From there on profits increased at approximately 13 cents per box dumped for every 10 percent increase in pack-out.

The average pack-out for grapefruit was 65.5 percent for which the theoretical profit per box dumped is 34 cents as against a corresponding figure of 79 cents at 100 percent pack-out, a difference of 45 cents. Green color accounted for 16 percent of grapefruit eliminated here; 16 percent of 45 cents is 7.2 cents per box dumped, as penalty for green color on these grapefruit. This figure is typical, not absolute, and would, of course, vary with day to day prices, the pack-out on each crop, etc. It is, however, quite representative, even though 1958-59 was a "good color year".

Summary

The relative importance in 1958-59 of various fruit blemishes contributing to low pack-outs of fresh citrus is presented as part of a continuing study. Particular attention was paid to the role of fruit color as a grade lowering factor. The "a" scale on the Hunter Color and Color Difference Meter provided a purely objective measurement of color of oranges, corresponding to grade specifications. Such measurements showed that the judgment of commercial fruit graders with respect to color standards tended to become more lenient with the development of the regreening season. No correlation was found between the Hunter "a" scale and color of grapefruit.

No correlation between net profit and pack-out was found for oranges due to an unusual price structure. Profits on grapefruit increased approximately 13 cents per box per 10 percent increase in pack-out. Fruit graded out for green color reduced potential profits by over seven cents per box of grapefruit.

Acknowledgments

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REFERENCES CITED

1. Baier, W. E. How the citrus industry measures it: VII Color. Calif. Citrog. **31**: 6, 11, 1945.
2. Caprio, J. M. Regreening of Valencia oranges: temperature relationships. Calif. Citrog. **40**: 287. 1955.
3. .... An analysis of the relation between regreening of Valencia oranges and mean monthly temperatures in Southern California. Proc. Am. Soc. Hort. Sci. **67**: 222-235, 1956.
4. Francis, F. J. A method of measuring the skin color of apples. Proc. Am. Soc. Hort. Sci. **60**: 213-220. 1952.
5. Grierson, W. The effect of pack-out on grower profits. Proc. Fla. State Hort. Soc. **70**: 21-28, 1957.
6. .... Causes of low pack-outs in Florida packinghouses. Proc. Fla. State Hort. Soc. **71**: 166-170, 1958.
7. .... and W. F. Newhall. Degreening conditions for Florida citrus. Proc. Fla. State Hort. Soc. **66**: 42-46, 1953.
8. .... and .... Degreening of Florida Citrus Fruits. Fla. Agr. Expt. Stn. Bull. 620 (in press).
9. Huggart, R. L., and F. W. Wenzel. Color differences of citrus juices and concentrates using the Hunter Color and Color Difference Meter. Food Technol. **9**: 27-29, 1957.
10. Stearns, C. R. Jr. and G. T. Young. The relation of climatic conditions to color development in citrus fruit. Proc. Fla. State Hort. Soc. **55**: 59-61, 1942.
11. Ting, S. V., John W. Sites and E. J. Deszyck. Measuring the internal color of Florida red and pink grapefruit with the Hunter Color and Color Difference Meter. Proc. Amer. Soc. Hort. Sci. **71**: 265-270, 1958.
12. .... and E. J. Deszyck. The internal color and carotenoid pigments of Florida red and pink grapefruit. Proc. Amer. Soc. Hort. Sci. **71**: 271-277, 1958.
13. . . . . . State of Fla. Citrus Fruit Laws. Chap. 601, Sections 16, 19, 21, 231 and 26. Revised 1957.
14. . . . . . Regulations pursuant to Chap. 601 (Citrus Code) Reg. 1, Section 1. (Fla. Citrus Commission, Lakeland, Fla.)
15. . . . . . U. S. Standards for Florida Grapefruit (17 F.R. 7408) 9/14/52. Production and Marketing Administration, U.S.D.A., Washington, D. C.
16. . . . . U. S. Standards for Florida Oranges and Tangelos (20 F.R. 7205) 10/14/55. Production and Marketing Administration, U.S.D.A., Washington, D. C.
17. . . . . Daily Market Bulletin, Fla. Citrus Mutual, Lakeland, Fla.

HESPERIDIN IN ORANGE JUICE AND PEEL EXTRACTS DETERMINED BY U.V. ABSORPTION

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Florida Citrus Experiment Station

Lake Alfred

Examination of the ultraviolet absorption of orange juice and peel extracts has revealed a more sensitive and specific technique for evaluating the hesperidin content of citrus products. The procedure is based on the ultraviolet absorbance of glucosides at 290-300 millimicrons (2) and is an extension of similar work by the authors on the naringin content of grapefruit juices (3, 4). It was anticipated that a more discriminatory analytical procedure would be of much interest and value to the citrus industry. Even though hesperidin does not have a bitter flavor that would materially affect the flavor of a juice

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as naringin can, there is a need for an analytical technique to follow and correlate changes brought about by such conditions as freeze damage, maturity, and the new pulp washing techniques. Likewise, analyses of peel extracts by this new procedure were expected to be more informative than techniques presently in use.

Procedure

A procedure for evaluating the hesperidin content of orange juice or peel extracts is as follows: to 50 ml. of the test solution is added 0.4 ml. of a 10 percent copper sulphate solution. The pH is adjusted to 3.8 ± 0.1 by the addition of one or two drops of concentrated hydrochloric acid or 4N sodium hydroxide and the sample is aerated for one hour. Aeration is performed with a sintered glass sparging tube connected to compressed air and immersed in the solution. The juice or extract is then diluted 20:1, with 99 percent isopropanol, by pipetting 0.5 ml. of the sample into a 10 ml. volumetric flask and diluting to the mark. The alcohol insoluble solids are precipitated and slowly coagulate. After a short time, a sparkling clear sample is obtained by filtering off the insolubles using vacuum, a Whatman No. 42 paper disc in a perforated gooch and a small amount of filter-aid such as filter-cel.

The clear diluted solution is evaluated for hesperidin content by placing a portion of the solution in a 10 mm. silica cell, which is placed in a Beckman DU quartz spectrophotometer with a hydrogen lamp. The instrument is adjusted to have a 0.15 mm. slit and sensitivity varied to give 100 percent transmission at 360 millimicrons (mu.) for 99 percent isopropanol. Absorbance readings are taken at wavelengths of 300 and 290 mu. and the quantity of hesperidin interpolated from the difference between the two readings. The approximate percentage of hesperidin is equivalent to this difference divided by ten, but is more accurately determined from a standard curve.

Experimental Results and Discussion

The similarity of the absorption peaks for hesperidin and naringin at 286 and 284 mu. (2) respectively, made it appear that hesperidin could be quantitatively determined by ultraviolet absorption as was naringin (4). The absorbance curves for numerous samples of orange juice were plotted using the U.V. absorption technique developed for naringin in grapefruit juice. The typical result was a curve similar to curve four of Figure 1. Orange juice was noted under these conditions to have a more pronounced secondary peak at 330 mu. than grapefruit juice, while the main absorption peak of hesperidin was found only as a shoulder of a much larger absorption peak occurring at a wavelength below 260 mu.

Effect of pH.—It has been shown previously in similar work with grapefruit juice that pH had a profound effect. Figure 1 shows a similar influence of pH on the absorbance curve of an orange juice sample. Absorbance was greatest at the lower pH values, but the sharpness of the main and secondary peak was reduced. Above pH 4.0, absorbance increased over the entire plotted range with the same disadvantage noted for the lower pH readings. A pH of 3.8 was finally chosen as a good compromise between overall absorption level and sharpness of the important hesperidin peak.

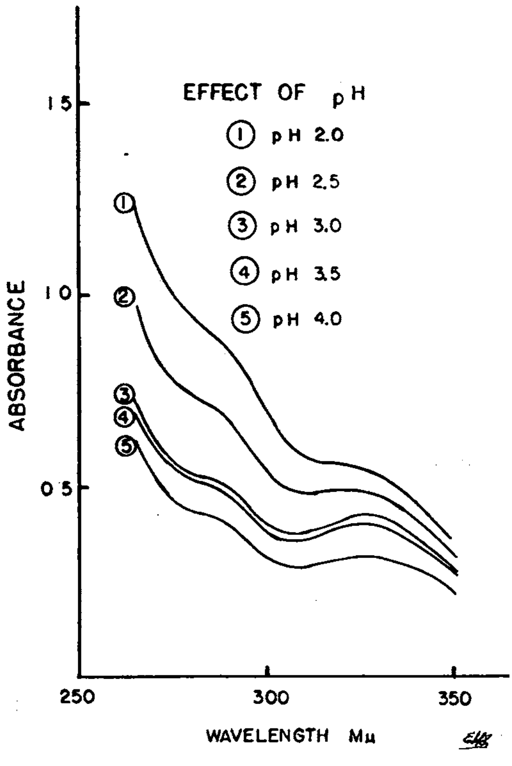

Fig. 1.—Effect of pH on ultraviolet absorbance of orange juice.

Effect of copper and aeration. — Since the hesperidin absorbance peak at 286 mu was in many cases only an indistinct shoulder when orange juice was analyzed by the earlier developed technique, attempts were made to modify the procedure. A previous plot of numerous juice constituents of grapefruit juice (4) showed the addition of ascorbic acid contributed a pronounced absorbance below 270 mu. with its peak value occurring at 245 mu. After investigating various methods of oxidizing the ascorbic acid normally present in orange juice, it was found that copper and aeration had the desired end effect of making the hesperidin peak more discernible.

The effects of copper and aeration are readily seen in Figure 2. Small additions of copper as copper sulphate gave a more distinct shoulder for the hesperidin peak of an orange juice sample while changing the overall absorption level very little. Subsequent aeration changed the hesperidin shoulder to a peak although this did not invariably happen. Aeration by itself was found to decrease continually the overall ultraviolet absorption for the first six hours and then suddenly to increase it greatly. At the longer periods of aeration, the hesperidin peak became more pronounced, but still remained as a shoulder rather than a true peak. After investigating various levels of copper sulphate, no improvement was apparent above 0.08 percent copper sulphate in orange juice, which is the equivalent of 0.4 ml. of a 10 percent copper sulphate solution being added to 50 ml. of single strength orange juice. When copper was added at this level, the subsequent aeration of the juice decreased overall absorption during the first six hours with no particular advantage being found beyond one hour.

Other attempts to improve the absorption peak of hesperidin in orange juice by using ion exchange resins, such as IRA-4B, IRA-400, and IRC-50, to remove preferentially interfering material were unsuccessful. Also tried with discouraging result were numerous chemical additives that had a chance of precipitating or coprecipitating unwanted U.V. absorbing impurities, among which were tannic acid, mercuric chloride, meta phosphoric acid, phospho-tungstic acid, and phospho-molybdic acid. An encouraging possibility of using iodine to oxidize ascorbic acid in orange juice was finally abandoned because too delicate a balance was needed, with excess iodine having an absorption peak of its own at 290 mu.

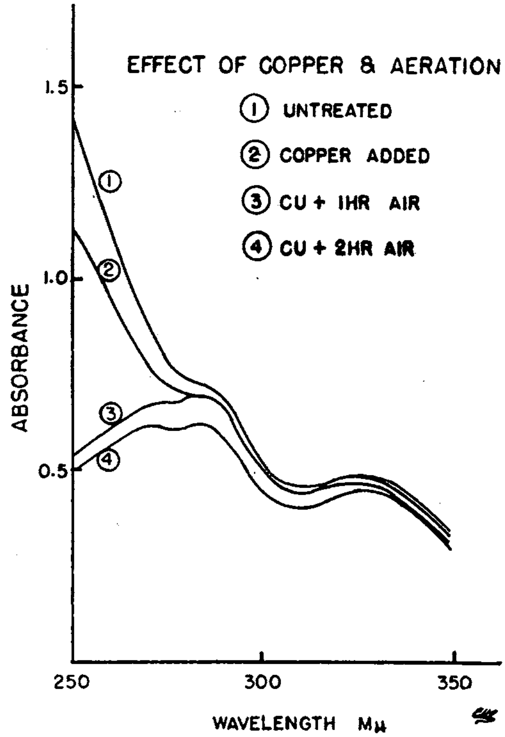

Fig. 2.—Effect of copper and aeration upon the ultraviolet absorption of an orange juice.

Effect and Measurement of Hesperidin Concentration.—In previous work (4) on the measurement of naringin content of grapefruit juice by ultraviolet absorption, it was found that naringin content could be correlated to the rate of absorbance change on the slope of the naringin peak. Hesperidin content can be measured in a similar manner. Lacking an absolute standard for test purposes, the accuracy of the following method rests on the premise that if equal absorbance readings are obtained at 290 and 300 mu. there is neither a hesperidin peak, nor in essence any hesperidin present.

A standard curve for the measurement of hesperidin content of orange juice was prepared by analyzing a diluted commercial orange concentrate to which varying amounts of hesperidin were added or photometrically subtracted. Results are presented in Figure 3, which shows the absorbance changes caused by adding 0.01, 0.02, and 0.03 percent hesperidin to the diluted orange concentrate sample that has previously been adjusted for pH and aerated in the presence of copper. In a similar manner 0.02, 0.03, and 0.04 percent hesperidin have been subtracted photometrically by adding the equivalent quantity of hesperidin to the blank. It will be noted that the subtraction of 0.03 percent hesperidin gave equal absorption at 290 and 300 mu. and would therefore by coincidence be equivalent to its analysis. A standard curve can be plotted from similar results by first interpolating to obtain the juice's hesperidin content and then plotting the 290 minus 300 mu. absorbance readings against hesperidin level. By Davis test (1) this same juice was found to have 0.089 percent hesperidin. Thus, here as well as in other analyses, it will be seen that Davis test results tend to be much larger than actually shown by ultraviolet absorption. This can be rationalized by remembering that the Davis test is a non-specific test that was originally designated for naringin, a compound that is almost three times more sensitive to the Davis test. Ascorbic acid was mentioned (1) as being a compound that would cause a five percent error when analyzing grapefruit juice. It would therefore follow that it is a much larger source of error when analyzing for the less sensitive hesperidin in orange juice.

Analyses of Orange Juices.—A comparison of the absorption curves of orange juices from different sources is shown in Figure 4. The two commercial concentrate absorption curves were plotted from analyses of 1958-59 production and are equivalent to the limits between which 22 samples of current production fell. There was a notable tendency for the early season samples to have a more pronounced secondary peak at 330 mu., as shown by curve four. The Citrus Experiment Station pilot plant sample presented has a lower overall absorption level than was usually found, but the sample greatly resembled hand reamed orange juices. Curve two was representative of the absorption curve obtained for ten commercial pulp washing extracts. The partic-

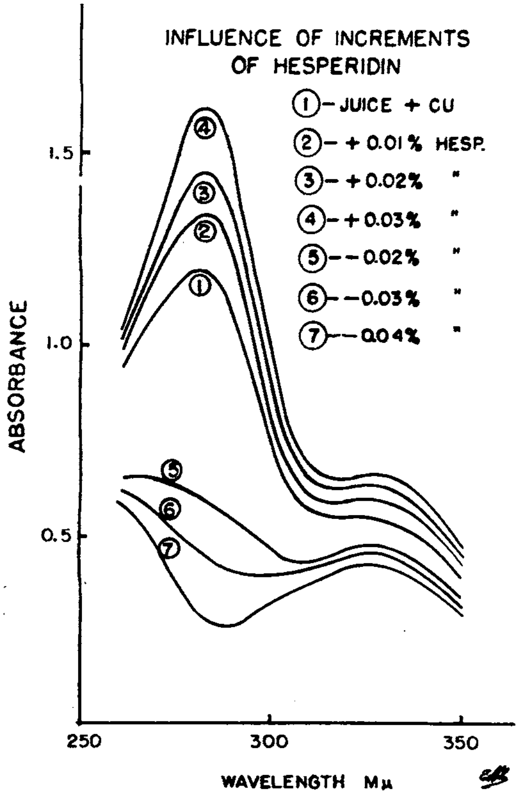

Fig. 3.—Influence of small increments of hesperidin on the ultraviolet absorption of orange juice.

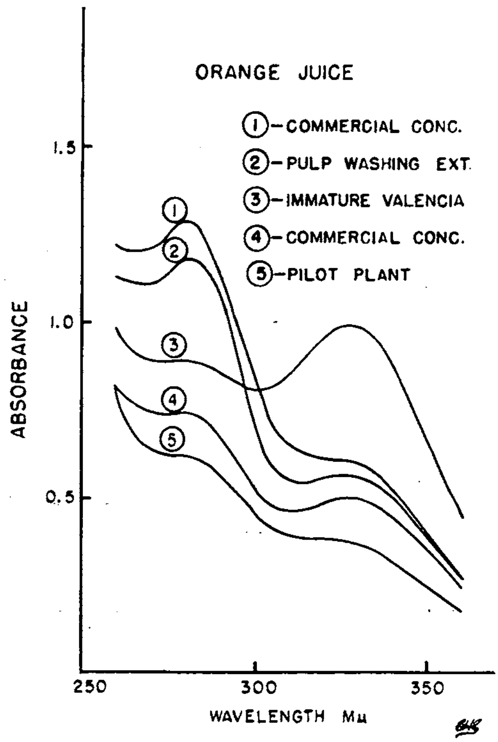

Fig. 4.—Ultraviolet absorption curves of orange juice from different sources.

ular sample shown had a Brix of approximately eight degrees and would therefore have, at the usual Brix of single strength orange juice, a much higher overall absorption and an even more pronounced hesperidin peak. The addition of pulp washing extracts to an orange juice therefore can be expected to increase the hesperidin peak as well as the overall absorbance level and would explain the wide absorbance differences among commercial samples. The juice from very immature Valencia oranges was considerably different in appearance. The greatly pronounced 330 mu. peak, however, was largely reduced by peeling the immature fruit prior to extracting the fruit.

Quantitative analyses for hesperidin in a variety of orange juice samples by this ultraviolet method have given consistently lower results than the Davis test. A comparison of analytical results on Citrus Experiment Station pilot plant samples, commercial concentrates, and commercial pulp washing extracts revealed that this ultraviolet technique could only find an average of 26, 27, and 24 percent, respectively, of the hesperidin found by Davis test. As might be expected, in some instances there was better agreement between these methods and it was usually coincidental with high hesperidin content as determined by U.V. technique. The overall range of agreement between the two methods varied from a low of 18 percent to a high of 53 percent. A very large part of this difference can be accounted for by the earlier mentioned non-specific nature of the Davis test. The lower U.V. results were further rationalized by the fact that hesperidin, which is very insoluble in water (0.002 percent at room temperature (5)), should not be found in higher concentration in orange juice than the more soluble naringin in grapefruit juice, all of which would tend to further substantiate the lower ultraviolet analysis to be more of the proper magnitude.

Analysis of Orange Peel Extracts.—The absorption curves for a number of extracts from orange peel are shown in Figure 5. The curve for orange peel vinegar represents a greatly diluted sample and was surprisingly similar to the absorption curve of the original orange peel press juice from which the vinegar was made. The orange juice vinegar absorption curve, which is shown for comparison, was noted to have a lower overall absorptin than the orange peel vinegar even though the latter was diluted five times more than the former. The absorption curve for an alkaline extract of orange peel (Fig. 5) represents an abstract that has been made for the purpose

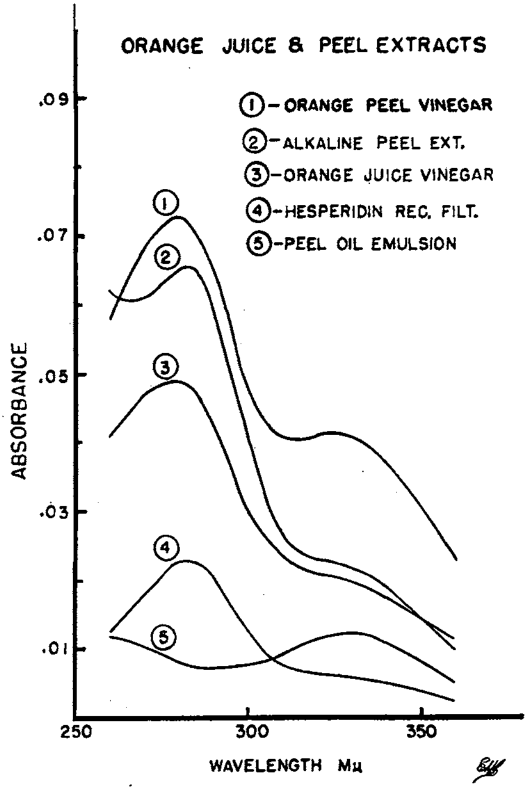

Fig. 5.—Ultraviolet absorption curves for various products from orange peel and juice.

of recovering hesperidin. Curve four shows the absorbance to be considerably less when the maximum quantity of hesperidin has been precipitated and filtered from this type extract. A peel oil emulsion was obtained from the surface of immature Valencia fruit with a spoon. The absorption curve for this emulsion, at a concentration of 0.025 percent shows the absence of hesperidin and the presence of a constituent responsible for the absorption at 330 mu. A similar effect in peel extracts and juices may be due to this substance.

By virtue of the higher levels of hesperidin in the peel extracts there was closer agreement between the analytical results by Davis test and the ultraviolet technique, with the latter method always showing less hesperidin to be present.

Summary and Conclusions

A new ultraviolet method for determining the hesperidin content of orange juice and peel extracts has been described to show the conditions necessary for best results. Quantitative results by this new ultraviolet technique consistently found less hesperidin in orange juice and peel extracts than by Davis test. Sufficient absorption differences and similarities were found in juice samples to suggest the possible use of this method to more fully evaluate the orange juice.

LITERATURE CITED

1. Davis, W. B. Determination of flavanones in citrus fruits. Anal. Chem. 19: 476-8. 1947.
2. Hendrickson, R. and J. W. Kesterson. Chemical analysis of citrus bioflavanoids. Proc. Fla. State Hort. Soc. 70: 196-203. 1957.
3. ————, ———— and G. J. Edwards. Effect of processing variables on U.V. absorption of grapefruit juices. Proc. Fla. State Hort. Soc. 71: 190-94. 1958.
4. ————, ———— and G. J. Edwards. Ultraviolet absorption technique to determine the naringin content of grapefruit juice. Proc. Fla. State Hort. Soc. 71: 194-198.
5. Merck Index of Chemicals and Drugs. 6th Ed. 1167 pp. 1952. Merck & Co., Inc.

SOME RECENT WORK ON CITRUS SECTIONS

Gray Singleton

Salada Shirriff Horsey Inc.

Plant City

For many years citrus sections have been a staple commodity. The market is not large but it is steady. If the pack is limited to what can be sold at a fair price a good profit is realized.

But the demand for sections does not increase in proportion to the sales of some other citrus products. Why this condition exists we do not know.

It may be a matter of quality. Early in the season the sections have a harsh, bitter

TABLE I

PACKED 4-20-53 CODE	TREATMENT	TASTE PANELS 5-5-53	9-21-53	7-15-55
42053-1	Dry pack, no juice, sugar or syrup	Stale	Stale	
42053-2	Juice pack 2 ounces by weight	Stale	Stale	
42053-3	42.8 Brix sucrose syrup 2 oz. by weight + 1/200 of 1% Ethyl Caffeate	Fresh	Fresh	Fresh
42053-4	Same as 3 except 1/100 of 1% Ethyl Caffeate	Fresh	Fresh	Fresh

After 2 years and 3 months, sections treated with Ethyl Caffeate looked and tasted like fresh fruit, others went stale quickly.

The use of Ethyl Caffeate is well known. Used here as control.

TABLE II
VACUUM PACK, FROZEN

PACKED 5-4-53 CODE	TREATMENT	TASTE PANEL 9-21-53
50453-1	Orange sections covered with orange juice	Stale
50453-2	Grapefruit sections covered with grapefruit juice	Stale
50453-3	Salad sections covered with blend juice	Stale
50453-4	Grapefruit sections covered with grapefruit juice exhaust before vacuum closure.	Stale