Each step in the procedure was tested for possible contribution to experimental error. A fourth chloroform extraction of each (6 samples) were combined and analyzed for Limonin. The Limonin value obtained was less than 0.5 ppm (or < 0.08 ppm per sample).

The quantitative acetonitrile dilution step was tested by adding known amounts of Limonin to chloroform, evaporating to dryness and adding 0.250 ml of acetonitrile. Recovery ranged between 98 - 103%.

A check of the combined (6 samples) of hexane showed no Limonin.

Reproducibility.—Seven replicate aliquots of a sample of commercial canned grapefruit juice were run through the procedure. The acetonitrile samples were injected in triplicate. Standard deviation on the twenty-one results was calculated.

Limonin content of one grapefruit sample in seven replicate analyses.

No.	ppm 3 Inj.	Ave. ppm	
1	2.0, 2.1, 2.3	2.1	
2	2.7, 2.8, 2.3	2.6	
3	2.0, 2.3, 2.3	2.2	Mean:- 2.30
4	2.8, 2.4, 2.8	2.6	Standard
5	2.0, 1.7, 2.0	1.9	Deviation:- 0.31
6	2.1, 2.4, 2.5	2.4	
7	2.1, 2.5, 2.1	2.2	

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NARINGIN ISOMERS AND LIMONIN IN CANNED FLORIDA GRAPEFRUIT JUICE

JAMES H. TATUM, JACK C. LASTINGER, JR., AND ROBERT E. BERRY

USDA Citrus and Subtropical Products Laboratory[1]
Winter Haven

Abstract. **Simplified thin-layer chromatographic (TLC) methods developed to estimate naringin, its isomer 7-β-rutinoside of naringenin and limonin allowed use of whole juice and simple measurements. These determinations, as well as Davis tests (glycosides or compounds that give a yellow color with base) were carried out on commercial canned grapefruit juice collected weekly throughout the season at two processing plants using different processes. Naringin ranged from about 218 to 340 ppm with a general trend to diminish toward midseason and increase again toward the end of the season. Limonin ranged from about 10 ppm to 2 ppm and generally lessened as the season progressed. Plant blending practices greatly influenced these values. Naringin values could be estimated by Davis value divided by 2.1. Bitterness was influenced as much by Brix/acid as by naringin and limonin levels.**

This study was undertaken to follow the fluctuations in naringin and limonin contents of com-

[1]One of the laboratories of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture. References to specific commercial products do not constitute endorsement.

mercially produced grapefruit juice throughout a season to establish seasonal ranges. Naringin and limonin both contribute to the bitterness of grapefruit juice (6, 7, 9). Bitterness frequently is cited as one of the principal deterrents to the marketing of canned grapefruit products. If fast and reliable methods could be developed for determining these compounds, standards governing their concentrations might be established. Such methods also would help in the blending of various juices to keep these compounds at acceptable levels.

Several methods have been developed and reported for determining the naringin content in grapefruit juice (3, 4) and several methods also have been developed for limonin in both orange and grapefruit juice (1, 10). All of these methods, however, are time consuming and complex. The purposes of the current study were two-fold: (1) to develop simplified procedures for determining naringin and limonin in grapefruit juice and (2) to measure their amounts in canned grapefruit juice throughout a season to indicate fluctuations and effects of commercial operations such as blending. These studies might make it easier to monitor citrus products on a routine basis and might provide information needed as a basis for establishing standards.

Materials and Methods

Samples. All juices used in this study were commercially produced, canned single-strength grapefruit juice. Some of the samples contained added sugar and some contained reconstituted grapefruit concentrate.

Naringin Determination. Baker-flex Polyamide 6 thin-layer sheets, 20 x 20 cm, (J. T. Baker Chemical Co., Phillipsburg, N.J.) were used for separation of naringin from its tasteless isomer by the method reported by Tatum and Berry (13). The whole untreated juice was spotted across the plate. The band was developed in solvent and dried. After development, the plates were sprayed with 1% $AlCl_3$ in ethanol. Naringin and its isomer, appearing as yellow fluorescent bands under UV light, were marked, sprayed with water, scraped and transferred to test tube. Davis reagent was added to develop a yellow color which was read using a colorimeter. Experimental samples were compared with standards and a blank. Details of the method have been previously reported (13).

Limonin Determination. Silica Gel G, 20 x 20 cm 250μ, (Analtech Inc., Newark, Delaware) plates were used for the separation of limonin using the method described by Tatum and Berry (14). These plates were scribed on 1-cm centers to form 20 individual bands. Whole untreated juice was spotted on 12 bands and a standard solution of limonin (containing 0.01 $\mu g/\mu l$ in acetonitrile) was spotted on five bands using 0.1 through 0.5 μg. Concentrations above 0.5 μg were difficult to differentiate. The plate then was developed in a solvent tank, dried, sprayed with 10% H_2SO_4 in ethanol and held at 125°C for 6 min. The plate was placed over a UV light and the unknown compared visually to the known standards. Details of the method were previously reported (14).

The Brix/acid ratios were determined at the Winter Haven U.S. Processed Foods Inspections Office on a weight-to-weight basis, following standard procedures.

In taste panels conducted by paired comparison, the 15-member experienced panel was asked to indicate which sample was more bitter. Each taster was presented 2 pairs of samples at each testing. Each sample of juice throughout the season was compared with the succeeding samples, i.e., the first sample collected was compared with the second sample then the third sample vs the fourth sample and so forth. Statistical evaluations were made according to the method of Krum (8). Taste panel results were evaluated and compared with analytical results to determine whether there appeared to be any relationship between limonin-naringin content and bitterness.

Results and Discussion

In samples from Plant A the naringin values (as shown in Table 1) were slightly higher at

Table 1. Naringin (1-2), 7-s-rutinoside of naringenin (1-6), total glycosides by Davis test and limonin contents of canned grapefruit juice from Plant A during the '71-72 season.

Date produced	Davis ppm	1-2 ppm	1-6 ppm	1-2 / 1-6	Davis / 1-2	Limonin ppm
11/27/71	668	307	107	2.9	2.2	10
12/6/71	674	311	102	3.0	2.2	10
12/13/71	674	296	91	3.2	2.3	10
12/20/71	529	254	92	2.8	2.1	6
1/17/72	457	221	75	2.9	2.1	7
1/24/72	566	242	78	3.1	2.3	6
2/4/72	514	254	84	3.0	2.0	6
2/11/72	571	265	76	3.5	2.2	4
2/16/72	474	218	69	3.2	2.2	5
2/18/72	588	293	93	3.2	2.0	3
2/25/72	580	268	90	3.0	2.2	4
2/29/72	633	283	98	2.9	2.2	4
3/20/72	528	249	90	2.8	2.1	3
3/23/72	600	282	95	3.0	2.1	3
3/31/72	586	280	92	3.0	2.1	5
4/14/72	638	326	110	3.0	2.0	6
4/28/72	624	289	91	3.2	2.2	2
5/12/72	732	340	110	3.4	2.2	4
5/19/72	746	318	92	3.5	2.3	2
Mean				3.1	2.15	

the beginning and end of the season; limonin values were high at the beginning and tended to decrease as the season progressed. Maier (9) in 1965 reported that limonin was still present in grapefruit 6 months after it had reached commercial maturity and found measureable amounts of limonin in juice produced in May. In samples from Plant B naringin and limonin values (Table 2) fluctuate in a manner that suggests that blending of grapefruit juice is effectively used, especially during the early season when naringin is probably a greater bitterness factor. Also, in Plant B, limonin values remained within a narrow range (2 to 6) throughout the season.

Tables 1 and 2 also indicate the relationship between the 1-2 (bitter) isomer and the 1-6 (nonbitter) isomer as well as between the bitter isomer and the Davis values as usually determined. In both plants there was considerable spread and fluctuation of values for both isomers through the season but most of the time the 1-2 isomer was in the range of 250 to 300 ppm. The nonbitter isomer ranged from about 90 to 100 in Plant A but was somewhat lower (around 75 to 90) in Plant B. The ratios of the 1-2 to 1-6 isomers ran, fairly consistently, a little over three with some slight trend to increase toward the end of the season.

Because the Davis test, which measures total glycosides, so often is relied upon as a quality control index for grapefruit juice, the ratio was determined between this value and the level of naringin (bitter isomer). This ratio (Tables 1, 2) was remarkably consistent around 2.1. In agreement with previous findings (5), Davis values, divided by 2.1, generally would give a fair index of concentration of the bitter isomer.

Kefford (11) reported limonin to be distinctly bitter at 2 ppm in orange juice. Kefford and Chandler (12) later reported about oranges, "The amount of limonin required in a juice before bitterness becomes detectable varies with the sweetness and acidity of the juice as well as the sensitivity of the taster." The threshold level of limonin in grapefruit juice has not been reported. In the present study limonin values from Plant A were as high as 10 ppm (definitely bitter) at the beginning of the season and decreased to 2 as the season progressed. In Plant B, throughout the season limonin values were in the mid range of 2 to 6 indicating that juice was blended or that processing factors were controlled.

To investigate the relationship of the test factors to flavor, 25 taste comparisons were conducted. In these paired comparison tests the tasters were asked to pick the more bitter sample. In 12 of these tests, one sample was significantly selected as more bitter than the other (Table 3). The juice containing the highest levels of naringin and/or limonin was expected to be judged more bitter. This relationship, however, was not always found. Inspection of the Brix/acid ratios

Table 2. Naringin (1-2), 7-8-rutinoside of naringenin, total glycosides by Davis test and limonin contents of canned grapefruit juice from Plant B during the '71-72 season.

Date produced	Davis ppm	1-2 ppm	1-6 ppm	1-2 / 1-6	Davis / 1-2	Limonin ppm
11/2/71	463	210	78	2.7	2.2	4
11/8/71	498	251	83	3.0	2.0	4
11/9/71	425	193	59	3.3	2.2	4
11/18/71	500	240	64	3.7	2.1	6
11/23/71	446	210	60	3.5	2.1	3
12/1/71	543	257	92	2.8	2.1	4
12/8/71	500	248	66	3.8	2.0	6
12/13/71	541	230	81	2.8	2.4	3
12/20/71	436	193	54	3.4	2.3	5
12/29/71	500	210	76	2.8	2.4	3
1/3/72	488	236	78	3.0	2.1	6
1/10/72	508	251	66	3.8	2.0	4
1/16/72	495	250	68	3.7	2.0	4
1/28/72	665	331	112	3.0	2.0	4
2/4/72	583	309	103	3.3	1.9	5
2/11/72	533	244	71	3.4	2.2	4
2/18/72	661	363	66	5.5	1.8	3
2/23/72	667	343	80	4.3	1.9	6
3/2/72	570	288	75	3.8	2.0	4
3/6/72	608	310	90	3.4	2.0	4
3/17/72	516	248	68	3.6	2.1	2
3/25/72	528	229	67	3.4	2.3	4
3/26/72	611	274	90	3.0	2.2	5
4/3/72	612	306	69	4.4	2.0	6
4/10/72	566	275	75	3.7	2.1	3
4/17/72	550	230	93	2.5	2.4	3
4/26/72	502	241	60	4.0	2.1	2
5/5/72	668	303	96	3.2	2.2	3
5/12/72	658	314	99	3.2	2.1	2
Mean				3.4	2.1	

Table 3. Paired comparison taste evaluations of canned grapefruit juice which showed significant differentiation of bitterness.

Naringin ppm	Limonin ppm	More[a] bitter	Wt/wt Brix/acid
240	6	3	11.6
257	4	27	10.2
230	3	25	8.0
210	3	3	9.1
210	3	7	9.1
251	4	23	8.9
251	4	9	8.9
331	4	21	8.0
331	4	28	8.0
244	4	2	12.5
244	4	0	12.5
343	6	30	8.5
343	6	26	8.5
310	4	4	9.5
230	3	22	8.5
303	3	8	9.4
311	10	27	8.3
254	6	3	7.9
221	7	8	11.2
254	6	22	8.1
282	3	8	9.2
326	6	22	9.3
340	4	8	11.5
318	2	22	9.1

[a]Tests were conducted on pairs as shown. This column shows number of tasters who judged this sample more bitter of this pair.

(Tables 3 and 4) indicated this factor probably influenced the judgment of the tasters as much or more than the limonin or naringin values. In 8 of the 12 tests with significant differentiations, the low-ratio sample also had the highest naringin and/or limonin. When taste panels could differentiate between two samples at a significant level the more bitter sample usually had the greatest naringin level (10 of 12); more, or at least equal, amounts if limonin (9 of 12) and generally had a lower ratio (10 of 12). As shown in Table 4, however, when the samples with higher limonin or naringin levels also had a higher Brix/acid ratio the differentiation of bitterness was not nearly so obvious. A few samples higher in naringin or limonin were chosen as more bitter by a majority of tasters, but not by enough to reach a 95% level of confidence. Apparently sweetness might have masked and sourness emphasized bitterness.

Table 4. Paired comparison taste evaluations of canned grapefruit juice which did not show significant differentiation of bitterness.

Naringin ppm	Limonin ppm	More[a] bitter	Wt/wt Brix/acid
210	4	12	11.6
240	6	18	11.6
257	4	11	10.2
230	3	19	8.0
310	4	14	9.5
363	3	16	8.8
229	4	14	9.1
306	6	16	9.1
306	6	18	9.1
230	3	12	8.5
303	3	20	9.4
314	2	10	11.1
307	10	13	8.5
311	10	17	8.3
254	6	18	8.1
293	3	12	8.7
293	3	11	8.7
283	4	19	8.6
283	4	15	8.6
249	3	15	8.2
249	3	19	8.2
280	3	11	9.2
326	6	18	9.3
289	2	12	9.3
289	2	14	9.3
340	4	16	11.5

[a]Tests were conducted on pairs as shown. This column shows number of tasters who judged this sample more bitter of this pair.

The principal points brought out by these taste results are: when both naringin and limonin were higher and Brix/acid was lower in a sample it was always judged most bitter. Conversely, when both naringin and limonin were lower and Brix/acid was higher it way always judged least bitter. Any other combination of these factors such as high naringin, low limonin and high Brix/acid ratio, etc., resulted in confusing results with little or no distinct determination of most bitter. These results indicate in many cases Brix/acid ratio may be more important than naringin and limonin content in determining quality of grapefruit juice. The overall relationship of bitterness to Brix/acid ratio requires further study before definite conclusions can be drawn.

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