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ARTICLE

Electron-Beam Irradiation Effects on Phytochemical Constituents and Antioxidant Capacity of Pecan Kernels [*Carya illinoinensis* (Wangenh.) K. Koch] During Storage

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Pecans kernels (Kanza and Desirable cultivars) were irradiated with 0, 1.5, and 3.0 kGy using electron-beam (E-beam) irradiation and stored under accelerated conditions [40 °C and 55−60% relative humidity (RH)] for 134 days. Antioxidant capacity (AC) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) assays, phenolic (TP) and condensed tannin (CT) content, high-performance liquid chromatography (HPLC) phenolic profile, tocopherol content, peroxide value (PV), and fatty acid profiles were determined during storage. Irradiation decreased TP and CT with no major detrimental effects in AC. Phenolic profiles after hydrolysis were similar among treatments (e.g., gallic and ellagic acid, catechin, and epicatechin). Tocopherol content decreased with irradiation (>21 days), and PV increased at later stages (>55 days), with no change in fatty acid composition among treatments. Color lightness decreased, and a reddish brown hue developed during storage. A proposed mechanism of kernel oxidation is presented, describing the events taking place. In general, E-beam irradiation had slight effects on phytochemical constituents and could be considered a potential tool for pecan kernel decontamination.

KEYWORDS: Pecan; phenolics; tannin; ellagic acid; E-beam irradiation; oxidation; fatty acid; tocopherol

INTRODUCTION

In 2004, the U.S. Food and Drug Administration (FDA) issued a press release regarding an outbreak of *Salmonella enteritidis* likely as a result of the consumption of raw almonds. This brought attention of potential foodborne illnesses as a result of the consumption of other nuts, such as pecans [*Carya illinoinensis* (Wangenh.) K. Koch], which are harvested with similar horticultural practices. At harvest, trees are shaken and nuts fall onto the ground. Nuts are then gathered in windrows and harvested by sweeping the orchard floor, thus increasing the risk of contamination with fungi, viruses, or bacteria and making sanitation a necessary step (*1*).

Food irradiation is mostly known as a food safety procedure and involves exposure of food to different sources of ionizing energy. Ionizing radiation can kill pathogenic microorganisms by direct or indirect DNA damage. Ionization of water molecules causes the loss of one electron, producing reactive species, such as hydrogen and hydroxyl radicals, which can cause lipid peroxidation (*2*). Ionizing energy sources include γ-rays, X-rays, and electron-beam (E-beam) β rays. Similar to X-rays, radioactive sources are not required for E-beam irradiation (*2*).

The high lipid content of pecan kernels (~65%) makes them susceptible to oxidation (*3*, *4*). However, no detrimental effects in oxidative stability or sensorial scores were detected when irradiating pecan kernels with up to 1 kGy using γ-rays to control aflatoxigenesis by *Aspergillus flavus* (*5*). Authors suggested the use of higher levels of ionizing irradiation to increase inhibition of post-harvest fungi. Furthermore, it has been suggested that irradiation may prevent oxidation through degradation of oxidative enzymes, such as lipoxygenases (*6*). Lipoxygenase has been identified as a cause of lipid peroxidation in pecan kernels and is inactivated by different procedures, such as steam and dielectric heating (*7*, *8*), which can be used to increase their shelf life.

Despite available studies on ionizing radiation and its effects on food components, there is no information regarding the effects of E-beam irradiation on phenolic compounds and other antioxidants present in pecan kernels. Pecan kernels have been found to have high antioxidant capacity and phenolic content (*9*, *10*), which may act against lipid peroxidation (*11*, *12*). In addition, there is abundant evidence in the literature suggesting the health benefits and prevention of chronic diseases by phenolic compounds.

The objective of the present study was to evaluate the effects of a potential decontamination E-beam irradiation treatment dose of 0−3.0 kGy on the antioxidant profile and quality parameters of pecan kernels during accelerated storage conditions.

MATERIALS AND METHODS

Chemicals and Apparatus. Solvents used were high-performance liquid chromatography (HPLC)-grade and purchased from Fisher Scientific (Houston, TX). Folin−Ciocalteu reagent, vanillin reagent,

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6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, fluorescein sodium salt (FL), and phenolic standards were purchased from Sigma Chemical Co. (St. Louis, MO). 2,2′-azobis(2-amidino-propane)-dihydrochloride (AAPH radical) was obtained from Wako Chemicals (Richmond, VA). Water of nanopure grade was used when needed. A Synergy HT plate reader and KC-4 version 3.4 analytical software (Bio-Tek Instruments, Inc., Winooski, VT) were used with different 96-well plates depending upon the fluorescence or absorbance measurements. A photodiode array spectrophotometer (Hewlett-Packard 8425A, Waldbronn, Germany) was also used to perform assays requiring absorbance measurements with greater liquid volumes.

Sample Preparation and E-Beam Irradiation. Pecan nuts were mechanically harvested during the fall of 2004 from trees of Kanza and Desirable cultivars. Kanza nuts were grown at the United States Department of Agriculture (USDA) Experiment Station located in Brownwood, TX. Desirable nuts were harvested from a commercial orchard located near Caldwell, TX. After harvest, approximately 20 kg of nuts per cultivar were stored at −5 °C. Before mechanical cracking and shelling, nuts were equilibrated for 24 h at 5 °C. After removal of rotten and necrotic kernels, pecan halves were stored at −80 °C in freeze-resistant plastic bags.

For irradiation treatments, kernels were transported to the National Institute for Electron Beam Food Research (Institute of Food Science and Engineering, College Station, TX), divided into ∼200 g sets, and placed in sealable plastic bags. Bags were laid over a cardboard tray, with kernels placed in single-layer beds. Two alanine dosimeters were inserted into two pecan halves per cardboard tray to monitor the amount of ionizing energy applied (*13*). Bags were treated with 0, 1.5, or 3.0 kGy using a single electron beam linear accelerator. Doses applied were such that the 1.5 and 3.0 kGy absolute minimum were achieved, including the ranged ±7.5% error margin for the material, transport system, accelerator, and dosimetry.

After irradiation, pecan bags were perforated and stored in an oven set at 40 °C and a relative humidity (RH) of 55−60% using a ReliON humidifier model RWM-975 (Southborough, MA). One bag of each treatment from both cultivars was extracted from the oven at 0, 7, 21, 55, 98, and 134 days of storage and analyzed for its oxidative status, phenolic profile, antioxidant capacity, tocopherol content, and fatty acid profile. No differences were observed in samples before and after treatment on day 0.

Oil from non-irradiated kernels of both cultivars was extracted using hexane and placed inside an open beaker at the same conditions as pecan kernels. The oxidative status by peroxide values was also determined in these oils.

Phenolic Compound Extraction. Kernels were chopped using a food processor and defatted by homogenizing samples with hexane (1:20, w/v) using an Ultraturrax T25 homogenizer (IKA Works, Wilmington, NC). After homogenizing, samples were filtered with a Buchner funnel and slow-filtration rate filter paper (FisherBrand P5, Fisher Scientific, Pittsburgh, PA). The cake was defatted 2 more times, and the remaining powder was dried at 35 °C under vacuum for 2 h. The powder was flushed with nitrogen and stored in a sealed container at −20 °C until analyses. Oil was obtained from filtrates after hexane was evaporated using a rotavapor. The oil was flushed with nitrogen and stored at −20 °C until analyses of the lipidic fraction.

Defatted pecan powders (1 g) were placed in 50 mL falcon tubes and homogenized with 20 mL of acetone/water (70:30) solution. Falcon tubes were capped, placed in an oscillatory shaker at 5 °C, and shaken overnight. After shaking, slurries were centrifuged at 18000*g* and supernatants were collected, flushed with nitrogen, and stored at −20 °C.

DPPH and ORAC Antioxidant Assays. DPPH free radical was used to measure antioxidant capacity (AC_{DPPH}) as described by Villarreal et al. (*10*). A standard curve was prepared using Trolox as the reference reagent. AC_{DPPH} was expressed as micrograms of Trolox per gram of defatted sample (μg of TE/g).

For the ORAC assay, a modification of the procedure described by Wu et al. (*9*) to measure hydrophilic antioxidant capacity (AC_{ORAC}) was used and adapted as described by Villarreal et al. (*10*). Results were expressed as micromoles of Trolox equivalents per gram of defatted kernel (μmol of TE/g).

Condensed Tannin (CT) Content, Total Phenolics (TPs), Phenolic Hydrolysis, and Reverse-Phase HPLC Analysis. Procyanidins or CTs were evaluated using the vanillin assay (*10*). Results were expressed as milligrams of catechin equivalents per gram of defatted sample (mg of CE/g). Six replicates of each sample were used.

TP analysis was performed by the Folin−Ciocalteu method as described by Villarreal el al. (*10*). A standard curve made with chlorogenic acid was elaborated to express TP as milligrams of chlorogenic acid equivalents per gram of defatted kernel (mg of CAE/g). Six replicates of each sample were analyzed. To determine the phenolic profile, extracts used for AC and TP assays were analyzed by HPLC with and without basic and acidic hydrolysis using the conditions described by Villarreal et al. (*10*). Standard curves of the identified compounds were elaborated by dissolving standards in methanol and injecting them into HPLC. Individual phenolic content was evaluated at 0 and 134 days of storage. Samples and standard curves were analyzed in triplicate.

Fatty Acid Profile and Tocopherol Content. Fatty acid methyl esters were analyzed in a Varian CP 3800 gas chromatograph (Palo Alto, CA) coupled with a Varian CP-8200 autosampler and a flame ionization detector (FID) using conditions described by Villarreal et al. (*10*). Fatty acids were identified and expressed as a percentage of the total fatty acid content.

The tocopherol content was determined as explained by Mendoza et al. (*14*). Pecan oil was weighted (∼0.5 g), and 2 mL of methanol was added. After the sample was vortexed for 1 min, 500 μL of hexane was added and vortexed again. Samples were then centrifuged; 1 mL of the top layer was carefully extracted and filtered using 0.2 μm PTFE filters; and 20 μL were injected into a Waters HPLC system (Waters Corp., Milford, MA). The HPLC system was equipped with a Lichrosorb Spherisorb ODS2 C18 column (5 μm particle size, 4.6 × 250 mm) and a guard column of the same chemistry (Waters Corp., Milford, MA). An isocratic flow of 1 mL/min of 100% methanol was used as the mobile phase. The concentrations of α-, β-, and γ-tocopherol standard solutions were determined as suggested by American Oil Chemists' Society (AOCS) official method Ce 8-89 (*15*). Peak spectra at 295 nm and retention times were used for identification and quantification. Only γ-tocopherol is reported because of minimal detection of other isomers. Values are reported as micrograms of tocopherol per gram of oil (μg of γ-Toc/g).

Oxidative Status. Oxidation of oils was assessed using the peroxide value (PV) and *p*-anisidine value. The AOCS Cd 8b-90 (*15*) protocol was followed for PV analyses; isooctane was used as a solvent as recommended by the methodology. Values were reported as milliequivalents of peroxide (O_2) per kilogram of oil (milliequiv of O_2/kg). The *p*-anisidine value was evaluated using the AOCS official method Cd 18-90 (*15*).

Lipoxygenase Activity. Enzymatic oxidation by lipoxygenases was evaluated as suggested by Villafuerte and Barrett (*16*). Pecan kernels were weighted and homogenized with cold acetone (−20 °C). The slurry was filtered through a Whatman number 1 filter, and this procedure was repeated until the filtrate was colorless. The cake was dried with nitrogen flow, and powders were used to evaluate enzymatic activity using the methylene blue assay and conjugate diene production (*16*).

Color. Pecan kernels were evaluated for their color after storage using a LabScan II model SN-12384 equipped with Universal Beta version 2.4 software (Hunter Associates Laboratories, Inc., Reston, VA). Three kernels from each treatment were placed in the color reader and evaluated using the CIELAB D10°/D65 as the scale and light source. Values are reported as lightness (*L*), redness (*a*), and yellowness (*b*).

Moisture. The moisture content was evaluated by placing 5 g of chopped pecan kernels in an Isotemp Vacuum Oven model 285A (Fisher Scientific, Houston, TX) set at 70 °C and −381 mmHg. After 24 h, the weight difference was recorded and reported as a moisture percentage.

Statistical Analysis. To determine statistical difference between means ($p \leq 0.05$), analysis of variation (ANOVA) and Tukey's honestly significant difference (HSD) were calculated using the SPSS statistical software package, version 11.5 (SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

TPs, CTs, and Phenolic Profiles. TPs in Kanza kernels decreased gradually during storage (**Figure 1A**). At day 134, controls and 1.5 and 3.0 kGy samples decreased ∼14, 20, and

19%, respectively, compared to the initial day (**Table 1**). For Desirable kernels, only the 1.5 kGy sample treatment showed a significant decrease in TP by ~15 % (**Figure 1B**).

CTs decreased in both pecan varieties, showing large differences among treatments within the first 98 days of storage, after which all treatments decreased with similar values among them (**Figure 2**). CTs in Kanza kernels decreased sharply with ~19 and 17% in 1.5 and 3.0 kGy samples, respectively, during the first 7 days of storage compared to controls, followed thereafter by a gradual reduction until day 134 (**Figure 2A** and **Table 1**). At day 134, controls and 1.5 and 3.0 kGy samples showed CT reductions of ~30, 37, and 33%, respectively, compared to day 0. Desirable kernels also showed a sharp reduction in CT for irradiated samples on the initial days and a gradual reduction thereafter (**Figure 2B**). At day 134 of storage, controls and 1.5 and 3.0 kGy samples showed CT reduction levels of ~34, 34, and 25%.

Reduction of CT because of irradiation has been reported in other studies (*17*, *18*). For example, Breitfellner et al. (*18*) found that irradiation induced a rapid degradation of catechin and other

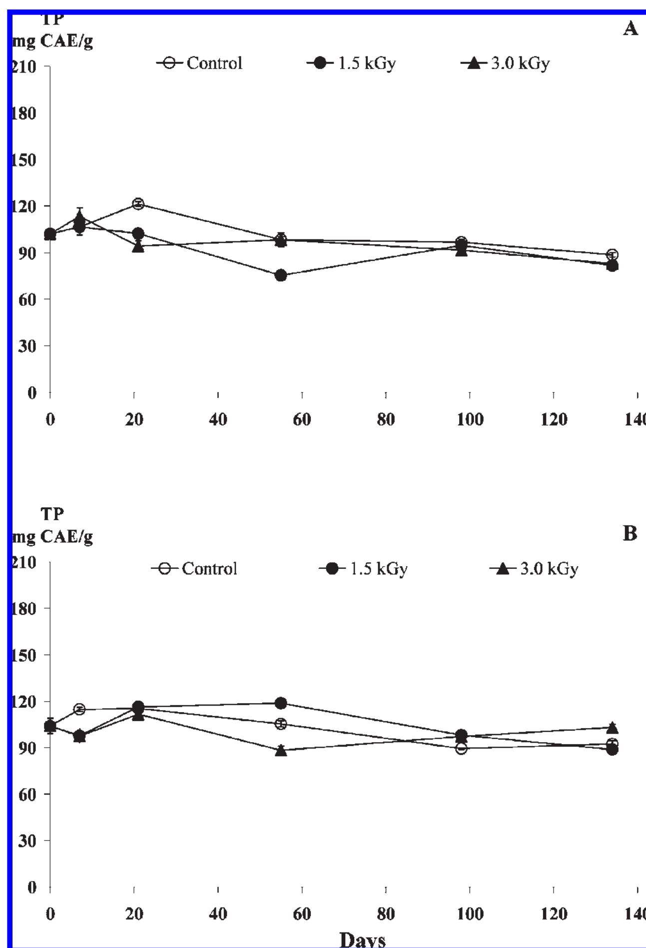

Figure 1. Extractable phenolic content (TP) determined by the Folin−Ciocalteu reagent for (A) 'Kanza' and (B) 'Desirable' kernels irradiated with 0, 1.5, and 3.0 kGy and stored at 40 °C and 55% RH.

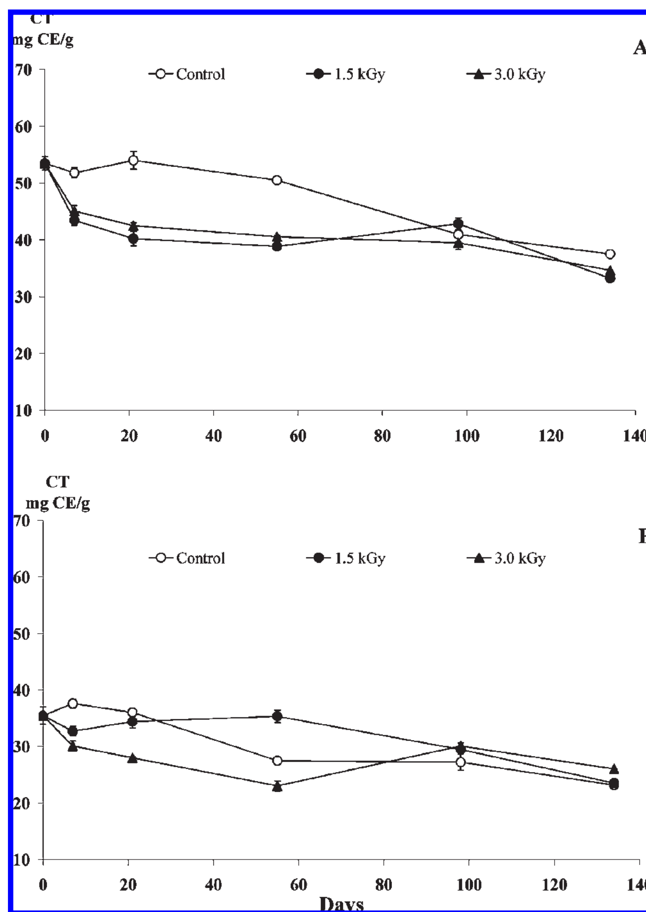

Figure 2. CT content determined with the vanillin−HCl assay for (A) 'Kanza' and (B) 'Desirable' kernels irradiated with 0, 1.5, and 3.0 kGy and stored at 40 °C and 55% RH.

Table 1. Total Phenolic Content, Antioxidant Capacity, Condensed Tannin Content, and γ-Tocopherol Content of Irradiated Pecan Kernels after 134 Days of Storage at 40 °C and 55% RH

		Kanza			Desirable		
analysis	day	0.0 kGy	1.5 kGy	3.0 kGy	0.0 kGy	1.5 kGy	3.0 kGy
TP[a]	0	102 ± 2 a[b]			104 ± 5 a		
	134	89 ± 1 b	82 ± 1 c	83 ± 2 bc	92 ± 2 ab	88 ± 1 b	103 ± 2 a
AC_{ORAC}[c]	0	532 ± 20 a			427 ± 22 a		
	134	467 ± 11 bc	429 ± 12 c	505 ± 7 ab	465 ± 10 ab	496 ± 7 bc	540 ± 9 c
AC_{DPPH}[d]	0	124 ± 3 a			134 ± 2 a		
	134	108 ± 4 b	121± 3 a	120 ± 1 ab	144 ± 4 a	119 ± 2 b	110 ± 5 b
CT[e]	0	53 ± 1 a			35 ± 2 a		
	134	37 ± 1 b	33 ± 1 c	35 ± 0 bc	23 ± 0 a	23 ± 0 b	26 ± 0 b
γ-Toc[f]	0	97 ± 1 a			74 ± 1 a		
	134	101 ± 5 a	69 ± 4 b	77 ± 6 c	89 ± 1b	66 ± 4 a	46 ± 0 c

[a] Total phenolic content expressed in milligrams of chlorogenic acid equivalent per gram of defatted sample. [b] Values are expressed as the mean of 6 replicates ± standard error (SE). Values with different letters are significantly different ($p = 0.05$). [c] Antioxidant capacity using the ORAC assay and expressed as μmoles of Trolox equivalent per gram of defatted sample. [d] Antioxidant capacity using the DPPH free radical expressed as milligrams of Trolox equivalent per gram of defatted sample. [e] Condensed tannin content using the vanillin assay expressed as milligrams of Catechin equivalent per gram of defatted sample. [f] γ-Tocopherol content expressed as micrograms of γ-tocopherol per gram of oil.

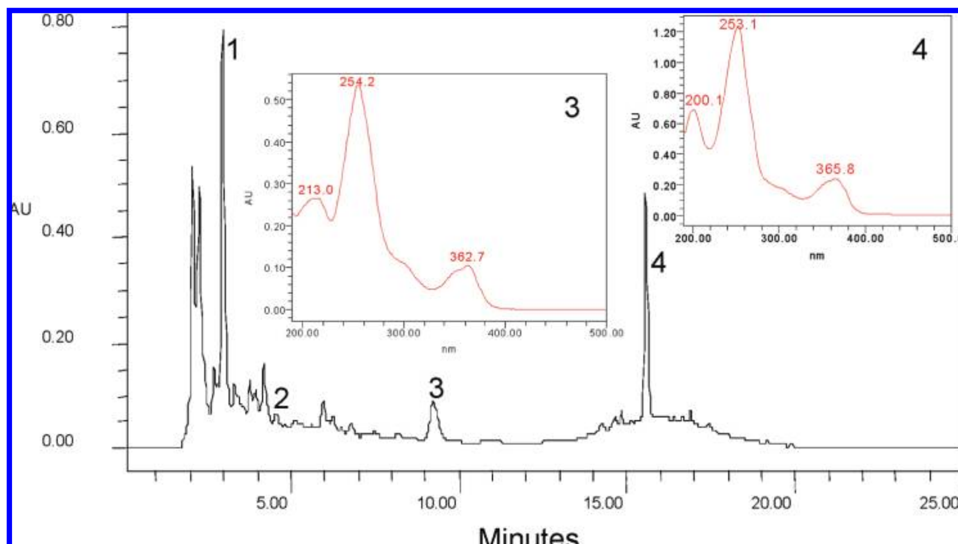

Figure 3. Typical HPLC phenolic profile at 280 nm from hydrolyzed extracts of non-irradiated Kanza kernels after 98 days of storage at 40 °C and 55% RH. Identified peaks correspond to (1) gallic acid (RT, 3 min), (2) catechin (RT, 4−5 min), (3) ellagic acid derivative (RT, 9−10 min), and (4) ellagic acid (RT, 15.5 min). Insets are UV−vis spectra for peaks 3 and 4. AU = absorbance units. The horizontal axis is the retention time (RT) in minutes, and nm = nanometer.

flavonoids in water solutions. However, such large degradation was not seen in irradiated strawberries with a similar dose range of 0−6 kGy (*17*), implying the presence of a protection mechanism of flavonoids in the fruit matrix. In the present study, the low water content of pecans (3−5%) might have avoided a greater formation of free radicals, providing a greater oxidative stability for these compounds.

Phenolic HPLC profiles for irradiated and non-irradiated samples from acetone/water (70:30) extracts were similar, and the obtained peaks were unable to be identified directly. After hydrolysis with a base (4 N NaOH) followed by an acid (6 M HCl), peaks for catechin, epicatechin (traces), gallic acid, an ellagic acid derivative, and ellagic acid were identified and similar chromatograms were observed for all samples. A typical HPLC chromatogram at 280 nm is shown for Kanza kernels in **Figure 3**. A large variation was found for the identified compounds (probably because of the extreme hydrolysis conditions), and differences or trends could not be confirmed when comparing control and irradiated samples. In general, the major peaks of gallic and ellagic acids in Kanza kernels showed levels of ∼1.9 and 0.9 mg/g of defatted kernel, respectively, while for Desirable kernels, values were ∼2 and 0.8 mg/g of defatted kernel, respectively. These values are similar to those previously reported for several pecan cultivars (*10*).

Phenolic compounds in the kernel pellicle are more exposed to environmental agents, such as oxygen and light, and are likely to oxidize at a faster rate than phenolics in the internal matrix. Senter et al. (*19*) stored pecans at 70 °C for 7 days and studied the changes in their phenolic constituents. Oxidation of leucoanthocyanidin and leucodelphinidin, flavan-3,4-diols, to their respective condensed tannins of varying degrees of polymerization was observed in the outer layers of kernels.

Antioxidant Capacity. Pecans were evaluated for their antioxidant capacity (AC) using the DPPH free radical (AC_{DPPH}) and the ORAC (AC_{ORAC}) assays during storage for 134 days (**Figures 4** and **5**). Unexpectedly, after 7 days of storage, we observed an increase in AC_{DPPH} for some treatments in both pecan varieties and, afterward, a gradual decrease until reaching values slightly lower or similar to the initial day (**Figure 4** and **Table 1**). The greatest initial increase in AC_{DPPH} on day 7 was observed for Kanza control kernels (42%) followed by 1.5 kGy

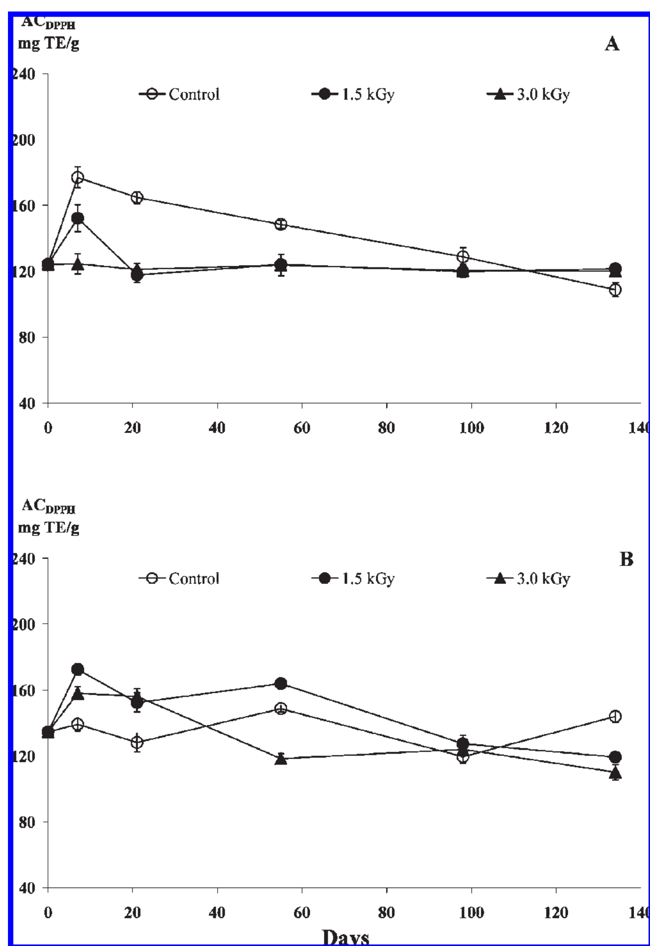

Figure 4. Antioxidant capacity for the DPPH assay (AC_{DPPH}) of (A) Kanza and (B) Desirable kernels irradiated with 0, 1.5, and 3.0 kGy and stored at 40 °C and 55% RH.

samples (23%). Samples treated with 3.0 kGy had no significant increase in AC_{DPPH} throughout storage (**Figure 4A**). Desirable kernels treated with 1.5 kGy samples had the greatest initial increase (28%) on day 7 followed by kernels treated with 3.0 kGy

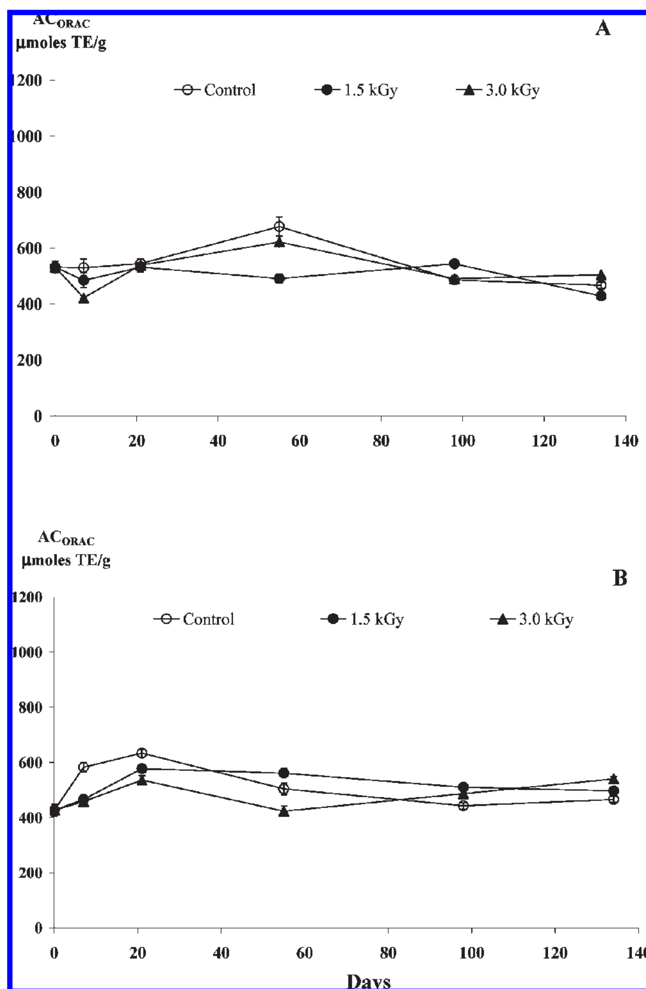

Figure 5. Antioxidant capacity for the ORAC assay (AC_{ORAC}) of (A) Kanza and (B) Desirable kernels irradiated with 0, 1.5, and 3.0 kGy and stored at 40 °C and 55% RH.

(18%) (**Figure 4B**). Control samples for Desirable kernels had no substantial increase throughout the experiment. By the end of the storage period (day 134), the final AC_{DPPH} for Kanza 1.5 and 3.0 kGy kernels did not differ from the initial values ($p > 0.05$), while for Desirable 1.5 and 3.0 kGy samples, the AC_{DPPH} decreased ~11 and 18%, respectively (**Table 1**).

Similarly, the AC_{ORAC} values for both pecan varieties also increased initially during storage and decreased afterward, reaching values slightly lower, similar, or slightly higher than the initial day (**Figure 5**). The AC_{ORAC} values for Kanza kernels gradually increased from day 7 to day 55 for controls and 3.0 kGy samples (27 and 17%, respectively) (**Figure 5A**). Desirable samples peaked on day 21 in all treatments (48, 35, and 25% for controls and 1.5 and 3.0 kGy samples, respectively), followed by a slow decrease (**Figure 5B**). In general, values obtained for both AC assays were retained throughout the experiment, suggesting high antioxidant activity of the compounds present at any given time during storage.

These results revealed unexpected responses of non-lipophilic antioxidants to storage conditions. Increases of AC values in both assays suggest the development of compounds with enhanced antioxidant activity in the initial period of storage and with differing mechanisms of action. The formation of compounds with increased ability to donate electrons or transfer hydrogen atoms is reflected in AC_{DPPH} and AC_{ORAC}, respectively (*20*).

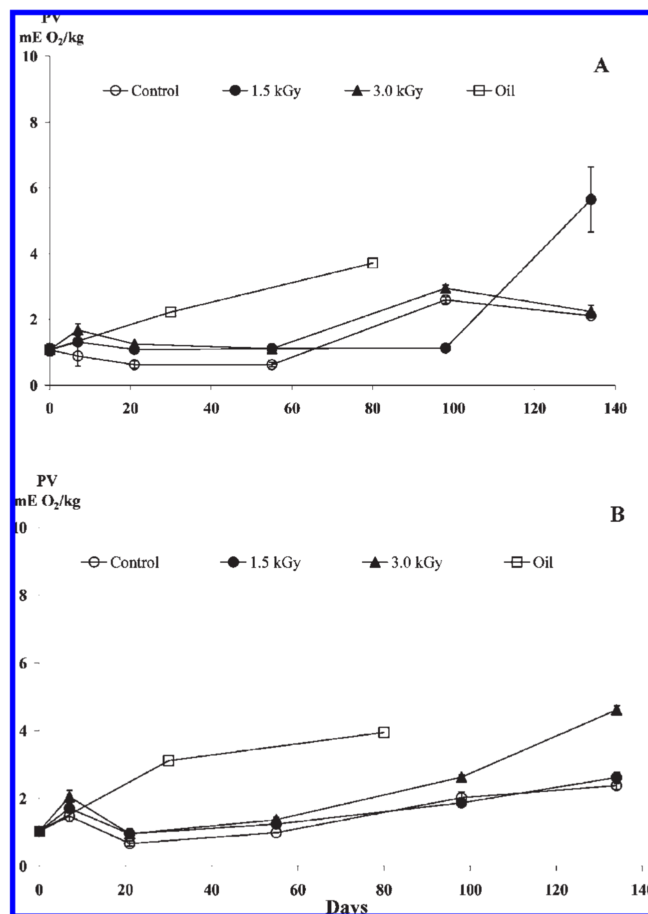

Figure 6. PVs of (A) Kanza and (B) Desirable kernels irradiated with 0, 1.5, and 3.0 kGy and stored at 40 °C and 55% RH. Oil extracted with hexane from non-irradiated Kanza kernels was stored together with pecan kernels.

Galloyl groups in epigallocatechin, gallocatechin gallate, and other flavonoids improve their antioxidant properties when compared to their non-galloylated versions (*21*). The presence of catechin and epicatechin in pecan kernels and the high content of gallic acid greatly increase the probability to find galloylated compounds, such as the ones mentioned above.

Furthermore, flavonoids can polymerize if given adequate conditions (*19*), and it has been suggested that these polymers may be more potent antioxidants than their monomers (*21*, *22*). The oxidation of flavonoids into polymers of varying degrees of polymerization has been reported in pecan kernels stored for 7 days at 70 °C (*19*). In the same study, authors reported that this oxidation is progressive, which suggests an increase in the degree of polymerization over time.

Peroxide Value (PV) and Tocopherol Content. PVs in both pecan varieties showed an increase for most treatments starting at day 55 of storage (**Figure 6**). Kanza kernels treated with 1.5 kGy had the greatest increase between 98 and 134 days (**Figure 6A**) as well as Desirable kernels irradiated with 3.0 kGy, which showed the greatest PV in that same window period of accelerated storage (**Figure 6B**). When analyzing *p*-anisidine values in both pecan varieties, results showed low values, indicating low production of aldehydes (data not shown).

Irradiation is known to inactivate lipoxygenase enzymes (*6*). The presence of lipoxygenases was evaluated according to other authors (*23*), but no quantification could be made in pecan kernels in the present study. Because PVs for irradiated samples were similar or higher than those of controls (**Figure 6**), our

results suggest that the oil oxidation observed was not enzyme-mediated.

One possible explanation for the PV increase in both pecan varieties would be related to the degradation of other antioxidant compounds, such as γ-tocopherol. In the present study, irradiated kernels had a lower content of γ-tocopherol after 134 days of storage compared to control samples. Kanza kernels showed a decrease of ~28 and 20% for 1.5 and 3.0 kGy samples, while controls showed no major changes (**Table 1**), and these levels were observed since day 21 (data not shown). Similarly, for Desirable kernels, the decrease was ~37% for 3.0 kGy samples, while controls and 1.5 kGy samples showed slight changes at the end of the storage period (**Table 1**). Once again, these levels were observed since day 21 (data not shown).

Interestingly, the PV increased for most samples after 55 days of storage, but no further decrease in the tocopherol content was observed after 21 days. This could be related to a higher rate of lipid oxidation within the outer cell layers of the kernel once the tocopherol has been depleted in that area; however, the average amount of tocopherol in the inner cell layers or bulk was not affected because of oxygen restriction through the kernel matrix.

Rudolph et al. (*24*) found a dramatic decrease of ~90% in the tocopherol content for pecan oil extracted with solvent and stored at 70 °C for 9 days. On the other hand, Yao et al. (*25*) and Fourie et al. (*26*) found only a slight decrease of $\sim\leq$ 20% in the tocopherol content of pecan kernels after ~1 year of storage at 30 °C.

In the present study, after 55 days of storage, PV levels for kernels were ~1 milliequiv of O_2/kg of oil for most samples (**Figure 6**); however, pecan oil extracted from kernels and stored under similar conditions as the kernels showed double the amount of peroxide values (~2 milliequiv of O_2/kg of oil) after only 30 days (**Figure 6**). These results indicate that protection against lipid oxidation was interrupted in extracted oil because of the kernel matrix removal. The disruption of kernel pellicle and lipid bodies and exposure to oxygen are the most reasonable explanations for this expected increased oxidation rate.

An alternative explanation to lipid protection against oxidation has been associated with phenolic antioxidants. Jurd et al. (*11*) studied thoroughly the composition of walnut (*Juglans regia*) pellicle and suggested a oxidative protection of kernel oil by the phenolic acids. Authors found that the amount of ellagic acid was up to 4% of the pellicle weight, mainly as pyrogalloyl glucose forms (*11*). Similarly, Senter et al. (*12*) found that the content of phenolic acids in pecan kernels was correlated to PVs and severely decreased (> 50%) after 12 weeks of storage.

However, phenolics are mainly located in the skin or pellicle and lipid bodies within the cells; thus, although correlation may take place, it does not necessarily mean cause and effect.

Fatty Acid Profile. The fatty acid profile of pecan oil was similar for both cultivars studied, including oleic (71%), linoleic (20%), palmitic (5%), stearic (2.5%), and linolenic (1%) acids. At the end of the 134 days of storage period, the fatty acid profile did not change for the control or irradiated samples despite that the PV had increased during storage. An increase in PV was probably due to the oxidation of minor fatty acid components, such as linolenic and eicosanoic acids, which oxidize faster (*27*) and have minimum effects on major fatty acid composition. Most of studies relating oxidation and fatty acid composition to pecan kernel quality have been performed in extracted oil and stored under accelerated conditions (*3*, *24*). However, as shown in the present study, oxidation of extracted oil differs from that of lipids in the kernel, and caution is recommended when estimating the shelf-life of pecan nuts by relating the quality of the whole kernel to previously extracted oil samples.

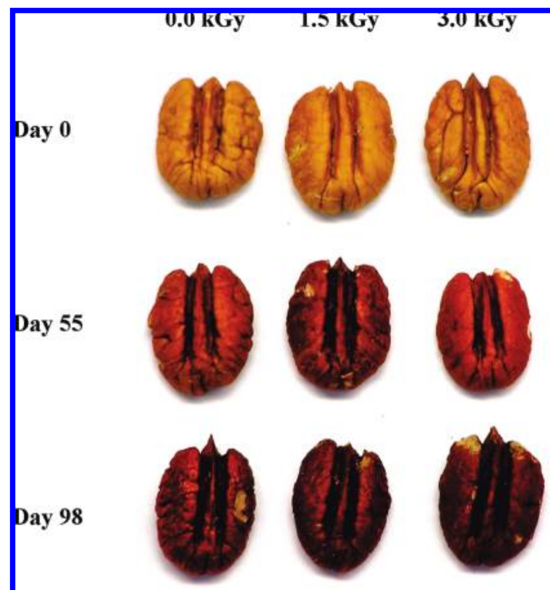

Figure 7. Color of 'Kanza' kernels irradiated with 0, 1.5, and 3.0 kGy and stored for 0, 55, and 98 days at 40 °C and 55% RH.

Color of Pecan Kernels. Kanza kernels at day 0 had "*L*", "*a*", and "*b*" values of ~47.7, 13.4, and 35.3, respectively. During storage "*L*" and "*b*" gradually decreased until reaching values at 134 days of 23.7 and 8.1, respectively, while "*a*" remained around 14.5. This response was similar for both control and irradiated kernels. As "*b*" decreased and "*a*" remained the same, this changed the kernel color toward a reddish hue, and as "*L*" decreased, kernel lightness decreased. These changes were associated visually from a yellowish to a reddish brown darkening of the stored Kanza kernels (**Figure 7**). Similarly, Desirable kernels had initial values of "*L*", "*a*", and "*b*" of ~34.5, 15.2, and 25.9, respectively. Once more, at day 134 of storage, "*L*" and "*b*" decreased to values of 23.5 and 10.4, respectively, while "*a*" remained without changes around 14.4. Desirable kernels also darkened during storage, with no difference between control and irradiated samples.

Pecan kernel color changes have been previously reported by Senter et al. (*19*), who found strong correlations between color and polymerization of leucoanthocyanidins (flavan-3,4-diols). These authors found that a decrease in lightness and yellowness was correlated with the formation of condensed tannins and that the rate of formation of these compounds depended upon cultivar (*19*, *12*, *28*). In the present study, this color change may be related to the observed apparent decrease in CT, which is most likely due to a polymerization process of flavonoids into condensed tannins.

A light-brown, golden color is considered to be an attribute of high-quality pecans. Furthermore, it is recommended to allow for a certain degree of color development, which will improve flavor and aroma, attributes known to be influenced by tannins (*29*). Some methods used for sanitizing pecans in large scale, such as soaking in chlorine water and roasting, increased the darkening rate in kernels (*30*). In the present study, irradiated kernels had no difference in color compared to controls, indicating that this technology is suitable for sanitizing kernels without decreasing their visual quality.

Proposed Mechanism of Pecan Kernel Oxidation. Many plants, including pecans, are known to have oil-bearing seeds. Oil accumulation in seeds occurs in many small organelles inside the cell cytoplasm called oil or lipid bodies (*31*). Lipid bodies in seeds tend to be small (often less than 1 μm in diameter), especially

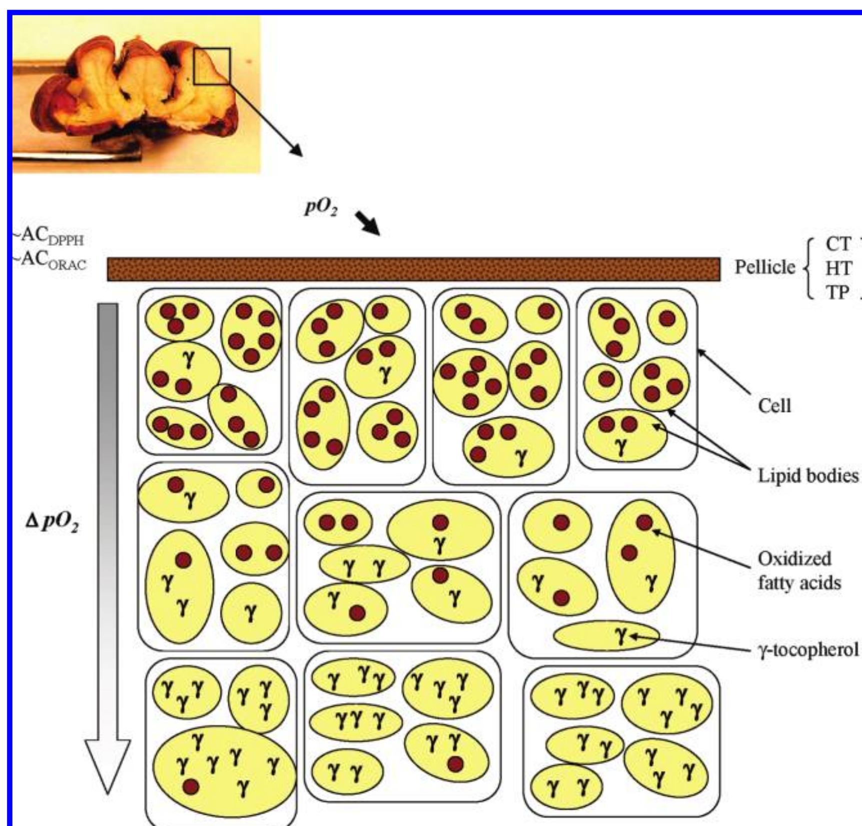

Figure 8. Oxygen transfer and oxidation of lipid bodies in pecan kernel external layers. pO_2, partial oxygen pressure; ΔpO_2, partial oxygen pressure gradient; AC_{DPPH}, antioxidant capacity measured by DPPH free radical; AC_{ORAC}, antioxidant capacity measured by the ORAC assay; CT, condensed tannin; HT, hydrolyzable tannin; TP, total extractable phenolic content.

when compared to the ones found in fruit mesocarp (olive, palm, and avocado; ≥20 μm) (*31*). Little information is available about the ultrastructure of pecan kernel parenchyma cells (flesh tissue) and the interface with pellicle tissue. Wakeling et al. (*32*) studied the microstructure of cells and lipid bodies in opalescent and non-opalescent pecan kernels (opalescence is a non-desirable browning of the kernel interior tissue) and found a well-defined and compact cell structure in non-opalescent kernels. This tight cell arrangement could provide protection against oxygen transfer through cells and into lipid bodies. A possible oxidation mechanism for kernels is hypothesized and shown in **Figure 8**. Several events will take place simultaneously as kernels oxidize during storage. The pellicle is exposed to air, causing phenolic compounds therein contained to oxidize and, in some cases, to polymerize (flavonoids), which will induce a change in the kernel color. At the same time, the lipid bodies located in parenchyma cells closer to the pellicle start oxidizing. Lipophilic antioxidants inside these lipid bodies, such as tocopherols, start degrading first, thus preventing fatty acids from oxidation in the early stages of storage. However, after tocopherol decreases in the external cell layers, peroxide values start increasing because of a progressive degradation of fatty acids located in these same cells. Because of the potential low oxygen permeability (e.g., diffusion and solubility) through kernel tissue (intercellular space, membranes, and lipid bodies), the overall tocopherol content and fatty acids from cells located deeper inside the kernels are not further affected. This could explain why peroxide values progressively increase with storage without significant further effects on the total tocopherol content. According to this proposed oxidation model, the physical location of fatty acids and tocopherols and oxygen permeability of kernels would play an important role in the oxidation process of pecans. This model may be used to understand irradiation treatment effects on different types of nuts, including almonds (*33*), hazelnuts (*34*), and pistachio (*35*), among others.

In conclusion, pecan kernels are difficult to process and sanitize with conventional methods (e.g., chlorine and hot water) without decreasing their quality. Irradiation treatments are currently in use to decontaminate different foods across the U.S. The present study showed that E-beam irradiation could be used as a decontaminating tool on pecan kernels with no major effects on antioxidant levels and color change, despite the observed reduction in total phenolics and condensed tannins. Some degree of lipid peroxidation was induced by the E-beam irradiation treatment; however, induction of peroxides was seen until advanced stages of storage in accelerated conditions. We hypothesize that the mechanism of protection of lipid oxidation was mainly due to small oxygen diffusion through the kernel tissue and the presence of tocopherol antioxidants in the lipid bodies. Further studies are needed to determine the composition of hydrolyzable and condensed tannins in pecan kernels.

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