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Electron-Beam Ionizing Radiation Stress Effects on Mango Fruit (*Mangifera indica* L.) Antioxidant Constituents before and during Postharvest Storage

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The effect of electron-beam ionizing radiation stress and storage on mango fruit antioxidant compounds was evaluated in a dose range of 1−3.1 kGy. Phenolic high-performance liquid chromatography (HPLC) profiles were not affected right after the irradiation process; however, an increase in flavonol constituents was observed after 18 days in storage (3.1 kGy). Total phenolics by the Folin Ciocalteu method and antioxidant capacity (ORAC) were not affected, while reduced ascorbic acid decreased ∼50−54% during storage (≥1.5 kGy). No major changes in carotenoid HPLC profiles indicated a delay in ripening of irradiated mangoes (1−3.1 kGy) compared to nonirradiated fruits. However, irradiation dose ≥1.5 kGy induced flesh pitting due to localized tissue death. A summary of the potential roles of reactive oxygen species generated by the irradiation stress on different antioxidant constituents of mango fruits is presented.

KEYWORDS: Ionizing radiation; *Mangifera indica* L.; phenolic compounds; carotenoids; ascorbic acid; ORAC

INTRODUCTION

The use of ionizing radiation as a food-processing technique has been known for many years (*1*), and many of its potential applications have been explored in the last decades, including sprout inhibition, insect disinfestation, shelf life enhancement, senescence or ripening delay, and microorganism elimination (*2*, *3*). The use of ionizing radiation in foods has been approved for gamma-rays (from ^{60}Co or ^{137}Cs sources), X-rays, and electron beam (*4*). Although all these processing techniques can ultimately break chemical bonds, remove electrons (forming ions, free radicals, or reactive oxygen species), and induce water radiolysis (*5*, *6*), the type of radiation used can potentially alter the processing outcome of the irradiated material (*7*).

Ionizing radiation has been reported to affect the cellular antioxidant status through the induction of reactive oxygen species (ROS) in living tissue (*8*, *9*). This oxidative stress can have an impact on the nutritional components of foods such as fruits and vegetables. Ionizing radiation can cause a stress that may affect the secondary metabolism of fresh produce. Recent work has shown that ionizing radiation may increase quercetin levels in onions (*10*), anthocyanins in strawberries (*11*), flavanones in grapefruits (*12*), and other phenolic compounds in oranges (*13*) and mushrooms (*14*).

The ionizing radiation-induced ROS may act as a signaling molecule, triggering the phenylpropanoid metabolism (*13*). In recent years, there has been increasing interest in the enhancement of antioxidant content in fruits and vegetables because of their health benefits (*15*); thus, one of the potential effects of using ionizing radiation can be the nutraceutical enhancement of fresh produce (*16*). Most of the work on ionizing radiation has focused mainly on food-safety (*6*) and food-quality issues (*5*). A complete understanding of the effect of ionizing radiation on the content of antioxidant phytochemicals and changes in antioxidant capacity is lacking (*17*). The purpose of this study was to characterize the changes induced in antioxidant constituents in mango fruits after ionizing radiation and during storage. The approach was to expose the fruit to a typical dose range of 1−3.1 kGy used for insect disinfestation and reduction of human pathogen microorganisms and to characterize the effects on phenolics, carotenoids, and ascorbic acid as well as antioxidant capacity. The generated information can be used to optimize the conditions for applying ionizing radiation treatments to maintain or enhance the nutritional supply of fruits.

MATERIALS AND METHODS

Plant Material and Irradiation Procedure. Mango fruits (*Mangifera indica* L. cv. Tommy Atkins) were purchased from a local retailer in College Station, TX, and stored overnight at 10 °C. Mangoes were individually labeled, taken to the electron-beam irradiation facility at the National Center for Electron Beam Food Research at Texas A&M University (College Station, TX), and placed on different open card box trays for each treatment performed. The fruits were then subject to different ionizing radiation doses at room temperature (1, 1.5, and 3.1 kGy). Control samples were not irradiated. The desired dose was obtained by varying the speed of the conveyor belt (0.3, 0.2, or 0.1 m/s, respectively) and measured with dosimetry

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Table 1. Phenolic Changes and Tryptophan in Irradiated and Nonirradiated Mango Fruit before and after 18 Days of Storage at 15 °C

				phenolic and tryptophan content[j] (mg/100 g of tissue)							
				day 0				day 18			
peak[a]	ret. time (min)	phenolic compound and tryptophan	visible spectra[b] (nm)	0 kGy	1 kGy	1.5 kGy	3.1 kGy	0 kGy	1 kGy	1.5 kGy	3.1 kGy
1[f]	4.84	tryptophan	217.2, 279.0	1.8 ab	1.2 b	2.0 a	1.7 ab	1.2 a	1.9 a	1.0 a*	1.2 a
2[g]	15.13	quercetin derivative[c]	202.8, 253.8, 356.4	0	0	0	0	0	0	0	0.1*
3[g]	15.29	quercetin derivative[d]	204.8, 254.1, 355.8	0	0	0	0	0.07 b*	0.14 b*	0.1 b*	0.28 a*
6[h]	16.04	gallic acid derivative[e]	216.0, 272.8	0	0	0	0	0.2 a*	0.1 a*	0.2 a*	0
8[h]	28.80	gallic acid derivative[e]	212.7, (279.3)	1.02 a	0.97 a	0.96 a	0.95 a	0.94 a*	0.76 a*	0.80 a*	0.79 a
9[i]	29.02	benzoic acid derivative	196.5, (209.0), 235.4, 286.3	5.7 a	5.6 a	5.4 a	5.0 a	5.9 a	5.3 a	4.9 a	5.5 a*
10[i]	29.73	benzoic acid derivative	195.4, 238.8, 285.0	14.3 a	12.8 a	12.4 a	12.7 a	14.5 a	13.3 a*	13.4 a*	14.5 a*
11[i]	30.64	benzoic acid derivative	196.9, 223.6, 272.7	2.5 a	2.0 a	1.9 a	1.8 a	1.9 a	1.5 a*	1.5 a*	1.5 a*
12[i]	31.73	benzoic acid derivative	194.4, (207.1), 236.9, 286.5	3.7 a	3.1 a	3.0 a	3.0 a	2.6 a	2.2 a*	2.2 a*	2.2 a*
13[i]	31.95	benzoic acid derivative	194.2, 238.9, 285.6	5.8 a	5.0 a	5.1 a	5.1 a	5.1 a	4.5 ab*	4.3 b*	4.2 b*

[a] Peak assignment corresponds to **Figures 1A** and **1B**. [b] Data in parentheses represents a shoulder at that wavelength. [c] Derivative corresponds to either glucoside or galactoside. [d] Tentative identification based on matching UV spectra with quercetin–*o*-rutinoside. [e] Tentative identification based on matching UV spectra with gallic acid. [f] Expressed as tryptophan equivalents. [g] Expressed as quercetin–*o*-rutinoside equivalents. [h] Expressed as gallic acid equivalents. [i] Expressed as benzoic acid equivalents. [j] Same letters within data (ab) show nonsignificant differences due to irradiation dosage at a given storage day ($P > 0.05$). An asterisk (*) indicates data that are significantly different after storage at a given irradiation dose ($P < 0.05$).

labels attached to the fruit surface. The 10 MeV electron-beam linear accelerator (Titan Corp., Lima, OH) was set with a dual-electron-beam fixture (from top and bottom), a dose rate of 0.8 kGy/s, and a scan width of 0.61 m.

Storage and Sampling. After irradiation, mangoes were stored at 15 °C and sampled at day 0, 5, and 18. Four different mango fruits were used as replicates per treatment (in average, 440 g each). After storage, fruit samples were cut and sampled according to the different phytochemical analyses. Mango slices were cut longitudinally to the mango pith (from stem end to blossom end), and the pericarp was removed from the slices. Slices were taken from all sides of the fruit and were then cut and sampled directly into 50 mL plastic tubes. Sample weights were recorded, and the plastic tubes were covered and stored at −80 °C until required for analysis. Each sample analyzed represents tissue from a single fruit. Each assay was sampled with 4 replications using one independent extraction per fruit.

Phenylalanine Ammonia Lyase Activity (PAL) and Phenolic Compounds. PAL activity was assayed using a 1 g sample in borate buffer (pH 8.5) containing β-mercaptoethanol and polyvinylpolypyrrolidone, while total soluble phenolics was assayed by the Folin Ciocalteu method using a 5 g sample in methanol solvent. Both methods were assayed according to Reyes et al. (*18*). The methanol extract used for total phenolic quantification was also used for high-performance liquid chromatography (HPLC) phenolic profile analysis, which was adapted from Hale (*19*). The HPLC system was a Waters 515 binary pump system, a Waters 717 plus autoinjector, and a Waters 996 photodiode array detector connected to a computer with Waters Millennium 3.2 software. Phenolic compounds were separated in a Waters Atlantis column (4.6 × 150 cm, 5 μm) fitted with a Waters Atlantis guard column (3.9 × 20 mm, 10 μm, 125 Å) and maintained at 40 °C using a SpectraPhysics SP8792 column heater. Separation was achieved with a gradient set at 1.0 mL/min with a mobile phase of water (pH 2.3 with HCl; solvent A) and acetonitrile (solvent B). The gradient was programmed for 60 min as follows (min/%A): 0/85, 5/85, 30/0, and 35/0, using a linear gradient for each step. The column was then reconditioned for 25 min before the next injection. Phenolic compounds were detected at 280 (phenolic acids), 320 (hydroxycinnamic acids), and 360 nm (flavonols). Standards used, including benzoic acid, quercetin, quercetin−*o*-rutinoside, quercetin glucoside, quercetin galactoside, gallic acid, and tryptophan, were purchased from Sigma Chemical Co. (St. Louis, MO).

Carotenoids. For carotenoid determination, 10 g of mangoes were homogenized with 25 mL of dichloromethane (DCM) containing 0.1% butylated hydroxytoluene (BHT) (*20*). Samples were kept in ice and under low light conditions throughout the assay. The homogenate was filtered through four layers of cheesecloth and centrifuged at 31 000 g at 2 °C for 15 min. The supernatant was collected and used for carotenoid HPLC profile analysis. From the clear supernatant, 5 mL were collected and placed in a Savant SVC-100H SpeedVac concentrator (Thermo Savant, Holbrook, NY) connected to a Cole-Parmer aspirator pump (model 7049-00; Cole-Parmer Instruments Co., Vernon Hills, IL) inside a cold room at 2 °C for 2 h. Samples were then resuspended in 1 mL of the extracting solvent. The determination of individual carotenoids was adapted from Hale (*19*) using the HPLC system used for phenolic profiles attached to a Waters YMC C30 carotenoid column (4.6 × 250 mm, 5 μm) maintained at 35 °C using a SpectraPhysics SP8792 column heater, with detection set at 450 nm. Carotenoids were separated using a gradient set at 1.0 mL/min with a mobile phase of 90:10:0.1 methanol/water/triethylamine (solvent A) and 6:90:0.1 methanol/methyl−*tert*-butyl ether/triethylamine (solvent B). The gradient was programmed for 73 min as follows (min/A%): 0/99, 8/99, 45/0, 50/0, and 53/99, using a linear gradient for each step. The column was then reconditioned for 20 min before the next injection. Carotenoid standards used including violaxanthin, neoxanthin, and β-carotene were purchased from Sigma Chemical Co. (St. Louis, MO).

Ascorbic Acid. Reduced ascorbic acid extraction and quantification was according to Reyes et al. (*18*) using 5 g samples with 25 mL of 3% citric acid. The HPLC system was a Thermo Finnigan P100 isocratic pump, a Spectra Physics Spectra 100 UV/vis variable-wavelength detector, and a HP3394 integrator.

Antioxidant Capacity. A modification of the procedure by Huang et al. (*21*) and Prior et al. (*22*) was used to determine the antioxidant capacity of mango extracts against peroxyl radicals (ORAC assay). The methanolic extract used for total phenolic content determination was used for ORAC measurements. Sample extracts (typically diluted 50 or 100 times), Trolox standard (40 μM), and reagents were dissolved in 75 mM potassium phosphate buffer, pH 7.4, and assayed on a Bio-Tek Synergy HT plate reader with automatic injectors (Bio-Tek Instruments, Inc., Winooski, VT) using a black, clear-bottom 96-well plate from Corning (Costar #3631, Corning, Inc., Corning, NY). The fluorescein stock solution (FLs) was prepared with 0.1125 g of fluorescein (FL) (Sigma-Aldrich, St. Louis,

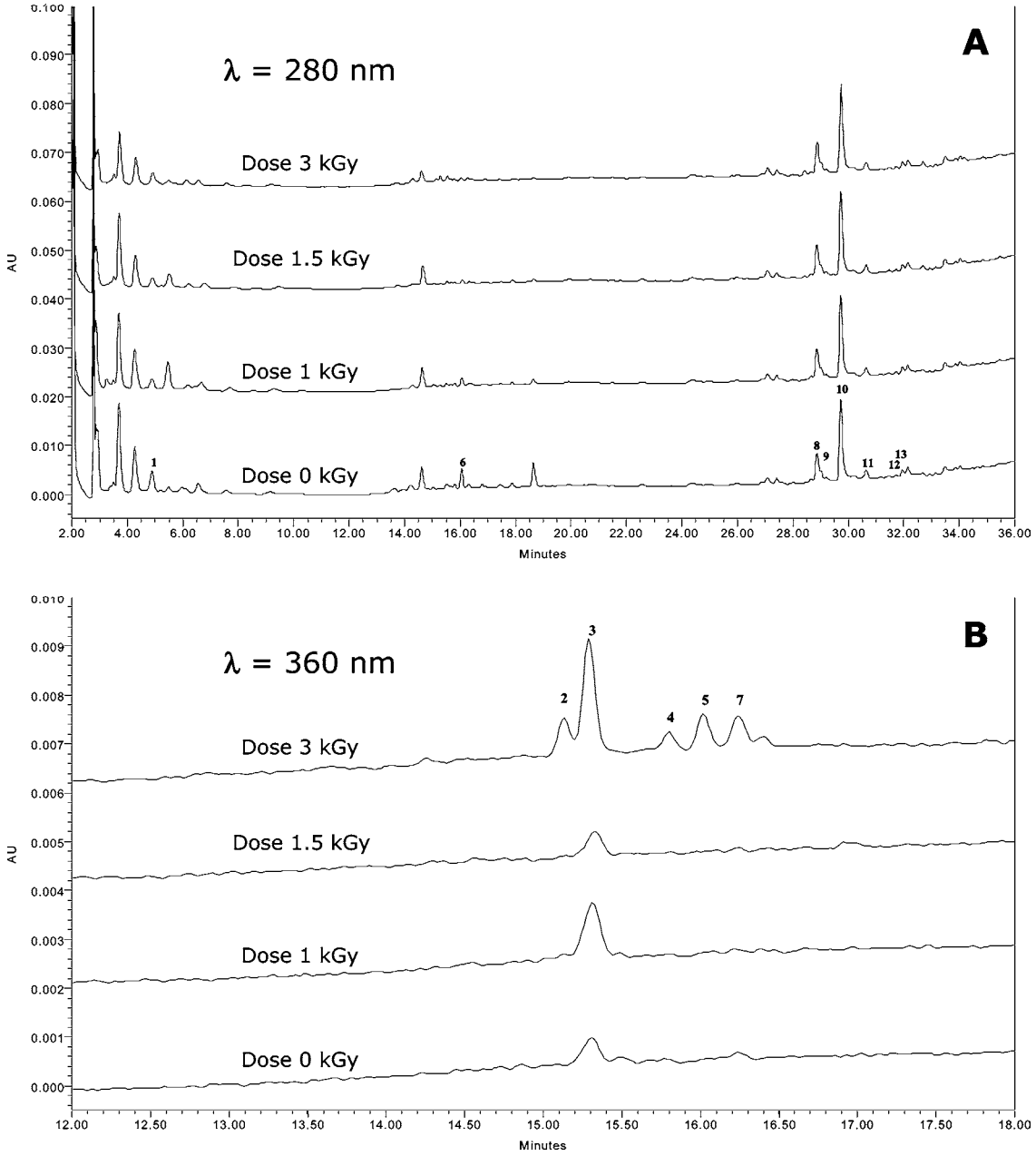

Figure 1. HPLC profile of phenolic compounds in irradiated and nonirradiated mangoes after 18 days of storage at 15 °C. Numbers in each peak refer to phenolics in **Table 1** and as explained in the Results and Discussion section. HPLC profiles are reported at 280 nm (**A**) and 360 nm (**B**).

MO) in 50 mL of the buffer. Next, 100 μL of FLs was diluted in 10 mL of the buffer (FL_2). After preparation, FLs and FL_2 solutions were stored at 2 °C. The assay was performed at 37 °C (plate reader chamber was previously incubated at this temperature for 30 min). Before the assay, a third FL solution (FL_3) was prepared by taking 400 μL of FL_2 to 25 mL. A water bath (to incubate FL_3 and ~15 mL of buffer) and a circulating air oven (to incubate the black plate and the reagent plastic containers for the autoinjector) were set at 45 °C for 30 min. After 30 min, samples were loaded in duplicate columns into the black plate (25 μL) (48 samples per run in 6 columns × 8 rows of the 96-well plate) with each row containing different samples or dilutions. Columns 5 and 6 were loaded with 25 μL of Trolox and the buffer (blank), respectively. The plate was then placed inside the circulating air oven for 15 min. At 15 min, the AAPH peroxyl radical solution (2,2′-azobis(2-amidinopropane)dihydrochloride; Wako Chemicals, Richmond, VA) was prepared right away by weighing 0.26 g of AAPH and completing to 10 mL with the incubated buffer. The FL_3 and AAPH solutions were then transferred into the incubated autoinjector plastic containers. Injectors were primed, and the plate reader was configured to inject 200 μL of FL_3, shaking plate at medium intensity for 3 s, injecting 75 μL of AAPH solution, and taking readings for 50 min every 1:27 min (35 cycles) without shaking during readings. The reading mode was fluorescence kinetic with injection (Ex/Em: 485/ 528), using bottom optics and sensitivity 46. Data was recorded with the KC-4 v3.4. rev 12 software and exported into Excel. Relative fluorescence ($f_i = F_i/F_1$, where F_i is fluorescence at cycle i) was calculated for all wells and kinetic readings, and the area under the curve (AUC) was calculated for the 35 cycles as AUC $= (f_1 + f_{35})/2 + (f_2 + f_3 ... + f_{33} + f_{34})$. The net area under the curve (NAUC) was calculated as AUC sample − AUC blank. ORAC value (expressed in μM) was then calculated as (NAUC sample/NAUC Trolox) × 40. The linear range of the assay was from 6.25 to 75 μM Trolox.

Table 2. Carotenoid Changes in Irradiated and Nonirradiated Mango Fruit before and after 18 Days of Storage at 15 °C

				carotenoid content[f] (μg/100 g of tissue)							
				day 0				day 18			
peak[a]	ret. time (min)	carotenoid	visible spectra[b] (nm)	0 kGy	1 kGy	1.5 kGy	3.1 kGy	0 kGy	1 kGy	1.5 kGy	3.1 kGy
1[c]	22.35	violaxanthin derivative	414.8, 439.1, 469.5	37 ab	46 a	29 ab	10 b	35 a	25 a	21 a	1 b*
2[c]	25.92	violaxanthin derivative	415.2, 439.5, 469.5	8 a	11 a	6 a	5 a	28 a*	13 ab	16 ab	3 b
3[c]	27.92	violaxanthin derivative	415.0, 440.9, 469.8	48 a	68 a	32 a	33 a	60 a	44 ab	58 a	8 b
4[d]	28.39	neoxanthin derivative	409.9, 435.5, 461.8	21 a	16 a	18 a	20 a	49 a*	25a b	32 ab	17 b
5[c]	28.98	violaxanthin derivative	416.0, 440.4, 469.8	251 a	381 a	203 a	183 a	531 a*	315 ab	389 a	51 b
6[d]	30.48	neoxanthin derivative	412.3, 435.5, 464.7	111 a	165 a	93 a	78 a	223 a*	148 ab	179 a	25 b
7[c]	33.38	violaxanthin derivative	416.6, 441.3, 470.7	28 a	30 a	16 b	16 b	21 a	19 a	25 a	9 a
8[c]	33.55	violaxanthin derivative	416.6, 441.3, 470.8	0	0	0	0	75 a*	39 b*	30 bc*	3 c*
9[c]	34.79	violaxanthin derivative	416.2, 441.0, 470.8	47 a	69 a	37 a	32 a	87 a*	55 ab	63 a	13 b
10[c]	36.05	violaxanthin derivative	415.3, 440.4, 469.9	25 a	31 a	18 a	14 a	98 a*	47 bc	64 ab	14 c
11[d]	36.61	neoxanthin derivative	412.8, 436.9, 466.3	20 a	23 a	16 a	11 a	50 a*	29 ab	40 a	8 b
12[e]	36.92	β-carotene	(433.1), 452.3, 478.4	562 ab	791 a	455 ab	340 b	823 a	613 ab	641 ab	382 b
			total	1159 a	1634 a	923 a	744 a	2078 a*	1371 ab	1556 ab	534 b

[a] Peak assignment corresponds to **Figure 2**. [b] Data in parentheses represents a shoulder at that wavelength. [c] Expressed as violaxanthin equivalents. [d] Expressed as neoxanthin equivalents. [e] Expressed as β-carotene. [f] Different letters within data (abc) show significant differences due to irradiation dosage at a given storage day ($P < 0.05$). An asterisk (*) indicates data that are significantly different after storage at a given irradiation dose ($P < 0.05$).

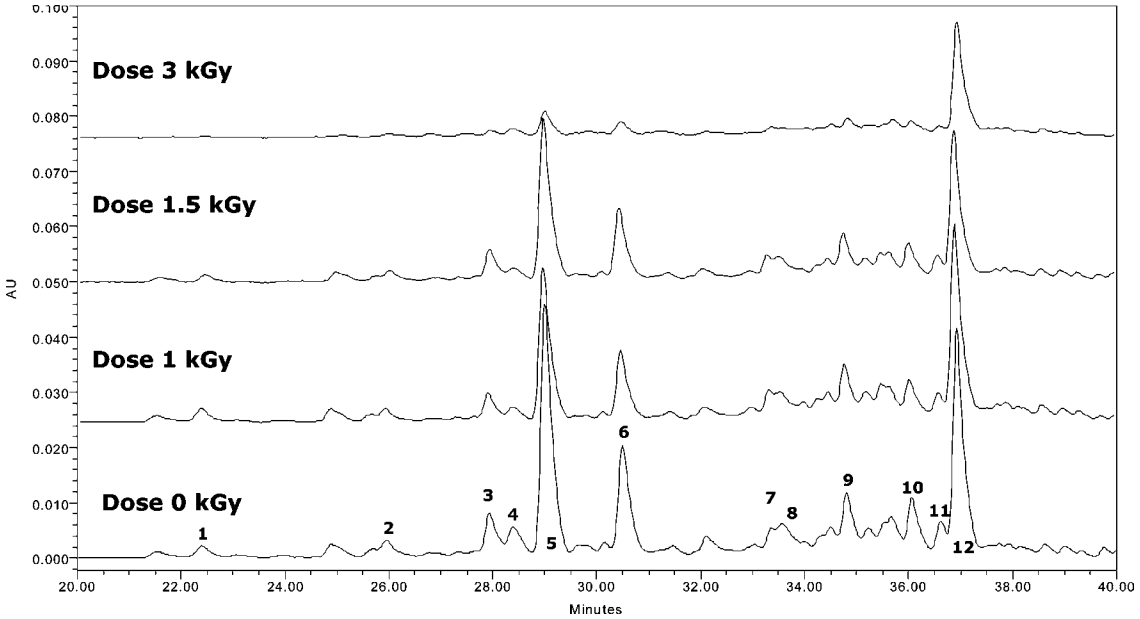

Figure 2. HPLC profile of carotenoids in irradiated and nonirradiated mangoes after storage at 15 °C for 18 days. Numbers in each peak refer to carotenoids listed in **Table 2**.

Dry Matter. Samples (5 g) were dried for 24 h in a vacuum oven set at 70 °C and 12 in. Hg vacuum (Isotemp vacuum oven model 285A; Fisher Scientific, Pittsburgh, PA). Storage and irradiation dose did not induce significant changes in the dry matter content of mangoes, which averaged at 17.0%. Therefore, all results were expressed on a fresh weight basis.

Graphs and Statistical Analysis. Summary statistics, graphs, and linear regressions were obtained using Microsoft Excel 2002. Statistical analyses were performed with the GLM procedure, and means were compared using Duncan's multiple range test ($\alpha = 0.05$) (The SAS System for Windows version 8.2; SAS Institute Inc., 1999).

RESULTS AND DISCUSSION

Phenolic HPLC Profiles and PAL Activity. The main phenolic compounds identified in control mango fruits at day 0 were phenolic acids (**Table 1**). Gallic acid and benzoic acid derivatives were tentatively identified because of similar spectra to the corresponding phenolic acids. These observations differ from those reported by Shieber et al. (*23*), where 80% of phenolic compounds corresponded to flavonol derivatives. Flavonol content was found to be dependent on mango maturity stage and cultivar selection (*24*).

Right after the irradiation treatments, no changes were observed in the phenolic profile of irradiated mango fruits compared to controls ($P > 0.05$). However, when control fruits were stored for 18 days, two peaks appeared ($P < 0.05$) corresponding to quercetin and gallic acid derivatives (peaks 3 and 6) and one gallic acid derivative decreased by ~8% (peak 8) compared to day 0 (**Table 1**). On the other hand, irradiated fruits showed decreases ranging from 16 to 21% and from 10 to 29% in gallic acid and benzoic acid derivatives (peaks 8 and 11−13), respectively, after storage compared to day 0 (**Table 1**). Typical phenolic HPLC profiles on day 18 for control and irradiated fruits are shown in **Figures 1A** and **1B**. Interestingly, it was observed that irradiation induced the accumulation of benzoic acid derivatives ranging from 4 to 14% (peaks 9 and 10) and of several flavonol compounds (peaks 2−5 and 7)

Table 3. Reduced Ascorbic Acid Content and Antioxidant Capacity Changes in Irradiated and Nonirradiated Mango Fruit before and after 18 Days of Storage at 15 °C[a]

	ascorbic acid content (mg of ascorbic acid/100 g)			ORAC values (μmol of Trolox equiv/g)	
dose	day 0	day 18	% decrease	day 0	day 18
0 kGy	19.99 ± 0.75 ab	14.90 ± 2.57 a	25%**	5.36 ± 1.00 a	5.58 ± 0.25 a
1 kGy	20.19 ± 5.29 ab	13.72 ± 4.77 a	32%	5.46 ± 0.45 a	6.31 ± 0.79 a
1.5 kGy	21.97 ± 4.67 a	10.91 ± 1.49 ab	50%**	5.46 ± 0.96 a	5.91 ± 0.81 a
3.1 kGy	15.65 ± 2.09 b	7.23 ± 1.69 b	54%**	4.99 ± 0.48 a	5.74 ± 0.73 a

[a] Data represents mean ± standard deviation ($n = 4$). Different letters within a column (ab) indicate significant differences between doses ($P < 0.05$). Two asterisks (**) indicates significant differences after storage (between columns) ($P < 0.05$).

Figure 3. Whole irradiated and nonirradiated mangoes before and after 18 days of storage at 15 °C.

during storage compared to day 0 (**Figure 1B**). The appearance of flavonol compounds in irradiated fruits was mainly observed at 3.1 kGy, and these were tentatively identified as quercetin derivatives because of similar spectra to quercetin glycosides (peaks 2 and 3). The role these flavonols may have in the cell is unknown, in part because it is unclear if these compounds were synthesized under the extreme noncommon abiotic stress or alternatively were the resultant byproducts of a disrupted cellular metabolism due to the excessive irradiation treatment.

PAL is the first enzyme involved in the biosynthesis of phenylpropanoid compounds and an increase in its activity would suggest that irradiation affected PAL gene expression. Results indicated that PAL activity for control and irradiated samples on day 0 was ~0.036 (μmol of *t*-cinnamic acid)/(g h) and showed no increase ($P > 0.05$) during storage when measured at days 5 and 18 (data not shown). However, the minor increases observed in phenolic compounds (peaks 2, 3, and 6) in control and irradiated fruits after storage strongly suggest that slight increases in PAL activity could have taken place and were missed when assayed in the present study. In previous studies, Tan and Lam (*25*) reported that mango phenolics increased when exposed to lower irradiation doses (0.25−1 kGy) through induction of PAL within 1−2 days of treatment. Similarly, Dubery (*13*) reported that ionizing radiation induced oxidative stress in citrus fruits, which stimulated a transient accumulation of phenolic compounds through PAL activation. It is important to consider that, apart from the synthesis of phenolic compounds via PAL activation, some compounds such as gallic acid or derivatives could also be synthesized through other means such as the Shikimate pathway (*26*).

In general, the total phenolic content of irradiated and control mangoes as measured by the Folin−Ciocalteu assay ranged from 102−108, 87−111, and 78−109 mg of chlorogenic acid/100 g at days 0, 5, and 18, respectively. The total phenolic content was not affected by irradiation dose and storage ($P > 0.05$), despite that few individual phenolic acids decreased and flavonols accumulated (**Table 1**).

Carotenoid HPLC Profiles. The main carotenoids identified in control mangoes at day 0 were violaxanthin derivatives (~47.7%), neoxanthin derivatives (~16.1%), and β-carotene (~36.2%) (**Table 2**). Violaxanthin and neoxanthin derivatives were tentatively identified because of similar spectra to violaxanthin (414.7, 438.9, and 468.7 nm) and neoxanthin (412.2, 436.7, and 464.5 nm) standards. These observations are in agreement with those of Mercadante el al. (*27*), who reported 59% and 27% content of violaxanthin and β-carotene, respectively. Furthermore, the contribution of violaxanthin can range from 63 to 72%, while those of β-carotene range around 11−12%, depending on the maturity state of the fruits (*28*).

There was no major effect of the irradiation treatments on the total carotenoid content ($P > 0.05$) and the carotenoid HPLC profile of mango fruits compared to control fruits on day 0, with the exception of a 42−46% reduction of a single violaxanthin derivative (peak 7, $P < 0.05$) at an irradiation dose ≥ 1.5 kGy, possibly due to an oxidation process (*29*). Exposure of foods to ionizing radiation induces the formation of ROS,

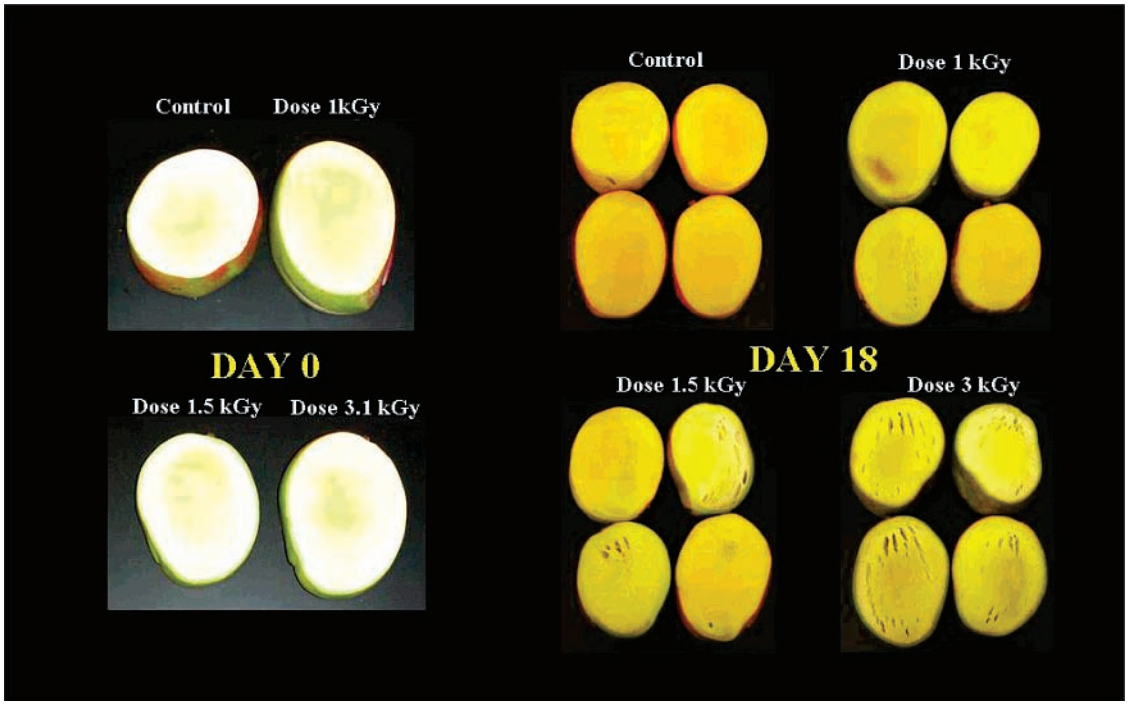

Figure 4. Effect of irradiation on the flesh of stored mangoes before and after 18 days of storage at 15 °C.

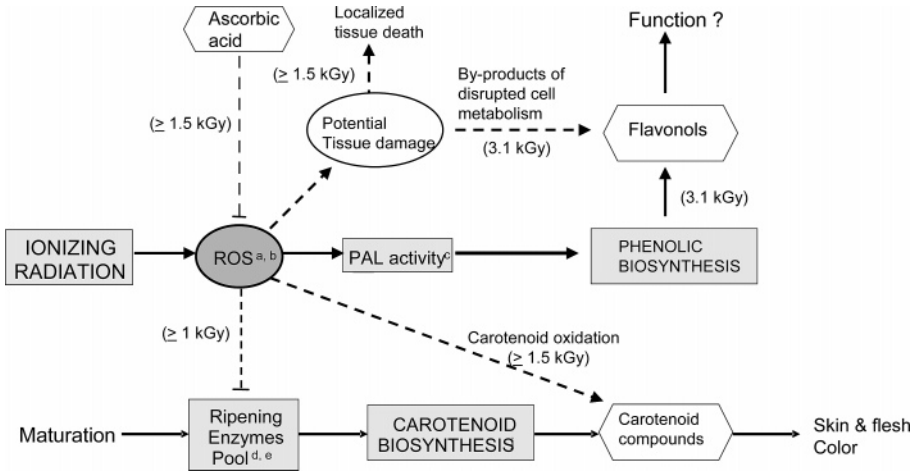

Figure 5. Diagram summarizing the effects of e-beam irradiation and storage on the antioxidant constituents present in mango fruits. Superscripts a–n refer to literature references as follows: (a) Lyng et al. (*8*); (b) Choe and Min (*9*); (c) Tan and Lam (*25*); (d) Dubery et al. (*31*); and (e) Frylinck et al. (*40*).

which can alter the carotenoid content through the formation of epoxides or peroxy radicals (*9*, *30*). On the other hand, storage of mango fruits induced ripening and an increase by ∼79% in total carotenoid content of control fruits ($P < 0.05$). The increase in individual carotenoids ranged from ∼111−292% and 100−150% in violaxanthin derivatives (peaks 2, 5, 9, and 10) and neoxanthin derivatives (peaks 4, 6, and 11), respectively. A new violaxanthin derivative appeared after storage (peak 8), while β-carotene (peak 12) content did not change ($P > 0.05$).Irradiated fruits after storage showed similar levels of total carotenoid content compared to fruits on day 0 ($P > 0.05$) due to no major changes in individual carotenoids, with the exception of a violaxanthin derivative (peak 8) that accumulated in lower levels compared to that in control fruits. In addition, there was a decrease in a violaxanthin derivative (peak 9) by 90% at 3.1 kGy irradiation dose (**Table 2**). These results suggest a biosynthesis inhibition of the majority of the individual carotenoids, rather than an oxidation process caused by the treatments. The delay in ripening of mango fruits caused by the irradiation treatment most likely affected key enzymes in the ripening process (*31*) because of effects on enzyme gene expression (**Figure 2**; **Table 2**).

Ascorbic Acid. Irradiation treatments did not affect reduced ascorbic acid content of mangoes at day 0 ($P > 0.05$). After storage, there was a decrease in reduced ascorbic acid content for control and irradiated fruits ranging from 25 to 54%, with a higher reduction for 3.1 kGy treated fruits (54%, $P < 0.05$) (**Table 3**), most likely due to a higher oxidative stress caused by the extreme irradiation dose. Ascorbic acid is present in plants as part of a series of antioxidant systems present in the cell to counteract oxidant stresses (*32*, *33*). The effect of ionizing radiation on the cellular antioxidant status has been reported to affect the ascorbate content of produce (*9*) such as strawberries (*34*), potatoes (*34*, *35*), and herbs and spices (*36*). The magnitude of this effect is dependent on the type of tissue (*34*) and the irradiation dosage (*37*). Similar effects on reduced ascorbic acid have been reported for other stresses such as wounding (*18*).

Antioxidant Capacity. Table 3 summarizes the effects of irradiation treatments on the antioxidant capacity of mango fruits before and after storage. Irradiation dosage did not induce changes in ORAC values after the irradiation treatment on day 0 ($P > 0.05$). Furthermore, the ORAC values for control and irradiated fruits were similar after storage to those of day 0 ($P > 0.05$). Interestingly, despite a significant decrease in ascorbic acid content during storage, this did not affect the ORAC values. In previous studies, it was reported that ascorbic acid did not contribute to antioxidant activity as much as phenolic compounds (*18*, *38*). In addition, the observed changes in total carotenoids for control fruits did not affect ORAC values as well. The ORAC assay used in the present study reflects the antioxidant activity of hydrophilic compounds only. In previous work, ORAC assays for hydrophilic and hydrophobic compounds showed that carotenoids in mango fruits only represented 1.4% of the overall antioxidant activity (*39*).

It has been shown previously that the antioxidant capacity of an extract is dependent on the profile (type and amount) of phenolic compounds (*18*); however, the slight changes observed in flavonol and phenolic acids in the present study were not enough to affect the overall antioxidant capacity. This suggests that the ORAC values did not change since there was no effect on total phenolic content by the irradiation treatments before and after storage (**Table 1**).

Quality Changes. Storage at 15 °C for 18 days induced ripening of mango fruits observed visually as a skin color change from green to yellow−red (**Figure 3**). However, irradiation of mango fruit delayed the ripening process (≥1.0 kGy), with fruit skins remaining green after the storage period. These color changes are associated to the changes observed in the HPLC carotenoid profile. Mangoes exposed to higher irradiation doses (≥1.5 kGy) were found to be more susceptible to tissue damage, showing peel scalding (**Figure 3**) and void formations (**Figure 4**), which is related to the death of the exposed cells (*40*) and possible subsequent cell wall fragmentation and breakdown (*41*).

In general, our results indicate that electron-beam ionizing radiation stress elicits several responses in the selected fruit before and during storage that are dose dependent. On the basis of the results from the present study and literature information, we present in **Figure 5** a diagram proposing a hypothesis on the mechanism of action of ionizing radiation mediated by ROS. ROS may act as a signaling molecule by triggering the phenylpropanoid metabolism through induction of PAL activity (*25*) and the biosynthesis of phenolic compounds. Alternatively, ROS may induce an increase in phenolic compounds as byproducts of a disrupted cellular metabolism due to the excessive treatment. ROS may also affect gene expression, inhibiting a pool of key enzymes responsible for the ripening process of the climacteric fruit (*31*, *40*). ROS may partially be modulated by ascorbic acid, acting as part of the antioxidant system present in the cell, as well as reacting with other scavengers including individual carotenoid compounds. Finally, ROS may damage cells (e.g., DNA damage and cell membrane lesions), causing localized tissue death. This scenario of events elicited by this noncommon or "artificial abiotic stress" may be more complex than those present in "common abiotic stresses" to which plants are normally exposed, such as wounding (*18*). In summary, the present study has shown there was very small or no effects on the antioxidant constituents of mango fruit right after irradiation; however, during storage, ascorbic acid decreased and carotenoid synthesis of the majority of individual carotenoids was inhibited. The total phenolic content was not affected, despite few reductions and increases observed in individual phenolic compounds, and this in turn was associated to no changes in the antioxidant activity of the fruit. Further studies are needed to verify if these responses are tissue dependent, including climacteric versus nonclimacteric fruit. Knowledge of the physiological secondary metabolism response of fresh produce, besides quality issues, is key to optimizing the irradiation treatment to deliver high-quality products with similar or enhanced functional properties.

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