Food Chemistry 111 (2008) 387–392

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Influence of pre- and post-harvest factors and processing on the levels of furocoumarins in grapefruits (*Citrus paradisi* Macfed.)

B. Girennavar, G.K. Jayaprakasha, Bhimanagouda S. Patil *

Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2119, USA

ARTICLE INFO

Article history:
Received 31 May 2007
Received in revised form 25 March 2008
Accepted 31 March 2008

Keywords:
HPLC
Seasonal variation
Furocoumarins
Processing
Hand squeezed

ABSTRACT

The changes in the levels of three furocoumarins such as dihydroxybergamottin (DHB), paradisin A and bergamottin in Rio Red and Marsh White cultivars of grapefruits were monitored from November to May. The levels of DHB and bergamottin in both varieties of grapefruits decreased as the season progressed except for the bergamottin in Marsh White grapefruit. Influence of growing location, processing and storage on the levels of these compounds were also evaluated. Among the varieties the highest levels of DHB (2.266 μg/ml) and bergamottin (2.411 μg/ml) were found in Flame grapefruit grown in Florida. The highest level of paradisin A was found in Rio Red grapefruit grown in California and the lowest levels were observed in Rio Red grapefruit grown organically in Texas. Hand squeezed juice contained 1.98, 1.06 and 3.03-fold more DHB, paradisin A and bergamottin, respectively as compared to processed juice. The levels of furocoumarins showed a decreasing trend in all the juices with progress of storage. Levels of furocoumarins were more in cartons container than the cans and cardboard container juices.

Published by Elsevier Ltd.

1. Introduction

Grapefruit juice has been shown to increase the bioavailability of many commonly used drugs such as felodipine (Bailey, Spence, Munoz, & Arnold, 1991), terfenadine (Clifford et al., 1997), cyclosporine, saquinavir and several other categories (Greenblatt, Patki, Von Moltke, & Shader, 2001). Increase in concentration of drug in plasma is clinically important and may lead to the risk of toxicity. The mechanism of grapefruit juice induced drug interaction are through inhibition of drug metabolizing enzyme cytochrome P450 3A4 (CYP3A4) (Eagling, Profit, & Back, 1999), intestinal membrane efflux transporter P-glycoprotein (Wang, Casciano, Clement, & Johnson, 2001) and organic anion transporting polypeptides (Dresser, Kim, & Bailey, 2005). Furocoumarins are the main grapefruit juice bioactive compounds responsible for inhibition of first pass metabolism of drugs. Edwards and coworkers reported that DHB found in grapefruit juice is a potent CYP3A4 inhibitor (Edwards, Bellevue, & Woster, 1996). Recently several furocoumarins and limonoids have been isolated, identified and tested for the inhibition of CYP3A4 enzyme from grapefruits (Girennaver, Jayaprakasha, Jadegoud, Nagana Gowda, & Patil, 2007; Girennavar, Poulose, Jayaprakasha, Bhat, & Patil, 2006; Guo, Fukuda, Ohta, & Yamazoe, 2000; Poulose, Jayaprakasha, Mayer, Girennavar, & Patil, 2007).

The health maintaining properties of citrus is due to the wide range of bioactive compounds. Grapefruit contains flavonoids, limonoid aglycones, glucosides, furocoumarins, ascorbic acid, folic acid, carotenoids, pectin and potassium. While some recent studies demonstrated changes in levels furocoumarins in commercial grapefruit juice (Fukuda, Gua, Ohashi, Yoshikawa, & Yamazoe, 2000; Widmer & Haun, 2005). To the best of our knowledge there are no reports on levels of three furocoumarins in fresh grapefruit as influenced by different seasons, locations, processing and storage. The aim of the present work was to investigate the changes in the levels of furocoumarins during the different seasons, influence of growing locations, processing effect, and post -harvest storage.

2. Experimental

2.1. Chemicals and supplies

All the solvents for extraction were ACS grade and for the quantitative analysis HPLC grade were obtained from EMD (EMD Chemicals Inc., Gibbstown, NJ). Rotary evaporators (Buchi Analytical Inc, New Castle, DE, USA), qualitative filter paper (VWR International West Chester, PA, USA) and HPLC Luna column (Phenomenex Inc., Torrance, CA, USA) were used for the study.

2.2. Seasonal study

Rio Red and Marsh White grapefruits were harvested from an orchard at the Texas A&M University-Kingsville, Citrus Center, Weslaco, Texas (Girennavar, Jayaprakasha, John, & Patil, 2008).

* Corresponding author. Tel.: +1 979 458 8090; fax: +1 979 862 4522.
E-mail address: b-patil@tamu.edu (B.S. Patil).

0308-8146/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2008.03.086

The fruits were harvested at 30 days interval starting from the month of November 2003 (early season) to May 2004 (late season). The fruits were juiced and stored at −80 °C until the analyses.

2.3. Postharvest storage study

Rio Red and Marsh White grapefruits were packed in boxes (total of 36 numbers), separately, each box contained 12–15 fruits. Boxes were stored at 24 °C and 9 °C with relative humidity between 90% and 95%. Furocoumarins were quantified for every two weeks. Four samplings were taken for the grapefruits stored at 9 °C, whereas three samplings were taken for fruits stored at 24 °C. Grapefruits were juiced using blender and quantified for furocoumarins.

2.4. Location study

Rio Red grapefruits were collected from Texas, Florida and California, while Marsh White grapefruits were collected from Florida and Texas during the month of March. Fruits were shipped from Florida and California through overnight mail. All the fruits were juiced and analyzed for the levels of furocoumarins immediately.

2.5. Juice storage study

Three single strength commercial Rio Red grapefruit juice products, such as cans (100% juice from concentrate), cartons (100% fresh juice and not from concentrate, can be stored for 60 days at refrigerated condition) and cardboards (100% juice from concentrate) were obtained from Texas Citrus Exchange (Mission, TX, USA). Total of 25 cans and cardboard containers and 20 cartons containers were used for the storage study. Cans and cardboard containers were stored at room temperature while cartons containers were stored at refrigerated conditions, simulating the grocery store conditions. Cans and cardboard containers were stored for 90 days while cartons containers were stored 60 days and every 15 days five containers were used for analysis.

2.6. Processing of juice

An industrial level processing of grapefruits was conducted to analyze the variations of levels of furocoumarins during juice. The Rio Red grapefruits were harvested from the Texas A&M University-Kingsville, Citrus Center at Weslaco and grouped into two batches. One batch of fruits from two bins of fruits were juiced in the lab and the other batch containing 48 bins of fruits were processed at Texas Citrus Exchange (Mission, TX) a commercial juice plant under commercial processing conditions. Sequence of events in the processing of grapefruits include (i) washing the fruits, (ii) puncturing the fruits to obtain peel oil, (iii) mechanical cutting of fruits into two halves, (iv) juicing the halves by automated juicers, (v) removing the pulp by straining the juice, (vi) pasteurizing the juice and (vii) automated packing of the juice into cans. Both types of juices were analyzed for the furocoumarin levels.

2.7. Furocoumarin standards

Marsh white grapefruit juice was extracted with ethyl acetate and concentrated under vacuum. The concentrated extract was broadly fractionated on open silica column chromatography and preparative HPLC was used to obtain DHB, paradisin A and bergamottin as previously described (Girennavar et al., 2006). The identified compounds were used for the calibration curves as described by Girennaver et al. (2007). The regression equations were used for the quantification of furorcoumarins in all the juice samples.

2.8. Sample preparation

Different juice samples (50 ml) were taken in a 250 ml separating funnel and 50 ml of ethyl acetate was added and mixed well for 5 min. The organic layer was separated carefully and this step was repeated twice. Extracts were pooled and the solvent was evaporated to dryness under vacuum using Buchi rotary evaporator. The entire ethyl acetate was dried and extract was dissolved in 1 ml of methanol and used for HPLC analysis.

2.9. HPLC analysis

The HPLC system consisted of a Thermo Electron Corporation P-400 quaternary HPLC pump (Thermo Electron Corporation San Jose, CA, USA), Membrane degasser, Spectra system AS3000 auto sampler and UV-600 PDS detector (Thermo separation products). Chromatographic separations were accomplished on Chemcosorb-5-ODS column (150 × 6.0 mm, 5 μm particle size) (Chemco-Pak, Osaka, Japan). Elution was carried out using methanol (A) water (B) multiple gradient conditions: 0 min, 60% A, 20 min 80% A, 25 min 80% A, 45 min 85% A, 50 min 90% A, 55 min 95% A, 60 min 100% A, 65 min 60% A the flow rate was set at 1.1 ml/min and elution was monitored with UV absorption at 240 nm.

2.10. Statistical analysis

The results presented in this study are the mean of 15 samples and standard deviations (SD). Dunnett's multiple comparisons test was used to determine the significance at $P < 0.05$. Analysis was performed using GraphPad Instat version 3.00 for windows.

3. Results

3.1. Seasonal variation of furocoumarins

DHB is the most abundant among the three furocoumarins in Rio Red and Marsh White varieties (Table 1). DHB levels were highest in the beginning of the grapefruit harvesting season and levels were decreased by the end of the season. A significant level of DHB was decreased from the beginning (43.2%) of the season to the end (39.8%) of the season in Rio Red and Marsh White grapefruit, respectively. Marsh White contains more DHB than Rio Red through out the season. Marsh White had 30.1% higher DHB during November and 34% higher end of the season (May) than Rio Red grapefruit. Paradisin A, a dimer probably derived from DHB by a dehydration reaction, levels was 10–15 times less than DHB. Paradisin A was 0.091 and 0.087 μg/ml at the beginning of the season and 0.081 and 0.076 μg/ml at the end of the season in Rio Red and Marsh White, respectively (Table 1). Bergamottin levels showed decreasing trend from the beginning of the season to the end of the season. However, bergamottin levels were significantly increased in February compared to January in Marsh White grapefruit. Among the two varieties, Marsh White contains average of 35.58% more bergamottin than Rio Red. Unlike DHB and bergamottin, there is no particular trend in the distribution of the paradisin A throughout the season.

3.2. Influence of growing location, cultivation method and variety on the levels of furocoumarins

Furocoumarins were quantified in five samples of two varieties of grapefruits grown at Texas, Florida and California. The concentration of dihydroxybergamottin, paradisin A and bergamottin in different varieties have been presented in Fig. 1. Marsh

Table 1
Influence of season on the levels of DHB, paradisin A and bergamottin in Rio Red and Marsh White grapefruits[a]

	November	December	January	February	March	April	May
Rio Red							
DHB	1.171 ± 0.053	1.031 ± 0.04	0.964 ± 0.024	0.895 ± 0.31	0.801 ± 0.049	0.709 ± 0.068	0.665 ± 0.031
Paradisin A	0.091 ± 0.003	0.089 ± 0.004	0.076 ± 0.005	0.083 ± 0.004	0.069 ± 0.001	0.079 ± 0.008	0.081 ± 0.005
Bergamottin	0.905 ± 0.006	0.881 ± 0.054	0.716 ± 0.022	0.696 ± 0.069	0.632 ± 0.081	0.583 ± 0.131	0.482 ± 0.071
Marsh White							
DHB	1.675 ± 0.039	1.562 ± 0.08	1.445 ± 0.042	1.202 ± 0.051	1.162 ± 0.024	1.091 ± 0.077	1.008 ± 0.047
Paradisin A	0.087 ± 0.005	0.093 ± 0.003	0.088 ± 0.002	0.078 ± 0.006	0.091 ± 0.005	0.083 ± 0.006	0.076 ± 0.008
Bergamottin	1.393 ± 0.051	1.253 ± 0.043	1.008 ± 0.109	1.283 ± 0.063	0.986 ± 0.029	0.863 ± 0.061	0.813 ± 0.046

[a] Mean expressed in μg/ml ± standard deviation, $n = 15$.

White grown in Texas contains 32.2% higher DHB compared to the same variety grown in Florida. Significantly higher levels of bergamottin were found in Marsh White grapefruit, which was grown in Florida as compared to Texas. No differences of paradisin A was observed in between both locations. Interestingly, among the three growing locations, when Rio Red grown in California, Texas and Florida, DHB levels were significantly higher in fruits grown in California compared the same grapefruit grown in Texas and Florida. Furthermore, Rio Red grapefruit grown in Florida had significantly higher paradisin A compared to the same grapefruit grown in Texas and California while there is no differences in the levels of bergamottin were observed among the three locations.

Furocoumarins were quantified and compared from conventionally and organically grown Rio red grape fruits. DHB levels were found to be higher in Rio Red grapefruit grown with conventional practices as compared to organically grown fruits (Table 2). Paradisin A levels were more in organically grown grapefruit compared conventionally grown grapefruit. No significant differences were observed in the levels of bergamottin levels in Rio Red grapefruit grown with conventional and organic practices. Among the grapefruit varieties grown at different locations, Flame grapefruit from Florida had the highest concentration of DHB, bergamottin and paradisin A, while lowest levels of DHB and paradisin A were observed in organically grown Rio Red grapefruits (Table 2).

Table 2
Influence of cultivation method and variety on the levels of furocoumarins in grapefruit[a]

Grapefruit	Furocoumarins levels (μg/ml)		
	DHB	Paradisin A	Bergamottin
Rio Red – conventional	0.989 ± 0.1158	0.0910 ± 0.004	0.754 ± 0.057
Rio Red – organic	0.627 ± 0.061	0.0669 ± 0.014	0.774 ± 0.074
Flame – Florida	2.266 ± 0.113	0.2089 ± 0.004	2.411 ± 0.116
Melgold – California	2.129 ± 0.068	0.0794 ± 0.012	1.451 ± 0.118

[a] Mean ± standard deviation, $n = 15$.

3.3. Processing effects on furocoumarin levels

Fig. 2 depicts the levels of furocoumarins in hand squeezed and commercially processed grapefruit juices. Results of the present study indicated that DHB and bergamottin content were 1.98 and 3.03 times higher in hand squeezed juice than processed juice, respectively. The levels of paradisin A were not significantly ($P < 0.005$) different between hand squeeze and commercially processed juices.

3.4. Postharvest storage on furocoumarins levels

The levels of furocoumarins decreased quite remarkably in both varieties stored at 24 and 9 °C (Table 3). The DHB content was

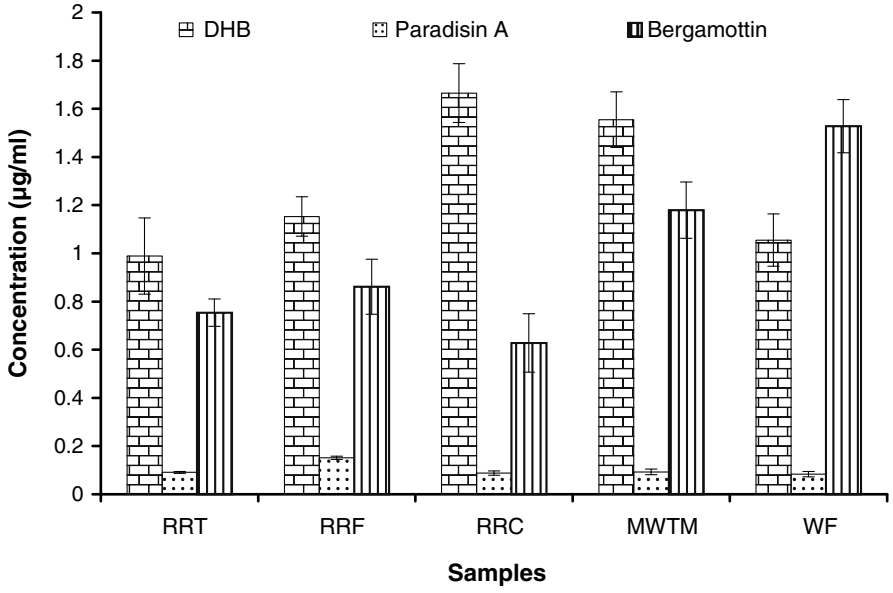

Fig. 1. Influence of location on bergamottin, paradisin A and DHB on the levels in Rio Red and Marsh White grapefruits. The codes in the panel are RRT, Rio Red from Texas, RRF, Rio Red from Florida, RRC, Rio Red from California, MWT, Marsh White from Texas and MWF, Marsh White from Florida. Data shown are the mean ± SD for 15 samples.

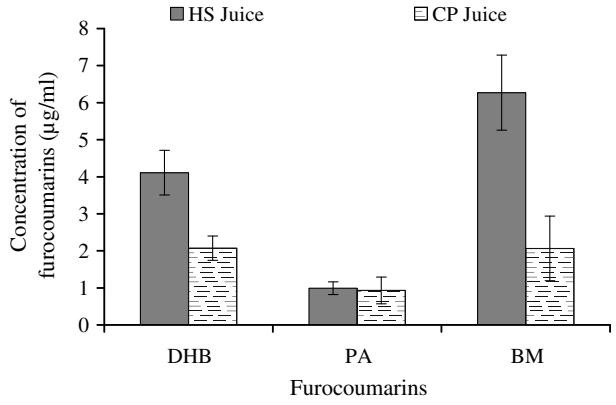

Fig. 2. Levels of furocoumarins in hand squeezed (HS) juice and commercial processed (CP) juice. Abbreviations on the panel: DHB, dihydroxybergamottin; PA, paradisin A; BM, bergamottin. The results represent mean ± SD for 15 samples.

decreased by 8.57% and 20.34% in Rio Red, while in case of Marsh White 7.37% and 16.15% stored at 9 °C and 24 °C, respectively. The most pronounced decrease (30.4%) was noticed for paradisin A in Rio Red stored at 24 °C. Bergamottin was relatively stable compound in both temperatures, with decrease of 4.1 and 12.2% in Rio Red and 2.9% and 2.9% in Marsh White at 9 °C and 24 °C, respectively. In general, bergamottin was more stable during post -harvest storage followed by DHB and paradisin A.

Table 4 depicts the levels of three furocoumarins in three kinds of processed juice in containers such as cans, cardboard and cartons. The levels of furocoumarins showed a decreasing trend in all three types of container with progress of storage time. Levels of furocoumarins were higher in cartons. After 90 days of storage levels of the furocoumarins decreased by 31.67%, 43.43% and 11.31% of DHB, paradisin A and bergamottin, respectively. Over a period of 90 days, levels of DHB, paradisin A and bergamottin in cardboard containers decreased by 33%, 57% and 32%, respectively. However, DHB, paradisin A and bergamottin were 24%, 32% and 35% lower over 60 days of storage in cartons grapefruit juice. The juice in the cartons was made with pulp and stored under refrigerated conditions with maximum storage time of 60 days.

Table 3
Influence of temperatures during storage on the levels of furocoumarins in Rio Red and Marsh White grapefruit juices*

Grapefruit	Storage temperature	Days	Levels of furocoumarins (μg/ml)		
			DHB	Paradisin A	Bergamottin
Rio Red	9 °C	0	1.201 ± 0.061[a]	0.09 ± 0.004[a]	1.005 ± 0.049[a]
		15	1.137 ± 0.078[a]	0.088 ± 0.002[a]	0.995 ± 0.062[a]
		30	1.108 ± 0.069[a]	0.087 ± 0.004[a]	0.978 ± 0.028[a]
		45	1.098 ± 0.068[a]	0.076 ± 0.006[b]	0.965 ± 0.37[a]
	24 °C	0	1.201 ± 0.061[a]	0.09 ± 0.001[a]	1.005 ± 0.049[a]
		15	1.091 ± 0.055[a]	0.077 ± 0.004[b]	0.909 ± 0.055[b]
		30	0.998 ± 0.014[b]	0.069 ± 0.007[b]	0.896 ± 0.081[b]
Marsh White	9 °C	0	1.704 ± 0.043[a]	0.092 ± 0.004[a]	1.437 ± 0.038[a]
		15	1.689 ± 0.076[a]	0.089 ± 0.006[a]	1.426 ± 0.04[b]
		30	1.618 ± 0.091[b]	0.086 ± 0.0052[a]	1.408 ± 0.044[c]
		45	1.587 ± 0.068[c]	0.085 ± 0.003[a]	1.397 ± 0.083[d]
	24 °C	0	1.704 ± 0.043[a]	0.092 ± 0.005[a]	1.437 ± 0.038[a]
		15	1.593 ± 0.099[d]	0.081 ± 0.003[a]	1.406 ± 0.019[e]
		30	1.467 ± 0.065[e]	0.071 ± 0.002[a]	1.396 ± 0.045[f]

Means with a different letter, differ for the levels of the particular compound in the fruits significantly, $P < 0.05$.
* Mean ± standard deviation, $n = 15$.

Table 4
Post-harvest storage effect on the levels of furocoumarins in grapefruit juice in different containers*

Type of containers	Days	Levels of furocoumarins (μg/ml)		
		DHB	Paradisin A	Bergamottin
Cans				
	0	2.065 ± 0.081[a]	0.099 ± 0.004[a]	1.255 ± 0.028[a]
	15	1.985 ± 0.092[b]	0.088 ± 0.003[b]	1.223 ± 0.046[b]
	30	1.885 ± 0.015[c]	0.078 ± 0.003[c]	1.213 ± 0.021[c]
	45	1.772 ± 0.064[d]	0.069 ± 0.004[d]	1.193 ± 0.037[d]
	60	1.598 ± 0.109[e]	0.065 ± 0.015[e]	1.166 ± 0.045[e]
	75	1.499 ± 0.089[f]	0.061 ± 0.028[f]	1.142 ± 0.013[f]
	90	1.411 ± 0.037[g]	0.056 ± 0.007[g]	1.1138 ± 0.049[g]
Cardboard				
	0	2.125 ± 0.073[a]	0.104 ± 0.008[a]	1.487 ± 0.071[a]
	15	1.985 ± 0.092[a]	0.097 ± 0.005[b]	1.354 ± 0.087[b]
	30	1.818 ± 0.108b	0.089 ± 0.001[c]	1.285 ± 0.051[c]
	45	1.789 ± 0.089[c]	0.076 ± 0.003[d]	1.116 ± 0.119[d]
	60	1.5612 ± 0.089[d]	0.074 ± 0.006[e]	1.099 ± 0.19[e]
	75	1.4511 ± 0.061[e]	0.072 ± 0.002[f]	1.037 ± 0.017[f]
	90	1.4198 ± 0.183[f]	0.066 ± 0.004[g]	1.004 ± 0.105[g]
Cartons				
	0	2.3146 ± 0.091[a]	0.1009 ± 0.006[a]	1.8655 ± 0.091[a]
	15	2.1485 ± 0.092[b]	0.0981 ± 0.013[a]	1.6813 ± 0.086[b]
	30	1.9915 ± 0.105[c]	0.0883 ± 0.008[b]	1.4513 ± 0.107[c]
	45	1.8212 ± 0.064[d]	0.0769 ± 0.017[c]	1.3193 ± 0.045[d]
	60	1.7598 ± 0.109[c]	0.0685 ± 0.009[d]	1.2168 ± 0.021[c]

Means with a different letter, differ for the levels of the particular compound in the containers significantly, $P < 0.05$.
* Mean ± standard deviation, $n = 15$.

4. Discussion

Furocoumarins are found as secondary metabolites in the plant kingdom and their exact roles in plants are not known. Nevertheless, these compounds seems to play some role in plant defense mechanism due to the induction of their biosynthesis following various stress events as well as their anti-microbial and anti-oxidative activities (Shimizu et al., 2005; Zobel, 1997). Furocoumarins are phytoalexins produced perhaps in response to external stimuli such as stress or pathogenic infection (Tietjen, Hunkler, & Matern, 1983). At any given time the level of these compounds reflect the physiological condition of the plant. The concentration of three furocoumarins such as xanthotoxin, bergapten and psoralen were measured by Zobel and Brown (1990) in whole leaves of Heracleum lanatum and in the extracts of leaf over vegetative season. Furocoumarin concentrations in the whole leaf varied, with highest concentration being in the month of April and sharp decline afterwards as leaf enlarged rapidly. It has been previously shown that celery grown near urban centers in California was found to stimulate accumulation of psoralen, bergapten, xanthotoxin and isopimpinellin (Dercks, Trumble, & Winter, 1989).

Naringin and limonin are the major bitter compounds of grapefruit juice and their variation is well documented during the growing season (Mansell, McIntosh, & Vest, 1983). Limonin content decreased rapidly in the early part of the season. Naringin content varied considerably among the cultivars and between crop years, in some cases increase in the later part of the growing season with commencement of rapid vegetative growth (Mansell et al., 1983). Genetic make up such as cultivar/variety, environmental conditions, developmental stage of plant also influence the content of plant secondary metabolites. Variations of furocoumarins and naringin have been reported in commercial grapefruit juice and in different parts of grapefruits (Fukuda et al., 2000; Ho, Saville, Coville, & Wanwimolruk, 2000). However, information related the pre- and post-harvest factors on fresh grapefruit furocoumarins is not available.

It has been shown that plants respond to oxidative stress by increasing the levels of antioxidants such as carotenoids and also by increasing some antioxidant enzymes (Walker & McKersie, 1993). It is possible that plant responds to these changing environmental conditions by changing the levels of secondary metabolites. Irradiation and other stress parameters have shown to increase the phenylalanine ammonia lyase (PAL) activity in citrus and other fruits (Ismail & Brown, 1979; Oufedjikh, Mahrouzm, Amiot, & Lacroix, 2002). PAL enzyme catalyzes the deamination of L-phenylalanine to form *trans*-cinnamic acid, a precursor for flavonoids and tannins (Dixon & Paiva, 1995). This is major enzyme involved in the metabolism of secondary metabolites and any changes in its activity leads to qualitative and quantitative changes in secondary metabolites. While environmental factors may have influence on furocoumarins and flavonoids, genetic factors might play a major role in variation of these bioactive compounds.

Wangensteen, Molden, and Christensen (2003) proposed that, during commercial manufacturing of juice, epoxybergamottin present in the grapefruit peel possibly re-distribute into the juice and by hydrolysis epoxybergamottin may be converted to more potent CYP3A4 inhibitor i.e. DHB. Interestingly, no increased levels of furocoumarins in the commercial grapefruit juice, commercial processed juice significantly contain lower levels of DHB and bergamottin compared to hand squeezed juice. Our results also seems to provide justification for the study where, fresh squeezed grapefruit juice has been shown to increase the bioavailability of terfanadine by two fold compared to commercially processed juice (Clifford et al., 1997). Commercial grapefruit juice is manufactured in a multi-step process, which includes washing, puncturing of peel to obtain essential oils and squeezing of the whole fruit to juice. Furthermore, the juice is heated during pasteurization process and finally essential oil, an important constituent of grapefruit peel, is added as a flavor enhancer to the commercially produced grapefruit juice.

We previously reported the isolation and characterization of bergamottin, 6′, 7′-dihydroxybergamottin and paradisin A from grapefruit juice. The identified compounds were tested for their inhibitory effects on human CYP3A4 and CYP1B1 microsomes using specific substrates such as dibenzylfluorescein and alkoxyresorufin. Paradisin A found to be the most potent CYP3A4 inhibitor with an IC_{50} of 1.2 μM followed by DHB and bergamottin (Girennavar et al., 2006). Edwards et al. (1996) reported that DHB found in grapefruit juice was a potent CYP3A4 inhibitor. Several furocoumarins such as bergamottin, epoxybergamottin, paradisin A, paradisin B and paradisin C have also shown to inhibit the CYP3A4 enzyme (Fukuda et al., 2000; Wangensteen et al., 2003). Certain dimers are more effective inhibitors of CYP3A4 enzyme compared to their respective monomers (Guo et al., 2000), but the dimer concentrations are very low in the juice which posses a challenge to isolate such compounds (Manthey & Buslig, 2005). Grapefruit juice induced drug interaction studies have shown wide variation in terms of pharmacokinetics and this is mainly due to the amounts of bioactive agents in the juice (Greenblatt et al., 2001). Inter individual variation in the levels of cytochrome P450 isozymes may also contribute to the observed variation in drug interaction. Genetic variability may influence drug absorption, drug metabolism and for drug interactions with receptors. This may account for about 20–40% of the inter-individual differences in drug metabolism and response.

5. Conclusions

Our results suggest that pre-harvest factors such as season, location and growing methods influences the levels of furocoumarins in grapefruits. Moreover, postharvest factors such as storage and processing significantly affect the levels of furocoumarins. Therefore, it is important to consider the pre- and post-harvest factors on the levels of these bioactive compounds. It is possible that these factors affecting furocoumarin levels will have major effect on drug bioavailability. The observed variability in the grapefruit-drug interaction could be partially due to the variability in the levels of these compounds between the different juice preparations due to pre and post-harvest factors and processing.

Acknowledgements

This project is based upon work supported by the USDA-CSREES # 2006-34402-17121 "Designing Foods for Health" through the Vegetable and Fruit Improvement Center. Authors would like to thank Mr. Elias Hernandez and field crew at the Texas A&M University-Kingsville, Citrus Center, Weslaco, for help in grapefruit harvesting and Mr. Manuel Ochoa at Texas Citrus Exchange, Mission, TX, for his generous help in processing of the grapefruits at industrial level for this study.

References

Bailey, D. G., Spence, J. D., Munoz, C., & Arnold, J. M. (1991). Interaction of citrus juices with felodipine and nifedipine. *Lancet, 337*, 268–269.

Clifford, C. P., Adams, D. A., Murray, S., Taylor, G. W., Wilkins, M. R., Boobis, A. R., et al. (1997). The cardiac effects of terfanidine after inhibition of its metabolism by grapefruit juice. *European Journal of Clinical Pharmacology, 52*, 311–315.

Dercks, W., Trumble, J., & Winter, C. (1989). Impact of atmospheric pollution on linear furocoumarin content in celery. *Journal of Chemical Evolution, 16*, 443–454.

Dixon, R. A., & Paiva, N. L. (1995). Stress – induced phenylaproponoid metabolism. *Plant Cell Tissue and Organ Culture, 7*, 1085–1097.

Dresser, G. K., Kim, R. B., & Bailey, D. G. (2005). Effect of grapefruit volume on the reduction of fexafenadine bioavailability: Possible role of organic anion transporting polypeptides. *Clinical Pharmacology and Therapeutics, 77*, 170–177.

Eagling, V. A., Profit, L., & Back, D. J. (1999). Inhibition of CYP3A4 mediated metabolism and P-glycoprotein-mediated transport of the HIV-1 protease inhibitor saquinavir by grapefruit juice components. *British Journal of Clinical Pharmacology, 48*, 543–552.

Edwards, D. J., Bellevue, F. H., & Woster, P. M. (1996). Identification of 6′, 7′-dihydroxybergamottin, cytochrome P450 inhibitor, in grapefruit juice. *Drug Metabolism and Disposition, 24*, 1287–1290.

Fukuda, K., Gua, L., Ohashi, N., Yoshikawa, M., & Yamazoe, Y. (2000). Amounts and variation in grapefruit juice of the main components causing grapefruit-drug interaction. *Journal of Chromatography B, 741*, 195–203.

Girennavar, B., Poulose, S. M., Jayaprakasha, G. K., Bhat, N. G., & Patil, B. S. (2006). Furocoumarins from grapefruit juice and their effect on human CYP3A4 and CYP 1B1 isoenzymes. *Bioorganic and Medicinal Chemistry, 14*, 2606–2612.

Girennaver, B., Jayaprakasha, G. K., Jadegoud, Y., Nagana Gowda, G. A., & Patil, B. S. (2007). Radical scavenging and cytochrome P450 3A4 inhibitory activity of bergaptol and geranylcoumarin from grapefruit. *Bioorganic and Medicinal chemistry, 15*, 3684–3691.

Girennavar, B., Jayaprakasha, G. K., John, L. J., & Patil, B. S. (2008). Variation of bioactive furocoumarins and flavonoids in different varieties of grapefruits and pummelo. *European Food Research and Technology, 226*, 1269–1275.

Greenblatt, D. J., Patki, K. C., Von Moltke, L. L., & Shader, R. I. (2001). Drug interactions with grapefruit juice: An update. *Journal of Clinical Psychopharmacology, 21*, 357–359.

Guo, L. Q., Fukuda, K., Ohta, T., & Yamazoe, Y. (2000). Role of furocoumarins derivatives on the grapefruit juice-mediated inhibition of human CYP3A activity. *Drug Metabolism and Disposition, 28*, 766–771.

Ho, C. H., Saville, D. J., Coville, P. F., & Wanwimolruk, S. (2000). Content of CYP3A4 inhibitors, naringin, naringenin and bergapten in grapefruit and grapefruit juice products. *Pharmaceutica Acta Helveticae, 74*, 379–385.

Ismail, M. A., & Brown, G. E. (1979). Postharvest wound healing in citrus fruit: Induction of phenylalanine ammonia lyase in injured 'Valencia' orange flavedo. *Journal of American Society of Horticulture Sciences, 104*, 126–129.

Mansell, R. L., McIntosh, C. A., & Vest, S. E. (1983). An analysis of the limonin and naringin content of grapefruit juice samples collected from Florida state test houses. *Journal of Agriculture and Food Chemistry, 31*, 156–162.

Manthey, J., & Buslig, B. S. (2005). Distribution of furocoumarins in grapefruit juice fractions. *Journal of Agriculture and Food Chemistry, 53*, 5158–5163.

Oufedjikh, H., Mahrouzm, M., Amiot, M. J., & Lacroix, M. (2002). Effect of γ-irradiation on phenolic compounds and phenylalanine – lyase activity during storage in relation to peel injury from peel of *Citrus clementia* Horticex. Tanaka. *Journal of Agriculture and Food Chemistry, 48*, 559–565.

Poulose, S. M., Jayaprakasha, G. K., Mayer, R. T., Girennavar, B., & Patil, B. S. (2007). Purification of citrus limonoids and their differential inhibitory effects on human cytochrome P450 enzymes. *Journal of Science Food and Agriculture, 87*, 1699–1709.

Shimizu, B. I., Miyagawa, H., Ueno, T., Sakata, K., Watanabe, K., & Ogawa, K. (2005). Morning glory systematically accumulates scopoletin and scopolin after interaction with Fusariun oxysporium. *Zeitschrift fur Naturforschung, 60c*, 83–90.

Tietjen, K. G., Hunkler, D., & Matern, U. (1983). Differential response of cultured parsley cells to elicitors from two non-pathogenic strains of fungi1. Identification of produced products as furocoumarins derivatives. *European Journal of Biochemistry, 161*, 401–407.

Walker, M. A., & McKersie, B. D. (1993). Role of ascorbate-glutathione antioxidant system in chilling resistance of tomato. *Journal of Plant Physiology, 141*, 234–239.

Wang, E. L., Casciano, C. N., Clement, R. P., & Johnson, W. W. (2001). Inhibition of P-glycoprotein transport unction by grapefruit juice psoralen. *Pharmaceutical Research, 18*, 432–438.

Wangensteen, H., Molden, E., & Christensen, H. (2003). Identification of epoxybergamottin as a CYP3A4 inhibitor in grapefruit peel. *European Journal of Clinical Pharmacology, 28*, 663–668.

Widmer, W., & Haun, C. (2005). Variation of furocoumarin content and new furocumarin dimers in commercial grapefruits (*Citrus paradise* Macf). *Journal of Food Science, 70*, 305–312.

Zobel, A. M. (1997). Coumarins in fruit and vegetables. In F. A. Tomas-Barberan & R. J. Robins (Eds.), *Phytochemistry of fruits and vegetables. Proceedings of the phytochemical society of Europe* (vol. 41, pp. 173–178). Oxford: Clarendon Press.

Zobel, A. M., & Brown, S. A. (1990). Seasonal changes of furocoumarin concentrations in leaves of *Heracleum lanatum*. *Journal of Chemical Evolution, 16*, 1623–1634.