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Received for review August 1, 1986. Accepted February 19, 1987.

## Quantitative Survey of Narirutin, Naringin, Hesperidin, and Neohesperidin in *Citrus*

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Narirutin, naringin, hesperidin, and neohesperidin concentrations in juices from 52 citrus cultivars were determined using HPLC. Neither naringin nor neohesperidin was detected in sweet orange (*Citrus sinensis*), tangerine (*Citrus reticulata*), tangerine-like hybrids, or most tangelos. Grapefruit (*Citrus paradisi*), sour orange (*Citrus aurantium*), and K-Early (a grapefruit-like tangelo) juices all contain similar amounts of naringin and widely varying amounts of neohesperidin. Therefore, naringin cannot be used as the sole indicator for the presence of grapefruit juice in orange juice. However, neohesperidin concentrations can be used to differentiate grapefruit, sour orange, and K-Early juices. Naringin concentrations in these cultivars ranged from 100 to 800, 150 to 350, and 150 to 200 ppm, respectively, whereas, neohesperidin concentrations ranged from 4 to 10, 100 to 200, and 600 to 950 ppm, respectively.

Although flavonoids are ubiquitous in the plant kingdom, there are several flavanone glycosides unique to *Citrus* and specific citrus cultivars. Swingle (1943) suggested that these glycosidic compounds might be useful taxonomic markers. In an early citrus chemotaxonomic study, Albach and Redman (1969) studied the flavanone glycoside content in whole fruit of 41 citrus cultivars representing 18 recognized citrus species and 49 hybrids. Subsequent investigators (Tatum et al., 1974; Kamiya et al., 1979; Anis and Aminuddin, 1981) used flavonoid patterns in leaves and fruit, as determined by TLC, to study citrus taxonomy. There is general agreement as to the major flavanones in most citrus cultivars, but perhaps due to the subjective estimation of the intensity of TLC spots, there is considerable disagreement as to the presence of minor flavanones in several citrus cultivars. Little, if any, flavanone chemotaxonomic work has been reported on citrus juices.

Historically, orange juice (*Citrus sinensis*) has commanded a higher price than grapefruit juice (*Citrus paradisi*). There is the temptation for those who supply and market citrus juices to add the lower priced juice, i.e., grapefruit juice, to the more expensive juice for financial gain. However, this would violate orange juice standards of identity in most countries. To guard against the addition of grapefruit juice to orange juice, Greiner and Wallrauch (1984) proposed using the presence of naringin

to indicate the addition of grapefruit juice. Using HPLC, they analyzed over 50 orange juice samples, three Murcott juices (an orange-tangerine hybrid), and eight tangerine juices without finding naringin (detection limit was 3 ppm). This confirmed the TLC work of Horowitz (1961) and Albach and Redman (1969) but conflicted with the work of Drawert et al. (1980) who reported between 30 and 40 ppm naringin in commercial orange juice.

The purpose of this study was to use the sensitivity and resolving power of HPLC to determine the concentrations of the major citrus flavanone glycosides, narirutin, naringin, hesperidin, and neohesperidin, in a systematic study of the major citrus cultivars produced in Florida. From such a survey, it would be possible to determine whether naringin is a natural component in any commercially significant orange cultivar. The survey will also determine whether cultivars added into orange juice under U.S. FDA Standards of Identity (USFDA, 1984) contain naringin, or other neohesperidosides. A final goal of the survey is to quantify the flavanone glycoside concentration levels in order to establish a data base from which it might be possible to differentiate various naringin-containing juices based upon these concentration profiles.

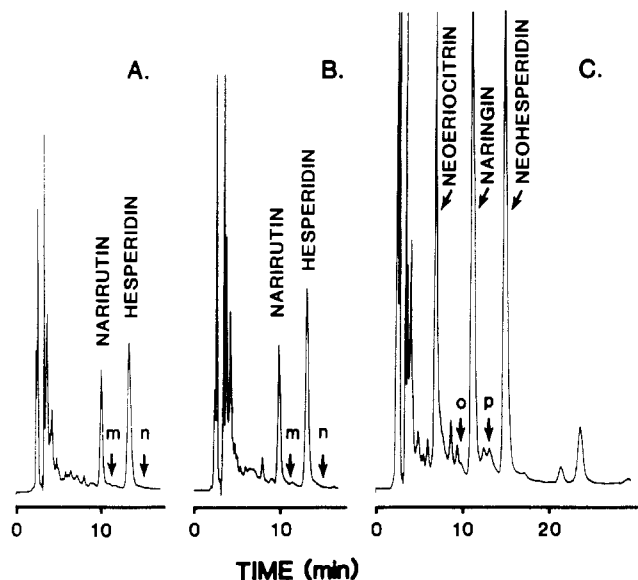
### MATERIALS AND METHODS

**Equipment.** The high-performance liquid chromatographic system consisted of a Waters (Milford, MA) Model M-6000A pump, a Model 710B WISP autosampler, and a Model 440 fixed-wavelength UV-vis detector. The column effluent was monitored at 280 nm. Chromatographic peaks were integrated by a Spectra-Physics (San Jose, CA) Model 4000 recording integrator.

**Chromatography.** A Du Pont (Wilmington, DE) Zorbax ODS (C-18) column 25 cm × 4.6 mm (i.d.) was used with a Brownlee (Santa Clara, CA) spheri-5 C-18 pre-co-

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**Figure 1.** Chromatograms from (A) navel orange juice (*C. sinensis*), (B) minneola tangelo, and (C) sour orange (*C. aurantium*). The major flavanone glycoside peaks have been labeled. Retention times for naringin, neohesperidin, narirutin, and hesperidin have been indicated by arrows labeled m, n, o, and p, respectively. Detection was at 280 nm. See text for experimental details.

lumn, 4.6 mm (i.d.)  $\times$  5 cm. The mobile phase consisted of 79.5% water, 20% acetonitrile, and 0.5% glacial acetic acid (v/v). Sample injection volume was 20  $\mu$ L. Solvents were degassed under vacuum in an ultrasonic bath. Flow rate was 1.0 mL/min.

**Reagents and Standards.** HPLC-grade Baker Analyzed acetonitrile was obtained from the J. T. Baker Chemical Co. (Phillipsburg, PA). Laboratory-deionized water was further purified on a Milli-Q (Millipore, Milford, MA) water purification system. Naringin, hesperidin, and neohesperidin were obtained from Sigma Chemical Co. (St. Louis, MO) and recrystallized before use. Authentic narirutin was obtained from Dr. James Fisher of the Florida Department of Citrus. Stock standard solutions (500 ppm) for all standards, except hesperidin, were dissolved in the chromatographic mobile phase. Hesperidin is extremely insoluble, and stock solutions were prepared by dissolving a weighed amount of hesperidin in a small amount of dimethylformamide (DMF) and diluted with enough acetonitrile to keep the DMF concentration at 20%. Working standards were prepared fresh daily by making the appropriate dilutions with chromatographic mobile phase to give a final concentration of 50 ppm naringin, 20 ppm hesperidin, and 50 ppm neohesperidin.

**Sample Preparation.** Fruit samples were obtained from the Florida Citrus Arboretum in Winter Haven, FL. Samples consisted of four to six fruit taken at even intervals around a single tree. Each fruit was halved and juiced with a Sunkist Type 6 juicer (Ontario, CA). Crude pulp and seeds were separated from the juice by passing the freshly extracted juice through a double layer of cheesecloth.

To prepare each juice sample for HPLC analysis, 3 mL of centrifuged juice is pipetted into a 10-mL syringe fitted with a conditioned Sep-Pak C-18 cartridge. The cartridge was preconditioned with 4 mL of methanol followed by 8 mL of water. The juice is slowly (0.3 mL/min) passed through the cartridge and then washed with 3 mL of 10% methanol to remove most of the sugars. The flavanone glycosides are slowly (1 drop/3–4 s) eluted into a 4-mL glass vial with 3 mL of methanol. The vial was septum

**Table I. Flavanone Glycosides of the Common Sweet Oranges (*C. sinensis*)**

cultivar	concentration, ppm			
	nari-rutin	naringin	hesperidin	neo-hesperidin
Bahianinha Navel	31	ND <sup>a</sup>	135	ND
Glen Navel	42	ND	162	ND
Hamlin	27	ND	122	ND
Khalily	28	ND	204	ND
Late Navel	56	ND	232	ND
Med. Blood	35	ND	180	ND
Parson Brown	18	ND	150	ND
Pera	41	ND	208	ND
Pineapple	34	ND	162	ND
Queen	65	ND	203	ND
Ruby Blood	38	ND	254	ND
Shamouti	47	ND	152	ND
Valencia	27	ND	151	ND
Vicieda	48	ND	169	ND

<sup>a</sup>ND = not detected (1 ppm detection limits).

sealed to prevent evaporation, and the contents were mixed prior to HPLC analysis.

Alternately, 2 mL of the same centrifuged juice was filtered through a 1.2- $\mu$ m filter and injected to check the recovery of flavanone glycosides by the above procedure.

## RESULTS AND DISCUSSION

Shown in Figure 1 are typical chromatograms for navel orange juice (*C. sinensis*), Minneola tangelo juice (a Duncan-Dancy hybrid; *C. paradisi*  $\times$  *Citrus reticulata*), and sour orange juice (*Citrus aurantium*). It can be clearly seen from Figure 1 that these orange and tangelo juices do not contain naringin or neohesperidin. Grapefruit and sour orange juices contain measureable amounts of these bitter neohesperidosides. Since both sour orange and grapefruit juices contain naringin, and since sour orange is legally allowed in orange juice, the claim that the presence of naringin in orange juice indicates that grapefruit juice has been illegally added (Greiner and Wallrauch, 1984; Galensa et al., 1986) is clearly incorrect and warrants clarification. To differentiate the addition of grapefruit juice vs. sour orange or other allowed cultivars, neohesperidin values must also be obtained. No additional experiments are required to determine juice neohesperidin concentrations. They may be obtained with the same chromatographic conditions as used for naringin. It is only necessary to extend the run time a few extra minutes to allow neohesperidin to elute.

**Chromatographic Recovery and Precision.** To determine the recovery of each of the four flavanone glycosides studied, peak areas from Sep-Pak samples were compared against those from direct injection of the filtered juice. Peak areas agreed within 2–3%, indicating there was no significant loss of flavanone glycosides due to sample preparation. Retention time precision was generally within 0.3% relative standard deviation, RSD ( $n = 4$ ). Peak area precision experiments conducted with 100 ppm naringin had a 0.99% RSD ( $n = 4$ ).

**Sweet Oranges (*C. sinensis*).** As shown in Table I, neither naringin nor neohesperidin could be detected in the juice of any major or minor sweet orange cultivar (detection limit was 1 ppm). The lack of these neohesperidosides confirms the earlier findings of Horowitz (1961), Albach and Redman (1969), Greiner and Wallrauch (1984), and Galensa et al. (1986). As shown in Figure 1, a few cultivars had one or two very small peaks in the general area where naringin would normally be found. Retention times for these extremely minor peaks were never at the correct retention time for naringin. The

**Table II. Flavanone Glycosides of Tangors, Tangelos, Grapefruit (*C. paradisi*), and Pummelo (*C. grandis*)**

cultivar	type	concentration, ppm			
		nari-rutin	narin-gin	hesperi-din	neo-hesperidin
Murcott	tangor	249	ND <sup>c</sup>	80	ND
Temple	tangor	150	ND	191	ND
Minneola	tangelo	54	ND	59	ND
Orlando	tangelo	17	ND	74	ND
Seminole	tangelo	18	ND	58	ND
Wekiwa	tangelo	100	ND	155	ND
K-Early <sup>a</sup>	tangelo	41	174	89	720
Sampson	tangelo	10	18	46	116
Canned <sup>b</sup>	grapefruit	124	419	16	10
Duncan	grapefruit	45	197	4	5
Foster	grapefruit	38	133	4	4
Marsh	grapefruit	35	152	4	5
Ruby Red	grapefruit	23	124	4	5
Starr Ruby	grapefruit	35	73	16	4
Chinese	pummelo	PT <sup>d</sup>	94	PT	ND
Liane	pummelo	ND	138	PT	ND
Sha-Ten-Yau	pummelo	ND	60	PT	ND
Siamese	pummelo	ND	33	PT	ND
Thong Dee	pummelo	ND	144	ND	ND
Wainwright	pummelo	PT	40	PT	ND

<sup>a</sup> Average of three samples. <sup>b</sup> Averages from 118 samples of Florida canned single-strength grapefruit juice from the 1977-1978 season. <sup>c</sup> ND = not detected (detection limits 1 ppm). <sup>d</sup> PT = possible trace (small peaks  $\approx$  1-3 ppm at approximately the correct retention time).

relative retention time for the most common minor peak was similar to that reported for prunin, naringenin 7- $\beta$ -glucoside (Park et al., 1983).

The 30-40 ppm naringin values reported by Drawert et al. (1980) in orange juice are difficult to explain in terms of misidentification of a minor peak, as the possible naringin peak would only have corresponded to 1-5 ppm naringin. However, the 30-40 ppm naringin values are very similar to narirutin values for many cultivars reported in Table I. Since Drawert et al. (1980) did not report narirutin values, it is possible they confused narirutin with its bitter isomer naringin. Alternatively, since they used a commercial orange juice for analysis, it is possible that they used juices that were already contaminated with grapefruit juice.

**Mandarin/Tangerines (*C. reticulata*).** U.S. Food and Drug Administration, USFDA, Standards of Identity for orange juice allow the addition of up to 10% tangerine or tangerine hybrids to be added to improve color (USFDA, 1984). The 12 mandarin and mandarin-like cultivars studied were Batangas, Dancy, Fremont, Honey, Lee, C. tachibana, Navelo, Nova, Page, Ponkan, Robinson, and Sunburst. Neither naringin nor neohesperidin was detected in any of these cultivars.

It is also interesting to note that none of the tangelo  $\times$  tangerine hybrids (Robinson, Nova, Lee) contained naringin or neohesperidin.

**Tangors and Tangelos.** Tangors are orange  $\times$  tangerine hybrids and tangelos are either tangerine  $\times$  grapefruit or tangerine  $\times$  pummelo hybrids. Since both are tangerine hybrids, up to 10% of these juices can be added to orange juice. Shown in Table II are the flavanone values for the two major tangors of commerce, namely Murcott and Temple. Also shown in Table II are the flavanone values for six tangelo cultivars. Tangelos appear to favor either the tangerine or grapefruit/pummelo parent in both physical appearance and flavanone composition. Most tangelos of commercial importance fall into the "tangerine-like" category. The only exception is the K-Early, which is one of two "grapefruit-like" tangelos found in Table II. ("Grapefruit-like" tangelos are cultivars whose

**Table III. Flavanone Glycoside Concentrations in Sour Orange (*C. aurantium*) Cultivars**

cultivar	concentration, ppm			
	nari-rutin	naringin	hesperi-din	neo-hesperidin
African	PT <sup>a</sup>	243	PT	97
Argentina	PT	136	PT	107
Bouquet de Fleurs	PT	362	PT	209
Chinotto	PT	160	PT	106
Seville	PT	244	PT	147
Willowleaf-Fla.	PT	151	PT	109
Willowleaf-Med.	PT	184	PT	123

<sup>a</sup> PT = possible trace (small peaks  $\approx$  1-3 ppm at approximately the correct retention time).

peel, flesh color, and flavor resemble grapefruit.) As shown in Figure 1, the flavanone glycosides of the "tangerine-like" hybrids are very similar to tangerines in terms of the levels of narirutin and hesperidin found and the lack of naringin and neohesperidin. However, just like grapefruit, the "grapefruit-like" tangelos contain both naringin and neohesperidin. Even though juices from these tangelos and grapefruit contain the same four flavanone glycosides, as seen in Table II, the relative proportions of these compounds differ dramatically. The important point is that even though most of the commercially important tangelos lack naringin, there is at least one cultivar in limited production that does contain appreciable amounts of naringin, namely K-Early. Clearly, the presence of naringin in orange juice cannot be used as an absolute indicator for the presence of grapefruit juice.

**Grapefruit (*C. paradisi*).** As seen in Table II, grapefruit contains all four flavanone glycosides studied, and naringin is the predominant flavanone. All major grapefruit cultivars of commercial interest have been included in the table. Most juices were obtained from hand-squeezed fruit. Juices from commercial extractors will contain higher amounts of flavanone glycosides because the peel and segment membranes contain higher concentrations of these compounds, which will be expressed in the juice as the fruit is squeezed harder. For the purposes of this study, hand-squeezed juices were used predominantly because the flavanoid contents were less variable. Flavanoids in juices from commercial extractors will depend on the type of extractor, extractor pressure, and finisher pressure (Attaway et al., 1972). However, for comparative purposes, average values from over 100 samples of commercial canned single-strength grapefruit juice from Florida are included in Table II.

On the average, the commercial juices contained from 2-4 times higher flavanoid concentrations than the hand-squeezed juices. But for all practical purposes, the relative proportions of these flavanoids are the same.

**Pummelo (*Citrus grandis*).** This cultivar is thought to be one of the parents of grapefruit (Young, 1986). As seen in Table II, these juices contain almost exclusively naringin with very little hesperidin and, interestingly, no neohesperidin. Pummelo is rarely, if ever, commercially juiced.

**Sour orange (*C. aurantium*).** This cultivar is rarely used as a juice source because it is too sour/bitter for most consumers. However, according to USFDA Standards of Identity (USFDA, 1984), it can be added to orange juice up to a maximum of 5%. As seen in Table III and Figure 1, this cultivar contains primarily flavanone neohesperidosides, naringin, and neohesperidin. Sour orange juice also contains neoeriocitrin. Like grapefruit, its major flavanoid is naringin. But unlike grapefruit, sour oranges contain almost equal amounts of neohesperidin. This

feature in addition to appreciable amounts of the relatively rare neohesperidin can be used as a means of distinguishing sour orange juices from grapefruit juice.

#### CONCLUSION

Since there are several cultivars that contain naringin and can legally be present in commercial orange juice, the view that naringin alone can be used to detect the presence of grapefruit juice is clearly incorrect. Other flavanone glycosides such as neohesperidin and neohesperidin must also be taken into consideration. Naringin concentrations in grapefruit, sour orange, and K-Early juices are reasonably similar. However, neohesperidin concentration ranges were unique for each of the three naringin-containing cultivar groups. Therefore, the concentration profile of naringin and especially neohesperidin could be used to distinguish between juices that may be legally added to orange juice (sour orange, K-Early) from those that may not (grapefruit juice).

**Registry No.** Naringin, 10236-47-2; narirutin, 14259-46-2; neohesperidin, 13241-33-3; hesperidin, 520-26-3.

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Received for review January 9, 1987. Accepted June 8, 1987.

## Analysis of Volatile Heteroatomic Meat Flavor Principles by Purge-and-Trap/Gas Chromatography-Mass Spectrometry

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A simple purge-and-trap/gas chromatography-mass spectrometry (GC-MS) procedure was developed and proven effective for the comprehensive analysis of nitrogen-, oxygen-, and sulfur-containing volatile compounds in ground roast beef, commercial beef flavor concentrate, and beef meat powder. These compounds, as released from the sample heated in the evaporator flask of a rotary evaporator, were effectively trapped in a Tenax-containing glass liner under 5 psi vacuum. The trap was then heated in an external inlet port, and the volatiles were purged into a GC-MS system for identification and concentration estimation. More than 50 heteroatomic, mostly cyclic, compounds were identified in these samples.

It has been repeatedly suggested (Vernin, 1982; Shibamoto, 1980; Ohloff and Flament, 1979; Maga, 1975, 1981, 1982; Maga and Sizer, 1973, 1974; Wasserman, 1979;

Dwivedi, 1975; Katz, 1981; KacLeod and Seyyedain-Ardebili, 1981) that heteroatomic compounds containing oxygen, nitrogen, and sulfur, mostly Maillard reaction products with cyclic structures (Bailey, 1983), are the principal constituents of meat flavors and aromas. Numerous methods, as summarized in the reviews cited above, for the identification and isolation of these compounds have been developed. These procedures, in general, required kilograms of sample and involved laborious and time-consuming extraction steps, followed by concentration of large volumes of combined extracts (Min et al., 1979; Mussinan et al., 1976; Chang et al., 1968; Tonsbeek et al., 1969, 1971; Watanabe and Sato, 1971; Mussinan et al., 1973; Wilson et al., 1973; Hirai et al., 1973; van der Ouweland and Peer, 1975).

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