Variation in Furanocoumarin Content and New Furanocoumarin Dimers in Commercial Grapefruit (*Citrus paradisi* Macf.) Juices

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ABSTRACT: Grapefruit (*Citrus paradisi* Macf.) sales and crop value have declined during the last decade. One reason is consumer concerns about possible drug interactions. Coadministered grapefruit increases the bioavailability of some medicines because it contains furanocoumarins that inhibit an intestinal enzyme (cytochrome P450-3A4 or CYP3A4) that normally metabolizes these drugs. Only drugs metabolized by intestinal CYP3A4 are significantly affected when taken with grapefruit juice, but the magnitude of the effect varies considerably between studies, indicating that there are differences in the amount of components responsible for CYP3A4 inhibition in commercial grapefruit juices. Content variation of 6,7-dihydroxybergamottin, bergamottin, and 6 furanocoumarin dimers were determined for 58 commercial grapefruit juices collected over 2 seasons. The content of 6,7-dihydroxybergamottin ranged from 0.2 to 7.7 ppm in all juices tested, and averaged 1.8 ± 0.85 ppm in the red compared with 2.9 ± 2.07 ppm for white grapefruit juices. Bergamottin content ranged from 1.6 to 7.3 ppm for all juices and averaged 3.4 ± 0.98 and 4.2 ± 1.23 ppm and in red and white grapefruit juices, respectively. Only 1 dimer varied significantly between the red and white juice types. Dihydroxybergamottin and 2 dimer compounds were significantly lower in shelf-stable or nonrefrigerated products compared with refrigerated products whereas bergamottin and 1 dimer compound occurred at higher levels in the shelf-stable products. Individual measured dimer compounds varied up to 60-fold in all juices, but the sum of all 6 dimers varied only 14-fold. The clinical significance in terms of CYP3A4 inhibition is uncertain. The presence of 3 new furanocoumarin dimers are also reported.

Keywords: grapefruit, CYP3A4, P450, furanocoumarin, psoralen, coumarin, drug interaction, *Citrus paradisi*

Introduction

Grapefruit (*Citrus paradisi* Macf.) juice, when consumed with certain orally administered medications, has been shown to increase their bioavailability. This interaction was 1st reported when grapefruit juice was administered with the calcium antagonist felodipene (Bailey and others 1989). After more than a decade of research, it has been shown that the interaction occurs because a number of grapefruit components cause inhibition of an intestinal P-450 enzyme, cytochrome P-450 3A4 (CYP3A4), a topic that has been well reviewed (Ameer and Weintraub 1997; Dresser and others 2000; Kane and Lipsky 2000; Greenblatt and others 2001; Dahan and Aftman 2004; Huang and others 2004). Although CYP3A4 is also present in the liver and has been well documented as being involved in drug-drug interactions, grapefruit juice has no effect on hepatic CYP3A4 unless consumed in very large quantities (Dresser and others 2000; Greenblatt and others 2001; Veronese and others 2003).

Naringin was initially believed to be the active CYP3A inhibitor in grapefruit juice (Bailey and others 1992; Fuhr and others 1993; Fuhr and Kummert 1995). Although naringin does have some inhibitory effect in vitro on CYP3A4, it has very little or no effect in vivo (Bailey, and others 1993a, 1993b, 1998). The furanocoumarins in grapefruit were 1st associated with CYP3A inhibition in vitro (Edwards and Bernier 1996; Edwards and others 1996) with 6′,7′-dihydroxybergamottin (DHB; shown in Figure 1). Since then, research has demonstrated that DHB is not the only component in grapefruit that causes CYP3A inhibition (Fukuda and others 1997; Guo and others 2000; Tassaneeyakul and others 2000), and it is now fairly well accepted that the components in grapefruit largely or completely responsible for the CYP3A4 inhibition are naturally occurring furanocoumarin monomers and dimers (Dresser and others 2000; Kane and Lipsky 2000). The structures shown in Figure 1 all have been identified as inhibitors of CPY3A4 activity. Dimer compounds identified are composed of 2 furanocoumarins either linked by joining the terpene sidechains (tail-to-tail) or by linking the terpene side chain to the ketone oxygen on the aromatic ring (head-to-tail). The Japanese researchers (Fukuda and others 1997; Guo and others 2000; Tassaneeyakul and others 2000) isolated and identified only 1 dimer with a head-to-tail linkage; however, Harris (1999) isolated and identified several naturally occurring head-to-tail furanocoumarin dimers from grapefruit juice, calling them orthospiroesters (OSEs). Harris has since claimed that the OSEs are the only significant active CYP3A4-inhibiting components in grapefruit juice and has been issued several patents on isolation, extract preparation, and their potential utilization in pharmaceutical applications (Harris 1998, 1999, 2001, 2002, 2003).

Several studies have identified and compared CYP3A4 inhibition activity between furanocoumarin monomer and dimers. DHB and the dimer compounds are approximately 10 and 200 times more active than bergamottin, respectively (Fukada and others 1997; Tas-

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Table 1—Range in component amounts, expressed as ppm, from grapefruit juice samples collected and tested in Japan[a]

	Tassaneeyakul and others (2000)		Guo and others (2000)	
Compound	Range (n = 28)	Variation	Range (n = 7)	Variation
6′,7′ DHB (GF-I-3)	—	—	0.3 TO 12.8	43 X
Bergamottin (GF-I-2)	1.9 to 7.4	4 X	2.4 to 10.0	4 X
GF-I-1 (tail/tail dimer)	ND to 0.39	—	0.02 to 0.44	15 X
GF-I-4 (tail/tail dimer)	0.08 to 0.44	5.5 X	0.07 to 0.37	5 X
GF-I-5 (epoxide)	—	—	0.17 to 0.27	1.6 X
GF-I-6 (orthospiroester)	—	—	ND to 6.31	—

[a]ND = not detected.

Table 2—High-performance liquid chromatography (HPLC) gradient profile

Time (min)	Water (%)	Acetonitrile(%)
0	90	10
5	90	10
30	20	80
40	20	80
41	5	95
50	5	95
53	90	90
65	90	10

saneeyakul and others 2000). These same studies also showed that even though the dimers were 200 to 500 times more potent than bergamottin, concentration of the dimers in grapefruit juice were considerably lower (Table 1). The content of these components in grapefruit juice has been shown to vary considerably in Japanese products. A study done on 7 commercial grapefruit juice samples collected in Japan found bergamottin content varied 4-fold, DHB content varied 43-fold, and 3 dimer compounds (structures, Figure 1) collectively varied greater than 65-fold (Guo and others 2000). A 2nd study comparing 28 commercial grapefruit juice samples from the market in Japan found approximately the same extent of variation (Tassaneeyakul and others 2000). Because the commercial grapefruit juices tested in Japan contained products from the United States, Australia, and Israel and were for the most part products reconstituted and packaged in Japan, it seemed appropriate to conduct a survey of commercial grapefruit juice products produced in the United States to determine furanocoumarin variation in U.S. products.

Materials and Methods

Chemicals

Bergamottin (5-geranoxypsoralen) and bergapten (5-methoxypsoralen) were purchased from Extrasynthase (Genay, France). The 6,7-dihydroxybergamottin was a gift from Antonio Montanari obtained by extraction and purification with preparative high-performance liquid chromatography (HPLC) from grapefruit peel. Water, ethyl acetate, and acetonitrile used were HPLC-grade. Commercial grapefruit juice samples were collected from the retail market throughout the United States as part of the Juice Monitoring and Adulteration Program at the State of Florida Dept. of Citrus. A total of 29 white and 29 red commercial grapefruit juices samples were collected over 2 seasons. They consisted of 25 red and 7 white pasteurized grapefruit juices not from concentrate, and 4 red and 22 white grapefruit juices reconstituted from concentrate. Samples were divided, placed into 125-mL amber plastic containers, frozen, and stored at –8 °C until analyzed.

Sample preparation

A 31.2-g amount of thoroughly mixed juice (equivalent to 30 mL) was weighed and extracted with 20 mL of ethyl acetate for 15 min with agitation. Samples were weighed to avoid problems associated with pipetting liquids containing pulp and other particulates. Layers were separated by centrifugation, and the ethyl acetate was decanted. Extractions were then repeated 3 times using 10 mL of ethyl acetate. The ethyl acetate extracts were combined, concentrated to near dryness in a rotary evaporator at 25 °C, transferred to a 5-mL volumetric flask with ethyl acetate, and made to volume. They were then filtered through a 0.45-μm nylon filter and analyzed immediately or stored at 4 °C overnight before analysis. Extractions were repeated for the grapefruit juice samples with the 10 highest and 10 lowest determined values for 6,7-DHB to confirm high and low values. Standard deviations between replicate extractions were less than 15%.

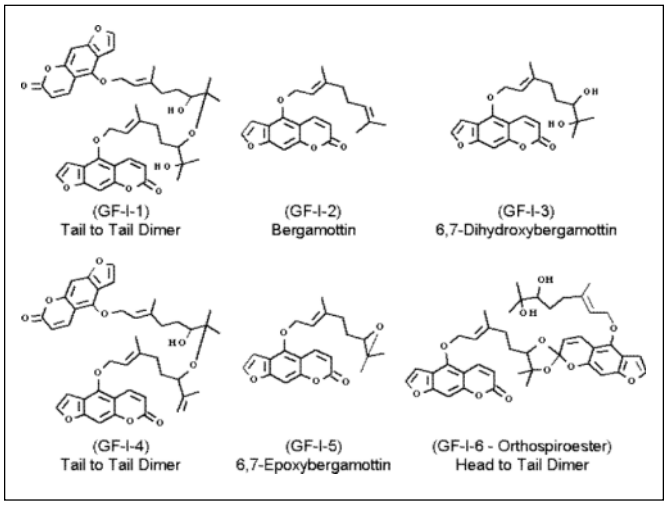

Figure 1—Structures of CYP3A4 active furanocoumarins from grapefruit juice (Guo and others 2000).

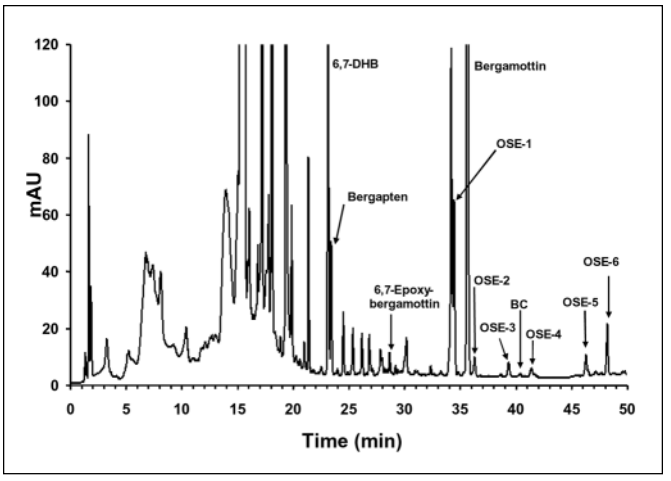

Figure 2—Chromatographic separation at 310 nm of a pasteurized white grapefruit juice with selected peak identifications. Conditions are as described in the text.

Pulp content

Pulp and particulate content were determined in each juice sample by measuring 25-g sample into a 30-mL centrifuge tube. Samples were then centrifuged at 14000 × *g* for 15 min, and the wet pellet was weighed after decanting the clear juice supernatant.

High-performance liquid chromatography analysis

Sample extracts were analyzed using a procedure described by Harris (1999). A Thermo Electron Corp. (San Jose, Calif., U.S.A.) Spectrasystem HPLC, which consisted of a P4000 pump, AS3000 autosampler with oven and sample tray temperature control, P6000 photodiode array detector (PDA), and Chromquest V3.0 system control and data analysis software, was used. Samples were analyzed in duplicate using 5-μL injections, water:acetonitrile gradient as listed in Table 2, and flow rate of 0.5 mL/min with a YMC J-Sphere M80 3 × 250 mm 4μ C-18 column (Waters, New Bedford, Mass., U.S.A.). Data were collected on the PDA from 200 to 360 nm, 1 s rise time, and 5-nm window. Quantification was performed using response factors determined for bergamottin and DHB at 310 nm.

Liquid chromatography-mass spectrometry analysis

Several samples were also analyzed on a Thermo Electron Corp. Finnegan LCQ Advantage LC-MS system. The column and chromatography conditions used were identical to those for HPLC analysis. Electrospray ionization was used with a spray voltage of 5 kV, ion transfer tube voltage of 12 V at 285 °C, nitrogen sheath and auxiliary gas flow rates of 26 mL/min and 13 mL/min, respectively, and a tube lens offset of 35 V. Positive ion scans were collected from 150 to 1400 amu.

Results and Discussion

Separation of a typical white grapefruit juice is shown in Figure 2 with furanocoumarin monomer and dimer compounds identified. Peaks eluting before 20 min are primarily flavonoids and hydroxycinnamic acids. Bergamottin, DHB, and bergapten were identified by comparison of retention times, UV spectra, and mass spectral data with those of authentic standards. Identification of epoxybergamottin was possible due to its abundance in grapefruit peel. Extraction and analysis of grapefruit peel samples allowed identification of epoxybergamottin from UV and mass spectral characteristics (M+1 355, m/e 203, 339). Epoxybergamottin was identified in the juice samples based on retention time, UV, and mass spectral characteristics. Another compound was tentatively identified as a bergamottin congener (BC) based on UV absorbance and mass spectral data. Epoxybergamottin and BC were not quantified because of their presence in such small amounts. Three orthospiroesters (OSEs) were identified by chromatographic retention (Harris 1999), UV absorbance (Harris 1999), and mass spectral characteristics (Fukuda and others 1997). OSEs differ from furanocoumarin dimers that are joined tail-to-tail by the more prominent UV absorbance at 245 nm (Figure 3a and 3b). An additional 3 peaks were also identified as OSEs based on mass spectral and UV absorbance characteristics. Mass spectral characteristics used to identify the 6 compounds as OSEs (Figure 4) were as follows: OSE1: MW 726 (M+1 727), m/e 203, 337, 355, 373, 557; OSE2: MW indeterminate, m/e 203, 337, 339, 355; OSE3: MW 708 (M+1 709), m/e 203, 337, 357; OSE4: MW indeterminate, m/e 203, 337, 339, 355; OSE5: MW 708 (M+1 709), m/e 203, 337, 355, 557; OSE6: MW 708 (M+1 709), m/e 203, 339, 355, 557. Values for m/e at 203 correspond to a protonated hydroxypsoralen, 337 and 339 are M–1 and M+1 ions, respectively, for bergamottin, and 355 corresponds to loss of water from the M+1 ion for DHB. The m/e 557 corresponds to an OSE after loss of a monoterpene alcohol side chain. Previously, Guo and others (2000) had reported only 1 naturally occurring head-to-tail furanocoumarin dimer present in grapefruit juice, and Harris (1999) reported retention data for only 3 head-to-tail dimer compounds.

Values for the components found in each red grapefruit juice sample are listed in Table 3 along with the range, average, and standard deviation for each component. Component values for white grapefruit juices are listed in Table 4. The amounts calculated are

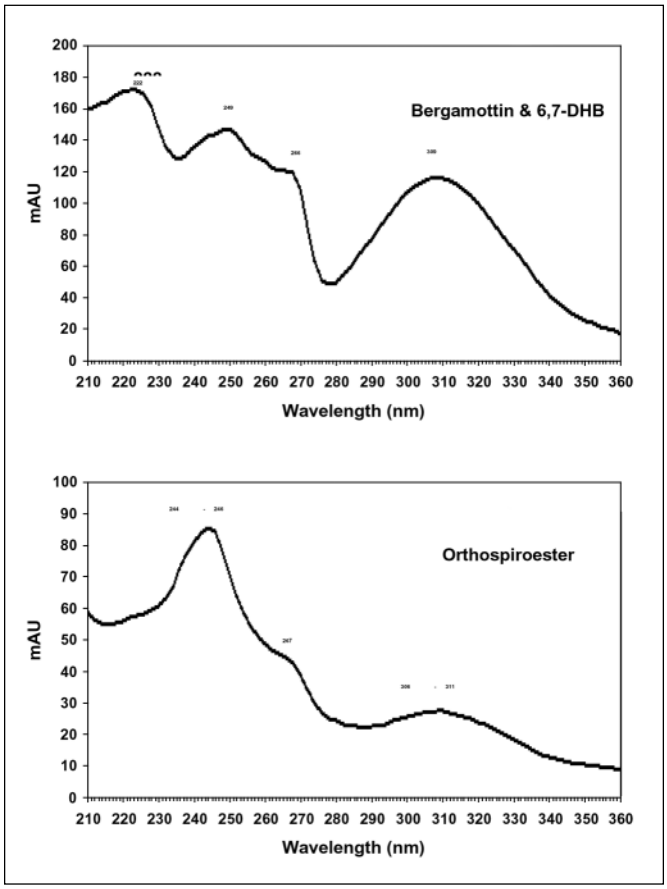

Figure 3—UV absorption spectra for (a) bergamottin and (b) 6,7-dihydroxybergamottin orthospiroesters.

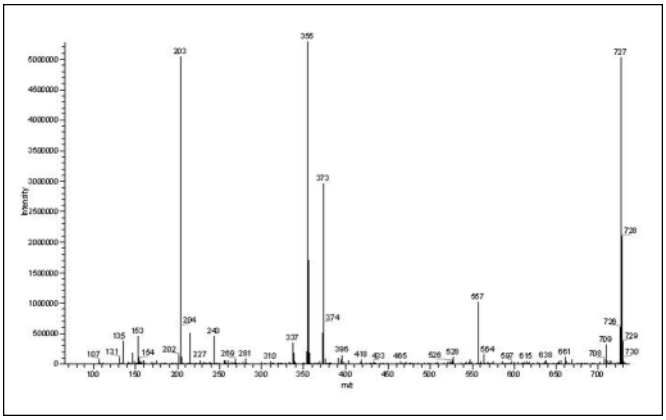

Figure 4—Mass spectra of orthospiroester 1 (OSE1) showing characteristic ions.

Table 3—Amounts and variation of components in red grapefruit juice samples

Red grapefruit		Compound amounts (ppm)							
Sample nr	Type	6,7-DHB	BERG	OSE1[a]	OSE2[a]	OSE3[a]	OSE4[a]	OSE5[a]	OSE6[a]
S1_G15	FC-NR	0.25	3.73	0.02	0.01	0.07	0.01	0.02	0.10
S2_G26	FC-R	1.34	1.56	0.12	0.01	0.02	ND	0.02	0.04
S1_G16	FC-R	0.22	2.19	0.03	ND	0.05	ND	0.11	0.04
S1_G17	FC-R	3.16	3.17	0.38	0.05	0.08	0.02	0.05	0.13
S1_G11	NFC-R	0.80	1.85	0.23	0.05	0.04	ND	0.03	0.10
S2_G10	NFC-R	1.56	2.36	0.08	0.01	0.04	0.01	0.01	0.06
S2_G02	NFC-R	1.26	2.69	0.42	0.07	0.06	0.01	0.06	0.14
S1_G13	NFC-R	1.08	2.76	0.31	0.06	0.06	0.01	0.05	0.16
S2_G19	NFC-R	1.64	2.78	0.17	0.03	0.04	0.01	0.01	0.09
S2_G03	NFC-R	2.80	2.92	0.36	0.07	0.05	0.01	0.05	0.11
S1_G14	NFC-R	1.15	2.94	0.44	0.08	0.07	0.03	0.09	0.19
S1_G02	NFC-R	1.89	3.01	0.33	0.07	0.07	0.02	0.07	0.16
S1_G29	NFC-R	1.38	3.07	0.45	0.08	0.08	0.03	0.10	0.21
S1_G12	NFC-R	1.24	3.09	0.37	0.07	0.07	0.01	0.06	0.17
S2_G24	NFC-R	2.01	3.22	0.35	0.06	0.05	0.01	0.05	0.12
S2_G09	NFC-R	2.53	3.28	0.47	0.07	0.07	0.02	0.07	0.18
S1_G05	NFC-R	1.19	3.32	0.49	0.09	0.08	0.03	0.07	0.20
S2_G07	NFC-R	1.53	3.32	0.14	0.02	0.06	0.01	0.02	0.07
S2_G14	NFC-R	2.17	3.35	0.73	0.10	0.10	0.02	0.11	0.21
S1_G23	NFC-R	1.15	3.37	0.19	0.04	0.07	0.02	0.03	0.11
S1_G01	NFC-R	1.72	3.56	0.48	0.07	0.07	0.02	0.08	0.18
S2_G06	NFC-R	2.80	3.56	0.26	0.04	0.05	0.01	0.04	0.12
S1_G30	NFC-R	1.85	4.40	0.61	0.12	0.13	0.04	0.12	0.31
S2_G23	NFC-R	2.54	4.53	0.47	0.07	0.08	0.02	0.08	0.21
S1_G19	NFC-R	2.32	4.56	0.93	0.13	0.13	0.08	0.16	0.33
S2_G01	NFC-R	2.41	4.56	1.01	0.16	0.12	0.03	0.19	0.33
S1_G28	NFC-R	1.93	4.65	0.74	0.15	0.14	0.04	0.16	0.34
S1_G20	NFC-R	2.21	4.67	0.77	0.13	0.11	0.04	0.14	0.33
S2_G11	NFC-R	4.12	6.19	1.22	0.17	0.18	0.12	0.21	0.47
Min		0.22	1.56	0.02	ND	0.02	ND	0.01	0.04
Max		4.12	6.19	1.22	0.17	0.18	0.12	0.21	0.47
Average		1.80	3.40	0.43	0.07	0.08	0.02	0.08	0.18
Std dev		0.85	0.98	0.29	0.05	0.04	0.03	0.05	0.10
Variation		19 X	4 X	62 X	—	9 X	—	21 X	12 X

[a]Classified as active by Harris, quantified as bergamottin @ 310 nm. FC-NR = not refrigerated from concentrate; FC-R = refrigerated from concentrate; ND = not detected; NFC-NR = not refrigerated and not from concentrate; NFC-R = refrigerated not from concentrate.

an average of at least 2 replicate analyses. Standard deviations for replicate injections was less than 8%. For samples with the 10 highest and 10 lowest values of DHB, amounts listed are the average of 2 sample extractions with replicate injections. Standard deviations were less than 15% for replicate extractions. Amounts of the OSE components listed were determined using the response factor for bergamottin (at 310 nm) and likely do not represent actual amounts present. These values are useful for comparing relative levels of OSEs between juices. Bergapten amounts were not determined as this standard was not obtained until the end of the study.

All red and white grapefruit juice samples were compared statistically using single factor analysis of variance (ANOVA) and 2-sample *t*-test, assuming unequal variances. DHB averaged 1.8 ± 0.85 ppm in the red juices compared with 2.9 ± 2.07 ppm in the white juice types and was significant at $P < 0.02$. Bergamottin (red = 3.4 ± 0.98 ppm versus white 4.2 ± 1.27 ppm) was also significantly different at $P < 0.01$. Only 1 OSE (OSE3) varied significantly between the red and white juice types. Looking at the samples types, it can be noticed that 25 of the 29 red type juice samples are products that were refrigerated not-from-concentrate juice, whereas 22 of the white juice products were reconstituted from concentrate. Additionally, all but 1 of the red juice types were refrigerated product, whereas 11 of the white grapefruit juices were shelf-stable or nonrefrigerated products. This makes any conclusions regarding the effects of color alone difficult because of additional processing done to the majority of white juice types during concentration and reconstitution steps. Several experiments were performed to test the effect of extraction settings, a pasteurization step, and concentration on furanocoumarin content (data not shown). Concentration, pasteurization, and squeeze pressure settings were found to not have a significant effect on the furanocoumarin content, although extreme evaporator and pasteurization temperatures were not tested. Preliminary data from a 2-year variety and maturity study that is almost complete suggest a variety effect. The variety Ruby Red consistently contained lower amounts of DHB and bergamottin compared with Marsh White, Flame, and Rio Red varieties. With approximately one half of the red grapefruit grown in Florida being Ruby Red, less DHB and bergamottin in juice from Florida red varieties can be expected compared with white juice types because Flame and Rio Red contained about as much of these compounds as the Marsh White variety. However, when the 25 refrigerated not-from-concentrate red and 6 refrigerated not-from-concentrate white juice samples were compared, no significant differences were seen for any of the furanocoumarins.

This would suggest other factors besides color having an effect on juice furanocoumarin content. When the refrigerated and nonrefrigerated shelf-stable juices were compared (Table 5), the shelf-stable juice type had significantly less DHB ($P < 0.01$), OSE1 ($P < 0.01$), and OSE2 ($P < 0.01$). There were also significantly higher amounts of bergamottin ($P < 0.01$) and OSE3 ($P < 0.01$) in the shelf-stable juices.

Like the results in the 2 Japanese studies (Guo and others 2000; Tassaneeyakul and others 2000), no correlation was found between furanocoumarin content and pulp amount in this study, which is

Table 4—Amounts and variation of components in white grapefruit juice samples

White grapefruit		Compound amounts (ppm)							
Sample nr	Type	6,7-DHB	BERG	OSE1[a]	OSE2[a]	OSE3[a]	OSE4[a]	OSE5[a]	OSE6[a]
S1_G06	FC-NR	0.69	3.56	0.09	0.02	0.09	0.01	0.02	0.08
S1_G27	FC-NR	2.55	3.63	0.35	0.06	0.07	0.01	0.06	0.15
S1_G22	FC-NR	0.27	3.93	0.05	0.01	0.12	0.01	0.03	0.09
S1_G03	FC-NR	2.50	4.35	0.28	0.05	0.13	0.01	0.07	0.20
S2_G28	FC-NR	0.28	4.85	0.01	0.01	0.15	0.01	0.04	0.13
S2_G20	FC-NR	0.63	5.07	0.12	0.02	0.12	0.01	0.06	0.17
S1_G18	FC-NR	0.49	5.30	0.10	0.03	0.18	0.01	0.05	0.21
S2_G15	FC-NR	1.10	5.67	0.14	0.04	0.16	0.02	0.05	0.19
S1_G31	FC-NR	1.99	7.26	0.25	0.04	0.24	0.03	0.11	0.34
S1_G25	FC-R	6.53	1.75	0.27	0.03	0.05	ND	0.04	0.08
S2_G27	FC-R	2.01	2.44	0.27	0.03	0.04	0.01	0.05	0.08
S2_G08	FC-R	4.04	2.61	0.32	0.04	0.04	0.01	0.05	0.11
S2_G21	FC-R	4.02	3.80	0.34	0.04	0.07	0.01	0.07	0.16
S1_G24	FC-R	4.13	3.89	0.65	0.07	0.10	0.01	0.10	0.19
S2_G05	FC-R	3.71	3.90	0.55	0.06	0.09	0.02	0.07	0.15
S2_G22	FC-R	4.96	3.93	0.61	0.09	0.09	0.02	0.09	0.17
S2_G18	FC-R	5.00	4.21	0.60	0.06	0.09	0.01	0.08	0.15
S2_G16	FC-R	4.81	4.24	0.69	0.07	0.11	0.01	0.09	0.18
S2_G04	FC-R	4.27	4.48	1.10	0.15	0.14	0.03	0.15	0.25
S2_G17	FC-R	5.64	4.52	0.68	0.12	0.10	0.01	0.11	0.22
S1_G04	FC-R	7.66	4.68	0.97	0.13	0.15	0.04	0.16	0.31
S1_G10	FC-R	2.05	6.72	0.33	0.06	0.22	0.04	0.10	0.31
S1_G09	NFC-NR	1.08	5.89	0.36	0.01	0.13	0.04	0.09	0.29
S1_G08	NFC-R	1.20	2.13	0.22	0.05	0.05	0.02	0.03	0.10
S1_G07	NFC-R	1.28	2.56	0.20	0.04	0.04	0.01	0.02	0.08
S1_G26	NFC-R	1.67	3.47	0.51	0.08	0.09	0.02	0.09	0.19
S1_G21	NFC-R	1.25	3.75	0.47	0.09	0.09	0.02	0.10	0.27
S2_G25	NFC-R	2.02	4.40	0.13	0.02	0.08	0.01	0.02	0.11
S2_G12	NFC-R	5.74	5.14	0.86	0.09	0.16	0.05	0.13	0.32
Min		0.27	1.75	0.02	0.01	0.04	ND	0.02	0.08
Max		7.66	7.26	1.10	0.15	0.24	0.05	0.16	0.34
Average		2.88	4.21	0.40	0.06	0.11	0.02	0.07	0.18
Std Dev		2.07	1.27	0.28	0.04	0.05	0.01	0.04	0.08
Variation		28 X	4 X	55 X	15 X	6 X	—	8 X	4 X

[a]Classified as active by Harris, quantified as bergamottin @ 310 nm. FC-NR = not refrigerated from concentrate; FC-R = refrigerated from concentrate; ND = not detected; NFC-NR = not refrigerated and not from concentrate; NFC-R = refrigerated not from concentrate.

Table 5—Probability statistics for shelf-stable compared with refrigerated juice types

	6,7-DHB	BERG	OSE1	OSE2	OSE3	OSE4	OSE5	OSE6
Shelf stable (*n* = 11)								
Min	0.25	3.56	0.01	0.01	0.07	0.01	0.02	0.08
Max	2.55	7.26	0.36	0.06	0.24	0.04	0.11	0.34
Average	1.08	4.84	0.16	0.03	0.13	0.02	0.05	0.18
S.D.	0.877	1.154	0.128	0.018	0.050	0.010	0.028	0.082
Refrigerated (*n* = 47)								
Min	0.22	1.56	0.03	ND	0.02	ND	0.01	0.04
Max	7.66	6.72	1.22	0.17	0.22	0.12	0.21	0.47
Average	2.63	3.56	0.47	0.07	0.08	0.02	0.08	0.18
S.D.	1.663	1.077	0.280	0.041	0.041	0.021	0.048	0.095
P value	<0.01	<0.01	<0.01	<0.01	<0.01	0.34	0.09	0.88

surprising, given that the dimers and bergamottin are associated with the particulate fraction of the juice. Studies are in progress to look at juice storage effects and how fruit maturity and variety affect furanocoumarin content in grapefruit products.

Conclusions

In conclusion, the red commercial grapefruit contained significantly less DHB, bergamottin, and 1 dimer compound than white juices tested. This is likely due in that most of the commercial red grapefruit grown in Florida is the Ruby Red variety. In a separate study, fresh fruit of Ruby Red variety was found to contain less of these compounds than marsh White, Rio Red, or Flame grapefruit varieties. Grapefruit juice storage at ambient temperature also had an effect on the amount of furanocoumarins present in commercial products. Shelf stable juices had significantly amount of furanocoumarins present in commercial products. Shelf stable juices had significantly lower amounts of DHB, OSE 1, and OSE 2; and higher amounts of bergamottin and OSE.

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Dept. of Agriculture.

References

Ameer B, Weintraub RA. 1997. Drug interactions with grapefruit juice. Clin Pharmacokinet 33:103–21.
Bailey DG, Arnold JM, Munoz C, Spence JD. 1993. Grapefruit juice-felodipine interaction: mechanism, predictability, and effect of naringin. Clin Pharmacol Ther 53:637–42.
Bailey DG, Arnold JM, Strong HA, Munoz C, Spence JD. 1993. Effect of grapefruit juice and naringin on nisoldipine pharmacokinetics. Clin Pharmacol Ther 53:589–94.
Bailey DG, Kreeft JH, Munoz C, Freeman DJ, Bend JR. 1998. Grapefruit juice-felodipine interaction: effect of naringin and 6′,7′-dihydroxybergamottin in humans. Clin Pharmacol Ther 64:248–56.
Bailey DG, Munoz C, Arnold JM, Strong HA, Spence JD. 1992. Grapefruit juice and naringin interaction with nitrendipine. Clin Pharmacol Ther 51:156.
Bailey DG, Spence JD, Edgar B, Baycliff CD, Arnold JM. 1989. Ethanol enhances the hemodynamic effects of felodipine. Clin Invest Med 12:357–62.
Dahan A, Altman H. 2004. Food-drug interaction: grapefruit juice augments drug bioavailability—mechanism, extent and relevance. Eur J Clin Nutrit 58:1–9.
Dresser GK, Spence JD, Bailey DG. 2000. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P4503A4 inhibition. Clin Phar-

macokinet 38:41–57.
Edwards DJ, Bellevue FH, Woster PM. 1996. Identification of 6'7'-dihydroxybergamottin, a cytochrome P450 inhibitor, in grapefruit juice. Drug Metab Dispos 24:1287–90.
Edwards DJ, Bernier SM. 1996. Naringin and naringenin are not the primary CYP3A4 inhibitiors in grapefruit juice. Life Sci 59:1025–30.
Fuhr U, Klittich K, Staib AH. 1993. Inhibitory effect of grapefruit juice and the active component naringinen on CYP1A2 dependent metabolism of caffeine in man. Br J Clin Pharmacol 35:431–6.
Fuhr U, Kummert AL. 1995. The fate of naringin in humans: a key to grapefruit juice-drug interactions? Clin Pharmacol Ther 58:365–73.
Fukuda K, Ohta T, Oshima Y, Ohashi N, Yoshikawa M, Yamazoe Y. 1997. Specific CYP3A4 inhibitiors in grapefruit juice: furocoumarin dimers as components of drug interaction. Pharmacogenetics 5:391–6.
Greenblatt DJ, Patki KC, Von Moltke LL, Shader RL. 2001. Drug interactions with grapefruit juice: an update. Clin Psychopharm 21:357–9.
Guo LQ, Fukuda K, Ohta T, Yamazoe Y. 2000. Role of furanocoumarin derivatives on grapefruit juice-mediated inhibition of human CYP3A activity. Drug Metab Dispos 28:766–71.
Harris J, inventor; Bioavailability Systems, LLC, assignee. 1998. Method for the preparation of a first-pass effective citrus-derived substance and product thereof. U.S. patent 5,820,915. Issued Oct 13, 1998.
Harris J, inventor; Bioavailability Systems, LLC, assignee. 1999. Safe citrus juice and process for preparation. U.S. patent 5,993,887. Issued Nov 30, 1999.
Harris J, inventor; Bioavailability Systems, LLC, assignee. 2001. Anti-first-pass effect compounds and citrus extract. U.S. patent 6,255,337. Issued July 3, 2001.
Harris J, inventor; Bioavailability Systems, LLC, assignee. 2002. Anti-first-pass effect compounds. U.S. patent 6,476,066. Issued Nov 5, 2002.
Harris J, inventor; Bioavailability Systems, LLC, assignee. 2003. Anti-first-pass effect compounds. U.S. patent 6,666,766. Issued Dec 9, 2003.
Huang SM, Hall SD, Watkins P, Love LA, Serabjit-Singh C, Betz JM, Hoffman FA, Honig P, Coates PM, Bull J, Chen ST, Kearns GL, Murray MD. 2004. Drug interactions with herbal products and grapefruit juice: a conference report. Clin Pharm Ther 75:1–12.
Kane GC, Lipsky JJ. 2000. Drug-grapefruit juice interactions. Mayo Clin Proc 75:933–42.
Tassaneeyakul W, Guo LQ, Fukuda K, Ohta T, Yamazoe Y. 2000. Inhibition selectivity of grapefruit juice components on human cytochrome P450. Arch Biochem Biophysics 378:356–63.
Veronese ML, Gillen LP, Burke JP, Dorval EP, Hauck WW, Pequignot E, Waldman SA, Greenberg HE. 2003. Exposure-dependent inhibition of intestinal and hepatic CYP3A4 in vivo by grapefruit juice. J Clin Pharm 43:831–9.