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Extraction and measurement of prominent flavonoids in orange and grapefruit juice concentrates

W.E. Bronner*, G.R. Beecher

Food Composition Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705, USA

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Abstract

A procedure has been developed for determining levels of the major flavonoids in orange and grapefruit juice concentrates. Following addition of an internal standard, commercial juice concentrates were extracted multiple times with methanol, centrifuged, then filtered. Filtrates were analyzed by high-performance liquid chromatography employing UV–Vis detection. Flavonoids present in the extracts were separated on a C_{18} column with an isocratic mobile phase consisting of water–acetonitrile–2-propanol–formic acid (158:23:19:0.2, v/v). A second separation of juice extracts on the same HPLC column with a water–tetrahydrofuran mobile phase (18:7, v/v) was used to confirm the identity of the flavonoids present.

By comparing UV–Vis spectra and retention times with commercial standards chromatographed under identical conditions, three flavanone glycosides were identified and quantitated. The two brands of orange juice concentrate examined were found to contain 120 and 150 mg hg^{-1} hesperidin (where hg corresponds to 100 g), along with 24 and 30 mg hg^{-1} narirutin. The two brands of grapefruit juice concentrate examined contained 62 and 68 mg hg^{-1} narirutin, and identical levels of naringin (200 mg hg^{-1}). Consistent with several previously published reports, these high levels indicate citrus can be a major source of flavonoids in the diet.

1. Introduction

Flavonoids are a class of naturally occurring and structurally related compounds found widely distributed in plants and plant foods. They have been assigned such diverse biological properties as antioxidant, anti-inflammatory, anti-allergic and anti-carcinogenic activity, as well as vitamin C sparing [1]. Recently, dietary flavonoids have been associated with reduced risk of coronary heart disease in an epidemiological study which places a new perspective on these food components [2]. Significant quantities have been detected in many foods common to our diets, although estimates of daily consumption differ considerably [1–3].

Orange juice and grapefruit juice (as well as the fruits, themselves) are highly consumed and, as such, are potentially major contributors to total dietary flavonoids. A number of publications have reported high levels of flavanone glycosides in these juices, although quantitative values showed some disparity [4–14]. While this may be primarily due to sample variability, it may also, to some degree, reflect differences in analytical methodologies. In one comparative study, the manner of juice pretreatment was shown to affect some quantitative results by as much as an order of magnitude, whereas in other instances nearly identical values were obtained [14].

* Corresponding author.

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Solvent extraction, solid-phase extraction, and simple filtration have all been used as preparative techniques for analyzing components in foods. Solid-phase cartridges are a convenient means of obtaining purified and concentrated extracts, often directly from the food product, while filtration has the advantages of speed and simplicity. In many applications, however, these extraction methods result in less than quantitative extraction. Consequently, the actual levels in foods may be substantially underestimated. In the case of orange and grapefruit juices, filtration or solid-phase extraction may be ineffective in removing flavonoids located in suspended juice solids, even though these may represent a large fraction of the total flavonoids present. In this kind of food matrix, solvent extraction seems a preferable alternative.

This report describes a method for the extraction and quantitation of the major flavonoids in commercial orange and grapefruit juice concentrates combining solvent extraction with liquid chromatography. Although several HPLC methods for the determination of flavonoid levels in citrus juices have been developed previously [6–9,12–14], the effect of sample preparation, itself, on the quantitative results has been little studied. Consequently, the effectiveness of various sample pretreatments, such as filtration, solid-phase extraction, and solvent extraction with water, aqueous alkali or organic solvents, is largely unknown. In contrast, by measuring the extent of extraction, the present study demonstrated effective extraction of flavonoids from juice concentrates.

2. Experimental[1]

2.1. Materials and instrumentation

Minute Maid and Giant brands of orange juice concentrate and grapefruit juice concentrate were purchased at local food stores. After thawing, concentrates were subdivided into 50-ml polypropylene tubes and stored in a freezer at −20°C. Naringin (naringenin-7-neohesperidoside) hydrate, hesperidin (hesperetin-7-rutinoside), and formic acid were purchased from Aldrich (Milwaukee, WI, USA). Naringin hydrate contained 6.02% H_2O by weight and was greater than 97% pure by TLC; hesperidin contained 3.81% H_2O by weight and was greater than 98% pure by TLC (as analyzed by Aldrich). Narirutin (naringenin-7-rutinoside) and rhoifolin (apigenin-7-neohesperidoside) were purchased from Indofine Chemical Company (Belle Meade, NJ, USA). Narirutin contained 3.45% H_2O by weight and was greater than 97% pure by HPLC (as analyzed by Indofine). HPLC grade acetonitrile and 2-propanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). HPLC grade tetrahydrofuran was purchased from Mallinckrodt Specialty Chemicals Co. (Chesterfield, MO, USA). Anotop 0.2-μm porosity syringe filters (25 mm diameter) and Alltech 1-μm porosity glass fiber syringe filters (25 mm diameter) were purchased from Alltech (Deerfield, IL, USA).

The HPLC system consisted of a Perkin-Elmer Series 4 liquid chromatograph (Norwalk, CT, USA), an Altex manual injection valve (Beckman, San Ramon, CA, USA) equipped with a 20-μl injection loop, a 25 cm × 4.6 mm I.D. Alltima column (Alltech Associates) packed with 5 μm C_{18} modified silica, a Hewlett-Packard 1040M series II diode-array detector, a Hewlett-Packard HPLC ChemStation and a Hewlett-Packard 3630A PaintJet printer (Palo Alto, CA, USA).

The centrifuge used was a Beckman Model J2-21 (Fullerton, CA, USA).

2.2. Standard solutions

Working solutions of hesperidin (0.47 mg ml^{-1}), naringin (2.1 mg ml^{-1}), narirutin (0.065 mg ml^{-1}), and rhoifolin (1.8 mg ml^{-1}) were prepared by dissolving commercial flavonoid standards in methanol. The rhoifolin solution was filtered through a 0.2-μm porosity Anotop filter to remove insoluble material. Stan-

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

dards were stored in dark amber glass bottles at room temperature following preparation and appeared stable during the period of this study. Structures of the flavonoid compounds are shown in Fig. 1.

2.3. Extraction efficiencies

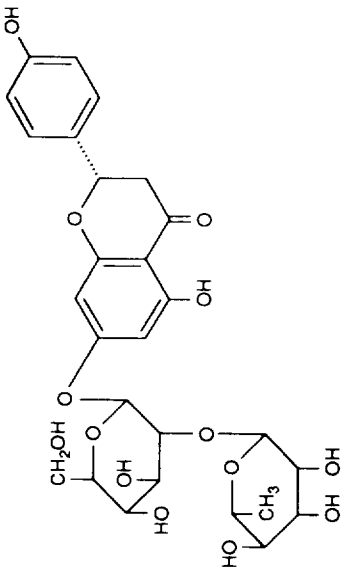
Factors which contribute to the efficiency of solvent extraction include choice of solvent, temperature, and number of extraction steps. In this study, the influence of these variables on the extraction of flavonoids from juice concentrates was examined. In a comparison of solubilities, small quantities each of naringin, hesperidin, and rhoifolin were added separately to methanol, 2-propanol, acetonitrile, and tetrahydrofuran. In all cases, these flavonoids were more soluble in methanol than in the other solvents examined (as indicated by viewing the amounts remaining undissolved), and subsequent juice samples were extracted with methanol. Completeness of extraction was monitored by measuring flavonoid levels in successive solvent fractions of juice concentrates extracted multiple times. The following procedures were employed.

A 0.5-ml volume of 1.8 mg ml^{-1} rhoifolin internal standard and 0.5 ml of methanol were added to approximately 1 g of thawed grapefruit juice concentrate. This mixture was vortex-mixed for 1 min, centrifuged at 25 000 *g* for 15 min, and the supernatant collected. An additional 0.5 ml of rhoifolin solution and 0.5 ml of methanol were added to the remaining solid. The solid material was broken apart with a spatula, vortex-mixed for 1 min, and centrifuged. A second supernatant was collected and the process repeated until a total of 5 supernatants were collected. A 1-ml volume of water was added to each extract 2 through 5. Each extract was vortex-mixed, filtered through a 1-μm porosity glass fiber filter, followed by a 0.2-μm porosity Anotop filter. Following analysis by HPLC, the naringin/rhoifolin and narirutin/rhoifolin absorbance ratios were used to calculate the fraction of flavanone recovered for each individual extraction step (Table 1).

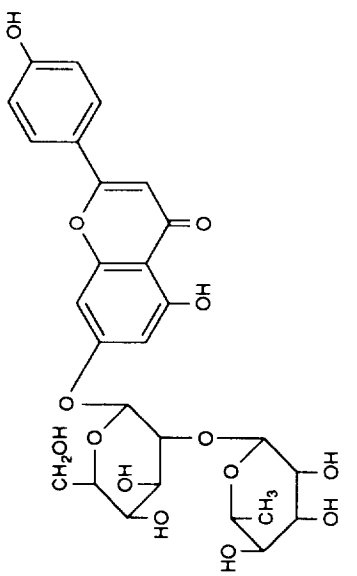
The above extraction procedure was found to be unsuitable for application to orange juice concentrate. Even five extractions resulted in incomplete recovery of hesperidin (see Table 1). Extraction efficiency was improved, however, by extracting at an elevated temperature. A 0.5-ml amount of rhoifolin solution and 3 ml of methanol were added to approximately 1 g of thawed orange juice concentrate. This was vortex-mixed for 30 s, warmed for 15 min in a 55°C water bath, and mixed an additional 30 s. The mixture was centrifuged at 25 000 *g* for 15 min and 3 ml of water were added to the supernatant collected. To the remaining solid, 0.5 ml of rhoifolin, 2 ml of methanol, and 1 ml of water were added. This was vortex-mixed for 30 s, warmed 15 min in the water bath, and vortex-mixed for another 30 s. Following centrifugation, 2 ml of water were added to the supernatant collected. To the remaining solid, 0.5 ml of rhoifolin solution, 1.5 ml of methanol, and 1 ml of water were added. This was vortex-mixed, warmed in the water bath, and mixed again. The mixture was centrifuged, and 3 ml of water were added to the supernatant collected. The three individual extracts were each vortex-mixed, then filtered through 1-μm porosity glass fiber filters, followed by 0.2-μm porosity Anotop filters. Following analysis by HPLC, the hesperidin/rhoifolin and narirutin/rhoifolin absorbance ratios were used to calculate the fraction of flavanone recovered for each individual extraction step.

2.4. Juice extraction

Frozen grapefruit juice concentrate was thawed briefly, and approximately 1-g aliquots weighed into centrifuge tubes. To each aliquot, 1.0 ml of 1.8 mg ml^{-1} rhoifolin was added as an internal standard. Each aliquot was vortex-mixed for 1 min and then centrifuged at 25 000 *g* for 15 min at room temperature. The supernatant was collected, and an additional 1 ml of methanol was added to the remaining solid. The solid material was broken apart with a spatula, vortex-mixed for 1 min, and again centrifuged. The second supernatant was added to the first, and the remaining solid re-extracted with another 1



NARINGIN



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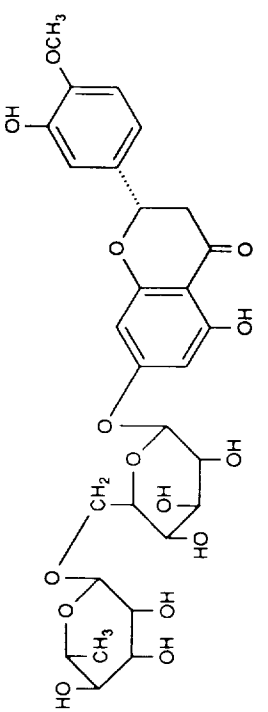
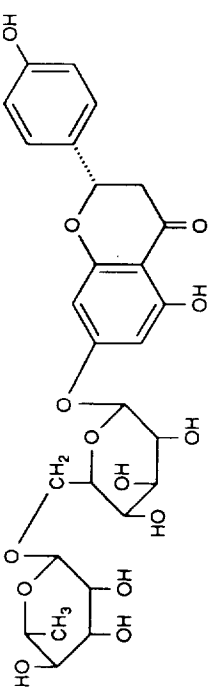


HESPERIDIN



NARIRUTIN

Fig. 1. Structures of hesperidin [15], naringin [16], narirutin [16], and rhoifolin [17].

Table 1
Percentages of prominent flavonoids in juice concentrate extracts[a]

Juice concentrate Exact number[b]	Flavonoid		
	Hesperidin	Naringin	Narirutin
Grapefruit (room temperature)			
extract 1		75	73
extract 2		23	22
extract 3		2	2
extract 4		3	
extract 5		1	
Orange (room temperature/55°C)			
extract 1	32/91		64/94
extract 2	19/8		17/6
extract 3	18/1		8
extract 4	17		7
extract 5	10		3
extract 6	4		

[a] Percentages are amounts extracted relative to the total quantity extracted from the individual juice concentrate aliquot. Values were calculated from ratios of analyte/internal standard chromatographic peak areas for single juice samples.
[b] Extract numbers indicate the particular extract in successive extractions of the same juice concentrate aliquot.
Methanol used as extraction solvent. Flavanone absorbances measured at 283 nm; rhoifolin at 335 nm.

ml of methanol following the same procedure. A 2-ml volume of water was added to the 3 combined methanolic extracts. The resulting mixture was vortex-mixed, filtered through a 1-μm porosity glass fiber filter, followed by a 0.2-μm porosity Anotop filter.

Frozen orange juice concentrate was thawed briefly, and approximately 1-g aliquots weighed into centrifuge tubes. To each aliquot, 0.4 ml of 1.8 mg ml^{-1} rhoifolin and 3 ml of methanol were added. After vortex-mixing for 30 s, each aliquot was warmed for 15 min in a 55°C water bath. Following additional mixing for 30 s, the mixture was centrifuged at 25 000 *g* for 15 min. The supernatant was collected, and 2 ml of methanol plus 1 ml of water were added to the remaining solid. This was vortex-mixed for 30 s, warmed 15 min in the water bath, vortex-mixed another 30 s, and then centrifuged. The supernatant was combined with the first juice extract and 4 ml of water were added. This was vortex-mixed, filtered through a 1-μm porosity glass fiber filter, followed by a 0.2-μm porosity Anotop filter.

A second set of grapefruit and orange juice concentrate samples having no internal standard was prepared for qualitative analysis, only. These samples were processed using the procedure described above, except no rhoifolin was added.

2.5. Recoveries from spiked samples

Recoveries of hesperidin, naringin, narirutin and rhoifolin added to orange and grapefruit juice concentrates were measured by comparing three sets of chromatograms: flavonoid standards, juice concentrates, and spiked juice concentrates. Standards (a single level for each flavonoid) were prepared by diluting measured volumes of working standards with water–methanol to a known final volume. The juice concentrate samples were weighed, extracted as previously described, and brought to the same final volume. Spiked juice concentrates were prepared by adding measured volumes of working standards to weighed amounts of juice concentrates. In each case the quantity of flavonoid added was approximately equal to that present in the juice, itself. The spiked juice concentrates were then extracted as previously described, and

diluted to the same final volume as the other samples.

Quantitative levels in the three sample sets were determined by fully loading the injection loop for each injection. The increased response due to addition of analyte into the juice concentrate (the difference in peak area between a spiked and non-spiked sample) was ratioed against the theoretical increase (the peak area expected from added standard alone) to calculate the recovery.

2.6. Chromatography

All separations were performed at ambient temperature by reversed-phase HPLC on a 25 cm C_{18} column using isocratic runs and premixed eluents. Initial examination of orange and grapefruit juice extracts with varied mobile phase composition indicated only two predominant flavonoids present in each juice. The extracts prepared just for qualitative analysis were analyzed using a water–tetrahydrofuran mobile phase (18:7) at a flow-rate of 0.4 ml min^{-1}. All other separations employed a water–acetonitrile–2-propanol–formic acid (158:23:19:0.2) mobile phase having a flow-rate of 0.6 ml min^{-1}.

Spectral scans were continuously collected from 210 to 450 nm during each run. Peak areas were measured from absorbance/time plots obtained at λ_{max}: naringin, hesperidin, and narirutin plots were integrated at 283 nm; rhoifolin at 335 nm.

3. Results and discussion

3.1. Extraction efficiencies and recoveries

The average recoveries of flavonoids spiked into orange juice concentrate were 106% hesperidin, 84% narirutin and 90% rhoifolin. In grapefruit juice concentrate recoveries were 100% naringin, 84% narirutin and 91% rhoifolin. These values represent duplicate analyses of two spiked samples for each flavonoid examined.

Measured quantities of hesperidin, naringin, and narirutin extracted from juice concentrates are listed in Table 1. Results indicate that in the first two methanolic fractions nearly all extractable material was removed from grapefruit juice concentrate at room temperature, and from orange juice concentrate at 55°C. In both cases, most material was removed in the initial extract. A comparison of orange juice extraction at room temperature and 55°C shows that hesperidin, in particular, was difficult to extract from this matrix without warming. In no case were any of the flavonoid glycosides fully extracted in a single step.

These findings suggest that a significant fraction of total flavonoids were initially present in the juice solids, and these were only partially removed in the first methanolic fraction. Complete extraction of remaining flavonoids required additional extraction stages. The improved extraction of hesperidin in orange juice at an elevated temperature may be indicative of flavonoid glycosides, in general, even though higher temperatures were unnecessary for effective extraction of grapefruit juice.

A minor problem associated with methanolic extraction of juice concentrates, even following centrifugation, was formation of cloudy mixtures, presumably due to suspended particulate matter. This occurred only after multiple extractions, however, and could be corrected by addition of a small percentage of water to the extraction solvent, if necessary. In the absence of water, solids tended to carry over into the supernatant and more readily clog filter units.

In previous work involving analysis of flavonoids in citrus, limited quantitative information regarding the extraction process, itself, has been reported. Except for recovery studies involving fortified samples [6–8,10,12,18], completeness of extraction from the juice matrix has been largely neglected. Trifirò et al. [14], however, in evaluating different methods of extraction, showed that simple filtration resulted in artificially low values for hesperidin in orange and lemon juices. The fact that even added methanol will not totally extract flavonoids from juice solids in a single step (Table 1) is consistent with this finding. Consequently, measured levels of flavo-

noids in citrus juices using only filtration for sample preparation [6,7,9,19] may well underestimate true values. No similar comparative study has been conducted for solid-phase extraction, although 92.5% recovery of hesperidin from orange juice [8] and quantitative recovery of four flavonoids added to citrus juice supernatants [18] have been reported. The effectiveness of solid-phase extraction of citrus juices in two other studies [5,20] is not known.

Methanol [21,22], methanol–2-propanol [4,10], acetone–hexane [4], dimethylformamide [12,13], hot water [14], and aqueous base [14] have all been used as solvents for extraction of flavonoids from citrus juices. Results of the present study suggest that some of these methods, particularly those employing organic solvents at elevated temperature [10,12,13,22] and/or using multiple extraction steps [4,10,21], may be quite effective. Additionally, the increased recovery of hesperidin from orange juice at a higher temperature supports earlier studies of flavonoid glycoside solubilities in aqueous media. Hesperidin, normally sparingly soluble at room temperature, is relatively soluble in boiling water [14]. Similarly, naringin solubility in water is strongly temperature dependent, increasing dramatically at higher temperatures [23].

3.2. Identification and quantitation

The major flavonoids in orange and grapefruit juice concentrates were identified by comparison with commercial standards. The closely matched spectra and retention times confirmed hesperidin and narirutin as major components in orange juice, and naringin and narirutin as major components in grapefruit juice. In addition, application of the peak purity software to the diode-array data indicated no impurities present in any of the chromatographic peaks of interest.

Representative chromatograms of orange and grapefruit juice extracts separated with the water–acetonitrile–2-propanol–formic acid mobile phase are shown in Fig. 2. The associated UV–Vis spectra (Fig. 3) are quite similar and are typical of flavanones, in general [24]. Only at the shorter wavelengths are some minor differences

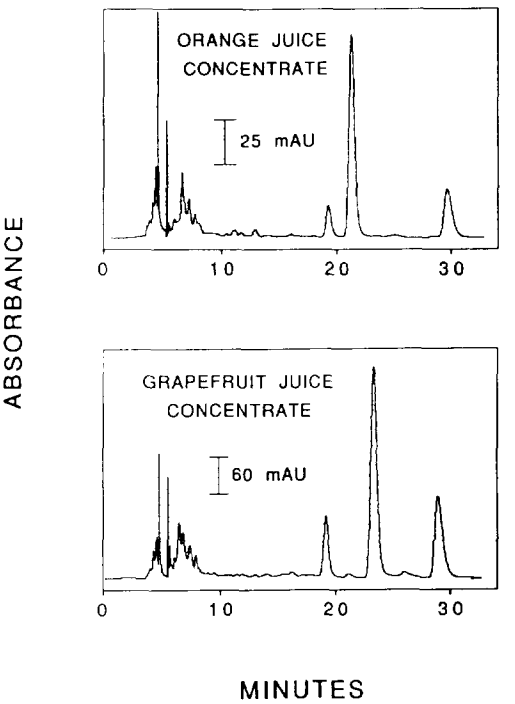


Fig. 2. Chromatograms of orange juice concentrate and grapefruit juice concentrate extracts. All chromatograms are shown with absorbances at 283 nm, whereas peak areas used for quantitative analysis were integrated at 283 nm for flavanone analytes and 335 nm for rhoifolin. Chromatograms show (in order of increasing retention) narirutin, hesperidin, naringin, and rhoifolin (internal standard). C_{18} column; mobile phase of water–acetonitrile–2-propanol–formic acid (158:23:19:0.2); flow-rate = 0.6 ml min^{-1}.

notable. Retention times (average of three runs) of the flavanones separated using the water–tetrahydrofuran mobile phase were 17.6, 22.5 and 26.6 min for hesperidin, narirutin and naringin standards, respectively (not shown). Under these run conditions, the spectra of the three citrus flavanones were, again, quite like one another and, also, similar to those obtained using the other mobile phase.

Rhoifolin, the internal standard added to the juice concentrates, is known to occur in grapefruit peel/pulp [25] and sour orange peel [17], but none was observed in chromatograms of orange or grapefruit juices unless added to the samples. In addition, no components identifiable as flavonoids were found among the group of

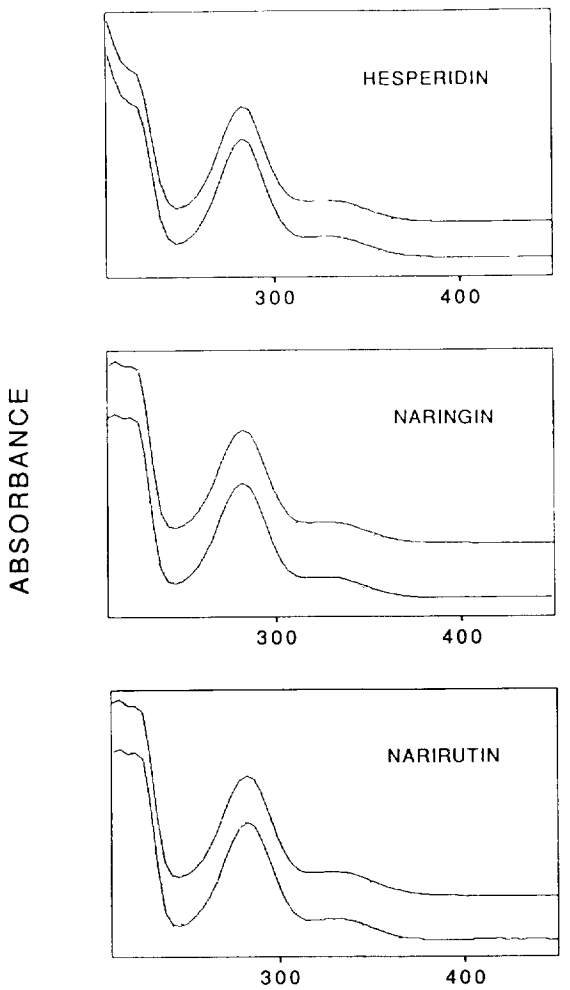


Fig. 3. Absorbance–wavelength spectra of hesperidin, naringin, and narirutin standards (lower tracings), and of corresponding chromatographic peaks from orange juice concentrate or grapefruit juice concentrate extracts (upper tracings) obtained from chromatograms in Fig. 2. Spectra were normalized and offset for purpose of comparison.

early eluting peaks seen in both juice chromatograms (Fig. 2).

Calibration plots of peak-area ratios vs. mass ratios for hesperidin/rhoifolin, naringin/rhoifolin, and narirutin/rhoifolin were obtained for solutions of the pure compounds. Each flavanone standard was prepared at four different concentrations (relative to rhoifolin) in aqueous methanol and analyzed in duplicate. Levels were chosen to bracket those found in the juice concentrates. A least squares linear fit was applied to each data set, the results of which are presented in Table 2. Weights of standards were corrected for percent water (as reported by the suppliers).

All plots were found to be linear across the ranges studied, with intercepts near the origin. Values for the standard errors and correlation coefficients listed in Table 2 indicate good precision was obtained using the internal standard chosen. In addition, results obtained for duplicate runs of the same extract (not listed) were highly reproducible. In contrast to the work of Mouly et al. [13], and Nogata et al. [18], who observed nearly identical response factors for hesperidin, naringin, and narirutin in water–acetonitrile–tetrahydrofuran–acetic acid and water–methanol–phosphoric acid mobile phases, respectively, noticeably different slopes for these three flavonoids were found in the present study.

Quantitative values for juice flavonoids were determined from chromatographic peak areas obtained from absorbance–time plots. Flavanone/rhoifolin absorbance area ratios were converted to flavanone/rhoifolin mass ratios using calibration plots (Table 2). Final concentrations were determined by factoring into calculations the quantity of juice initially aliquoted and the amount of internal standard added.

In orange juice concentrates 120/150 mg hg^{-1} hesperidin and 24/30 mg hg^{-1} narirutin were measured for Giant/Minute Maid brands, respectively. (Calculated values for prepared orange juices, based on diluting 1 part concentrate with 3 parts water, were 31/38 mg hg^{-1} hesperidin and 6.0/7.4 mg hg^{-1} narirutin.) In grapefruit juice concentrates 200/200 mg hg^{-1} naringin and 62/68 mg hg^{-1} narirutin were measured for Giant/Minute Maid brands, respectively. (Calculated values for prepared grapefruit juices, based on diluting 1 part concentrate with 3 parts water, were 49/51 mg hg^{-1} naringin and 16/17 mg hg^{-1} narirutin.) These flavonoid levels are averages of 6 measurements (duplicate analyses of 3 extracts from the same batch of juice

Table 2
Statistical data for calibration plots[a]

	Hesperidin	Naringin	Narirutin
Number of data points	8	8	8
Range[b]	0.49–4.9	0.21–2.1	0.069–0.69
Slope	0.96	0.86	0.61
Intercept	−0.056	−0.016	0.004
Standard error of estimate	0.054	0.014	0.017
(Correlation coefficient)^2	0.999	1.000	0.990

[a] Results derived from linear regression analyses of analyte/internal standard chromatographic peak-area ratios (ordinates) plotted vs. analyte/internal standard mass ratios (abscissae).
[b] Ranges given are for analyte/internal standard mass ratios.
Flavanone absorbances measured at 283 nm; rhoifolin at 335 nm.

concentrate) expressed to 2 significant figures; 1 hg equals 100 g.

The flavonoid levels reported here are in the same general range as the values reported in most previous publications on the subject. Hesperidin in orange juice has been quantitated at 11 [5], 9.8–12.0 (Valencia) and 5.4–10.0 (Hamlin) [7], 31.5–58.3 [8], 7.64–21.9 [9], 29.3–91.5 and 23.5–40.7 [12], and 20–163 and 19.5–350 mg hg^{-1} [14]. Narirutin levels in orange juice have been reported at 1.7–15.3 [8], 2.63–5.42 [9], and 3.69 (Valencia), 8.51 (navel), 4.33 (blood), 8.03 (Thompson), 3.97 (Malta), 5.1–12.8 and 3.0–8.4 mg hg^{-1} [12]. Naringin in grapefruit juice has been quantitated at 23.2–59.9 [4], 21 [6], 30.6 [10], 35.5–46.7 [11], 33.1 (white), 15.91 (pink), 27.54 (red), 25.16 (green), 20.5–20.6, and 11.3–48.1 [12], 13.8–22.7 and 33.6–67.5 [13], and 29.6 and 27.8 mg hg^{-1} [14]. Narirutin levels in grapefruit juice have been reported at 9.6–13.4 [4], 12.4 [10], 10.57 (white), 5.61 (pink), 7.60 (red), 17.86 (green) and 3.3–16.1 [12], and 5.9–7.3 and 10.3–12.2 mg hg^{-1} [13]. Obviously, the large variability in food sample composition precludes direct comparison of results and makes method accuracies difficult to assess. Nonetheless, the high flavonoid content of orange and grapefruit juices, in general, indicates that comparatively large quantities could be consumed in a typical diet.

Based on the levels of hesperidin, naringin, and narirutin found in the Giant/Minute Maid brand juices studied here, a single 8 fluid ounce serving (the standard serving size for fruit juices designated by the Food and Drug Administration [26]) of orange juice would contain 73/90 mg hesperidin (36/44 mg hesperetin) and 14/18 mg narirutin (6.6/8.3 mg naringenin), while 8 fluid ounces of grapefruit juice would contain 120/120 mg naringin (54/57 mg naringenin) and 38/40 mg narirutin (17/19 mg naringenin). These quantities are a substantial fraction of Kühnau's estimate of 1 g daily intake of flavonoid glycosides [1] and exceed the 23–25 mg flavonoid aglycones per day reported by Hertog and coworkers [2,3] who did not include flavanones in their totals. Daily intake of these citrus flavanones could easily exceed that of both β-carotene and vitamin E.

In recent surveys of the American diet, average individual daily consumption of orange and grapefruit juices was estimated as 48 and 4.3 g, respectively [27]. This corresponds to an average daily consumption of 15/18 mg hesperidin, 2.1/2.2 mg naringin and 3.6/4.3 mg narirutin, based on the values obtained for the Giant/Minute Maid juices, respectively. This is higher than the flavanone level reported in the Dutch diet, and up to 50% of the combined total for flavonols and flavones [2,3]. Nonetheless, these quantities are a small fraction of Kühnau's estimate of total flavonoid intake, and do not fully account for the discrepancy in dietary flavonol and flavone levels reported by Hertog and co-workers [2,3] and the levels of all 4-oxo-flavonoids (flavonols, flavones and flavanones) reported by Kühnau [1].

4. Conclusions

The presence of high levels of hesperidin, naringin, and narirutin in orange and grapefruit juice concentrates allows direct HPLC analysis of juice extracts. In the present study, essentially complete extraction of these flavanone glycosides was achieved by extracting multiple times with methanol. For orange juice concentrate, extraction at an elevated temperature resulted in improved recoveries. Flavonoid glycosides were easily separated and detected by reversed-phase HPLC and UV–Vis detection without the need for hydrolysis or derivatization.

Accurate quantitative data are essential to assessing the health benefits of flavonoids in the diet. In addition, the ability to differentiate authentic fruit juices from adulterated products based on flavonoid levels is valuable from a commercial standpoint. The methods presented enable accurate determination of the principal flavonoids in orange and grapefruit juices, and may well be applicable to other citrus not examined in this study. Although the effects of flavanones in humans have not yet been established, these compounds are potentially important dietary components. The increasing evidence that flavonoids may be valuable constituents of the diet underscores the importance of determining their levels in food products.

Acknowledgements

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