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Variation of Flavonoids and Furanocoumarins in Grapefruit Juices: A Potential Source of Variability in Grapefruit Juice−Drug Interaction Studies

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Grapefruit juice (GFJ) has been found to interact with several medications, increasing their oral bioavailability and the risk of toxicity. Inhibition of CYP3A4 in the small intestine by flavonoids (such as naringin and naringenin) and furanocoumarins (including bergamottin and 6′,7′-dihydroxybergamottin) present in GFJ seems to be the predominant mechanism, although P-glycoprotein and influx transporters in the small intestine are also involved. The quantity of interactive compounds ingested may affect the magnitude and mechanism of the food−drug interaction. Therefore, these four compounds were quantified by HPLC analysis in commercially available and fresh-squeezed GFJ and in grapefruit tissues. Considerable variability in naringin (174−1492 μmol/L), bergamottin (1.0−36.6 μmol/L), and 6′,7′-dihydroxybergamottin (0.22−52.5 μmol/L) was observed, whereas naringenin could not be detected. White grapefruit showed higher concentrations of naringin and furanocoumarins located in the albedo and flavedo compared with red varieties. Findings from this study suggest considering concentrations of components with a potential for drug interactions in GFJ−drug interaction studies. The concentration of potentially contributing compounds may crucially influence the magnitude of observed interaction and impair direct comparison of studies in which different juices have been used.

KEYWORDS: Grapefruit; flavonoids; furanocoumarins; HPLC; grapefruit−drug interaction; cytochrome P-450

INTRODUCTION

The health benefits of fruits and vegetables have been confirmed by many studies (*1*). Epidemiological data reveal an association between diets rich in fresh fruits and vegetables and a decreased risk of cardiovascular diseases and certain forms of cancer (*2*−*5*). Grapefruit juice (GFJ), a citrus beverage consumed by many households in the United States labeled with the American Heart Association's "Healthy Heart Check", contains compounds that may both reduce atherosclerotic plaque formation (*1*, *6*, *7*) and inhibit cancer cell proliferation (*8*, *9*). However, unlike other citrus fruit juices, grapefruit juice has been demonstrated to interact with a variety of prescription medications, raising the potential for concern regarding its concomitant consumption with drugs (*10*, *11*). Most notable are its effects on cyclosporine, some calcium antagonists, and statins, leading to elevation of the serum concentrations of these drugs from 1.5- to 15-fold after oral administration (*10*−*12*). For some drugs such as felodipine, nicardipine, and halofantrine the increased drug concentrations have been associated with an increased frequency of dose-dependent adverse effects (*13*−*15*).

The major mechanism for grapefruit−drug interaction is the inhibition of the drug-metabolizing enzyme cytochrome P-450 3A4 (CYP450 3A4) in the small intestine, resulting in a significant reduction of the presystemic metabolism of drugs (*12*). Concomitantly, a rapid decline in the enterocyte CYP450 3A4 levels also was observed (*16*). However, 8 oz (∼237 mL) of regular-strength GFJ three times a day did not affect liver CYP3A4 activity, colon levels of CYP3A5, or small bowel concentrations of P-glycoprotein, villin, CYP1A1, and CYP2D6 (*17*). The intestinal CYP450 3A4 appears to be subjected to a mechanism-based inactivation by compounds present in GFJ (*12*).

Studies using distinct drugs (*18*−*23*) have reported that GFJ also inhibits the intestinal P-glycoprotein (P-gp), a energy-dependent membrane efflux transporter which restricts the absorption of a wide range of substrates such as anticancer and anti-HIV drugs, cyclosporine, digoxin, talinolol, and erythromycin by carrying them from the enterocyte back to the gut lumen (*24*, *25*). However, the clinical relevance of such interaction is still a controversial issue.

Although co-administration of GJF and digoxin (0.5 mg), a P-gp substrate, in healthy subjects had promoted a slight increase (1.1-fold) in the mean plasma concentration time curve of

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digoxin (AUC_{0-24}) compared with water (*26*), it should not be deduced as the absence of interaction between GFJ and P-gp (*27*). Because digoxin has a high oral bioavailability (70−80%), even the total inhibition would be expected to enhance the digoxin oral bioavailability by only 1.2−1.3-fold compared with water (*27*). Therefore, further trials using different substrates are required to clarify such interaction and its clinical implication.

Another important effect of GFJ is the inhibition of the influx transporters in the small intestine, such as the organic anion-transporting polypeptides (OATPs) and organic cation transporters (OCTs), which can mediate the cellular uptake of a large number of structurally unrelated compounds (*28*−*33*) and may be the source of a number of food−drug interactions. In fact, administration of 300 mL of regular-strength GFJ with fexofenadine (120 mg) or talinolol (50 mg) in healthy subjects decreased the mean AUC and the peak plasma concentration (C_{max}) by around 50 and 60%, respectively, compared with the same volume of water (*30*, *33*). Because both drugs are P-gp and OATP substrates and GFJ decreased rather than enhance their oral bioavailability, it seems that some constituents of GFJ preferentially inhibited the intestinal uptake transport instead of the P-gp (*29*, *33*).

Among GFJ components, the flavonoids naringin, naringenin, quercetin, kaempferol, and the furanocoumarins bergamottin and 6′,7′-dihydroxybergamottin and its dimers bergapten, bergaptol, and 6′,7′-epoxybergamottin have been suggested to contribute to GFJ−drug interaction (*12*, *16*, *22*, *34*, *35*). The concentrations of flavonoids and furanocoumarins in GFJ are expected to show considerable variability depending on the origin, fruit variety, maturity, quality of raw material, manufacturing procedures, and storage conditions (*36*−*40*). The amount of active ingredients ingested may be an important factor influencing the mechanism, magnitude, and reproducibility of the grapefruit−drug interaction (*41*), compromising the comparison of data gained in different clinical studies.

A clinical trial with healthy subjects has revealed that the AUC and the C_{max} of felodipine were increased by about 73 and 138% compared with water, respectively, when a single oral dose (10 mg) of the drug was administrated with 200 mL of GFJ (prepared by diluting 50 mL of frozen concentrate with 150 mL of tap water) (*42*). However, in a similar study coadministration of the same oral dose of felodipine and 250 mL of a commercial GFJ increased the AUC and C_{max} values by 305 and 228%, respectively (*43*). Although the variability in magnitude of the interaction might be at least partially explained by inherent differences in enterocyte CYP3A4 content (*17*, *43*), it is also possible that more concentrated or larger volumes of grapefruit juice caused a more pronounced interaction. Furthermore, differences in the concentrations of drug-interacting compounds in the juices used in both studies may have contributed to the discrepancy of results. This points out the potential role of the concentration of components causing drug interactions in juices used in clinical trials.

The objective of this study was to compare the contents of the specific flavonoids (naringin and naringenin) and furanocoumarins (bergamottin and 6′,7′-dihydroxybergamottin) (**Figure 1**) in commercially available and fresh-squeezed GFJ as well as in different tissues of grapefruit in order to assess the potential influence of the variability of these juice components on the variability of data gained in drug interaction studies. These compounds have been already described as the most abundant present in GFJ and play a role in the GFJ−drug interaction (*40*, *44*, *45*).

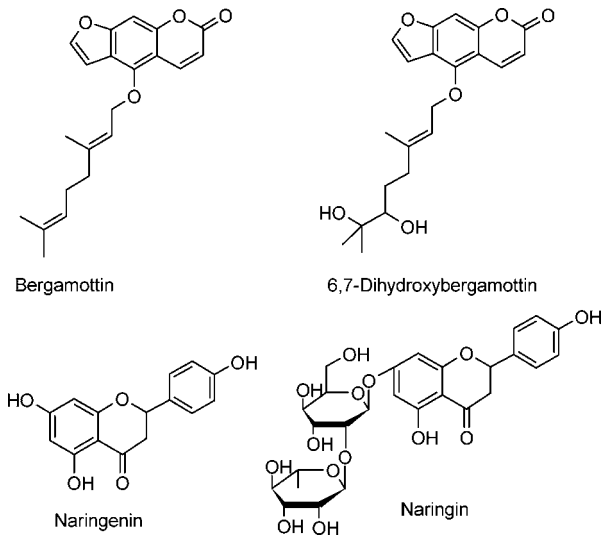

Figure 1. Structures of flavonoids and furanocoumarins present in grapefruit juice.

MATERIALS AND METHODS

Sample Preparation. Twenty-nine commercially available GFJ samples and two types of fresh grapefruit (white and red) were purchased at local supermarkets. Concentrations of flavonoids (naringin and naringenin) and furanocoumarins (bergamottin and 6′,7′-dihydroxybergamottin) in fresh grapefruit were determined in the flavedo layer (0.1 g), pulp (0.1 g), albedo (1.0 g), seeds (1.0 g), and fresh-squeezed juice. Fruit tissues were first homogenized with 6 mL (Flavedo and pulp) or 12 mL (albedo and seeds) of purified water using a PowerGen homogenizer (Fischer Scientific, Pittsburgh, PA) prior to the extraction step. The freshly squeezed juices were obtained by using an HJ 29 handy juicer (Black & Decker, Towson, MD). Samples were analyzed by a Shimadzu VP series HPLC system (Kyoto, Japan) equipped with an SPD-M10Avp diode array detector, an LC-10ATvp solvent delivery unit, an SIL-10AF autosampler, a CTO-10Avp column oven, an SCL-10Avp system controller, a DGU-14A on-line degasser, an FCV-10ALvp low-pressure gradient unit, and Class VP 7.2 SP1 chromatographic software. Additionally, the peak purity software (Class VP 7.2 SP1 chromatographic software, Shimadzu) was applied to the diode array data to test for impurities in all of the chromatographic peaks of interest. All data collected for each sample were reported as means ± SEM for $n \geq 3$.

Determination of Flavonoids (Naringin and Naringenin). The GFJ and the homogenate of tissues (200 μL) were mixed with cold methanol (400 μL), vortexed for 1 min, and centrifuged at 2500*g* for 15 min, as previously described (*38*). After filtration through a 0.45 μm PVDF membrane filter (Millipore Corp., Bedford, MA), the supernatant (25 mL) was injected and analyzed at 285 nm. The flow rate and the temperature were set to 0.5 mL/min and 35 °C, respectively. Mobile phases A and B consisted of water (pH 2.4) (adjusted with orthophosphoric acid) and water (pH 2.4) (adjusted with orthophosphoric acid)/methanol (40:60), respectively. The 250 × 4.6 mm i.d., 5 μm, Lichrospher RP-18 column and Lichrospher 100 RP-18 guard column (Merck KGaA, Darmstadt, Germany) were initially equilibrated during 30 min with solvent A. After sample injection, an initial isocratic run for 5 min was followed by a linear gradient from 100% of A at 5 min to 100% of B at 55 min. This condition was maintained until 70 min and then returned to 100% of A, which was kept constant during 5 min before proceeding to the next injection.

Extraction of Furanocoumarins (Bergamottin and 6′,7′-Dihydroxybergamottin). GFJ and the homogenate of tissues (3 mL) were mixed with ethyl acetate (2 mL). The extraction was performed by shaking the mixtures four times over 30 min. The mixture was centrifuged at 3200*g* for 20 min; the organic phase was collected and evaporated under vacuum. The residue was reconstituted with 600 μL of a DMSO/methanol solution (1:3 v/v).

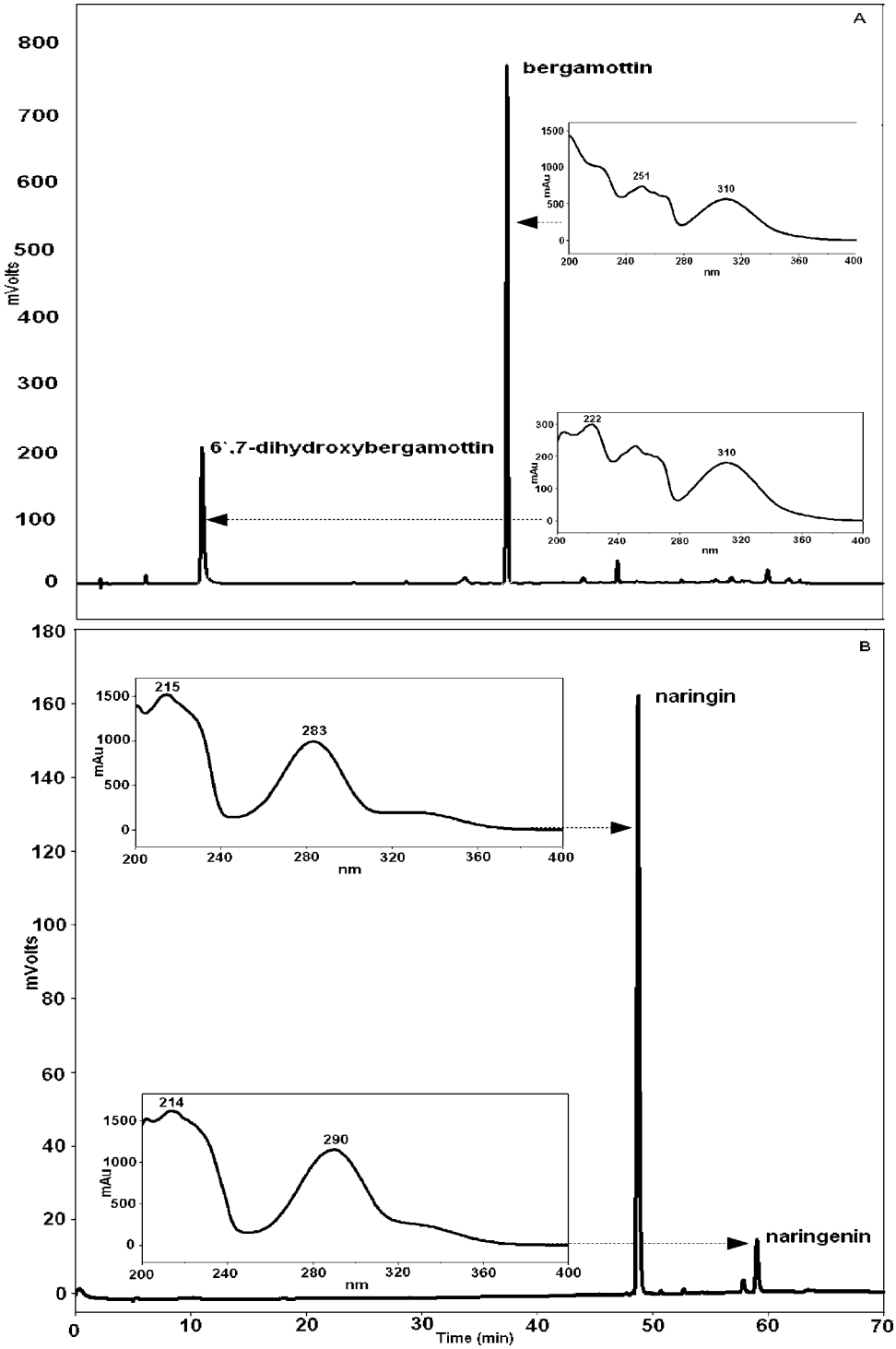

Figure 2. HPLC separation and absorbance-wavelength spectra of (**A**) furanocoumarins 6′,7′-dihydroxybergamottin and bergamottin and (**B**) flavonoids naringin and naringenin.

The reconstituted residues were filtered through a 0.45 μm PVDF membrane filter (Millipore Corp.). Volumes of 25 μL of each sample were injected and analyzed at 310 nm. The flow rate and the temperature were set to 1 mL/min and 35 °C, respectively. Solvents A and B consisted of water and methanol, respectively. The column and guard column (as used for flavonoids) were initially equilibrated with mobile phase consisting of a mixture of solvents A and B (45:55), respectively. Twenty minutes after injection, solvent B was increased linearly from 55 to 100% in 20 min. This condition was maintained for 5 min, after which the system returned to the original mobile phase and was equilibrated for a further 5 min before the next injection.

Calibration and Recovery. Stock solutions of each component were prepared in DMSO. Five different concentrations of standard solutions of naringin (from 50 to 450 μmol/L) and naringenin (from 10 to 50 μmol/L) (Roth GmbH & Co., Karlsruhe, Germany) were prepared in methanol/water (1:1). Six different concentrations of bergamottin (from 2.5 to 250 μmol/L) and 6′,7′-dihydroxybergamottin (from 1 to 100 μmol/L) were prepared in DMSO/methanol (1:3). All solutions were filtered through a 0.45 μm PVDF membrane filter (Millipore Corp.). Bergamottin and 6′,7′-dihydroxybergamottin were kind gifts obtained from Dr. John Manthey at the USDA, SAA Citrus and Subtropical Products Laboratory, Winter Haven, FL. The recovery of each compound was evaluated by adding known amounts of pure compounds to orange juice, from which they are naturally absent (*46*), and extracting them under the same conditions used in the grapefruit samples. The analytical results for extracted samples at three concentra-

Table 1. Naringin, Bergamottin, and 6′,7′-Dihydroxybergamottin Contents in Different Brands of Grapefruit Juice Sold in Florida

grapefruit juice	variety	lot	concentration (μmol/L) naringin	bergamottin	6′,7′-dihydroxybergamottin
brand A	pink	L1	777.6 (4.3)[a] h–k[b]	9.76 (0.5) klm	0.44 (0.01) hij
		L2	772.3 (10.1) h–k	9.03 (0.2) lmn	0.44 (<0.01) hij
brand B[c]		L1	878.9 (32.4) gh	8.79 (1.0) lmn	0.65 (0.04) hij
		L2	939.3 (34.1) fgh	12.13 (0.6) jkl	1.07 (0.02) hij
brand C		L1	668.5 (28.1) i–l	5.98 (0.02) mno	0.35 (<0.01) hij
		L2	654.6 (6.3) i–l	14.24 (0.1) h–k	0.76 (0.02) hij
brand B[c]	white	L1	1,262 (42.1) bc	18.81 (1.3) fgh	1.14 (0.1) hij
		L2	1,492 (44) a	25.86 (1.0) de	2.21 (0.1) hij
brand C		L1	820.4 (43.9) ghi	21.95 (0.5) ef	1.03 (0.03) hij
		L2	589.9 (14) lm	28.04 (0.3) cd	2.29 (0.3) hij
brand D[c]		L1	915.6 (8.8) fgh	15.54 (0.9) g–j	3.37 (0.2) g–j
		L2	651.8 (15.4) i–l	14.71 (0.7) g–j	1.34(0.2) hij
brand E[d]		L1	810.9 (58.5) hij	31.77 (1.1) bc	45.04 (1.8) b
		L2	1,056 (22.6) def	34.37 (1.6) ab	49.01 (2.5) ab
		L3	1,318 (36.3) b	36.34 (1.6) a	52.51 (2.8) a
brand F[c]		L1	1,216 (57) bcd	17.14 (0.4) ghi	0.73 (0.05) hij
		L2	980.6 (7.8) efg	25.42 (0.7) de	1.15 (0.03) hij
brand C	red	L1	174.1 (2.8) o	2.95 (0.03) op	0.28 (<0.01) ij
		L2	238.9 (3.8) o	5.06 (0.1) nop	0.28 (0.01) ij
brand F[c]		L1	234.0 (3.1) o	3.59 (0.3) op	0.22 (<0.01) j
		L2	303.6 (12.6) no	4.02 (0.1) op	0.25 (<0.01) ij
brand D[c]		L1	667.5 (15.7) i–l	13.56 (0.2) g–j	14.57 (0.4) c
		L2	648.4 (14.8) jkl	14.16 (0.20) g–j	17.92 (0.7) c
brand G		L1	303.9 (5.1) no	5.17 (0.3) m–p	0.25 (<0.01) ij
		L2	291.5 (2.8) o	2.58 (0.2) op	0.24 (0.02) ij
brand H		L1	469.4 (18) mn	12.19 (0.5) i–l	4.55 (0.05) j–i
		L2	1,147 (97) cde	12.73 (0.2) jkl	7.38 (0.02) efg
brand I		L1	584.4 (10) lm	19.18 (1.3) fg	8.74 (0.4) def
		L2	613.4 (18.8) klm	18.92 (0.9) fg	12.10 (0.8) cd
fresh squeezed	white		312.6 (9.5) no	0.97 (<0.01) qr	4.71 (0.1) fgh
	red		245.7 (28.3) o	3.10 (0.1) p	10.28 (0.6) cde

[a] Values are means ($n = 3$) with SEM in parentheses. [b] Values with the same letter(s) are not significantly different (Tukey–Cramer multiple comparison, $p \leq 0.05$). [c] From concentrate. [d] Frozen from concentrate.

tions were compared with unextracted standards that represent 100% recovery.

Statistical Analysis. Data were analyzed by one-way analysis of variance (ANOVA) with JMP5 software (SAS Institute Inc., Cary, NC, 1996). Mean separation was conducted using the Tukey−Cramer HSD comparison for all pairs ($p \leq 0.05$). Values with different letters are significantly different ($p \leq 0.05$). The linearity of the standard curves was determined by linear regression using the least-squares method and expressed in terms of correlation coefficient (r). The intra- and interday precision and accuracy of the quantification were measured by replicate analyses ($n = 3$) of three different concentrations of standards on the same day and on alternate days. The precision was based on the calculation coefficient of variation (CV%), and the accuracy was expressed as percent of the found amount compared to the theoretical one. The limits of quantification (LOQ) and detection (LOD) were expressed on the basis of the mean values of the intercept (Y_{bl}), the standard deviation (S_{bl}) of the blank responses, and the slope (b) of the calibration curve (eqs 1 and 2) (*47*).

$$\mathrm{LOQ} = \frac{Y_{bl} + 3S_{bl}}{b} \quad (1)$$

$$\mathrm{LOD} = \frac{Y_{bl} + 10S_{bl}}{b} \quad (2)$$

The concentration of each compound in the juice and tissues was expressed as the mean of the three determinations ± SEM. Asterisks indicate significant differences for white grapefruit compared to red grapefruit ($p \leq 0.05$).

RESULTS AND DISCUSSION

Calibration and Recovery. The methods provided good resolutions between naringin and naringenin as well as between bergamottin and 6′,7′-dihydroxybergamottin. The wavelengths 285 and 310 nm used to quantify flavonoids and furanocoumarins at their maximum absorption, respectively, were confirmed by their UV spectra (**Figure 2**).

There was no endogenous interference from GFJ or orange juice in either assay, indicating specificity of both methods to the tested compounds. The results of the analysis with the peak purity software suggested the absence of impurities for all compounds. Peaks of the flavonoids and furanocoumarins had similar retention times and UV spectra (200−400 nm) in all samples when compared to the standards.

The precisions intra- and interday for both flavonoids and furanocoumarins were satisfactory with CV values between 0.55 and 6% (*48*). The accuracy of the assay was between −7 and 14.8% for all compounds tested at three different concentrations. The LODs for naringin, naringenin, bergamottin, and 6′,7′-dihydroxybergamottin were 0.47, 0.56, 0.01, and 0.04 μmol/L, respectively, whereas the LOQs were 1.25, 1.11, 0.02, and 0.05 μmol/L. The recoveries from orange juice spiked samples were between 97.7 and 106% (data not shown). Therefore, the analytical methods applied were suitable to quantify the predominant flavonoids and furanocoumarins in the juice and in different tissues of the grapefruit.

Quantification of Naringin, Naringenin, Bergamottin, and 6′,7′-Dihydroxybergamottin in Grapefruit Juices. The values found for all compounds (**Table 1**) are in the same general range as those reported in previous publications except for naringenin, which was absent in all samples tested. In contrast to a previous study in which concentrations of naringenin were found from 19.5 to 595 μmol/L in 8 different brands of GFJ (*40*), we did

not detect this flavonoid in any of the 14 brands of juices analyzed, which was also in agreement with other authors (*49*). However, a great variability of naringin (from 174 to 1492 μmol/L), bergamottin (from 1.0 to 36.6 μmol/L), and 6′,7′-dihydroxybergamottin (from 0.22 to 52.5 μmol/L) contents was observed among all tested brands of GFJ. Although Bronner and Becheer (*44*) found similar concentrations of naringin (~850 μmol/L) in two different brands of GFJ (Giant and Minute Maid), a large variability of naringin, bergamottin, and 6′,7′-dihydroxybergamottin in GFJ has also been confirmed by different authors with ranges of 218−2062 μmol/L (*40*), 2.0−28.3 μmol/L (*20*), and 9.1−42 μmol/L (*20*), respectively. In the fresh-squeezed GFJ the highest concentration of naringin (312.6 μmol/L) was measured in the white variety, whereas the red fruit provided the highest contents of bergamottin (3.1 μmol/L) and 6′,7′-dihydroxybergamottin (10.3 μmol/L).

In the pink GFJ (A−C) the concentrations of 6′,7′-dihydroxybergamottin intra- and interlot were not statistically different ($p > 0.05$) and brand B showed a higher content of naringin when compared with the other two brands, although the variability of this flavonoid between the lots was not significant. Additionally, the content of bergamottin in lot 2 of brand C was ~2.4-fold higher than in lot 1. Intralot comparisons revealed that the content of naringin was significantly different ($p < 0.05$) in all brands of white GFJ used in this study. The white brands D and E showed similar concentrations of bergamottin within lots. In red juices only brand H showed significant intralot variability of naringin and 6′,7′-dihydroxybergamottin.

The concentrations of bergamottin and 6′,7′-dihydroxybergamottin in brand E (frozen from concentrate) were significantly higher when compared to all other juices. Although there is a lack of detailed information regarding the stability of furanocoumarins (bergamottin and 6′,7′-dihydroxybergamottin) present in GFJ, brand E is sold as a frozen product, which may possibly have prevented extensive degradation of furanocoumarins compared to juices stored at room temperature.

In general, the white GFJ showed the highest levels of naringin, bergamottin, and 6′,7′-dihydroxybergamottin with mean concentrations of 1010 ± 87, 24.5 ± 2.3, and 14.5 ± 6.7 μmol/L, respectively. Additionally, the red variety showed higher amounts of bergamottin (9.5 ± 1.8 μmol/L) and 6′,7′-dihydroxybergamottin (5.6 ± 1.9 μmol/L) compared to the pink variety, although the mean concentration of naringin was ~1.7-fold lower.

Localization of Naringin, Naringenin, Bergamottin, and 6′,7′-Dihydroxybergamottin in Grapefruit. A comparison of concentrations and distribution of the target compounds between fresh white and red grapefruit varieties (**Figure 3**) revealed that extracts from white grapefruit showed higher concentrations of naringin (3152 μg/g), bergamottin (19.8 μg/g), and 6′,7′-dihydroxybergamottin (106 μg/g) located in the albedo and flavedo. The lowest concentrations were found in the seeds and pulp of red grapefruit. These results are in agreement with the values found in the commercial juices where, in general, the white grapefruit juices showed the highest contents of naringin, bergamottin, and 6′,7′-dihydroxybergamottin compared to the pink or red varieties. A similar study was conducted to determine the content of the dimers of bergamottin and 6′,7′-dihydroxybergamottin in the white and red grapefruits (*39*).

Bergamottin and 6′,7′-dihydroxybergamottin were able to inhibit the human microsomal CYP3A-mediated testosterone 6β-hydroxylation by half at 22 and 2 μmol/L, respectively (*45*). With regard to their effects on P-gp activity, bergamottin (10 μmol/L) and 6′,7′-dihydroxybergamottin (33 μmol/L) have been

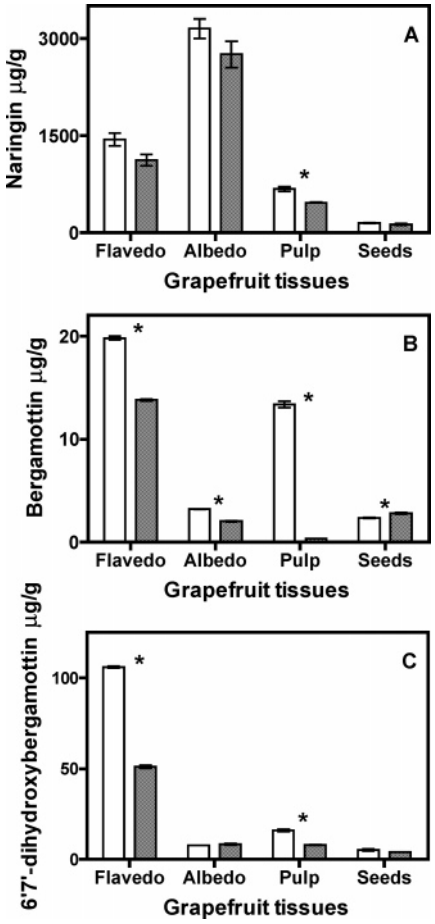

Figure 3. Distribution of naringin, bergamottin, and 6′,7′-dihydroxybergamottin in different tissues of white (open bars) and red (shaded bars) grapefruits. Values are means ± (SEM) ($n = 3$). Asterisks indicate significant differences between values of white compared to red grapefruit.

reported to reduce the activity of P-gp in vitro by 58 and 50%, respectively (*29*, *49*). In addition, 6′,7′-dihydroxybergamottin has been shown to be a potent inhibitor of rat OATP3 ($IC_{50} = 0.28$ μmol/L) (*29*). Although the flavonoid naringin has been excluded as the principal CYP3A4 inhibitor in GFJ (*49*, *51*, *52*), it promoted a significant inhibition on P-gp and OATP activity in vitro at concentrations of 1000 and 5 μmol/L, respectively (*29*, *31*). Naringin has also been shown to be hydrolyzed into the more potent CYP3A4 inhibitor naringenin by the gastrointestinal microflora located preferentially in the distal part of the small intestine and in the colon (*53*). The clinical relevance of this interaction is uncertain because most of the drug absorption takes place in the small intestine. Overall, the concentrations of naringin, bergamottin, and 6′,7′-dihydroxybergamottin in the samples analyzed in this study appear to be high enough to considerably decrease CYP3A4, P-gp, and OATP activities. Other GFJ constituents (not tested in this study) have been shown to inhibit the CYP3A4 activity, such as the dimeric compounds ($IC_{50} < 1$ μmol/L) (*45*) and epoxybergamottin ($IC_{50} = 4.2$ μmol/L) (*55*). Additionally, the flavonoids quercetin and kaempferol have been shown to inhibit the organic cation transporters (OCT) by half at 32 and 38 μmol/L, respectively (*31*).

In general, the GFJ−drug interactions studies are characterized by a wide variability in the pharmacokinetics and pharmacodynamics data among patients from the same study, as well as between different studies. Variations in the intestinal concentrations of CYP3A4 among individuals seem to contribute

to the differences in the intensity of this interaction (*55*). In addition, it was speculated that the high variability of components in GFJ may also increase the risk of an interaction in patients who drink GFJ habitually and appear to be equilibrated during a drug therapy, if the brand or even the lot is switched during drug therapy with a susceptible drug (*11*). However, further studies comparing the effect of different brands on the magnitude of the GFJ−drug interaction are needed to clarify this issue. Additionally, the correlation between the content of the interacting compounds in a GFJ preparation and their inhibitory effects would help to estimate the role of each component on the overall GFJ-mediated inhibition and also to predict and circumvent such interactions (*56*).

On the basis of the demonstrated variability and lack of information regarding the profile flavonoids and furanocoumarins of juices used for human trials, a direct comparison between different studies is impaired. Therefore, to minimize prevalent variability in GFJ−drug interaction trials, it is important to correlate measured endpoints to the concentrations of those grapefruit components that are considered to be relevant for the interaction with drugs.

In future studies investigating grapefruit−drug interactions, concentrations of compounds with a suspected interaction should be considered. When possible, juices of very similar composition should be used for studies designed for direct comparison. In addition, for animal studies the concentrations of critical compounds may be adjusted by supplementation to normalize the administered juices.

ABBREVIATIONS USED

CYP, cytochrome; DMSO, dimethyl sulfoxide; GFJ, grapefruit juice; LOD, limit of detection; LOQ, limit of quantification; OATPs, organic anion-transporting polypeptides; P-gp, P-glycoprotein; OCT, organic cation transporters; AUC, area under the plasma concentration time curve; C_{max}, peak plasma concentration; IC_{50}, concentration able to inhibit 50% of the normal enzyme activity.

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