Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 1404–1411
Food Chemistry
www.elsevier.com/locate/foodchem

# Ionizing radiation and marketing simulation on bioactive compounds and quality of grapefruit (*Citrus paradisi* c.v. Rio Red)

Jairam Vanamala, Greg Cobb, Julio Loaiza, Kilsun Yoo, Leonard M. Pike, Bhimanagouda S. Patil *

*Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133, United States*

Received 29 June 2006; received in revised form 3 May 2007; accepted 8 May 2007

**Abstract**

Bioactive compounds in citrus fruits have been shown to be protective against chronic diseases such as cancer and heart disease, but their levels may be affected by postharvest treatments such as storage and irradiation. In this study, grapefruits were exposed to gamma irradiation at 0, 150 and 300 Gy and then stored at 10 °C for 36 d, followed by an additional 20 d at 20 °C. Flavonoid content, terpenoid content, quality (acidity and total soluble solids) and phenylalanine ammonia-lyase (PAL) activity were evaluated at regular intervals during storage. Irradiation and storage affected ($P \leqslant 0.05$) the levels of bioactive compounds in grapefruit; however, the effect of storage was prominent. Irradiation differentially affected the flavonoid content of pulp and peel. Fruits exposed to 300 Gy had higher ($P \leqslant 0.01$) narirutin content in peel compared to control fruits at 12 and 56 d after storage. While storage increased the D-limonene and myrcene content in all treatments, control fruit had higher terpenoid content at the end of the storage. PAL activity was found to be in traces in the peel. In general, irradiation or storage had no considerable effect on total soluble solids; however, acidity decreased ($P \leqslant 0.05$) with storage.


*Keywords:* Grapefruit; Irradiation; Storage; Phenylalanine ammonia-lyase; Flavonoids; Terpenoids

## 1. Introduction

Fruit fly infestation is a global problem with devastating effects on more than 100 fruit species, thus restricting fruit distribution among countries and even within the country. Common quarantine treatment for most fruits against fruit flies is methyl bromide fumigation. However, methyl bromide is planned to be phased out by 2015 as it is toxic to humans and harmful to the ozone layer. In recent years, much effort has been directed towards developing alternative methods to methyl bromide fumigation. Following the success in Hawaii of using low-dose irradiation as a quarantine treatment against fruit flies (Moy & Wong, 2002), irradiation is being considered around the world as a potential alternative to methyl bromide (Hallman, 1999; Hallman & Martinez, 2001). Ionizing radiation sterilizes or kills insects and pathogenic microorganisms such as *Escherichia coli* by damaging their DNA (Delincee & Soika, 2002). However, low-dose irradiation with γ-rays results in the intracellular generation of reactive oxygen species (ROS) and hydrogen peroxide ($H_2O_2$) in plant tissues (Kovacs & Keresztes, 2002), which may alter the phytochemical antioxidant content of fruits and vegetables (Patil, Vanamala, & Hallman, 2004; Vanamala et al., 2005).

Citrus fruits are rich sources of bioactive compounds such as ascorbic acid, terpenoids and flavonoids (Mouly, Gaydou, & Auffray, 1998; Vanamala et al., 2005). Flavonoids are polyphenolic compounds which are categorized into flavonols, flavones, flavanones, isoflavones, and catechins. Flavanones are found abundantly in citrus fruit, mainly as flavonoid glycosides, and are thus an important

* Corresponding author. Address: 1500 Research Parkway, Suite A 120, TAMU, College Station, TX-77845 United States. 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 © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.05.042

source of these compounds in the human diet (Mouly et al., 1998). Flavonoids possess antioxidant (Peterson & Dwyer, 1998) and blood lipid lowering activities (Kurowska, Borradaile, Spence, & Carroll, 2000; Vinson et al., 2002). We and others have previously reported that citrus fruits may protect against chronic diseases such as cancer (Deschner, Ruperto, Wong, & Newmark, 1991; Leonardi et al., 2004; Liu et al., 2001; Poulose, Harris, & Patil, 2005; Poulose, Harris, & Patil, 2007; Tian, Miller, Ahmad, Tang, & Patil, 2001; Yang et al., 2000; Vanamala et al., 2006), heart diseases (Yu et al., 2005) and osteoporosis (Deyhim et al., 2006; Deyhim, Lopez, Gonzalez, Garcia, & Patil, 2006).

'Rio Red' grapefruits from the Rio Grande Valley in Texas are exported to other states such as California, Florida, and Arizona, and even to other countries like Japan. Exports account for 40% of market value and generate about $9.3 million per year for Texas grapefruit farmers. The farming community recognizes the need for developing an alternative quarantine treatment for fruit flies. A low dose of gamma irradiation as a quarantine treatment against fruit flies was recently developed for citrus fruit (Hallman & Martinez, 2001). A minimum dose of 58 or 69 Gy was suggested for sterilization of fruit fly larvae. However, during commercial scale operations, fruit could receive up to three times the minimum absorbed dose for quarantine purposes (Hallman & Martinez, 2001) as irradiation is applied to packed and ready to be shipped pallet loads of produce to minimize the cost of radiation. Thus, when fruits in the center of the pallet receive minimum required dose, the outermost fruits which are close to the radiation source may receive approximately three times more radiation. Thus, fresh produce should tolerate three times the required dose of radiation for quarantine purposes.

Accumulating evidence suggests that irradiation in the range of 150–300 Gy on citrus fruit influences parameters such as acidity, total soluble solids (TSS) and appearance (Hallman & Martinez, 2001; Moshonas & Shaw, 1984; Selles, Maarse, & Bemelmans, 1986). However, the effect of irradiation and storage-induced oxidative stress on grapefruit bioactive compounds in the edible portion (pulp) and peel of 'Rio Red' grapefruit has not been fully elucidated. The purpose of this study was to evaluate the effect of oxidative stress induced by proposed quarantine doses of irradiation and simulated storage on the content of grapefruit bioactive compounds.

## 2. Materials and methods

### 2.1. Fruit Samples

Grapefruits (*Citrus paradisi* c.v. Rio Red) were collected from an orchard at the Texas A&M University-Kingsville, Citrus Center's South Farm, and the fruits were washed and waxed in a commercial facility. All fruits were of a similar maturity stage and a size of 96. Thirty-six fruits were exposed to irradiation (300 or 150 Gy) 3 d after harvest, and the same number of fruits served as a control. After irradiation, fruit were stored at 10 °C for 36 d followed by an additional 20 d at 20 °C to simulate marketing conditions. Pulp (devoid of seeds) and peel samples were collected from six fruit at 12 d intervals (three intervals) under low-temperature storage followed by 10 d (two intervals) under ambient temperature (20 °C). Samples were stored at −80 °C until analyzed for flavonoid content, terpenoids, total soluble solids (TSS), and titratable acidity in fruit pulp and flavonoid content and phenylalanine ammonia-lyase (PAL) activity in peel.

### 2.2. Irradiation treatment

Irradiation treatments were carried out with a Cesium ($^{137}$Cs) self-contained dry-storage irradiator (Husman Model 521A, Isomedix, Inc., Whippany, NJ) at the United States Department of Agriculture facility in Mission, TX. Fruit were irradiated at a dose of 150 and 300 Gy with a centreline-absorbed dose of about 40 Gy min$^{-1}$. The fruit temperatures were measured before (20.5 °C; SEM = 0.22; $n = 15$) and after (21.9 °C; SEM = 0.27; $n = 15$) irradiation using non-contact thermometers (Rayteck Raynger ST Series, Santa Cruz). The non-irradiated fruits that served as control were also transported (20 °C) to the irradiation facility, with the aim of exposing them to similar conditions as that of irradiated fruits.

### 2.3. Standards

Naringin (naringenin-7-rhamnosidoglucoside, NAR) and narirutin (naringenin-7-rutinoside, NAT) were purified in our laboratory according to the established procedures (Raman, Jayaprakasha, Cho, Brodbelt, & Patil, 2004). Didymin (isosakuranetin-7-rutinoside, DID), neohesperidin (hesperitin-7-neohesperidoside, NEH) and poncerin (Isosakuranetin-7-neohesperidoside, PON) were obtained from Indofine Chemical Company, Inc. (Hillsborough, NJ). Myrcene and D-limonene were purchased from Sigma Chemical Co. (St. Louis, MO). Dimethyl formamide (DMF) and acetonitrile (ACN) were obtained from VWR Scientific Products (Houston, TX).

### 2.4. Flavonoid analysis and quantification

Flavonoids in grapefruit pulp samples were analyzed by the method of Mouly et al. (Mouly et al., 1998), with some modifications. Fresh pulp (5 g) was homogenized with 20 mL (25 mL for peel) of dimethylformamide and subsequently a 1.5 mL aliquot was centrifuged at 7500 g for 20 min. The supernatant (20 μL) was injected into the high-pressure liquid chromatography (HPLC) system by an autosampler. Separation of flavonoid compounds was performed using Altima C-18 column (Alltech Associates, Deerfield, IL; 250 mm × 4.6 mm) and a guard column. A binary solvent system of ACN and water with 4% acetic acid was programmed to start at 0% and end at 70% CAN concentration for a 65 min period. The flavonoid

peaks were detected at 280 nm and were identified by matching spectra and retention times with those of commercially obtained standards. Quantification was performed using known concentrations of external standards.

### 2.5. Phenylalanine ammonia-lyase (PAL) extraction and assay

PAL activity was determined as previously described (Ke & Saltveit, 1986) in three replicate samples from frozen peel tissue. For the assay, PAL was extracted from 1.5 g sample with 16 mL of 50 mM sodium phosphate buffer (pH 8.5) containing 0.02 M β-mercaptoethanol and 0.15 g polyvinylpolypyrrolidine (PVPP). PAL activity was determined by measuring the absorbance of *trans*-cinnamic acid at 290 nm over a period of 1 h at 40 °C. The reaction mixture contained 5 mL of enzyme preparation, and 0.55 mL of L-phenylalanine (100 mM). A mixture containing 5 mL of enzyme preparation and 0.55 mL of water served as a blank.

### 2.6. Terpenoid analysis and quantification

Fresh grapefruit pulp (15 g), 50 mL distilled water (24 °C), and 200 μL of 5% acetone were placed in a 540 mL plastic jar (The Bel–Art Scienceware Company, Pequannock, NJ) and blended for 2.5 min at medium speed using an Osterizer food blender (Oster, Milwaukee, WI.). The plastic jar had a hole at the bottom which was covered by a rubber stopper for a sample port. Headspace gas samples (1 mL) were drawn using a Hamilton Syringe (Reno, NV) and injected into a GC (Perkin–Elmer 8700 Model) equipped with a flame ionization detector (FID).

Operating conditions for the GC were as follows: injector and detector temperature, 250 °C; air and $H_2$ pressure, 138 and 105 kPa, respectively; and 30 mL/min of helium as carrier gas. A glass column (2 mm ID and 270 cm long) packed with 80% Carbowax 1500 on Chromosorb WAW-HMDS 80/100 mesh was used for the separation. Oven temperatures were maintained at 50 °C for 0.5 min and raised to 130 °C at the rate of 10 °C/min (total run time of 8.5 min). Standard retention times were used to confirm the identity of volatile components. External standards were injected, and standard curves were constructed for quantification of the compounds in the samples.

### 2.7. Statistical analysis

The data was analyzed using a $3 \times 6$ factorial design with radiation dose and storage time intervals as factors and fruit weight as covariate in a GLM model of SAS (SAS., 2002). Treatment means were compared by least significant difference (LSD) at 5% probability levels.

## 3. Results and discussion

Grapefruit weight was significantly reduced with storage time ($P < 0.01$) irrespective of treatment. No significant ($P > 0.05$) interaction between irradiation dose and storage time was observed for fruit weight or any of the compounds tested in this study (data not shown).

### 3.1. Influence of irradiation and storage on flavonoid content and PAL activity

Fig. 1a and b shows HPLC chromatograms of flavonoids in grapefruit pulp and peel, respectively. These chromatograms show NAT, NAR, NEH, DID and PON along with the other peaks, which were not identified in this study. Fig. 1(c) shows the GC chromatogram of terpenoids (D-limonene and myrcene) in the grapefruit pulp samples. In our study, different flavonoids in the peel and pulp responded differently to irradiation dose and storage. Naringin content in grapefruit pulp was lower ($P = 0.012$) in fruits exposed to 150 Gy compared to 0 and 300 Gy (Fig. 2). These results indicate that irradiation (150 Gy) induced *de novo* synthesis of naringin may not be sufficient to counteract oxidative losses due to irradiation. Kakegawa, Hattori, Koike, and Takeda (1991) reported UV induction of flavonoid synthesis in cell cultures through regulation of two key enzymes phenyl ammonia lyase (PAL) and chalcone synthase (CHS). However, irradiation had no effect on naringin content of peel (Fig. 3). Even though naringin content was significantly reduced at initial storage period (12 d), the overall effect of storage on narigin content of either peel or pulp was not significant.

The narirutin content of the pulp was not affected by irradiation or storage (Fig. 2). Fruits exposed to 300 Gy irradiation had higher ($P = 0.01$) narirutin content in peel compared to fruits exposed to 0 Gy irradiation. Storage significantly increased narirutin content in the peel, even though significant decline was observed at initial (12 d) storage period (Fig. 3).

Fruits treated with 150 Gy irradiation had significantly lower poncerin content in the pulp compared to the fruits exposed to 0 and 300 Gy (Fig. 4). These results suggest that irradiation (150 Gy) induced *de novo* synthesis of poncerin may not be sufficient to counteract losses due to irradiation induced oxidative stress (Kakegawa et al., 1991). Fruits exposed to 300 Gy had higher ($P = 0.026$) poncerin content in the peel compared to control fruits (Fig. 5). Poncerin content in pulp was not affected by storage; however, a decline ($P = 0.016$) was observed in peel at initial (12 d) storage period, even though no significant differences were observed at the end of the storage compared to 0 d of storage.

Irradiation doses of 0 and 300 Gy resulted in higher ($P = 0.02$) didymin content in pulp compared to 150 Gy (Fig. 4). These results indicate that didymin degradation due to oxidative stress induced by radiation at 150 Gy was greater than *de novo* synthesis. Didymin content in peel was higher ($P = 0.001$) in fruits exposed to 300 Gy than 0 Gy (Fig. 5). Didymin content declined with the storage time, reaching lower levels ($P \geqslant 0.05$) after 24 d of storage at low temperature compared to initial levels. Moreover, significant reduction in didymin content was also observed

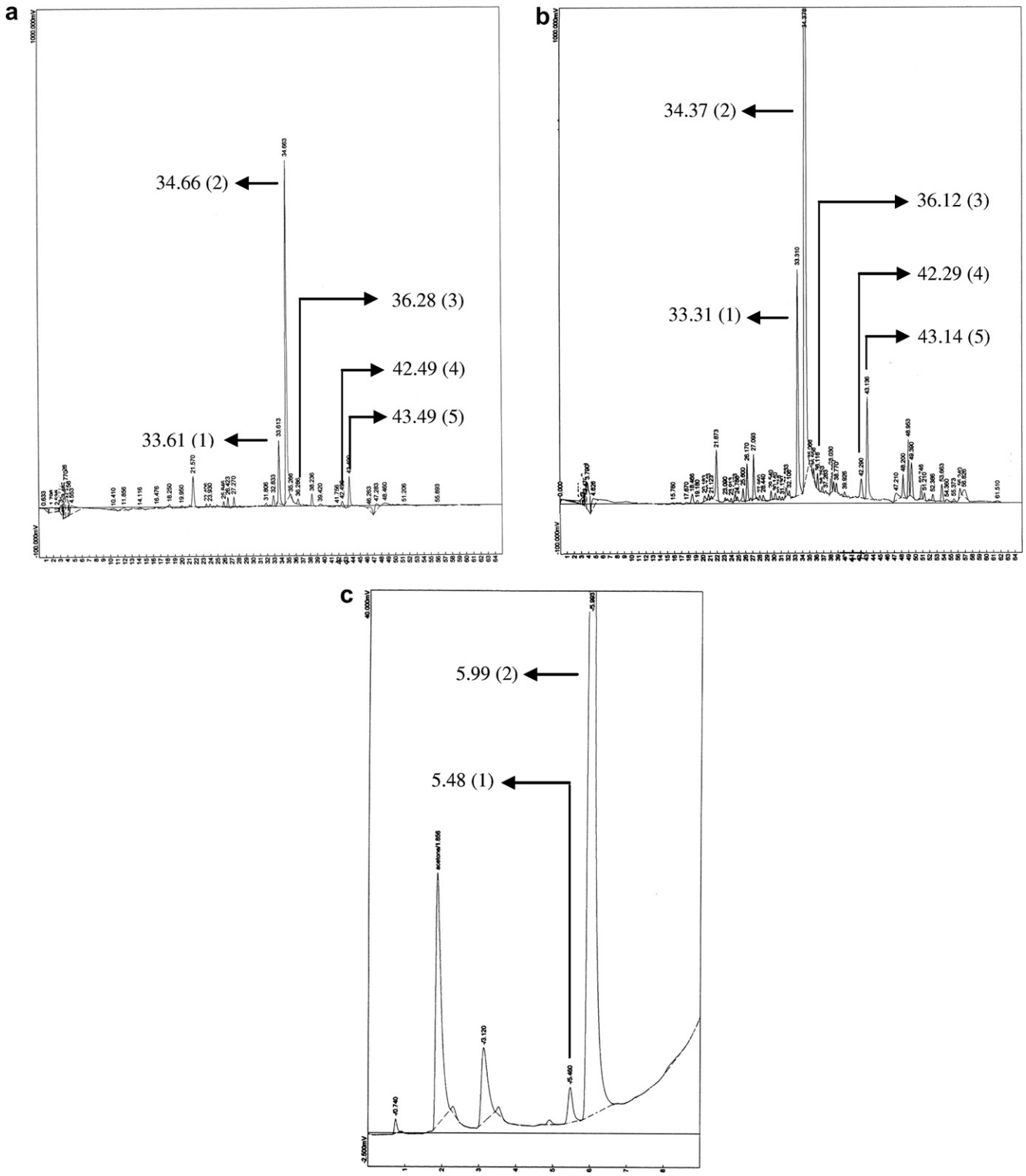


Fig. 1. (a and b) HPLC chromatograms of flavonoids from grapefruit pulp and peel using C-18 column and U.V. detection at 280 nm, respectively. Peaks are labeled 1–5 (corresponding retention times clarified in call-out boxes): 1, NAT; 2, NAR; 3, NEH; 4, DID; and 5, PON. (c) GC Chromatogram of terpenoids from grapefruit pulp using a glass column packed with 80% Carbowax 1500 on Chromosorb WAW-HMDS 80/100 mesh. Peaks are labeled 1 and 2 (corresponding retention times clarified in call-out boxes): 1, Myrcene and 2, D-Limonene.

immediately after transferring the fruits to marketing simulation. Interestingly, didymin content in peel increased ($P = 0.038$) with storage.

Irradiation had no significant effect on neohesperidin content of either pulp or peel (Fig. 6). Storage also had no significant effect on neohesperidin content in the pulp.

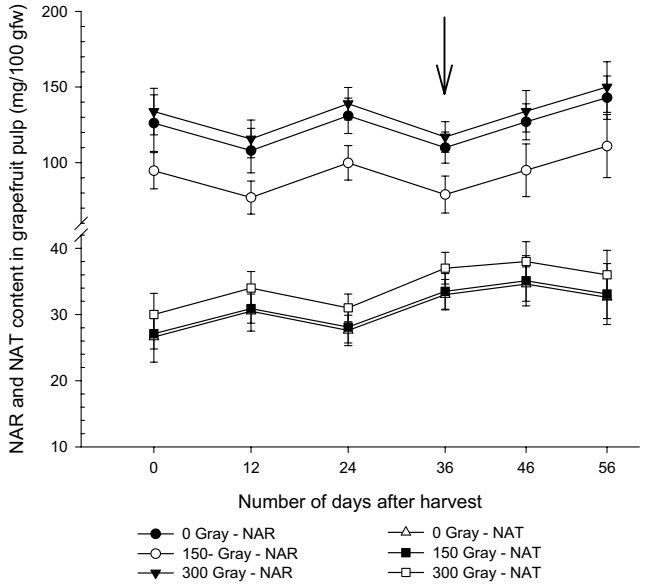


Fig. 2. Irradiation and storage effects on naringin (NAR) and narirutin (NAT) content of 'Rio Red' grapefruit pulp. Same letter for the lines indicates no significant differences for irradiation dose regardless of storage. "Arrow" indicates time of transfer from low temperature (10 °C) storage to ambient (20 °C) conditions.

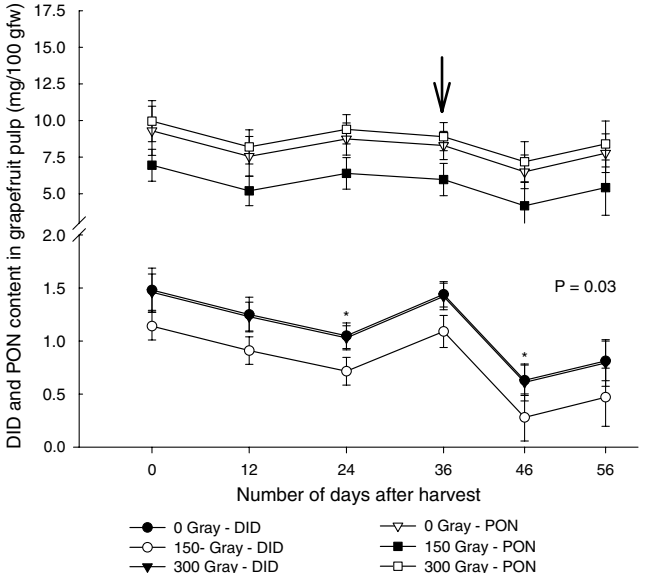


Fig. 4. Irradiation and storage effects on didymin (DID) and poncerin (PON) content of 'Rio Red' grapefruit pulp. *Indicates storage times which are significantly different from 0 d of storage regardless of irradiation dose. "Arrow" indicates time of transfer from low temperature (10 °C) storage to ambient (20 °C) conditions.

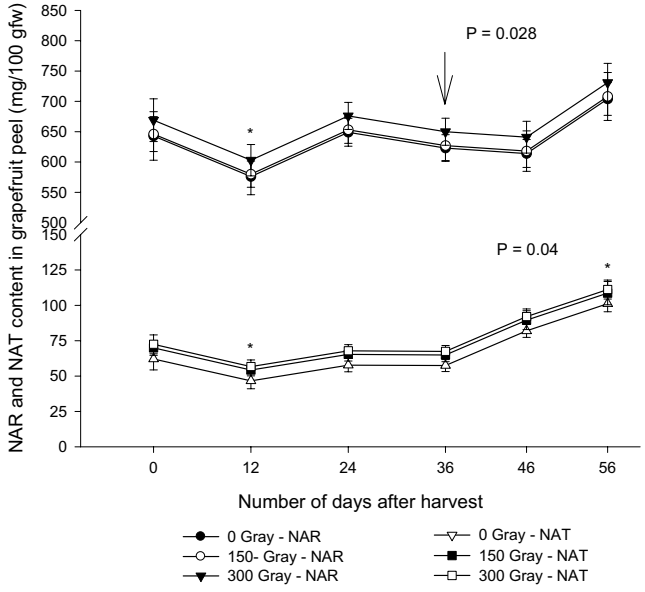


Fig. 3. Irradiation and storage effects on naringin (NAR) and narirutin (NAT) content of 'Rio Red' grapefruit peel. *Indicates storage times which are significantly different from 0 d of storage regardless of irradiation dose. "Arrow" indicates time of transfer from low temperature (10 °C) storage to ambient (20 °C) conditions.

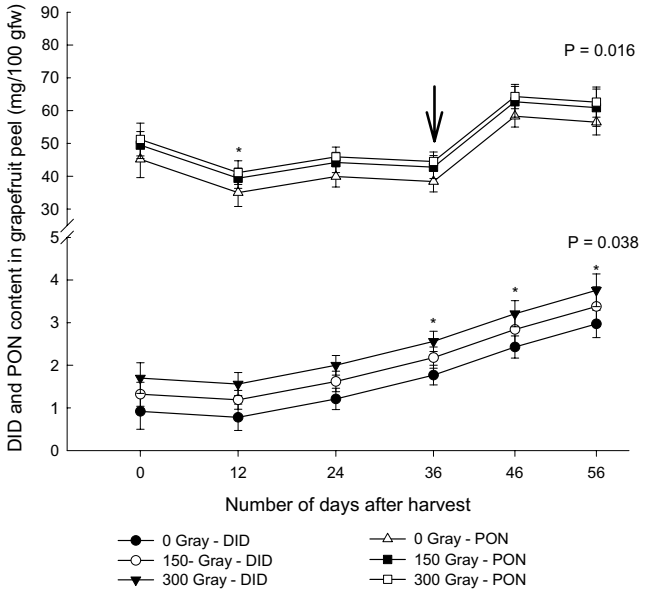


Fig. 5. Irradiation and storage effects on didymin (DID) and poncerin (PON) content of 'Rio Red' grapefruit peel. *Indicates storage times which are significantly different from 0 d of storage regardless of irradiation dose. "Arrow" indicates time of transfer from low temperature (10 °C) storage to ambient (20 °C) conditions.

However, neohesperidin content was significantly ($P = 0.006$) increased in the peel at the end of the storage compared to 0 d.

Gamma radiation (Riov, 1971) and storage (Oufedjikh, Mahrouz, Amiot, & Lacroix, 2000) have been found to stimulate phenolic biosynthesis in citrus fruit. PAL catalyzes the first reaction of the biosynthesis of flavonoids and a large group of other phenolic compounds such as lignins and coumarin in fruit (Dixon & Paiva, 1995). Previous research suggests that temperature may be the regulator for stimulation or reduction of PAL activity (Lafuente, Zacarias, Martinez-Tellez, Sanchez-Ballesta, & Granell, 2003). Furthermore, PAL activity was induced in Fortune mandarins stored at low temperature (Martinez-Tellez &

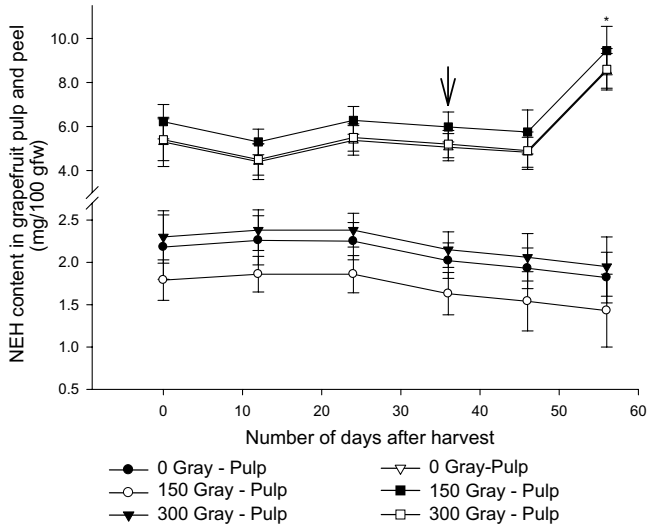


Fig. 6. Irradiation and storage effects on neohesperidin (NEH) content of 'Rio Red' grapefruit pulp and peel. *Indicates storage times which are significantly different from 0 d of storage regardless of irradiation dose. "Arrow" indicates time of transfer from low temperature (10 °C) storage to ambient (20 °C) conditions.

Lafuente, 1997). Apples stored at 10 °C had 2 times higher PAL activity as compared to that in apples stored at 24 °C (Faragher, 1983). However, we found that PAL activity in the peel of grapefruit at commercial maturity was very low (data not shown). Earlier studies also reported that PAL activity decreases sharply as the fruit increases in size during early months of growth, and only a trace of PAL activity could be found in fruits weighing 190 g (Maier & Hasegawa, 1970). In our study, average fruit weight was 420 g and this might be the reason for undetectable levels of PAL activity. As it is difficult to measure the activity of PAL in mature grapefruit, quantification of changes in protein levels of PAL using immunoblotting technique in future studies may provide insight into the effect of irradiation and storage on this important enzyme.

Grapefruit pulp (naringin, poncerin, and didymin) and peel (naringin, narirutin, poncerin and neohesperidin) flavonoid content decreased numerically or significantly ($P \leqslant 0.05$) after 12 d compared to 0 d at 10 °C. Low temperature storage induces oxidative stress in fruit, including citrus fruit (Sala, 1998). It is possible that rapid reduction in flavonoid content after12 d of storage may be due to utilization of flavonoids to scavenge reactive oxygen species produced by low temperature stress. Our results are in agreement with those of Oufedjikh et al. (2000) who showed a numerical reduction in hesperidin (a flavonoid) content in irradiated Clementine mandarin fruit at 7 d after low temperature storage.

Variation in flavonoid content at different doses of irradiation treatment (Oufedjikh et al., 2000) and storage time (Patil et al., 2004) may be due to difference in the loss of flavonoids by gamma irradiation and low temperature induced oxidative stress, and *de novo* synthesis of flavonoids.

### 3.2. Influence of irradiation and storage on terpenoid (D-limonene and myrcene) content

Terpenoids are the largest group of natural products in plants, and several of them are biologically active (Wagner & Elmadfa, 2003). Monoterpenes such as D-limonene derived from citrus fruits have been shown to possess chemopreventive properties against mammary, liver, and/or lung carcinogenesis (Reddy et al., 1997).

The content of D-limonene and myrcene in grapefruit pulp at the end of the storage was not significantly different from the 0 d of storage (Fig. 7). The content of D-limonene was numerically lower after 12 d of storage compared to 0 d of storage. However, a gradual increase in D-limonene content was observed after 12 d after storage and the D-limonene content at the end of the storage was higher ($P = 0.01$) compared to 12 d of storage. Non-irradiated (0 Gy) fruit had significantly higher D-limonene ($P = 0.005$) and myrcene ($P = 0.04$; Fig. 7) contents than fruits exposed to 150 Gy irradiation. Nunez-Selles et al. (Nunez-Selles, Maarse, & Bemelmans, 1986) reported numerical reduction in the D-limonene, myrcene and other volatile compounds in irradiated (1000 Gy) grapefruit stored for 28 d at 12 ± 1 °C.

### 3.3. Irradiation and storage effect on quality of grapefruit

Irradiation or storage did not result in considerable changes of the content of total soluble solids in grapefruits (Fig. 8). However, there was a decline ($P = 0.009$) in acid content during storage (Fig. 8). Fruit respiration continues even at low temperature, and organic acids are preferred

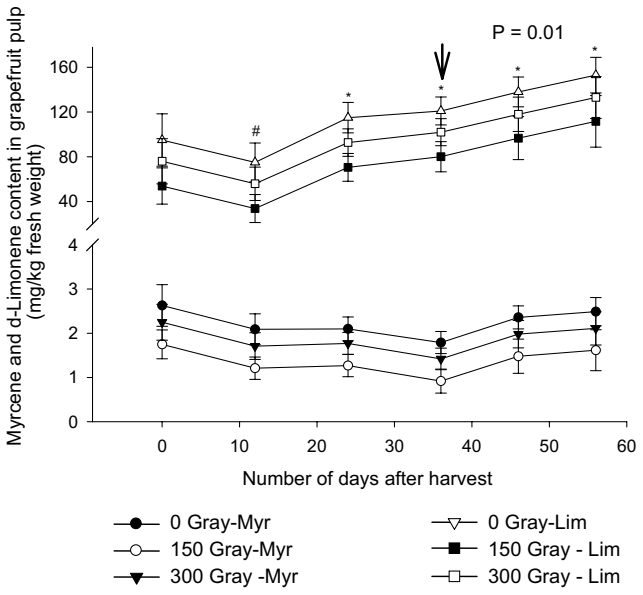


Fig. 7. Irradiation and storage effects on D-limonene and Myrcene content of 'Rio Red' grapefruit pulp. *Indicates storage times which are significantly different from 12 d of storage regardless of irradiation dose. "Arrow" indicates time of transfer from low temperature (10 °C) storage to ambient (20 °C) condition.

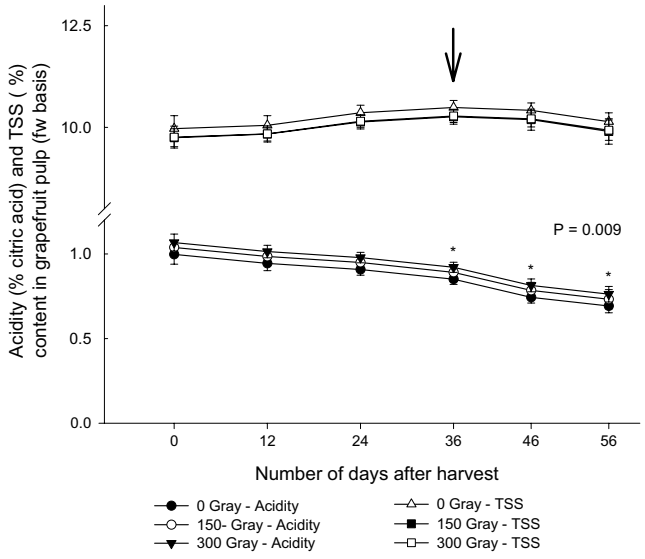


Fig. 8. Irradiation and storage effects on acidity and TSS content of 'Rio Red' grapefruit pulp. *Indicates storage times which are significantly different from 0 d of storage regardless of irradiation dose. "Arrow" indicates time of transfer from low temperature (10 °C) storage to ambient (20 °C) conditions.

substrates during respiration (Ladaniya, Singh, & Wadhawan, 2003). Thus, reduction in acidity due to storage could be attributed to continued respiration during prolonged storage. Fruits exposed to 300 Gy of irradiation had higher ($P = 0.012$) acidity compared to the control (0 Gy). A previous study reported that at the end of 60 d storage, higher acid content was observed in asparagus irradiated at 1, 1.5 and 2 kGy (Lescano, Narvaiz, & Kairiyama, 1993).

In summary, our results suggest that low-dose irradiation at 300 Gy enhanced or maintained the flavonoid concentration in the pulp during storage and did not have deleterious effects on the quality. Our data also suggests that irradiation at 300 Gy can be a viable quarantine treatment for grapefruit as it causes insignificant damage to the quality of grapefruit while promoting some bioactive compounds like flavonoids. However, it is important to understand the net effect of irradiation on the chemopreventive ability of grapefruit utilizing functional *in vivo* models before considering irradiation as a quarantine method.

## Acknowledgements

We are grateful to Dr. Nancy D. Turner and Dr. Lavanya Reddivari for useful suggestions during manuscript preparation. This project is based upon work supported by the USDA-CSREES-IFAFS # 2001-52102-02294 and USDA # 2005-34402-14401 "Designing Foods for Health" through the Vegetable and Fruit Improvement Center.

## References

Delincee, H., & Soika, C. (2002). Improvement of the ESR detection of irradiated food containing cellulose employing a simple extraction method. *Radiation Physics and Chemistry, 63*, 437–441.

Deschner, E. E., Ruperto, J., Wong, G., & Newmark, H. L. (1991). Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. *Carcinogenesis, 12*, 1193–1196.

Deyhim, F., Garcia, K., Gonzalez, J., Lopez, E., Ino, S., Garcia, M., et al. (2006). Citrus juice modulates bone strength in male senescent rat model of osteoporosis. *Nutrition, 22*, 559–563.

Deyhim, F., Lopez, E., Gonzalez, J., Garcia, M., & Patil, B. S. (2006). Citrus juice modulates antioxidant enzymes and lipid profiles in orchidectomized rats. *Journal of Medicinal Foods., 9*, 422–426.

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

Faragher, J. D. (1983). Temperature regulation of anthocyanin accumulation in apple skin. *Journal of Experimental Botany, 34*, 1291–1298.

Hallman, G. J. (1999). Ionizing radiation quarantine treatments against tephritid fruit flies. *Postharvest Biology and Technology, 16*, 93–106.

Hallman, G. J., & Martinez, L. R. (2001). Ionizing irradiation quarantine treatment against Mexican fruit fly (diptera: Tephritidae) in citrus fruits. *Postharvest Biology and Technology, 23*, 71–77.

Kakegawa, K., Hattori, E., Koike, K., & Takeda, K. (1991). Induction of anthocyanin synthesis and related enzyme activities in cell cultures of *Centaurea cyanus* by UV-light irradiation. *Phytochemistry, 30*, 2271–2273.

Ke, D., & Saltveit, M. E. (1986). Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in iceberg lettuce. *Hortscience, 21*, 1169–1171.

Kovacs, E., & Keresztes, A. (2002). Effect of gamma and UV-b/c radiation on plant cells. *Micron, 33*, 199–210.

Kurowska, E. M., Borradaile, N. M., Spence, J. D., & Carroll, K. K. (2000). Hypocholesterolemic effects of dietary citrus juices in rabbits. *Nutrition Research, 20*, 121–129.

Ladaniya, M. S., Singh, S., & Wadhawan, A. K. (2003). Response of 'Nagpur' mandarin, 'Mosambi' sweet orange and 'Kagzi' acid lime to gamma radiation. *Radiation Physics and Chemistry, 67*, 665–675.

Lafuente, M. T., Zacarias, L., Martinez-Tellez, M. A., Sanchez-Ballesta, M. T., & Granell, A. (2003). Phenylalanine ammonia-lyase and ethylene in relation to chilling injury as affected by fruit age in citrus. *Postharvest Biology and Technology, 29*, 309–318.

Leonardi, T., Vanamala, J., Taddeo, S. S., Murphy, M. E., Patil, B. S., Wang, N., et al. (2004). Apigenin and naringenin suppress high multiplicity aberrant crypt foci formation and cell proliferation in rat colon. *FASEB Journal, 18*, A348.344.

Lescano, G., Narvaiz, P., & Kairiyama, E. (1993). Gamma irradiation of asparagus (*Asparagus officinalis*, var. Argenteuil). *Lebensmittel-Wissenschaft und-Technologie, 26*, 411–416.

Liu, Y., Ahmad, H., Luo, Y., Gardiner, D. T., Gunasekera, R. S., McKeehan, W. L., et al. (2001). Citrus pectin: Characterization and inhibitory effect on fibroblast growth factor-receptor interaction. *Journal of Agricultural and Food Chemistry, 49*, 3051–3057.

Maier, V. P., & Hasegawa, S. (1970). L-phenylalanine ammonia-lyase activity and naringenin glycoside accumulation in developing grapefruit. *Phytochemistry, 9*, 139–144.

Martinez-Tellez, M. A., & Lafuente, M. T. (1997). Effect of high temperature conditioning on ethylene, phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase activities in flavedo of chilled "Fortune" mandarin fruit. *Journal of Plant Physiology, 150*, 674–678.

Moshonas, M. G., & Shaw, P. E. (1984). Effects of low-dose gamma irradiation on grapefruit products. *Journal of Agricultural and Food Chemistry, 32*, 1098–1101.

Mouly, P., Gaydou, E. M., & Auffray, A. (1998). Simultaneous separation of flavanone glycosides and polymethoxylated flavones in citrus juices using liquid chromatography. *Journal of Chromatography A, 800*, 171–179.

Moy, J. H., & Wong, L. (2002). The efficacy and progress in using radiation as a quarantine treatment of tropical fruits – A case study in Hawaii. *Radiation Physics and Chemistry, 63*, 397–401.

Nunez-Selles, A. J., Maarse, H., & Bemelmans, J. M. H. (1986). Flavor changes in gamma irradiated grapefruit. *Food Chemistry, 21*, 183–193.

Oufedjikh, H., Mahrouz, M., Amiot, M. J., & Lacroix, M. (2000). Effect of gamma -irradiation on phenolic compounds and phenylalanine ammonia-lyase activity during storage in relation to peel injury from peel of *Citrus clementina* hort. Ex. Tanaka. *Journal of Agricultural and Food Chemistry, 48*, 559–565.

Patil, B., Vanamala, V., & Hallman, G. (2004). Irradiation and storage influence on bioactive compounds and quality of early and late season 'Rio Red' grapefruit (citrus paradisi macf). *Postharvest Biology and Technology, 34*, 53–64.

Peterson, J., & Dwyer, J. (1998). Flavonoids: Dietary occurrence and biochemical activity. *Nutrition Research, 18*, 1995–2018.

Poulose, S. M., Harris, E. D., & Patil, B. S. (2005). Citrus limonoids induce apoptosis in human neuroblastoma cells and have radical scavenging activity. *Journal of Nutrition, 135*, 870–877.

Poulose, S. M., Harris, E. D., & Patil, B. S. (2007). Antiproliferative effects of citrus limonoids against human neuroblastoma and colonic adenocarcinoma cells. *Nutrition and Cancer, 56*, 103–112.

Raman, G., Jayaprakasha, G. K., Cho, M., Brodbelt, J. S., & Patil, B. S. (2004). Isolation of structurally similar citrus flavonoids by flash chromatography. *Analytical Letter, 37*, 3005–3016.

Reddy, B. S., Wang, C. X., Samaha, H., Lubet, R., Steele, V. E., Kelloff, G. J., et al. (1997). Chemoprevention of colon carcinogenesis by dietary perillyl alcohol. *Cancer Research, 57*, 420–425.

Riov, J. (1971). 6,7-Dimethoxycoumarin in the peel of gamma-irradiated grapefruit. *Phytochemistry, 10*, 1923–1924.

Sala, J. M. (1998). Involvement of oxidative stress in chilling injury in cold-stored mandarin fruits. *Postharvest Biology and Technology, 13*, 255–261.

Selles, A. J. N., Maarse, H., & Bemelmans, J. M. H. (1986). Flavour changes in gamma irradiated grapefruit. *Food Chemistry, 21*, 183–193.

Tian, Q., Miller, E. G., Ahmad, H., Tang, L., & Patil, B. S. (2001). Differential inhibition of human cancer cell proliferation by citrus limonoids. *Nutrition and Cancer – An International Journal, 40*, 180–184.

Vanamala, J., Cobb, G., Turner, N. D., Lupton, J. R., Yoo, K. S., Pike, L. M., et al. (2005). Bioactive compounds of grapefruit (*Citrus paradisi* c.v. Rio Red) respond differently to postharvest irradiation, storage, and freeze drying. *Journal of Agricultural and Food Chemistry, 53*, 3980–3985.

Vanamala, J. V., Leonardi, T., Patil, B. S., Taddeo, S. S., Murphy, M. E., Pike, L. M., et al. (2006). Suppression of colon carcinogenesis by bioactive compounds in grapefruit. *Carcinogensis, 27*, 1257–1265.

Vinson, J. A., Liang, X., Proch, J., Hontz, B. A., Dancel, J., & Sandone, N. (2002). Polyphenol antioxidants in citrus juices: In vitro and in vivo studies relevant to heart disease. *Advances in Experimental Medicine and Biology, 505*, 113–122.

Wagner, K. H., & Elmadfa, I. (2003). Biological relevance of terpenoids. Overview focusing on mono-, di- and tetraterpenes. *Annals of Nutrition and Metabolism, 47*, 95–106.

Yang, K., Lamprecht, S. A., Liu, Y., Shinozaki, H., Fan, K., Leung, D., et al. (2000). Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. *Carcinogenesis, 21*, 1655–1660.

Yu, J., Wang, L., Walzem, R. L., Miller, E. G., Pike, L. M., & Patil, B. S. (2005). Antioxidant activity of citrus limonoids, flavonoids, and coumarins. *Journal of Agricultural and Food Chemistry, 53*, 2009–2014.