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Ethylene degreening modulates health promoting phytochemicals in Rio Red grapefruit

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ABSTRACT

In the current study, we examined the effects of postharvest degreening and storage on phytochemicals in Rio Red grapefruit. Grapefruits were degreened with 3.5 μl/l of ethylene at 21 °C and 80% relative humidity for 72 h, while non-degreened fruits were used as the control. Furthermore, the grapefruits were stored at 11 °C for 3 weeks and then at 21 °C for 2 weeks. Degreening improved the peel colour of the grapefruit without affecting total soluble solids or acidity of the juice. Degreened fruits had significantly more ascorbic acid after 35 days of storage. Degreening had no significant effect on the levels of carotenoids, limonoids and flavonoids as compared to the non-degreened fruits, after 35 days of storage. However, after 7 days, degreened fruits had more limonin and flavonoids and less furocoumarin, namely 6′,7′-dihydroxybergamottin. Overall, ethylene treatment had a significant effect on the phytochemical contents of Rio Red grapefruit, especially after 7 days of storage.

1. Introduction

Several studies have shown the potential health benefits of fruits and vegetables and their important roles in human health (Slavin & Lloyd, 2012). The 'My Plate' food guide developed by the USDA also emphasizes on increasing the consumption of fruits and vegetables (USDA, 2011). Increased awareness about the role of phytochemicals in improving health has increased consumer demand and broadened the market for functional foods (Annunziata & Vecchio, 2013; Krystallis, Maglaras, & Mamalis, 2008). The media and food processing companies have increased their interest in fruits and vegetables with higher levels of antioxidants, promoting fruits such as pomegranates, acai berries and blueberries. Citrus is a major fruit crop that is eaten fresh and used in processing. Citrus fruits are rich sources of vitamin C although recent research has focused on other citrus phytochemicals. Several previous studies used cell culture and animal models to demonstrate various health-promoting properties of citrus phytochemicals, including anti-inflammatory (García-Lafuente, Guillamón, Villares, Rostagno, & Martínez, 2009), anti-proliferative effect on human neuroblastoma cells (Poulose, Harris, & Patil, 2006), anti-carcinogenic activity in human colon, breast, pancreatic and prostate cancer cells (Kim, Jayaprakasha, Vikram, & Patil, 2012), cholesterol lowering (Kurowska et al., 2000) and cardioprotective effects (Vinson et al., 2002).

Grapefruit is one of the important citrus crops grown in the United States. Grapefruit (*Citrus paradisi*, Macf.), a hybrid between sweet orange (*Citrus sinensis*) and pummelo (*Citrus grandis*), originated in the Jamaica around the 18th century. The United States ranks second in grapefruit production and fourth in grapefruit export in the world (FAS, 2014). Of the total grapefruit acreage under cultivation in the United States in 2013–2014, 24% is in Texas (NASS, 2014). Rio Red, a bud sport mutant with red flesh, is the main variety grown in the Rio Grande Valley in South Texas. Grapefruit contains several health beneficial secondary metabolites including lycopene, β-carotene, limonoids, flavonoids, ascorbic acid, folic acid, sterols, volatiles and furocoumarins, which are influenced by various postharvest treatments (Chaudhary, Jayaprakasha, Porat, & Patil, 2012, 2014; Girennavar et al., 2008).

One of the most common postharvest treatment in early season grapefruits and other citrus fruits is ethylene degreening. The gaseous hormone ethylene regulates many physiological responses in plants and is commonly known as the ripening hormone, due to its role in fruit ripening. Cold temperatures, especially minimum night temperatures (Iglesias et al., 2007), trigger ethylene production and initiate normal ripening in citrus. The Star Ruby and Rio Red grapefruit cultivars require temperatures below 13–14 °C to begin the natural degreening of the peel (Porras, Brotons, Conesa, &

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Manera, 2014). During the early season (October), the temperature may not fall below the required level to initiate the colour change. In addition, warm temperatures interfere with chlorophyll degradation and carotenoid accumulation (Iglesias et al., 2007). Thus, early season grapefruits, harvested in October and November, are often degreened using ethylene gas to change their peel colour from green to orange/red. Moreover, ethylene degreening will affect different metabolic pathways and the levels of certain phytochemicals will vary significantly. In our previous study a significant amount of phytochemicals were influenced in the edible part of Star Ruby grapefruit after ethylene degreening (Chaudhary et al., 2012). Thus, it is imperative to study the influence of ethylene on the health beneficial compounds in the juice sacs of Rio Red grapefruit. Therefore, the main objective of the current study was to investigate the effect of artificial ethylene degreening on the levels of phytochemicals, including ascorbic acid, carotenoids, limonoids, flavonoids and furocoumarins present in Rio Red grapefruit juice vesicles.

2. Materials and methods

2.1. Plant materials

Rio Red grapefruits of uniform size were harvested from three different blocks (replications) from a commercial grove in the Rio Grande Valley in South Texas (November 19, 2008).

2.2. Chemicals

Reagent grade butylated hydroxytoluene (BHT), metaphosphoric acid, L-ascorbic acid, lycopene, β-carotene, narirutin, naringin, didymin, poncirin, limonin, and 6′,7′-dihydroxybergamottin (DHB) were procured from Sigma Aldrich Co. (St. Louis, MO, USA). Sodium hydroxide was purchased from EMD Chemicals (Gibbstown, NJ, USA). Analytical grade solvents were used for quantitative analysis (Fisher Scientific Research, Pittsburgh, PA, USA).

2.3. Degreening treatment

Fruits collected from each block (400 fruits) were randomly assigned to two groups of 200 fruits; one group was used for ethylene treatment (degreened fruits) and other was used as a control without any ethylene treatment (non-degreened fruits). Fruits were degreened with 3.5 μl/l (ppm) ethylene for 72 h at 21 °C and 80% relative humidity in a commercial degreening room.

At the packing house, both non-degreened (control) and degreened fruits were passed through treatments consisting of a dump sprayer line with 0.02% chlorine buffered at pH 7.0, a high pressure washing system with 0.02% chlorine at pH 7.0, pre-drier brushes and a spray with 0.085 ml/l peracetic acid. The grapefruits were waxed with Decco Pearl Lustr (Decco, Cerexagri Inc. Monrovia, CA) containing 2 g/kg imazalil and 3.5 g/kg thiabendazole and then transferred to the Vegetable and Fruit Improvement Center, Texas A&M University, College Station (Texas, USA). Grapefruits were stored under market-simulated conditions for 3 weeks at 11 °C followed by 2 weeks of storage at 21 °C. Samples were collected every 7 days. Each treatment consisted of three replications containing 200 fruits per replication (fruits collected from 3 different blocks). Furthermore, from each replication, three subsamples were prepared ($n = 3$ replications $\times$ 3 subsamples = 9). In the current study all parameters (except for peel colour) had a single sample set for day 0 analysis.

2.4. Juice sample preparation

Juice subsamples were prepared by blending three peeled grapefruits. The juice samples were stored at −80 °C until further phytochemical analysis. All phytochemical analysis and fruit quality parameters (except peel colour) were conducted on the juice sacs/juice samples of Rio Red grapefruit.

2.5. Peel colour measurements

The peel colour of the non-degreened and degreened fruits was measured with a Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan). Before recording the sample measurements, the instrument was calibrated every week, using the white calibration plate (Calibration Plate CR-A43, Minolta Cameras, Osaka, Japan). Peel colour was measured for 90 grapefruits (30 fruits per replication, for 3 replications) per treatment. Fruits were circled with a black marker on their equatorial side (three readings per fruit) and the hue angle was measured within these circles at weekly intervals. The results are expressed as hue angles, with a hue angle of 90° indicating yellow, 60° indicating orange and 30° indicating red colour (Chaudhary et al., 2012).

2.6. Total soluble solids and titratable acidity

Total soluble solids (TSS) were measured using a hand refractometer (American Optical Corp., South Bridge, MA, USA) and expressed as °Brix. A DL 22 Food and Beverage analyzer (Mettler Toledo, Columbus, OH, USA) was used to measure the titratable acidity of juice. Grapefruit juice (5 ml) was mixed with 50 ml of nanopure water and titrated against 0.1 N NaOH.

2.7. Ascorbic acid determination

Ascorbic acid was extracted using meta-phosphoric acid according to our previously developed method (Chebrolu, Jayaprakasha, Yoo, Jifon, & Patil, 2012). Extracted samples were injected into an HPLC for ascorbic acid determination at 254 nm (Chebrolu, Jayaprakasha, Yoo et al., 2012). Each sample was injected three times and the ascorbic acid contents were expressed as mg/100 ml.

2.8. Carotenoids analysis

Extraction of carotenoids was achieved using chloroform, as per our previously established method (Chaudhary et al., 2012). BHT was added to chloroform (0.2%) to prevent oxidation of carotenoids. All extractions were conducted in the dark, using yellow light to avoid degradation of carotenoids. An Agilent HPLC 1200 Series (Foster City, CA, USA) system consisting of a solvent degasser, quaternary pump, autosampler, column, oven and diode array detector was used for quantification. A Gemini 5 μm C-18 column (250 mm × 4.6 mm i.d.) (Phenomenex, Torrance, CA, USA) with a guard cartridge was used for separating carotenoids. Elution was carried out using a mobile phase of acetonitrile (A) and isopropyl alcohol (B). Carotenoids were detected at 450 nm and quantified using external standard calibration. Three injections per sample were carried out.

2.9. Analysis of limonoids, flavonoids, and furocoumarins

2.9.1. Sample preparation

Each juice sample (10 g) was extracted using 15 ml of ethyl acetate by vortexing and homogenizing for 5 min (Chaudhary et al., 2012). The organic layer was separated and the residue was re-extracted twice. All extracts were pooled and the solvent