Three-Dimensional Distribution of Limonin, Limonoate A-Ring Monolactone, and Naringin in the Fruit Tissues of Three Varieties of *Citrus paradisi*

Cecilia A. McIntosh*,† and Richard L. Mansell‡

Department of Biological Sciences, East Tennessee State University, Johnson City, Tennessee 37614-0703, and Department of Biology, University of South Florida, Tampa, Florida 33620

Limonin and naringin are the two major bitter compounds in *Citrus paradisi* (grapefruit), and tissue-specific patterns of their distribution are well-established. This study was undertaken to determine the distribution of these compounds within Duncan, Marsh, and Thompson Pink tissues using three-dimensional fruit dissection (600−900 samples per fruit) and highly specific radioimmunoassays for limonin and naringin quantification. Results from a GLM ANOVA showed that there was no radial distribution pattern of limonin or naringin accumulation in these fruit. There were statistically significant differences in the axial distribution of these compounds within the fruit tissues. The limonin concentration in flavedo, albedo, outer segment membrane, and juice vesicles increased toward the distal end of the fruit. Naringin concentrations in flavedo, albedo, and outer segment membranes tended to be higher in the center portion of the fruit. Limonoate A-ring monolactone levels are also reported.

Keywords: *Grapefruit; limonin; limonoate A-ring monolactone; naringin; axial distribution*

INTRODUCTION

Limonin is a bitter triterpenoid dilactone derivative which is found in major *Citrus* cultivars such as grapefruit, Navel orange, and Shamouti orange. This compound has been one of the most extensively studied of the limonoids due to its abundance and its importance in processed citrus fruit, especially juices. Previous studies have shown that lemon, *C. limon*, leaves are able to synthesize limonin from nomilin, and ultimately this compound or its nonbitter precursor, limonoate A-ring monolactone, is subsequently transported to the fruit where it accumulates in the seeds, carpellary membranes, and the central pith (Hasegawa and Hoagland, 1977; Hasegawa et al., 1980, 1986; McIntosh et al., 1982; Ronneberg et al., 1995).

Grapefruit also contains a second bitter compound, the flavanone diglycoside, naringin. Naringin is less bitter than limonin, but since it is the most abundant flavonoid in grapefruit, it, too, greatly affects the quality of the fruit products. In low concentrations, it can be involved in organoleptic responses through its synergistic interaction with limonin (Guadagni et al., 1973, 1974a,b).

Chandler et al. (1976) investigated the effect of rootstock, variety, and fruit end on the changes of limonin content of maturing oranges. This was done in order to determine the factors affecting a limonin degrading enzyme which had been found in orange albedo. Results of this study indicated that variety had the greatest effect on the limonin content. In addition, it was found that limonin and soluble solids accumulated in the distal end of both Navel and Valencia oranges. In an earlier study, Ting (1969) found that titratable acidity, soluble solids, total nitrogen, sugar, and vitamin C each showed both radial and axial patterns of distribution in grapefruit and oranges. While the gross distribution of limonin and naringin in the fruit tissues of grapefruit has been studied and tissue-specific patterns have been noted (McIntosh et al., 1982; Mansell and Weiler 1980; Jourdan et al., 1985a), little is known about the distribution of these compounds within tissues.

Results from a previous study of the distribution of limonin in grapefruit tissues of nine grapefruit cultivars showed that the concentration range within each tissue varied more than 10-fold (McIntosh et al., 1982). The observed ranges in concentration could be due to random events. If there is a pattern of accumulation within each tissue, however, the observed ranges in concentration could be due to the location from which samples were taken. The present study was conducted to determine the axial and radial distribution of limonin, limonoate A-ring monolactone, and naringin within the fruit of three grapefruit cultivars (Duncan, Marsh, and Thompson Pink) and to elucidate the ranges of concentration of these compounds within fruit tissues.

MATERIALS AND METHODS

Fruit samples of three varieties of *Citrus paradisi* (Duncan, Marsh, and Thompson Pink) were obtained from the variety block at the University of Florida Experiment Station in Lake Alfred, FL. Fruit were harvested in February (the earliest fully mature stage) from mature (20+ years old) trees. One fruit was taken from the north side of each tree, the side of the fruit nearest the tree trunk was marked, and each fruit was weighed. The fruit were sliced in cross-sections in 1 cm increments from the proximal (stem) to the distal end. The segment with the mark for the inner side of the fruit was labeled segment 1, and the numbering continued in a counterclockwise manner. Each slice was weighed, and the flavedo, albedo, outer portion of the segment membrane, the membrane on the right side of the segment, juice vesicles, and seeds for

* Author to whom correspondence should be addressed (e-mail mcintosc@access.etsu-tn.edu).
† East Tennessee State University.
‡ University of South Florida.

S0021-8561(97)00057-5 CCC: $14.00

Table 1. Summary of Average Total ppm Limonin and Average ppm Naringin in Grapefruit Tissue

	Duncan			Marsh			Thompson		
tissue	ppm limonin	ppm naringin	*n*	ppm limonin	ppm naringin	*n*	ppm limonin	ppm naringin	*n*
juice vesicles	3.3	377	118	7.4	376	83	7.0	295	96
flavedo	6.1	2701	140	42	4319	108	15.3	3634	108
albedo	11.6	13010	116	65	15595	107	27.2	13590	108
back membrane	216	27330	121	422	19819	84	234	17994	95
pith	103	14270	12	525	13285	9	477	17603	11
side membrane	306	11448	116	860	8035	84	644	7861	96
seeds	1885	323	50	1188	2677	26	1252	295	19

Table 2. Average Percent of Total Limonin That Was Limonoate A-Ring Monolactone[a]

tissue	Duncan	Marsh	Thompson
flavedo	52.1 ± 1.3 (140)	78.9 ± 1.0 (108)	58.9 ± 1.4 (108)
albedo	51.6 ± 0.9 (140)	61.4 ± 1.1 (108)	60.4 ± 0.9 (108)
back membrane	53.5 ± 1.0 (121)	62.9 ± 1.0 (84)	60.9 ± 0.9 (95)
side membrane	52.3 ± 0.9 (118)	58.2 ± 1.2 (84)	56.1 ± 1.0 (96)
juice vesicles	6.9 ± 1.4 (117)	15.0 ± 2.2 (84)	14.6 ± 1.9 (96)
pith	61.6 ± 4.3 (12)	57.6 ± 3.0 (10)	63.0 ± 1.8 (10)
seeds	83.9 ± 2.8 (51)	64.0 ± 3.4 (26)	70.6 ± 4.3 (10)

[a] Data are average percent ± SE (*n*).

each segment were dissected and weighed. The central pith of each slice was dissected out as one sample. Each sample was labeled as to the grapefruit variety, slice, segment, and tissue, and samples were frozen (−20 °C) until ready for extraction. (All fruits were dissected within 2 days of harvest.) This sampling technique resulted in 650−900 tissue samples per grapefruit.

Immediately before assay, samples were extracted with 5.0 mL of 0.1 M Tris-HCl, pH 8.0, for 30 min in a boiling water bath after which the tissue was crushed with a glass rod and re-extracted for another 30 min. Samples were diluted 50−500-fold with water and with 0.01 N HCl for the limonin and limonoate A-ring monolactone analyses (McIntosh and Mansell, 1983) and 5000−50000-fold with water for naringin. Assays were performed using the appropriate ^{3}H-radioimmunoassay (RIA) method (Weiler and Mansell, 1980; McIntosh and Mansell, 1983; Jourdan et al., 1985a,b).

Statistical analyses were done on the IBM mainframe computer at the University of South Florida. Statistical programs were taken from the compatible SAS pack (SAS Institute, Inc., Raleigh, NC), and the standard critical values were obtained from Zar (1974).

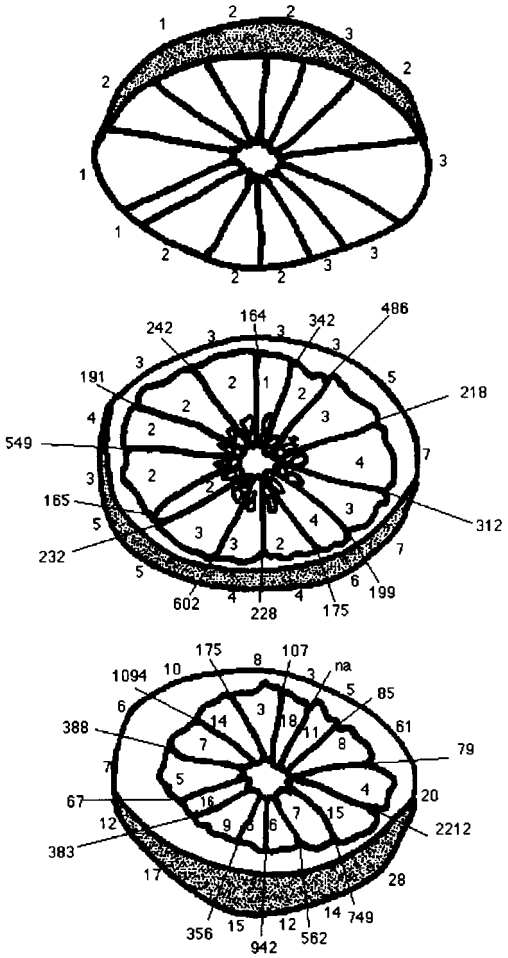

Figure 1. Limonin concentration (total limonin = limonin + limonoate A-ring monolactone) in juice vesicles, side segment membrane and flavedo of the proximal, central, and distal Duncan fruit slices. Data are ppm limonin in tissue. This clearly indicates the lack of a radial pattern of limonin accumulation and indicates the potential for an axial distribution. Marsh and Thompson Pink fruit showed similar trends.

RESULTS AND DISCUSSION

Differences in accumulation patterns of limonin and naringin between different grapefruit tissues are well-documented. Ranges of concentration of these compounds within a single tissue raised additional questions which were addressed in this study. First, is there a specific pattern to the distribution of these compounds between different fruit segments (radial distribution)? Second, is there any pattern of distribution from the proximal (stem) to the distal portions of the fruit (axial distribution)? A three-dimensional dissection of the Duncan, Marsh, and Thompson Pink fruit followed by extraction and assay of each individual sample was used to address these questions.

Limonoate A-ring monolactone has been identified as a naturally occurring nonbitter precursor of limonin that can spontaneously convert to limonin under acidic conditions (Maier and Beverly, 1968; Maier and Margileth, 1969). Since specificity of the [^{3}H]RIA for limonin permits the quantification of limonoate A-ring monolactone when performed as described in Materials and Methods, the concentration of this compound in fruit tissues was also determined.

In order to establish whether or not the fruits used in this study were representative, the average concentration of total limonin (both exogenous and limonoate A-ring monolactone) and naringin in the tissues was determined. Table 1 summarizes these results. The tissue-to-tissue relationship of limonin concentration on

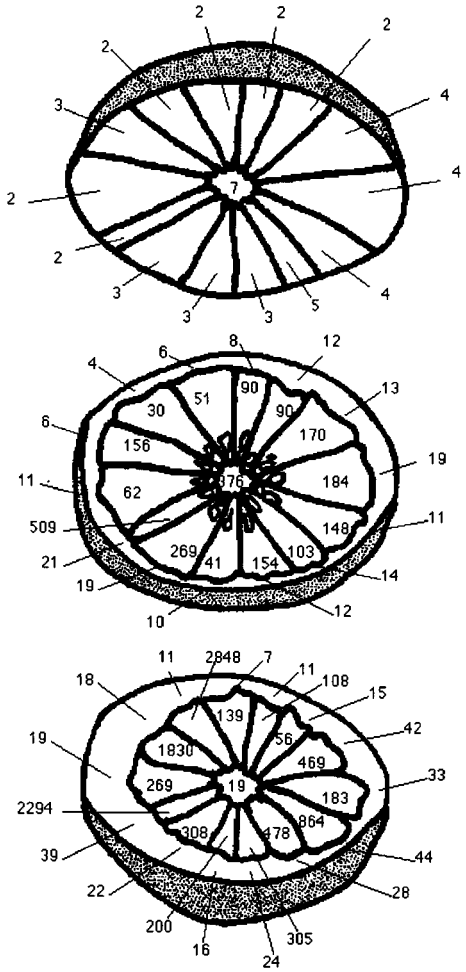

Figure 2. Limonin concentration (total limonin, cf. Figure 1) in albedo, pith, and outer (back) segment membrane of the proximal, central, and distal Duncan fruit slices. This clearly indicates the lack of a radial pattern of limonin accumulation and indicates the potential for an axial distribution. Marsh and Thompson Pink fruit showed similar trends.

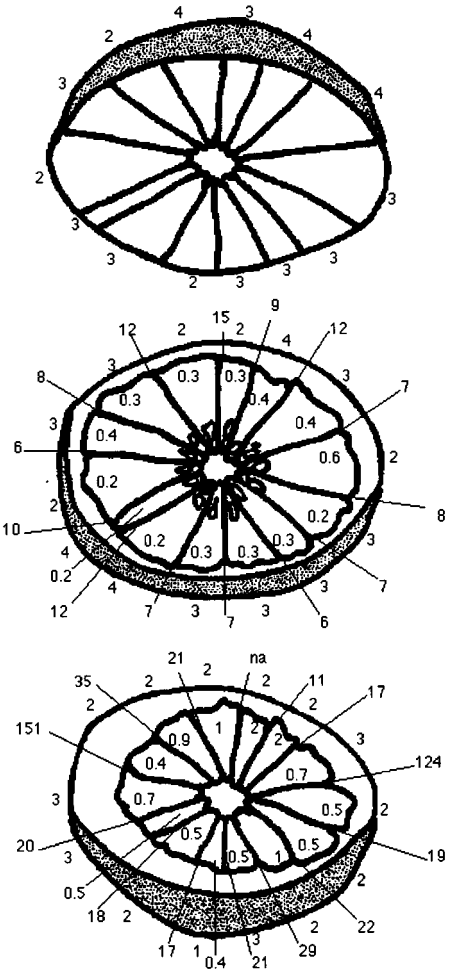

Figure 3. Naringin concentration (in thousands of ppm) in juice vesicles, side segment membrane, and flavedo of the proximal, central, and distal Duncan fruit slices.

a whole fruit basis is the same as that reported in earlier distribution studies (McIntosh et al., 1982; Weiler and Mansell, 1980). The pattern of naringin concentration is also similar to that seen in earlier work (Jourdan et al., 1985a).

Table 2 shows the average percent of the total limonin that was due to limonoate A-ring monolactone in the tissues of each grapefruit variety. Flavedo, albedo, and segment membranes of Duncan grapefruit showed similar limonoate A-ring monolactone content at about 52%. Duncan juice vesicles had the lowest limonoate A-ring monolactone content at 6.9%, probably due to the acidic environment in this tissue that would serve to favor formation of the lactone form of the D-ring resulting in limonin. Duncan seeds had the highest proportion of limonoate A-ring monolactone at 83.9%. Tissues of the other fruit showed similar trends with the exception of Marsh flavedo which also had a high percent of limonoate A-ring monolactone (78.9%). In all cases, the low standard errors for each tissue indicate that these values were nearly constant from segment-to-segment and from slice-to-slice within a given tissue within a given fruit. Because of the consistency in the percent limonoate A-ring monolactone for each tissue, all further limonin data refers to "total limonin", i.e., the combined accumulation of limonin and limonoate A-ring monolactone.

The existence of a radial distribution pattern in the limonin and naringin content in Duncan, Marsh, and Thompson grapefruits was investigated using a general linear model analysis of variance (GLM ANOVA). The GLM ANOVA was set up to test the hypothesis that the limonin concentrations in samples of a given tissue were the same in all segments versus the alternate hypothesis that there was an inequality somewhere ($\alpha = 0.01$). A data transformation of $\log(x+1)$ was used to meet the basic assumptions of the model. Differences due to the slice (axial differences) were partitioned out of the error term in order to test only the differences due to the segments and the results showed that this was the correct design. Results from the ANOVA showed that there were no statistically significant differences in the limonin concentrations in a given tissue taken from different segments of the same slice for any of the three fruit varieties. Figures 1 and 2 show the limonin content in fruit tissues of the proximal, central, and distal Duncan fruit slices and clearly indicate the potential for an axial distribution and the lack of a radial pattern of limonin distribution.

Identical analyses were performed using the naringin concentration data with the results showing the same general trend. Partitioning out of differences due to the slice was the correct design, and there were no statistically significant differences in naringin concentration in a given tissue within the same slice ($\alpha = 0.01$). Figures 3 and 4 show the naringin content (in thousands of ppm) for samples taken from the proximal, central,

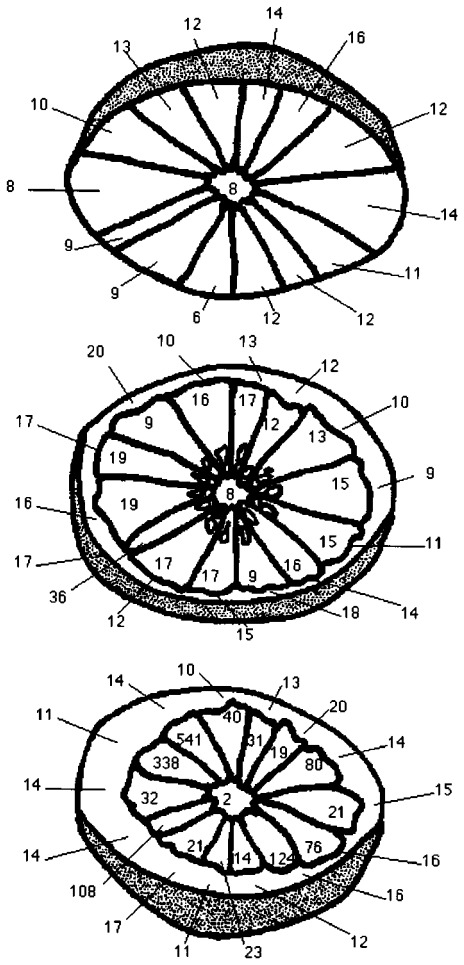

Figure 4. Naringin concentration (in thousands of ppm) in albedo, pith, and outer (back) segment membrane of the proximal, central, and distal Duncan fruit slices.

and distal slices of Duncan grapefruit; Marsh and Thompson fruits showed similar trends.

It is apparent that there was no radial distribution pattern of limonin and naringin accumulation in the grapefruit tested. Therefore, in a comparison of concentration values for a given tissue in the different 1 cm slices (axial distribution), the values for each tissue from each slice were averaged. GLM ANOVA results indicated that there was a statistically significant difference in the limonin and naringin content of tissues from different fruit slices of Duncan, Marsh, and Thompson grapefruit ($\alpha = 0.01$).

Figure 5 shows the limonin and naringin concentration in flavedo of the different slices of these three grapefruit varieties. The concentration of limonin in Duncan flavedo was 2.1 parts per million (ppm) in the proximal end of the fruit and increased to 16 ppm in the distal slice; in Marsh grapefruit the range was 12−207 ppm and in Thompson grapefruit 5.8−72 ppm. While there was a general overall trend of increasing levels toward the distal fruit end, Marsh and Thompson showed the most dramatic increase in the most-distal slice. The pattern of naringin concentration in flavedo from the proximal to the distal ends of the fruit was 2878−2396 ppm in Duncan, 4065−4290 ppm in Marsh, and 2971−2881 ppm in Thompson (4342 ppm in the next-to-last slice); overall a pattern of slight increase toward the center of the fruit with a slight decrease in the distal end.

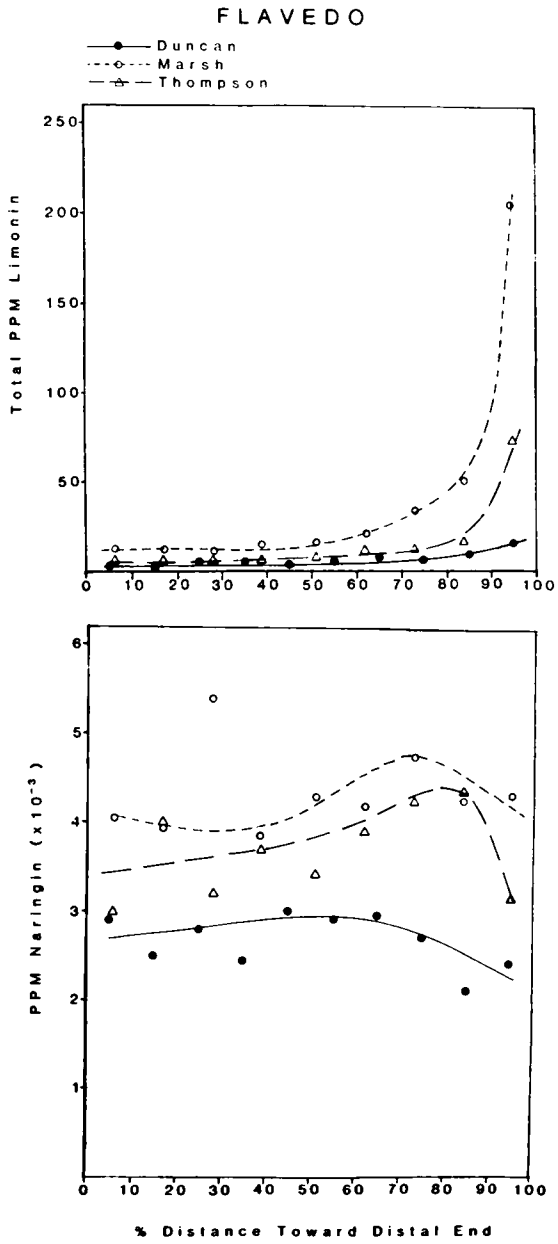

Figure 5. Axial distribution pattern of limonin (ppm) and naringin (thousands of ppm) concentration in the flavedo of Duncan (●), Marsh (○), and Thompson Pink (△) grapefruit.

Axial distribution of limonin and naringin concentration in Duncan, Marsh, and Thompson albedo is presented in Figure 6. Duncan albedo contained 2.9 ppm limonin in the proximal slice and increased to 21 ppm in the distal slice; Marsh had 12.9−234 ppm and Thompson 9.3−99 ppm. This shows a similar trend (as compared to flavedo) toward higher limonin content toward the distal fruit end, and once again, Marsh and Thompson fruit showed the most dramatic increase in the two most distal slices. The naringin concentration in albedo increased slightly toward the center slice of the fruit and decreased again at the distal end. This pattern was also similar to that seen in flavedo. Overall, naringin concentration from the proximal to the distal ends of the fruit was as follows: 11212−13991 ppm in Duncan, 15186−14756 ppm in Marsh, and 11127−13866 ppm in Thompson.

Segment membranes were dissected into two pieces, the portion at the back of the segment nearest the albedo ("back membrane") and the portion bordering the

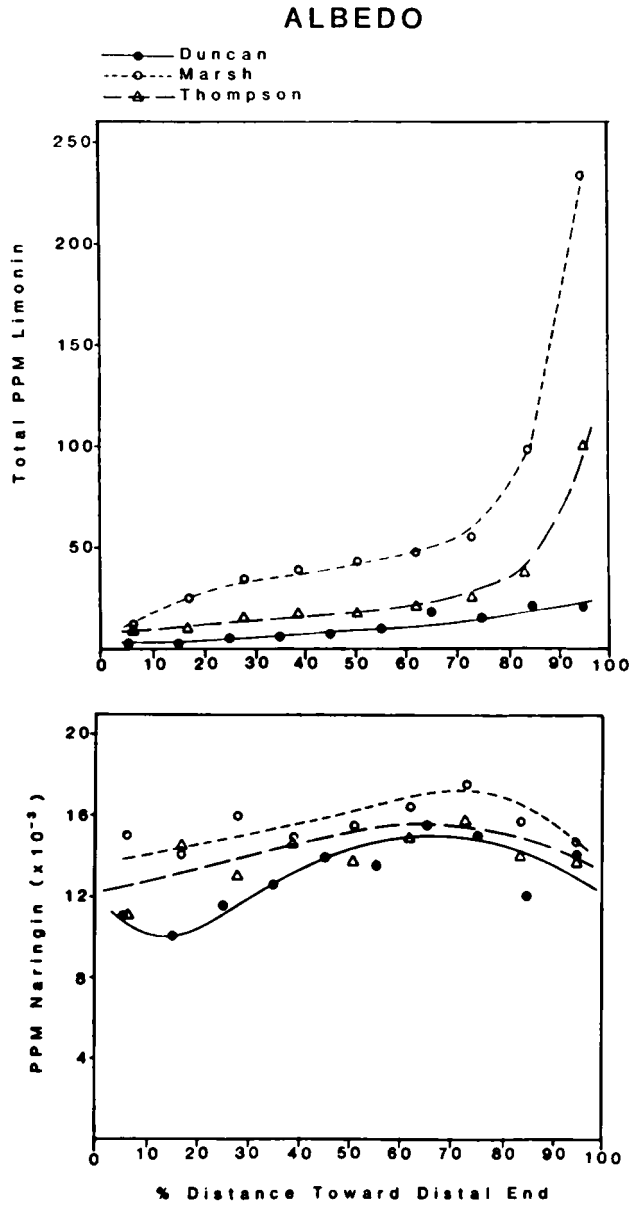

Figure 6. Axial distribution pattern of limonin (ppm) and naringin (thousands of ppm) concentration in the albedo of Duncan (●), Marsh (○), and Thompson Pink (△) grapefruit.

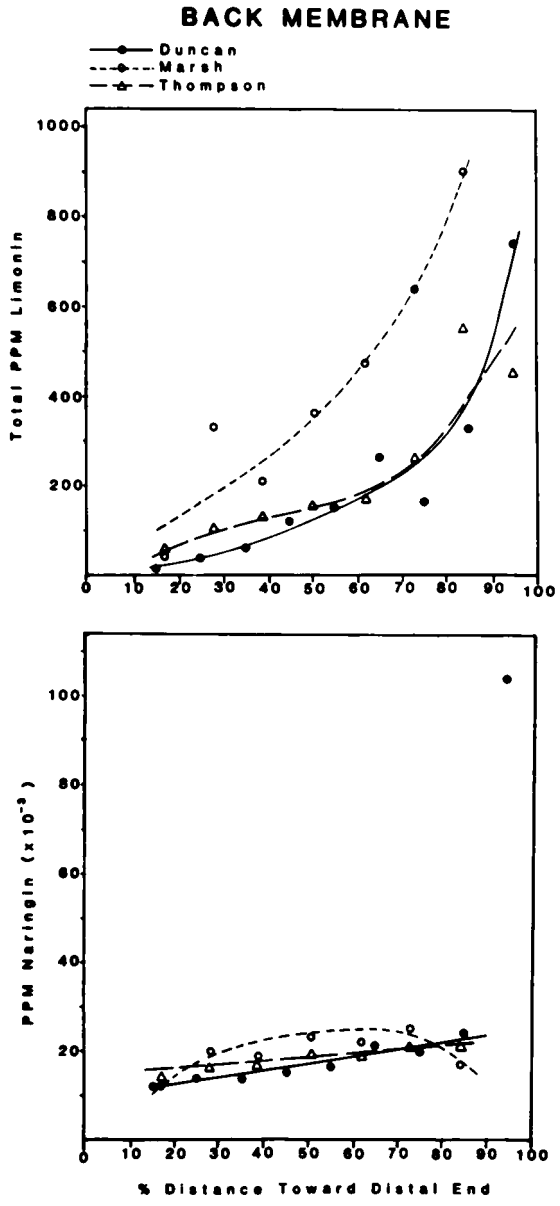

Figure 7. Axial distribution pattern of limonin (ppm) and naringin (thousands of ppm) concentration in the back (outer) segment membranes of Duncan (●), Marsh (○), and Thompson Pink (△) grapefruit.

right side of the segment ("side membrane"). Limonin concentration in the back membrane (Figure 7) showed a steady increase from the stem to the distal end in all three fruit varieties. The back segment membrane limonin concentration went from 14.8 ppm in the proximal end of the Duncan fruit to 739 ppm in the distal end; 42−896 ppm in Marsh, and 54−446 ppm in Thompson. The back segment membrane of Duncan showed a trend of slightly increasing naringin content from 12 156 ppm in the proximal (stem) slice toward the distal end with a dramatic increase in the most-distal slice (104 830 ppm) due to the content of two particular segments (cf. Figure 3). Marsh back segment membranes contained 12 684 ppm in the proximal end increasing to 25 125 ppm 70% of the distance toward the distal end and then decreasing to 16 929 ppm in the distal end; Thompson had 14 372 ppm naringin in the proximal end and 16 772 ppm in the distal end.

The overall pattern of increasing limonin concentration near the distal fruit end was evident in Duncan side segment membranes, but was not found in the side segment membranes of Marsh or Thompson (Figure 8).

The limonin concentration for the side segment membrane in Duncan was 22 ppm in the stem slice and 583 ppm in the distal. In Marsh and Thompson fruit the limonin concentration increased from 25 ppm in the stem end and peaked at about 60−70% of the distance toward the distal end. The limonin concentration then decreased in the two most-distal slices, to 1005 ppm in Marsh and 689 ppm in Thompson. The naringin concentration in side segment membranes (Figure 8) showed an initial decrease from the proximal to the second slice followed by increasing concentration toward the distal end of the fruit. Overall, the Duncan grapefruit had 10 538 ppm naringin in the side membranes of the proximal end, increasing to 39 322 ppm in the distal end; Marsh values were 6318−9291 ppm, Thompson had 5133−12772 ppm.

The axial limonin distribution pattern of juice vesicles followed the trend seen in flavedo, albedo, and back segment membranes, i.e., an increase in limonin concentration toward the distal end of the fruit. There was 1.1 ppm limonin in juice vesicles at the proximal end of

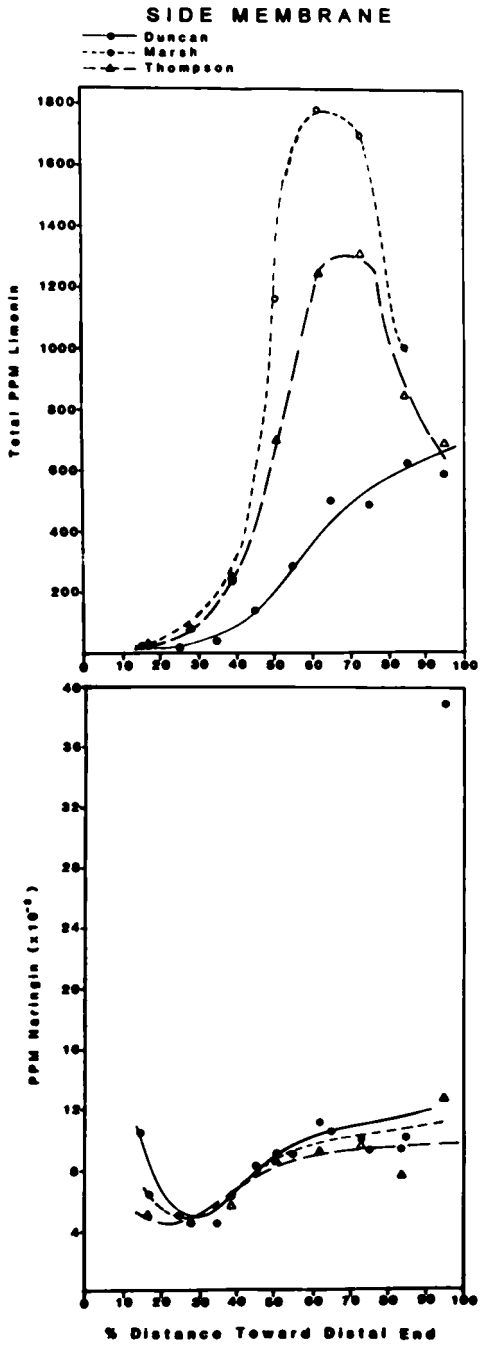

Figure 8. Axial distribution pattern of limonin (ppm) and naringin (thousands of ppm) concentration in the side segment membranes of Duncan (●), Marsh (○), and Thompson Pink (△) grapefruit.

the Duncan fruit to 9.2 ppm in the distal end; 1.9 (starting at the second slice) to 15.3 ppm in Marsh, and 2.2−13.5 ppm in Thompson. Naringin levels in the different slices showed a much less distinct pattern. From the proximal to the distal ends of the fruit (Figure 9), there was 361−827 ppm in Duncan juice vesicles (279 ppm in the next-to-last slice), 331−360 ppm in Marsh, and 304−422 ppm in Thompson (280 ppm in the next-to-last slice). In each fruit, the level of naringin was relatively constant in juice vesicles with the exception of one slice in each fruit.

There was only one pith sample per slice for each fruit, and in Duncan the limonin concentration was 7 ppm in the proximal end of the fruit increasing to 448 ppm 60% of the distance toward the distal end then decreasing to 19 ppm in the distal end of the fruit

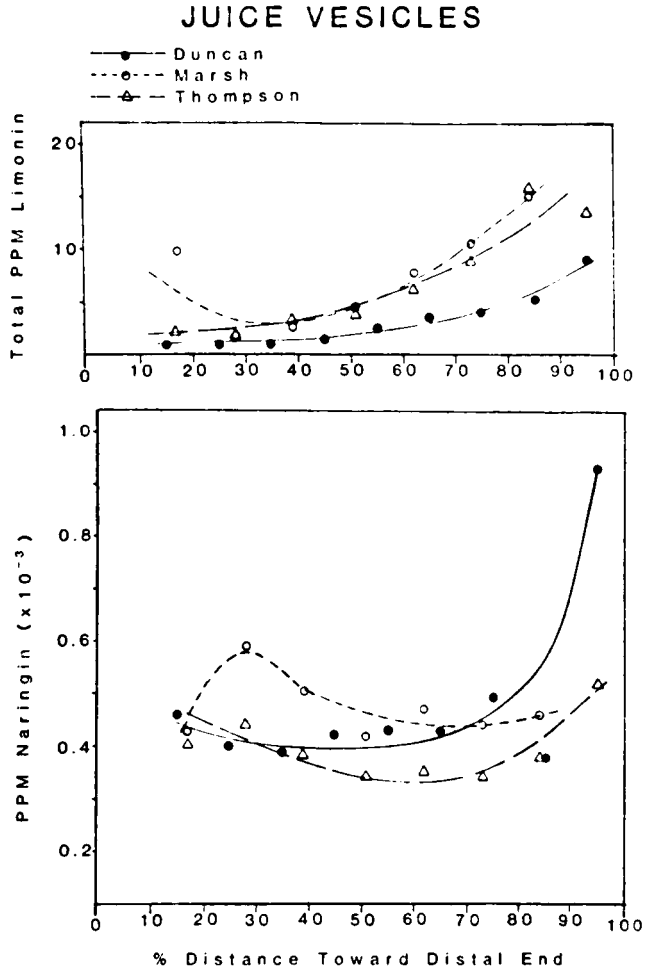

Figure 9. Axial distribution pattern of limonin (ppm) and naringin (thousands of ppm) concentration in the juice vesicles of Duncan (●), Marsh (○), and Thompson Pink (△) grapefruit.

(Figure 10). Marsh pith contained 11 ppm limonin in the stem end, 1999 ppm in the middle of the fruit, and 529 ppm in the distal end; Thompson had 12 ppm in the proximal end increasing to 1435 ppm two-thirds of the distance toward the distal end, and 202 ppm in the distal end of the fruit. This pattern is similar to that seen in side segment membranes of Marsh and Thompson grapefruit. There is no obvious distribution pattern for the naringin concentration in the pith (Figure 10). Concentrations of naringin in this tissue ranged from 2329 to 23 520 ppm in Duncan, from 9060 to 18 234 ppm in Marsh, and from 13 600 to 35 597 ppm in Thompson.

Figure 11 shows the limonin and naringin concentration in seeds from these grapefruit. Duncan is a much seedier variety; therefore more seed samples were available for this fruit. Most of the seeds were found in the central portion of the fruit with the concentration of limonin decreasing toward the distal end of the fruit in both Duncan and Thompson and increasing toward the distal end of the fruit in Marsh. Sample sizes ranged from one to four seeds near the ends of the fruit and from 10 to 14 seeds in the center slices. The naringin concentration in seeds (Figure 11) decreased slightly from the most proximal samples to the most distal samples, from 659 to 334 ppm in Duncan, was perhaps highest in the middle of the Marsh fruit (only three data points), and increased from 456 to 2585 ppm in the Thompson fruit.

Overall, the pattern of limonin distribution in grapefruit was stronger than that of naringin. For example,

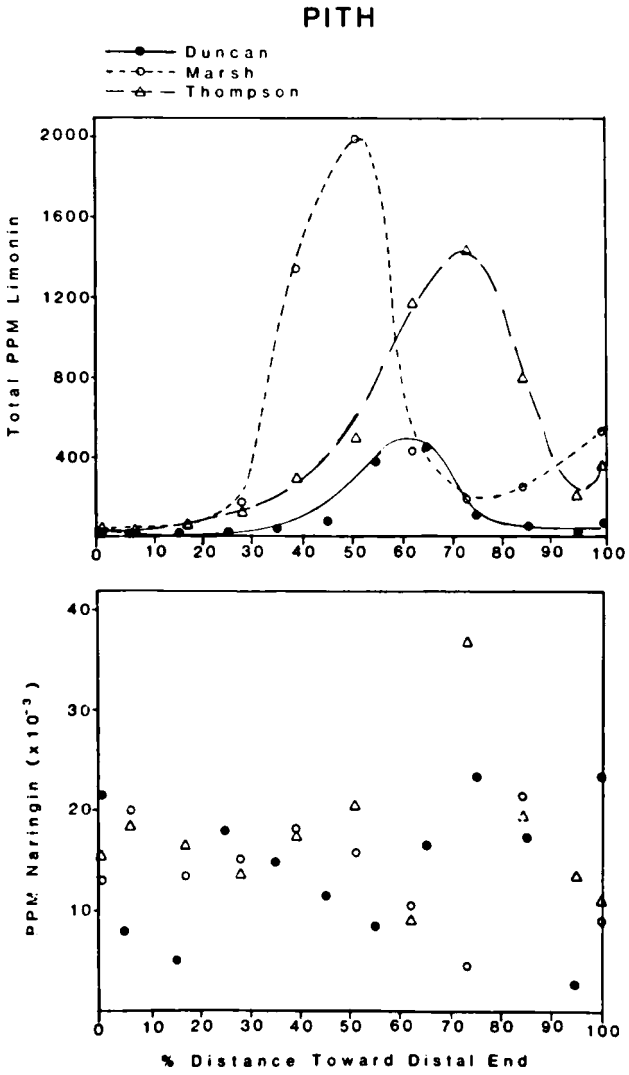

Figure 10. Axial distribution pattern of limonin (ppm) and naringin (thousands of ppm) concentration in the pith of Duncan (●), Marsh (○), and Thompson Pink (△) grapefruit.

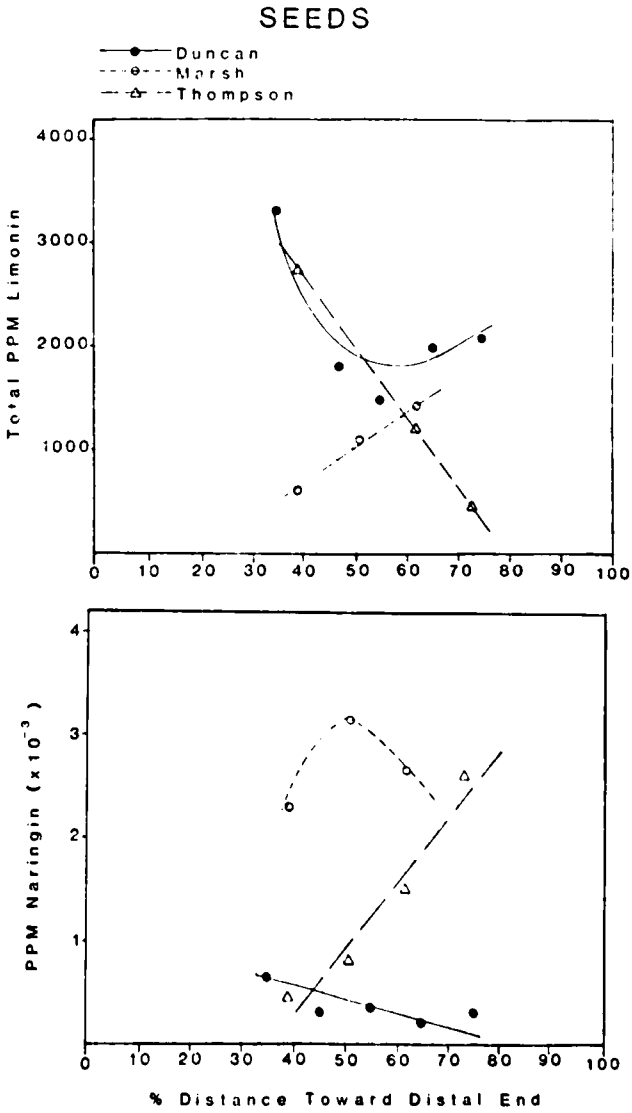

Figure 11. Axial distribution pattern of limonin (ppm) and naringin (thousands of ppm) concentration in the seeds of Duncan (●), Marsh (○), and Thompson Pink (△) grapefruit.

in the outer tissues (flavedo, albedo, and back segment membrane) there was a definite trend of increasing limonin concentrations toward the distal end of the fruit. This was also apparent in the juice vesicles. The side segment membranes and the pith both showed the highest limonin concentrations in the center slices of the fruit. Changes in limonin concentration were more dramatic in the less-seedy Marsh and Thompson fruit which also had the highest limonin concentration in all fruit tissues except the seeds (Figures 5−11). Naringin levels showed less axial variation with the exception of the juice vesicles (increasing toward the distal end; Figure 9) and the seeds (Figure 11).

Recent investigations into localization of biosynthesis of naringin in *Citrus* have shown that the most abundant levels of enzymes occur in young leaves (Bar-Peled et al., 1993; McIntosh and Mansell, 1990). Biosynthetic enzyme levels were much lower in growing fruits and undetectable in mature fruit (Bar-Peled et al., 1993). Information on the localization of limonin-synthesizing enzymes is not yet available. The results on limonin and naringin axial distribution patterns presented in this paper indicate that there is some translocational and/or depositional control of limonin and naringin in grapefruit. The dynamics of translocation or deposition of these compounds in the fruit should be the focus of future investigations.

ACKNOWLEDGMENT

We acknowledge R. L. Rouseff and S. F. Martin of the Florida Department of Citrus Research Station for providing the fruit used in this study, and S. E. Vest for technical assistance.

LITERATURE CITED

Bar-Peled, M.; Fluhr, R.; Gressell, J. Juvenile-Specific Localization and Accumulation of a Rhamnosyltransferase and Its Bitter Flavonoid in Foliage, Flowers, and Young Citrus Fruits. *Plant Physiol.* **1993**, *103*, 1377−1384.

Chandler, B. V.; Nicol, K. J.; von Biedermann, C. Factors Controlling the Accumulation of Limonin and Soluble Constituents Within Orange Fruits. *J. Sci. Food Agric.* **1976**, *27*, 866−876.

Guadagni, D. G.; Maier, V. P.; Turnbaugh, J. G. Effects of Some Citrus Constituents on Taste Thresholds for Limonin and Naringin Bitterness. *J. Sci. Food Agric.* **1973**, *24*, 1277−1288.

Guadagni, D. G.; Maier, V. P.; Turnbaugh, J. G. Some Factors Affecting Sensory Thresholds and Relative Bitterness of Limonin and Naringin. *J. Sci. Food Agric.* **1974a**, *25*, 1199−1205.

Guadagni, D. G.; Maier, V. P.; Turnbaugh, J. G. Effect of Subthreshold Concentrations of Limonin, Naringin, and

Sweeteners on Bitterness Perception. *J. Sci. Food Agric.* **1974b**, *25*, 1349−1354.

Hasegawa, S.; Hoagland, J. E. Biosynthesis of Limonoids in Citrus. *Phytochemistry* **1977**, *16*, 469−471.

Hasegawa, S.; Bennett, R. D.; Verdon C. P. Limonoids in Citrus Seeds: Origin and Relative Concentration. *J. Agric. Food Chem.* **1980**, *28*, 922−925.

Hasegawa, S.; Herman, Z.; Orme, E.; Ou, P. Biosynthesis of Limonoids in Citrus: Sites and Translocation. *Phytochemistry* **1986**, *25*, 2783−2785.

Jourdan, P. S.; Weiler, E. W.; Mansell, R. L. Naringin Levels in Citrus Tissues I. Comparison of Different Antibodies and Tracers for the Radioimmunoassay of Naringin. *Plant Physiol.* **1985a**, *77*, 896−902.

Jourdan, P. S.; McIntosh, C. A.; Mansell, R. L. Naringin Levels in Citrus Tissues II. Quantitative Distribution of Naringin in *Citrus Paradisi* Macfad. *Plant Physiol.* **1985b**, *77*, 903−908.

Maier, V. P.; Beverly, G. D. Limonin Monolactone, the Nonbitter Precursor Responsible for Delayed Bitterness in Certain Citrus Juices. *J. Food Sci.* **1968**, *33*, 488−492.

Maier, V. P.; Margileth, D. A. Limonoic Acid A-ring Lactone, a New Limonin Derivative in *Citrus*. *Phytochemistry* **1969**, *8*, 243−248.

Mansell, R. L.; Weiler, E. W. Radioimmunoassay for the Determination of Limonin in *Citrus*. *Phytochemistry* **1980**, *19*, 1403−1407.

McIntosh, C. A.; Mansell, R. L. Distribution of Limonin During the Growth and Development of Leaves and Branches of *Citrus paradisi*. *J. Agric. Food Chem.* **1983**, *31*, 318−325.

McIntosh, C. A.; Mansell, R. L. Biosynthesis of Naringin in *Citrus Paradisi*: UDP-Glucosyltransferase Activity in Grapefruit Seedlings. *Phytochemistry* **1990**, *29*, 1533−1538.

McIntosh, C. A.; Mansell, R. L.; Rouseff, R. L. Distribution of Limonin in the Fruit Tissues of Nine Grapefruit Cultivars. *J. Agric. Food Chem.* **1982**, *30*, 689−692.

Ronneberg, T. A.; Hasegawa, S.; Suhayda, C.; Ozaki, Y. Limonin Glucoside β-Glucosidase Activity in Lemon Seeds. *Phytochemistry* **1995**, *39*, 1305−1307.

Ting, S. V. Distribution of Soluble Components and Quality Factors in the Edible Portion of Citrus Fruits. *J. Am. Soc. Hortic. Sci.* **1969**, *94*, 515−519.

Weiler, E. W.; Mansell, R. L. Radioimmunoassay of Limonin Using a Tritiated Tracer. *J. Agric. Food Chem.* **1980**, *28*, 543−545.

Zar, J. H. *Biostatistical Analysis*; Prentice-Hall: Englewood Cliffs, NJ 1974.

Received for review January 22, 1997. Revised manuscript received May 16, 1997. Accepted May 20, 1997.[⊗] This work was supported by a research grant from the Florida Department of Citrus awarded to R.L.M.

JF970057D

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.