Fully-Methoxylated Flavones in Florida Orange Juices

M. K. Veldhuis, Lyle James Swift, and W. Clifford Scott

Frozen concentrated orange juices commercially produced from 1952 to 1968 were analyzed for five fully-methoxylated flavones: nobiletin, tangeretin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, tetra-O-methylscutellarein, and sinensetin (5,6,7,3′,4′-pentamethoxyflavone). Concentrations of individual flavones were all below 2 ppm. The taste thresholds of the flavones were determined in a synthetic medium and found to range from 15 ppm for tetra-O-methylscutellarein to 46 ppm for nobiletin. A mixture of the five flavones in proportion to their occurrence in peel juice was not detected in orange juice at 24 ppm. The total concentration of the five orange juice flavones found in this study averaged 4.2 ppm and never exceeded about 7 ppm. It was concluded that these substances are not important contributors to the flavor of orange juice.

For several years, Swift conducted a study of orange peel juice components that might contribute to the development of off-flavors if accidentally incorporated into juice products. The neutral fraction of the benzene extract of expressed peel juice was studied in detail because it was more bitter than either the lactone or acidic fractions, and the most plentiful compounds found in this fraction were fully methoxylated flavones. In 1967 he devised a convenient tlc method for their estimation in peel juice, and, for this study, adapted it to the analysis of commercially produced orange juice.

Only those flavones present in the juice serum were determined, since it seemed likely that these were the most important from a flavor standpoint. The five flavones considered were nobiletin, tangeretin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, tetra-O-methylscutellarein, and sinensetin (5,6,7,3′,4′-pentamethoxyflavone). The taste threshold of a typical mixture of these flavones in orange juice was determined and compared with the values observed in commercial juices and in peel juices.

EXPERIMENTAL

Samples for Analysis. The principal series of samples were frozen concentrates from the quality control laboratory reserve of a single manufacturer who used Brown extractors. Included were early-season concentrates from 1961 to 1968 inclusive (1967 missing), midseason concentrates 1952 to 1968 inclusive (1960 and 1967 missing), and late season concentrates from 1952 to 1968 inclusive (1958 and 1960 missing). In order to test the effect of extractor type, a limited number of concentrates were obtained from a manufacturer using FMC extractors.

Analysis. The cans of frozen concentrate were thawed, opened, and stirred thoroughly. Sixty milliliter portions of concentrate were diluted with 180 ml of water after which 5 g of filter-aid were added and the mixture filtered by suction through a pad of filter-aid. The soluble solids content of the filtrate was determined by refractometer. To 100 ml of filtrate were added 5 g of solid sodium hydroxide and the mixture shaken until solution was complete. After standing for 30 min the alkaline mixture was then extracted with 25 ml of benzene and next by additional 10-ml portions of this solvent until a drop spotted on filter paper showed no fluorescence, usually about three times. The combined benzene extracts were then evaporated to dryness and the residues taken up in 1 ml of benzene. From these 1 ml volumes of extract 300 μl portions were streaked on 20 × 20 cm silica gel plates. From this point the published analytical method was followed (Swift, 1967). The flavone contents of the juices were calculated from the μg found and these values adjusted to a 12% solids basis.

Taste Thresholds of Individual Flavones. The synthetic medium in which the flavone thresholds were evaluated was made up as follows: Trisodium citrate dihydrate 6.4 g, citric acid monohydrate 24 g, carboxymethyl cellulose 8.5 g, gum arabic 5.3 g, glucose 306 g, and sucrose 613 g. This mixture was made to volume of 8 l. This solution had a final pH of about 3.5 which is comparable to that of orange juice. The flavones were dissolved in ethanol to give stock solutions containing 2 mg of flavone per ml and were added to the synthetic medium to provide different concentrations. The same proportion of ethanol was added to portions of the medium for use as controls.

The triangular method of presentation was used in presenting samples to the laboratory panel.

Taste Evaluation of Flavones in Orange Juice. An alcoholic solution containing the five flavones in a typical peel juice proportion was prepared, the total concentration being 100 mg per ml. This was added to the water used to reconstitute orange concentrate to give final flavone concentrations equivalent to various levels of peel juice addition. The various mixtures were submitted to the laboratory taste panel in triangular taste tests to determine the threshold level.

U.S. Fruit and Vegetable Products Laboratory, Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Winter Haven, Fla. 33880

Table I. Yields of Frozen Concentrated Orange Juice Per 85 Lb Box of Fruit (Averages for all Florida Plants As Reported by the Florida Canners Association)

Season	Yield Gal/box	Season	Yield Gal/box	Season	Yield Gal/box
1950–51	1.33	1956–57	1.47	1962–63[a]	1.09
51–52	1.40	57–58[b]	1.30	63–64	1.57
52–53	1.43	58–59	1.52	64–65	1.63
53–54	1.36	59–60	1.51	65–66[c]	1.24[d]
54–55	1.45	60–61	1.51	66–67	1.36[d]
55–56	1.43	61–62	1.57	67–68	1.35[d]
				68–69[e]	1.13

[a] Major freeze: Dec. 14, 1962. [b] Major freeze: Dec. 12–14, 1957 and Feb. 4, 1958. [c] Major freeze: Jan. 31, 1966. [d] Gals of 45° Brix concentrate: all previous yields reported as 42° Brix; also, juice yields reduced by industry regulations. [e] Moderate freezes: December 16 and 17, 1968, and February 5, 1969.

Table II. Taste Threshold of Flavones in a Synthetic Medium and Their Concentrations in Peel Juice

Flavone	Taste Threshold (ppm)	Ave. conc. in peel juice[a] (ppm)
Tangeretin	33	13
Tetra-O-methylscutellarein	15	32
Heptamethoxyflavone	28	22
Nobiletin	46	91
Sinensetin	30	85
Total		243

[a] Swift (1965, 1967).

RESULTS AND DISCUSSION

Table I shows the seasonal average yields for all fruit varieties in all Florida concentrate plants during the period covered by this study. It can be seen that there was a rather regular increase in yield over the period of 1950 to 1966. This was due to the use of more efficient equipment and methods for the extraction of juice and to the introduction of pulp washing. Yields were lower in the 1957–58, 1962–63, and 1965–66 seasons because of freezes in the citrus production areas. Freeze damage to fruit causes it to dry and produce less juice; also after freezes, extraction and finishing must be less vigorous in order to avoid high viscosity, gelation, and the formation of lumps in the concentrate. In 1965–66, the Florida Citrus Commission started a program of controlled yield, eliminated pulp washing in the manufacture of frozen concentrated orange juice, and simultaneously raised the

Table III. Occurrence of Fully Methoxylated Flavones in Commercial Frozen Concentrated Orange Juice Prepared with Brown Extractors, Expressed as ppm of Juice Reconstituted to 12° Brix

Season	Tangeretin	Tetra-O-methyl scutellarein	Heptamethoxy-flavone	Nobiletin	Sinensetin	Total of 5 flavones
			Early Season Samples			
1960–61	0.30	0.40	0.65	1.20	1.10	3.65
1962–63	0.20	0.30	0.60	1.00	0.85	3.00[a]
1963–64	0.45	0.50	0.70	1.25	1.00	3.90
1964–65	0.35	0.50	0.70	1.20	1.00	3.75
1965–66	0.45	0.50	0.80	1.30	0.80	3.85
1966–67	0.30	0.40	0.60	1.40	1.20	3.95
1968–69	0.60	0.55	0.90	1.50	0.80	4.35
			Midseason Samples			
1951–52	0.60	0.70	1.40	1.70	1.70	6.10
1952–53	1.25	0.40	1.60	1.65	1.30	6.20
1953–54	1.00	0.45	1.00	1.45	1.30	5.20
1954–55	0.50[b]	0.55[b]	0.60	0.90	0.80[b]	3.35[b]
1955–56	0.25[b]	0.30[b]	0.50	0.80	0.70[b]	2.60[b]
1956–57	0.85	1.05	1.55	1.60	2.05	7.10
1957–58	0.80	0.50	0.80	1.10	0.80	4.00[a]
1958–59	0.30	0.40	0.65	0.95	0.70	3.00
1960–61	0.20	0.35	0.60	1.10	0.85	3.15
1961–62	0.50	0.45	0.65	1.20	0.85	3.65
1962–63	0.55	0.30	0.85	1.25	1.05	4.00[a]
1963–64	0.25	0.25	0.40	0.80	0.50	2.25
1964–65	0.30	0.40	0.65	1.15	0.85	3.25
1965–66	0.40	0.50	0.80	1.25	1.10	4.10[a]
1967–68	0.30	0.50	0.60	1.35	1.00	3.75
			Late Season Samples			
1951–52	0.40	0.80	1.25	1.70	1.35	5.55
1952–53	0.45[b]	0.70[b]	1.05	1.65	1.30	5.20[b]
1953–54	0.40	0.50	0.65	1.10	0.95	3.60
1954–55	0.35[b]	0.45	0.75	1.25	1.30	4.10[b]
1955–56	0.35[b]	0.45[b]	0.55	1.25	1.05	3.60[b]
1956–57	0.30[b]	0.45	0.75	1.20	1.10	3.75[b]
1958–59	0.35[b]	0.35	0.60	0.95	0.80	3.10[b]
1960–61	0.30	0.50	0.60	1.25	1.00	3.65
1961–62	0.30	0.50	0.70	1.25	1.20	3.95
1962–63	0.45	0.75	1.05	1.95	1.75	5.90[a]
1963–64	0.40	0.50	0.70	1.40	1.05	4.05
1964–65	0.45	0.75	1.05	1.75	1.45	5.40
1965–66	0.45	0.60	0.95	1.60	1.45	5.50[a]
1966–67	0.50	0.70	0.90	1.65	1.25	5.00
1967–68	0.40	0.60	0.70	1.35	1.05	4.05

[a] Indicates seasons in which severe freezes occurred. [b] Some interfering substance or substances distorted the spectra of these samples so that accurate absorption maxima could not be obtained.

Table IV. Flavone Content of Miscellaneous Concentrate in ppm of 12° Brix Juice, and Fruit Parts, ppm Wet Basis

Sample	Tangeretin	Tetra-O-methyl-scutellarein	Heptamethoxy-flavone	Nobiletin	Sinensetin	Total of five flavones
FMC Extracted FCOJ Seasonal Composite						
1956–57	0.40	0.40	0.80	1.20	1.15	3.95
1962–63	0.35	0.65	1.20	1.90	1.55	5.65
1963–64	0.35	0.50	0.85	1.40	1.25	4.35
1964–65	0.45	0.60	0.85	1.70	1.10	4.75
Retail Pack						
7/1/67	0.30	0.65	0.65	1.00	0.80	3.45
5/4/68	0.35	0.85	0.70	1.40	1.10	4.35
5/1/69	0.45	0.70	0.95	1.85	1.45	5.45[a]
Market Purchase						
Tangerine	3.50	0.30	0.45	5.67	0.95	10.80
Grapefruit	0	0.05	0.15	0.35	0.05	0.65

[a] Indicates seasons in which severe freezes occurred.

standard concentration from 42 to 45° Brix. These moves combined to produce a substantial reduction in gallons of concentrate obtained per box of fruit, as is shown in Table I. The yield figures in this table will be discussed further along with the analytical results.

Results of the evaluation of flavor thresholds of the five individual flavones in synthetic medium are shown in Table II. Tetra-O-methylscutellarein was the most potent, having a taste threshold of 15 ppm while nobiletin was the least flavorful with a taste threshold of 46 ppm. Their average concentrations in peel juices collected in 1963–64 are also shown in this table.

The taste evaluation of the combination of the five flavones in proportion to their occurrence in peel juice indicated that the panel could not detect in orange juice 24 ppm of the flavones. This is approximately equivalent to the quantity found in a 10% peel juice–90% orange juice mixture. At a level of 20% simulated peel juice (48 ppm), the flavones were detected with a significance of 0.1%. Since juices expressed from orange peel were detected at the 3–8% level when added to orange juice of good quality (Swift, 1965), and the range in orange juice was only up to 7.10 ppm, it was not considered necessary to determine more closely the taste threshold of the combined flavones in orange juice.

Results of analysis of commercial concentrates prepared from juice obtained with the Brown extractor are shown in Table III. The concentration of the flavones are given to the nearest 0.05 ppm. In those cases where interfering substances were encountered, the concentrations are indicated as being less than the value given. One can be sure that not more than the indicated amounts of the flavones were present.

Total concentration of the five flavones ranged from 2.25 to 7.1 ppm. Individual flavones were found in roughly similar proportions, relative to each other, as they had been found to occur previously in peel juice (Swift, 1967) but the amounts found were far below their taste threshold levels either singly or as a group.

Table IV shows results of analyses of commercial concentrates packed over a period of seven seasons in a plant using FMC extractions. Flavone contents are expressed as ppm of juice reconstituted to 12° Brix, The first four samples were composites of the entire season's pack, while each of the last three were of regular late-season production. By comparison with the data in Table III, it is seen that the flavone content is similar for corresponding seasons. These samples had total flavone contents ranging from 3.45 to 5.65 ppm, well within the range of the larger group in Table III.

In comparing the yield data in Table I with flavone values in Tables III and IV, no consistent relationship is apparent. The gradual increase in yield from 1950–51 to 1964–65 was not accompanied by a significant increase in flavones. The values at the end of the period were approximately the same as those at the start, and the values in general are dispersed in a manner one would expect from random samples of natural products.

During seasons in which there were freezes (footnote, Tables III and IV) the total flavones averaged 4.54 ppm, slightly more than the 4.24 ppm average observed for all seasons, but not nearly enough to reach the flavor threshold of more than 24 ppm. The reductions in yields during these seasons may have been a factor in preventing the flavone values from rising higher.

The average values for the total of the five flavones in six samples of whole peel juice calculated from previous publications (Swift, 1965, 1967) was 243 ppm and it is observed that this value is over 50 times the average value of 4.24 ppm in reconstituted organge juice. A considerable quantity of flavones was present in the peel juice, but the methods of juice extraction used commercially did not result in enough peel flavones in the juice to affect flavor, even with increased yield.

On the basis of the large differences in flavone content of peel compared to commercial juices and the lack of a definite increase in flavones with increased yield, the data tends to indicate that the principal source of increased yield was improved recovery of juice from sacs and rag, rather than from peel. The data also suggests that in searching for compounds that might be adverse to flavor and related to yield, the search should not be restricted to the peel.

Also shown in Table IV are the flavone contents of commercially produced tangerine and grapefruit concentrates reduced to 12° Brix. Data on the tangerine juice is of interest because of the extremely high tangeretin and nobiletin content, and on grapefruit juice because it was very low in all of the flavones.

ACKNOWLEDGMENT

The cooperation of the Winter Garden Citrus Cooperative and the Florida Citrus Canners Cooperative in supplying some of the concentrate samples used in this study is acknowledged.

LITERATURE CITED

Swift, L. J., J. Agr. Food Chem. **13**, 282 (1965).
Swift, L. J., J. Agr. Food Chem. **15**, 99 (1967).

Received for review February 11, 1970. Accepted May 4, 1970.