly attribute part of the pigments found in flavedo to colorless portions of the peel. Since we analyzed only flavedo layers our carotenoid figures, if expressed on a fresh weight basis, would be much higher than those found in the literature.

Carotenoid data may be expressed on a "per cm²" basis. This type of calculation was selected since the quantitative separation of the albedo from the external flavedo layer is not required and it may be more suitable to express data obtained from a thin layer on a bidimensional basis.

Seasonal changes in total carotenoid content and external color. The simultaneous changes in external color and pigment content of orange flavedo during several months (from the stage of young fruitlet in July to that of overripe orange the following May) are described in Fig. 1. It shows the course of changes in reflectance of fruit surface (curve a for redness, curve b for yellowness), as well as changes in chlorophyll and total carotenoid content of flavedo. The abscissa represents the number of days from full bloom. The latter occurs around April 15, but may deviate from this date by as much as 2 weeks.

The period covered can be generally divided into 3 stages, according to external color of the fruit:

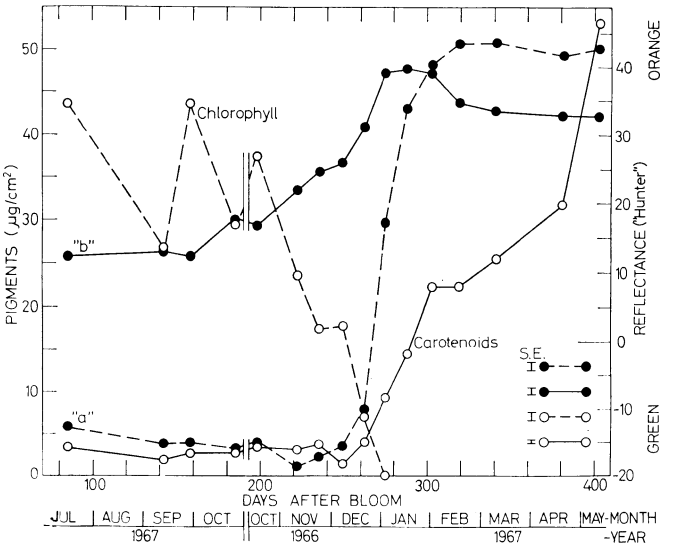

Fig. 1. Seasonal changes in external color, chlorophyll and total carotenoid content of Shamouti orange flavedo.

a) The green stage extends from fruit set to early December when commercial picking starts. The chlorophyll level decreases throughout this period, declining at a much faster rate between 200 and 250 days after full bloom, when there is practically no chlorophyll left. Accordingly, yellowness (Fig. 1, curve b) increases in spite of the fact that the carotenoid content is almost constant during this period.

b) The stage of visible color change occurs between December and mid-February. This period is characterized by the rapid increase in total carotenoid content of flavedo, together with increase in both a and, to a lesser extent, b values. It should be noted that the total carotenoid content increases during this period from 2.5 to 22.5 μg/cm²—a 9 fold increase. Around the middle of this period when values of about 30 and 40 are attained for a and b respectively, satisfactory market color is achieved; internal quality becomes fully satisfactory several days earlier.

c) During the stage of constant external color from early March to May (or about 340 to 400 days after bloom), while both a and b remain constant, considerable additional increases in total carotenoid content of flavedo are recorded. About 360 days after bloom, the commercial picking season of 'Shamouti' comes to an end, and the additional increase in carotenoids recorded during at least 40 more days occurred when the fruit was decidedly overripe, though turgid and of normal appearance. During this stage the pigment content increased 2-fold as compared to the end of the second stage and attained 53 μg/cm².

Such a continuous increase of carotenoids well after the end of the picking season was also found by Miller et al. (11) for 'Parson Brown' and 'Pineapple' oranges, and by Lewis and Coggins (8) for the 'Washington' navel orange. These workers did not report on parallel determinations of external color. The fact that the continuous increase in pigment content found by us is not matched by a simultaneous change in reflectance values was observed in each of the seasons studied. This feature has not been mentioned by others; it might be explained by an accumulation of pigments in the deeper flavedo layers, which would not contribute to the external color. Some of our preliminary observations seem to confirm that the

thickness of flavedo layers increases during the late resumption of peel growth, known to begin around mid-November (7). External color is therefore not a valid index of pigment content and standards of pigment content of peel can be established only with regard to known fruit age.

Seasonal changes in the carotene/xanthophyll ratio. During the entire season, xanthophylls were found to constitute the bulk of the carotenoid mixture, in accordance with previous work (2, 3).

Fig. 2 shows the amounts of carotenes and xanthophylls as percentage of total carotenoids for the same period covered by Fig. 1. There is a continuous increase in the percentage of xanthophylls, which is also matched by an increase in absolute xanthophyll level from 2.3 to 52.5 μg/cm². On the other hand, the relative decrease in carotenes is matched by an absolute decrease from 1.1 to 0.5 μg/cm². It should be noted that these quantitative changes are accompanied by compositional changes of the fractions, as shown by shifts in absorption maxima from 443 to 436 nm for the xanthophyll fraction and from about 448 to 425 nm for the carotenes. A study of the changes in composition of these 2 fractions during ripening is being carried out at present. The above quantitative trends are in accordance with the findings of Young and Erickson (12) who were able to correlate color improvement in orange peel, as the result of temperature treatments, with the increase in xanthophyll content. A high relative amount of xanthophylls in orange peel at full maturity has also been shown by Curl and Bailey (2, 3) by careful quantitative determinations. These findings clearly disprove the statements found in the earlier literature (5, 11) about an increase of carotene/xanthophyll ratio in mature orange peel.

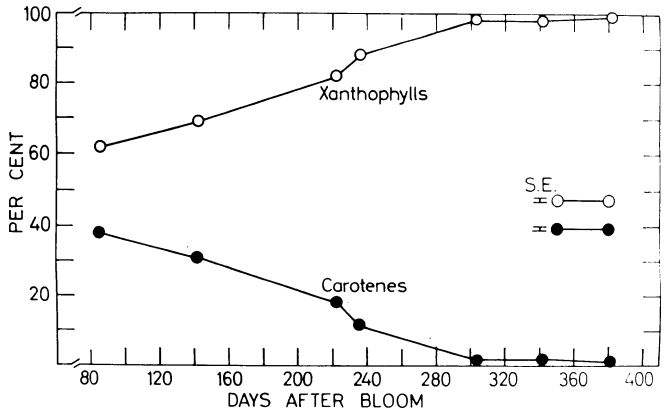

Fig. 2. Seasonal changes in carotenes and xanthophylls of 'Shamouti' orange flavedo as percentage of total carotenoids.

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