UNEQUAL DISTRIBUTION OF SOLUBLE SOLIDS IN THE PULP OF CITRUS FRUITS[1]

E. T. BARTHOLOMEW AND WALTON B. SINCLAIR

(WITH SIX FIGURES)

Introduction

The nature of the citrus fruit is such that it affords many interesting problems of research for the plant physiologist. The pulp of the citrus fruit, unlike that of most edible fruits, is divided into segments (carpels) the walls of which are not readily permeable; and each segment is composed of hundreds of units (vesicles, juice sacs) the walls of which are still more impermeable, especially laterally (**18**).

In the past, investigators have drawn many conclusions that were based entirely on results obtained by analyzing a whole plant or the major portion of a plant. While the results of such analyses have their value, they obviously omit many interesting and vital details. The results reported in this paper illustrate the value of making detailed analyses of the different tissues of a given portion of a plant. They are a part of the results of a line of research which is being carried on at the present time in this laboratory. This concerns the effects of rootstocks, maturity, insecticides, and the physiological disorder ''granulation'' on the composition of citrus fruit.

Materials and methods

Valencia and Navel oranges of different sizes and of different stages of maturity were used in these tests, primarily; but a few grapefruit were tested also. All mature fruits were peeled, the segments isolated, and the juice from each stem and stylar segment half, or from each entire segment, was tested for the components mentioned in later sections of this paper. When a fruit was too young to be peeled and to have the segments isolated, the entire fruit pulp was bisected transversely. The peel of such fruits was first cut away with a knife, care being taken to leave a minimum of the peel and to cut away a minimum of the pulp.

Juice was extracted from the whole or halved segments of the mature fruits with a modified, old-style lemon squeezer. From the pulp halves of the young fruits, the juice was extracted by subjecting each half to a pressure of 6,000 pounds in a hydraulic press. Aliquots of the juice were centrifuged for three minutes at 1,700 r.p.m. before being tested.

Total soluble solids were determined with an Abbé refractometer. Total acidity was determined in terms of citric acid by titrating with standardized

[1] Paper no. 423, University of California Citrus Experiment Station.

solutions of NaOH, with phenolphthalein as indicator. The pH values were determined with a Beckman glass electrode pH meter.

Sugar determinations were made by the HAGEDORN and JENSEN method (**13, 14**) as modified by BLISH (**4, 5**). The strength of the reagents employed by BLISH was satisfactory for determining the reducing and total sugars as glucose, when the values ranged from 3 to 10 mg. in 10 ml. of citrus juice. The samples were diluted when necessary, so that the values fell within this range. This method was used because comparative tests showed that it was more rapid than the best of the copper reduction methods and, at the same time gave accurate results. Because potassium ferricyanide is subject to gradual change on standing, the glucose factor was redetermined just before making each group of tests. The values were determined by checking against a sample of glucose furnished by the National Bureau of Standards.

Results

SOLUBLE SOLIDS IN CARPELLARY SEGMENTS OF CITRUS FRUITS

SOLUBLE SOLIDS IN STEM AND STYLAR HALVES OF SEGMENTS.—SUTHERST (**20**), CHACE and CHURCH (**6**), BAKER (**2**), and HAAS and KLOTZ (**12**), who made an extensive study of polarity gradients, found that the concentration of soluble solids is noticeably greater in the stylar end of certain mature citrus fruits than in the stem end. This was especially true in the Valencia orange.

As is well known, other conditions being equal, the higher the concentration of soluble solids in a liquid, the lower the temperature required to freeze it. Therefore, in cutting many thousands of mature Valencia oranges during the study of granulation [a physiological disorder of Valencia fruits (**3**)], it was surprising to find that during the previous winter one or more segments in some of the fruits had been frozen at the stylar end and not at the stem end.

In order to find an answer to this apparent anomaly, an effort was made to correlate this condition with the distribution of soluble solids in the juice of the stem and stylar ends of the fruit during the winter months before the fruit was mature. Valencia and Navel oranges and also a few grapefruits were tested. This study entailed the making of almost 3,000 refractometric readings in addition to a large number of acid, sugar, and other determinations. Most of the fruits were tested on the day they were picked or on the following day.

In the localities from which the different fruits were obtained, the Valencias mature from the last of March to the first of May; Navels, from the middle of November to the latter part of December; and grapefruit, from the last of April to the first of June. All of these citrus fruits set at approximately the same time; therefore Valencias and grapefruit mature in

12 to 14 months and Navels in 8 to 9 months. The dates of maturity have an important bearing on the interpretation of the data to be presented.

The results of determining the total soluble solids in separate segment halves of Valencias, Navels, and grapefruits are presented in table I. These

TABLE I

DISTRIBUTION OF SOLUBLE SOLIDS IN STEM AND STYLAR HALVES OF CARPELLARY SEGMENTS OF CITRUS FRUITS IN DIFFERENT STAGES OF MATURITY

DATE FRUIT WAS PICKED AND TESTED	APPROXIMATE AGE OF FRUIT	NUMBER OF FRUITS	NUMBER OF SEGMENTS	PERCENTAGE OF SEGMENTS		
				TOTAL SOLUBLE SOLIDS HIGHER IN STEM THAN IN STYLAR HALVES	TOTAL SOLUBLE SOLIDS HIGHER IN STYLAR THAN IN STEM HALVES	TOTAL SOLUBLE SOLIDS EQUAL IN STEM AND STYLAR HALVES
			Valencia oranges			
	mo.			%	%	%
1937						
Nov. 1, 2	6.0	8	81	47	44	9
Dec. 27, 28	8.0	12	131	22	63	15
1938						
Jan. 5, 7, 8	8.0	9	95	25	63	12
Jan. 14–17	8.5	5	51	20	68	12
Feb. 4, 16	9.0	12	121	7	87	6
Mar. 11, 18	10.5	12	120	0	99	1
Mar. 21	11.0	12	129	1	99	0
June 28	14.0	10	106	0	100	0
1939						
Jan. 18	8.5	14	138	4	95	1
			Navel oranges			
1937						
Oct. 25–28	6.0	12	130	31	53	16
Dec. 22	8.0	6	63	0	97	3
1938						
Jan. 10, 11, 31	8.5	8	76	1	99	0
Feb. 2, 9, 23, 24	9.5	7	69	0	99	1
			Grapefruit			
1938						
Jan. 12–14	8.5	6	71	9	78	13
Feb. 14, 28	9.5	3	39	0	97	3

data show that, as a rule, the more mature the fruit, the greater the percentage of segments in which the total soluble solids were higher in the stylar than in the stem halves. For example, only 44 per cent. of the segments of the Valencias picked November 1 and 2, 1937, (6-month-old fruits), showed a concentration of total soluble solids higher in stylar than in stem halves, whereas practically 100 per cent. of the segments of the fruits picked from

March 11 to June 28, 1938 (10- to 14-month-old fruits), showed this condition. Similar trends were exhibited by the Navels and grapefruits.

The ages of the fruits given in table I are only approximate. The setting and maturing dates may be as much as a month or six weeks earlier or later one year than another. For instance, the Valencias tested January 14–17, 1938, were less mature than those tested January 18, 1939. This was shown, not only by the fact that on the latter date a larger percentage of the stylar halves of the segments contained the higher concentration of soluble solids (table I), but by the fact that these fruits had a considerably higher concentration of soluble solids (data not included in table) than those tested January 14–17, 1938.

From the data (table I) one may logically conclude that the concentration of total soluble solids in the stylar half of the citrus fruit does not noticeably exceed that in the stem half until the fruit is nearing maturity. It should be pointed out, however, that even in the youngest of the Valencias tested (6-month-old fruits), 44 per cent. of the segments had a higher concentration of total soluble solids in stylar than in stem halves, and 9 per cent. had equal concentrations in both halves. A similar condition existed in the Navel orange segments.

The diagrams in figures 1, 2, 3, and 4 illustrate the differences in concentration of total soluble solids in segments of Valencias about five months before maturity (fruit no. 103, fig. 1) and about two months after maturity (fruit no. 84, fig. 2), and in Navels about two months before maturity (fruit no. 92, fig. 3) and about two months after maturity (fruit no. 47, fig. 4). These two Valencias and two Navels showed the greatest extremes of all the fruits tested. Valencia no. 103 (fig. 1) had only one segment (no. 6) which

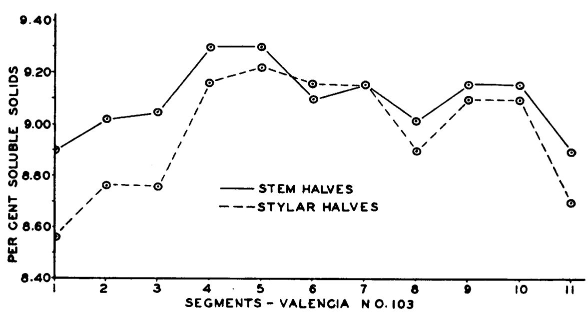

FIG. 1. Concentrations of total soluble solids in the stem and stylar halves of carpellary segments of an immature Valencia orange. With two exceptions, the concentrations are higher in the stem halves.

had a higher concentration of total soluble solids in its stylar than in its stem half. Segment 7 had the same concentration in both halves (9.16 per cent.), but all the other segments had lower concentrations in the stylar than in the stem halves. Navel no. 92 (fig. 3) showed a condition similar to that in Valencia no. 103 (fig. 1), except that four segments (nos. 1, 2, 9, and 10)

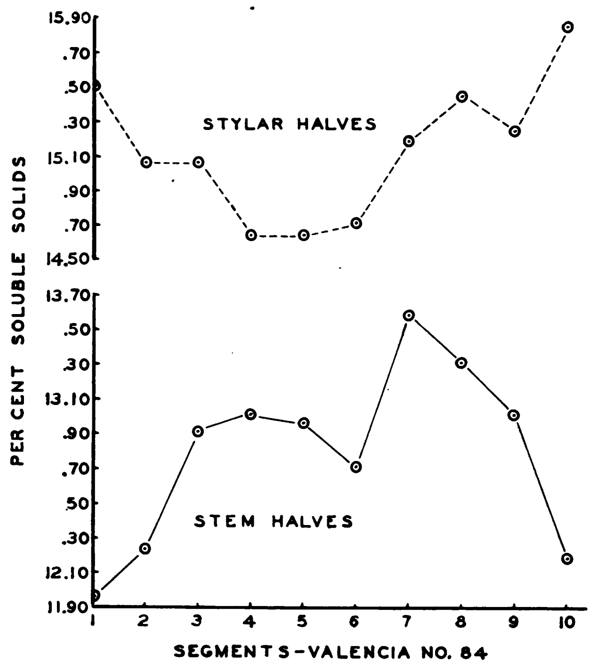

FIG. 2. Concentrations of total soluble solids in the stem and stylar halves of the carpellary segments of a mature Valencia orange. The concentrations are much lower in the stem than in the stylar halves, and there is a great difference in concentrations, not only in different segments, but in different stem and stylar segment halves.

had equal concentrations of soluble solids in both halves, and in no segment was the concentration higher in the stylar than in the stem half. The mature Valencia (fruit no. 84, fig. 2) and Navel (fruit no. 47, fig. 4) not only showed great differences in concentration in different stem and stylar segment halves, but showed much greater concentrations in all stylar halves than in stem halves. In the mature Valencia, segment 10 showed the great-

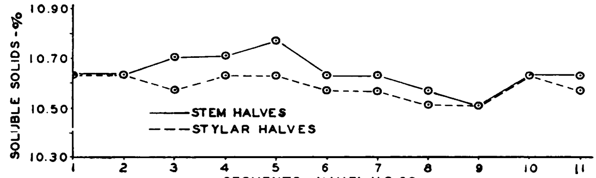

FIG. 3. Concentrations of total soluble solids in the stem and stylar halves of the carpellary segments of an immature Navel orange. The concentrations are higher in the stem halves of all segments except 1, 2, 9, and 10, in which they are equal in the two halves.

est difference between the concentration of soluble solids in the stem and stylar halves; the concentration in the stem half was 12.18 per cent. and that in the stylar half, 15.84 per cent., a difference of 3.66 per cent. A comparison of halves of segment 2 of the mature Navel showed the concentration in

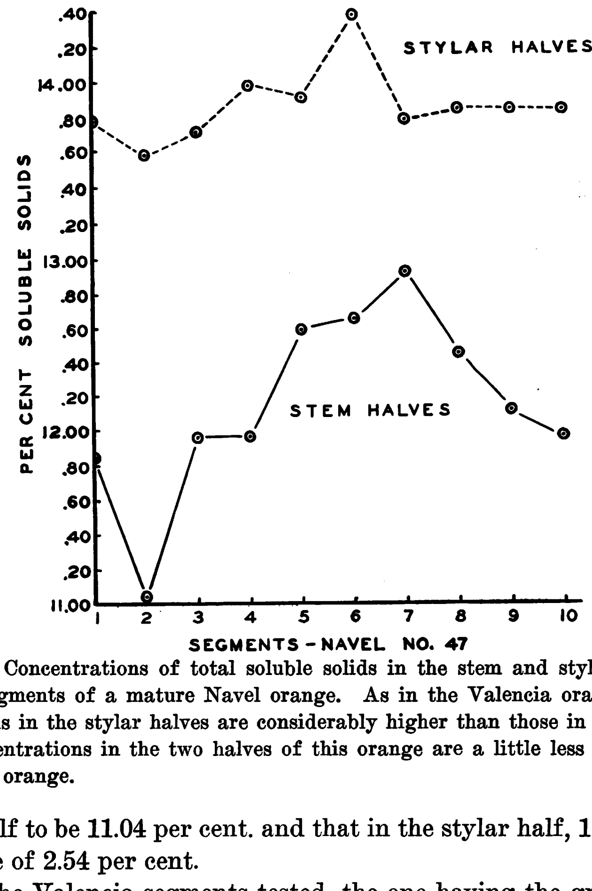

FIG. 4. Concentrations of total soluble solids in the stem and stylar halves of the carpellary segments of a mature Navel orange. As in the Valencia orange (fig. 2), the concentrations in the stylar halves are considerably higher than those in the stem halves; but the concentrations in the two halves of this orange are a little less than in those of the Valencia orange.

the stem half to be 11.04 per cent. and that in the stylar half, 13.58 per cent.; a difference of 2.54 per cent.

Of all the Valencia segments tested, the one having the greatest concentration of soluble solids in the stem half over that in the stylar half was from fruit no. 135, picked January 18, 1938. In this segment the concentration in the stem half was 8.70 and in the stylar half, 7.93 per cent; a difference of 0.77. The average difference for all Valencia segments that had the higher concentration in the stem halves was 0.22 per cent. The segment having the greatest concentration in the stylar half over that in the stem half was from fruit no. 84 (fig. 2), picked June 2, 1938. The average difference for all Valencia segments that had the higher concentration in the stylar halves was 0.76 per cent.

The concentration of soluble solids in Navel segments showed a stem-half high of 12.11 per cent. and a stylar-half low of 11.31 per cent. for all segments having the greater concentration in the stem halves, a difference of 0.80 per cent. The average difference for these segments was 0.17 per cent. The stem-half low for all segments having the greater concentration in the stylar halves was 11.04 per cent. and the stylar-half high, 13.58 per cent; a difference of 2.54. The average difference for these segments was 0.33 per cent. The results of tests of grapefruit segments were similar but are not reported here because only a comparatively few fruits were tested and these were practically all of the same age. It should be borne in mind that the differences and averages given for Valencias and Navels refer to both immature and mature fruits.

One might expect to find that in immature fruits the concentration of soluble solids would be about equal in the stem and stylar halves of the segments, even though there is a much greater concentration in the stylar halves by the time the segments mature. Table I shows that there were comparatively few segments in which the concentration of soluble solids was equal in the two halves. On the other hand, the results of a limited number of tests on whole citrus fruits indicate that in the earlier stages of development the concentration of soluble solids, though not greatly, may be measurably higher in the stem half than in the stylar half. Of 13 Valencias about three and one-half months old, 9 had an average of 0.59 per cent. higher concentration of soluble solids in the stem halves than in the stylar halves of the pulp, 1 had equal concentrations in the two halves, and 3 had an average of 0.31 per cent. higher concentration in the stylar halves than in the stem halves. Of 23 Navels of similar age, 21 had an average of 0.23 per cent. higher concentration in the stem halves than in the stylar halves, and 2 had equal concentrations in the two halves. None of the 23 Navels had a higher concentration in the stylar half than in the stem half of the fruit.

The original records show that of those fruits in which the concentration of soluble solids was higher in the stem halves than in the stylar halves of the whole fruits, some had (*a*) the higher concentration of both acids and sugars in the stem half; others had (*b*) acids higher in the stem half and sugars higher in the stylar half; or (*c*) acids higher in the stem half and sugars equal in both halves; or (*d*) acids equal in both halves, but sugars higher in the stylar half; or (*e*) acids higher in the stylar half, but sugars equal in both halves. The last condition mentioned, (*e*), indicates that soluble solids other than acids and sugars are partially responsible for the conditions mentioned (*a*) to (*d*), especially in immature fruits.

When the comparative concentrations of total soluble solids in the stem and stylar segment halves were determined (table I), the total acids (in terms of citric acid) and total sugars (in terms of glucose) were also determined in the two halves of some of the segments. Because of the great amount of work that would have been involved, not all segment halves were tested for acids and sugars. The tests were made on the stem and stylar halves of 37 segments from both immature Valencias and immature Navels and on stem and stylar halves of 20 segments from mature Navels.

The approximate ages of the fruits from which these segments were taken and the results of the tests are given in table II. The data on total soluble solids are from the stem and stylar halves of whole fruits, while the data on acids and sugars are from segment halves.

TABLE II

AVERAGE PERCENTAGE OF TOTAL SOLUBLE SOLIDS IN FRUIT HALVES AND DISTRIBUTION OF ACIDS AND SUGARS IN CARPELLARY SEGMENTS OF IMMATURE VALENCIA ORANGES AND OF IMMATURE AND MATURE NAVEL ORANGES

FRUITS TESTED	AVERAGE TOTAL SOLUBLE SOLIDS IN FRUIT HALVES		PERCENTAGE OF SEGMENTS					
			ACIDS (IN TERMS OF CITRIC ACID)			SUGARS (IN TERMS OF GLUCOSE)		
	STEM HALVES	STYLAR HALVES	HIGHER IN STEM THAN IN STYLAR HALVES	HIGHER IN STYLAR THAN IN STEM HALVES	EQUAL IN STEM AND STYLAR HALVES	HIGHER IN STEM THAN IN STYLAR HALVES	HIGHER IN STYLAR THAN IN STEM HALVES	EQUAL IN STEM AND STYLAR HALVES
	%	%	%	%	%	%	%	%
Immature Valencias (8–9.5 months old)	10.22	10.54	91	9	0	18	81	1
Immature Navels (6 months old)	9.86	9.90	60	30	10	26	58	16
Mature Navels (9.5 months old)	12.29	13.30	9	91	0	0	100	0

Although the first two lots of fruit mentioned in table II were immature, the average total soluble solids was slightly higher in their stylar halves than in their stem halves. It may be stated, however, that even in these fruits, 27 per cent. of the segments had a slightly higher concentration of soluble solids in the stem than in the stylar halves. The higher concentration in the stylar halves of the last lot of Navels mentioned in the table was to be expected because the fruits were mature.

The data in table II show that although the acid content was higher in 91 and 60 per cent., respectively, of the stem segment halves of the immature Valencias and immature Navels, total sugars were higher in 81 and 58 per cent., respectively, of the stylar segment halves of the same fruits. Of the mature Navels, 91 per cent. had the higher acid content and 100 per cent. had the higher sugar content in the stylar segment halves. These and other data in the table show that as a result of shifting or of some other process, there is a pronounced difference in the distribution of soluble solids in immature and in mature citrus fruits.

SOLUBLE SOLIDS IN ENTIRE SEGMENTS.—The differential response to low temperatures was not confined to the stem or stylar halves of the different segments. Many fruits were found in which an entire single segment had been frozen and had collapsed, whereas the other segments were unaffected. The apparent explanation for this condition was found by refractometric determinations of the total soluble solids in individual segments of many Valencia and Navel fruits.

In making approximately 1,500 tests, it was found that comparatively great differences may exist between the concentrations in the segments of a given fruit. For example, in a mature Valencia fruit (no. 75) picked June 28, 1938, one segment contained 16.11 per cent. soluble solids, while the two adjacent segments contained 13.41 and 15.44 per cent., respectively. Another segment in a different portion of the same fruit contained only 12.61 per cent.—a difference of 3.50 per cent. between the highest and lowest concentrations in the segments of that fruit.

In the stem half of one of the segments in this fruit (no. 75) the concentration of soluble solids was only 11.38 per cent., while in the stylar half of one of the other segments, the concentration was 16.98 per cent., a difference of 5.60. The differences mentioned for this fruit were greater than for any other fruit tested, but almost all the other fruits were less mature than this one.

SOLUBLE SOLIDS IN NORTH AND SOUTH SEGMENTS OF FRUITS.—Each fruit, before it was picked, was marked to designate its north or south side as it hung on the tree. It was also marked to indicate from which side of the tree it was taken and whether it was an inside or an outside fruit.

When the tabular data on concentration of total soluble solids in different segments was examined an interesting, but as yet unexplained, phenomenon was discovered. Of fruits picked from the outside of the trees and about 4 to 6 feet from the ground, 80 out of 88 Valencias, 25 out of 33 Navels, and 8 out of 9 grapefruits (or 87 per cent. of the 130 fruits) had a higher total soluble-solids content in their three north segments than in their three south segments. This condition held whether the fruits were borne on the north, south, east, or west sides of the trees, provided they were outside fruits.

Figure 5 shows the comparative concentrations of total soluble solids in each of the segments of 3 of the 80 Valencia oranges that showed a higher concentration in their three north than in their three south segments. The vertically hatched columns represent the three north, and the stippled

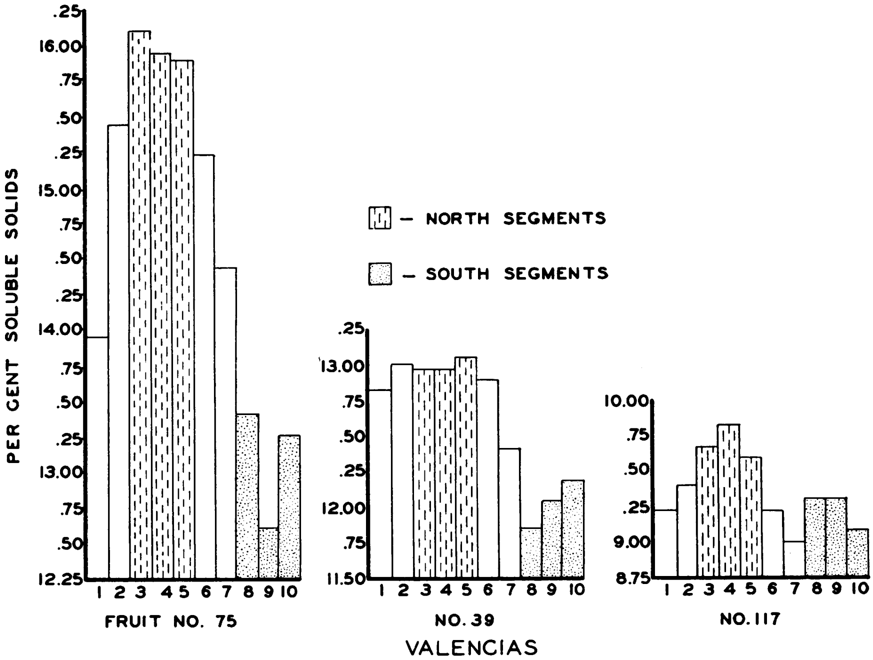

FIG. 5. Comparative concentrations of total soluble solids in the three north and the three south carpellary segments of three Valencia oranges. This figure shows the great difference in concentrations in the different segments of a given fruit.

columns, the three south segments in each fruit. The average total soluble solids for the three north segments of Valencia no. 75 was 15.98 per cent. and for the three south segments, 13.09 per cent. Averages for the three north and the three south segments of fruits nos. 39 and 117 were 12.98 and 12.02 per cent. and 9.69 and 9.23 per cent., respectively. Valencia no. 75 was picked June 28; no. 39, February 12; and no. 117, December 28. The indications are that the more mature the fruit, the greater is the difference in concentration of soluble solids in the segments, and, also, the greater is the north-south polarity of concentrations in the fruit.

Figure 6 represents the comparative total soluble solids in each of the segments of 3 of the 25 Navel oranges that showed a higher concentration in their three north than in their three south segments. The differences, however, were not so great in the Navels as in the Valencias. Navel no. 89 (fig. 6) was more mature than Valencia no. 117 (fig. 5), and Navel no. 46 (fig. 6) was less mature than Valencia no. 75 (fig. 5). Navels nos. 46, 33, and 89 were picked February 24, February 9, and October 27, respectively. The average total soluble solids for the three north segments of Navel no. 46 was 14.39 per cent. and for the three south segments, 12.91 per cent. Averages for the three north and the three south segments of Navels nos. 33 and 89 were 12.20 and 11.71 per cent. and 11.00 and 10.66 per cent., respectively.

While in 80 out of 88 Valencias and in 25 out of 33 Navels the three north segments contained greater concentrations of soluble solids than the three south segments, attention should be called to the fact that the segment which contained the highest concentration was not always on the north (fruit no.

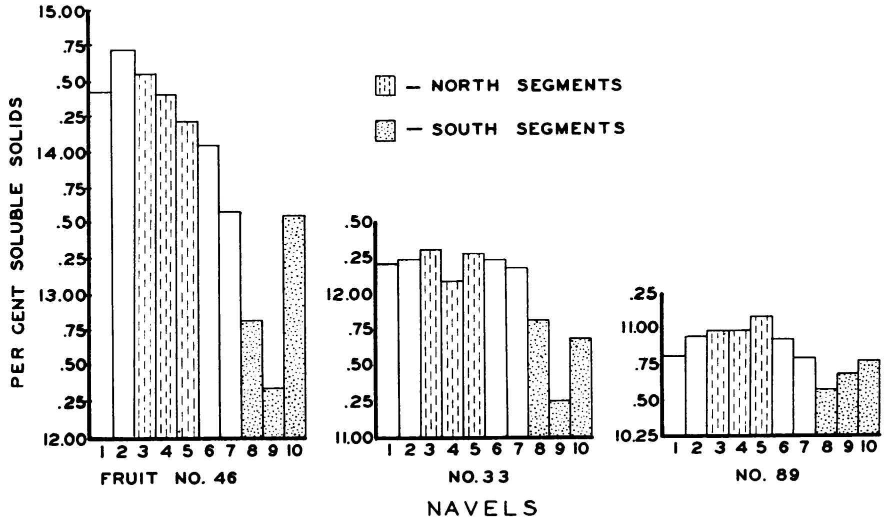

FIG. 6. Comparative concentrations of total soluble solids in the three north and the three south segments of three Navel oranges. The segment differences are similar to those in the Valencias (fig. 5), but are a little less.

46, segment 2, fig. 6), and the one that contained the lowest concentration was not always on the south side of the fruit (fruit no. 117, segment 7, fig. 5). As a rule, however, the concentration of soluble solids in the two segments adjacent to the group of three north segments was higher than that in the two segments adjacent to the group of three south segments. Figures 5 and 6 illustrate this condition.

The north-south polarity concentrations were determined in only six inside fruits, all Valencias, time not permitting the making of more such tests. Two of the six fruits showed higher concentrations of soluble solids in the three north segments than in other segments, and four showed higher concentrations in the three south segments. The exposures of inside fruits are variable; for some fruits may be heavily shaded, while others may receive almost as much light as those on the outside.

Just what significance lies in the fact that, as a rule, the north half of an outside citrus fruit contains a higher concentration of soluble solids than its south half, can be determined only by further study. The fact is interesting from a physiological viewpoint, but with the data at hand, the condition is as difficult to explain as it is interesting.

SIZE OF SEGMENTS AND CONCENTRATION OF SOLUBLE SOLIDS.—Even by a casual observation it can be determined that the segments in the pulp of a citrus fruit are not all equal in size. Some segments are comparatively large; others may extend the entire length of the pulp, but be only about a fourth to a half as thick as the largest; and in occasional fruits, especially in navels and grapefruits, a segment may extend only a half or two-thirds of the length of the fruit and be very thin. The results of the tests showed that there is no direct relation between the size of the segment and the concentration of total soluble solids which it contains.

SOLUBLE-SOLIDS CONCENTRATION AND THE FREEZING POINT OF CITRUS JUICE

Since the concentration of soluble solids in the stylar halves of many segments of immature and newly matured citrus fruits was equal to, or lower, than that in the stem halves of the same segments (table I, figs. 1 and 3), it is not surprising that freeze injury was found in the stylar as well as in the stem halves. Nor is it surprising that freeze injury was found in one segment of a given fruit and not in another when, as shown in figures 2, 4, 5, and 6, there was such a difference in concentration of soluble solids in the different entire segments. These conditions prevail in the fruit at the time of the year when it is most likely to be subjected to freezing temperatures. Later, as already stated and as found by other investigators (**2**, **6**, **12**, **20**, **22**), the concentration of soluble solids is noticeably higher in the stylar half than in the stem half of the fruit; and it seems logical to conclude that if the fruit were subjected to freezing temperatures at this time, the injury would probably register first in the stem half.

The concentration of the total soluble solids in expressed sap does not, alone, determine the exact temperature at which this sap would have frozen while present in the tissues. Articles by other investigators in recent years have presented much data on this point, but they will not be reviewed here. Time and equipment were not available when these tests were made, to determine the freezing point of the juice *in situ* in the segments or segment halves.

A few tests were made, however, to determine whether the concentration of soluble solids in the juice expressed from immature and mature citrus-pulp tissues would give a reliable clue to the freezing-point trends of the expressed juice. The results of these tests are shown in table III. The fruits were not frozen before the juice was extracted. The peels were cut away from the immature Navels, as explained previously; the pulps were bisected transversely, and each half was subjected to 6,000 pounds pressure. The mature Valencias were bisected transversely without peeling, and the

TABLE III

SOLUBLE SOLIDS IN JUICE AND FREEZING-POINT DEPRESSION OF JUICE FROM STEM AND STYLAR HALVES OF IMMATURE NAVEL AND MATURE VALENCIA ORANGES

FRUIT NUMBER	SOLUBLE SOLIDS IN JUICE		FREEZING-POINT DEPRESSION OF JUICE	
	STEM HALF OF FRUIT	STYLAR HALF OF FRUIT	STEM HALF OF FRUIT	STYLAR HALF OF FRUIT
Navel oranges (immature)				
	%	%	°C.	°C.
1	9.07	8.95	0.989	0.980
2	9.01	8.80	0.979	0.963
3	9.15	8.95	1.001	0.997
4	9.61	9.15	1.026	1.006
5	9.61	9.35	1.048	1.019
6	9.77	9.77	1.079	1.034
7	9.35	9.35	0.966	0.962
8	9.50	9.43	0.974	0.994
9	9.69	9.49	0.981	0.967
10	9.50	9.33	0.976	0.859
11	9.89	9.43	0.968	0.960
12	9.07	8.80	0.978	0.955
Valencia oranges (mature)				
13	16.44	19.66	1.935	2.512
14	15.06	17.46	1.863	2.200
15	14.06	16.27	1.590	1.828
16	16.29	17.58	2.055	2.379
17	15.24	17.44	1.780	2.084
18	16.25	17.51	1.902	2.118
19	14.90	16.70	1.719	2.010
20	15.11	17.69	1.809	2.287

juice was reamed from each half. Soluble-solids and freezing-point determinations were made on the juice thus obtained from the two lots. The Navels used in these tests were about 4 months old, and the Valencias, about 17.5 months old. Valencias were used because no mature Navels were available at the time these tests were made.

The data in table III show that the concentration of soluble solids was slightly higher in the stem than in the stylar halves of all the immature fruits except nos. 6 and 7, in which soluble solids were equal in the two halves. The freezing points were also slightly higher for the juice from the stem halves of all the fruits except no. 8.

The extreme differences in concentrations of soluble solids and freezing-point depressions for the juice from the immature Navels were small, 1.09 per cent., and 0.175° C., respectively; but if the differences between the stem and stylar halves are considered, they appear to be significant, since the results by the two methods show the same trends. On the other hand, when the relationship between the soluble solids and the freezing-point depressions is considered without reference to the differences in concentrations in the stem and stylar halves of the fruit, some discrepancies are evident. For example, the juice from the stem halves of fruits 4 and 5 had the same percentage of soluble solids but different freezing-point depressions.

The results of the tests on the mature Valencias (table III) show not only a marked increase in concentration of soluble solids over that of the immature Navels, but also concentrations much greater in the stylar than in the stem halves of the fruits. As would be expected, these increased concentrations resulted in much greater depressions of the freezing points. Here again small discrepancies may be found, however, and a straight line would not be obtained if the percentages of soluble solids were plotted against the freezing-point depressions.

The apparent discrepancies which appear in the results of these tests (table III) may have been caused by experimental error and by soluble inorganic constituents which had a marked effect on the freezing-point depression and but little effect on the refractive index of the solution. Or they may have been caused by the presence of colloidal material which registered in the refractive index but had little or no effect on the freezing-point depression.

As was previously stated, in earlier tests fruits were found in which freeze injury was evident in the stylar and not in the stem halves of the segments. These findings, together with those given in table III, indicate that if immature Navels are subjected to the minimum temperature which will cause injury, the stylar halves of the segments will be injured first.

Color differences in centrifuged juice

The supernatant portion of the centrifuged juice from the two halves of a segment did not always have the same depth of color. Data of this kind were kept on the segments of only 80 of the fruits tested, and most of these segments were from mature or nearly mature fruits. Eighteen of the fruits showed no difference in the color of the juice from the stem and from the stylar halves of the segments. Of the 654 segments of the other 62 fruits, 33 (5 per cent.) had darker juice in the stylar halves than in the stem halves; 328 (50 per cent.) had the darker juice in the stem halves; there was no appreciable difference, however, in the color of the juice from the halves of the remaining 293 segments, There were 5 fruits in which the juice from the stem half of every segment was darker than that from the stylar half.

Differences in juice color were more prevalent and more noticeable in mature than in immature fruits and were found in all three varieties of citrus fruits tested—Valencia, Navel, and grapefruit. In addition to color there were often similar differences in the juice from the whole segments of a fruit.

The degree of any color contrast was not accurately determined by the use of photoelectric or similar equipment, but was determined simply with the unaided eye. Where there was a color difference in the juice from different portions of mature fruits, it appeared that the amount of carotene present might be the controlling factor. In immature fruits the juice was pale gray with a greenish tinge. Contrasts in juice of this kind appeared to be owing to differences in opacity rather than in color.

As yet, no attempt has been made to isolate the substance or substances that are responsible for the color differences, nor is any special significance attributed at this time to their existence. Their significance, if any, and their explanation await further and more accurate tests. These data are included merely to give added evidence of the desirability and value of studying small portions of a given plant.

Discussion

The refractometer was used for determining the dry weights of the citrus juices, expressed in terms of total soluble solids, because preliminary tests showed that the results obtained by this method were as accurate as those obtained by the dry-weight method and were much more rapidly determined. To test the dry-weight method, 25-ml. aliquots of juice were dried in vacuum at 72 to 73 cm. and at a temperature of 55° C. The results of the preliminary tests are given in table IV. In three of the five tests, the results obtained

TABLE IV

COMPARISON OF PERCENTAGES OF SOLUBLE SOLIDS IN CITRUS JUICE, DETERMINED BY DRYING IN VACUUM AND WITH REFRACTOMETER

EXPERIMENT NUMBER	SOLUBLE SOLIDS IN JUICE	
	DETERMINED BY DRYING IN VACUUM*	DETERMINED WITH REFRACTOMETER
1	11.70	11.83
2	11.76	11.91
3	11.58	11.57
4	9.34	9.33
5	10.10	10.26

* Twenty-five-ml. aliquots of juice dried in vacuum at 72 to 73 cm. and at temperature of 55° C.

with the refractometer are slightly higher than those obtained by the dry-weight method. It is not likely that the differences are significant, but if they are, it is probable that the results obtained with the refractometer are the more accurate because of the time element and the effect of the vacuum on the results given by the dry-weight method. The results compare very satisfactorily with those of GORTNER and HOFFMAN (**10**), who used the refractometer for the determination of moisture as well as for the determination of colloidal material in expressed plant saps. Therefore, the determination of the soluble solids by the refractive index of citrus juice is considered to be a rapid and an accurate method for determining its dry weight.

An unequal distribution of soluble solids has been found in fruits other than citrus. LALL (**17**) and ARCHBOLD and BARTER (**1**) have shown that the most highly colored half of an apple, when cut from stem to calyx, and the calyx half of the transversely cut apple, may contain a higher concentration of soluble solids than the opposite halves. HARDING (**15**) found the concentration of soluble solids was highest in the skin of the Jonathan apple, with a gradual decrease toward the pith. TUCKER (**21**) and SCOTT (**19**) found an unequal distribution of soluble solids in different varieties of melons.

It is possible that migration or redistribution of soluble solids in such fruits as apples and melons could be more easily explained than similar processes in citrus fruits. The segment (carpel) walls in citrus fruits, especially the walls of the vesicles which contain the soluble solids, have a high degree of impermeability. REED (**18**) used different dyes on fresh vesicles and on those that had been treated with such substances as alcohol, ether, NaOH, and HCl and concluded that the lateral walls of the vesicles contained suberin or cutin, which made them comparatively impermeable. He found that the walls of the stalk and of the distal tip of the vesicle were more permeable than those of the body of the vesicle. From these results it would appear that to migrate from the stem end to the stylar end of the fruit, soluble solids would have to pass out of each vesicle stalk, in which there is no developed vascular tissue, through the segment wall, and into the surrounding parenchymatous tissue of the peel. From there they would have to migrate slowly through this tissue toward the stylar end of the fruit or would have to find their way to one of the bundles of the peel, where they would be transferred more rapidly. All vascular bundles of the citrus fruit are separated from the walls of the segments by at least several layers of parenchymatous tissue, except where they pass through the inner walls and into the ovules.

That the segment and vesicle walls of citrus fruits are comparatively impermeable is further indicated by the work of CURTIS and CLARK (**8**), who found that when the two opposite sides of apples and tomatoes were kept at different temperatures, there was a movement of water, in vapor form, from the warm to the cool side. No such movement of water could be induced in oranges and potatoes by the same treatment. For a more extended discussion of the work on apples, see CURTIS (**7**). It may be mentioned here that similar tests had been made on oranges at the Citrus Experiment Station before it was known that CURTIS and CLARK had done their work. The results were the same as those obtained by these men. The nature of the tissues in the pulp of the orange is such that the movement of water is probably prevented largely by the comparative impermeability of the walls rather than merely by the absence of air spaces, as CURTIS and CLARK concluded.

Several other theories might be advanced to explain the presence of a higher concentration of soluble solids in the stylar than in the stem end of mature citrus fruits. For example, there may be an uneven rate of respiratory activity in the two ends of the fruit as it matures, or the soluble solids may naturally continue to flow in greater abundance to the tissues that have been most recently in a meristematic condition. The navel portion of the Navel orange contains a higher concentration of soluble solids than any other portion of the mature fruit, and it is the youngest portion of the Navel fruit.

Inasmuch as there is no transfer of substance from one segment to another and each segment behaves principally as a separate unit, it is not surprising that one segment may have a higher concentration of soluble solids than another, even the adjoining one, in the same fruit. This condition fits in very well with the theory that each carpellary segment of the pulp of a citrus fruit represents a modified leaf, because no two leaves on a twig contain the same concentration of soluble solids. It is not the purpose of this paper, however, to present any argument for or against any theory concerning the ontogeny of citrus or other fruits. For recent discussions of three different theories on this subject, see EAMES and MACDANIELS, (**9**); HAYWARD, (**16**); and GREGOIRE, (**11**).

Sufficient data are not yet available to explain the north-south polarity of concentration of soluble solids in citrus fruits. That the different amounts of light received by the two sides of the fruit may be a factor is indicated by the fact that the difference in concentrations in the north and south halves of the fruit is usually greater in the fruits from the south than in those from the north side of the tree. Just why the shaded side of the fruit on the south side of the tree and the exposed side of the fruit on the north side of the tree should have the highest concentration is difficult to explain, unless one concludes that the south side of the south, east, and west fruits receive more direct light and, therefore, more heat, and that the resulting increase in respiration causes a decrease in total concentration of soluble solids in the south half over that in the north half of the fruit. This could not be true for the north fruit however, unless one were to conclude that the south half, though shaded, was warmer than the north half.

A study of the vascular system of the citrus fruit does not afford any direct explanation for the condition under discussion. A transection through the center of the fruit shows as many vascular bundles in the center of the fruit as there are segments (carpels). Each bundle, as a rule, lies near the inner point of contact of each two adjacent segment walls. Branches from these bundles pass into surrounding parenchymatous tissue and into the ovules, but they do not pass through or even contact the segment walls except at the point of attachment of the ovules. One large bundle may be seen in the mesocarp (spongy parenchyma of the peel) in the vicinity of the center of the external wall of each segment; another, in a similar position but in the depression opposite the juncture of two segment walls. Branches from these main bundles pass into all parts of the mesocarp of the peel, but they do not penetrate or contact the tissues of the segment walls. The lack of direct contact of any of the vascular bundles with any of the vesicles, or even with the segment wall which surrounds the vesicles, would at least lead one to question the theory that the differences in concentration of soluble solids in different segments or parts of segments, are due to the difference in amounts of food or food materials supplied by the different vascular bundles of the fruit. It is probable that an explanation would more likely be found by a study of conditions inherent in the segments themselves.

Summary

Mature oranges and grapefruit have a considerably higher concentration of total soluble solids in their stylar than in their stem halves. Immature fruits may have equal amounts in both halves or may have the higher concentration in their stem halves. The latter condition suggests an explanation for the fact that the stylar half of an immature fruit segment may be injured by low temperature while the stem half may remain uninjured.

The concentration of total soluble solids in the segments of a given fruit may differ greatly, especially if the fruit is mature. This may be largely because of the comparatively impermeable nature of the walls of the segments and juice vesicles.

Of 130 fruits tested, 87 per cent. had a higher concentration of total soluble solids in their three north than in their three south segments. This condition was found whether the fruits were borne on the north, south, east, or west side of the tree, provided they were exposed, outside fruits.

Apparently the concentration of soluble solids in a segment of a citrus fruit is not governed by the size of the segment.

The total soluble-solids content of expressed citrus juice, determined with the refractometer, appears to be a reliable index to the temperature at which such juice will freeze.

In the citrus fruits tested, the color of the juice was not the same in all the segments of a given fruit, nor was the color always the same in the stem and stylar halves of the segments.

Appreciation is expressed for assistance rendered by B. E. Janes and C. C. Papke during parts of this investigation.

University of California
Citrus Experiment Station
Riverside, California

LITERATURE CITED

1. ARCHBOLD, H. K., and BARTER, A. M. Chemical studies in the physiology of apples. XV. The relation of carbon dioxide output to the loss of sugar and acid in Bramley's seedling apples during storage. Ann. Bot. **48**: 957–966. 1934.
2. BAKER, THADDEUS H. Maturity studies of Texas grapefruit. Texas Citriculture **10**(2): 6, 12–13. 1933.
3. BARTHOLOMEW, E. T., SINCLAIR, WALTON B., and TURRELL, F. M. Granulation of Valencia oranges. California Agr. Exp. Sta. Bul. (In press.)
4. BLISH, M. J. Report on diastatic value of flour. Jour. Assoc. Off. Agr. Chem. **16**: 497–504. 1933.
5. ———————. Report on diastatic value of flour. Jour. Assoc. Off. Agr. Chem. **17**: 394–399. 1934.
6. CHACE, E. M., and CHURCH, C. G. Maturity data on the California Washington Navel orange. California Citrograph **15**: 534, 569. 1930.
7. CURTIS, OTIS F. Vapor pressure gradients, water distribution in fruits, and so-called infra-red injury. Amer. Jour. Bot. **24**: 705–710. 1937.
8. ———————, and CLARK, ALFRED K. The effect of a temperature gradient on the distribution of water in apples, tomatoes, oranges, and potatoes. [Abstract.] Proc. Amer. Soc. Hort. Sci. **35**: 160. 1937.
9. EAMES, ARTHUR J., and MACDANIELS, LAURENCE H. An introduction to plant anatomy. McGraw-Hill Book Co., Inc. New York. 1925.
10. GORTNER, ROSS AIKEN, and HOFFMAN, WALTER F. Determination of Moisture content of expressed plant tissue fluids. Bot. Gaz. **74**: 308–313. 1922.
11. GREGOIRE, V. La valeur morphologique des carpelles dans les Angiospermes. Bull. Acad. Roy. de Belg. **17**: 1286–1302. 1931.
12. HAAS, A. R. C., and KLOTZ, L. J. Physiological gradients in citrus fruits. Hilgardia **9**: 181–217. 1935.
13. HAGEDORN, H. C., and JENSEN, B. N. Zur Mikrobestimmung des Blutzuckers mittelst Ferricyanid. Biochem. Zeitschr. **135**: 46–58. 1923.
14. ———————, and ———————. Die Ferricyanidmethode zur Blutzuckerbestimmung. Pt. 2. Biochem. Zeitschr. **137**: 92–95. 1923.
15. HARDING, PAUL L. Distribution of total soluble solids and catalase in different parts of Jonathan apples. Jour. Agr. Res. **53**: 43–48. 1936.

16. HAYWARD, HERMAN E. The structure of economic plants. The Macmillan Co., New York. 1938.

17. LALL, GIRDHARI. Chemical studies in the physiology of apples. XIV. A method of estimating chemical change and rate of respiration in stored apples. Ann. Bot. **48**: 273–292. 1934.

18. REED, H. S. Absorption of dyes by citrus fruits and their juice sacs. (Unpublished project notes on file at the University of California Citrus Experiment Station, Riverside.) 1931.

19. SCOTT, G. W. Variation in soluble solids within individual fruits of the cantaloupe and related melons. [Abstract.] Proc. Amer. Soc. Hort. Sci. **33**: 523. 1935.

20. SUTHERST, W. F. Sugar content of oranges. California Cultivator **36**: 612. 1911.

21. TUCKER, LOWELL R. Soluble solids in the watermelon. Plant Physiol. **9**: 181–182. 1934.

22. WOOD, J. F., and REED, HAROLD M. Maturity studies of Marsh seedless grapefruit in the lower Rio Grande Valley. Texas Agr. Exp. Sta. Bull. **562**. 1938.