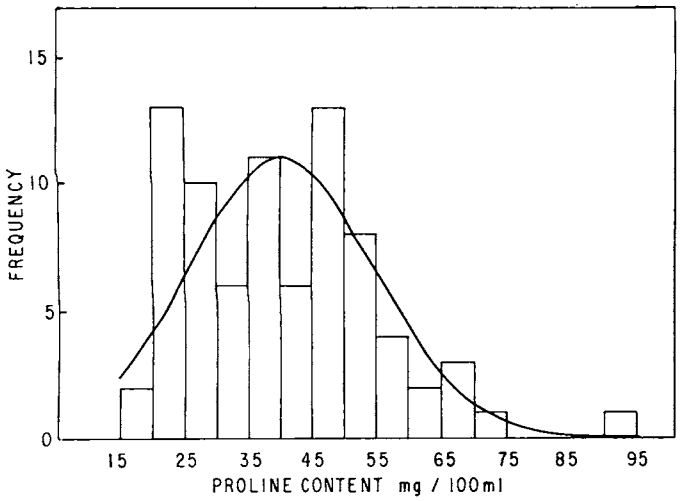

Fig. 3. Frequency distribution of proline content in Florida canned grapefruit juice. Mean = 39.1 mg/100 ml; standard deviation = 14.6 mg/100 ml; n = 80.

same shortcomings of many other juice characteristics used for this purpose. However, in the 100 samples of orange juice analyzed, the mean was 107.8 mg/100 ml with a standard deviation of 21.5 mg/100 ml. A confidence at the level of 95% could be expected that the juice should not have a value below the 2 standard deviations of the mean, or 63.8 mg/100 ml for a 12.8° Brix orange juice. The amount must be adjusted for juices reconstituted to lower Brix values. The use of proline content as an index for grapefruit juice will be even less meaningful because of its low values and high standard deviation (14.6 mg/100 ml).

Products containing significant amount of juices derived from less mature early and mid-season Florida oranges may have low proline content. With proportioned amount of the late season orange juice added to blend for Brix to acid ratios and for color, the resulting product may reach the minimum values for this amino acid as proposed by Bielig *et al.* (1) and Koch (5, 6).

The simplified method of determining proline could give the quality control laboratories an additional tool for monitoring the characteristics of the juice. The reagents are easy to prepare and stable. The diethyleneglycol used to prepare the ninhydrin reagent is generally available in most citrus quality control laboratories for the Davis test (4).

The ninhydrin reagent will also produce a red color with hydroxyproline and orinthine. No hydroxyproline has ever been reported to occur in citrus, and orinthine is only about 0.5-1.5% of proline as determined in orange juice (2, 6).

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TANGERETIN CONTENT OF FLORIDA CITRUS PEEL AS DETERMINED BY HPLC

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Additional index words. albedo, flavedo, dietary fiber, flavones.

Abstract. **Eleven varieties of Florida citrus have been analyzed for peel tangeretin contents using a new HPLC method. Highest tangeretin concentrations are found in the flavedo. Of the varieties tested, the average flavedo contributes to only 42.5% of the fresh peel weight, yet it contains 87.5% of the total amount of tangeretin in the peel. Mandarin peel had the greatest tangeretin concentration (183 ppm) while lemon peel had the least (.06 ppm). Orange peel contained, on the average, slightly greater amounts of tangeretin than grapefruit peel. The tangeretin content of any citrus peel based product can be increased or decreased dramatically depending on the variety and the portion of the peel used as a starting material.**

Tangeretin is one of several methoxylated flavones found in citrus and has been found in all of the component parts of the Valencia orange (4). It has been reported by Robbins

to be one of the methoxylated flavones to reduce erythrocyte sedimentation *rate* (red blood cell aggregation) (3). He suggests that tangeretin as well as other citrus methoxylated flavones may be beneficial because they reduce high blood viscosity and may ultimately reduce the risk of coronary heart disease. However, not all the reports on tangeretin are favorable. Jones et al (1) found tangeretin to have a cytotonic potency against zebra-fish embryo, yet Schwartz and Rate (6) found tangeretin to protect rat liver against certain cytotoxic agents. Stout et al (7) found tangeretin to be completely nontoxic to the mouse, rat and dog. However, when crude tangeretin was repeatedly injected into pregnant rats, most of the young died within three days. The results of this latter study are subject to question because no pathological examination was made to establish the cause of death. There were no abortions and no physical abnormalities. Furthermore, the conclusions of this study were based on the results from only eight female rats and apparently no control group. Since these reports cast some suspension as to the general safety of tangeretin, the Food and Drug Administration (FDA) has been reluctant to grant approval to any product which might contain tangeretin as an included ingredient.

Recently dried citrus peel has shown some promise as a source of natural dietary fiber in baking flours. It also increases the moisture holding ability and the resistance to spoilage of the final product. However, there is currently no information in the literature on peel tangeretin content of various citrus varieties. Therefore, the purpose of this paper was to determine the tangeretin content in the peel of various citrus cultivars. The tangeretin content of the albedo and flavedo was also determined separately for each variety in order to determine the distribution of tangeretin within citrus peel. Another purpose in analyzing the citrus peel components separately was to determine whether ultimate tangeretin content can be lowered if only one portion of the peel was used.

Materials and Methods

Reagents and Standards. UV grade solvents purchased from Burdick and Jackson Labs were used for extractions and as chromatographic mobile phases. Distilled, deionized, filtered (0.22 μm) water was also used as a mobile phase solvent.

An authentic tangeretin sample was obtained from Lyle J. Swift, formerly of the U.S. Fruit and Vegetable Products Laboratory, Winter Haven, Fla. Chromatographic analysis indicated that it could be used without further purification.

Apparatus and Chromatographic Conditions. The chromatographic system consisted of a Waters Associates Model 6000A pump, U6K injector and 440 UV detector with a 313 nm filter kit. Separations were carried out on a DuPont Zorbax C_8 column, 25 cm x 4.6 mm I.D. The mobile phase consisted of tetrahydrofuran (THF): acetonitrile: water (22:6:72) at a flow rate of 1.5 ml/min. The system was operated at ambient temperatures (22-25°C).

Sample Preparation. Samples were prepared from 4-12 commercially mature fruit taken from four quadrants of the tree. The varieties of citrus fruit used in this study are shown in Table 1. Each fruit was quartered along the central axis and the flavedo and albedo from opposite quarters were separated using a surgical scalpel. Total sample weight as well as the weight of all the separated flavedo and albedo was recorded. Each portion of the peel was thoroughly mixed to be certain that the 20g sample used for analysis was representative.

Tangeretin was extracted from the peel by grinding each sample in 100 ml of methanol with a Sorvall grinder.

Table 1. Tangeretin content of Florida citrus peel.

Variety	Tangeretin concentration (ppm)		
	Flavedo	Albedo	Total peel
Duncan grapefruit	15.0	.4	5.8
Mott grapefruit	29.2	1.6	6.8
Thompson grapefruit	19.0	4.0	7.9
Valencia orange	46.8	3.2	27.4
Pineapple orange	25.8	2.5	12.8
Parson Brown orange	21.2	1.2	8.2
Temple orange	13.1	1.6	8.8
King mandarin	75.1	10.0	40.6
Dancy tangerine	—	—	182.8
Eureka lemon	T	.06	.06
Sweet lime	.89	.07	.51

The finely ground peel was filtered with Whatman #1 filter paper and washed with 10 ml of fresh methanol. The filtered extract was placed in a round bottom flask and evaporated to dryness. The residue was redissolved in 2.00 ml of absolute ethanol and filtered through a 1.2 μ filter. It was then placed in a septum sealed vial and refrigerated if not analyzed the same day.

Results and Discussion

A typical HPLC separation of tangeretin from the other components in grapefruit peel is shown in Fig. 1 for the case of Mott grapefruit. The advantages of this procedure over the older TLC procedure in terms of speed and accuracy have been previously discussed (5). The HPLC procedure used is general in that other methoxylated flavones besides tangeretin can also be analyzed. The disadvantage of this procedure is that it takes about 30 min for each analysis. If only the tangeretin peak was of interest the time could be easily shortened by increasing the proportion of tetrahydrofuran in the mobile phase.

The large tangeretin peak in Fig. 2 is typical of mandarin varieties. This chromatogram is from the total peel of Dancy tangerine. The thin albedo layer could not be easily separated from the flavedo in the thin skinned mandarin varieties, therefore only total peel was analyzed.

The results of the HPLC tangeretin analyses for eleven varieties of citrus grown in Florida are given in Table 1. Dancy tangerine has the greatest concentration of tangeretin. This should not be surprising as tangeretin was first isolated and identified from tangerine oil (2), and was generally thought (but never shown) to reside in the oil sacs. The data in Table 1 suggest that the heaviest concentration of tangeretin is in the flavedo layer which is also rich in oil sacs. Much lower amounts of tangeretin are found in the albedo suggesting that tangeretin is not confined to the oil sacs alone.

The use of solvent extractions to reduce the tangeretin content in citrus peel based products has been proposed. However, these additional steps would be costly and time consuming. Using the data in Table 1 it can be seen that similar if not superior results can be achieved simply by using albedo alone instead of whole peel. On the average there is an 80% reduction in tangeretin concentration if albedo is used instead of whole peel. In Duncan grapefruit the tangeretin concentration can be reduced 93% by using albedo alone. While dramatically reducing tangeretin content this approach would also allow for the recovery of the commercially valuable oil from the unused flavedo.

Tangeretin content in total peel varies considerably between various citrus varieties (see Table 1). Mandarins and mandarin like hybrids generally have the greatest tangeretin concentrations. Tangeretin concentration in the

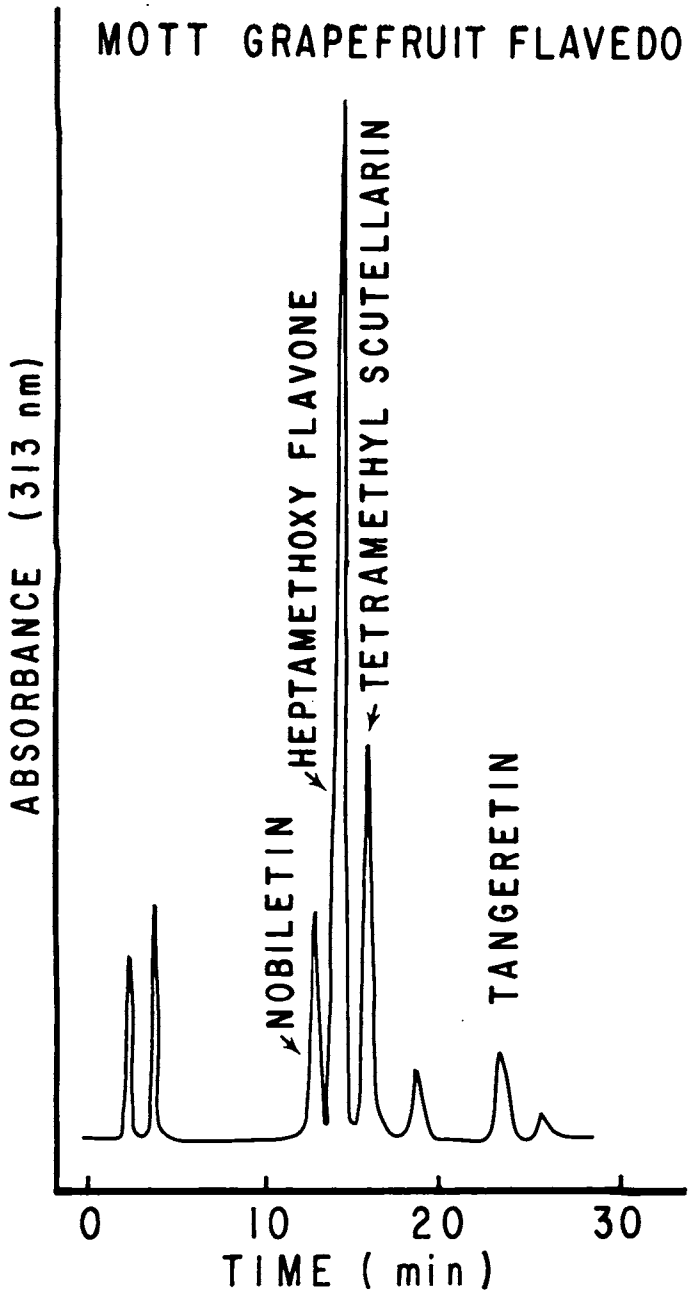

Fig. 1. 5 μl of Mott grapefruit flavedo extract; Zorbax C_8 column; mobile phase: tetrahydrofuran: acetonitrile: water (22:6:72 v/v): flow rate: 1.5 ml/min.

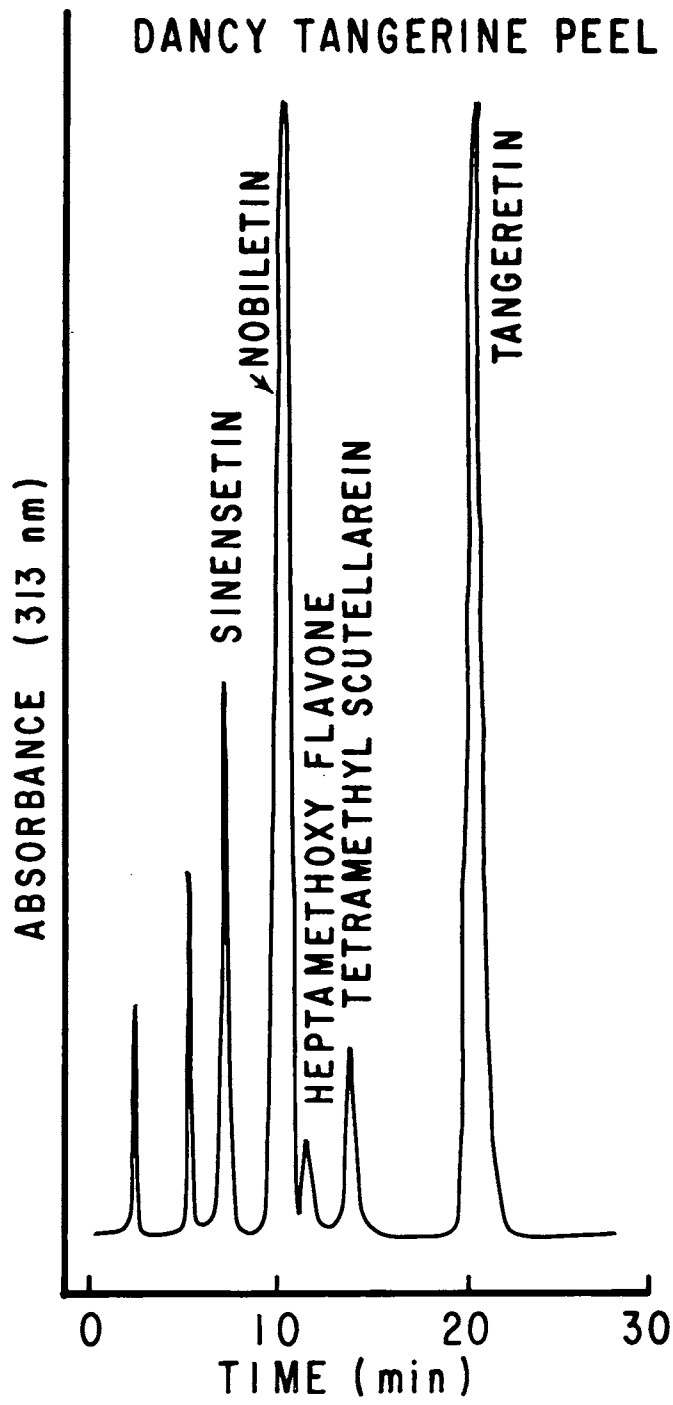

Fig. 2. 5 μl of Dancy tangerine peel extract; Zorbax C_8 column; mobile phase: tetrahydrofuran: acetonitrile: water (22:6:72 v/v); flow rate: 1.5 ml/min.

Temple orange is surprisingly low considering its mandarin parentage. With an 8.8 ppm tangeretin concentration, it more closely resembles the true oranges rather than either the King mandarin, 40.6 ppm, (also a tangor) or the Dancy tangerine, 182.8 ppm, (a true mandarin). Lemons and limes have the lowest tangeretin concentrations while oranges and grapefruit have somewhat higher values. There are appreciable differences among a given citrus group as well. Valencia orange (27.4 ppm) has more than twice the tangeretin content of either the Pineapple (12.8 ppm) or Parson Brown orange (8.2 ppm). The tangeretin content was surprisingly consistent (range 5.8 to 7.9 ppm) in the three grapefruit varieties tested. No grapefruit peel contained as much as even the least concentrated orange peel. Thus whole peel tangeretin concentration was always greater in oranges than grapefruit.

While Table 1 shows the *concentrations* of tangeretin in albedo, flavedo and whole peel, Table 2 shows the relative distribution of the *amounts* of tangeretin within citrus peel. This latter table also shows the relative proportion of albedo and flavedo within the peel of each variety. Using average values from Table 2, the flavedo contributes to only 42.5% of the fresh weight of the peel, yet it contained 87.5% of the total amounts of tangeretin in the peel. On the other hand the albedo, which comprised over half of the peel by weight contained an average of only 12.5% of the total peel tangeretin. It should be noted that Dancy tangerine and Eureka lemon have been omitted from Table 2 because the albedo could not be separated from the flavedo in the case of Dancy tangerine. The Eureka lemon was omitted because only a trace of tangeretin was found in the flavedo. In the Duncan grapefruit 95.7% of the total

tangeretin content can be removed from the peel if the flavedo is removed, with only a 37.5% loss in peel weight. The Duncan grapefruit is somewhat of an exception, however. Even though about 90% of the tangeretin is located in the flavedo and can be removed if the flavedo is removed, as much as 62.8% of the peel will be lost if this is done. This is because the flavedo constitutes the major portion of the peel in some varieties (see Table 2).

Table 2. Relative distribution of tangeretin in Florida citrus peel.

Variety	Flavedo Wt. %	Flavedo % Total tang.	Albedo Wt. %	Albedo % Total tang.
Duncan grapefruit	37.5	95.7	62.5	4.3
Mott grapefruit	18.8	80.8	81.2	19.1
Thompson grapefruit	26.1	62.7	73.9	37.3
Valencia orange	55.5	94.8	44.5	5.2
Pineapple orange	46.9	89.0	53.1	11.0
Parson Brown orange	34.9	90.7	65.1	9.3
Temple orange	62.8	93.5	37.1	6.4
King mandarin	46.9	86.9	53.1	13.1
Sweet lime	53.5	93.6	46.5	6.4
Average	42.5	87.5	57.5	12.5

The differences between flavedo and albedo tangeretin concentrations varies considerably among varieties. Using just grapefruit as an example, Duncan flavedo tangeretin levels are almost forty times greater than the corresponding albedo levels. Thompson grapefruit flavedo is only 4.7 times more concentrated than is albedo. The source of this eight fold difference is found by comparing the flavedo and albedo concentrations found in Table 1. While the flavedo layers have similar tangeretin concentrations, there is ten times more tangeretin in Thompson albedo than Duncan albedo. Thus, tangeretin levels can be further reduced if a variety with low albedo tangeretin level is used.

Conclusion

Tangeretin is found in all portions of citrus peel but is primarily concentrated in the flavedo. If it is desirable to reduce final tangeretin from citrus peel based dietary fiber, it can be readily accomplished by either of two ways. Tangeretin content may be lowered as much as 98% by using albedo alone instead of whole peel. While some extra cost would be incurred in removing the flavedo, some of this cost could be recouped by recovery of the commercially valuable oil. The second approach to reduce tangeretin (with little or no added expense) would be to avoid the use of varieties that are particularly high in tangeretin content such as the mandarins, preferably using varieties that have low albedo tangeretin content.

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THE INFLUENCE OF COLOR ON CONSUMER PREFERENCES FOR FLORIDA FROZEN CONCENTRATED GRAPEFRUIT JUICES[1]

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Abstract. **During the 1978-79 citrus season, 5 frozen concentrated grapefruit juices were prepared for a consumer test, the only variable being different levels of visual colors. *Taste tests were* conducted to determine the effect of color on acceptance, preference and flavor ratings of the juices as individual juices or when juices were compared with other juices. Color was found to have a significant effect in acceptance of paired juices. In general, yellowish-white to brownish-yellow (chamois) juices were preferred over either white or pink juices. A bias was found between male and female consumers.**

Pink grapefruit sell very well in the fresh fruit markets. The 'Star Ruby', a newly introduced deep red variety is reported in the "Citrograph" (1) to have become the number two grapefruit in Texas production ahead of the 'Marsh Pink' and the 'Marsh White'. The success of pink fruit in Texas has not gone unnoticed in Florida. Florida is now reported to have some 28% of its grapefruit in pinks (2). With pink fruit production come the problems that have been reported in Texas. Mature pink grapefruit juice has a tendency to develop a distinct "muddy brown" color when processed. Reuther, et al. (9) reported that until processing methods are perfected to retain the natural color of the lycopene pigments which are responsible for the red color in pink fruit, utilization of the pigmented varieties would be largely restricted to fresh fruit outlets.

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