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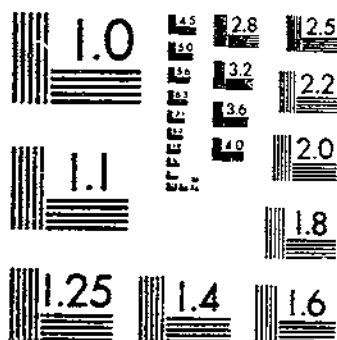
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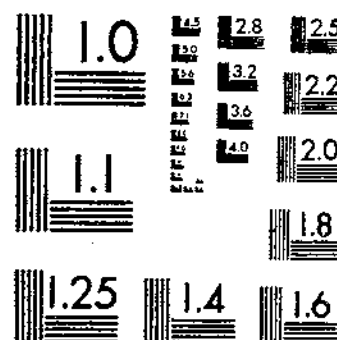
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A PHYSIOLOGICAL STUDY OF CAROTENOID PIGMENTS AND OTHER CONSTITUENTS IN
MILLER, E. Y. WINSTON, J. R. FISHER, D. F. 1206 11

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**UNITED STATES
DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.**

A Physiological Study of Carotenoid Pigments and Other Constituents in the Juice of Florida Oranges¹

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INTRODUCTION

During the course of earlier work by the present writers on the pigments in citrus rinds (1936-37), preliminary analyses indicated that much of the yellow color in the orange flesh is due to ether-soluble or carotenoid pigments. Studies on seasonal changes in oranges have been conducted at the Orlando, Fla., United States Department of Agriculture Horticultural Field Laboratory for the past 5 years, and it is believed that a presentation of the results with respect to carotenoid pigments in the juice of the fruits under investigation will materially assist in a proper appraisal of quality and food value of Florida oranges.

Carotenoid pigments are of especial interest because a number of them are converted into vitamin A in the animal body. A deficiency of this vitamin lowers resistance to respiratory diseases and may cause nutritional "night blindness," a disease in which the victim may see distinctly during the day or in bright light but sees poorly at night or in faint light.

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² The writers wish to acknowledge the assistance of A. Peyton Musselwhite and Francis L. Ingley in the collection of data for this bulletin.

In the preliminary work it was noted also that different varieties of oranges contained different quantities of these carotenoid pigments and that some varieties, like Temple, King, Dancy tangerine, and Owari satsuma, contained exceptionally large quantities. It is believed that these highly pigmented varieties of oranges should be called to the attention of dietitians seeking additional sources of vitamin A.

LITERATURE REVIEW

The literature on carotenoid pigments in orange fruits is not very extensive. Vermast (6)³ in 1931 reported that the flesh of *Citrus aurantium* L. contained 0.36 mg. of carotene and 1.08 mg. of xanthophyll per 100 gm. of fresh material. In 1936 Zechmeister and Tuzson (8) reported that 660 gm. of the flesh of the orange (*Citrus aurantium*) contained 1.6 mg. of cryptoxanthin and 2.8 mg. of other oxygen-containing polyenes (calculated as lutein). Ahmad, Mullick and Mazumdar (1937)(1) stated that orange juice contains 0.3 to 0.4 mg. of carotene per 100 gm. In 1938 Taylor and Witte (5) analyzed 164 lots of oranges purchased on the open market in New York City. These investigators reported the following carotene content expressed as milligrams per liter of juice: Florida assorted, 0.32; Florida Pineapple, 0.34; Florida Valencia, 0.57; California Navel, 1.07, and California Valencia, 1.65.

Other studies of pigments in citrus fruits that have been reported have been limited to the purification and identification of these compounds.

MATERIALS AND METHODS

DESCRIPTION OF SAMPLES

The fruits studied in these experiments included most of the more common commercial varieties of sweet oranges (*Citrus sinensis* Osbeck) and several of the less common ones. In addition, there were several representatives of the mandarin types (*Citrus nobilis* Lour.).

The early varieties studied were Sixteen-to-One,⁴ Parson Brown, Hamlin, and Washington Navel. The first two mature during October and November. The season for Hamlin is usually given as October, November, and later, and the Washington Navel ripens during the period of October to January. The most popular midseason variety in Florida is the Pineapple, its season extending from November through February. Other varieties that ripen in this same period are Conner, Homosassa, and Jaffa. The Ruby is listed in the period February to March. The most extensively planted late variety is the Valencia, which ripens during the period of March to June.

The seasons for the mandarin types studied are Owari (satsuma), October to November; Dancy (tangerine), November to January; Temple (probably an inter-species hybrid), January to April; and King, March to April.

Comparisons were made of the effect of both sour orange (*Citrus aurantium*) and rough lemon (*Citrus limonia* Osbeck) rootstocks on Parson Brown, Hamlin, Pineapple, Valencia, and tangerine oranges. The same variety was collected in two or three different sections of

³ Italic numbers in parentheses refer to Literature Cited, p. 31.

⁴ Name used locally for an undescribed early variety of sweet orange.

the State. The plots selected for the experimental work consisted entirely of commercial groves whose history was well known. The trees had had adequate care for a number of years and were in good condition. A more detailed description of the samples and the conditions surrounding them is presented in table 1. The samples marked "Near Orlando" were grown within a radius of 10 miles of that city, which is located near the center of the State. The soil types were mostly Norfolk fine sand. They are referred to locally as "high pine sandy soils." The one plot in south-central Florida was also typical of high pine land. The soils in the plots in the north-central section (Marion County) and on the east coast contain more organic matter, and are usually referred to as hammock. As a rule, rough lemon rootstock is used on the lighter soils and the sour orange stock on the heavier ones.

These investigations were conducted at Orlando, Fla., during the citrus season 1938-39 and 1939-40. The first samples were collected in September, and collections were made once a month thereafter until June. Data on juice constituents other than pigments were obtained on three lots of fruit in 1936-37, and these are included in the section on maturity.

TABLE 1. Summarized description of samples

Variety	Rootstock	Age of trees	Locality
		Years	
Parson Brown	Rough lemon	16-18	Near Orlando.
Do	Sour orange	17-18	Do.
Do	do	About 30	North-central Florida.
Do	do	About 30	East coast.
Hamlin	Rough lemon	16-18	Near Orlando.
Do	Sour orange	About 10	North-central Florida.
Sixteen-to-One	Rough lemon	16-17	Near Orlando.
Washington Navel	Sour orange	About 7	North-central Florida.
Pineapple	Rough lemon	8-10	Near Orlando.
Do	Sour orange	8-10	North-central Florida.
Do	do	15	East coast.
Do	do	30	Do.
Romossassa	Rough lemon	14-16	Near Orlando.
Conner	Sour orange	23-24	Do.
Jaffa	do	About 30	North-central Florida.
Ruby (Blood)	do	40 or more	Near Orlando.
Valencia	Rough lemon	About 12	South-central Florida.
Do	Sour orange	About 17	East coast.
Do	do	About 27	Near Orlando.
Do	Rough lemon	33-35	Do.
Dancy tangerine	do	About 20	Do.
Do	Sour orange	About 15	North-central Florida.
Temple	do	About 20	Near Orlando.
Do	do	About 15	East coast.
Owari satsuma	Rough lemon	40 or more	Near Orlando.
Do	Grapefruit	About 12	Do.
King	Rough lemon	About 15	Do.

An especial effort was made to obtain plots in which the history of the trees was definitely known. In several instances the source of the budwood could be traced back to the original seedling that gave rise to the variety. Parson Brown oranges from north-central Florida were collected from the Carney grove at Oklawaha, Marion County. The budwood had come from what was considered the best one of the original Parson Brown seedlings at Webster. The Pineapple oranges from this same section of the State were grown in the Crosby-Wartman grove at Citra, and these had been budded from the original Pineapple orange trees in the J. B. Owens grove. The Temple oranges from near Orlando were collected from the Bennett grove at

Minerville. These trees had been budded from the original Temple tree discovered at Winter Park.

Prior to the commercial picking date, samples of fruit were collected from 10 trees. Two trees in each plot were reserved for subsequent samples. Oranges were collected once a month, beginning with September. A sample consisted of 75 to 100 fruits, gathered from all sides of the trees in order to obtain uniform sizes representing the average for the tree. This sample was then reduced to 50 fruits showing the most uniformity in size. The average weight, size, and rind color were recorded. The fruits were then halved, and the juice was extracted by means of a fruit press during the early part of the season and by hand after the commercial picking stage of development was reached. The juice was filtered through a 16-mesh strainer. Flavor and color of the composite juice were recorded. The following determinations were made immediately: Total soluble solids, hydrogen-ion concentration, total acidity, and ascorbic acid. The methods of analysis were identical with those described by Harding, Winston, and Fisher (2).

TOTAL CAROTENOIDS IN THE JUICE

The only method for determining carotenoid pigments in orange juice known to the writers at the time this work was begun was the one described by Taylor and Witte (5). This was an adaptation of Guilbert's hot-saponification method recommended for determining carotene in forage crops. Peterson, Hughes, and Freeman (4) had simplified the Guilbert method which was itself the one the writers tried first. It was found unsatisfactory for the following reasons: (1) Refluxing the juice with alcoholic potash produces caramelization, and the resultant darkened color makes it difficult to extract the pigments completely; (2) duplicate extractions do not always agree, apparently because of incomplete saponification; (3) the Guilbert method, designed for forage crops, does not quantitatively extract cryptoxanthin, which has been shown by Zechnmeister and Tuzson to be the main precursor of vitamin A in oranges.

After considerable experimentation with the methods already mentioned, which proved to be unsatisfactory, the writers developed a satisfactory method for determining total carotenoid pigments in the juice. It will be described in detail as finally adopted by the writers in the following paragraphs.

The ether-soluble carotenoid pigments exist in the orange juice as served to the consumer either in colloidal dispersion or intimately associated with fragments of the juice sacs. An attempt was made closely to simulate the juice as actually consumed in beverage form. This was accomplished by first passing the juice through a 16-mesh sieve and then by removing a 25-ml. aliquot by means of a pipette with the tip filed to make a larger opening than usual.

The aliquot was transferred to a 1-ounce ointment jar and frozen in the freezing compartment of a mechanical refrigerator. The purpose of freezing was twofold. First, this permitted storing the pigment samples without loss while the less stable constituents were being determined; and second, the ice crystals could be utilized as an abrasive in macerating the sample. This was done the next day when the frozen sample was transferred quantitatively to a stone mortar. The

grinding was necessary to separate the carotenoid pigments as completely as possible from the cell fragments. Sand was not suitable for this purpose, because it was found to interfere seriously with filtering and extracting.

The aliquot of juice was transferred to a Büchner funnel, and the bulk of the aqueous portion was filtered off by suction (but not to dryness). When the solution showed a sirupy consistency, the aqueous filtrate was transferred from the filter flask to another flask and redistilled acetone was added to the filter. This was repeated until no additional color was extracted. The acetone extract was transferred to a separatory funnel containing 50 ml. of purified petroleum ether, and the aqueous filtrate, previously removed from the filter flask, was added to the acetone-petroleum ether mixture. The water-soluble pigments and the acetone were removed from the petroleum ether by washing first with 1-percent sodium carbonate and then with distilled water. The petroleum-ether extract, containing the total carotenoid pigments, was dried over anhydrous sodium sulfate made up to volume and these were determined colorimetrically as described in a previous publication (3). The results are reported as total carotenoids in milligrams per liter of juice, although the colorimeter employed was standardized with only the β -carotene crystals.

The total carotenoid reading presumably includes values for carotene, xanthophyll, and cryptoxanthin. Inasmuch as xanthophyll possesses no vitamin A potency, it is believed that the value of the data would be enhanced if the results were presented so as to indicate some of the component fractions of the total carotenoids.

XANTHOPHYLL

The xanthophyll fraction was removed by washing the petroleum-ether extract with 92-percent methanol until the washings appeared colorless. Ninety-two-percent methanol was used because Wiseman et al. (7) have shown that over 99 percent of the xanthophyll can thus be removed from a mixture of carotene and xanthophyll in petroleum ether. After this treatment the petroleum-ether extract was dried over anhydrous sodium sulfate made up to definite volume, carotene was then determined colorimetrically and the xanthophyll by difference.

SAPONIFIABLE FRACTION

In all of the methods consulted, the plant material is saponified prior to assay for carotene. It was found in the present work that saponification removes an additional fraction (possibly cryptoxanthin) from the petroleum ether extract, and in order to make a thorough comparison of the methods an aliquot was saponified after xanthophyll had been removed. The procedure was as follows: Another 25-ml. aliquot was treated with 5 ml. of saturated methyl alcoholic potash and held in the refrigerator overnight. The next day the alkali was washed out with water, and the epiphasic petroleum ether was washed thoroughly with 92 percent methanol. The petroleum-ether solution was dried over anhydrous sodium sulfate made up to definite volume and the pigment was determined colorimetrically. This fraction, remaining after the removal of xanthophyll and the saponifiable pigments, is customarily reported as carotene.

In table 2 will be found a comparison of the results obtained with the new method and with the Peterson-Hughes-Freeman method for determining carotenoid pigments in plant material. The latter method was modified slightly in that after the hot saponification the pigments were first extracted with cold acetone before transferring to petroleum ether, instead of extracting directly with the petroleum ether.

TABLE 2.—Comparison of methods for extraction of carotenoid pigments from orange juice

PETERSON-HUGHES-FREEMAN HOT SAPONIFICATION

Sample No.	Total carotenoids	Total carotenoids less xanthophyll	Total carotenoids less xanthophyll and saponifiable fraction
	Mg./l.	Mg./l.	Mg./l.
1.....	5.79	3.76	1.60
2.....	5.60	3.78	1.78
3.....	5.06	3.84	1.30
4.....	5.19	3.87	.96
5.....	5.50	3.82	1.14
6.....	5.36	3.54	.88
7.....	4.79	3.50	.86
8.....	4.72	3.45	.86
9.....	4.92	3.53	.88
10.....	4.88	3.50	1.08
Average.....	5.17	3.66	1.13

COLD ACETONE EXTRACTION

Sample No.	Total carotenoids	Total carotenoids less xanthophyll	Total carotenoids less xanthophyll and saponifiable fraction
	Mg./l.	Mg./l.	Mg./l.
1.....	6.08	4.81	1.20
2.....	6.01	4.72	1.12
3.....	6.42	4.96	1.12
4.....	6.34	4.73	1.05
5.....	5.66	4.84	1.14
6.....	6.20	4.81	.98
7.....	6.44	4.73	1.18
8.....	5.95	4.80	1.04
9.....	6.21	4.88	1.44
10.....	6.06	4.62	1.40
Average.....	6.14	4.79	1.17

Ten replicate samples of Valencia orange juice were analyzed by each method. It will be readily seen that the extraction with cold acetone yielded higher values in all fractions. Furthermore, less variable replications were obtained when the new method was used. With the hot saponification method the results for total carotenoids ranged from 4.70 to 5.79 mg. per liter, whereas with the second method the values ranged from 5.66 to 6.44 mg. per liter.

RESULTS

SEASONAL CHANGES IN PIGMENTS

Figure 1 is a graph depicting seasonal changes in the pigments of the juice of early varieties of oranges. This group includes Parson Brown and Hamlin on both sour orange and on rough lemon rootstock, Washington Navel on sour orange stock, and Sixteen-to-One on rough lemon rootstock. All those on rough lemon rootstock and one

Parson Brown on sour orange were grown near Orlando, one Parson Brown on sour orange stock was grown on the east coast, and all others came from north-central Florida. Viewing the graph as a whole, it will be seen that the carotenoid pigments in the juice gradually increased during the season. In most of the cases the highest

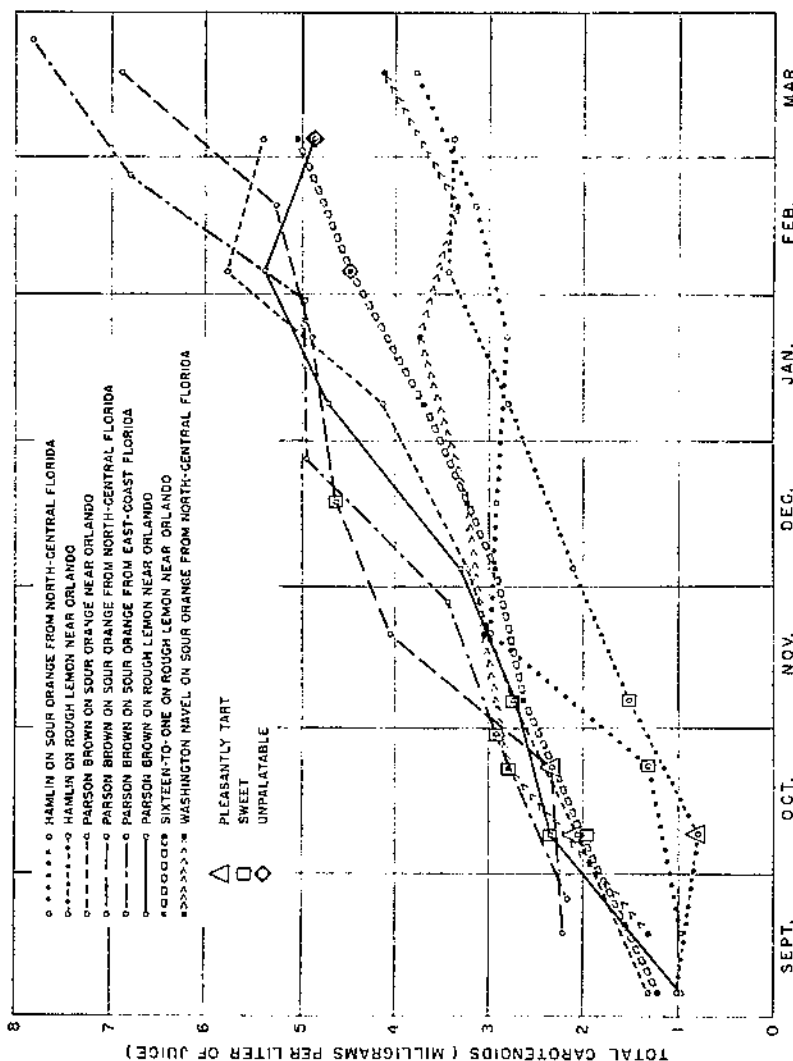


FIGURE 1.—Seasonal changes in carotenoid pigments in, and flavor of, the juice of early Florida oranges, 1939-40.

value shown occurred in March; in a few others the peak was attained earlier. Fluctuations are apparently due to sampling errors. When these early varieties had attained their greatest pigmentation, their pigment content was from three to five times that shown in the September samples. The Parson Browns showed the highest maxima, and the east coast and north Florida samples of this variety were

higher than those grown near Orlando. The Parson Brown on rough lemon root from Orlando increased from 0.97 mg. per liter of juice to 5.39 mg. per liter, whereas the lot on sour orange stock from the same grove showed an increase from 1.32 to 5.78 during the season. The other two lots of Parson Browns showed higher carotenoid pigments almost from the beginning. The north Florida lot showed a range of from 2.21 to 6.89 mg. per liter, while those on the east coast ranged from 2.17 to 7.82 mg. per liter.

The Hamlins on both rootstocks were comparatively low in carotenoid pigments throughout the season. However, like other varieties they increased their quantities of juice pigments if left on the tree long enough, although they never attained as high maxima as the Parson Browns. It is significant that in late October and early November, when these early varieties are normally harvested, the Hamlins were quite low in carotenoids. Their average for the October sample was 1.027 mg. per liter as compared with 2.40 for all the others, and the vast differences among the other early varieties had not begun to appear. By January the Hamlins had attained approximately as much pigment as the Parson Browns showed during their own shipping season. The Washington Navel and Sixteen-to-One varieties at the last sampling showed higher carotenoid pigments than did the Hamlins, although their maxima did not equal those of the Parson Browns.

Flavor tests made in the laboratory indicated that the juices of all these varieties were either pleasantly tart or sweet in the samples collected between October 9 and November 6. Commercial picking of all these varieties occurred between October 5 and November 20. It is evident therefore that maximum carotenoid content of the juice was not reached until long after the fruits had attained their most palatable stage of maturity. This was especially true of varieties like the Washington Navel and Sixteen-to-One, which were notably low in acid and which became too sweet and even insipid in February and March.

In figure 2 will be found a comparison of the juice pigments in the midseason varieties—Pineapple on sour orange and rough lemon rootstocks, Ruby, Conner, and Jaffa on sour orange, and Homosassa on rough lemon. The Pineapple on sour orange rootstock was grown on the east coast, the Jaffa in north-central Florida, and the others were produced near Orlando. These midseason varieties showed a seasonal increase in carotenoid pigments similar to that in the early varieties, the one exception being the Jaffa. This variety showed an increase in carotenoid pigments up to November, remained almost constant from this time until January, and increased only slightly after that. More specifically, in September the juice of oranges of this variety contained 1.24 mg. total carotenoids per liter; in November it had 2.52 mg., but by February it had increased only to 2.74 mg. per liter. The maximum value attained was 3.11. During this period the Pineapple on rough lemon showed an increase in pigment from 0.86 to 5.40 mg. per liter whereas the same variety on sour orange stock increased from 1.70 to 5.66 mg. per liter. Throughout the season the Homosassa variety showed somewhat lower quantities of carotenoid pigments in the juice than did the Pineapple, ranging from 0.95 to 4.08 mg. per liter.

The juices of these varieties were considered pleasantly tart or sweet sometime during the months of November, December, or January. The shipping season for these varieties usually extends from the middle of November through January. It is evident, then, that they contain a higher pigment content when shipped than do the earlier

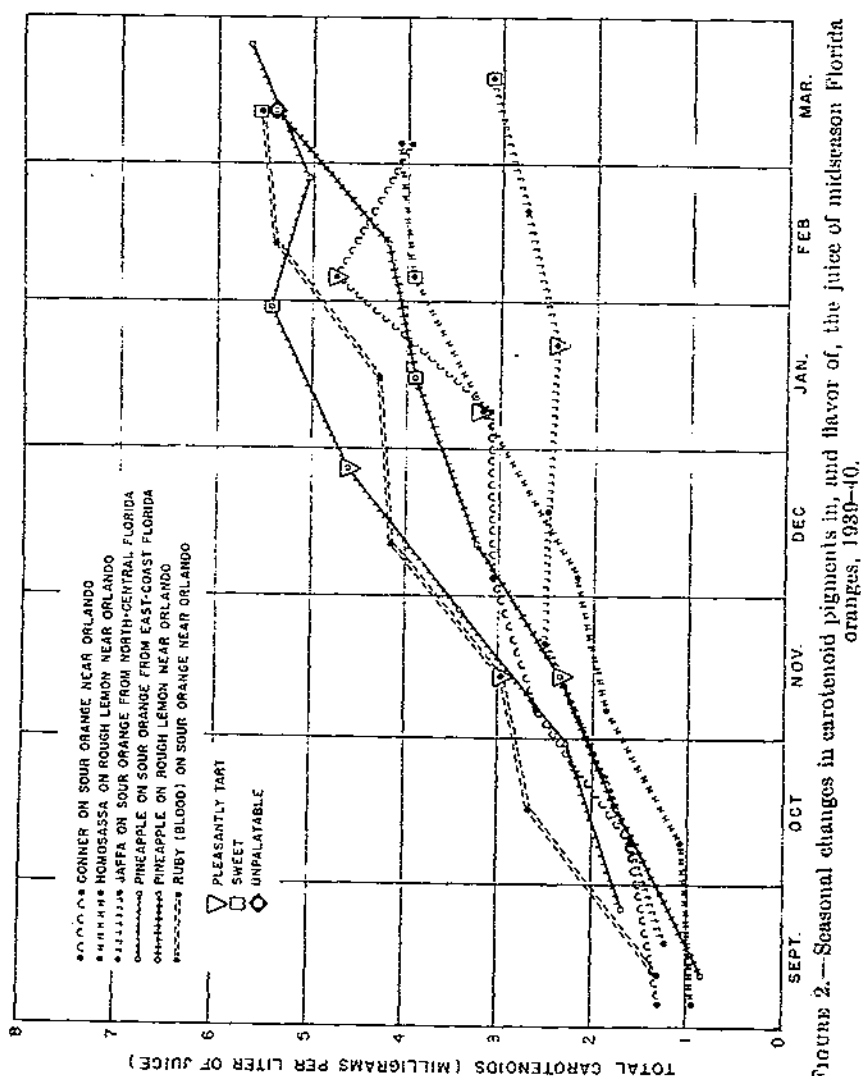


FIGURE 2.—Seasonal changes in carotenoid pigments in, and flavor of, the juice of midseason Florida oranges, 1939-40.

varieties. Likewise they may increase in carotenoid pigment even after attaining maximum flavor. The Pineapple orange on rough lemon stock illustrates the point. It was considered pleasantly tart in November, sweet in January, but by the time it had attained its maximum pigmentation (March 11) it had developed an undesirable oversweet and insipid flavor.

Figure 3 shows the seasonal changes in carotenoid pigments in the juice of Valencia oranges. These being representative of a late variety, the period of observation was extended through May. The lots included fruits grown on sour orange rootstock from the east

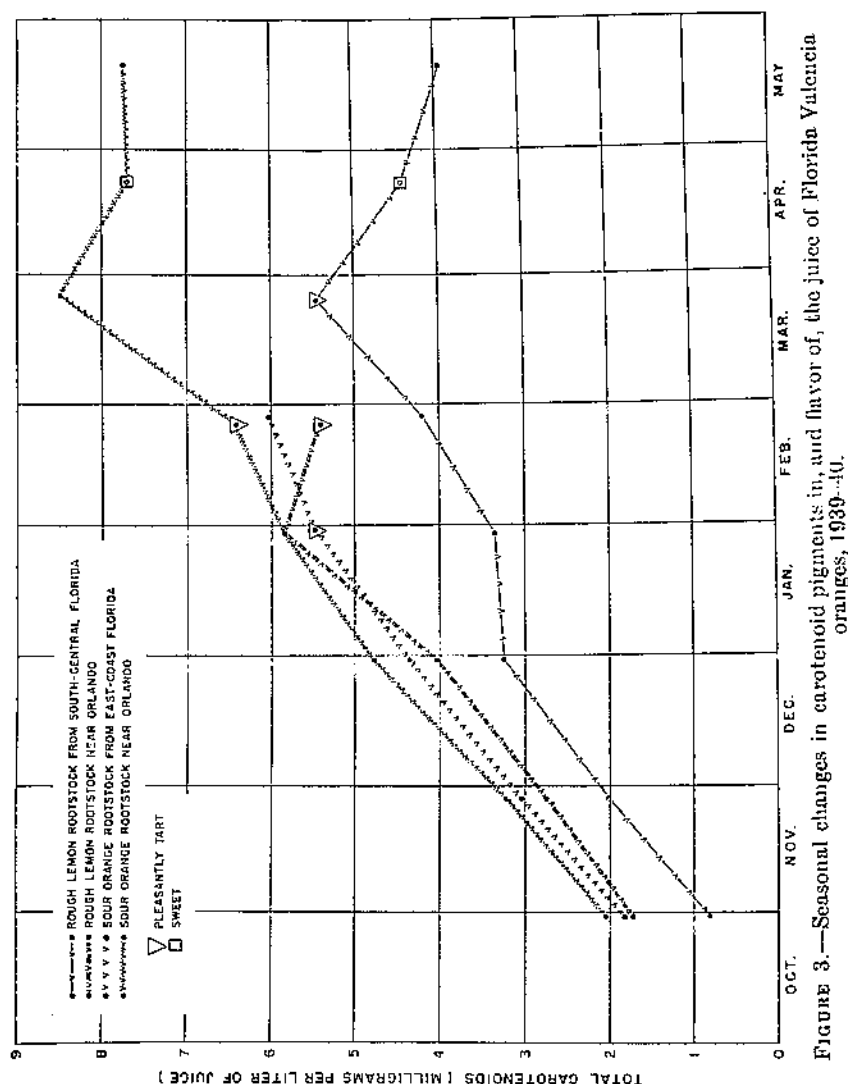


Figure 3.—Seasonal changes in carotenoid pigments in, and flavor of, the juice of Florida Valencia oranges, 1939-40.

coast, a series on rough lemon stock from Polk County (south-central Florida), and series on sour orange and on rough lemon rootstock grown near Orlando. These samples showed a seasonal rise in carotenoids like that found in the early and midseason varieties, but the peak was usually followed by a decline. The sour orange lot from near Orlando began in November with 2.05 mg. per liter, increased to 8.48 by March 26, then began falling. The rough lemon lot from

south-central Florida reached its peak in March with 5.45 mg. per liter, when it began to decrease. The other two lots were not sampled after commercial picking because of the growers' failure to hold them beyond this period. However, the rough lemon lot from Orlando had already begun to show a decline in carotenoid pigments in the

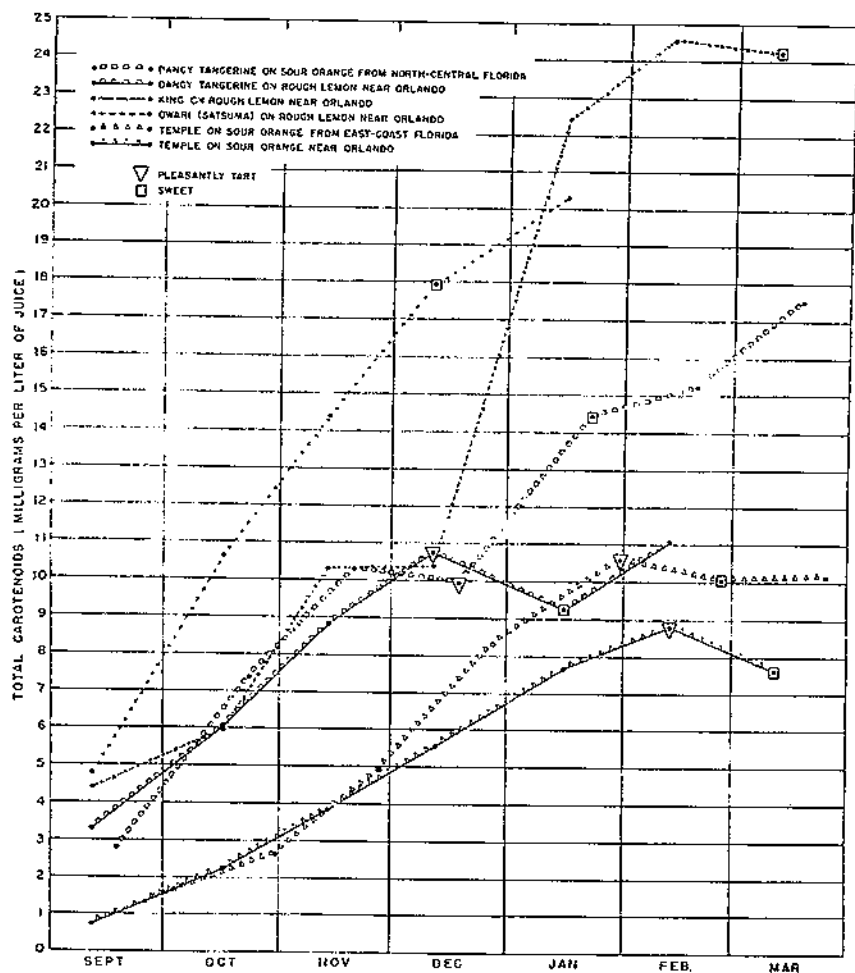


FIGURE 4. Seasonal changes in carotenoid pigments in, and flavor of, the mandarin type of Florida oranges, 1939-40.

juice beginning with the March sample. As the shipping season for Valencias normally extends from March to June, it appears that this variety may be marketed at the peak of its pigmentation, but doubtless most of the fruit is picked after the juice color has begun to decline, as is always the case with rind color.

Figure 4 shows the seasonal changes in pigments in the juice of the mandarin types of oranges. The lots studied were Dancy tangerine on sour orange and on rough lemon rootstocks, two lots of Temple on sour orange rootstock, Owari satsuma on rough lemon, and the

King orange on rough lemon. The tangerines on sour orange stock were collected in north-central Florida, one lot of the Temple on the east coast, and all the remainder from near Orlando. As would be expected from the deep color of the flesh of this type of fruit, these mandarin varieties showed very high carotenoid content right through

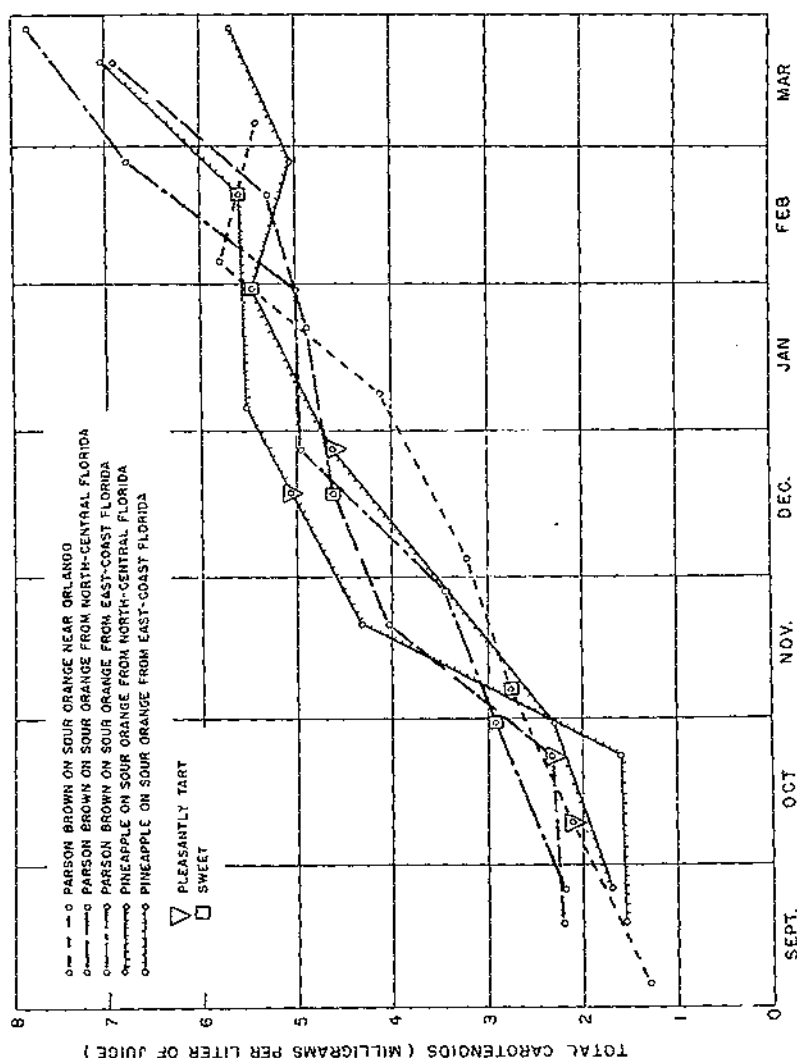


FIGURE 5.—Seasonal changes in carotenoid pigments in, and flavor of, the juice of Parson Brown and Pineapple oranges from different localities, on sour orange rootstock.

the season. The tangerines on rough lemon ranged from 3.34 to 11.03 mg. per liter, and those on sour orange stock showed a range of from 2.78 to 17.52 mg. per liter. The two lots of Temple reached peaks of 8.78 and 10.56 mg. per liter, the Owari a little over 20 mg. per liter, while the King—the highest of them all—reached a maximum of 24.52 mg. of carotenoids per liter of juice.

EFFECT OF LOCALITY ON PIGMENTATION

Figure 5 gives a comparison of the juice pigments in a given variety of orange on one rootstock as grown in different parts of the State. Parson Brown oranges on sour orange rootstock were collected near Orlando, on the east coast, and in north-central Florida, and Pineapple oranges on the same rootstock from the east & east and north-central Florida. The greatest divergence between the curves appears in the middle of the sampling period, i. e., about December. In other words, all lots started out with about the same quantity of pigment and attained approximately the same maxima, but those grown in the northern part of the State increased in pigmentation more rapidly. A deeper colored rind is generally found on oranges produced in the northern part of the State, and it would seem that this holds also for the flesh during the shipping season. In commercial practice, these relatively early oranges are not permitted to remain on the tree as long as these lots were held, so that these varieties rarely develop their maximum flesh pigmentation. The curves show a tendency toward more rapid increase in carotenoids during November in the northern region, as compared with the other regions. The rate of increase slows down in December, and the lead is largely lost by the end of the month. Unaccountably, the Parson Brown oranges from Orlando do not show their greatest rate of increase until January.

ROOTSTOCK AND PIGMENTS

Harding, Winston, and Fisher (2) have called attention to the effect of rootstock on quality of oranges, and the question has arisen concerning the effect of rootstock on juice pigments. In figure 6 some of the early and midseason oranges previously discussed have been grouped so as to compare the same varieties on two different rootstocks, sour orange and rough lemon. Because of the suitability of these rootstocks for different types of soil, it was not possible, as a rule, to find both rootstocks in the same planting, or even in the same general region. The Hamlin on sour orange rootstock was grown in north-central Florida, and the Pineapple on sour orange was grown on the east coast on soils to which the sour orange rootstock is especially adapted. The Hamlin and Pineapple oranges on rough lemon, as well as the Parson Brown lots on both rootstocks, were all collected near Orlando. The Parson Brown lots afford the best comparison, because they were produced in the same grove and on the same soil type.

There was no significant difference in the amounts of the pigments in the Parson Brown variety on the two rootstocks. The rough lemon lot ranged from 0.97 to 5.39 mg. per liter and the sour orange lot from 1.32 to 5.78. The Hamlins on both rootstocks were notably lower than the other varieties, and fairly uniform, except for one "sour orange rootstock" determination in November. The Pineapple oranges on rough lemon grown near Orlando were consistently lower in pigment than those on sour orange from the east coast. Soil and climatic factors may have had an influence here. Figure 3, which includes only Valencias on two rootstocks, illustrates the same points brought out in figure 5. The Valencias on sour orange and on rough lemon rootstock from near Orlando were both produced in the same

grove on what is locally called high pine sandy soil. The curves for these two lots are very similar. The "rough lemon" lot from south-central Florida was also produced on the same type of soil in the "ridge section" but gave distinctly lower readings, whereas the

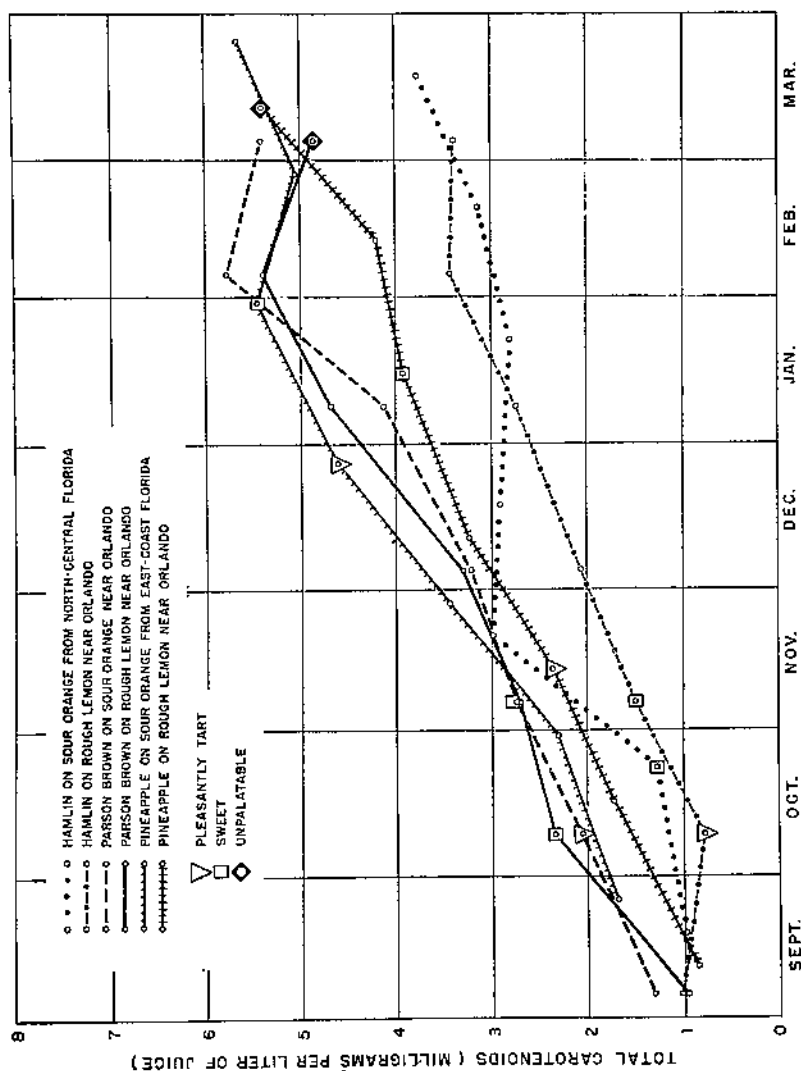


Figure 6.—Seasonal changes in carotenoid pigments in, and flavor of, the juice of early and midseason oranges grown on two rootstocks, 1939-40.

Valencias on sour orange rootstock grown on the east coast closely paralleled the two lots from Orlando.

MAXIMUM CAROTENOID CONTENT OF JUICE OF ALL VARIETIES

A better idea of the relative carotenoid content of the juice of all lots of oranges studied can be gained from figure 7. The values represent the maximum attained by each lot during the period of sampling, which in some instances extended beyond the commercial marketing

season. It will be seen that the carotenoid content of the mandarin varieties as a whole is much higher than that of the fruits of the sinensis or sweet orange type. The average of the maxima for all mandarins was 16.12, as compared with 5.45 for the others.

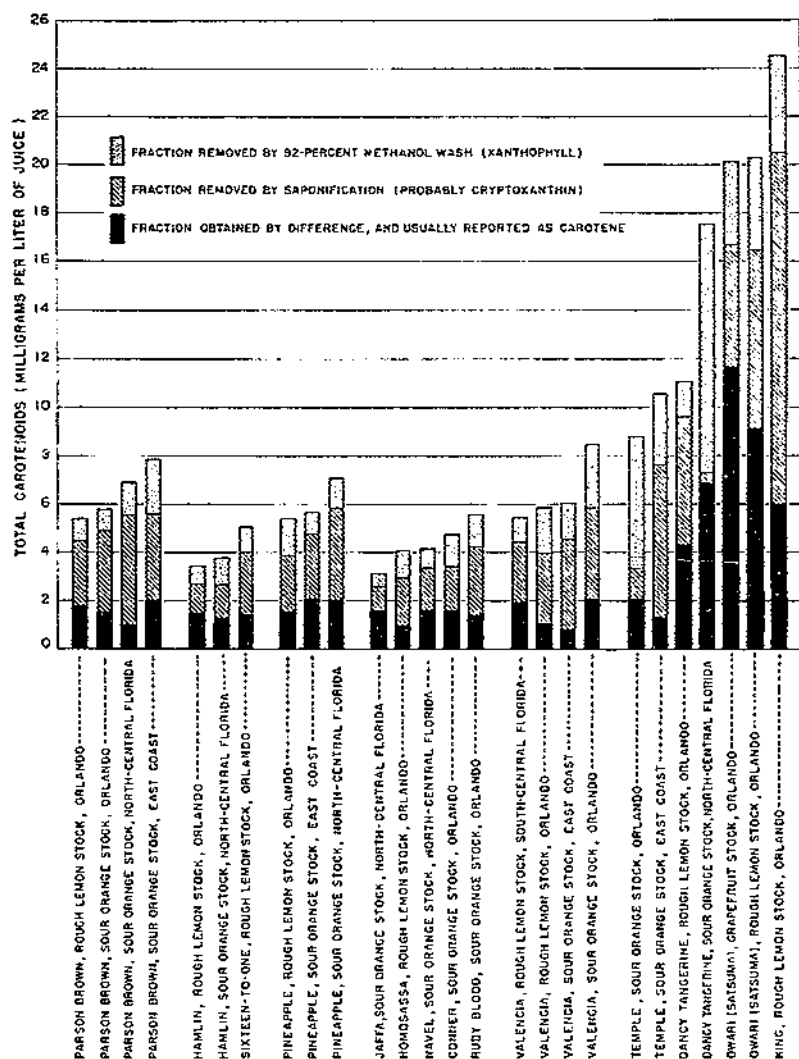


FIGURE 7.—Maximum carotenoid content of juice of all varieties.

The bars showing total carotenoids are divided into three parts, representing fractions of the pigment. The first subdivision (at the top) represents the portion that is removed by 92 percent methanol and should correspond to the xanthophyll fraction. Since xanthophyll has been reported to possess no vitamin A potency, it was thought that the data might be made more useful by indicating the amount of this fraction present. The second portion of the bar represents that removed by cold saponification and 92 percent methanol extraction.

The lower portion represents the carotene fraction. Much of the data on carotene content of orange juice reported by others is based on a method which would determine only this last fraction.

CAROTENOIDS AND OTHER CONSTITUENTS OF THE JUICE

In studying the relationship of the carotenoid pigments to maturity of oranges, it was necessary to assemble data on other juice constituents that are known to change in quantity with the advance of the season. These data were all collected at the same time the pigment analyses were made. Because of the increasing demand for such information, the detailed results of this phase of the investigation are presented here.

The seasonal changes in carotenoids and other juice constituents for all varieties studied in 1939-40 are presented in table 3. Table 4 gives the effect of two rootstocks on juice constituents over a period of 2 years.

TABLE 3.—Seasonal changes in carotenoid pigments and other constituents in, and flavor of, the juice of Florida oranges, 1939-40

Variety and description of sample	Date collected	Total soluble solids	Total acids	Solids-to-acid ratio	Active acidity	Ascorbic acid	Total carotenoids	Flavor
		Percent	Percent		pH	Mg/ml	Mg/ml	
Parson Brown orange on rough lemon rootstock collected near Orlando.	Sept. 5	7.77	1.090	7.13	3.47	0.514	0.97	Sour.
	Oct. 9	8.72	.662	13.16	3.80	.596	2.31	Sweet.
	Nov. 6	9.20	.551	16.60	4.00	.531	2.71	Do.
	Dec. 4	9.71	.516	18.80	4.06	.523	3.30	Do.
	Jan. 8	10.65	.491	21.70	4.31	.517	4.68	Do.
	Feb. 5	10.95	.420	26.07	4.18	.435	5.30	Do.
Parson Brown orange on sour orange rootstock collected near Orlando.	Mar. 4	10.82	.351	30.55	4.33	.401	4.85	Sweet; old.
	Sept. 5	8.88	1.229	7.23	3.39	.601	1.32	Sour.
	Oct. 9	9.62	.749	12.83	3.76	.469	2.05	Pleasantly tart.
	Nov. 6	10.20	.687	14.84	3.87	.557	2.75	Sweet.
	Dec. 4	10.98	.558	19.60	4.06	.550	3.22	Do.
	Jan. 8	12.00	.529	22.68	4.20	.558	4.13	Do.
Parson Brown orange on sour orange rootstock collected in north-central Florida.	Feb. 5	12.95	.489	26.50	4.12	.475	5.78	Do.
	Mar. 4	12.62	.418	30.35	4.24	.428	5.41	Do.
	Sept. 18	9.05	1.284	7.75	3.25	.615	2.21	Slightly sour.
	Oct. 23	10.53	.818	12.87	3.73	.592	2.33	Pleasantly tart.
	Nov. 20	11.30	.708	15.73	3.78	.559	4.04	Do.
	Dec. 18	12.71	.672	17.43	3.91	.559	4.61	Do.
Parson Brown orange on sour orange rootstock collected on east coast.	Jan. 22	12.17	.575	21.17	4.20	.563	4.88	Do.
	Feb. 19	12.49	.455	27.46	4.41	.447	5.27	Do.
	Mar. 18	13.00	.445	29.22	4.17	.461	6.89	Do.
	Sept. 25	9.78	1.176	8.32	3.45	.564	2.17	Tart.
	Oct. 30	11.09	.860	12.90	3.69	.602	2.91	Sweet.
	Nov. 27	11.10	.738	15.08	3.78	.557	3.44	Do.
Hamlin orange on rough lemon rootstock collected near Orlando.	Dec. 27	11.90	.680	17.50	3.90	.592	4.95	Do.
	Jan. 30	12.51	.582	21.55	3.87	.569	4.98	Do.
	Feb. 26	13.50	.558	24.19	4.00	.522	6.77	Do.
	Mar. 25	13.56	.465	29.31	4.25	.568	7.82	Sweet.
	Sept. 5	8.31	1.199	6.93	3.29	.586	1.03	Sour.
	Oct. 9	9.01	.816	11.05	3.54	.565	.78	Pleasantly tart.
Hamlin orange on sour orange rootstock collected in north-central Florida.	Nov. 6	9.60	.762	12.75	3.67	.565	1.51	Sweet.
	Dec. 4	9.90	.710	13.90	3.75	.520	2.07	Sweet; watery.
	Jan. 8	9.96	.602	14.30	3.75	.509	2.76	Do.
	Feb. 5	10.25	.611	16.77	3.92	.465	3.43	Do.
	Mar. 4	10.52	.548	19.20	4.04	.464	3.30	Do.
	Sept. 18	8.21	1.042	7.88	3.35	.485	.98	Slightly sour.
Sixteen-to-One orange on rough lemon rootstock collected near Orlando.	Oct. 23	8.53	.782	10.91	3.50	.478	1.27	Sweet.
	Nov. 20	9.10	.714	12.74	3.60	.464	2.99	Do.
	Dec. 18	8.95	.651	13.68	3.68	.444	2.92	Do.
	Jan. 22	9.77	.611	15.99	3.80	.473	2.81	Do.
	Feb. 19	9.67	.463	23.74	3.99	.318	3.14	Do.
	Mar. 18	9.92	.342	29.00	4.12	.341	3.77	Do.
Sixteen-to-One orange on rough lemon rootstock collected near Orlando.	Sept. 5	7.98	1.217	6.55	3.25	.670	1.22	Sour.
	Oct. 9	8.71	.630	13.83	3.89	.643	2.01	Sweet.
	Nov. 6	9.00	.573	15.70	3.89	.602	2.62	Do.
	Dec. 4	9.70	.552	17.50	3.99	.560	2.93	Do.
	Jan. 8	10.00	.493	20.28	4.17	.561	3.70	Do.
	Feb. 5	10.25	.474	21.62	4.10	.534	4.45	Sweet; insipid.
	Mar. 4	10.02	.405	24.75	4.25	.600	5.06	Do.

TABLE 3.—Seasonal changes in carotenoid pigments and other constituents in, and flavor of, the juice of Florida oranges, 1939-40—Continued

Variety and description of sample	Date collected	Total soluble solids	Total acids	Solids-to-acid ratio	Active acidity	Ascorbic acid	Total carotenoids	Flavor
		Percent	Percent		pH	Mg/ml	Mg/ml	
Washington Navel orange on sour orange rootstock collected in north-central Florida.	Sept. 18	8.42	.863	9.75	3.56	0.450	1.31	Slightly sour.
	Oct. 23	9.02	.705	13.59	3.75	.407	2.76	Sweet.
	Nov. 20	10.80	.634	17.03	3.81	.435	3.06	Do.
	Dec. 18	11.05	.638	17.32	3.87	.403	3.22	Do.
	Jan. 22	11.67	.575	20.30	3.99	.413	3.73	Do.
	Feb. 19	11.51	.377	30.56	4.18	.279	3.36	Very sweet; insipid.
	Mar. 18	12.30	.318	38.69	4.23	.266	4.12	Do.
	Sept. 11	7.87	1.476	5.34	3.29	.589	.56	Sour.
	Oct. 16	8.79	1.081	8.11	3.45	.640	1.73	Tart.
	Nov. 13	9.70	.952	10.19	3.65	.644	2.34	Pleasantly tart.
Pineapple orange on rough lemon rootstock collected near Orlando.	Dec. 11	10.64	1.010	10.54	3.67	.634	3.24	Do.
	Jan. 15	10.92	.884	12.35	3.81	.633	3.93	Pleasantly tart to sweet.
	Feb. 12	10.92	.846	12.91	3.81	.582	4.21	Sweet.
	Mar. 11	11.13	.784	14.20	3.85	.600	5.40	Off-flavor.
	Sept. 18	8.11	1.338	6.06	3.27	.498	1.55	Sour.
	Oct. 23	8.72	.836	9.32	3.55	.466	1.57	Tart.
	Nov. 20	11.00	1.082	10.16	3.43	.579	4.33	Do.
	Dec. 18	11.30	1.014	11.14	3.52	.551	5.05	Pleasantly tart.
	Jan. 5	11.90	.908	11.92	3.57	.561	5.51	Do.
	Jan. 22	11.97	.942	12.71	3.68	.563	5.02	Pleasantly tart to sweet.
Pineapple orange on sour orange rootstock collected in north-central Florida.	Feb. 19	12.32	.810	15.21	3.79	.489	5.57	Do.
	Mar. 18	12.90	.824	20.68	3.93	.501	7.04	Sweet.
	Sept. 25	9.08	1.642	5.90	3.12	.656	1.70	Slightly sour.
	Oct. 30	10.70	1.265	8.53	3.33	.677	2.29	Tart.
	Nov. 27	11.82	1.158	10.21	3.52	.672	3.43	Do.
	Dec. 27	12.70	1.138	11.24	3.69	.672	4.63	Pleasantly tart.
	Jan. 30	13.34	.988	13.50	3.61	.665	5.45	Sweet.
	Feb. 26	12.80	.884	14.47	3.73	.494	5.05	Do.
	Mar. 25	14.06	.802	17.53	3.91	.558	5.66	Do.
	Sept. 18	9.15	1.403	6.52	2.78	.466	1.24	Sour.
Jaffa orange on sour orange rootstock collected in north-central Florida.	Oct. 23	9.02	1.678	9.21	3.80	.458	1.90	Tart.
	Nov. 20	10.50	1.054	9.96	3.42	.441	2.52	Do.
	Dec. 18	10.80	1.062	10.17	3.34	.422	2.40	Do.
	Jan. 22	11.47	9.42	12.17	3.47	.444	2.42	Pleasantly tart.
	Feb. 19	11.12	.650	16.95	3.74	.329	2.74	Do.
	Mar. 18	11.70	.533	21.96	3.86	.316	3.11	Sweet.
	Sept. 11	8.88	1.353	6.56	3.33	.448	1.31	Slightly sour.
	Oct. 16	9.50	1.028	9.33	3.42	.418	2.62	Tart.
	Nov. 13	10.68	1.046	10.11	3.40	.492	2.98	Pleasantly tart.
	Dec. 11	11.14	.996	11.19	3.50	.490	4.15	Do.
Ruby orange on sour orange rootstock collected near Orlando.	Jan. 15	11.70	.856	13.66	3.58	.478	4.27	Do.
	Feb. 12	12.84	.938	13.69	3.77	.465	5.39	Do.
	Mar. 11	12.64	.686	11.42	3.80	.432	5.55	Sweet.
	Sept. 5	8.88	1.810	4.88	3.14	.512	1.20	Sour.
	Oct. 9	9.21	1.262	7.30	3.32	.473	1.61	Do.
	Nov. 6	9.70	1.032	9.39	3.40	.480	2.60	Tart.
	Dec. 4	10.46	1.098	9.50	3.48	.480	3.05	Do.
	Jan. 8	10.90	.950	11.47	3.66	.489	3.11	Slightly tart.
	Feb. 5	11.25	.804	13.99	3.76	.433	4.75	Pleasantly tart.
	Mar. 4	11.25	.690	16.30	3.91	.337	3.91	Off-flavor.
Conner orange on sour orange rootstock collected near Orlando.	Sept. 5	7.71	1.353	5.69	3.29	.499	.95	Very sour; astringent.
	Oct. 9	8.31	.955	8.70	3.50	.563	1.08	Tart.
	Nov. 6	8.50	.864	9.95	3.57	.503	1.85	Do.
	Dec. 4	9.08	.814	11.15	3.65	.474	2.17	Do.
	Jan. 8	9.30	.792	11.75	3.67	.461	3.19	Pleasantly tart.
	Feb. 5	9.35	.740	12.80	3.70	.438	3.95	Sweet.
	Mar. 4	9.62	.668	14.40	3.84	.410	4.08	Do.
	Oct. 30	8.40	1.777	4.72	3.03	.500	.81	Very sour; astringent.
	Nov. 27	9.41	1.530	6.15	3.12	.576	2.01	Sour.
	Dec. 30	9.99	1.387	7.20	3.37	.546	3.25	Do.
Valencia orange on rough lemon rootstock collected in south-central Florida.	Jan. 29	10.84	1.152	9.41	3.34	.510	3.86	Tart.
	Feb. 26	11.30	1.260	8.75	3.34	.455	4.19	Do.
	Mar. 25	11.34	1.036	10.95	3.62	.440	5.45	Pleasantly tart.
	Apr. 22	10.89	.810	13.45	3.63	.377	4.42	Sweet.
	May 20	10.69	.649	16.48	3.96	.278	3.84	Do.

TABLE 3.—Seasonal changes in carotenoid pigments and other constituents in, and flavor of, the juice of Florida oranges, 1939-40—Continued

Variety and description of sample	Date collected	Total soluble solids	Total acids	Solids-to-acid ratio	Active acidity	Ascorbic acid	Total carotenoids	Flavor
		Percent	Percent		pH	Mg/100	Mg/100	
Valencia orange on sour orange rootstock collected on the east coast.	Oct. 30	9.47	1.879	5.04	2.98	0.569	1.83	Very sour; astringent.
	Nov. 27	10.10	1.688	6.03	3.11	.538	3.04	Sour.
	Dec. 30	10.97	1.616	6.78	3.23	.536	4.35	Do.
	Jan. 30	11.64	1.407	8.27	3.26	.540	5.48	Tart to pleasantly tart.
	Feb. 26	12.60	1.574	8.00	3.23	.522	6.02	Do.
Valencia orange on sour orange rootstock collected near Orlando.	Oct. 30	9.60	1.984	4.83	2.97	.551	2.05	Very sour; astringent.
	Nov. 27	10.40	1.800	5.78	3.02	.578	3.24	Sour.
	Dec. 30	11.49	1.586	7.24	3.28	.591	4.78	Do.
	Jan. 29	11.54	1.383	8.34	3.30	.542	5.87	Do.
	Feb. 24	11.72	1.514	7.74	3.25	.498	6.42	Pleasantly tart.
Valencia orange on rough lemon rootstock collected near Orlando.	Mar. 26	12.24	1.350	9.07	3.41	.533	8.48	Do.
	Apr. 22	12.16	.969	12.55	3.58	.491	7.70	Sweet.
	May 20	12.87	.858	15.00	3.83	.485	7.74	Do.
	Oct. 30	8.97	1.859	4.82	2.95	.592	1.73	Immature.
	Nov. 27	9.51	1.606	5.92	3.01	.576	2.76	Sour.
Dancy tangerine on rough lemon rootstock collected near Orlando.	Dec. 30	10.30	1.410	7.31	3.27	.579	4.03	Do.
	Jan. 29	11.54	1.383	8.31	3.30	.512	5.80	Do.
	Feb. 24	10.87	1.266	8.65	3.23	.513	5.41	Pleasantly tart.
	Sept. 11	9.58	4.192	2.28	2.55	.291	3.31	Very sour; astringent.
	Oct. 16	9.89	2.524	3.91	2.72	.339	6.04	Do.
Dancy tangerine on sour orange rootstock collected in north-central Florida.	Nov. 13	10.00	1.456	6.87	3.01	.340	8.81	Tart.
	Dec. 11	11.04	1.008	10.06	3.32	.339	10.72	Pleasantly tart.
	Jan. 15	12.20	.976	12.50	3.47	.282	9.21	Sweet.
	Feb. 12	13.44	.936	14.36	3.57	.231	11.03	Do.
	Sept. 18	9.91	3.140	3.15	2.56	.294	2.78	Very sour; astringent.
Temple orange on sour orange rootstock collected near Orlando.	Oct. 23	10.25	1.750	5.86	2.86	.300	7.35	Tart.
	Nov. 20	11.30	1.260	8.96	3.17	.324	10.26	Do.
	Dec. 18	12.40	1.174	10.56	3.25	.340	9.91	Pleasantly tart.
	Jan. 22	13.70	.904	15.15	3.55	.345	14.41	Pleasantly tart to sweet.
	Feb. 19	15.22	.377	40.37	4.13	.139	15.22	Sweet.
Temple orange on sour orange rootstock collected on east coast.	Mar. 18	17.81	.674	26.42		.134	17.52	Do.
	Sept. 11	8.58	2.750	3.11	2.91	.502	.72	Very sour; astringent.
	Oct. 15	9.20	1.091	4.60	2.88	.482	2.23	Do.
	Nov. 13	10.00	1.014	6.19	3.15	.539	3.80	Sour.
	Dec. 11	10.74	1.412	7.61	3.27	.515	5.53	Do.
Owari satsuma orange on rough lemon rootstock collected near Orlando.	Jan. 15	11.97	1.291	9.25	3.40	.555	7.62	Slightly tart.
	Feb. 12	12.54	1.008	12.44	3.64	.420	8.78	Pleasantly tart.
	Mar. 11	13.34	.978	13.64	3.68	.465	7.60	Sweet.
	Sept. 25	9.58	2.912	3.29	2.74	.512	1.36	Very sour; astringent.
	Oct. 30	10.07	1.886	5.66	3.02	.537	2.43	Sour.
Owari satsuma orange on grapefruit rootstock collected near Orlando.	Nov. 27	11.00	1.554	7.05	3.16	.534	4.85	Do.
	Dec. 27	11.66	1.220	9.56	3.36	.527	5.32	Tart to pleasantly tart.
	Jan. 30	12.54	1.114	11.26	3.43	.536	10.56	Pleasantly tart.
	Feb. 26	13.17	.987	13.34	3.53	.513	10.01	Pleasantly tart to sweet.
	Mar. 25	13.14	.822	15.98	3.73	.514	10.14	Do.
King orange on rough lemon rootstock collected near Orlando.	Sept. 11	9.78	3.488	2.80	2.69	.202	4.77	Very sour; astringent.
	Oct. 16	9.99	1.036	5.13	2.91	.195	10.57	Do.
	Nov. 13	10.28	1.154	8.99	3.29	.273	14.39	Tart.
	Dec. 11	11.54	1.008	11.54	3.40	.267	17.90	Sweet.
	Jan. 15	12.20	.886	13.76	3.64	.204	20.30	Do.
King orange on rough lemon rootstock collected near Orlando.	Nov. 13	9.97	.874	11.41	3.38	.330	15.60	Pleasantly tart.
	Dec. 11	11.54	1.008	11.45	3.46	.267	15.44	Sweet.
	Jan. 15	11.70	.894	13.08	3.53	.394	20.16	Do.
	Sept. 11	8.18	2.955	2.76	2.72	.230	4.39	Very sour; astringent.
	Oct. 16	8.39	2.074	4.04	2.76	.206	5.09	Do.
King orange on rough lemon rootstock collected near Orlando.	Nov. 13	8.67	1.662	5.41	3.01	.242	10.28	Do.
	Dec. 11	9.55	1.424	6.70	3.35	.217	10.36	Sour.
	Jan. 15	10.94	1.234	8.87	3.35	.197	22.40	Slightly sour.
	Feb. 12	12.04	1.109	10.04	3.43	.199	24.52	Tart.
	Mar. 11	13.73	1.214	11.31	3.52	.157	24.24	Sweet.

TABLE 4.—Effect of two rootstocks on juice constituents of Florida oranges, 1938-39 and 1939-40¹

Variety and season	Month collected	Total soluble solids		Total acids		Solids-to-acid ratio		Active acidity		Ascorbic acid		Flavor	
		Rough lemon stock	Sour orange stock	Rough lemon stock	Sour orange stock	Rough lemon stock	Sour orange stock	Rough lemon stock	Sour orange stock	Rough lemon stock	Sour orange stock	Rough lemon stock	Sour orange stock
Parson Brown orange, 1938-39.	September	Percent 7.52	Percent 8.18	Percent 1.502	Percent 1.808	5.01	4.53	pH 3.35	pH 3.20	Mg/ml 0.631	Mg/ml 0.740	Sour; astringent	Sour; astringent.
	October	8.87	10.17	.888	1.482	10.00	6.80	3.84	3.46	.633	.735	Pleasantly tart	Slightly sour.
	November	9.07	10.97	.850	1.064	11.38	10.31	3.75	3.48	.571	.660	do	Pleasantly tart.
	December	10.07	12.83	.716	.932	14.06	13.77	3.08	3.75	.582	.758	Sweet	Do.
	January	10.50	12.41	.646	.708	16.35	15.55	4.02	3.88	.561	.717	do	Sweet.
	February	10.98	12.37	.644	.716	17.05	17.28	3.94	3.92	.550	.723	do	Do.
Parson Brown orange, 1939-40.	September	7.77	8.88	1.090	1.229	7.13	7.23	3.47	3.39	.514	.601	Sour	Slightly sour.
	October	8.72	9.62	.662	.774	13.16	12.83	3.80	3.76	.506	.469	Sweet	Pleasantly tart.
	November	9.20	10.20	.554	.687	16.60	14.84	4.00	3.87	.531	.557	do	Sweet.
	December	9.71	10.98	.516	.558	18.80	19.60	4.06	4.06	.523	.550	do	Do.
	January	10.65	12.00	.491	.529	21.70	20.68	4.31	4.20	.517	.558	do	Do.
	February	10.95	12.95	.420	.489	26.07	26.50	4.18	4.12	.435	.475	do	Do.
Hamlin orange, 1938-39	March	10.82	12.62	.354	.416	30.05	30.35	4.33	4.24	.401	.428	Sweet; old	Do.
	September	6.62	7.27	1.316	1.236	5.03	5.88	3.39	3.17	.520	.470	Sour; astringent	Sour; astringent.
	October	7.57	8.35	1.002	.868	7.57	9.02	3.55	3.73	.489	.463	Sour	Tart.
	November	8.62	8.91	.936	.738	9.21	12.10	3.57	3.87	.446	.458	Pleasantly tart	Pleasantly tart.
	December	9.23	8.58	.814	.786	11.34	10.92	3.87	3.75	.466	.415	do	Do.
	January	8.61	9.16	.751	.706	11.47	12.98	3.93	3.78	.458	.430	Pleasantly tart to sweet.	Do.
Hamlin orange, 1939-40.	February	8.92	10.40	.788	.644	12.09	16.15	3.91	4.03	.457	.448	do	Do.
	September	8.31	8.21	1.109	1.042	6.93	7.88	3.29	3.38	.586	.485	Sour	Slightly sour.
	October	9.01	8.53	.816	.782	11.05	10.91	3.54	3.50	.565	.478	Pleasantly tart	Sweet.
	November	9.60	9.10	.752	.714	12.75	12.74	3.67	3.60	.565	.464	Sweet	Do.
	December	9.90	8.95	.710	.654	13.90	13.68	3.75	3.68	.520	.444	Sweet; watery	Watery.
	January	9.90	9.77	.602	.611	14.30	15.99	3.75	3.80	.509	.473	do	Do.
Pineapple orange, 1938-39	February	10.25	9.57	.611	.403	16.77	23.74	3.92	3.90	.465	.318	do	Watery; off-flavor.
	March	10.52	9.92	.548	.342	19.20	20.00	4.04	4.12	.464	.341	do	do
	September	6.92	7.52	1.570	1.610	4.41	4.67	3.29	2.96	.553	.667	Very sour; astringent	Very sour; astringent.
	October	8.02	8.63	1.211	1.292	6.62	6.68	3.42	3.53	.567	.630	Sour	Sour.
	November	8.67	9.39	1.076	1.058	8.06	8.87	3.53	3.67	.503	.551	Tart	Tart to pleasantly tart.
	December	9.73	10.43	.986	1.022	9.87	10.21	3.73	3.85	.582	.583	Tart to pleasantly tart	Pleasantly tart.
Pineapple orange, 1939-40	January	9.81	10.37	.912	.894	10.76	11.65	3.90	3.89	.563	.556	Pleasantly tart	Do.
	February	9.92	11.57	.900	.872	11.02	13.27	3.85	3.87	.561	.534	do	Do.

¹ Parson Brown on both rootstocks were grown near Orlando; other varieties on rough lemon stock grown near Orlando; those on sour orange stock (except Pineapple noted in footnote 2) grown in north-central Florida.

TABLE 4.—Effect of two rootstocks on juice constituents of Florida oranges, 1938-39 and 1939-40—Continued

Variety and season	Month collected	Total soluble solids		Total acids		Solids-to-acid ratio		Active acidity		Ascorbic acid		Flavor	
		Rough lemon stock	Sour orange stock	Rough lemon stock	Sour orange stock	Rough lemon stock	Sour orange stock	Rough lemon stock	Sour orange stock	Rough lemon stock	Sour orange stock	Rough lemon stock	Sour orange stock
Pineapple orange, 1939-40	September	Percent 7.87	Percent 8.11	Percent 1.476	Percent 1.338	5.34	6.06	pH 3.29	pH 3.27	Mg/ml 0.589	Mg/ml 0.498	Sour	Sour.
	October	8.79	8.72	1.084	.936	8.11	9.32	3.45	3.55	.640	.466	Tart	Tart.
	November	9.70	11.00	.952	1.082	10.19	10.16	3.65	3.43	.644	.579	Pleasantly tart	Do.
	December	10.64	11.30	1.010	1.014	10.54	11.14	3.67	3.52	.654	.551	Pleasantly tart to sweet	Pleasantly tart.
	January	10.92	11.97	.884	.942	12.35	12.71	3.81	3.68	.633	.563	Sweet	Pleasantly tart to sweet.
	February	10.92	12.32	.846	.810	12.91	15.21	3.81	3.79	.583	.489	do	Sweet.
Pineapple orange, 1939-40 ²	March	11.13	12.90	.784	.624	14.20	20.68	3.85	3.93	.600	.501	do	Do.
	September	7.87	9.68	1.476	1.642	5.34	5.90	3.29	3.12	.589	.656	Sour	Slightly sour.
	October	8.79	10.79	1.084	1.265	8.11	8.53	3.45	3.33	.640	.677	Tart	Tart.
	November	9.70	11.82	.952	1.158	10.19	10.21	3.65	3.52	.644	.672	Pleasantly tart	Do.
	December	10.64	12.79	1.010	1.138	10.54	11.24	3.67	3.59	.654	.672	Pleasantly tart; sweet.	Pleasantly tart.
	January	10.92	13.34	.884	.988	12.35	13.50	3.81	3.61	.633	.668	Sweet	Sweet.
Dancy tangerine, 1938-39	February	10.92	12.80	.846	.884	12.91	14.47	3.81	3.73	.583	.494	do	Do.
	March	11.13	14.06	.784	.802	14.20	17.53	3.85	3.91	.600	.558	do	Do.
	September	7.87	8.70	2.464	2.983	3.19	2.92	2.68	2.73	.310	.280	Very sour; astringent.	Very sour; astringent.
	October	8.95	9.60	1.252	1.466	7.15	6.55	3.55	3.24	.311	.328	Sour	Sour.
	November	9.46	10.46	.824	.986	11.50	10.60	3.56	3.51	.300	.270	Pleasantly tart to sweet	Pleasantly tart.
	December	10.16	11.21	.814	.840	12.48	13.35	3.57	3.64	.279	.298	do	Pleasantly tart to sweet.
Dancy tangerine, 1939-40	January	10.56	11.73	.615	.696	17.17	16.85	3.70	3.72	.213	.231	Very sweet	Very sweet.
	February	12.74	13.39	.598	.733	21.30	18.27	4.01	3.91	.174	.243	do	Do.
	September	9.58	9.91	4.192	3.140	2.28	3.15	2.55	2.56	.291	.294	Very sour; astringent.	Very sour; astringent.
	October	9.89	10.25	2.524	1.750	3.91	5.86	2.72	2.86	.339	.300	do	Tart.
	November	10.00	11.30	1.456	1.260	6.87	8.96	3.01	3.17	.349	.324	Tart	Do.
	December	11.04	12.40	1.098	1.174	10.06	10.56	3.32	3.25	.339	.340	Pleasantly tart	Pleasantly tart.
Dancy tangerine, 1939-40	January	12.20	13.70	.976	.904	12.50	15.15	3.47	3.55	.282	.345	Sweet	Pleasantly tart to sweet.
	February	13.44	15.22	.936	.377	14.36	40.37	3.57	4.13	.231	.139	do	Sweet.
	March		17.81		.674		26.42				.134		

² Samples on sour orange rootstock were from east coast of Florida.

In general the data confirm those reported by Harding, Winston, and Fisher (2). Parson Brown oranges grown on sour orange rootstock contained a greater percentage of soluble solids and acids than did those grown on rough lemon rootstock. During the period in which the analyses were made, the rough lemon lot showed a range in solids of 7.77 to 10.82 percent, whereas those on sour orange stock contained 8.88 to 13.56 percent solids. The range of acidity in the rough lemon lot was between 0.354 and 1.090 percent as compared with 0.416 and 1.284 percent for the sour orange lots. Higher ascorbic acid (vitamin C) was found in the fruit when the trees were grown on sour orange stock. Hamlin and Sixteen-to-One showed lower percentages of soluble solids than the other early varieties. The two Hamlin lots ranged from 8.21 to 10.52 percent soluble solids and the Sixteen-to-One from 7.98 to 10.25 percent. The Washington Navel, though fairly high in soluble solids, was low in acid, ranging from 0.318 to 0.863 percent during the season sampled. This variety developed a sweet flavor early in the season but lacked the tartness usually found in other varieties as a result of their higher acidity. The Hamlin oranges on sour orange rootstock were collected from a thickly planted hammock grove where there was considerable shading, and it was probably on this account that these did not show the customary superiority over fruit of the same variety on rough lemon stock, insofar as solids and acids are concerned.

The seasonal variation in solids and acids in these early varieties over a 2-year period is shown in figure 8. The consistently low percentage of soluble solids in the Hamlin variety is pronounced. In November, when the Parson Browns showed 10.49 and 11.30 percent solids, the Hamlins contained 8.91 and 9.10 percent solids. By the end of the sampling period and later than the customary shipping season, the Hamlins contained approximately 10 to 10.5 percent soluble solids, whereas the Parson Browns had increased to between 12 and 13 percent solids. The low acidity of the Washington Navel as compared with its solids content is indicated here. The break in the curve for the Washington Navel in 1938-39 indicates a change to another grove in the same section of the State, after exhausting the supply of samples in one grove.

It will be observed in table 3 that the Pineapple oranges on rough lemon rootstock were grown near Orlando, and of the two lots on sour orange rootstock, one was collected on the east coast and the other in north-central Florida. Although the last lot was higher in total solids than those grown near Orlando, the east coast fruit was still higher in these constituents. The intermediate position of the north Florida fruit is doubtless due to shading, as was the case with the Hamlin lots on sour orange stock previously mentioned. Both varieties were collected from the same hammock grove in north Florida. The Pineapple oranges on rough lemon rootstock showed a range in total solids from 7.87 to 11.13 percent during the sampling season; those on sour orange stock, from 8.11 to 14.06 percent. The range of acids was from 0.784 to 1.476 percent for rough lemon rootstock and from 0.624 to 1.642 percent for sour orange stock. The east coast lot was generally higher than the other lots in total and ascorbic acids.

Total soluble solids in the Homosassa orange were rather low for a midseason variety, ranging from 7.71 to 9.62 percent. Other midseason varieties showed soluble solids content comparable to that of

the Pineapple variety; i. e., Ruby orange, 8.88 to 12.84 percent, Conner, 8.88 to 11.25 percent, and Jaffa, 9.15 to 11.70 percent. The last-named variety was relatively high in both solids and acids throughout the sampling season.

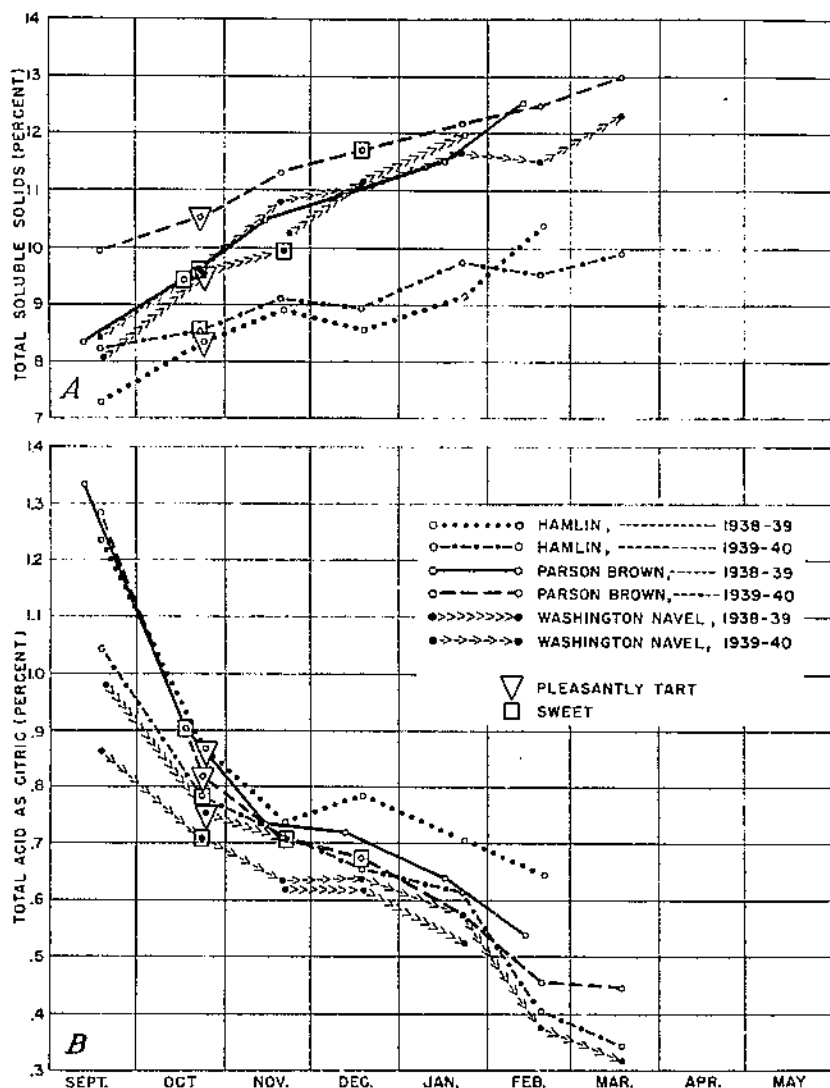


FIGURE 8.—Seasonal changes in flavor and in total soluble solids (A) and in total acid as citric (B) in early and midseason Florida oranges during two seasons. Fruit grown on sour orange rootstock in north-central Florida.

Figure 9 gives a comparison of Pineapple oranges from one source for three seasons and from another source for two seasons, with regard to total soluble solids and to total acidity. The high percentage of solids and of acids in the east coast fruit (see p. 13) is shown. The values for these constituents in other lots were fairly uniform. The

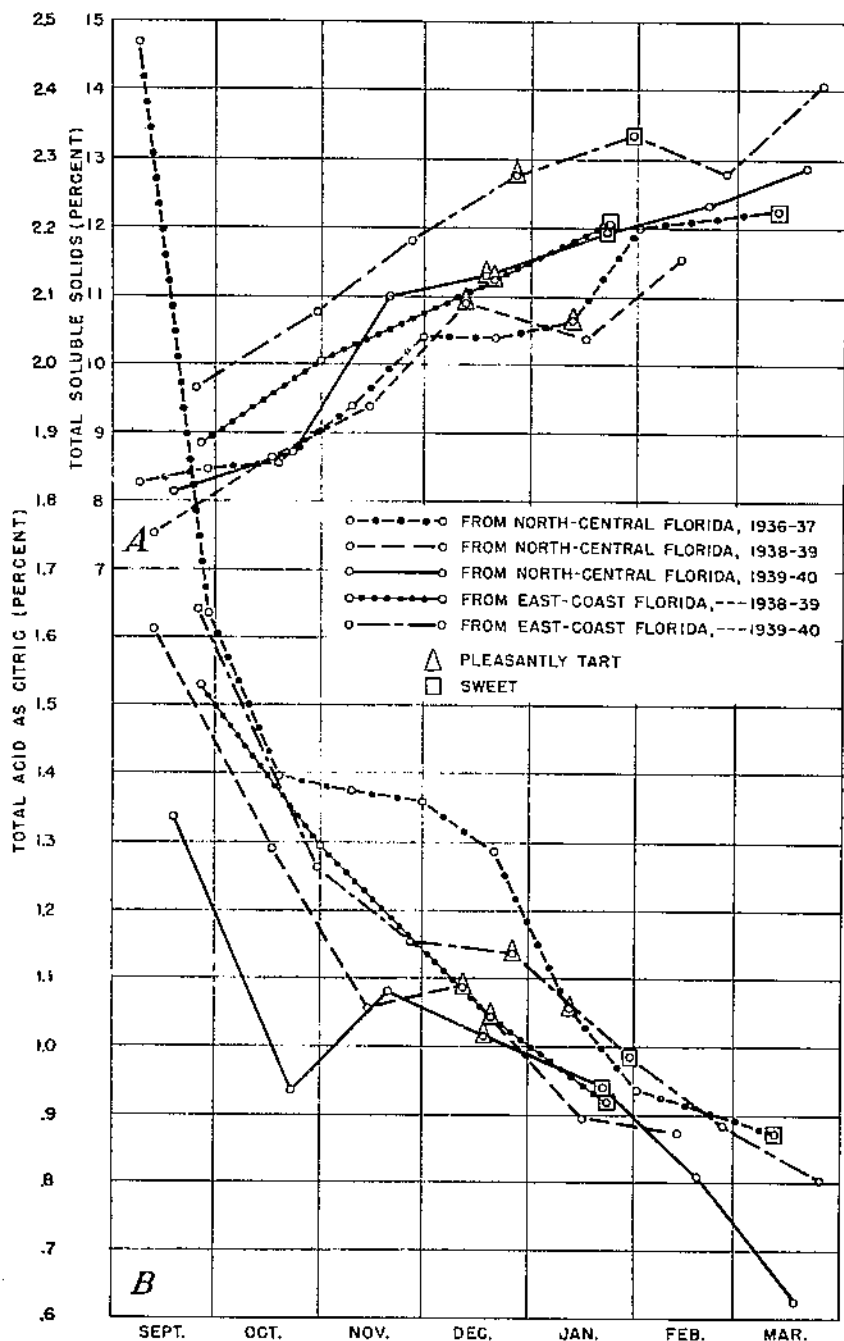


FIGURE 9.—Seasonal changes in flavor and in total soluble solids (A) and total acid as citric (B) of Pineapple oranges from one source for three seasons and from another source for two seasons. All fruit was grown on sour orange rootstock.

results for soluble solids in the lot from north Florida were fairly consistent for the 3-year period. The acidity was a little higher for the 1936-37 season in these fruits.

The Valencia oranges (table 3) resembled the early and midseason varieties in their seasonal changes in total soluble solids and total acids. Those grown on sour orange rootstock contained higher soluble solids and higher acids than those grown on rough lemon stock. There was not a great difference in the ascorbic acid content of the lots grown on the two rootstocks.

Figure 10 affords a comparison of Valencias grown in three different parts of the State and on two rootstocks. The figure shows seasonal changes in solids and acids for Valencia oranges on sour orange rootstock grown near Orlando and on the east coast and for the same variety on rough lemon rootstock from near Orlando and from south-central Florida. It will be seen that the rootstock exerted greater effect on the constituents than did the locality in which the fruit was grown. The two lots from near Orlando afford an excellent comparison of the rootstock effect, because they were both obtained from the same grove, which is on a soil to which both rootstocks are adapted, thus reducing the variables frequently encountered in comparing two lots from different sections. The curves for total acidity following the January sampling show a little irregularity. This is doubtless due to the fact that many fruits were frozen during the latter part of January. On an occasion like this the older fruit may drop prematurely, leaving a larger percentage of oranges maturing from later blooms.

The oranges of the mandarin group are characterized, among other qualities, by a deeper orange color in flesh and rind and a higher soluble-solids content of the juice. The latter point is emphasized in figure 11 where seasonal changes are shown for solids and acids in Dancy tangerines and Temple oranges. The Dancy tangerines on sour orange rootstock were grown in north-central Florida in soil to which the rootstock is well suited. The Temples on sour orange and the Dancy on rough lemon rootstock were obtained near Orlando from a grove in which the rough lemon rootstock does well. The Owaris (satsuma) were grown near Orlando. In the November 1938 samples (table 4) the juice of both lots of tangerines was considered pleasantly tart to sweet. At this time the rough lemon lot contained 9.46 percent solids and 0.824 percent acids; the one on sour orange stock showed 10.46 percent solids and 0.986 percent acids. The solids: acid ratio for the former was 11.5 and 10.6 for the latter. The figures for solids and acids also indicated the desirable effect of the sour orange rootstock as compared with the rough lemon.

The Temple orange was high in solids and very high in acids, as will be seen from figure 11. This variety was considered pleasantly tart on February 20. Analysis of the juice showed 12.39 percent solids, 1.262 percent acids and a solids: acid ratio of 9.82 at this time. It is significant that this ratio was lower than that for the tangerines on rough lemon (11.4) and on sour orange stock (10.6) when they were considered sweet. In other words, in these three instances, the higher the solids the lower the ratio, which suggests that the ratio is not in itself an adequate index of quality. Both high solids and a not too low acid content are essential to high quality in citrus fruit.

Another characteristic of mandarin type oranges is their low ascorbic

acid content as compared with that of the sweet orange type (*Citrus sinensis*). In table 3 the ascorbic acid for Dancy tangerines showed a

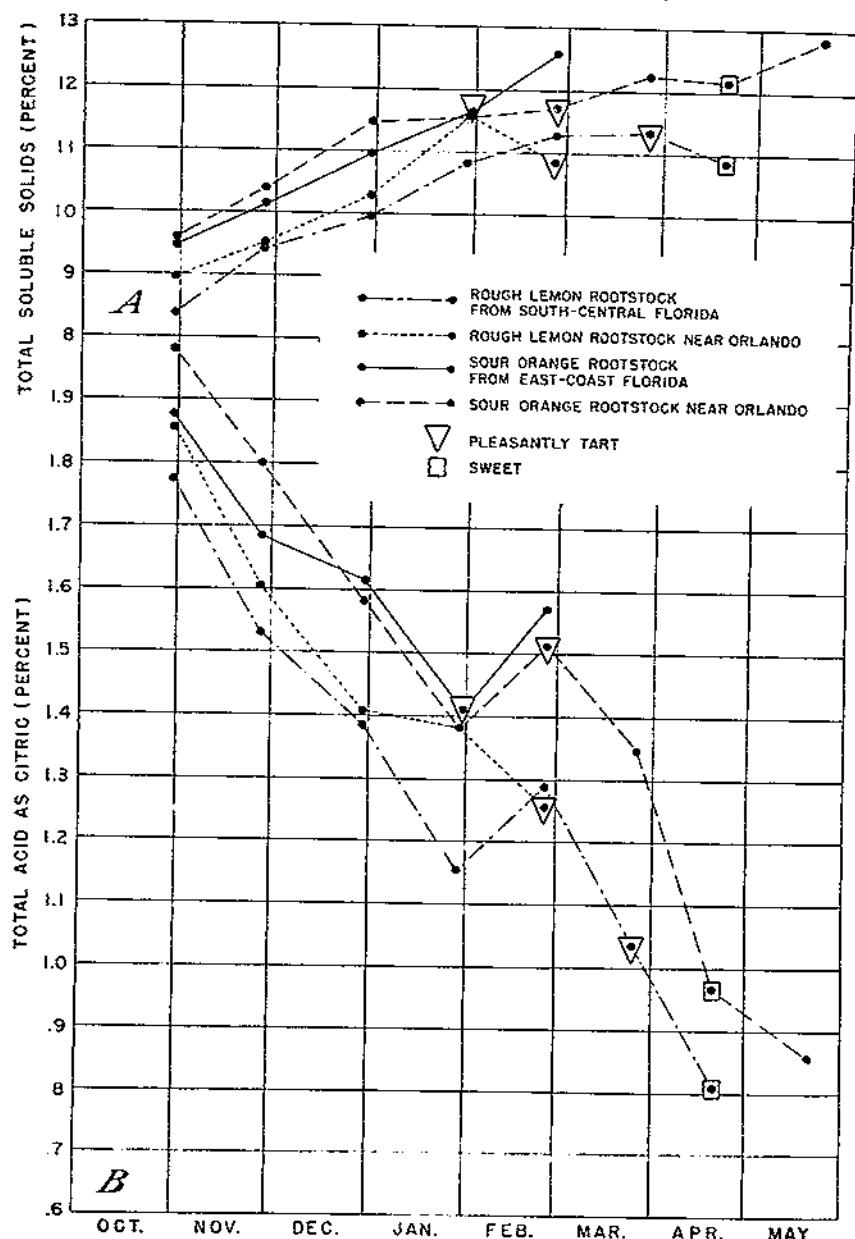


FIGURE 10.—Seasonal changes in flavor and in total soluble solids (A) and in total acids as citric (B) of Florida Valencia oranges.

range from 0.134 to 0.349 mg. per ml., for Owari satsumas from 0.202 to 0.364, and for Kings from 0.157 to 0.242. The Temple, a notable exception, showed a range of from 0.420 to 0.539 mg. per ml. In

fact, this variety compares favorably with the sweet orange in this constituent, and this fact further suggests its hybrid nature.

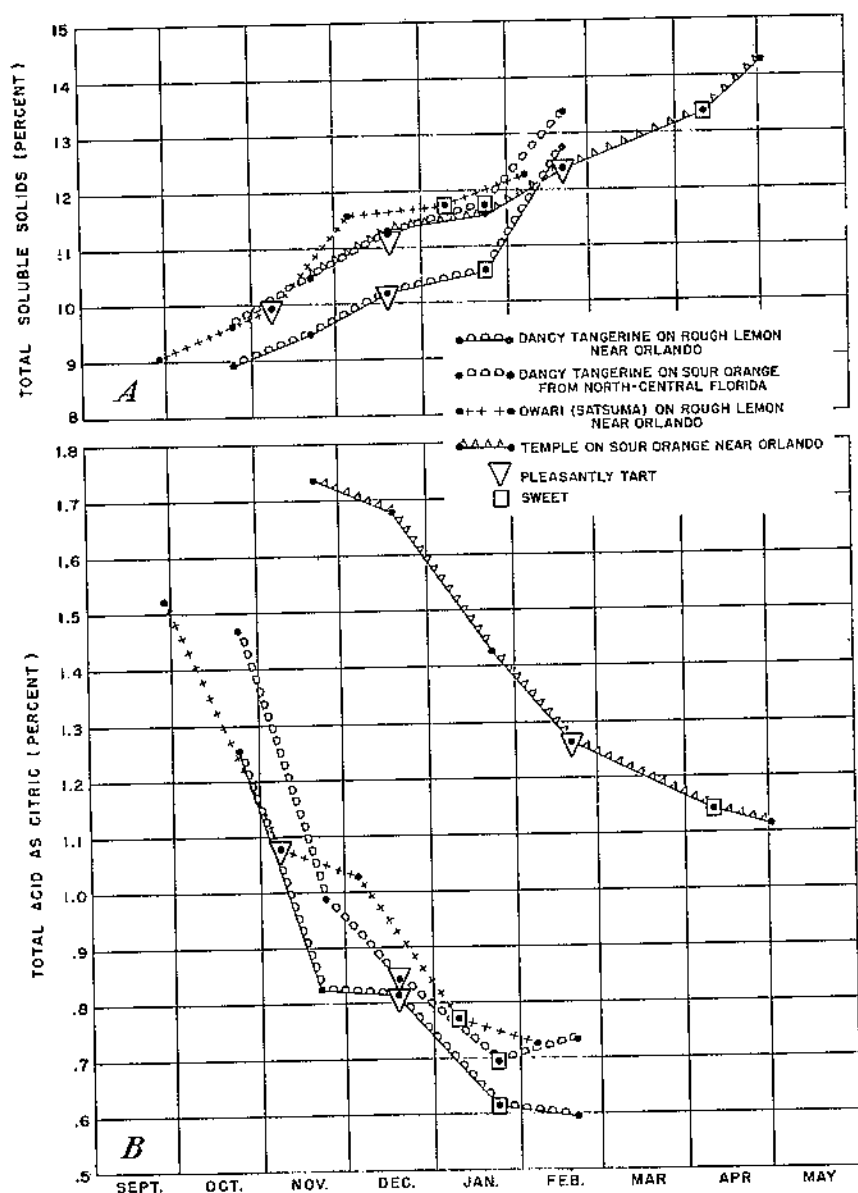


FIGURE 11.—Seasonal changes in flavor and in total soluble solids (A) and in total acid as citric (B) of mandarin types of Florida oranges (1938-39).

Figure 12 shows the differences usually found in the ascorbic acid content of different varieties. The Pineapple lot grown on the east coast showed the highest ascorbic acid content during most of the season. This was the same lot that showed high solids among several

lots of Pineapple. Again is evidenced the effect of the wider spacing of trees with resultant increase in sunlight. The curve next below this presents results on Pineapple oranges grown on rough lemon stock near Orlando. The Hamlin variety was noticeably low in

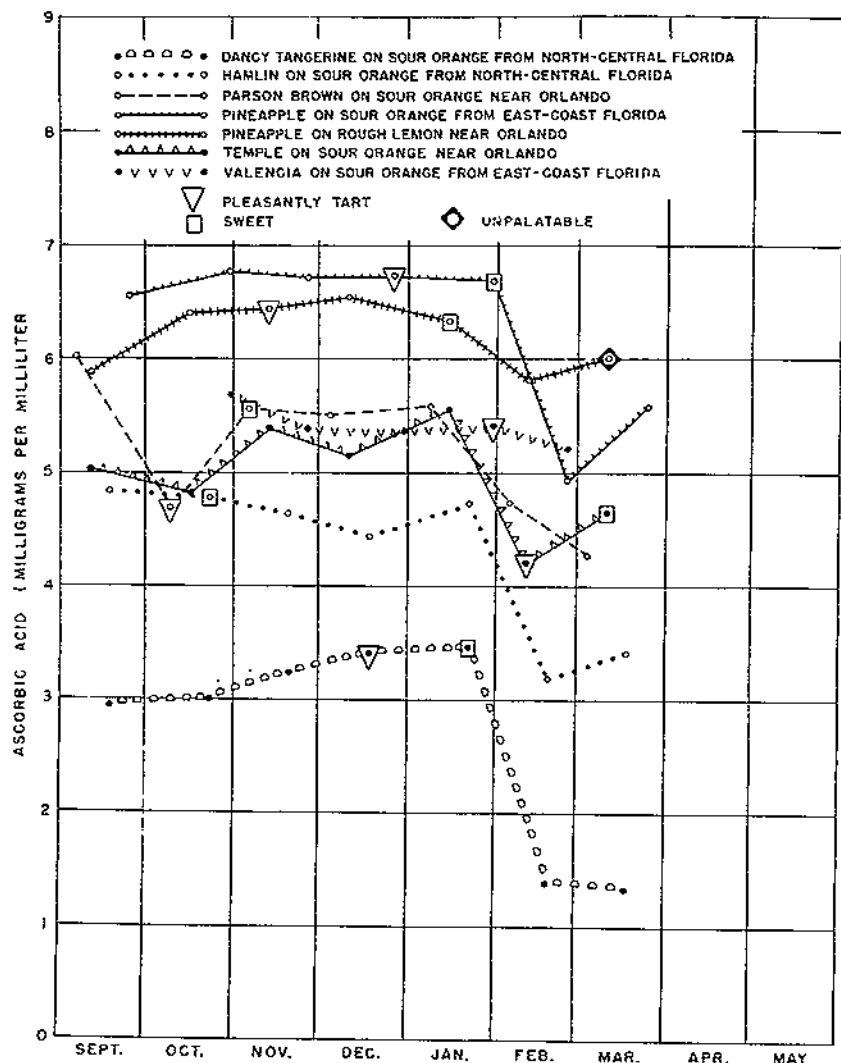


FIGURE 12.—Seasonal changes in flavor and in ascorbic acid content of several varieties of Florida oranges grown in different localities.

ascorbic acid. Parson Brown and Valencia varieties seemed to take an intermediate position among the commercial varieties. It will be noted that the Temple variety fell in this intermediate group of oranges. The results for the Dancy tangerines were typical for ascorbic acid in mandarin varieties. Ascorbic acid in all varieties showed considerable fluctuation during the season, with a pronounced tendency to decrease toward the end.

The pH values for active acidity (table 3) generally showed a consistent increase for all lots and all varieties during the sampling season. Occasional fluctuations, believed to be due to sampling error, were not nearly as evident in these figures as in those for other constituents.

From the combined results in tables 3 and 4, it would seem that the seasonal increases in carotenoid pigments were definitely a part of the ripening processes. These increases were quite generally accompanied by similar increases in total soluble solids and the solids-to-acid ratio, as well as by a decrease in total acids, in active acidity, and in ascorbic acid. There was no specific quantity of pigment in any variety at any time that could be taken as an index of maturity, nor was there any definite relationship between total carotenoids and other constituents when the fruit reached its most palatable stage. It has been suggested that fruits with pale-colored flesh at the time of marketing are lower in both solids and acids. This was found true for some, but not for all varieties. Hamlin, Homosassa, and Sixteen-to-One oranges were relatively low in carotenoid pigments, solids, and acids; on the other hand, the Jaffa orange was high in solids and acids and low in carotenoid pigments. In fact, this last-mentioned variety, when grown on sour orange stock, is considered a superior variety because it maintains a high solids and a high acid content throughout the season.

DISCUSSION

It seems probable that the carotenoid pigments in the juice of oranges must play a role in the general metabolism of the fruit. Their seasonal increase in the early and midseason varieties followed the same general trend as that of the total soluble solids. In varieties like Hamlin, Sixteen-to-One, and Homosassa, a low pigment content was associated with a low percentage of solids in the juice. The Jaffa, on the other hand, was characterized by a high percentage of solids and a low percentage of carotenoids, but the curves showing progressive changes in both of these constituents were very similar, suggesting a close relationship between the rates of formation.

Some of the observations are difficult to explain, as, for example, the continued increase in pigmentation after the juice has passed its most palatable stage. Some of this apparent increase may be due to dehydration of the fruit.

A highly colored rind in citrus fruits is usually associated with considerable pigmentation in the juice. This is especially true of the mandarin types. There are exceptions, however, as in the case of the Hamlin variety, which may develop a deep rind color without increasing the carotenoids in the juice. It is not understood why conditions that increase the pigmentation of the rind should not also affect the color in the flesh.

The blood oranges, of which Ruby is a type, though externally pale in color, contained almost as much pigmentation in the juice as the Parson Brown or Pineapple varieties. The term "blood" is applied because at a certain stage of maturity red, bloodlike spots appear in the flesh. The red color is due to a water-soluble pigment (anthocyanin) related to that found in apples, cherries, roses, etc. There is no record of any other citrus fruit having an anthocyanin pigment. The pigment responsible for the color in pink grapefruit is lycopene, an isomer of carotene, found most abundantly in the tomato fruit.

The seasonal increase in carotenoids in the juice of oranges should be of significance from a nutritional standpoint. Because several of the carotenoid pigments are precursors of vitamin A, it might be inferred that the potency of the fruit in regard to this vitamin would also increase with maturity. For instance, one lot of Parson Brown oranges contained 2.33 p. p. m. carotenoids in October and 4.04 p. p. m. in November; in fact it was shown that the carotenoids were increasing during most of the sampling season. This indicates that the results of a vitamin A assay may vary with the stage of maturity. Also, the varieties should show considerable differences in vitamin A potency if carotenoid pigment content is a criterion.⁵

Of importance in this connection is the high pigmentation of the mandarin types of oranges. The maximum carotenoid content of these oranges averaged nearly three times that of the sweet oranges. If they are found to be proportionately as high in vitamin A potency this would more than compensate for their relatively low ascorbic acid content.

Strangely enough these mandarin varieties constitute a small proportion of the oranges consumed in this country. Many of them do not find their way to the market in any great volume. It seems that there should be an outlet for many of these fruits as dietary supplements for both man and animals. Cannery wastes, which consist mainly of grapefruit refuse, have been converted into cattle feed for many years. These fruits, including the rinds, are almost totally lacking in precursors of vitamin A. This suggests the possibility of reinforcing the feed with citrus types higher in vitamin A potency.

In making a comparison between seasonal changes in pigments and other constituents of the juice, a great deal of information on factors influencing flavor has been accumulated. The importance of high percentages of solids and of acids as well as the influence of locality and rootstock has been presented. It has been demonstrated in these and in other investigations that for the most part fruit produced on the sour orange rootstock has higher acid and higher soluble solids content than does that on rough lemon rootstock. It must be borne in mind, however, that juice quality may be influenced by a number of factors. In the present report instances have been cited in which shade may reduce both soluble solids and ascorbic acid in fruit and thus sometimes make the fruit from trees grown on sour orange rootstock appear inferior to that from trees on rough lemon with ample sunlight.

There is another point which should be considered in a discussion of this kind; namely, that there is an element of time involved in determining dessert quality. This is further emphasized by the fact that juice quality is frequently a matter of individual preference. It has been the experience of the writers that any of the varieties of oranges tested may be acceptable and pleasing to the consumer if presented at the optimum stage of maturity. This may well be illustrated with the Hamlin orange which is notably low in solids and is considered by many to be an inferior variety. In the present investigations the lots of the Hamlin picked in October 1939 showed a range of 8.53 to 9.01 percent of soluble solids, whereas the fruits left

⁵ This is substantiated by the results of biological assays for vitamin A made on fruits of the same plots by Lela E. Bocher, Bureau of Home Economics. Parson Brown oranges harvested on October 23 showed 42 International Units of vitamin A per 100 ml. juice, and 80 units when harvested November 20. Pineapple oranges contained 75 units of vitamin A per 100 ml. on January 5 and about 175 units on January 22.

on the trees ultimately attained a percentage of soluble solids in the 9-to-10 range. That the Hamlin is often shipped in October may account for its apparent unpopularity.

Varieties like the Sixteen-to-One and the Washington Navel are characterized by a sweet flavor due to their low acid content. Sweetness in oranges appears to be preferred by residents of the northern part of the country. Winter visitors to Florida have often expressed a preference for Parson Brown oranges in the experimental plots comparatively late in the season, long after the trade had shipped all of the early oranges out of the State. Most persons resident in Florida prefer more acid in their oranges and naturally choose those with a pleasantly tart flavor.

It is true that a high percentage of soluble solids and a not too low acid content contribute toward quality in citrus fruits. Temple on sour orange stock and the majority of Florida seedling oranges fall in this category and are usually considered above the average, but even these must have attained optimum maturity to be most acceptable.

The Jaffa orange on sour orange stock is peculiar in that it will attain a high solids and high acid content early in the season and maintain a high percentage of these constituents for a considerable period. That is, the increase in solids and loss of acids proceeds rather slowly. Usually considered a midseason variety, its shipping season has been known to overlap both the early and the late seasons.

The period in which the varieties were first considered pleasantly tart during the two seasons from 1938 to 1940 is indicated below. It must be remembered that collections were made but once a month so that these dates do not indicate when the fruit became pleasantly tart but merely when the first sample was found to have attained this stage.

List of varieties studied and the time when the samples were first found to be pleasantly tart

Variety:	Period in which first pleasantly tart sample was collected
Parson Brown	Oct. 9–Nov. 17.
Sixteen-to-One	
Washington Navel.....	
Hamlin	Nov. 13–Dec. 27.
Pineapple.....	
Ruby.....	
Owari (satsuma)	Nov. 7–Dec. 11.
Dancy tangerine.....	Nov. 11–Dec. 18.
Homosassa	Jan. 9–Feb. 5.
Conner.....	
Temple.....	Jan. 30–Feb. 20.
Valencia.....	Feb. 24–Mar. 25.

SUMMARY

A study was made of the seasonal changes in total carotenoid pigments, total soluble solids, total acids, active acidity, and ascorbic acid in the juice of Florida oranges of both the sweet (*Citrus sinensis*) and the mandarin (*Citrus nobilis*) types.

Fruits were collected from central, north-central, south-central, and the east-coast sections of the State. The Parson Brown, Hamlin, Pineapple, Valencia, Dancy tangerine and Owari satsuma varieties were grown on two different rootstocks, and the Sixteen-to-One,

Washington Navel, Conner, Homosassa, Jaffa, Ruby, Temple, and King on one only.

A new and more accurate method for determining total carotenoid pigments in citrus juices is described.

During the period of these observations (September to March), the early and midseason oranges showed a gradual increase in carotenoid pigments. Valencia oranges (late) showed an increase in pigment in the juice up to February or March, and this was usually followed by a decline.

Seasonal changes in the mandarin type of oranges for the most part were similar to those in the sweet orange type, but the mandarin varieties were much higher in carotenoid pigments than other varieties.

Ripening in all varieties was characterized by an increase in soluble solids and in pH values, and a corresponding decrease in total acids and in ascorbic acid.

Other conditions being equal, oranges grown on sour orange rootstock contained higher percentages of solids and of acids than those grown on rough lemon stock, but the degree of pigmentation was apparently not affected by rootstock.

The amount of carotenoid pigments in the juice generally varied with the locality in which the fruit was grown, there being a suggestion that low pigment content was associated with soil of low fertility.

Degree of pigmentation in the juice was not an index of stage of maturity nor always of quality. The early and midseason fruits were marketable before attaining maximum percentages of pigments, and Valencias were frequently marketed after decline had set in.

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<i>Farm Security Administration</i>	C. B. BALDWIN, <i>Administrator</i> .
<i>Federal Crop Insurance Corporation</i>	LEROY K. SMITH, <i>Manager</i> .
<i>Forest Service</i>	EARLE H. OLAPP, <i>Acting Chief</i> .
<i>Bureau of Home Economics</i>	LOUISE STANLEY, <i>Chief</i> .
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This bulletin is a contribution from

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END