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Influence of Dietary Vitamin E on Behavior of *Listeria monocytogenes* and Color Stability in Ground Turkey Meat following Electron Beam Irradiation

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ABSTRACT

There is growing concern that the free radical scavenging effect of antioxidants added to meats might reduce the antimicrobial effectiveness of ionizing radiation. A study was conducted to determine the effect of vitamin E on the behavior (growth) of *Listeria monocytogenes* and color stability in turkey meat following electron beam irradiation. Raw ground turkey breast meat from birds fed diets containing 0 (control), 50, 100, and 200 IU/kg of vitamin E was inoculated with a five-strain mixture of *L. monocytogenes* to give approximately 10^7 CFU/g. Inoculated samples were irradiated at 0, 0.5, 1, and 2 kGy and stored aerobically (12 days) or under vacuum (42 days) at 4°C. *L. monocytogenes* survivors were determined by plating samples on modified Oxford medium and counting colonies on modified Oxford medium plates after 48 h at 35°C. Meat color was measured using a colorimeter. Irradiation at 2.0 kGy resulted in an approximately 3.5-log reduction of initial numbers of *L. monocytogenes.* There were no significant differences in *D*-values (decimal reduction times) for *L. monocytogenes* in meat irrespective of vitamin E treatment ($P > 0.05$). Also, vitamin E treatments did not affect growth of the pathogen in aerobic or vacuum-packaged samples following irradiation ($P > 0.05$). Compared with controls, irradiated meat from birds fed 100 or 200 IU/kg of vitamin E demonstrated significant improvement in color stability (lightness and redness values) during aerobic storage ($P < 0.05$). Dietary vitamin E (100 to 200 IU/kg) has good potential for improving the color stability of turkey meat without compromising the microbial safety of the irradiated product.

Listeria monocytogenes is a psychrotrophic enteric pathogen of major food safety concern. It has been implicated in several outbreaks traced to contaminated cheese, other dairy products, and various types of meat, including turkey meat *(7).* Of all the bacterial foodborne pathogens, *L. monocytogenes* has the second highest case fatality rate (20%) and the highest hospitalization rate (90%) *(17).* Because of its relatively high fatality rate and the uncertainty of the infectious dose for immunocompromised individuals, U.S. regulatory agencies established a zero tolerance for *L. monocytogenes* in cooked and ready-to-eat foods *(30).* Therefore, the elimination of this pathogen from foods by use of ionizing irradiation has been proposed.

Irradiation of fresh raw poultry meat at doses up to 3 kGy was approved by the Food and Drug Administration to extend shelf life and to inactivate foodborne pathogens in this meat product *(31).* Several studies have demonstrated the efficacy of irradiation for inactivating *L. monocytogenes* in poultry meat *(12, 15, 18, 25).* Mead et al. *(16)* demonstrated a 4-log cycle reduction in *L. monocytogenes* on artificially inoculated chilled broiler carcasses following irradiation with 2.5 kGy. Huhtanen et al. *(15)* investigated the resistance of seven strains of *L. monocytogenes* to gamma irradiation (2 kGy) in deboned chicken meat and reported a 4-log cycle reduction on the numbers of this pathogen. Patterson *(25)* reported that the radiation resistance (*D*-value [decimal reduction time]) of four strains of *L. monocytogenes* in ground poultry meat ranged from 0.42 to 0.55 kGy.

Microbial inactivation by irradiation involves damage to the DNA. Radiation damage to DNA occurs directly via deposition of energy into this macromolecule and/or indirectly by free radicals from the radiolysis of water *(18, 20).* A major concern associated with free radical production in irradiated meats is reduced meat quality from off-odor production, undesirable flavors, and color changes *(2, 9, 22, 23).* Since consumer responses to these radiation-induced quality changes are negative *(1, 11)*, there is growing interest among meat processors in the use of antioxidants such as vitamin E to alleviate undesirable changes in meat quality that result from irradiation.

Vitamin E is a natural antioxidant that, when supplied in the diet, becomes incorporated in the subcellular membrane and is effective in scavenging free radicals formed during irradiation *(10).* Ahn et al. *(2)* reported a threefold increase in vitamin E levels in breast muscle of turkeys when fed 200 IU of vitamin E per kg of feed. Also, dietary vitamin E has been shown to extend the color shelf life and reduce lipid oxidation in fresh beef *(3, 14)* and pasteurized beef ham *(13).* Based on these studies, the use of vitamin E seems to be an ideal approach for increasing the quality

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of irradiated meat; however, the addition of this antioxidant to meats poses a major food safety concern because its free radical scavenging action may compromise the lethal action of free radicals against foodborne pathogens during irradiation.

To our knowledge, there are no published reports on the combined effect of ionizing irradiation and dietary vitamin E supplementation on the radiation resistance and growth of *L. monocytogenes* in turkey meat. Accordingly, the main objective of this study was to evaluate the effects of dietary vitamin E on the survival and growth of *L. monocytogenes* in refrigerated (4°C) aerobic or vacuum-packaged ground turkey meat following electron-beam irradiation. A secondary objective was to determine the effect of dietary vitamin E on color stability of irradiated ground turkey meat during aerobic storage at 4°C.

MATERIALS AND METHODS

Sample preparation. Twelve-week-old, large, white turkeys were fed diets that contained 0 (control), 50, 100, or 200 IU/kg of dl-α-tocopheryl acetate. The feeding process lasted until the animals reached 16 weeks of age. At the end of the feeding trial, birds were slaughtered following U.S. Department of Agriculture guidelines *(29).* Breast muscles were deboned from the carcasses 24 h after slaughter and then were ground twice using a 3-mm plate. The breast samples were vacuum packaged and stored at −20°C for 1 month. After thawing at 4°C for 24 h, samples were prepared by aseptically weighing 10 g of ground meat from each dietary treatment into vacuum bags (Cryovac B-2540, Cryovac Sealed Air Corp., Duncan, S.C.; water vapor transmission = 0.5 to 0.6 g at 100°F, 100% relative humidity per 100 in^2/24 h; oxygen transmission rate = 3 to 6 cm^3 at 40°F/m^2/24 h at 0% relative humidity).

Measurement of vitamin E concentration. The α-tocopherol content was measured in breast muscle following the method of Du and Ahn *(11)*. The internal standard used to quantify vitamin E was 5-α-cholestane.

Bacterial strains and culture conditions. A five-strain mixture of *L. monocytogenes*, including strains H7962 serotype 4b, H7762 serotype 4b, H7969 serotype 4b, H7764 serotype ½a, and Scott A NADC 2045 serotype 4b, was used in this study. *L. monocytogenes* Scott A was obtained from Dr. Irene Wesley at the National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa. All other strains were obtained as clinical isolates from the multistate outbreak of 1998 to 1999 (Centers for Disease Control and Prevention, Atlanta, Ga.). Each culture was maintained as frozen (−70°C) stock in brain heart infusion broth (Difco, Becton Dickinson, Sparks, Md.) supplemented with 10% glycerol until used. Before each experiment, individual stock cultures were transferred twice in 10 ml of tryptic soy broth (Difco, Becton Dickinson) supplemented with 0.6% yeast extract (Difco, Becton Dickinson) and incubated at 35°C for 20 h.

Preparation of inoculum. Equal amounts of each culture were combined to prepare a five-strain mixture of *L. monocytogenes.* Cells from the mixture were harvested by centrifugation (10,000 × *g*, 10 min, 4°C) in a Sorvall Super T21 centrifuge (DuPont Instruments, Willmington, Del.) and washed once in sterile 0.85% (wt/vol) NaCl (saline). The cell pellet was suspended in fresh saline, and this suspension was used as the inoculum.

Sample preparation and inoculation. Samples (10 g) of ground turkey meat were each inoculated with 0.1 ml of the five-strain mixture to give a final cell concentration of approximately 2.0×10^7 CFU/g. Each bag was manually massaged for 30 s to evenly mix the inoculum into the meat. The bags were then vacuum sealed using a Multivac A 300/51 vacuum packaging machine (Multivac Sepp Haggenmuller, GmbH & Co., Wolfertschwenden, Germany) and stored at 4°C overnight (18 h) before irradiation.

Irradiation treatment and dosimetry. Inoculated ground turkey meat samples were irradiated at the Iowa State University Linear Accelerator Facility, which has a MeV CIRCE III Linear Electron Accelerator (MeV Industrie S.A., Jouy-en-Josas, France). Samples (4°C) were irradiated in duplicate at four target doses (0, 0.5, 1.0, and 2.0 kGy) at a dose rate of 88.1 kGy/min in the electron beam mode at an energy level of 10 MeV. Each target average dose is an arithmetic average of doses determined at the surfaces of the irradiated meat samples.

Alanine dosimeters, 5 mm in length by 5 mm in diameter, were placed on the top and bottom surfaces of one of the duplicate meat samples. Immediately after irradiation, the dosimeters were read using a 104 Electron Paramagnetic Resonance Instrument (Bruker Instruments Inc., Billerica, Mass.) to determine the absorbed doses.

Microbiological analysis. After irradiation, approximately half of the irradiated samples were aseptically opened using sterile scissors to render the packages aerobic. The open top of each package was loosely folded and closed with a metal clip. Both aerobic and vacuum-packaged samples were held at 4°C and analyzed within 2 h following irradiation to determine numbers of *L. monocytogenes* survivors. Turkey meat samples stored under aerobic conditions were analyzed in duplicate at 0 (2 h), 3, 6, 9, and 12 days of storage (4°C); vacuum-packaged samples were analyzed after 0, 14, 28, and 42 days. Each 10-g sample was mixed with 90 ml of sterile 0.1% peptone water and homogenized for 60 s at medium speed using a Seward Stomacher 400 Lab blender (Seward Ltd., London, England). Serial dilutions of the meat slurry were prepared in 0.1% peptone water, and 0.1-ml aliquots of appropriate dilutions were surface plated, in duplicate, on modified Oxford medium (Difco, Becton Dickinson). All inoculated agar plates were incubated aerobically at 35°C, and typical *L. monocytogenes* colonies were counted at 48 h.

Calculation of *D*-values. The *D*-value, the radiation dose (kGy) that produces 90% reduction in numbers of viable cells, was determined by graphing the log number of *L. monocytogenes* survivors per gram versus radiation dose (kGy) using Microsoft Excel 98 Software (Microsoft Inc., Redmond, Wash.). The line of best fit for the data was determined using linear regression analysis *(24).* The *D*-value was obtained by calculating the negative reciprocal of the slope of the regression curve.

Calculation of growth rate. Plate count (log CFU per gram) was plotted against time (days), and the best fit line for the data was obtained using linear regression analysis *(24).* Growth rate was determined by calculating the slope of the best fit line.

Color measurement. Samples of irradiated ground turkey meat were also evaluated to determine changes in color stability. Color values were measured on the surface of packaged samples using a Hunter LabScan Colorimeter (Hunter Associated Laboratories Inc., Reston, Va.) that had been calibrated against black and white reference tiles with the same packaging materials used for samples. The CIE lightness (L^*), redness (a^*), and yellowness

TABLE 1. *Effect of dietary vitamin E on populations of* Listeria monocytogenes *in nonirradiated (0 kGy) and irradiated (2 kGy) ground turkey breast meat stored aerobically at 4°C*[a]

Dietary vitamin E (IU)	Population of *L. monocytogenes* (log CFU/g)											
	0 kGy						2 kGy					
	Storage day					Growth rate (SEM)	Storage day					Growth rate (SEM)
	0	3	6	9	12		0	3	6	9	12	
0	7.42	7.96	8.24	8.48	8.43	0.08 (0.01)	4.33	1.89	1.00	1.48	2.22	0.20 (0.01)
50	7.29	7.82	8.07	8.09	8.29	0.08 (0.02)	3.83	1.59	1.08	1.08	1.56	0.08 (0.02)
100	7.29	7.81	8.36	8.39	8.56	0.10 (0.01)	3.71	1.37	1.12	1.00	2.06	0.15 (0.05)
200	7.42	8.07	8.48	8.57	8.85	0.11 (0.01)	3.71	1.18	1.00	1.08	1.48	0.09 (0.05)

[a] Means of growth rates within a column with same irradiation dose are not significantly different ($P > 0.05$).

(*b**) values were obtained using an illuminant A (light source). Area view and port size were 0.25 and 0.40 in., respectively. One color reading was taken from each side of a meat sample.

Statistical analysis. All experiments were conducted twice, and each experiment had two samples per treatment. Thus, the means of *D*-values, bacterial populations (log CFU per gram), and Hunter color values were calculated from four replicate samples for each treatment. Analysis of variance was performed with the General Linear Models procedure of the Statistical Analysis System software program (SAS Institute Inc., Cary, N.C.) *(26)*. Differences were considered statistically significant at $P < 0.05$ unless otherwise stated. Differences among variables were tested for significance using Tukey's honestly significant different multiple-comparison test. Mean values and standard error of the means are reported.

RESULTS AND DISCUSSION

Accumulation of vitamin E. The dietary levels of vitamin E used in this experiment were chosen because their use is practical for the poultry industry from an economic standpoint. In terms of conversion, 1 IU equals 1 μg/g. The amount of α-tocopherol in turkey breast muscle increased as the dietary vitamin E increased; however, this increase was not exactly linear. The levels of α-tocopherol incorporated in the breast meat from birds fed 50, 100, and 200 IU of vitamin E were 1.64, 2.24, and 3.47 μg/g, respectively (data not shown). These concentrations show an increase up to fourfold when compared with the control (0 IU) where the amount incorporated was 0.85 μg/g.

Radiation resistance of *L. monocytogenes*. The radiation resistance (*D*-values) of *L. monocytogenes* in ground meat from turkeys fed 0, 50, 100, and 200 IU of vitamin E ranged from 0.56 to 0.59 kGy. There were no significant differences ($P > 0.05$) in *D*-values of *L. monocytogenes* in turkey meat irrespective of the level of dietary vitamin E used in this study.

Preliminary studies in our laboratory indicated that exogenous addition of 1.5% (wt/wt) of vitamin E to ground turkey breast meat significantly ($P < 0.05$) increased the radiation resistance of *L. monocytogenes* in this meat product (data not shown). In a similar study, Stecchini et al. *(28)* demonstrated that the addition of the antioxidant carnosine (1.5%, wt/wt) to ground turkey meat resulted in a significant ($P < 0.05$) increase in the resistance of *Aeromonas hydrophila* to gamma radiation at 0.5 kGy. These findings indicate that the addition of antioxidants to turkey meat can increase the radiation resistance of foodborne pathogens. Certainly, the concentrations of added vitamin E used in our preliminary studies or of carnosine used in the study reported by Stecchini et al. *(28)* were approximately 50 times higher than levels present in turkey meat in the present study. Therefore, the relatively low levels of vitamin E incorporated in turkey meat from birds fed diets that contained up to 200 IU of this antioxidant are inadequate to protect *L. monocytogenes* from the lethal effects of ionizing radiation at 2 kGy.

Growth of *L. monocytogenes*. The effect of vitamin E on the growth of *L. monocytogenes* in aerobically stored ground turkey meat at 4°C is shown in Table 1. Under aerobic conditions, the growth rate of *L. monocytogenes* in nonirradiated and irradiated meat from birds fed vitamin E was not significantly different from the growth rate of the pathogen in the control samples ($P > 0.05$). On average, the population of *L. monocytogenes* in nonirradiated samples increased from approximately 7.4 (at day 0) to approximately 8.5 log CFU/g (at day 12). This represents an approximately 1-log CFU/g increase in *L. monocytogenes* populations after 12 days of refrigerated storage.

Immediately following irradiation (2 kGy), an approximately 3.5-log CFU/g reduction in initial populations of *L. monocytogenes* was achieved. *L. monocytogenes* survivors decreased by approximately 2.39 log CFU/g at day 3 of storage and remained relatively constant for 9 days followed by a slight increase at day 12. Changes in the population were not related to the amount of vitamin E present in the meat samples, indicating that dietary vitamin E up to 200 IU had no marked effect on the growth of this pathogen in aerobically stored turkey meat at 4°C. To our knowledge, there are no published studies that show the correlation between dietary vitamin E levels and growth of *L. monocytogenes* in turkey meat following irradiation. A few studies have shown the effect of dietary vitamin E on survival of this pathogen or that of natural bacterial flora during aerobic storage of nonirradiated meat samples. For example, Cabedo et al. *(5)* inoculated ground beef from animals fed 1,000 and 2,000 IU of vitamin E with *L. monocytogenes* and stored it aerobically for 10 days at 4°C. A

TABLE 2. *Effect of dietary vitamin E on populations of* Listeria monocytogenes *in nonirradiated (0 kGy) and irradiated (2 kGy) vacuum-packaged ground turkey breast meat stored at 4°C*[a]

Dietary vitamin E (IU)	Population of *L. monocytogenes* (log CFU/g)									
	0 kGy					2 kGy				
	Storage day				Growth rate (SEM)	Storage day				Growth rate (SEM)
	0	14	28	42		0	14	28	42	
0	7.42	8.57	8.17	7.78	−0.03 (0.00)	4.3	3.73	6.24	7.94	0.20 (0.05)
50	7.29	8.33	8.25	7.99	−0.01 (0.01)	3.8	3.33	4.46	7.76	0.16 (0.01)
100	7.28	8.60	8.26	8.10	−0.02 (0.00)	3.7	3.77	5.32	8.19	0.16 (0.03)
200	7.41	8.63	8.40	8.06	−0.02 (0.00)	3.7	3.40	4.95	7.86	0.14 (0.01)

[a] Means of growth rate within a column with same irradiation dose are not significantly different ($P > 0.05$).

variation of approximately 0.7 log CFU/g in *L. monocytogenes* counts was observed in control and high vitamin E ground meat during the storage period, indicating no major growth or treatment variation. Other studies have also demonstrated no significant changes in growth of spoilage microflora in aerobically stored beef from animals fed vitamin E *(3, 4, 8, 13)*.

A similar trend in growth of *L. monocytogenes* was observed in vacuum-packaged turkey meat samples (Table 2). There were no significant differences in growth rates of this pathogen in nonirradiated and irradiated samples irrespective of dietary vitamin E treatment ($P > 0.05$). Vitamin E, up to 200 IU, did not reduce or enhance the growth of *L. monocytogenes*. On average, the population of *L. monocytogenes* in nonirradiated meat increased approximately 0.6 log CFU/g after 42 days of storage under vacuum. Irradiation (2 kGy) decreased initial numbers of the pathogen by approximately 3.37 log CFU/g. After 14 days of storage, the population increased steadily until it reached approximately 7.94 log CFU/g at day 42. As in aerobic conditions, the growth rate of *L. monocytogenes* during refrigerated storage of vacuum-packaged samples was unaffected by the amount of dietary vitamin E. These results are comparable to those reported by Buys et al. *(4)*, who vacuum packaged meat from calves fed diets supplemented with 500 IU of vitamin E per day and then held the meat at 4°C for 7 days. The researchers found no statistically significant ($P > 0.05$) differences in microbial numbers in dietary vitamin E meat samples compared with control (40 IU/kg of vitamin E). Cannon et al. *(6)* also reported similar results in pork. The authors vacuum packaged precooked pork from hogs fed supplemental vitamin E (1,000 IU/kg) and stored it at 2°C for up to 56 days. No differences ($P > 0.05$) in total plate count between high vitamin E treatments and control (no vitamin E) were observed.

Color changes. The surface CIE color values of aerobically packaged ground turkey breast meat were compared based on effects of irradiation dose, storage time, and vitamin E treatment (Table 3). Irradiation increased the *a** value of the meat, and this increase was irradiation dose dependent. The redness was also significantly influenced by dietary

TABLE 3. *CIE color values of aerobically packaged ground turkey breast meat affected by dietary vitamin E and irradiation during storage at 4°C*[a]

Irradiation	Day 0					Day 5				
	Dietary vitamin E (IU):					Dietary vitamin E (IU):				
	0	50	100	200	SEM	0	50	100	200	SEM
*L** value										
0 kGy	54.3	56.1	54.1	54.7	1.2	55.1	55.7	54.8	52.7	1.2
2 kGy	59.0	58.1	53.3	55.6	2.2	57.9 A	57.3 A	53.1 BC	53.2 C	1.0
SEM	2.9	0.5	1.4	1.5		1.4	1.6	0.4	0.5	
*a** value										
0 kGy	10.5 AY	10.6 AY	11.0 AY	11.3 AY	0.2	9.5 A	9.1 AY	9.7 AY	11.1 B	0.3
2 kGy	13.9 ABZ	13.5 AZ	14.6 BCZ	15.4 CZ	0.3	10.0 A	10.1 AZ	10.9 AZ	12.3 B	0.3
SEM	0.2	0.2	0.3	0.3		0.2	0.1	0.2	0.5	
*b** value										
0 kGy	18.1 Y	17.5	17.1	18.0	0.6	16.3 Y	16.2 Y	17.3	17.1	0.6
2 kGy	16.1 ABZ	17.9 AC	15.5 B	18.3 C	0.5	19.8 Z	17.6 Z	17.4	17.5	0.7
SEM	0.3	0.6	0.6	0.6		0.6	1.1	0.5	0.2	

[a] Different letters (A through C) within a row are significantly different ($P < 0.05$). Different letters (Y and Z) within a column are significantly different ($P < 0.05$).

vitamin E. The color of irradiated breast meat from turkeys fed 200 IU/kg of vitamin E was much redder than the control (0 IU) at days 0 and 5 of storage. Nam and Ahn *(21)* attributed the increased red color in irradiated turkey meat to the formation of a carbon monoxide–myoglobin complex. They reported that carbon monoxide was one of the gas compounds generated by irradiation. Carbon monoxide–myoglobin is less readily oxidized to brown metmyoglobin than is oxymyoglobin because of the strong binding strength of carbon monoxide to the iron-porphyrin site on the myoglobin molecule. The carbon monoxide–myoglobin complex gives a stable bright or light bright red color with consistently high *a** values *(27)*.

Regardless of irradiation and dietary vitamin E treatments, the color *a** values of ground turkey breast meat decreased after 5 days of storage under aerobic conditions. This decrease was most likely due to oxidation of the heme pigments *(2, 21)*. The color of irradiated meat, however, was still redder than that of the nonirradiated meat. The *L** value decreased when ground turkey breast meat was irradiated at 2 kGy, and this decrease was significant after 5 days of storage. Low values for lightness implicate darker color in the surface of the meat. The *L** and *a** values are importantly related. Having increased *a** values and decreased *L** values indicate that the color of the meat was dark red-pink, which implies freshness, and therefore, is very attractive to the consumer. With respect to yellowness, the *b** values did not change consistently under the effect of irradiation or vitamin E treatments. Similar results have also been previously reported *(19, 22, 23)*.

The results of this study indicate that supplementing the diet of turkeys with vitamin E at doses up to 200 IU/kg does not cause increased radiation resistance of *L. monocytogenes* in the ground turkey breast meat. However, vitamin E at 100 to 200 IU/kg of feed for turkeys is effective in improving color stability of irradiated or nonirradiated turkey breast meat during aerobic storage at 4°C. Dietary vitamin E (100 or 200 IU/kg) shows good potential for improving the color stability of turkey breast meat without compromising the microbial safety of this product during irradiation at 2 kGy.

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