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Modulation of flavanone and furocoumarin levels in grapefruits (*Citrus paradisi* Macfad.) by production and storage conditions

Kranthi K. Chebrolu [a], J. Jifon [b,*], Bhimanagouda S. Patil [a,*]

[a] Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, Texas A&M University, College Station, TX, USA
[b] Texas A&M AgriLife Research Center, Weslaco, TX, USA

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ABSTRACT

Grapefruits grown under organic or conventional systems were analyzed for 6,7-dihydroxybergamottin (DHB) and flavanones using HPLC, and DPPH activity and ORAC using a micro-plate reader. Grapefruits harvested in November 2008 (E-1) and February 2010 (E-2) were stored at room temperature (RT) and 9 °C for four weeks. Higher levels of DHB were observed in conventional grapefruits during the second (4.7 ± 0.2 μg/g), third (1.5 ± 0.2 μg/g) and fourth (2.5 ± 0.2 μg/g) week of storage at room temperature in E2. Among flavonoids analyzed, narirutin (666.7 ± 33.9 μg/g), neohesperidin (17.5 ± 1.3 μg/g), didymin (75.5 ± 5.6 μg/g) and poncirin (130.8 ± 10.4 μg/g) levels were significantly higher ($P \leqslant 0.05$) in organic grapefruits over conventional grapefruits at harvest and storage in E-1. Although DPPH levels were moderately correlated with grapefruit flavanone content, variability in the individual flavanone activity was pronounced, resulting in non-significant differences in antioxidant activity between organic and conventional grapefruits.

1. Introduction

Grapefruit flavanones have demonstrated a wide array of health promoting properties, especially against heart diseases (Middleton, Kandaswami, & Theoharides, 2000; Mink et al., 2007). The American Heart Association has given a "healthy heart check" symbol for several commercially available grapefruit juices due to their preventive properties against coronary heart disease (Cerda, Robbins, Burgin, Baumgartner, & Rice, 1988; my.american.heart.org, 2011). Similarly, several *in vitro* studies have also demonstrated grapefruit's anti-cancer properties (Chidambara, Kim, Vikram, & Patil, 2012; Juskiewicz, Zdunczyk, Wroblewska, Oszmianski, & Hernandez, 2002; Vanamala et al., 2006). In general, these flavanone levels are correlated with fruit antioxidant capacity. Flavanones are ubiquitously produced in different plant parts, such as flowers, fruits, seeds and leaves, and play a key role in plant signaling, defense against ultra violet radiation, microbes and herbivory. Flavanone levels are also greatly influenced by growing conditions (production systems and climate) and plant genetics. Despite these health benefits, grapefruit consumption is not popular among patients under certain medications due to its potential for drug interactions.

Grapefruit furocoumarins interact with several drug classes, including antiepileptics, antihistamines, antimalarials, antiarrhythmics, cardiovascular agents (verapamil, amlodipine, felodipine and nicardipine), statins (atorvastatin, lovastatin and simvastatin), corticosteroids and several others (Bistrup, Nielsen, Jeppesen, & Dieperink, 2001; Dresser, Kim, & Bailey, 2005; Fuhr et al., 2002; Garg, Kumar, Bhargava, & Prabhakar, 1998; Goosen et al., 2004; Hukkinen, Varhe, Olkkola, & Neuvonen, 1995; Kantola, Kivisto, & Neuvonen, 1998; van Agtmael, Gupta, van der Graaf, & van Boxtel, 1999). Among grapefruit furocoumarins, dihydroxybergamottin (DHB) was identified as one of the primary candidates responsible for grapefruit drug interaction. Consequently, several reports on grapefruit drug interactions have led to consumer apprehension over grapefruit consumption and health benefits (Bellosta, Paoletti, & Corsini, 2004; Hansten, 2003). Strategies to alter the levels of these compounds in fruits include application of edible fungi, such as *Agaricus bisporus*, *Monascus purpureus* and *Pleurotus sapidus* (Myung, Narciso, & Manthey, 2008), fruit irradiation (Girennavar et al., 2008b) and breeding new grapefruit cultivars (Chen, 2011). Modulation of the pre- and post-harvest factors may alter levels of these compounds. The objective of this study was to characterize variations in the levels of coumarins, flavanones (SF.1) and antioxidant capacity in grapefruits as a function of production system (organic or conventional) and pre-retail storage conditions.

* Corresponding authors.
E-mail addresses: jljifon@ag.tamu.edu (J. Jifon), b-patil@tamu.edu (B.S. Patil).

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2. Materials and methods

2.1. Fruit harvest and sampling

Rio Red grapefruits with uniform color, size and maturity were selected from four quadrants of trees. The harvesting and sampling followed the same protocol as our previously published organic grapefruit study (Chebrolu, Jayaprakasha, Jifon, & Patil, 2012). The first experiment (E-1) was conducted in November 2008 and the second experiment (E-2) was conducted in February 2010. Organic grapefruits were harvested from South Texas Organics Citrus orchards (Mission, TX, USA) while conventional grapefruits were harvested from Rio Queen Citrus Farms (Mission, TX, USA). The certified organic Rio Red grapefruit orchard is located three miles away from the conventional orchard. The fruits were harvested in the morning, processed and packed by noon. The packed grapefruits were shipped overnight to Texas A&M University, College Station, TX. The grapefruits were kept at room temperature (RT) and 9 °C for four weeks. The fruits were collected every week from grapefruit boxes and analyzed for coumarins, flavanones and antioxidant capacity. Every week, 27 grapefruits were taken out from storage boxes to prepare nine grapefruit samples for each treatment by blending three individual fruits together. This study compared the levels of coumarins, flavanones DPPH radical scavenging activity and ORAC of organic and conventional grapefruits. The weather, crop production inputs, and sampling methods were presented in our previous publication (Chebrolu et al., 2012).

2.2. Dihydroxybergamottin analysis

Ten grams of grapefruit pulp was taken in a 50 ml centrifuge tube and extracted with 20 ml of ethyl acetate. The organic fraction was separated and the residual juice was re-extracted with 10 ml of ethyl acetate. The two organic extracts were pooled and evaporated to dryness. The extract was reconstituted with 5 ml of DMSO. Dihydroxybergamottin levels were quantified after slight modification of our previous reported method (Girennavar, Jayaprakasha, Jifon, & Patil, 2008a). The analysis was conducted using an analytical HPLC system consisting of a Perkin-Elmer series 200 pump, PDA detector (235C) and an autosampler (Perkin-Elmer, Norwalk, CT, USA). The separation was carried out on a C-18, 5 μm Gemini column (250 mm × 4.6 mm i.d.) attached to a guard column (Phenomenex, Torrance, CA, USA). DHB was eluted by a gradient mobile phase of 0.03 M phosphoric acid (A) and acetonitrile (B) with a constant flow rate of 1 ml/min throughout the analysis. The peak detection was carried out at 320 nm and analysis was carried out by Turbo chrome software (Perkin-Elmer, Norwalk, CT, USA). Each sample was analyzed in triplicate and each treatment had nine samples.

2.3. Flavanone analysis

The sample preparation for flavanone analysis was conducted according to the previously published method (Chebrolu, Jayaprakasha, Jifon, & Patil, 2011). Rio Red grapefruit juice (3 ml) was extracted with 6 ml of DMSO and the mixture was vortexed for 5 s. Later, the samples were centrifuged at 4600 rpm for 10 min. Approximately 1 ml of supernatant from the centrifuge tube was passed through a 0.45 μm acrodisc syringe filter into an amber glass vial and 6 μl was injected into the HPLC. The separation of flavanone was performed using a Finnigan Surveyor Plus, HPLC system (West Palm Beach, FL, USA). The HPLC system was equipped with a PDA Plus detector coupled to a quaternary LC Pump Plus system and a Surveyor Plus auto-sampler (25 μl sample loop with valco fittings). A C-18 Hypersil Gold column (100 mm × 4.6 mm i.d. and 5 μm particle size) was used to separate all five flavanones from grapefruit juice. The standard flavanones were purchased from Sigma Chemicals (St. Louis., MO, USA). Peaks were detected at 280 nm, and the analysis was carried out using Chromquest 5.0 software. Chromatographic separations were performed with a gradient mobile phase consisting of 3 mM phosphoric acid prepared in nanopure water (A) and 100% acetonitrile (B). The flavanones were eluted with the following solvent gradient: 0–4.5 min, 80% A; 11.6 min, 70% A; 13 min, 42% A; and 19.6 min, 80% A. The column was equilibrated for 5 min before successive injections.

2.4. Sample preparation for grapefruit antioxidant activity

Ten grams of grapefruit juice were mixed with 20 ml of methanol and extracted for 12 h on a mechanical shaker. The organic

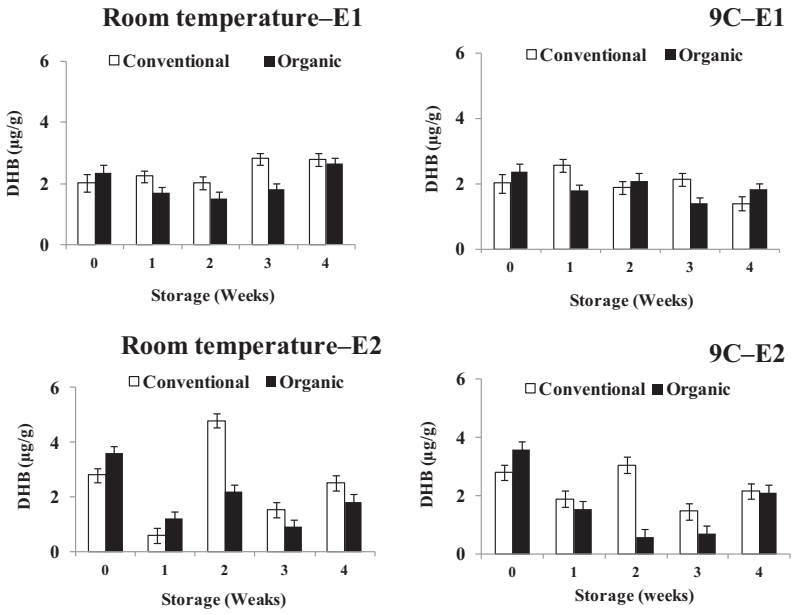

Fig. 1. DHB levels from organic and conventional grapefruit from nine individual samples (each sample is a mixture of three fruits) processed in E-1 and E-2 at room temperature and 9 °C.