average bitterness ratings suggesting a strong relationship between bitterness and acid (sourness). Four of the six low Brix/acid ratio plants also had below average flavor and above average bitterness. One of the two remaining low ratio plants (plant K) had exactly average flavor and bitterness, but the last low ratio plant, plant E, had the highest flavor score. Therefore B/A ratio is often but not always associated with below average flavor scores.

High quality grapefruit juice is usually associated with low bitterness. As average flavor scores increase during a season both bitterness and limonin decrease whereas naringin increases. Therefore naringin concentration is of questionable use as a bitterness indicator. Juices from below average flavor score plants were characterized by high limonin, high acid and low Brix/acid ratio.

Literature Cited

1. Attaway, J. A. Factors influencing the flavor of grapefruit juice. *Proc. Int. Soc. Citriculture* 3:816-820.
2. Carter, R. D., B. S. Buslig and J. A. Cornell. 1975. Statistical inferences on processed orange juice quality from the 1972-73 and 1973-74 juice definition program. *Proc. Fla. State Hort. Soc.* 88: 358-370.
3. Davis, W. B. 1947. Determination of flavonones in citrus fruits. *Anal. Chem.* 19:476-478.
4. Dougherty, M. H. and J. F. Fisher. 1977. Quality of commercial, canned, single-strength grapefruit juice produced in Florida during the 1975-76 and the 1976-77 citrus seasons. *Proc. Fla. State Hort. Soc.* 90:168-170.
5. Rouseff, R. L. and J. F. Fisher. 1980. Determination of limonin and related limonoids in citrus juices by high performance liquid chromatography. *Anal. Chem.* 52:1228-1233.
6. USDA Standards for Grades of Grapefruit Juice. 1968.

Proc. Fla. State Hort. Soc. 93:289-293. 1980.

AN ANALYSIS OF LIMONIN, BRIX AND ACID CONTENT IN GRAPEFRUIT SAMPLES COLLECTED FROM THREE STATE TEST HOUSES[1]

RICHARD L. MANSELL AND CECELIA A. MCINTOSH
Department of Biology,
University of South Florida,
Tampa, Florida 33620

Additional index words. RIA, Duncan grapefruit, Marsh grapefruit, Pink grapefruit, juice processing.

Abstract. **Grapefruit juice samples from three State Test Houses were analyzed for limonin, Brix, acid, and pounds juice per box. The results showed that there was no relation between any of these parameters except for acid and Brix, and acid and Brix-acid ratio. In addition, statistically significant differences were observed between the various cultivars sampled and between each of the State Test Houses.**

Limonin is the intensely bitter triterpenoid dilactone which is present throughout the Rutaceae but is of major importance only in certain citrus fruits, grapefruit and navel orange, as well as their processed products. For most of the other qualitative characteristics important in citrus, e.g. Brix, acid and solids, standardized, accurate and simple assay methods have been developed. For limonin, however, such a test has not existed until recently (1, 2) and therefore the relationship between limonin content and cultivar, Brix, acid or Brix-acid ratio could not readily be determined.

With the development of a radioimmunoassay (RIA) for limonin, it became possible to do studies which heretofore were not possible (3), and new horizons in citrus quality research have become apparent. In this report we will present the first in a series of studies which were designed to aid in our understanding of the relationship between limonin content in the various grapefruit cultivars being brought into the processing plants and to determine whether the content of this bitter principle was correlated with any of the other qualitative parameters which were being routinely assayed.

Materials and Methods

For this study samples of grapefruit juice were obtained from mid-December, 1979 through early March 1980 from the State Test Houses located at three different processing plants in west-central Florida. The juice was collected from the same batches which were used for Brix and acid determination, and the samples were collected by State Test House personnel. Each sample was stored in a 1.5 ml plastic vial which contained sodium azide to retard microbial growth. Each vial was marked according to the load number (representing a random sample from approximately 500 boxes of fruit) and date. The samples were picked up from the Test Houses weekly and assayed by the tritium-RIA method (2). The juice was centrifuged at 2,500 x g for 15 minutes to sediment particulate matter, and the supernatant was diluted 100 fold with distilled water. 0.1 ml aliquots were assayed without further purification. Each sample was assayed in duplicate, and an average value was calculated on a parts per million (ppm) basis. Corresponding data of pounds juice per box, percent acid, degree Brix and Brix-acid ratio for each load was provided from the Test House records at the Florida Department of Agriculture office in Winter Haven.

Statistical analyses were done on a IBM-370 mainframe computer located at the University of South Florida and statistical programs were taken from the compatible SAS pack (4) and the standard critical value numbers were obtained from (5).

Results and Discussion

For the initial analysis, the values for all the samples were combined, and the mean, standard deviation and range was determined for each of the qualitative parameters (Table 1). An average limonin concentration of 6.76 ppm was found for the 1,058 samples analyzed, and the range of concentration was from 1.35 to 13.8 ppm. The same analytical results for pounds juice per box, percent acid, degree Brix, and Brix-acid ratio are also presented. A Pearson

[1]This research was supported from a Florida Department of Citrus grant to RLM. The authors gratefully acknowledge the cooperation and support of the Test House employees and supervisors for the sample collections and to Mr. Marshall Dougherty for his help in coordinating the collections and providing the data for the correlation studies.

Table 1. Mean values and correlation coefficients for all grapefruit juice samples.

	All Juice Samples n = 1058			
	Mean	std dev	Min	Max
PPM limonin	6.76	1.954	1.35	13.80
lbs juice/box	40.21	2.560	23.71	48.30
Acid %	1.24	0.167	0.71	1.77
Brix°	10.14	0.918	8.12	13.25
B/A ratio	8.25	0.900	6.10	13.60

	Correlation Coefficients					
	PPM	Date	lbs juice	Acid	Brix	B/A
PPM limonin	1.000	0.028	0.266	−0.255	−0.220	0.121
Date processed		1.000	−0.032	0.264	0.270	−0.103
lbs juice/box			1.000	−0.418	−0.187	0.374
Acid %				1.000	0.643	−0.715
Brix°					1.000	0.061
B/R ratio						1.000

correlation matrix was also calculated for the collective juice samples, but only the percent acid and degree Brix, and the percent acid and Brix-acid ratio showed any strong correlative relationship.

For the second test, the values of limonin concentration for all the samples collected from a single Test House were plotted as a function of time, and the results are presented in figures 1-3. The resulting scatter diagrams reveal that the grapefruit coming into each Test House was extremely variable during the 110 days of sampling. Test House 1 showed a tendency toward a declining limonin concentration over time, but this same tendency was not observed for the other two Test Houses. Test House 2 exhibited the least amount of scatter, whereas the greatest degree was found in Test House 3. Thus, it is apparent that the distribution pattern for each Test House is distinct from the other two.

The data from each Test House was also analyzed separately, and the results are presented in Tables 2-4. Test House 1 had the highest mean ppm for limonin and the lowest was found in Test House 2. Differences were also observed for the other qualitative parameters. A Pearson correlation coefficient analysis showed that limonin was not related to any of the other parameters, and the only rela-

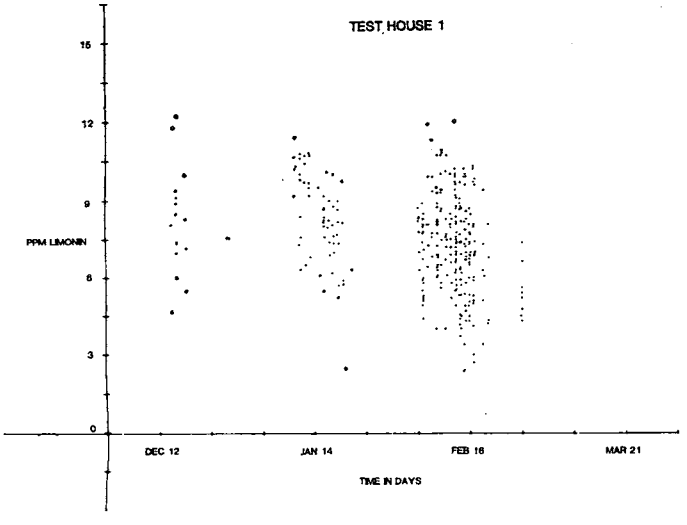

Fig. 1. Scatter plot of individual grapefruit juice samples from Test House 1.

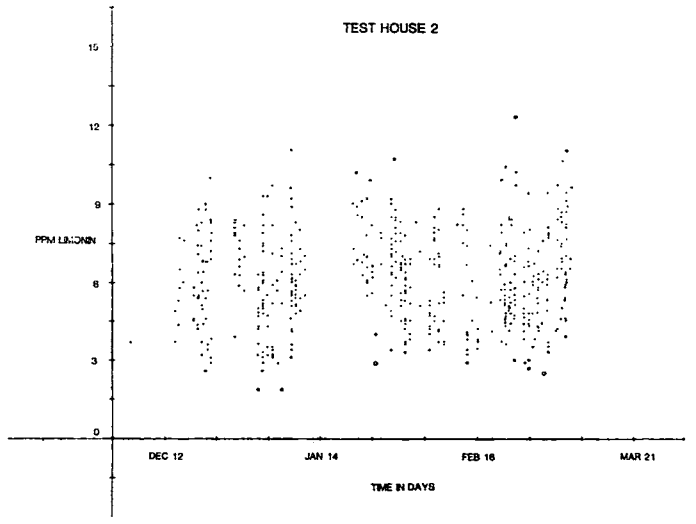

Fig. 2. Scatter plot of individual grapefruit juice samples from Test House 2.

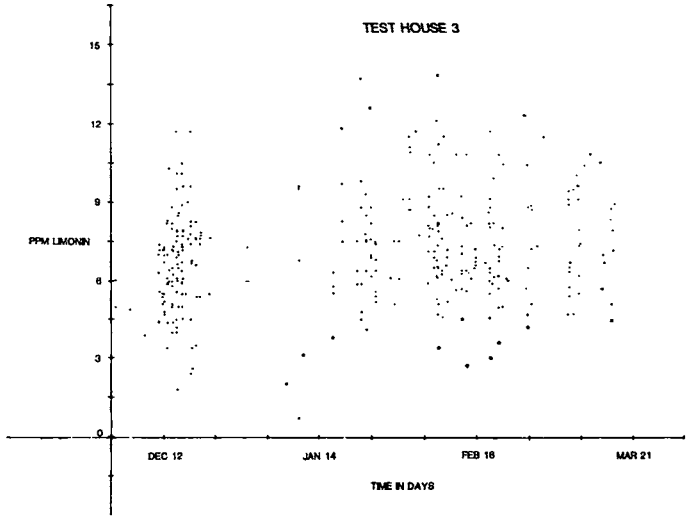

Fig. 3. Scatter plot of individual grapefruit juice samples from Test House 3.

Table 2. Mean values and correlation coefficients for grapefruit juice samples from Test House 1.

	Test House 1 n = 344			
	Mean	std dev	Min	Max
PPM limonin	7.52	1.857	2.38	12.30
lbs juice/box	40.98	2.435	32.70	47.63
Acid %	1.22	0.155	0.71	1.71
Brix°	9.88	0.949	8.12	13.25
B/A ratio	8.13	0.773	6.50	13.60

	Correlation Coefficients					
	PPM	Date	lbs juice	Acid	Brix	B/A
PPM limonin	1.000	−0.289	0.091	−0.238	−0.306	−0.001
Date processed		1.000	0.063	0.233	0.154	−0.177
lbs juice/box			1.000	−0.262	0.009	0.378
Acid %				1.000	0.714	−0.634
Brix°					1.000	0.071
B/A ratio						1.000

Table 3. Mean values and correlation coefficients for grapefruit juice samples from Test House 2.

	Test House 2 n = 488			
	Mean	std dev	Min	Max
PPM limonin	6.09	1.782	1.35	12.30
lbs juice/box	39.25	2.300	29.66	44.71
Acid %	1.29	0.164	0.85	1.77
Brix°	10.19	0.917	8.21	12.78
B/A ratio	7.89	0.681	6.10	10.20

Correlation Coefficients

	PPM	Date	lbs Juice	Acid	Brix	B/A
PPM limonin	1.000	0.042	0.203	−0.261	−0.235	0.125
Date processed		1.000	−0.007	0.235	0.358	0.028
lbs juice/box			1.000	−0.386	−0.338	0.229
Acid %				1.000	0.765	−0.703
Brix°					1.000	−0.093
B/A ratio						1.000

Table 4. Mean values and correlation coefficients for grapefruit juice samples from Test House 3.

	Test House 3 n = 272			
	Mean	std dev	Min	Max
PPM limonin	7.05	1.959	1.78	13.80
lbs juice/box	41.09	2.538	23.71	48.30
Acid %	1.15	0.142	0.83	1.74
Brix°	10.33	0.822	8.60	12.86
B/A ratio	9.02	0.914	6.80	12.90

Correlation Coefficients

	PPM	Date	lbs juice	Acid	Brix	B/A
PPM limonin	1.000	0.161	0.240	−0.013	−0.016	0.011
Date processed		1.000	−0.080	0.288	0.405	0.010
lbs juice/box			1.000	−0.372	−0.149	0.321
Acid %				1.000	0.579	−0.720
Brix°					1.000	0.135
B/A ratio						1.000

tions observed were those between percent acid and both Brix and Brix-acid ratio.

In order to determine whether the observed differences between the individual Test Houses were the result of the type of fruit being processed, each of the three cultivars was analyzed separately. The results are presented in Tables 5-7. This study showed that Duncan had the lowest average concentration of limonin (6.03 ppm), whereas the pink seedless had the highest (7.47 ppm), and the Marsh seedless was intermediate. Duncan fruit also yielded the lowest pounds juice per box, and the pink seedless produced the highest. Both Duncan and Marsh seedless showed a correlation between percent acid and Brix and between percent acid and Brix-acid ratio. Pink seedless, however, showed a correlation between only the latter two parameters.

In order to ascertain whether the differences existed among the individual Test Houses as well as among the three cultivars, the data was analyzed according to both the Test Houses and the individual cultivars. The results of this analysis are presented in Table 8. The values show that the ppm limonin and pounds juice for Duncan grapefruit especially, vary from one Test House to another. However, a similar variation pattern appeared in the other two

Table 5. Mean values and correlation coefficients of Duncan grapefruit juice samples.

	Duncan Seedy n = 394			
	Mean	std dev	Min	Max
PPM limonin	6.03	1.858	1.35	12.10
lbs juice/box	38.93	2.288	32.07	44.64
Acid %	1.34	0.149	0.71	1.77
Brix°	10.46	0.859	8.2	13.25
B/A ratio	7.83	0.711	6.1	13.6

Correlation Coefficients

	PPM	Date	lbs juice	Acid	Brix	B/A
PPM	1.000	0.067	0.284	−0.258	−0.152	0.187
Date processed		1.000	0.041	0.231	0.372	0.043
lbs juice/box			1.000	−0.275	−0.145	0.233
Acid %				1.000	0.682	−0.674
Brix°					1.000	0.054
B/A ratio						1.000

Table 6. Mean values and correlation coefficients of Marsh grapefruit juice samples.

	Marsh Seedless n = 537			
	Mean	std dev	Min	Max
PPM limonin	7.15	1.929	1.78	13.80
lbs juice/box	40.66	2.222	29.66	46.81
Acid %	1.18	0.149	0.85	1.68
Brix°	9.92	0.925	8.12	13.08
B/A ratio	8.41	0.853	6.50	11.30

Correlation Coefficients

	PPM	Date	lbs juice	Acid	Brix	B/A
PPM	1.000	−0.013	0.070	−0.082	−0.173	−0.067
Date processed		1.000	−0.094	0.374	0.206	−0.270
lbs juice/box			1.000	−0.313	−0.069	0.329
Acid %				1.000	0.623	−0.648
Brix°					1.000	0.185
B/A ratio						1.000

Table 7. Mean values and correlation coefficients of pink grapefruit juice samples.

	Pink Seedless n = 127			
	Mean	std dev	Min	Max
PPM limonin	7.47	1.896	2.41	12.00
lbs juice/box	42.27	2.712	23.71	48.30
Acid %	1.14	0.125	0.83	1.57
Brix°	10.09	0.772	8.51	11.81
B/A ratio	8.89	1.020	6.90	12.90

Correlation Coefficients

	PPM	Date	lbs juice	Acid	Brix	B/A
PPM	1.000	0.028	0.227	0.013	−0.108	−0.090
Date processed		1.000	0.052	0.107	0.407	0.213
lbs juice/box			1.000	−0.162	−0.187	0.025
Acid %				1.000	0.312	−0.743
Brix°					1.000	0.386
B/A ratio						1.000

Table 8. Mean values and ppm limonin correlation coefficients of grapefruit juice samples partitioned according to cultivar and Test House.

	Test House											
	1				2				3			
Mean	Duncan	Marsh	Pink	Overall	Duncan	Marsh	Pink	Overall	Duncan	Marsh	Pink	Overall
PPM	7.11	7.94	7.60	7.52	5.42	6.81	6.66	6.09	6.73	7.03	7.39	7.05
lbs juice	40.48	40.60	42.95	40.98	38.22	41.15	39.25	39.25	38.92	41.36	41.79	41.09
Acid %	1.27	1.19	1.17	1.22	1.38	1.21	1.29	1.29	1.30	1.12	1.12	1.15
Brix°	10.20	9.56	10.02	9.88	10.51	9.17	10.19	10.19	10.84	10.25	10.18	10.33
B/A	8.04	8.01	8.62	8.13	7.63	7.50	7.89	7.89	8.40	9.13	9.14	9.02
n =	110	134	54	308	241	245	2[z]	488	43	158	71	272
	Correlation Coefficients[y]											
PPM	1.00	1.00	1.00		1.00	1.00	—		1.00	1.00	1.00	
lbs juice	−0.171	−0.038	0.265		0.109	−0.013	—		0.169	0.266	0.203	
Acid %	−0.237	−0.254	0.010		−0.180	−0.025	—		0.223	−0.030	0.005	
Brix°	−0.222	−0.315	−0.241		−0.140	−0.107	—		0.194	−0.024	−0.042	
B/A	0.087	0.003	−0.163		0.084	0.111	—		−0.097	0.027	−0.050	

[z]n = 2 not large enough to run a correlation matrix.
[y]correlation with ppm limonin.

cultivars. The correlation coefficients of this analysis showed no relationship between any of the parameters measured.

Since it had been established that there were apparent differences between each of the Test Houses and also between the cultivars, additional statistical analyses (RCB ANOVAS) were done to determine whether the observed differences were significant. The results were determined to a 95% probability level and the resulting values and conclusions are presented in Tables 9 and 10. In Table 9 the hypothesis that there were no differences between cultivars was tested, with differences due to Test House partitioned out of the error term of the ANOVA, and in all cases (for each parameter tested) this hypothesis was found to be invalid. The same analysis was also done (Table 10) testing the hypothesis that all the Test Houses were equal, with differences due to cultivar partitioned out of the error term of the ANOVA, and the test results showed that this hypothesis is also incorrect.

The SNK conclusions, which test for the location of statistically significant differences, are that each of the cultivars are different from each other in pounds juice per box,

Table 9. Summary of RCB ANOVA's and SNK's test results for cultivars.

Summary of RCB ANOVA and SNK[z]

H_o: Duncan Seedy = Marsh Seedless = Pink Seedless
H_1: Inequality somewhere

Parameter tested	RCB ANOVA results[y]	SNK conclusions[x]
ppm limonin	accept H_1	Duncan ≠ Marsh = Pink
lbs juice/box	accept H_1	Duncan ≠ Marsh ≠ Pink
% Acid	accept H_1	Duncan ≠ Marsh = Pink
°Brix	accept H_1	Duncan ≠ Marsh ≠ Pink
B/A ratio	accept H_1	Duncan ≠ Marsh ≠ Pink

[z]Student-Neuman-Keuls test for the location of statistically significant differences.
[y]Differences due to test houses are partitioned out of the error term in order to test only the differences due to fruit variety. In all cases, the randomized complete block analysis of variance (RCB ANOVA) showed that this was the correct experimental design. CI = 95%, n = 1058.
[x]Where appropriate, varieties are listed in ascending order of mean parameter values.

Table 10. Summary of RCB ANOVA's and SNK's test results for Test Houses.

Summary of RCB ANOVA and SNK[z]

H_n: Test House 1=2=3
H_1: Inequality somewhere

Parameter tested	RCB ANOVA results[y]	SNK conclusions[x]
ppm limonin	accept H_1	Test House 2 ≠ 3 ≠ 1
lbs juice/box	accept H_1	Test House 2 ≠ 1 = 3
% Acid	accept H_1	Test House 3 ≠ 1 ≠ 2
°Brix	accept H_1	Test House 2 ≠ 1 ≠ 3
B/A ratio	accept H_1	Test House 2 ≠ 1 ≠ 3

[z]Student-Neuman-Keuls test for the location of statistically significant differences.
[y]Differences due to the different varieties of fruits are partitioned out of the error term in order to test only the differences due to test house. In all cases, the randomized complete block analysis of variance (RCB ANOVA) showed that this was the correct experimental design. CI = 95%, n = 1058.
[x]Where appropriate, test houses are listed in ascending order of mean parameter values.

Brix, and Brix-acid ratio. For ppm limonin, and percent acid, the Marsh seedless and pink seedless are not significantly different from each other but are different from Duncan. The SNK conclusions for the individual Test Houses are that each is significantly different from the others with the exception of the pounds juice per box for Test Houses 1 and 3.

In this study we have analyzed completely randomized grapefruit samples for five different qualitative parameters. The results of the limonin analyses have shown that there is no correlation between the concentration of this bitter principle and any of the other parameters. In addition, we have found that each of the cultivars is quite variable and different from the other two. Similar results were found with respect to the individual Test Houses.

In evaluating this data, however, it is important to remember that each of the juice samples originated from trees of unknown geographic locality, nutritional status, age and

rootstock. Since the fruit was harvested at specific times (when a Brix-acid ratio of near 8 was reached), this study does not reflect the natural maturation kinetics which would occur with a single tree or grove. In addition, this initial study was done for only 110 days of a single harvest season. Since it is generally agreed and accepted that grapefruit quality is variable from one year to the next, it will be important to continue this study for several seasons and also ultimately include the effects of the unknown parameters, listed above, into future analyses. Only then can a true measure of the inherent variation which occurs within the grapefruit population be presented.

Literature Cited

1. Mansell, Richard L. and Elmar W. Weiler. 1980. Radioimmunoassay for the Determination of Limonin in Citrus. *Phytochemistry* 19:1403-1407.
2. Weiler, Elmar W. and Richard L. Mansell. 1980. Radioimmunoassay of Limonin Using a Tritiated Tracer. *J. Agric. Food Chem.* 28(3):543-545.
3. Mansell, Richard L. and Elmar W. Weiler. 1980. Immunological Tests for the Evaluation of Citrus Quality. *Am. Chem. Soc. Symposium Series.*
4. *SAS User's Guide: 1979 edition.* SAS Institute, Inc., Raleigh, N.C. in conjunction with the SAS computer pack.
5. Zar, Jerrold H. 1974. *Biostatistical Analysis.* Prentice-Hall, Inc. Englewood Cliffs, N.J.

Proc. Fla. State Hort. Soc. 93:293-295. 1980.

COLOR AND PROCESSING CHARACTERISTICS OF 'STAR RUBY' GRAPEFRUIT[1]

S. V. Ting, R. L. Huggart and M. A. Ismail
Florida Department of Citrus,
Agricultural Research and Education Center,
P. O. Box 1088, Lake Alfred, FL 33850

Additional index words. Grapefruit juice color, processing.

Abstract. **'Star Ruby', a new cultivar of red grapefruit, was found to have excellent color both in the flesh and in the juice, even in late season. The color is sensitive to heat, but the juice, after pasteurization and concentration, retains sufficient color so that it may be used to enhance the color of pink grapefruit juice.**

The red color of the flesh of the pink and red grapefruit is known to fade with fruit maturity (4, 5). As a result, when the flavor quality of the fruit is at its best, the red color may have decreased to a very low level. The juice from such fruit becomes unacceptable as a pink grapefruit juice because of its low color, yet appears to be too reddish to meet white grapefruit juice color standards. Processing these juices with white grapefruit juice yields a muddy brown colored product which has poor appearance on account of its color.

There has been considerable demand for red colored grapefruit as fresh fruit and as processed products. A recent finding in new varieties is the 'Star Ruby' cultivar which has strikingly red colored flesh. This grapefruit was originated in Texas. It was grown from irradiated seeds of the 'Hudson', a seedy red grapefruit with relatively coarse texture (3). The highly colored 'Star Ruby' fruit is essentially seedless and has not been reported to revert to its parent seedy characteristics. The fruit has been well received in the fresh fruit trade and ranks second to the standard 'Ruby Red' grapefruit in number of cartons shipped out of Texas in 1978 (1). This paper describes the seasonal change of the flesh and juice color of the 'Star Ruby' and compares it with that of 'Ruby Red' grapefruit. The effect of heating, pasteurization, and concentration on the color of the juice of the 'Star Ruby' grapefruit and its potential use to increase the color of other grapefruit juices were also studied.

[1]Florida Agricultural Experiment Stations Journal Series No. 2737.
The authors wish to thank Dr. Mortimer Cohen and Mr. Robert Pelosi of the Agricultural Research Center, Fort Pierce, FL for providing the fruit used in this study.

Materials and Methods

Sampling. For determining the color change of the flesh and juice, 20 'Star Ruby' grapefruit were obtained at monthly intervals beginning November, 1978 and ending in June, 1979 from the Agricultural Research Center (ARC), University of Florida, at Fort Pierce. Six fruit taken from each sample were used for measuring flesh color, and the remainder for the study of juice color and other juice characteristics.

Fruit used to study the effect of heat pasteurization on the color of the juice were obtained from the Fort Pierce ARC in December, 1977. Two 90-lb. boxes of the fruit were washed and the juice extracted.

For the preparation of concentrate, a 10-box sample was obtained from the same source in January, 1979. These fruit were picked from 'Star Ruby' trees on several rootstocks from a rootstock experiment plot at the Fort Pierce ARC. These trees are about 6 years old and do not bear many fruit on each tree. The fruit was also brought to the Center at Lake Alfred and washed before extraction.

'Ruby Red' grapefruit for color comparison studies were picked at regular intervals from an experimental plot at the Center in Lake Alfred. The samples for flesh and juice color studies consisted of 20 fruit each.

Flesh color measurement. Fruit used for flesh color measurements were cut transversely into halves. The halved fruit was inverted on a glass plate over the aperture of a Hunter Color and Color Difference Meter (HCDM) model D25D. The Hunter "a" and "b" values were determined at two positions on each half of the fruit as described by Ting and Deszyck (5). The readings of all the stem halves and those of the stylar end halves were averaged separately, and the calculated Hunter "a"/"b" values were averaged for each monthly samples.

Juice extraction. The juice was extracted from fruit on an FMC In-line juice extractor, model 091B, with a 0.025 inch finishing tube using 16 lb. air pressure. The juice from the first 5 fruit of each sample was not used in the juice color measurement studies in order to minimize the color dilution from the residue in the finishing tube from previous samples. The °Brix, total acidity, and juice yield are determined in the usual manner (6).

The juice used in processing studies was extracted on an FMC In-line juice extractor model 591 with a 0.040 inch finishing tube. The juice was then passed through an FMC model 35 finisher with a 0.020 inch screen.