INVESTIGATION ON THE DEVELOPMENT OF COLOR IN CITRUS FRUITS

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It is not necessary to remind members of a horticultural society that the coloring (degreening) of citrus fruits is still fraught with many difficulties. This is true not only in the spring when Valencia oranges and Marsh grapefruit are being marketed, but may apply also to early and midseason varieties during the fall and winter. Again, even if degreening with ethylene has been successful, it must not be overlooked that decay is greatly stimulated by the ethylene treatment of fruit, even when the best known methods are employed.

During recent years great advances have been made in the construction of coloring rooms and in the improvement of the mechanical equipment, so that it is no exaggeration to say that this phase of the degreening operation is very modern and up-to-date. However, up to the present almost no studies have been made on the coloring process from the standpoint of the biological reactions occurring in the fruit itself. This is no doubt due to the fact that these reactions, being processes of living matter, are very complex and require highly specialized methods and expensive equipment for their proper investigation. As a consequence little is known concerning the fundamentals of the problem, and until more is learned of this phase of it little hope can be entertained for improving the degreening process. The investigations herein reported were undertaken and are being continued for the purpose of determining what changes take place in the fruit as it undergoes the degreening process, and it is hoped that as a result of these studies a more satisfactory method may be eventually developed.

THE COLOR IN THE ORANGE RIND

An orange owes its rind color for the most part to a group of pigments that dissolve in ether and are called "plastic pigments." There are two types of these: the green, called chlorophylls, and the yellow, called carotenoids. The first term means, literally, "leaf green." The word "carotenoid" signifies "like carotene," and the latter was named from the common carrot from which it was first isolated. The yellow pigments may be further subdivided into a group which dissolve in petroleum ether, called carotenes, and a group soluble in wood alcohol, called xanthophylls. In crystalline form carotenes are orange-red, whereas xanthophylls are yellow. Once in solution, however, they are difficult to distinguish by color alone. Carotenes are of additional interest because they are converted into vitamin A by animals, including man, and frequently the vitamin A potency of foods is measured by their carotene content. It has been known for some time that green leaves contain the yellow as well as the green pigments, but that the former are not visible unless the leaf becomes diseased, or aged, or is deprived of light for some time. It is now known that both the yellow and the green pigments are also present in the rind of a green orange. By means of chemical analyses and a specially designed instrument we have determined the actual quantities of these substances in orange rinds of several varieties during the maturing season. TABLE 1 shows the results of some of these analyses.

TABLE 1

Pigment Content of Orange Rinds in Fall and Spring of the Year

Date	Variety	(parts per million)	
		Total Chlorophyll	Total Carotenoids
9/29	Parson Brown	43.50	12.25
2/1	Parson Brown	0.0	52.10
10/18	Pineapple	50.0	10.27
2/22	Pineapple	0.0	85.35
11/11	Valencia	30.0	25.75
2/23	Valencia	0.0	55.95

It will be seen from the table that in the fall while the oranges are still green in color the yellow pigments are present to some extent also. That is, in September when the rind of the Parson Brown orange contained 43.5 parts per milllion of chlorophyll it contained 12.25 parts per million of carotenoids. Pineapple oranges in October contained 50 parts per million of chloryphyll and 10.27 parts per million of carotenoids. Valencias, sampled in November, showed 30 parts per million of chlorophyll and 25.75 parts per million of carotenoids.

INCREASE IN YELLOW PIGMENTS AS THE SEASON ADVANCES

A question frequently asked is, "Has the fruit attained its maximum yellow pigment when it is mature green?" These investigations have shown that the answer to this question is, "No." The carotenoids start increasing before the chlorophyll has disappeared, and the increase continues right through the season. Sometimes the maximum may not be attained until long after the fruit is ordinarily marketed and after it has lost its prime dessert quality. The increase in yellow pigments between the fall and spring samplings is also shown in TABLE 1. Between September and February the carotenoid content of Parson Brown rinds increased from 12.25 to 52.10 parts per million. Pineapple orange rinds showed an increase from 10.27 to 85.35 parts per million and the Valencias an increase from 25.75 to 55.95 parts per million during the winter months.

The idea that oranges appear better colored as the season advances is not new of course, but until these quantitative determinations were made one could not be certain whether there was an actual increase in carotenoid content, or a conversion of one fraction to another or that these pigments merely became concentrated near the surface of the rind.

EFFECT OF ETHYLENE GAS ON PIGMENTS

Thus far we have discussed the subject only from the standpoint of the degreening of oranges that occurs on the tree. What happens when the fruits are degreened with ethylene? It is obvious of course that chlorophyll is removed by this process. But what about the yellow pigments? The results of some of the studies along this line are shown in TABLE 2. Several hundred Valencia oranges were divided into three comparable lots. A sample from one lot was analyzed immediately, another after four days in ethylene, and a third at the end of eight days in ethylene. The results in Table 2 show that no significant change in the amount of carotenoids is produced by degreening oranges with ethylene. The total carotenoids found were 14.9 parts per million before degreening, 14.7 after four days, and 15.2 after eight days of ethylene treatment The slight differences are doubtless the result of variation between samples.

TABLE 2

Effect of Ethylene on Carotenoids in the Rind of Valencia Oranges

Days in Ethylene	Total Carotenoids (parts per million)
0	14.9
4	14.7
8	15.2

PIGMENT CHANGES IN CITRUS FRUITS OTHER THAN ORANGES

During these investigations it was noted that limes lemons and grapefruit do not behave like oranges in regard to pigment changes. It has been found that the carotenoid pigments are present in the rinds of mature green fruits of this type but that they diminish when the fruit is degreened. This is true whether the degreening is permitted to occur on the tree or is hastened by means of ethylene. It will be noted in TABLE 3 that Villa Franca lemons contained 9.15 parts per million of carotenoids in the rind when green but only 3.9 when degreened with ethylene and 3.35 when allowed to degreen on the tree. Similar figures for the Key lime were 11.19, 5.75, and 3.65 respectively. Grapefruit likewise showed a decrease in carotenoids as a result of degreening. The rather low carotenoid content in the second lot of grapefruit in TABLE 3 is due to the fact that it represented a pale type of grapefruit. Strange as it may sound then, a lime, a lemon or a grapefruit while still green in color, contains more of the carotenoid pigments than a fruit permitted to attain full color on the tree. This explains why grapefruit degreened with ethylene in the fall may have a deeper yellow color than those left on the tree to degreen during the winter.

TABLE 3

Pigment Changes in Limes, Lemons and Grapefruit

Variety	Total Carotenoids (parts per million)		
	Mature Green	Degreened with Ethylene	Degreened on Tree
Villa Franca Lemon	9.15	3.90	3.35
Key Lime	11.19	5.75	3.65
Grapefruit	11.19	5.85	....
Grapefruit	4.8	....	trace

ETHYLENE A STMULANT OF NATURAL PROCESSES

Looking at the results thus far obtained, it would appear that on picked fruit ethylene serves as a stimulant to natural processes. If limes, lemons, and grapefruit are destined to lose their carotenoids if allowed to remain on the tree, then a similar loss of carotenoids because of the ethylene treatment can hardly be referred to as unnatural. Oranges, on the other hand, contain the same amount of these yellow pigments after ethylene treatment as before but the green color is destroyed.

In nature the decomposition of chlorophyll has been shown to be due to the action of an enzyme. It is believed that ethylene causes degreening of citrus fruits not by direct action on the chlorophyll but by stimulation of the enzymatic action. Other workers have found that ethylene is evolved in the normal life processes of certain plant tissues including a number of fruits such as the apple, banana, peach and pear. During the past year we conducted experiments in the laboratory at Orlando which demonstrated that citrus fruit may now be added to the list of those that produce ethylene. Consequently, the effect of ethylene treatment of oranges, limes, lemons and grapefruit can no longer be regarded as unnatural.

ETHYLENE TREATMENT OF ORANGES ONLY BRINGS OUT COLOR ALREADY PRESENT

That it is impossible to bring out color in oranges with ethylene if it is not already present is illustrated by a recent experience of the writers. A prominent grower phoned in that he was having difficulty coloring tangerines in his coloring room. An inspection of the gassed fruit revealed that about half of it was well-colored and half poorly colored, which suggested that some of the fruit must have come from the inside of the tree. The field foreman confirmed this. Inasmuch as this was early in the season it seemed probable that the fruit from the inside branches of the tree had not yet manufactured as high a carotenoid content as the outer fruit and consequently the degreening with ethylene produced merely a pale yellow color. To test the accuracy of this diagnosis comparable samples of "inside" and "outside" fruit were degreened in the coloring rooms at the laboratory, and the results were identical with those obtained in the packing house. At the same time pigment analyses were made on similar lots of fruit. These analyses have been supplemented with others and typical results are presented in TABLE 4.

It will be readily seen that the fruits from the outside branches contained 50 per cent more yellow pigments than those from the inside.

TABLE 4

Effect of Position on Tree on Carotenoids in the Rind of Tangerines, November, 1938

Position on Tree	Total Carotenoids
Outside	90.0
Inside	60.0

SUMMARY

(1) Color in orange rinds is produced by two groups of ether-soluble pigments—chlorophylls (green) and carotenoids (yellow).

(2) Degreening on the tree results in a loss of chlorophylls and an increase in carotenoids in the rind of the orange.

(3) Ethylene degreening of oranges serves to remove the chlorophyll without affecting carotenoid content.

(4) Limes, lemons and grapefruit differ from oranges in that carotenoid pigments tend to decrease along with chlorophyll when they degreen. This is true whether they are degreened on the tree or are degreened with ethylene.

(5) When oranges are treated with ethylene it is impossible to bring out any more carotenoid pigment than is present at the beginning of the coloring process.

THE ASCORBIC ACID (VITAMIN C) CONTENT OF JUICE OF THE PRINCIPAL VARIETIES OF FLORIDA ORANGES

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A comprehensve investigation has been made on the seasonal changes, chemical trends and varietal characteristics of the leading commercial varieties of Florida oranges, as well as some of the less common ones, during their development and ripening. The findings are based on a systematic study made from 1935 to 1938 inclusive, but the results reported herein are for the last two seasons only.

The oranges used in the investigation and obtained from Central Florida were—Parson Brown, grown on rough lemon and sour orange rootstocks; Boone's Early, "Sixteen-to-One," Hamlin, Homosassa, Jaffa and Pineapple, grown on rough lemon rootstock; Conner's Seedless and Lue Gim Gong, on sour orange rootstock; Seedlings; and Valencia, grown on rough lemon, sour orange, grapefruit, Cleopatra and sweet orange rootstocks and Valencia grown on both rough lemon and sour orange rootstocks on Merritt Island, Florida East Coast.

The information obtained included data on seasonal changes in weight of the fruit, rind and flesh colors, diameter, thickness of rind, palatability of the juice as determiend by taste, volume of juice, buffer capacity, pH, total acidity, total solids, sucrose and reducing sugars and ascorbic acid content.

In the present paper only the results on the ascorbic acid content of the juice of the principal varieties of oranges are presented; the complete data on ascorbic acid of all the varieties of oranges tested are being published in the Proceedings of the American Society for Horticultural Science for 1938. Data on the other constituents and their seasonal changes correlated with ascorbic acid will be published as a technical bulletin of the United States Department of Agriculture.

RELATION OF ASCORBIC ACID TO THE HUMAN DIET

About 1933 vitamin C was isolated and identified, and inasmuch as the properties were antiscorbutic it is now commonly referred to as "ascorbic acid"; however, "cevitamic acid" is sometimes used. If sufficient amounts of this vitamin are not included in the human diet, abnormalities of the bones occur and the disease known as "scurvy" results.

A very interesting account of vitamin C malnutrition was noted in South Africa where the Banter gold mine laborers work under hot, humid conditions. Cases of scurvy and subscurvy occur with some frequency, despite the fact that the daily diet provided has been known to contain adequate amounts of vitamin C. The expla-