HILGARDIA

*A Journal of Agricultural Science Published by
the California Agricultural Experiment Station*

VOLUME 9

JANUARY, 1935

NUMBER 3

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COPPER CONTENT OF CITRUS LEAVES AND FRUIT IN RELATION TO EXANTHEMA AND FUMIGATION INJURY

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PHYSIOLOGICAL GRADIENTS IN CITRUS FRUITS

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INTRODUCTION

THE VARIOUS PORTIONS OF CITRUS FRUITS, particularly the regions near the calyx and styler ends, differ not only in their morphology but also in their physical and chemical characters. The active acidity of various organs of plants forms a gradient that has been given considerable attention by Haas,⁽¹¹⁾ Gustafson,^(9, 10) and Hurd-Karrer.^(17, 18) The present paper presents evidence of the existence of physical and chemical differences and gradients in citrus and other fruits, and attempts to explain these differences in citrus fruits by a consideration of the structure and function of the parts concerned.

The functions of the vascular system of plants are support, and the conduction and distribution of solutes. Soluble materials that remain in the vascular stream after lateral absorption by the cells adjacent to the tracheal tubes, concentrate in the tissues near the terminals of the vascular strands. Cells in this region necessarily receive the surplus of the solutes. The location of these terminals is not always easily recognized because of branching and anastomosing, but the concentrations of ions as revealed by chemical analyses may indicate regions in which numerous vascular strands terminate. Ross⁽²⁶⁾ has described the analogy of the morphology of the fruit carpels and that of the citrus leaf. The main lateral veins of the leaf join at the margin and proceed to the leaf tip, which region, as shown by Haas,⁽¹³⁾ becomes the principal place of deposition for soluble materials. It will be shown that similar regions are found in the citrus fruit, the structure of which is composed of a number of modified leaves coalesced to form the whole. Mature leaves on twigs will be considered only as they may affect the gradients in the fruits.

Susceptibility to abscission decreases as fruit matures, injuries due to malnutrition manifesting themselves by other symptoms. Localization of these injuries in certain parts of the fruit suggested a study of the physical and chemical composition of the susceptible and resistant parts.

¹ Received for publication July 26, 1934.

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Analyses showed the existence of relatively large differences. Furthermore, lesions caused by certain fungi, injuries due to fumigation and oil spray, and physiological breakdowns, such as granulation and storage spots, are more commonly found on and in the calyx end of the fruit than elsewhere, while certain other fungus spots and endoxerosis occur more commonly in the stylar end. One of the principal objects of this investigation was to find whether there are, in the fruits, differences in the physical or chemical composition which might serve as a basis for explaining the characteristic localization of the lesions above mentioned.

While there is considerable information available concerning the composition and nutrition of leaves and twigs, there has been little correlation of this information with the nutrition of the fruit.

Analyses of citrus fruit by previous workers have been concerned largely with total acids, oil content, proteins, reducing and total sugars, total ash of the whole fruit, and the sugars and acid to total solids ratio of the juice. These are discussed in the publications of Chace, Tolman, and Munson,⁽⁴⁾ Chace, Wilson, and Church,⁽⁵⁾ Wilson and Young,⁽²⁹⁾ Chace and Church,^(2, 3) Poore,⁽²⁴⁾ and Collison.⁽⁶⁾

METHODS OF ANALYSIS

Fruit juices were extracted in some instances by means of a hand extractor and in others with a motor-driven extractor, only one method, however, being employed in a set of comparative determinations. The extracts were centrifuged at high speed for 15 minutes to free them from suspended material. Any oil that collected at the top was discarded and the supernatant liquid decanted into clean, dry glassware.

Density of the fruit juice was determined by weighing in an adjusted Gay-Lussac specific gravity bottle of approximately 25-cc capacity, and relating this weight to the weight of an equal volume of distilled water.

The capacity of juices to fix iodine was determined by adding 25 cc of an iodate-iodide solution (60 grams KI and 5.4 grams KIO₃ per liter) and 20 cc 5N H₂SO₄ to 100-cc samples, shaking, and allowing them to stand 10 minutes. At the end of that period the free iodine remaining was titrated against N/10 sodium thiosulfate solution, using starch as an indicator. The result was subtracted from a blank determination in which 100 cc of distilled water was used instead of fruit juices. This value multiplied by the iodine equivalent of the thiosulfate solution represented the iodine fixed. The procedure is taken from a portion of the method for reducing sugars by Shaffer and Hartmann.⁽²⁷⁾

All fruit samples used for analysis were debuttomed and wiped free of dust. The analytical methods employed in the determination of inorganic constituents were those used by Haas⁽¹³⁾ in previous work. The

results were calculated on a dry-weight and ash basis and in some cases on a fresh-weight basis. Some of these computations have been omitted in order to conserve space in the tables, although they may be readily recalculated from the given data.

All peel and pulp samples of fruit were dried at 80° C without the loss of juice from the pulp. This was done by separating the fruit at considerable intervals of space on clean, heavy paper in well-ventilated ovens. When sufficiently dry the pulp mass of a single fruit was laid between layers of heavy paper, broken open, and further dried. Unless otherwise stated, the seeds were removed from the pulp prior to grinding the sample.

To obtain the sap of the peel, the peel was carefully removed, placed in paraffined bags, frozen 18 hours at a temperature of 20° F, thawed, subjected to 25,000 pounds pressure per square inch in a hydraulic press, and centrifuged.

The osmotic values of the juices were obtained from the freezing-point depression corrected for the degrees of under-cooling, according to the method of Harris and Gortner.⁽¹⁶⁾

The relative oil content of the peel of the calyx and styler ends of fruit was determined by a modified Soxhlet extraction of equal surfaces of peel. Equal numbers of disks were cut from the two ends, weighed, and placed into extraction thimbles with other materials in the following order: a thin layer of pure asbestos fibers, a layer of a mixture of pure silica sand and anhydrous CuSO_4 sufficient to absorb the water of the sample, another layer of asbestos, the sample disks of fruit peel, and another layer of asbestos on the top. The prepared sample was extracted for 48 hours with anhydrous ether in a weighed flask. The disks were removed, ground with quartz sand in a mortar, replaced in the extraction thimble with the aid of anhydrous ether, and extracted for an additional period of 24 hours. The condenser thimble and thimble holder were removed, and the ether was allowed to evaporate at a low temperature. The flask and oil were then weighed.

The method used in the determination of the relative permeability of fruits was that adapted by Klotz.⁽²¹⁾

The rate of evaporation or water loss from the styler and calyx halves of fruit was measured by direct weighing. In one procedure the fruit was cut along the equatorial line and the cut surfaces were dipped in powdered boric acid to retard the growth of microorganisms. The fruit was then placed with cut surfaces downward on glass plates covered with paraffin paper. In a second method the evaporation was excluded from one end or the other by dipping in paraffin of low melting point, as was done by Markley and Sando⁽²³⁾ with apples. The fruits, with un-

paraffined ends upward, were set in small cardboard cylinders on glass plates, and groups of a dozen or more fruits weighed together. It may be mentioned here that the equatorial line was determined by measuring with a flexible celluloid ruler half the surface distance from the center of the cut stem to the center of the styler scar. It was found by actual volume measurements, using a water displacement method, that the halves thus determined were remarkably similar in volume.

GRADIENTS IN COMPOSITION OF CITRUS PULP JUICE

H-ion Concentration, Specific Gravity, Carotinoids, and Iodine Fixation.—There were no consistent differences in the pH or the buffer action of the juice of calyx and styler halves of mature Valencia orange pulp. In three lots of 35 oranges each, the juice of both halves gave the values: 3.85, 3.90, and 4.20, respectively. In another similar lot of 35 oranges the pH of the juice of the calyx halves was 4.15 as compared with 3.80 for that of the styler halves. The juice of both halves of lemons in the silver stage showed pH values of 2.55. These results for lemons are in agreement with those of Bartholomew.⁽¹⁾

TABLE 1
SPECIFIC GRAVITY OF LEMON, GRAPEFRUIT, AND ORANGE JUICES

Type of fruit	Calyx half		Styler half	
	<i>sp. gr.</i>		<i>sp. gr.</i>	
Early silver-stage lemons.....	1.0347		1.0343	
Immature Marsh grapefruit.....	1.0397		1.0400	
Mature seedling grapefruit.....	1.0380		1.0423	
	Calyx quarter	Combined intermediate quarters	Styler quarter	
	<i>sp. gr.</i>	<i>sp. gr.</i>	<i>sp. gr.</i>	
Mature Valencia oranges.....	1.0434	1.0524	1.0591	

The data for the specific gravity at 21° C (using water as standard) of the juice of the different portions of ripe and of green fruit are given in table 1.

The juice samples were obtained in each case from 50 fruits collected at Riverside on November 4, 1932. No significant differences were found in the halves of either lemons or green grapefruit. In both the ripe seedling grapefruit and mature Valencia oranges the specific gravity was greater in the styler than in the calyx portion.

Frequently the authors observed marked differences in the intensity of the yellow color in numerous samples of centrifuged Valencia orange

juice. Quantitative determinations were made of the carotinoid content of the juice, which was obtained with a hand extractor from the calyx and styler quarters of pulp.

Fifty cc of centrifuged juice was run into a separatory funnel, 25 cc of chloroform was added, and the whole thoroughly shaken and centrifuged. The yellow pigments which dissolved in the lower chloroform layer were compared in a Dubosq microcolorimeter. When the weaker concentration was taken as unity the carotinoid content of the juice of the styler quarter was found to be 1.54 times that of the juice of the calyx quarter. In the preparation of orange juice for the market, the reduced concentration of desirable yellow pigments and sugar in the calyx quarter may warrant the discarding of that portion.

Recently Joslyn and Marsh⁽²⁰⁾ have found that as orange juice deteriorates its iodine-titration value decreases. The writers have determined the iodine-fixation capacity of the juices of the pulp of the calyx and styler portions of 50 citrus fruits in each case. The juice of the calyx half of mature and immature Valencia oranges and of immature Marsh seedless grapefruit was found to have a greater iodine-fixing power than that of the styler portion, while the iodine-fixing power of the juice of the styler portion of mature seedy Marsh grapefruit and of immature lemons and Washington Navel oranges slightly exceeded that of the calyx portion. The iodine-fixation power therefore varies with the degree of maturity, the species, and the portion of the pulp from which it is obtained.

Total and Amino Nitrogen Content of Valencia Orange Juice.—Determinations of total nitrogen, including nitrates (Ranker⁽²⁵⁾) were made of the centrifuged juice of calyx and styler quarters of fruit collected at Riverside on November 19, 1931. Two lots of 50 fruits each supplied the juices for the two sets of determinations. The results are expressed as grams of nitrogen per million cubic centimeters of juice. In the first lot the total nitrogen in the juice of the styler quarter averaged 1,447 grams and that in the juice of the calyx quarter, 1,508 grams. In the second lot the values obtained were 1,534 and 1,630 grams, respectively. The amino nitrogen content of the juices of both quarters showed no differences: 2 cc of juice contained 1.12216 mg of amino nitrogen at 22° C and 726.7 mm. Copeman⁽⁷⁾ has shown that during the ripening of Washington Navel oranges there is no significant increase in the nitrogen or ash content of the juice.

Inorganic Constituents of Juice of Mature Valencia and Navel Oranges.—Copeman⁽⁷⁾ found that during the ripening of Washington Navel oranges, calcium and phosphate decreased in the juice while potassium increased. Table 2 shows the gradient in dry matter of the juice

of 50 Valencia oranges. In general the percentage of dry matter increases from the calyx to the styler end, as was the case with the sugars referred to later in this paper. In contrast, the percentage of total ash of the juice decreases from the calyx to the styler end. Of the individual

TABLE 2
INORGANIC COMPOSITION, TOTAL SUGARS (AS REDUCING SUGARS), AND REDUCING SUGARS, IN MATURE VALENCIA ORANGE JUICE
(Expressed as Grams per Million cc of Juice)

Section of pulp	Dry matter	Ash	Ca	Mg	K	Na	Inorganic PO ₄	Total sugars	Reducing sugars
October 1, 1931									
Calyx half.....		3,920	146	152	1,552	519	668		
Stylar half.....		3,551	120	168	1,282	312	756		
October 6, 1931									
Calyx half.....	86,708	3,972	204	146	1,634	502	608		
Stylar half.....	104,643	3,606	138	134	1,329	311	712		
October 14, 1931									
Calyx half.....	100,630	4,737	152	157	2,112	602	645		
Stylar half.....	131,473	4,303	130	162	1,836	483	752		
October 15, 1931								October 17, 1931	
Calyx quarter.....	80,389	5,606	148	180	2,590	737	671	64,176	
Second quarter from calyx end.....	106,309	4,731	131	167	2,190	560	632	79,576	
Third quarter from calyx end.....	145,939	4,501	98	157	1,900	496	702	96,513	
Stylar quarter.....	155,473	4,517	72	152	1,901	448	788	95,680	
October 23, 1931								October 24, 1931	
Calyx quarter.....	77,494	4,975	160	163	2,396	641	803	54,996	30,320
Stylar quarter.....	118,421	4,107	98	168	1,643	430	870	98,013	51,676

ash constituents, calcium, potassium, and sodium increase in concentration from the styler to the calyx end, while inorganic phosphate increases in the opposite direction. The results show large concentrations of potassium and inorganic phosphate and relatively lower concentrations of calcium and magnesium in the juice. However, from the standpoint of absolute values, the calcium and magnesium concentrations are fairly high and are of approximately the same magnitude.

TABLE 3
AVERAGE INORGANIC COMPOSITION OF 50-CC ALIQUOTS OF MATURE WASHINGTON NAVEL ORANGE JUICE

Section of pulp	Dry matter, in grams	As a per cent of dry matter					As a per cent of ash					Grams per million cc of juice							
						Inor- ganic PO ₄					Dry matter					Inor- ganic PO ₄	Ash		
		Ca	Mg	K	Na		Ca	Mg	K	Na		Ca	Mg	K	Na				
Fifty Navel oranges from Riverside, March 8, 1932																			
Calyx quarter.....	4.6090	0.14	0.18	2.95	0.62	0.87	6.29	2.22	2.75	46.49	9.83	13.71	129	160	2,690	569	794	5,795	
Second quarter from calyx end.....	5.0655	0.08	0.14	2.14	0.38	0.56	4.68	1.70	3.11	45.08	8.97	11.82	79	145	2,159	429	566	4,745	
Third quarter from calyx end.....	5.3781	0.06	0.11	1.91	0.42	0.87	4.11	1.60	2.77	45.07	9.33	13.41	67	116	2,048	425	609	4,423	
Stylar quarter.....	5.8054		0.12	1.93	0.42	0.64	4.17	1.47	2.77	45.96	10.08	15.52	72	136	2,198	483	744	4,839	
Fifty extremely large jumbo Navel oranges from young trees at Hemet, January 21, 1932																			
Calyx quarter.....	5.7954			2.47	0.57		5.30	2.48	2.44	47.24	10.94	9.60	154	152	2,863	663	595	6,148	
Stylar quarter.....	6.0204			2.00	0.43		4.36	1.73	2.49	45.74	9.90	12.83	91	131	2,403	520	672	5,248	
Secondary fruit.....	5.9186			2.07	0.49		4.53	1.03	2.27	44.97	11.77	15.86	55	122	2,415	527	853	5,372	

Table 3 gives the results of similar analyses of the pulp juice of mature Washington Navel oranges of commercial size, and also of extremely large jumbo Washington Navel oranges and of their small, secondary fruit found at the navel end. The dry matter of the juice of the pulp of oranges of commercial size shows an increase from the calyx to the stylar end as in Valencia orange juice. The percentages of calcium, magnesium, sodium, potassium, and total ash in the dry matter increase from the stylar to the calyx end. Inorganic phosphate is higher at the calyx and stylar ends than in the middle portion.

The grams of dry matter in 1,000,000 cc of juice show an increase from the calyx to the stylar end, and the grams of ash from the center toward both ends. Based on total ash, the percentages of calcium increase from the stylar toward the calyx end.

The results shown in table 3 for the juices of 50 extremely large Washington Navel oranges and of their small, secondary fruits are somewhat different from those obtained from commercial fruits. The concentrations of the dry matter in the juices of the various portions of the pulp are similar and are relatively high. The concentrations of ash, sodium, and potassium in the dry matter of the juice of the secondary fruit more nearly resemble those of the stylar end than those of the calyx end. The concentrations of calcium and magnesium in the juice show an increase from the secondary fruit to the calyx end, and those of potassium from the stylar to the calyx end. The increase in inorganic phosphate is from the calyx to the secondary fruit which is in the same direction as that found in the Valencia orange.

Total and Reducing Sugars in Valencia Orange Juice.—Total sugars were determined as reducing sugars after HCl hydrolysis, by use of the Shaffer and Hartmann⁽²⁷⁾ iodometric method. The juice was secured from the calyx and stylar halves of each of three lots of 50 mature Valencia oranges which were secured at Riverside (two lots on October 6, 1931, and a third lot on October 15, 1931). For the calyx half, the average total sugar content of the three lots was 73,567 grams per million cc of juice, and for the stylar half, 94,133 grams. As will be discussed later, this is one of the chief factors that account for the lower loss of water from the stylar portion of oranges.

The sugar content of the juice of mature Valencia oranges varies, according to the age of the fruit, the health of the tree, and other factors. Table 2 gives more complete data on the gradient in total and reducing sugars of the juice of the various portions of the pulp. These data were secured from two lots each consisting of 50 fruits obtained at Riverside. The determinations were made respectively on the juice of the calyx, stylar, and two middle quarters of the pulp and show a concentration in-

crease from the calyx to the styler portion. This sugar gradient is so striking as to be easily recognized by taste.

Total Sugars in Juice of Lemons and Grapefruit.—Determinations were made of the total sugars in the juice of lemons in the silver stage, Marsh grapefruits which were nearly full-sized, but green, and overmature seedling grapefruits obtained at Riverside on November 2, 1932.

TABLE 4
TOTAL SUGARS, AS DEXTROSE IN JUICE OF LEMONS AND GRAPEFRUIT

Type of fruit	Number of fruits	Grams sugar per million cc in calyx halves	Grams sugar per million cc in styler halves
Silver-stage lemons.....	42	26,580	28,365
Green Marsh grapefruits.....	26	55,780	58,025
Overripe seedling grapefruits.....	25	70,810	79,870

In every case (table 4) there were more total sugars in the juice of the styler half than in the calyx half, although the differences were not as large as those in Valencia oranges (table 2).

Fig. 1.—Acidity, dry matter, total sugars, and osmotic pressure in different portions of lemon and orange fruit and osmotic pressure of leaf sap.

Osmotic Pressure.—Osmotic pressure of pulp juice of lemons (silver stage) and of mature Valencia oranges was determined as described in "Methods of Analysis." Figure 1 shows that the juice of the calyx half of the lemon pulp had an osmotic pressure of 12.96 atmospheres and that of the styler half 13.08 atmospheres. The juices of Valencia orange showed far greater differences in osmotic pressure than those of

lemon. Figure 1 indicates that the juice of the calyx quarter had an osmotic pressure of 13.49 atmospheres, that of the middle half 16.40 atmospheres, and that of the styler quarter 18.34 atmospheres. The variations in osmotic pressure were largely the result of the differences in the sugar content of the juice of different portions of the pulp. This is evident from table 2, which shows the greatest inorganic content in the calyx portion. The significance of the gradient in osmotic pressure will be discussed later.

TABLE 5
INORGANIC COMPOSITION OF PEEL AND PULP OF MATURE LEMONS AND GRAPEFRUIT

Portion of fruit	Per cent of dry matter in fresh weight	As a per cent of dry matter					
		Ash	Ca	Mg	Na	K	Inorganic PO ₄
Twenty-six Eureka lemons, September 5, 1930							
Calyx half of peel.....	20.85	3.75	1.15	0.14	0.09	0.39	0.15
Stylar half of peel.....	20.30	3.69	1.02	0.10	0.05	0.58	0.19
Twelve Eureka lemons, March 22, 1931							
Peel.....		4.94	1.26	0.13	0.18	0.92	0.28
Pulp, no seed.....		4.61	0.38	0.12	0.45	1.92	0.54
Peel and pulp, no seed.....		5.31	0.98	0.15	0.33	1.39	0.42
Twenty-five Marsh grapefruits, June 17, 1932							
Calyx half of peel.....	15.78	3.14	0.63	0.10	0.15	0.82	0.25
Stylar half of peel.....	17.08	2.89	0.53	0.08	0.13	0.81	0.33
Calyx half of pulp.....	8.39	3.52	0.28	0.12	0.28	1.42	0.64
Stylar half of pulp.....	8.98	3.22	0.21	0.11	0.27	1.26	0.70

GRADIENTS IN COMPOSITION OF CITRUS PULP

Inorganic Constituents of Pulp of Lemons, Grapefruit, and Valencia Oranges.—The inorganic composition of the pulp of lemons and of grapefruit is shown in table 5. The percentages of calcium and potassium of the calyx halves of pulp of grapefruit were greater than those of the styler halves while the phosphate was greater in the styler halves.

Data are given in table 6 for the inorganic composition of the calyx and styler halves of pulp of 16 Valencia oranges. Even though the percentage of dry matter is greater in the styler half, the percentages of ash, calcium, magnesium, potassium, and sodium are greater in the dry matter of the calyx half. The percentages of calcium, potassium, and sodium are higher in the ash of the calyx half, while those of magnesium

and inorganic phosphate are greater in that of the stylar half. When the peel is included with the pulp, the results are not appreciably altered.

The percentages of total sulfur and phosphorus were determined in the dry matter of the seed-free pulp of mature Valencia oranges collected in San Diego County. The total sulfur content was found to be 0.072 per cent, and the total phosphorus content 0.129 per cent.

Additional data are given in table 6 on the pulp of Valencia oranges from Riverside County. These oranges were prepared for analysis immediately after being picked and were not coated with paraffin as were those used in another experiment (table 6). On a dry-matter basis the percentages of ash, calcium, potassium, and sodium are greater in the calyx half, while the percentage of inorganic phosphate is greater in the stylar half.

The analysis of the pulp of 79 Valencia orange fruits collected on the same day in Orange County showed for the ash constituents in the dry matter gradients similar to those found in the fruits from Riverside County. The percentages of the several ash constituents of the fruit pulp from both locations were also similar, with the exception that the ash content of the Riverside-County fruit was 20 per cent greater than that from Orange County. This was due largely to the greater amounts of potassium, phosphate, and sodium in the Riverside-County fruit. In the calyx and stylar halves of the dry pulp of Orange-County fruit, the respective percentages were 1.46 and 1.16 for potassium; 0.39 and 0.24 for sodium; and 0.550 and 0.580 for inorganic phosphate. These percentages may be compared with those for the Riverside-County fruit given in table 6. The reasons for the differences between the fruits from the two locations are at present unknown.

Equatorial Radial Gradient in Ash of Valencia Oranges.—Two lots each consisting of 20 mature Valencia oranges obtained on June 29, 1932, were peeled and the calyx and stylar quarters of the pulp were cut away and discarded. The central core and the outer membrane of the pulp were removed. The pulp was separated into an inner and outer portion by cutting parallel to the core and making an inner cylinder with a radius equal to about two-thirds of the radius of the pulp. After the seeds were removed the two portions of pulp were dried to constant weight and ashed. The percentages of ash in two samples of the dry matter of the central portion were 4.06 and 3.94, while those for two samples of the outer portion were 2.92 and 2.96. An explanation of the direction of this ash gradient toward the central core is found in the arrangement of the juice vesicles in the pulp. The outer layers of the carpels are united along the whole length of their edges to form the rind, while the inner layers fold toward the core of the fruit to form the seg-

TABLE 6
INORGANIC COMPOSITION OF PEEL AND PULP OF VALENCIA ORANGES FROM RIVERSIDE, 1931

Portion of orange	Fresh weight, in grams	Per cent of dry matter in fresh weight	As a per cent of dry matter						As p.p.m. of dry matter	
			Ash	Ca	Mg	K	Na	Inorganic PO ₄	Fe	Mn
One hundred fruits, August 5										
Calyx quarter of peel.....	1,030.5	19.46	5.02	1.01	0.16	1.26	0.29	0.240	77	5.8
Second quarter of peel from calyx end.....	1,013.5	22.60	4.28	0.70	0.11	1.31	0.32	0.265		5.3
Third quarter of peel from calyx end.....	1,025.5	25.30	3.92	0.61	0.09	1.24	0.27	0.300	96	5.0
Stylar quarter of peel.....	804.5	27.72	3.87	0.59	0.08	1.19	0.26	0.365		6.1
Calyx half of pulp.....	2,790.5*	11.65	4.56	0.32	0.13	1.94	0.45	0.620		3.6
Stylar half of pulp.....	2,779.5*	11.46	4.52	0.32	0.13	1.89	0.47	0.645		3.7
.....	2,760.5*	13.11	3.65	0.24	0.12	1.42	0.37	0.670		3.3
.....	2,782.5*	13.01	3.70	0.23	0.12	1.47	0.36	0.675		4.2
Fourteen fruits, August 20										
Calyx half of peel and pulp.....	917.0	14.94	3.74	0.51	0.14	1.18	0.16	0.500		
Stylar half of peel and pulp.....	913.0	16.87	3.15	0.39	0.11	1.06	0.13	0.550		
Group A. Sixteen fruits, air-dried from August 20 to October 10. First lot of fruit, with calyx half of peel paraffined on August 20										
Calyx half of peel.....		33.36†								
Stylar half of peel.....	222.0†	28.38	3.65	0.73	0.10	0.89	0.17	0.410	117	
Calyx half of pulp.....	797.5†	10.60	4.24	0.31	0.14	1.75	0.41	0.800	252	
Stylar half of pulp.....	833.0†	12.42	3.32	0.22	0.12	1.23	0.26	0.750		3.2

* Sample consisted of pulp of 50 fruits.

† After air-drying.

TABLE 6—(Continued)

Portion of orange	Fresh weight, in grams	Per cent of dry matter in fresh weight	As a per cent of dry matter						As p.p.m. of dry matter	
			Ash	Ca	Mg	K	Na	Inorganic PO ₄	Fe	Mn
Second lot of fruit of Group A, with stylar half of peel paraffined on August 20										
Calyx half of peel.....	186.5†	30.83	4.08	1.00	0.12	0.69	0.12	0.280	362	
Styilar half of peel.....		35.81†								
Calyx half of pulp.....	724.5†	11.15	4.32	0.36	0.14	1.68	0.44	0.655		
Styilar half of pulp.....	779.0†	12.58	3.44	0.27	0.12	1.32	0.29	0.740	61	
Group B. Sixteen fruits, air-dried from August 20 to October 10. First lot of fruit, with calyx half of peel paraffined on August 20										
Calyx half of peel.....		32.21†								
Styilar half of peel.....	245.5†	30.35	3.61	0.72	0.10	0.86	0.17	0.400	149	
Calyx half of pulp.....	857.0†	12.72	3.75	0.31	0.08	1.49	0.33	0.690	79	
Styilar half of pulp.....	834.0†	14.57	3.05	0.24	0.10	1.17	0.22	0.680		
Second lot of fruit of group B with stylar half of peel paraffined on August 20										
Calyx half of peel.....	191.0†	31.28	4.16	0.87	0.14	0.84	0.16	0.320	192	
Styilar half of peel.....		36.53†								
Calyx half of pulp.....	768.0	12.96	3.80	0.32	0.12	1.50	0.35	0.660	34	3.6
Styilar half of pulp.....	800.5	14.43	2.98	0.25	0.11	1.12	0.27	0.600		3.1

† After air-drying.

ments, including their separating membranes. The juice vesicles arise as modified trichomes on the inner surface of the mesocarp, their free terminals extending toward the central core. The inside portion of pulp therefore consists largely of the termini of the juice sacs in which, as the analyses show, the ash accumulates somewhat as in the tip of a leaf.

Inorganic Constituents of Calyx Ends of Granulated and Control Valencia Oranges.—In the observations made late in November, 1931, it was frequently found that portions of the pulp of the calyx end were

TABLE 7

INORGANIC CONTENT OF GRANULATED AND CONTROL CALYX-END EIGHTHS OF VALENCIA ORANGE PULP (NO PEEL OR CORE), NOVEMBER 25, 1931

Number of eighths	Fresh weight, in grams	Dry matter as per cent of fresh weight	As a per cent of dry matter						As parts per million of dry matter	
			Ash	Ca	Mg	K	Na	Inorganic PO ₄	Fe	Mn
92 (control).....	1,174.0	10.78	5.16	0.38	0.15	2.12	0.45	0.85	81	4.0
145 (granulated).....	1,541.5	9.89	6.13	0.45	0.11	2.55	0.61	0.84	71	5.3

severely granulated. A large number of such affected calyx-end portions were compared chemically with a similar number of unaffected calyx-end portions. The results given in table 7 show that the affected pulp contained the higher percentages of water. The percentages of ash, calcium, potassium, and sodium were higher in the dry matter of the granulated pulp, while those for magnesium and iron were lower. When calculated as percentages of the ash, the granulated pulp contained the smaller percentages of calcium, magnesium, and inorganic phosphate. If we assume that the percentages of ash in the unaffected styler pulp of granulated and of control fruits are similar, it is evident that a steeper gradient exists in granulated fruit.

Total Sugars of Grapefruit.—Determinations of the total sugars were made with the dried pulp of the calyx and styler halves of 25 Marsh grapefruits collected at Riverside on June 17, 1932. The seeds were removed in the preparation of the samples. The fresh and dry weights of the calyx pulp were 3,518 and 295.25 grams, respectively, which is equivalent to 8.39 per cent of dry matter, 10.87 per cent of the total dry matter being sugars. The fresh and dry weights of the styler pulp were 3,420 and 307 grams, respectively, corresponding to 8.98 per cent of dry matter, 13.12 per cent of the total dry matter being sugars.

GRADIENTS IN COMPOSITION OF CITRUS PEEL

Oil Content of Calyx and Styler Ends.—Determinations of the oil content of calyx and styler disks of equal surface area of Valencia orange peel were made on September 25, 1931, as described under "Methods of Analysis." The results showed a greater amount of oil in the styler portion.

In each case the white portion of seven disks was cut back to make the calyx and styler peel of equal thickness. The average results of four

Fig. 2.—Oil glands in Valencia orange peel. *A*, Outside surface of calyx end; *B*, inside surface of calyx end; *C*, inside surface of styler end; *D*, outside surface of styler end.

closely agreeing determinations were for the calyx and styler ends, respectively, 0.1103 and 0.1699 grams of oil.

In another analysis, using steam distillation, oil was extracted from 70 disks of equal surface area of the calyx and styler peel. The weight of oil residue found for the calyx peel was 0.0110 gram, while that for the styler peel was 0.0142 gram. Figure 2 shows the difference in number and size of oil-bearing glands when thin slices of orange peel were photographed by transmitted light.

Inorganic Constituents.—When fruit was exposed on a table from August 18 to September 1, 1931, the gradient for the dry matter in the quarters of peel was still evident, but because of the desiccation the percentages were on a higher level. Fruit with leafy twigs attached and exposed similarly, showed a similar gradient but on a slightly higher level. However, it will be noted that little additional water was lost on account of the leafy twigs.

The results of the inorganic analysis of Valencia orange peel given in table 8 show a gradient in the percentages of dry matter. The percentages of ash, calcium, magnesium, and iron in the dry matter increase

TABLE 8

DRY MATTER AND INORGANIC COMPOSITION OF PEEL AND PULP OF LOTS OF 26 VALENCIA ORANGES FROM RIVERSIDE, AUGUST 18, 1931

	Fresh weight, in grams	Per cent of water	Per cent of dry matter in fresh weight	As a per cent of dry matter						In dry matter, p.p.m.	
				Ash	Ca	Mg	K	Na	Inorganic PO ₄	Fe	Mn
Control fruit prepared for analysis immediately after being picked*											
Calyx quarter of peel.....	270.00	78.33	21.67	3.85	1.06	0.170	0.43	0.08	0.22	192	
Second quarter of peel from calyx end.....	253.50	73.77	26.23	3.11	0.79	0.110	0.49	0.10	0.26	122	5.0
Third quarter of peel from calyx end.....	284.50	70.30	29.70	2.95	0.72	0.090	0.57	0.14	0.33		
Stylar quarter of peel.....	205.00	69.27	30.73	2.98	0.69	0.090	0.61	0.14	0.38		5.2
Calyx half of pulp.....	1,394.00	87.37	12.63	3.37	0.32	0.108	1.25	0.30	0.60		
Stylar half of pulp.....	1,383.50	85.15	14.85	2.65	0.24	0.091	0.91	0.25	0.60		
Fruit without leafy twigs attached; exposed August 18 to September 1 at room temperatures†											
Calyx quarter of peel.....	227.00	68.94	31.06	3.72	1.01	0.162	0.52	0.11	0.24		6.0
Second quarter of peel from calyx end.....	221.00	68.78	31.22	3.13	0.76	0.100	0.53	0.09	0.28	160	
Third quarter of peel from calyx end.....	213.00	67.84	32.16	3.06	0.72	0.090	0.60	0.13	0.32	156	
Stylar quarter of peel.....	211.00	66.82	33.18	3.12	0.66	0.090	0.67	0.16	0.42		5.0
Calyx half of pulp.....	1,496.00	89.37	10.63	3.99	0.36	0.126	1.46	0.38	0.71		3.2
Stylar half of pulp.....	1,521.00	88.20	11.80	3.17	0.26	0.096	1.13	0.22	0.73		2.7
Fruit with leafy twigs attached; exposed August 18 to September 1 at room temperatures‡											
Calyx quarter of peel.....	186.00	65.86	34.14	3.94	1.04	0.180	0.44	0.11	0.25	162	
Second quarter of peel from calyx end.....	192.00	65.36	34.64	3.26	0.80	0.120	0.53	0.08	0.30	149	
Third quarter of peel from calyx end.....	193.00	64.25	35.75	3.20	0.76	0.100	0.63	0.10	0.36	107	
Stylar quarter of peel.....	158.00	63.29	36.71	3.32	0.74	0.100	0.67	0.12	0.47	142	
Calyx half of pulp.....	1,215.00	88.15	11.85	3.79	0.39	0.120	*1.39	0.26	0.67		3.5
Stylar half of pulp.....	1,310.00	85.91	14.09	2.98	0.30	0.100	1.10	0.21	0.66	38	3.0

* Fresh weight, 3,832 grams.

† Fresh weight on August 18, 4,322 grams; fresh weight on September 1, 3,927 grams. Fresh and dry weights in table as of September 1.

‡ Fresh weight on September 1, 3,299 grams. Fresh and dry weights in table as of September 1.

toward the calyx end, while those for sodium, potassium, and inorganic phosphate increase toward the stylar end. When the results are expressed as per cent of ash, the directions of the gradients remain unchanged.

The percentages of total sulfur and phosphorus were determined in the dry matter of the peel of mature Valencia oranges collected later in San Diego County, the data of which are not shown in table 8. For the stem half the total sulfur was found to be 0.038 per cent and for the tip half 0.052 per cent. In the case of total phosphorus the percentages were 0.093 and 0.095, respectively.

The results for the peel of calyx and stylar halves of lemons collected at Riverside September 5, 1930, show in table 5 that the percentages of ash, calcium, magnesium, and sodium in the dry matter are greater in the calyx half, while the percentages of potassium are greater in the stylar half. The gradients of the inorganic constituents are therefore in a direction similar to those obtained for the peel of Valencia oranges (tables 6 and 8).

The percentages (table 5) of calcium in the dry matter and ash of the peel of lemons collected on March 22, 1931, at Riverside, exceed those found in the pulp, while those of potassium and inorganic phosphate are less than those found in the pulp. The percentages of calcium, magnesium, and sodium are greater in the dry matter or ash of the calyx halves while those of potassium and phosphate are greater in the dry matter or ash of the stylar halves. The directions of gradients of the inorganic constituents of the dry matter and ash are the same as those for Valencia orange (table 8).

Results with the calyx and stylar halves of peel of grapefruit collected June 17, 1932, at Riverside, show (table 5) that the percentage of dry matter is greater in the stylar half, as was found in Valencia oranges. Except for the potassium in the dry matter, the percentages of inorganic constituents in the dry matter and ash are in the same direction as in lemons and Valencia oranges.

Table 6 shows gradients in the inorganic constituents of the peel of Valencia oranges collected at Riverside on August 5. Similar determinations were made on 79 Valencia oranges collected on the same day in Orange County, the results for which showed similar gradients. The percentages of ash, potassium, and sodium in the peel of Orange-County fruits, however, were much less than those in the Riverside fruit, being 3.23 to 3.79 for ash, 0.65 to 0.80 for potassium, and 0.08 to 0.18 for sodium.

Radial and Polar Gradients of Inorganic Constituents of Valencia Oranges.—Since orange peel is thickest at the calyx end, it was divided

not only into calyx and stylar portions, but also radially into the gland-bearing (outer) and white (inner) portions. Determinations were made of the inorganic composition of these various portions of peel of two lots each consisting of 20 Valencia oranges collected from the fertilizer plots at the Citrus Experiment Station on June 29, 1932. Lot B was secured from a plot that received urea but no potassium or covercrop.

TABLE 9
INORGANIC COMPOSITION OF GLAND-BEARING (OUTER) AND WHITE (INNER)
PORTIONS OF LOTS OF 20 CALYX AND STYLAR HALVES
OF VALENCIA ORANGE PEEL

Portion of peel	As a per cent of dry matter					
	Ash	Ca	Mg	Na	K	Inor- ganic PO ₄
Lot A						
Outer portion of calyx half.....	4.19	0.98	0.18	0.11	0.76	0.35
Inner portion of calyx half.....	3.05	0.90	0.67	0.02	0.34	0.20
Outer portion of stylar half.....	3.67	0.77	0.12	0.10	0.86	0.48
Inner portion of stylar half.....	2.72	0.81	0.06	0.02	0.36	0.18
Lot B						
Outer portion of calyx half.....	3.71	0.95	0.18	0.09	0.56	0.28
Inner portion of calyx half.....	2.53	0.76	0.67	0.02	0.29	0.11
Outer portion of stylar half.....	3.42	0.75	0.11	0.15	0.77	0.45
Inner portion of stylar half.....	2.39	0.67	0.07	0.03	0.36	0.17

Lot A was from a plot that received urea and sulfate of potash, but no covercrop. Since potash is the variable in the two plots it is possible that differences in composition of the fruit may be related to the potash fertilization of the soil.

In both lots of fruit (table 9) the gland-bearing portion contained a greater percentage of ash than the white portion, and the values for the calyx half were greater than those for the stylar. Moreover, the percentage of ash in the dry matter was greatest in lot A.

In both lots of fruit the percentage of calcium in the dry matter was greater in the calyx than in the stylar half, and in most cases was greater in the gland-bearing portion of the peel than in the white portion. In the fruit examined, the percentages of calcium in the dry matter were larger in lot A.

The percentages of magnesium in the dry matter were approximately the same in lots A and B. It should be mentioned here that in the calyx

half there was nearly four times as great a percentage of magnesium in the dry matter of the white portion as in the gland-bearing portion, and in the styler half there was only twice as great a percentage.

In every case a larger percentage of potassium was found in the dry matter of the gland-bearing portion, and there was a larger percentage in the gland-bearing portion of the styler half than in that of the calyx half. The greater percentages of potassium in the dry matter of the peel of the styler half confirm previous results (table 8). Since it has been shown that the percentages of sugars are less in the dry matter of the peel of the styler half, it appears that on a dry-matter basis the percentages of potassium bear no positive relation to the sugar content. However, there was a positive relation of inorganic phosphate and nitrogen to sugar.

The percentages of potassium in the gland-bearing portion were highest and varied the most, while those of potassium in the white portion were relatively constant. In fruit from lot A the percentages of ash in the gland-bearing portion of the peel were much higher than in fruit from lot B.

In every case the percentages of inorganic phosphate were greatest in the gland-bearing portion, in which portion the percentages were higher for the styler than for the calyx end.

The percentages of calcium in the ash vary but little, and are higher in the white portion of the peel. In the gland-bearing portion they are higher in the calyx than in the styler half. The percentages of magnesium and sodium in the ash are greater in the gland-bearing portion.

The percentages of potassium in the ash are greater in the gland-bearing portion of the peel. The gland-bearing and white portions have higher percentages in the styler than in the calyx half.

It has been shown that the gland-bearing portion of the calyx half of Valencia-orange peel contains a smaller percentage of potassium than that of the styler half (table 9). Valencia orange fruits obtained from trees grown in sand cultures that received a culture solution lacking potassium, showed a breakdown and slight gumming in the peel of the calyx half (fig. 3). The outer portion of the peel appeared to be the only part affected. This is of interest because of the frequent assertion that potash improves among other things the quality of the rind.

In these studies of gradients the greatest concentration of potassium usually was not found where the concentration of sugars was greatest. While the role of potassium in the peel, pulp, leaf, or trunk is but little understood, it has been suggested that potassium is concerned with the synthesis of carbohydrates and proteins, although some investigators have found no such relation (Janssen and Bartholomew⁽¹⁹⁾). Taka-

hashi⁽²⁸⁾ has maintained that potash fertilization increased the sugar content of citrus fruit. However, for prunes, Lilleland⁽²²⁾ found that although potash fertilization of the soil increased the potassium con-

Fig. 3.—Effect of a deficiency of potassium in sand cultures on peel of Valencia orange. Upper row: magnesium substituted for potassium in culture solution. Lower row: left, calcium substituted for potassium in culture solution; right, control (complete culture solution).

tent of the leaves and fruit, there was no increase in the sugar content of the fruit.

Total Sugar and Starch Content of Calyx and Styler Halves.—The peel of the calyx halves of 26 Eureka lemons collected September 5, 1930, at Riverside, contained 20.85 per cent dry matter and 14.59 per cent total sugars in the dry matter, while the peel of the styler halves contained 20.30 and 11.91 per cent dry matter and total sugars in the dry matter, respectively.

Similar determinations were made with grapefruit and Valencia-orange peel. The values obtained for the calyx halves of peel of 25 Marsh grapefruits collected at Riverside were 15.78 per cent dry matter and 28.77 per cent total sugars in dry matter, and for the styler halves were 17.08 and 27.89 per cent dry matter and total sugars in dry matter.

Determinations of total sugars in the dried peel of two lots of Valencia oranges collected July 20, 1931, at Riverside, gave 25.74 and 28.16 per cent, respectively, for the calyx halves, and 22.66 and 23.67 per cent for the styler halves.

Fig. 4.—Starch in mature Valencia orange peel. *A*, Tip end, radial section; *B*, stem end, radial section; *C*, tip end, tangential section; *D*, stem end, tangential section.

On other samples of dry matter prepared from disks of equal surface area cut from the calyx and styler ends of the peel of Valencia oranges collected on October 14, 1931, at Riverside, determinations of total sugars showed 25.21 and 24.61 per cent, respectively.

The relative amounts of starch in the calyx and styler peel of mature Valencia oranges obtained at Riverside on October 15, 1931, may be seen in figure 4, in which fresh, thin sections were stained for equal periods in a solution of iodine and potassium iodide. The reaction for starch appears stronger in the styler peel. The concentration gradients for sugars and starch in the peel, while small, are the reverse in direction of those for sugars in the pulp.

Osmotic Pressure.—The osmotic pressures of the juices of lemon peel were obtained as described under "Methods of Analysis." Figure 1 shows the osmotic pressure of the juice of the peel from the calyx halves of 50 lemons (silver stage) as being 15.08 atmospheres and that for the juice of the peel from the styler halves as being 14.26 atmospheres.

The values obtained for the osmotic pressure of the juice of the peel from the calyx and stylar halves of 50 ripe Valencia oranges are shown in figure 1. The value for the calyx halves was 20.00 atmospheres and that for the stylar halves was 24.58 atmospheres. The increased osmotic pressure in the stylar halves cannot be ascribed to an increased percentage of total sugars, because the percentage of total sugars is slightly less in the stylar peel. From the data in table 8 it appears likely that the increased osmotic pressure may be a result of differences in the water content of the halves of peel.

WATER CONTENT OF VARIOUS PORTIONS OF CITRUS FRUITS

Determinations were also made of the water content and dry matter of disks of equal diameter cut from the stem and stylar quarters of mature Valencia orange rind collected September 25, 1931. A sufficient amount of the white (inner) portion was cut from the thicker disks of the calyx end to make them approximate the thickness of those from the stylar end. The 12 disks of the calyx end gave a fresh weight of 10.2006 grams; and dry matter 20.70 per cent of the fresh weight, whereas the 12 disks of the stylar end gave a fresh weight of 8.6170 grams; and dry matter 28.69 per cent of the fresh weight. Thus it is evident that the disks of the calyx end had a greater fresh weight, yet contained less dry matter than those of the stylar end. Further confirmation of these results is reported in table 10.

TABLE 10
WATER CONTENT OF VALENCIA ORANGE PEEL*

Number of disks	Half	Fresh weight, grams	Dry weight, grams, 100° C	Water as per cent of fresh weight
200	Calyx.....	178.50	38.31	78.54
200	Stylar.....	125.25	37.94	69.71
335	Calyx.....	269.07	50.08	81.39
335	Stylar.....	197.63	55.18	72.08

* Disks of equal surface area (11/16-inch diameter) and natural thickness.

The loss of volatile oils from the rind during heating complicates these determinations so that the values must be regarded as only relative and not absolute. The rind of a given surface of the calyx end is thicker and weighs considerably more than that of a similar surface of the stylar end, yet they closely approximate each other in dry matter per unit of surface. The extra thickness of the peel of the calyx end may be due principally to the swelling that results from its greater power to imbibe water.

The writers have shown that more water is lost through the rind of the calyx end of Valencia oranges than through that of the styler end, and later in this paper will report further data in partial explanation of these differences.

The water content of the calyx half of mature Valencia orange pulp is shown in tables 6 and 8 to exceed that of the styler pulp.

The uniformity of the water content of the various portions of lemon peel is seen in table 5.

Determinations of the water content of the two halves of 120 freshly picked tree-ripe lemons showed 84.41 per cent in the calyx halves and 84.0 per cent in the styler halves. Since the fruit was picked shortly following a 2-day period of heavy rain, the trees should not have been suffering from a water deficit. Similar results were obtained by Bartholomew⁽¹⁾ with determinations made in January, February, and March.

In the case of grapefruit peel and pulp the calyx half contains more water than the styler half (table 5). Thus the water content of grapefruit more nearly parallels that of the orange than the lemon.

GRADIENTS IN PERMEABILITY OF CALYX AND STYLAR HALVES OF CITRUS

It has been shown that the calyx half of lemons and Valencia oranges loses water more rapidly than the styler half, and it was therefore of interest to study the loss of other constituents from the fruit. This was accomplished by measuring the rate of decolorization of the KMnO_4 by materials that exosmose from fruit into water.

A comparison was made of the relative permeability of calyx and styler halves of green lemons, green Marsh grapefruit, and Valencia oranges. The lots were paraffined as described under "Methods of Analysis" and placed in 2,000 cc of tap water. The data are given in table 11.

The data show that the differences in permeability of the halves of green lemons are small. The calyx half shows only a slightly greater permeability. These results are in agreement with the observations that the various portions of the peel differ but little in their susceptibility to storage breakdowns and fungus invasion.

The results for grapefruit and Valencia oranges show that the calyx half has a distinctly greater permeability than the styler half, which is in agreement with the loss of water by evaporation from the halves of Valencia oranges.

Since the permeability of citrus fruit increases with increasing age (Klotz⁽²¹⁾) and since the calyx half is more permeable than the styler

half, the indications are that the calyx portion has aged more rapidly. It is known, moreover, that the calyx half is more subject to certain diseases and physiological breakdowns than the stylar half. The importance of methods of studying changes in the permeability of different portions as well as in whole fruit becomes greater where such changes can be related to other gradients.

TABLE 11

TIME REQUIRED TO MATCH KMnO_4 STANDARD IN PERMEABILITY TESTS OF CALYX AND STYLAR HALVES OF LEMONS, GRAPEFRUIT, AND ORANGES

Treatment of fruit halves	Time immersed, hours	Minutes and seconds required to match standard				
Green lemons from Riverside, October 28, 1932; lots of 20 fruits each, three determinations						
Calyx half not paraffined.....	16	15:23	12:22			
Stylar half not paraffined.....	16	15:23	12:22			
Calyx half not paraffined.....	66	1:30	1:50	1:30		
Stylar half not paraffined.....	66	1:42	1:40	1:55		
Nearly full-grown green Marsh grapefruit from Riverside, November 1, 1932; lots of 10 fruits each, four determinations						
Calyx half not paraffined.....	48	3:16	2:10	3:00	3:15	
Stylar half not paraffined.....	48	9:46	11:10	10:00	9:51	
Valencia oranges from Riverside, October 27, 1932; lots of 20 fruits each, five determinations						
Calyx half not paraffined.....		1:52	2:15	2:00	2:05	1:55
Stylar half not paraffined.....		7:12	5:00	7:20	7:40	7:20

The method for estimating permeability was used in a preliminary experiment to determine the effect of hydrocyanic acid gas on lemon fruit. Ten (300 size) silver-stage lemons were obtained directly from the trees at Riverside and were fumigated with 15 cc HCN in a chamber (100 cubic feet) for 35 minutes. This and the control fruit were placed in tap water at 20° to 21° C for 16 hours. Aliquots of the solution containing the fumigated fruit required 4 minutes to decolorize the KMnO_4 , while aliquots of the solution containing the control fruit required 11½ minutes. This suggests that the permeability of the peel of lemons is increased by the process of fumigation.

The relative effect of hydrocyanic acid gas on the permeability of the two halves of Valencia oranges was next determined. Ten fruits collected March 25 were used in each of four lots. The half not under consideration was paraffined before exposure to the gas. In each case the fruits were immersed in 2 liters of distilled water for 22 hours, and the

rate of KMnO_4 decolorization was estimated and compared with control fruit similarly paraffined but not fumigated. The time required (hours and minutes) to match the KMnO_4 standard was as shown in the following tabulation:

Calyx half not paraffined; stylar half paraffined; fruit fumigated.....	1:15
Calyx half not paraffined; stylar half paraffined; fruit not fumigated (control).....	11:05
Stylar half not paraffined; calyx half paraffined; fruit fumigated.....	3:25
Stylar half not paraffined; calyx half paraffined; fruit not fumigated (control).....	14:30

The data again show that the calyx half of the unfumigated (control) fruit had a greater permeability than the stylar half. The effect, how-

TABLE 12

WATER LOSS THROUGH PEEL OF HALVED FRUITS WITH CUT SURFACE ON PARAFFINED PAPER, 1930

Half	Number of fruits	Water loss after 5 days as a per cent of original weight	Water loss after 8 days as a per cent of original weight
Mature Valencia oranges			
Calyx.....	31	4.80	12.90
Stylar.....	31	3.88	9.74
Lemons (silver stage)			
Calyx.....	48	9.34	13.32
Stylar.....	48	7.40	12.21

ever, of the hydrocyanic acid treatment was not only to increase the permeability of both halves but also to increase that of the calyx half more than that of the stylar half. The results correspond with the degree of injury from hydrocyanic acid treatment of Valencia oranges in the field where the calyx half is ordinarily the first portion to be injured.

GRADIENTS IN WATER LOSS FROM CITRUS FRUITS

A knowledge of the water relations of leaves, twigs, and portions of the fruit may lead to a better understanding of the interrelations of organic and inorganic substances within these organs. The physiological diseases called granulation and endoxerosis have been considered in connection with the water relations of the fruit, especially with the gradient existing between the calyx and the stylar ends. At the initiation of this work, the writers investigated the source, causes, and pre-

vention of water loss in fruit, and the bearing of tissue structure and composition upon these factors.

Permeability of Peel.—Water losses through the peel of halved lemons and Valencia oranges were first determined, without the use of boric acid antiseptic on the cut surfaces. Although breakdown due to micro-organisms eventually occurred, later work with boric acid confirmed the preliminary results. Table 12 shows that in every case but one the peel

TABLE 13
WATER LOSS OF VALENCIA ORANGE HALVES, PEEL AND PULP, OBTAINED AUGUST 5, 1931; BUTTONS PARAFFINED

Half	Fresh weight, grams	Total loss of weight, grams				Dry weight as per cent of fresh weight
		1 day	3 days	6 days	9 days	
Orange County						
84 calyx.....	5,651	107	292	543	702	13.08
84 stylar.....	6,040	100	275	497	632	15.29
Riverside County						
100 calyx.....	7,097	141	389	701	907	12.71
100 stylar.....	7,745	127	350	616	775	14.87

of the calyx half of both citrus species permitted a greater water loss than that of the stylar half.

Sixteen fruits (table 6, group A), with calyx half of peel paraffined, lost 182 grams of water from August 20 to October 10, while the second lot of 16 fruits, with stylar half of peel paraffined lost 258.5 grams of water. In table 6, group B, the water losses were 199 and 359 grams, respectively. These results emphasize the greater water loss from the calyx half of Valencia oranges. Table 13 shows similar results with Valencia oranges from two sources, Riverside and Orange counties. The cut surfaces in this experiment were protected by boric acid powder. Table 13 also shows that the dry weight as a percentage of the fresh weight is less in the calyx halves than in the stylar halves, which means there is more water in the calyx half than in the stylar half. These results will be discussed more fully later.

Water losses from the two halves of lemons and Valencia oranges studied by the method of paraffining either half to the equatorial line are shown in tables 14 and 15.⁵ Here again more water is lost through

⁵ The water loss from the nonparaffined fruit surface (grams per square centimeter) may be calculated by dividing the water loss after any given period by the area of half of the surface of 16 fruits.

TABLE 14
WATER LOSS, WATER CONTENT, AND SURFACE AREA OF 16 VALENCIA ORANGES FROM RIVERSIDE*

Half paraffined†	Weight of fruit before being paraffined (grams)	Total loss of water from fruit, grams						Water content of fruit halves, grams		Area of surface of 16 fruits sq. cm.
		21.5 hrs.; rel. hum. 46; 84° F	116.0 hrs.; rel. hum. 46; 90° F	188.0 hrs.; rel. hum. 70; 80° F	284.0 hrs.; rel. hum. 86; 77° F	356.0 hrs.; rel. hum. 79; 75° F	452.0 hrs.; rel. hum. 68; 77° F	Stylar	Calyx	
Stylar.....	4,619.0	29.0	161.0	240.0	276.5	306.5	363.0	1,919	1,965	2,362 (halves)
Calyx.....	4,871.5	19.5	114.0	174.5	201.5	225.5	270.0	2,025	2,072	2,421 (halves)

* Experiment started at 4 p.m., August 20, 1931.

† Cut-off stems paraffined in both cases.

‡ Rain.

TABLE 15
RELATIVE WATER LOSSES FROM CALYX AND STYLAR HALVES OF 100 TREE-RIPE LEMONS, FEBRUARY 3, 1932
(Paraffined Half Placed on a Collar on Plates of Glass; Button Paraffined)

	Weight of freshly picked fruit before being paraffined, grams	Water loss as per cent of original fresh weight							
		22 hrs.	49 hrs.	139 hrs.	188 hrs.	287 hrs.	353 hrs.	497 hrs.	792 hrs.
Evaporation from calyx half when stylar half of fruit is paraffined.....	10,010.50	0.80	1.94	3.96	5.05	6.75	7.87	10.60	12.18
Evaporation from stylar half when calyx half of fruit is paraffined.....	9,821.00	0.72	1.79	3.81	4.93	6.52	7.57	10.16	11.67

the peel of the calyx half than through that of the stylar half. The interrelations of the two halves must be considered when this method is employed. Any water lost from the paraffined half must make its exit through the uncovered peel of the opposite half. As has been shown, there are greater amounts of osmotically active compounds in the pulp of the stylar half than in that of the calyx half, which means there should be a greater resistance to movement of water from the stylar end toward the calyx end. It would be expected that this condition would have a retarding effect on water loss from the calyx half in fruit where the stylar half was paraffined. The fact that more water escaped from the calyx half indicates that the rind of that portion was more permeable to water and contained a smaller concentration of osmotically active substances (fig. 1) than the rind of the stylar half. Measurements not only of water loss but also of permeability and osmotic pressure confirm these assumptions.

It is noted from tables 14 and 15 that the differences in water losses from the polar halves of the fruit are much greater with Valencia oranges than with lemons. These differences parallel the relatively greater degree of sensitivity of the calyx region of the Valencia orange to certain rind affections. The differences are much less noticeable in the case of lemons.

Effects of Leafy Twigs on Water Losses During Wilting.—A better understanding of the significance of water gradients within the fruit may be obtained by studying the influence of leaves and twigs on the rate of water loss in fruits. Three lots, each consisting of 26 mature Valencia oranges, were picked August 18, 1931. Two groups were cut from the tree in the usual manner with fruit clippers, while the third group was cut so that an 18-inch twig bearing leaves was left attached to each fruit. The first lot was worked up at once, the fruit being peeled and the rind divided into calyx, stylar, and two middle quarters for comparison of water content and chemical composition. The pulp was divided into calyx and stylar halves, weighed, dried, and analyzed. The second and third lots were left on a table until September 1, 1931, when they were divided and treated as was the first group.

The water content of peel and pulp of these oranges when picked, and after 14 days in the laboratory, can be calculated from table 8. The losses from the peel were relatively larger than from the pulp.

There was considerable loss from the peel whether or not leafy twigs were attached, and the loss was slightly less in the case of attached leafy twigs. The greatest loss was from the calyx quarter and the least from the stylar quarter of the rind. The per cent of water in the calyx end of the peel was greatest and was found to decrease progressively to a mini-

mum at the styler end. Conversely the styler quarter of the peel had the most dry matter and the calyx quarter the least. Similarly the styler half of the pulp contained a greater percentage of dry matter and less

TABLE 16

EFFECT OF GRADIENTS IN OSMOTIC PRESSURE ON THE WATER LOSS FROM FRUITS AT VARIOUS STAGES OF MATURITY

Maturity of fruit	Number of fruits	Treatment of fruit	After (days)	Water loss as per cent of original fresh weight
Lemons				
Green.....	17	Control, debuttomed.....	0	0.00
	15	With shoots, but with leaves removed.....	3	2.25
	15	With shoots and leaves attached; leaves badly wilted after 3 days (dry weight of leaves, 57 grams).....	3	5.12
Jumbo green.....	15	Control, debuttomed.....	0	0.00
	15	Buttons not sealed.....	4	1.83
	15	Buttons sealed with paraffin.....	4	0.79
	15	With shoots, but with leaves removed; shoots not sealed.....	4	3.75
	15	With shoots, but with leaves removed; shoots sealed.....	4	3.43
	13	With shoots not sealed; leaves attached and badly wilted after 4 days (dry weight of leaves, 60 grams).....	4	4.49
Light green.....	15	With shoots sealed; leaves attached and badly wilted after 4 days.....	4	4.13
	14	Control, debuttomed.....	0	0.00
	14	With shoots, but with leaves removed.....	4	4.23
Silver stage.....	12	With shoots and leaves attached.....	4	6.02
	14	Control, debuttomed.....	0	0.00
	15	With shoots, but with leaves removed.....	3	4.80
	15	With shoots and leaves attached; (dry weight of leaves, 74 grams).....	3	5.66
Oranges				
Green Washing-ton Navel.....	15	Control, debuttomed.....	0	0.00
	14	With shoots, but with leaves removed.....	3	4.72
	14	With shoots and leaves attached; leaves badly wilted after 3 days.....	3	9.85
Mature Valencia	32	Control, debuttomed.....	0	0.00
	30	With shoots, but with leaves removed.....	2	0.43
	31	With shoots and leaves attached; leaves badly wilted after 2 days (dry weight of leaves, 79 grams).....	2	1.28

water than the calyx half. The pulp of the second and third groups had smaller percentages of dry matter than that of the first group. These reductions in dry matter may be due to material lost in respiration and volatilization.

Significance of Gradients in Osmotic Pressure.—The effect of changes in the maturity of the fruit in resisting water loss is explained, in part at least, by the reduced surface in relation to the volume of the fruit (Haas⁽¹²⁾) and by the increase in the osmotic pressure of the fruit cells, due to the storage of osmotically active solutes such as sugars and organic acids. For similar reasons mature leaves would have more power to withdraw water from fruit than immature leaves. Halma and Haas⁽¹⁵⁾ have shown that the osmotic pressure of sap of leaves of a given age varies greatly throughout a single day. Seasonal changes in soil and air temperature were found by Haas and Halma⁽¹⁴⁾ to affect the concentration of solutes in mature leaves. For periods of a given length of time under the same environmental conditions, mature lemon leaves lost more water per unit area than orange leaves. These water losses were directly related to the osmotic pressure of the leaf saps.

Table 16 shows a reduction of the gradient between the osmotic pressure of leaves and fruit as indicated by a lessening of the power of the leaves to pull water from the fruit as the fruit matures.

Figure 1 gives the approximate range of osmotic values of the sap of mature lemon and orange leaves. A comparison of these values with those for the peel and pulp of the calyx and styler halves of 50 fruits indicates the possible effect of leaves in influencing water loss from fruit. Since the osmotic value of the sap of the styler end of lemon peel is less than that of the calyx end, the former would tend to lose water more rapidly than the latter whether due to the withdrawal by the leaves or to direct evaporation from the fruit surface.

If the injury called endoxerosis or internal decline of lemon fruit is induced by a deficiency of water, the significance of the osmotic values of the various portions of the fruit becomes apparent. The first manifestation thus far discovered of the presence of this trouble in lemons is the appearance of pentosans and gums in the peel at the styler end. Then follow the breakdown and drying of the pulp and core tissue with the accompanying pink to brown discoloration (Fawcett and Lee,⁽⁸⁾ p. 422).

The invariable first appearance of the trouble at the styler end may be accounted for in the following manner: While the osmotic value and sugar content of the pulp juice of the styler portion are slightly greater than those of the calyx end, the osmotic value and sugar content in the peel of the styler portion, where the internal decline begins, are lower than the values for the peel of the apparently unaffected calyx end. This indicates that the styler peel would be the first to suffer from a water deficit.

The pulp of the styler region, even though it has a slightly higher osmotic value than that of the calyx end, is the first portion of the pulp to be affected. A consideration of the anatomy of the fruit may make this understandable. Each juice vesicle of the pulp is attached to the periphery of the locular membrane just under the peel by means of a slender stalk or filament. Water is supplied from the vascular tissue of the peel. If that tissue is plugged with hydrophilic gum and pentosans, it is evident the supply of water to the underlying pulp is cut off and injury follows.

The influence of leaves and twigs on water loss from immature orange fruit was much more significant than in the case of mature fruit (table 16). This suggests the possibility that the injury called juice-sac granulation, which is most severe at the calyx end, may take place in immature fruit during periods of water deficit and become manifest as the fruit matures.

From figure 1 it is seen that the pulp of the calyx region has a smaller osmotic value than the remaining portions of the pulp and would therefore give up water more readily and be the first portion injured by a water deficit. The minimum osmotic value of the mature leaf sap is considerably greater than that of the pulp juice of the calyx end of the mature orange.

Diffusion from the Peel of Calyx and Styler Regions.—Further information was gained from determinations on the amount of material which diffused inwardly when oranges were cut in halves and the pulp replaced with water.

Intact halves of orange peel were secured by removing the pulp from the halved fruit with a spoon. The peel was washed rapidly with distilled water and wiped dry. The styler scar and button were sealed with paraffin and the halves with open end upward were supported on short pieces of mailing tube. The halves were nearly filled with distilled water and covered with watch glasses. At the end of several days the water was analyzed for dry matter; 3,400 p.p.m. was found in the water from the calyx halves and 6,500 p.p.m. in that from the styler halves.

In a repetition of this method of measuring diffusion, tap water was used instead of distilled water. The fresh weight of 13 calyx halves was 553.5 grams, and the dry weight after diffusion had taken place was 80.25 grams or 14.5 per cent of the fresh weight. The fresh weight of 13 styler halves was 583.5 grams, and the dry weight after diffusion had taken place was 86.25 grams or 14.8 per cent of the fresh weight. It has been shown that the per cent of dry matter in the fresh peel of the styler half is greater than that of the calyx half. However, it is seen that after diffusion the values are nearly equal, being 14.5 and 14.8 per cent,

respectively, which means that more material was lost from the stylar than from the calyx half. Here the thin-walled cells of the white portion of the peel are in immediate contact with the solvent, while in later experiments on exosmosis from the intact fruit, the cutinized heavier-walled cells of the epidermis are in contact with the solvent.

The water loss through the depulped calyx and stylar halves of peel was determined when the halves were nearly filled with tap water. The halves were usually weighed individually in order to avoid the complete loss of the experiment as a result of a single leaky peel. Tap water was used instead of distilled water because it approaches being a balanced solution and therefore obviates much of the toxicity of distilled water. However, as will be shown, the difference between the concentration of tap water and orange juice is so great that toxic effects due to plasmolysis result. This was evident in an experiment in which the halves were filled with tap water. Thirty-eight stylar and 6 calyx halves showed greater water losses than the opposite halves of the same fruits.

As shown by further studies these results with tap water cannot be explained by a consideration of osmotic values alone. In whole oranges the peel is in contact with the pulp, the juice of which is extremely concentrated as compared with tap water. Moreover, the thinness of the stylar peel may contribute to the rapidity of the injurious effect of tap water. It is possible that under the conditions brought about by the contact with tap water, the stomata, which are closer together in the stylar peel, may, if not plugged, function more noticeably as pores in a septum because of the absence of concentrated orange juice. If instead of tap water we use a more concentrated solution such as orange juice in the halves, we approach more nearly the conditions as they exist in the orange.

Valencia orange juice was used instead of tap water to fill the peel halves in the following experiment. Where both halves contained the same composite juice of the whole pulp, there was a greater loss of water through the calyx peel, 26 calyx halves and only 1 stylar half showing greater water losses than the opposite halves of the same fruits.

The loss was also greater through the calyx peel when calyx-peel halves were filled with juice of calyx pulp and stylar-peel halves with juice of stylar pulp, the water losses from 17 calyx halves and only 1 stylar half exceeding those from the opposite halves. However, when calyx-peel halves were filled with juice of stylar pulp and stylar-peel halves with juice of calyx pulp, the loss was greater through the stylar peel.

These results emphasize the importance of osmotic concentration in the regulation of water loss from fruit. The greater concentration of

the juice from the stylar half of pulp caused less water to be lost through the peel, regardless of which half of the peel contained it. This minimizes the importance of stomata composition, and permeability of the peel as regulators of the water loss in fruit, and emphasizes the importance of osmotic concentration of the pulp juice.

Distribution of Stomata and Oil in Calyx and Stylar Peel of Valencia Oranges.—The distances separating stomata about a mother stoma were measured with stained and unstained cleared mounts. One hundred fourteen measurements at the stylar end showed the average distance between stomata to be $133.6\ \mu$, while 81 measurements at the calyx end showed it to be $172.6\ \mu$. Forty counts showed an average of 14.4 stomata per microscopic field at the calyx end and 17 at the stylar end. Thus the stomata about a mother cell appear to be closer together at the stylar than at the calyx end. From these results a greater water loss would be expected at the stylar than at the calyx end. Measurements of actual water loss are contrary to such expectations, and therefore indicate that water loss from stomata of citrus fruits is of minor importance when compared with the total water loss from the surface of fruits.

The larger amount of oil in the stylar peel may be a factor in decreasing the water loss from the stylar peel. However, as we have seen, the amount of oil in the peel and the number of stomata are only of secondary importance in comparison with the rôle played by the osmotic pressure of the juices of pulp, peel, and leaves in regulating water loss from fruit.

SUMMARY

The pH and buffer action of the juice of the calyx and stylar halves of mature Valencia orange pulp showed but small differences.

No significant differences were found in the specific gravity of the pulp juice of halves of either lemons or immature grapefruit. In both mature seedling grapefruit and mature Valencia oranges the specific gravity was least in the calyx and greatest in the stylar portion.

The carotinoid content of the juice of the stylar quarter of mature Valencia oranges was 1.54 times that of the juice of the calyx quarter. The juice of the calyx half of Valencia oranges had a greater iodine-fixing power than that of the stylar portion.

The total nitrogen in the juice of the stylar quarter of mature Valencia orange pulp slightly exceeded that in the juice of the calyx quarter. The amino nitrogen content of the juice of both quarters showed no differences. In the juice of Valencia and Washington Navel oranges the percentage of dry matter increased and that of total ash decreased from the calyx to the stylar end.

The pulp juice of mature Valencia oranges had a concentration of approximately 72,000 grams of total sugars in 1,000,000 cc at the calyx half and approximately 95,000 grams in 1,000,000 cc at the styler half. The gradient in total sugar was so striking as to be easily recognized by taste. The pulp juice of the styler halves of lemons and grapefruit contained more total sugars than that of the calyx halves, although the differences were not as large as those in Valencia oranges.

The pulp juice of the styler halves of lemon had a slightly higher osmotic pressure than that of the calyx halves, while in Valencia orange the differences were much larger and in the same direction as in lemon.

Based on either fresh material or dry matter the calyx halves of Valencia oranges and grapefruit contain greater percentages of calcium and potassium and, in the ash, less phosphate than in the styler halves.

A radial equatorial gradient was found in the ash of the pulp of Valencia oranges, the ash increasing toward the central core. Granulated pulp of Valencia oranges was found to have a higher percentage of water in the fresh material and higher percentages of ash, calcium, potassium, and sodium in the dry matter than that of control fruits.

The pulp of the calyx halves of mature grapefruit contained 10.87 per cent of total sugars in the dry matter while that of the styler halves contained 13.12 per cent.

A greater amount of oil was found in the peel of the styler end of mature Valencia oranges than in that of the calyx end.

The percentages of ash, calcium, magnesium, and iron in the dry matter of Valencia orange peel increased toward the calyx end, while those of sodium, potassium, and phosphate increased toward the styler end.

A larger percentage of potassium was found in the dry matter of the gland-bearing portion of the peel of mature Valencia oranges than in the white portion, and a greater percentage in the gland-bearing portion of the styler half than in that of the calyx half. In mature Valencia oranges a positive relation was found between phosphate and nitrogen, on the one hand, and sugar, but none between potassium and sugar. The percentages of potash in the gland-bearing portion of the peel of mature Valencia oranges from trees on potash-fertilized soil were higher than in fruit from trees on soil that received no potash.

The percentages of phosphate were greatest in the gland-bearing portion of mature Valencia orange peel. In this portion the percentages were higher for the styler than for the calyx end. Valencia oranges from trees grown in sand cultures deficient in potassium showed breakdown of the peel.

The total sugars in the calyx halves of lemon peel (silver stage) were 14.59 per cent of the dry matter, while those of the styler halves were 11.91 per cent. The calyx halves of grapefruit peel contained 28.77 per cent of total sugars in the dry matter while the styler halves contained 27.89 per cent. In mature calyx halves of Valencia orange peel the dry matter contained 26.95 per cent total sugars while the styler halves contained 23.17 per cent. In mature Valencia oranges the color reaction for starch appeared stronger in the styler than in the calyx peel.

The osmotic pressure of the juice of the peel of the calyx halves of lemon (silver stage) was 15.08 atmospheres and that of the juice of the peel of the styler halves 14.26 atmospheres. The corresponding values obtained for mature Valencia oranges were 20.00 and 24.58 atmospheres, respectively.

By measuring the rate of decolorization of KMnO_4 by materials that exosmose from fruit into water, small differences were found in the permeability of the halves of green lemons. The calyx halves of grapefruit and Valencia oranges had a greater permeability than the styler halves. These and other differences may be related to certain diseases or physiological breakdowns.

The effect of hydrocyanic acid treatment of Valencia oranges was not only to increase the permeability of both halves but also to increase that of the calyx half more than that of the styler half.

More water was lost through the peel of the calyx half than through that of the styler half of lemons and Valencia oranges. The effect of leaves on the loss of water from fruits is considerably greater with immature than with mature fruits. The effect of increasing maturity of the fruit in resisting water loss is due to the increase in osmotic pressure that results from the storage of osmotically active solutes such as sugars and organic acids. The possible roles of osmotic pressure in certain physiological diseases of citrus fruit are described. The osmotic pressure of the juice of the calyx and styler halves of pulp of mature Valencia oranges was able to regulate the water loss from the peel of detached oranges.

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