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Influence of Electron-Beam Irradiation on Bioactive Compounds in Grapefruits (*Citrus paradisi* Macf.)

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Phytochemical levels in fruits and vegetables can be affected by several postharvest factors. In the present study, the effect of electron-beam (E-beam) irradiation was studied on grapefruit bioactive compounds. 'Rio Red' and 'Marsh White' grapefruits were irradiated with E-beam at 0, 1.0, 2.5, 5.0, and 10.0 kGy. Changes of various bioactive compounds, such as vitamin C, flavonoids, carotenoids, furocoumarins, and limonoids, were measured. The acidity decreased slightly with an increasing E-beam dose, whereas the total soluble solids were increased. Irradiation did not affect the vitamin C content at 1 kGy; however, doses beyond 1 kGy significantly reduced the vitamin C content. Lycopene and β-carotene did not change significantly from the irradiation. Lycopene levels decreased as the E-beam dose increased, while the β-carotene content slightly increased. Dihydroxybergamottin levels exhibited a decreasing trend, while the bergamottin content did not change. Naringin, a major flavonoid of grapefruit, showed a significant increase over the control at 10 kGy in both 'Rio Red' and 'Marsh White'. Nomilin continued to decrease with an increasing dose of E-beam irradiation, while limonin levels remained the same at all of the doses. Low-dose E-beam irradiation has very little effect on the bioactive compounds and offers a safe alternative to existing postharvest treatments for the disinfection and decontamination of grapefruits.

KEYWORDS: Vitamin C; limonoids; flavonoids; furocoumarins; carotenoids; postharvest effect; HPLC; E-beam

INTRODUCTION

Several epidemiological studies established a positive correlation between high intake of fruits and vegetables and low incidence of cardiovascular disease, certain forms of cancer, and other chronic diseases (*1−4*). Grapefruit (*Citrus paradisi* Macf.) is a rich source of bioactive compounds, such as vitamin C, carotenoids, limonoids, flavonoids, furocoumarins, folic acid, pectin, and potassium. These compounds may serve as chemopreventive agents in addition to their numerous other health benefits (*5*). Our group investigated that limonoids induce apoptosis in human neuroblastoma cells (*6, 7*). Further, obacunone and limonin reduces the incidence of adenocarcinomas induced by azoxymethane in male F344 rats by suppressing cell proliferation and enhancing apoptosis (*8*). Our recent study also demonstrated that grapefruit furocoumarins, such as dihydroxy-

bergamottin (DHB), paradisin A, and bergamottin, are involved in drug interaction by inhibiting CYP 3A4 activity (*9*).

The levels of bioactive compounds in fruits and vegetables can be altered by postharvest treatments, such as irradiation, storage, and freeze-drying (*10*). Ionizing radiation is one of the techniques to inactivate human pathogens and reduce the spoilage of fruits and other foods (*11−13*). The number of food items approved for irradiation is currently increasing worldwide (*11*). In the United States, the Food and Drug Administration has approved low-level irradiation of food and food-products to reduce the incidence of illness resulting from pathogenic microorganisms (*14*). World organizations, such as the Food and Agriculture Organization (FAO), International Atomic Energy Agency (IAEA), and World Health Organization (WHO), have endorsed irradiation of fruits and vegetables as a means to control foodborne diseases (*15*). In fact, irradiation, commonly referred to as cold pasteurization, is less environmentally and nutritionally harmful than most other traditional practices. However, irradiation can affect the food quality, odor, and flavors (*16*). Thus, the objective of this study was to investigate the influence of E-beam irradiation on pH, total soluble solids (TSSs), vitamin C, carotenoids, furocoumarins,

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10942 *J. Agric. Food Chem.*, Vol. 56, No. 22, 2008

Girennavar et al.

flavonoids, and limonoids in 'Rio Red' and 'Marsh White' varieties of grapefruits.

MATERIAL AND METHODS

Chemicals. All of the solvents used for the extraction were ACS-grade. For quantitative analysis, HPLC-grade solvents were obtained from EMD (EMD Chemicals, Inc., Gibbstown, NJ). Ascorbic acid, lycopene, β-carotene, naringin, and naringenin were purchased from Sigma (St. Louis, MO). DHB, bergamottin, limonin, and nomilin were purified according to our published methods (*9, 17*).

Samples. 'Rio Red' and 'Marsh White' grapefruits were harvested from an orchard at Texas A&M University−Kingsville Citrus Center located in Weslaco, TX, during the month of December 2005.

E-Beam Source, Irradiation, and Dose Calculation. 'Rio Red' and 'Marsh White' grapefruits were subjected to ionizing radiation ranging from 0 (control), 1.0, 2.5, 5.0, and 10.0 kGy using two separate 10 MeV Linac accelerators (top and bottom) located at the National Center for Electron Beam Food Research (Texas A&M University, College Station, TX). The time of exposure (dose accumulation) was adjusted accordingly to treat the fruit samples with 1, 2.5, 5, and 10 kGy $\pm$ 7.5% dose levels. Six fruits of 'Rio Red' and 'Marsh White' were packed in cardboard boxes of $24 \times 20 \times 6$ in. dimension. Absorbed irradiation dose was determined using certified Harwell Alanine dosimeter pellets placed on the top and bottom of the fruits when the fruits were irradiated, and the target fruits were irradiated at different doses. Three boxes were used for each treatment. Fruits were juiced using blenders after the irradiation and stored at −80 °C until the analysis.

Determination of Acidity and TSSs. Acidity of juice samples was measured by an Acuumet pH reader (Fisher Scientific, Pittsburgh, PA), and TSSs were measured using a hand refractometer (American Optical Corporation, Buffalo, NY).

Calibration Graphs. Stock solutions (3.90, 7.81, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 μg/mL) of vitamin C and carotenoids (lycopene and β-carotene) were prepared in 3% *meta*-phosporic acid and chloroform, respectively (*18, 19*). Stock solutions of DHB, bergamottin, and naringin were prepared in methanol. Aliquots (25 μL) of six different concentrations (equivalent to 2.5, 5, 7.5, 10, 15, and 20 μg) of DHB, bergamottin, and naringin were injected onto high-performance liquid chromatography (HPLC) as described by our earlier method (*20*). Stock solutions (3.90, 7.81, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 ppm) of limonin and nomilin were prepared in acetone. Elution was carried out to obtain peak area responses as described by Vikram et al. (*21*). The calibration curves for each compound were prepared by plotting the concentration of each compound versus the peak area.

Vitamin C Analysis. HPLC analysis was carried out as per the published method (*22*). A total of 2 mL of grapefruit juice was mixed with 10 mL of 3% metaphosporic acid and homogenized for 5 min. The homogenate was filtered. An aliquot of 1 mL was filtered through a 0.45 μm membrane (Pall Corporation, Ann Arbor, MI). A total of 20 μL of sample was injected into HPLC system. An AlphaBond Amino C-18 column (300×3.9 mm) (Alltech, Deerfield, IL) was used for the separation and quantification of vitamin C. The mobile phase used was acetonitrile/water (70:30, v/v) with 1.15 g/L of $(NH_4)H_2PO_4$ at a flow rate of 1 mL/min. The vitamin C peak was detected at 254 nm with a retention time of 6.49 ± 0.04 min.

Determination of Carotenoids. Carotenoids were determined by HPLC using an isocratic mobile phase with a flow rate of 1 mL/min (*23*). A total of 10 mL of grapefruit juice was mixed with 50 mL of acetone, 5 mL of hexane, and 50 mL of water and mixed for 10 min. The hexane layer was separated and dried under nitrogen. The residue was dissolved in 1 mL of acetone and filtered through a 0.45 μm membrane filter. A total of 10 μL of extract was injected onto a Waters Sperisorb ODS-2 5 μm column (250×4.6 mm). Elution was carried out with 65% A [aqueous acetonitrile (1:9 water/acetonitrile) with 1 mL of triethyl amine/L] and 35% B (ethyl acetate with 1 mL of triethyl amine/L).

Quantification of Furocoumarins. A total of 50 mL of juice was extracted with 50 mL of ethyl acetate in a 250 mL separating funnel for 5 min. The organic layer was collected carefully, and extraction was repeated twice more. Extracts of each sample were pooled and concentrated under vacuum to dryness. The dried residue was reconstituted in methanol for HPLC analysis. Volumes of 95 μL of each samples were injected, and elution was carried out at room temperature using (A) methanol and (B) water as a mobile phase. The gradient conditions were as follows: 0 min, 60% A; 20 min, 80% A; 25 min, 80% A; 45 min, 85% A; 50 min, 90% A; 55 min, 95% A; and 60 min, 100% A. The flow rate was set at 1.1 mL/min, and detection was carried out at 240 nm with a photodiode array detector (*9*).

Determination of Flavonoids. A total of 1 mL of grapefruit juice was mixed with 2 mL of methanol and filtered. An aliquot of 1 mL sample was prepared by filtering through a 0.45 μm membrane. Amounts of 10 μL were injected onto HPLC for quantification. A Luna C-18, 3.7 μm particle size (Phenomenex, Inc., Torrance, CA) column with dimensions of 150×4.6 mm was used. The elution of flavonoids was carried out at room temperature using aqueous methanol under gradient conditions. Starting with 0 min, 35% methanol and 65% water; 5 min, 50% methanol and 50% water; 15 min, 75% methanol and 25% water; 20 min, 80% methanol and 20% water; 25 min, 100% methanol, and at 30 min, initial conditions of 35% methanol and 65% of water were maintained. The flow rate was set at 0.6 mL/min, and flavonoids were monitored at 280 nm with a photodiode array detector.

Quantification of Limonoid Aglycones. Juice (10 mL) was extracted with 10 mL of ethyl acetate in a separating funnel. Then, 50 μL of extract was injected onto a Gemini (Phenomenex, Torrance, CA) column and eluted with (C) acetonitrile and (D) water as follows: 0 min 20% A and C; 20 min, 35% C; 40 min, 42% C; 50 min, 55% C; 55 min, 100% A; 57 min, 100% A; and 60 min, 20% A. The flow rate was set at 1 mL/min, and limonoids were detected at 210 nm.

Method Validation. Calibration curves were constructed by plotting the peak area of standard Vs concentration. Regression equations were determined with coefficients >0.998, >0.965, >0.948, >0.998, >0.999, >0.972, >0.999, and >0.998 for vitamin C, lycopene, β-carotene, DHB, bergamottin, naringin, limonin, and nomilin (**Figure 1**), respectively.

Statistical Analysis. Mean values and standard deviations (SDs) were reported. The Dunnett multiple comparison test was used to determine the significance at $p \leq 0.05$, using GraphPad Prism software, version 4.03, for Windows (GraphePad Software, San Diego, CA).

RESULTS AND DISCUSSION

Acidity and TSSs. Figure 2 depicts the effect of E-beam on acidity and TSSs of 'Rio Red' and 'Marsh White' grapefruit juice. As E-beam doses increased, acidity of the juice decreases and TSSs increased slightly as compared to the control. There was no significant change in the acidity between the treatments and two varieties of grapefruit.

Degradation of Vitamin C. Figure 3 depicts the levels of vitamin C in different treatments of E-beam irradiated 'Rio Red' and 'Marsh White' grapefruit juices. The vitamin C concentration in 'Rio Red' at 1 kGy treatment showed a 0.77% decrease in 'Marsh White' and a 1.26% decrease in 'Rio Red'. As the dose of the irradiation increased, the vitamin C content in both varieties decreased significantly. A remarkable decrease was observed at 10 kGy in both varieties of juices; 'Rio Red' showed a 53.52% decrease, while 'Marsh White' showed a 50.03% decrease in total vitamin C concentration. Vitamin C undergoes degradation during processing of juice and is also affected by storage duration conditions, time, and temperature (*24−27*). Oxidation of ascorbic acid proceeds both aerobic and anaerobic pathways and depends upon several factors, including oxygen, heat, and light (*28*). Degradation products of vitamin C, along with amino acids, leads to the formation of brown pigments, which is another problem contributing to quality loss in citrus juices during storage (*29*).

Variations in Lycopene and β-Carotenoid Content. The concentration of lycopene and β-carotene in the 'Rio Red' and 'Marsh White' grapefruit juices with different doses of E-beam
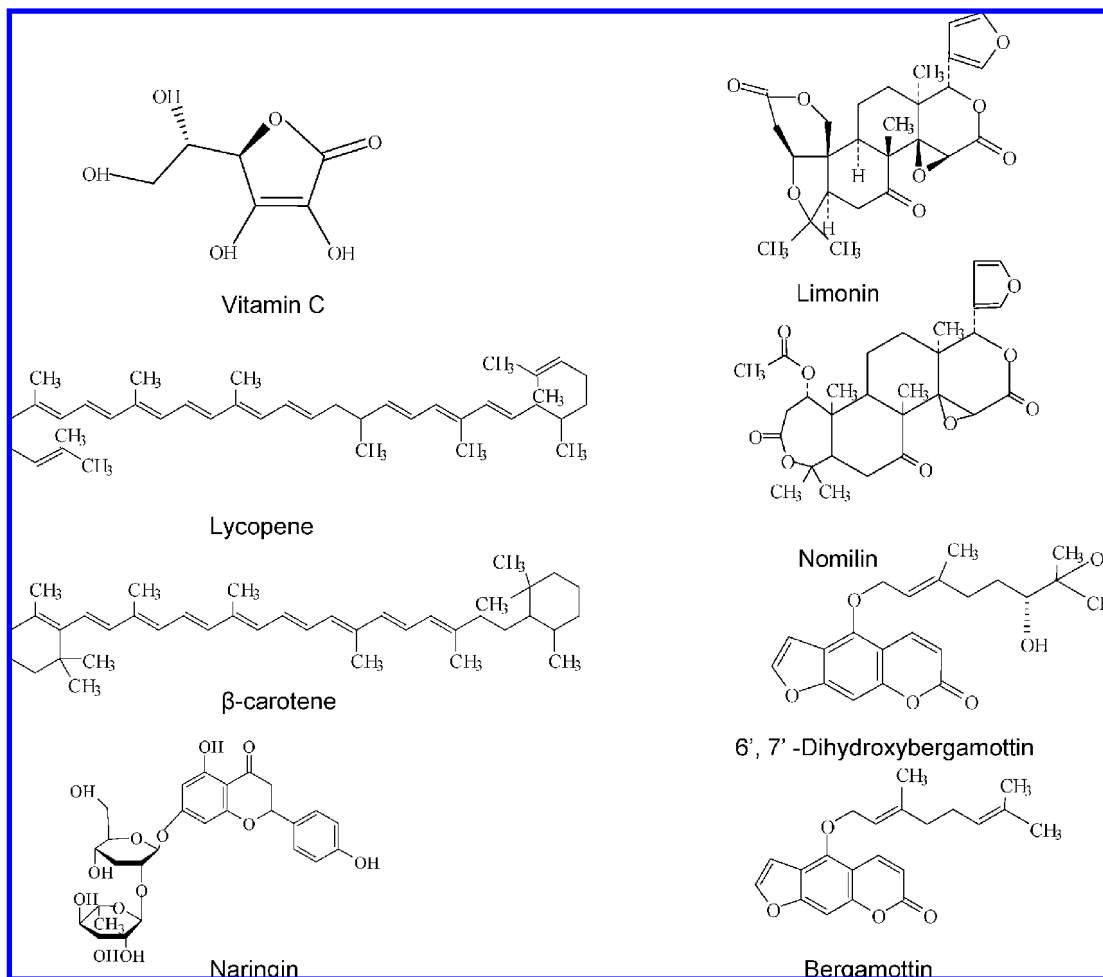
Figure 1. Structures of the bioactive compounds quantified in the present study.
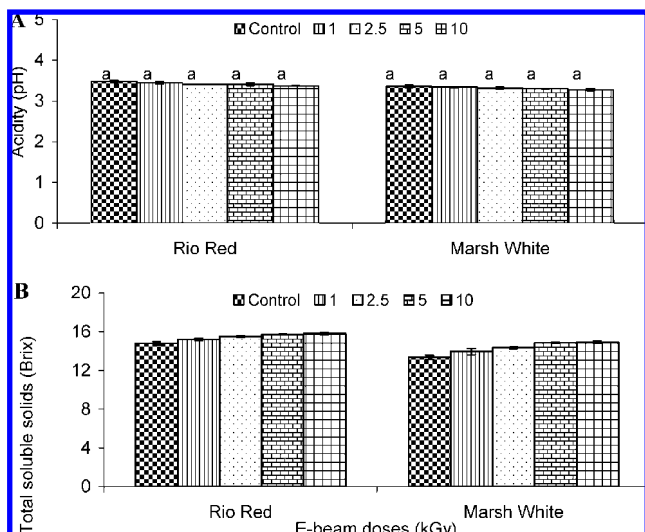

Figure 2. Effect of E-beam irradiation on (A) acidity and (B) TSSs in 'Rio Red' and 'Marsh White' grapefruit juice at different doses. Values are mean ± SD; $n = 3$. Different letters indicate significance at $p \leq 0.05$.
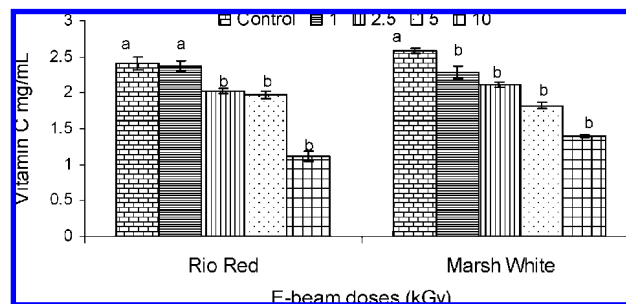

Figure 3. Degradation of vitamin C in 'Rio Red' and 'Marsh White' grapefruit juice after E-beam irradiation at different doses. Values are mean ± SD; $n = 3$. Different letters indicate significance at $p \leq 0.05$.

the lycopene content in late season fruit is significantly lower than that of early season fruit (*30*), especially from October to May (*31*). Fruit pulp attains the highest color early in the season and decreases as the season progresses (*32*). γ irradiation doses of 10 and 20 kGy did not affect β-carotene levels (*33*). However, other types of irradiation, freeze-drying, season, and storage do affect the carotenoids content (*10*). It has been shown that plants respond to oxidative stress by increasing the levels of antioxidants, such as carotenoids, and by increasing some antioxidant enzymes (*34*).

Changes in Furocoumarins. **Figure 5** depicts the effect of E-beam irradiation on DHB and bergamottin concentrations. DHB levels showed a significant decreasing trend from 1 to 10 kGy in both varieties. 'Rio Red' fruits exposed to 1 kGy showed a 10.21% decrease over the control, while juice exposed to 10
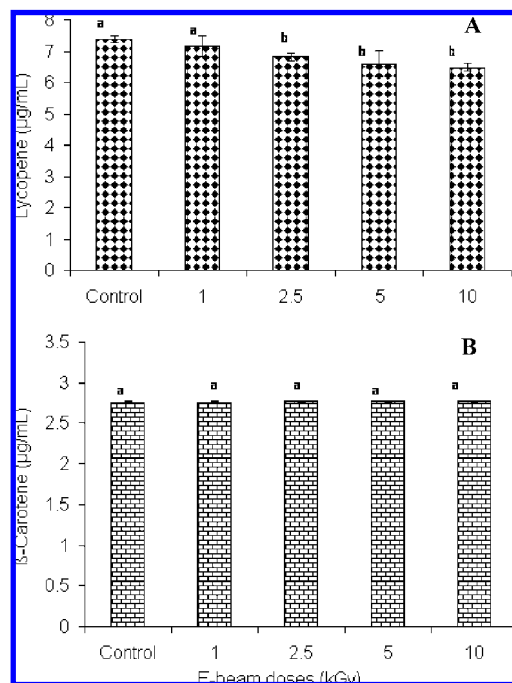
irradiation has been presented in **Figure 4**. In 'Rio Red' grapefruit, the lycopene concentration decreased by 2.98 and 2.33% at 1 and 10 kGy, respectively, as compared to untreated 'Rio Red' grapefruit. Conversely, β-carotene levels did not show significant change at all doses of irradiation. Further, lycopene and β-carotene were not found in 'Marsh White' grapefruit juice in both treated and untreated samples. Studies have shown that

10944 *J. Agric. Food Chem.,* Vol. 56, No. 22, 2008

Girennavar et al.

Figure 4. Effect of E-beam irradiation on (A) lycopene and (B) β-carotene in 'Rio Red' and 'Marsh White' grapefruit juice. Values are mean $\pm$ SD; $n = 3$. Different letters indicate significance at $p \leq 0.05$.
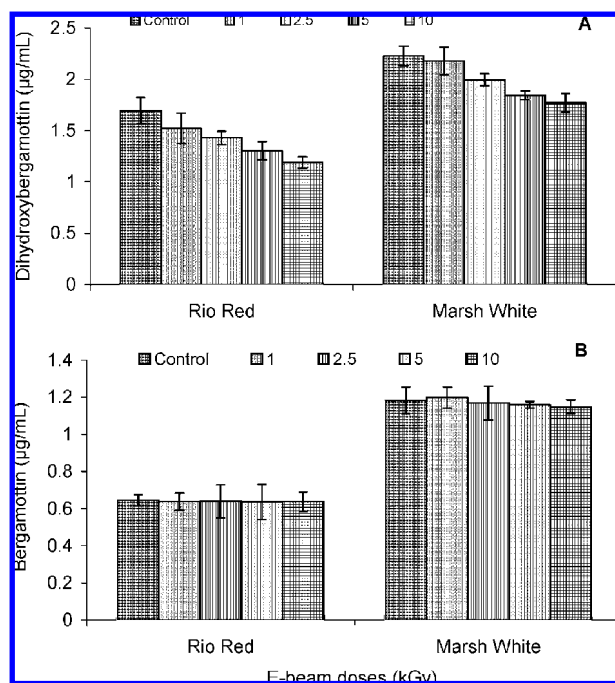

Figure 5. Variation of (A) dihydroxybergamottin and (B) bergamottin from 'Rio Red' and 'Marsh White' grapefruit juice after the E-beam irradiation at different doses. Different letters indicate significance at $p \leq 0.05$.

kGy showed a significant (29.87%) decrease. 'Marsh White' showed a noticeable change (2.23%) at 1 kGy dose, whereas at 10 kGy dose, a significant (20.54%) decrease was observed. Interestingly, we could not find any significant change in the bergamottin concentration in both varieties at all of the doses of E-beam irradiation.

Furocoumarins are phytoalexins produced by plants, and their concentration varies based on the variety, location, processing, and origin. The content of furocoumarins in grapefruit juice has been shown to vary considerably. Tassanyakula and co-workers
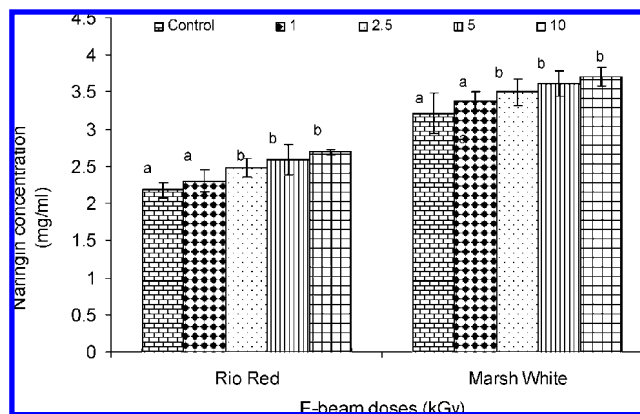

Figure 6. Changes in the naringin concentration during E-beam irradiation at different doses for 'Rio Red' and 'Marsh White' grapefruit juice. Values are mean $\pm$ SD; $n = 3$. Different letters indicate significance at $p \leq 0.05$.

reported 4-fold variations of bergamottin, 43-fold variations of DHB, and 65-fold variations of other related furocoumarins in the commercial grapefruit juice (*35*). Fukuda et al. (*36*) reported that furocoumarin levels in white grapefruit were higher than in red grapefruit juices and the highest level of the compounds were found in the fruit meat. While environmental factors have some influence on furocoumarins and flavonoids, genetic factors might play a major role in variation of these bioactive compounds.

Variations of Flavonoids. The levels of naringin in 'Rio Red' and 'Marsh White' as influenced by E-beam irradiation have been presented in **Figure 6**. Concentrations of naringin were shown significantly higher in both varieties of grapefruits. At higher dose (10 kGy) of naringin increased significantly by 18.90 and 15.37% in 'Rio Red' and 'Marsh White', respectively. Naringin is the major flavonoid component responsible for grapefruit juice bitterness. Irradiation has been shown to increase the phenylalanine ammonia lyase (PAL) activity in citrus and other fruits (*37, 38*). PAL enzymes catalyze the deamination of L-phenylalanine to form *trans*-cinnamic acid, a precursor for flavonoids and tannins (*39*). Irradiation-induced *de novo* synthesis of naringin by PAL may be responsible for the increase in the content of naringin in treatments over the control.

Levels of Limonoids. Figure 7 depicts the effect of irradiation on the concentration of limonin and nomilin in 'Rio Red' and 'Marsh White' grapefruit juices. Nomilin showed a decrease in the concentration with an increase in dose. At 1 kGy treatment, nomilin decreased by 7.19 and 4.27%, whereas at 10 kGy, the concentration was significantly reduced by 19.02 and 21.56% in 'Rio Red' and 'Marsh White', respectively. Interestingly, limonin did not show any significant change in the concentration with irradiation. In our previous results, we have shown that γ irradiation coupled with freeze-drying influenced limonoid aglycones significantly (*10*).

Health benefits of bioactive compounds may not have any practical significance if the irradiation makes the fruits unmarketable and unacceptable. Citrus fruits are an important source of vitamin C in the human diet. Studies have shown that loss of vitamin C is minimal when citrus fruits are exposed to irradiation doses up to 1 kGy (*40*). Our study also demonstrates that irradiation at 1 kGy does not affect the vitamin C content in 'Rio Red'. At higher doses, it reduced the vitamin C content considerably. Vitamin C degradation can cause browning, which leads to the problem of quality loss during high-dose irradiation. Vitamin C degradation products react with amino acids, leading
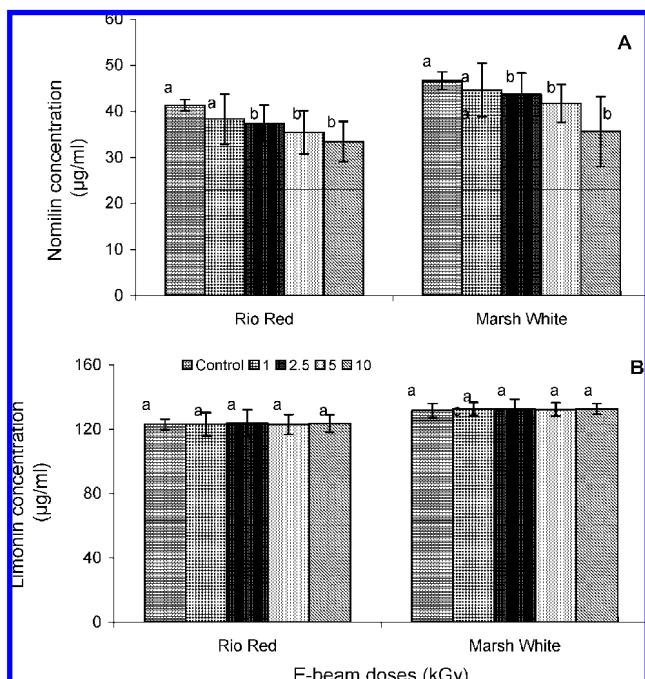
Figure 7. Effect of E-beam irradiation on (A) nomilin and (B) limonin in 'Rio Red' and 'Marsh White' grapefruit juice. Values are mean ± SD; n = 3. Different letters indicate significance at $p \leq 0.05$.

to the formation of brown pigments. Hydroxymethylfurfurol is one of the decomposition products of vitamin C and is a suggested precursor of brown pigments (*26*).

Conclusion. Low-dose E-beam irradiation has very little effect on the bioactive compounds, while higher doses tend to reduce the vitamin C concentration significantly. Naringin tends to increase with an increasing dose of irradiation, while the total carotenoid level tends to remain constant. Low-dose E-beam irradiation offers a safe alternative to existing postharvest treatments for the disinfection and decontamination of fruits.

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