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Seasonal Variation of Bitterness Components, Pulp, and Vitamin C in Texas Commercial Citrus Juices

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The bitter components, naringin (in grapefruit) and limonin (in oranges and grapefruit), were quantitated along with pulp, pH, acid, °Brix, oil, vitamin C (ascorbic acid), and color in single-strength and reconstituted concentrated juices packed by the major Texas commercial processors over three seasons. By December, for orange, and by March, for grapefruit, no juice contained more than 6 ppm of limonin. The 3-year mean for oranges was 3 ppm of limonin and for grapefruit 7 ppm of limonin and 585 ppm of naringin (by the Davis test). In both juices, limonin concentration decreased rapidly as the season progressed; naringin concentration remained steady until spring when it began to increase. There was found to be no linear correlation between pulp content of either juice and the concentration of the bitter components. Pulp content of orange juice varied considerably and consistently between plants. The three-year means of vitamin C in grapefruit and orange juices were 31.3 and 43.8 mg/100 mL, respectively.

Excessive bitterness due to limonin in orange juice or limonin and/or naringin in grapefruit juice lowers acceptability and sales of the products (Maier, 1969; Maier et al., 1980). Whereas basic studies on the nature of both limonin "delayed bitterness" and naringin bitterness have been extensive (Maier et al., 1977, 1980; Horowitz and Gentili, 1977), little quantitative data are available on seasonal trends of these components in the commercial citrus pack. Tatum et al. (1972) and Tatum and Berry (1973) determined respectively naringin and limonin in grapefruit juice and limonin in orange juice; both juices were commercially packed in Florida. Carter et al. (1975) examined limonin and 29 other analytical indicators of quality in Florida orange juice processed under simulated commercial conditions.

Excessive bitterness can be reduced by adjustment of processing parameters, by blending, or by the newer methods of removing the bitter components physically or enzymatically (Maier et al., 1977, 1980; Horowitz and Gentili, 1977); however, the relative concentrations of the components must be known if these methods are to be evaluated and applied commercially. Therefore, we studied the seasonal variation of bitterness components and other major quality factors of commercially prepared orange and grapefruit single-strength and concentrated juices from Texas-grown fruit over three consecutive seasons.

MATERIALS AND METHODS

Sample Source. The three principle citrus processing plants in the South Texas citrus belt provided triplicate samples of finished products direct from their processing lines. Samples were obtained at both one-fourth and three-fourths of the way through the day's run. Sampling was repeated at ~3-week intervals while the plants were in operation. The earliest samples were obtained in November and the latest in June. All samples were frozen as received and analyzed at one time at the end of the season. Concentrates were reconstituted to 12 °Brix for orange and 10.5 °Brix for grapefruit. Samples were collected during the 1976–1977, 1977–1978, and 1978–1979 seasons. Grapefruit samples consisted primarily of the Ruby Red variety; in 1978 one plant provided some samples prepared from Marsh. Orange juice was derived mainly from Marrs oranges from November through January with minor amounts from Hamlin, Joppa, and Pineapple varieties. Valencia contributed small percentages of juice in January and by March was the sole source.

Analyses. Suspended pulp, pH, acid, and °Brix were determined by the standard methods of the industry as compiled by Praschan (1975). Recoverable oil was assayed by AOAC (1975) method 22.103. Other determinations were for vitamin C (Nelson and Sommers, 1945), naringin (Davis, 1947), and the limonin TLC method of Tatum and Berry (1973), using the solvent system listed as no. 8 (benzene–hexane–acetone–acetic acid, 65:22:10:3 v/v). Orange juice color was determined both by the Hunterlab citrus colorimeter (Model D45) according to Huggart et al. (1969) and by the USDA (1963) method which involved visual comparisons between samples and plastic color standards. Grapefruit juice color was determined by a Gardner color difference meter (Model XL-10) (Harding and Fisher, 1945).

Two samples from each time and date of collection were analyzed separately, and all analytical determinations were run twice on each sample.

Sampling and analyses of data were designed for a three-factor analysis of variance with months being a split plot. Years were used as replications and samples within a year were used for determining sampling error.

RESULTS AND DISCUSSION

The quality factors of pH, acid, °Brix, acid/°Brix ratio, oil content, and the various color values found in this study were all within the normal seasonal ranges and trends which are well documented (Nagy et al., 1977) and appreciated. As important as those factors are to overall juice quality, their absolute values and trends will not be considered here. These quality factors for all sampling dates at the three plants are reported in the supplementary material for this paper (see paragraph at end of paper regarding supplementary material). A summary of the analysis of variance on the dependent variables is shown in Table I for grapefruit juice and Table II for orange juice.

In grapefruit juice, values for pH, acid, and oil show a significant interaction with the processing plant. This interaction may be due to a variation in fruit quality received by the plants, since they are located at ~30-km intervals along an east–west line through the production area. The plants themselves differ in the type of equipment used in their processing lines. Since several differ-

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Table I. Level of Significance of Quality Factor Interactions for Grapefruit Juice

source	pulp	pH	acid	ratio	oil	vit. C	color	naringin	limonin	°Brix[a]
year	NS[b]	0.002	0.006	NS	0.002	NS	0.04	0.0001	NS	0.003
month	NS	NS	0.02	NS	NS	NS	NS	NS	0.04	NS
plant	0.05	0.009	0.01	NS	0.02	NS	NS	0.009	NS	NS
month × plant	NS	NS	NS	NS	0.02	NS	NS	NS	NS	NS

[a] Plant C values excluded. [b] No significant interaction.

Table II. Level of Significance of Quality Factor Interactions for Orange Juice

source	pulp	pH	acid	ratio	oil	vit. C	Hunterlab color[a] CR	Hunterlab color[a] CY	Hunterlab color[a] points	McBeth[b] color	limonin	°Brix[c]
year	NS[d]	NS	NS	NS	NS	NS	0.01	0.01	0.01	0.01	0.01	0.004
month	NS	NS	0.02	NS	NS	NS	0.01	0.01	0.01	0.01	0.01	0.03
plant	0.0001	NS	NS	NS	NS	0.002	0.01	NS	0.01	NS	NS	NS
month × plant	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Hunterlab Model D-45 citrus colorimeter readings: CR = citrus redness; CY = citrus yellowness. [b] U.S. Department of Agriculture visual method of color estimation. [c] Plant C values excluded. [d] No significant interaction.

Table III. Three-Year Monthly Means of Pulp, Limonin, and Naringin in Grapefruit Juice from Three Plants

factor	plant	Nov	Dec	Jan	Feb	March	April	May	av
pulp, %, SE = 1.27	A	6.2	7.5	9.3	9.3	10.4	10.1	10.3	8.8
	B	8.3	10.0	10.8	10.3	12.9	13.4		11.0
	C	10.5	11.0	10.8	10.1	9.8	10.1	10.8	10.3
	av	8.3	9.5	10.3	9.9	11.0	11.2	10.6	10.0
limonin, ppm, SE = 0.9	A	11.5	11.0	8.9	7.9	4.9	3.7	3.1	7.3
	B	11.3	8.8	7.6	6.9	5.1	3.9		7.3
	C	9.8	9.1	10.7	7.1	5.4	3.8	3.1	7.0
	av	10.9	9.6	9.1	7.3	5.1	3.8	3.1	7.2
naringin, ppm, SE = 34	A	538	561	489	565	688	670	801	616
	B	722	530	424	705	763	777		653
	C	363	443	492	437	538	609	661	506
	av	541	511	468	569	663	685	731	585

ences in equipment, procedures, and managerial skills exist between plants, it would be unwise to attribute differences in quality factors to any single processing plant characteristic. Plant C produces only frozen concentrated juices while plants A and B supplied us only with samples of their single-strength juices. Since the concentrates from plant C were reconstituted to uniform °Brix values (10.5 for grapefruit and 12.0 for orange), this may account for some of the interaction between quality factors and plant.

Grapefruit Juice: Pulp, Naringin, and Limonin. From Table I it can be seen that only marginally significant pulp–plant interaction exists while a strong naringin–plant interaction occurs and limonin shows a significant plant interaction. In Table III the monthly means for these factors are listed by plant. The pulp from plant A is consistently lowest during the November through February period and always less than that of plant B. Yet plants A and B show no consistent differences between either limonin or Davis-test naringin values. Plant C, although having similar pulp and limonin values as plants A and B, has significantly lower Davis-test naringin values than plants A and B. There was found to be no linear correlation between pulp and limonin or Davis-test naringin values. Pulp content in itself then is not related to either limonin or naringin content of the juice which must then be dependent on other processing parameters.

Limonin content decreased almost linearly ($r = 0.85$) from November to May with the exception of plant C in January. This high January average was due to a single sample of concentrate prepared from the "Star Ruby" variety which was run during the 1976–1977 season. That sample yielded reconstituted juice with a limonin concentration of 16.6 ppm. The other concentrate samples were prepared from either the "Ruby Red" or "Marsh" varieties.

Davis-test naringin values remained relatively steady from November thru February after which they experienced an ~20% increase. It must be emphasized that the Davis test is not specific for naringin (Horowitz and Gentili, 1959) but provides a convenient and widely accepted estimate or naringin in citrus juices. Naringin content is known to vary widely between crop years (Albach et al., 1981), and this is illustrated by the strong interaction (Table I) between Davis-test naringin value and year.

There was considerable variation in limonin values and in naringin values between sampling times on the same date, the standad deviation being 0.48 and 32.2, respectively. This indicates the importance of proper sampling.

Because of the known contribution of limonin to juice bitterness (Maier et al. 1977), it can be concluded from the data in Table III that during the early and midseason, limonin may make an important contribution toward total grapefruit bitterness and any perceived decline in bitter taste as the season progresses is most likely due to decreasing limonin concentration.

Because of the complexities of the bitter taste sensation due to limonin and naringin in citrus juices and the variation in taster sensitivity (Maier et al., 1980), we did not report taste tests on the samples being studied. Of necessity the industry must employ nonsubjective measures of juice quality whenever possible.

Orange Juice: Pulp and Limonin. From Table II orange juice limonin content is seen to have a highly sig-

Table IV. Three-Year Monthly Means of Pulp and Limonin in Orange Juice from Three Plants

factor	plant	month						
		Dec	Jan	Feb	March	April	May	av
pulp, %, SE = 1.10	A	16.4	16.3	16.3	11.3	14.9	14.3	15.5
	B	20.7	21.4	21.5	20.5	20.5	20.3	21.1
	C	13.8	12.9	11.5	11.5	11.7	11.5	12.6
	av	17.0	16.9	16.4	14.4	15.7	15.4	16.4
limonin, ppm, SE = 0.43	A	4.7	4.1	3.3	3.1	2.8	2.1	3.4
	B	4.5	4.0	2.7	2.6	2.3	1.7	3.0
	C	4.3	3.4	5.7	3.7	2.8	2.0	3.6
	av	4.5	3.8	3.6	3.2	2.7	1.9	3.3

Table V. Three-Year Monthly Means of Vitamin C in Grapefruit and Orange Juices from Three Plants

juice	plant	vitamin C concn, mg/100 mL							
		Nov	Dec	Jan	Feb	March	April	May	av
grapefruit, SE = 2.14	A	30.0	31.1	30.5	29.4	32.5	29.8	28.5	30.3
	B	31.5	32.1	37.0	34.9	31.3	32.2		33.2
	C	34.0	32.6	26.7	31.6	32.2	29.3	27.5	30.6
	av	31.8	31.9	31.4	32.0	32.0	30.4	28.0	31.3
orange, SE = 2.08	A		46.7	45.0	48.3	45.0	41.8	39.0	44.3
	B		47.5	47.3	46.8	46.5	48.0	49.5	47.6
	C		42.7	39.3	41.8	40.0	37.3	36.0	39.5
	av		45.6	43.9	45.6	43.8	42.4	41.5	43.8

nificant interaction between both year and month. This means that limonin content varies between similar harvest dates in different years and as was seen with grapefruit also changes with the month of harvest. No significant interaction was noted between limonin content and plant.

Pulp, however, showed a very strong interaction with plant. From Table IV it can be seen that plant B has orange juice with about 27% more pulp than that of plant A and 40% more than that of plant C. In contrast, plant B has orange juice with about 11% less limonin than that of plant A and 18% less than that of plant C. As was true with grapefruit juice, orange juice pulp has no correlation with limonin content.

The November values for limonin were not included in Table IV due to insufficient samples for statistical analyses. These November samples ranged from a high of 8.2 ppm to a low of 4.3 ppm. A steady downward trend is seen to persist throughout the season. The high value of 5.7 ppm found in orange juice produced in February at plant C may be due to the processing of some "Valencia" oranges which had not yet reached full maturity and consequently had higher limonin content (Maier et al., 1980).

Although individuals differ in their sensitivity to limonin bitterness (Maier et al., 1980), the range of limonin concentrations found in the December through May orange juice samples would not constitute a "bitterness problem" with serious economic consequences as has been recognized among orange juice from other varieties, on other rootstocks, in different citrus producing areas.

The sample variation of limonin concentration due to sampling at two different times during the day's production run was 0.34 (standard deviation), less than that found with grapefruit. The standard deviation between samples taken at the same time was much less.

Vitamin C in Grapefruit and Orange Juice. No significant interactions were found to exist between vitamin C content of grapefruit juice and year, month, or plant (Table I). Vitamin C content of orange juice did show a significant interaction with plant (Table II).

Monthly averages of vitamin C by plant for both grapefruit and orange juices are found in Table V. Vitamin C in both juices tends to decrease during April and May; the values remained fairly steady during the early and midseason.

No significant difference in grapefruit juice vitamin C is apparent between plants. With orange juice, however, vitamin C is consistently lower in the reconstituted concentrate from plant C. This may be due to the concentrate being reconstituted to 12 °Brix which is often more dilute than the original juice.

It is noteworthy that the highest vitamin C is found in that orange juice which also had the highest pulp. Nagy (1980) cites evidence that orange pulp contains about the same concentration of vitamin C, on a weight bases, as juice, while albedo contains ~4 times as much.

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Supplementary Material Available: Tables containing the average values of quality factors from duplicate determinations on each of two samples of grapefruit and orange juice from each plant at different times and dates over three seasons (18 pages). Ordering information is given on any current masthead page.

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Annual and Seasonal Changes in Naringin Concentration of Ruby Red Grapefruit Juice

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Juices from Ruby Red grapefruit (*Citrus paradisi* Macf.) from five different groves in the South Texas citrus belt were assayed for the flavonoid bitter component naringin during five successive seasons from 1968–1969 to 1972–1973. The Davis method was used for assaying naringin. Naringin concentration of juice from the same grove and trees fluctuated during the season and varied considerably between crop years. Climatic variations between crop years strongly influenced the Davis-test naringin value and thus bitterness in grapefruit juice extracted by simulated commercial methods. Location also influenced naringin concentration in some but not all crop years. Juice naringin concentration oftentimes increased during February, March, or April after the onset of rapid vegetative growth. Although seasonal trends tended to persist among all groves during any one crop year, the trends and absolute amounts of naringin values showed little consistency between crop years.

The bitterness of grapefruit is due to components of two chemical classes: limonoids and flavonoids (Maier, 1969). Limonin bitterness only develops after juice is extracted from the fruit, and the intensity is greater in juice from fruit harvested early rather than late in the harvest season (Maier et al., 1977).

Among the flavonoids that causes bitterness—naringin, neohesperidin, and poncirin—naringin is by far the most important (Hagen et al., 1965; Horowitz and Gentili, 1977). Because naringin bitterness is one of the main factors that determine acceptability of canned grapefruit juice, several surveys of its concentration have been made (Maurer et al., 1950; Kesterson and Hendrickson, 1953; Tatum et al., 1972; Dougherty et al., 1977; Dougherty and Fisher, 1977; Ting and McAllister, 1977). Horticultural and climatic factors which might influence naringin concentration were not considered. Maurer et al. (1950) and Kesterson and Hendrickson (1953) surveyed the naringin content of different varieties at about monthly intervals during the course of a single season. Maurer et al. suggested that the naringin content of juice from 12 different Texas-grown grapefruit varieties showed rises in juice naringin concentration toward the end of the season. Kesterson and Hendrickson failed to note these rises in the Texas survey and indicated that their own survey of five grapefruit varieties grown in Florida showed no significant decrease in juice naringin concentration until past peak maturity in April. They found no correlation between juice naringin concentration and °Brix, percent acid, or °Brix/acid ratio.

Maurer et al. found that in juice from 12 varieties, naringin concentration varied between 580 and 1880 ppm in mid-October down to a range of 130–390 ppm at the last analysis in late January. The Florida juice surveyed by Kesterson and Hendrickson showed maxima of 300–370 ppm at any time from November through February for the different varieties. Minima of 150–190 ppm were confined to the last two analyses in April or May. These results contrast with those of Tatum et al. (1972), who found the lowest values mostly toward the beginning of the season and the highest values toward the end. They used the same nonspecific Davis test (Davis, 1947) as Maurer et al. and Kesterson and Hendrickson; their Davis-test naringin values of commercially prepared Florida grapefruit juice ranged from 425 to 746 ppm during one season.

All three of these reports refer to data from a single season and therefore have little predictive value for naringin levels in future crops from the same locality. Data for those seasons might not have been typical. Naringin content might be unusually high in certain years, as suggested by the expression "bitter grapefruit years" used by those knowledgeable in the art of grapefruit juice manufacture. Also, the method of juice extraction markedly affects the juice naringin concentration (Dougherty et al., 1977), and the juices in the three reports were not extracted by a standardized procedure.

Hagen et al. (1966) and Tatum et al. (1972) reported that the Davis test does not give a true measure of naringin concentration in grapefruit juice. These authors used sophisticated and time-consuming analytical methods based on thin-layer chromatography and spectral analysis to determine the concentration of the minor flavanones present in grapefruit juice. They independently concluded that the Davis-test method yielded apparent concentrations which were ~2.1 times the true narginin concentration. The authors reported, however, that the Davis method gave apparent naringin concentrations which were reasonably proportional to the true naringin concentration, and thus gave fair indexes of naringin concentration, and did accurately reflect changes in flavanone glycoside concentration. Hendrickson and Kesterson (1957) studied methods of assaying citrus flavanones and derivatives and

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