

Effect of Gamma Radiation on Levels of α -Tocopherol in Red Meats and Turkey*

ABSTRACT

The effect of low dose ionizing radiation on free α -tocopherol levels in beef, pork and lamb longissimus dorsi muscle and on turkey leg and breast muscle were determined. The samples were irradiated in air with a ^{137}Cs source at eight dose levels between 0 and 9.4 kGy at 5°C. Irradiation resulted in a significant decrease in α -tocopherol levels in all of the meats studied. There were no statistically significant differences in the rate of loss of tocopherol due to species, with the exception of turkey breast. The rate of loss of tocopherol in turkey breast tissue was greater than the other meats. The information obtained in this study may be of use for 'chemiclearance' purposes since the relative effects due to species variation were examined.

INTRODUCTION

The Congress of the United States (1958) legislated that the use of ionizing radiation to process foods was deemed to be analogous to the introduction of an additive. As a consequence all foods treated with ionizing radiation, with the exception of fruits and vegetables irradiated at or below 1 kiloGray (kGy), must be reviewed and approved by the US Food and Drug Administration (FDA). With regard to meat, the FDA already has approved the use of ionizing radiation to process fresh pork to eradicate *trichinella spiralis* (Federal Register, 1986), and to treat prepackaged fresh or frozen poultry to control foodborne pathogens (Federal Register, 1990). Future work on the approval of other meats may be facilitated if the concept of 'chemiclearance' (Basson, 1977; Taub, 1981) is valid. Chemiclearance is a concept based on studies which indicate that effects of radiation are influenced by dose, that it is possible to extrapolate findings from

*Reference to a brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

higher doses to a lower dose, and that the irradiation of similar commodities under comparable irradiation conditions will affect foods in a similar manner.

To determine whether the concept of chemiclearance is valid with respect to muscle tissue, and to obtain information for a possible petition for the commercial irradiation of beef, a study was initiated to examine and compare the effects of ionizing radiation on the *Longissimus dorsi* muscle of pork, beef and lamb, and on turkey leg and breast tissue. The meats were subject to low dose ionizing radiation at 5°C, and the effects on nutrients, microbial flora and other chemical and physical parameters were determined. This is a report on the effects of ionizing radiation on free α -tocopherol. Vitamin E is the most labile of the fat soluble vitamins (Knapp & Tappel, 1961), and therefore the most sensitive indicator of the effects of radiation on the non-polar compounds. In addition, to appraise some gross physical effects of ionizing radiation on tissue, a test which measured ease of expression of cellular fluid was employed.

MATERIALS AND METHODS

Reagents

D- α -Tocopherol (5,7,8-trimethyltolcol) and D- γ -tocopherol (7,8-dimethyltolcol) were used as purchased from Eastman Kodak Co. (Rochester, NY, USA). D- δ -Tocopherol (8-methyltolcol) 90%, obtained from Sigma Chemical Co. (St. Louis, MO, USA), was purified using semi-preparative μ Porasil HPLC column (Waters, Milford, MA, USA). Tetrahydrofuran (THF) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and butylated hydroxytoluene (BHT) (99%, Aldrich) was recrystallized from methanol. BHT, an antioxidant, was added to cyclohexane at a concentration of 100 mg/l prior to extraction. Anhydrous sodium sulfate was of analytical grade (Fisher Scientific, Valley Forge, PA, USA), cyclohexane and isooctane (EM Science, Paulsboro, NJ, USA), Omnisolve grade, were used as received.

Materials

All of the meats were obtained from an abattoir 24–48 h after slaughter. In all instances the muscle tissues were processed upon receipt by removal of skin if present, and removal of all visible adipose and connective tissue. To obtain homogeneous samples, the muscles were cut into thin strips, diced, frozen with dry ice pellets and then ground in a Hobart Mill (Cincinnati, OH, USA) to approximately 2 mm³. The samples were stored overnight in a refrigerator (4°C) for removal of carbon dioxide by sublimation. The following day, approximately 75 g of sample were sealed into air permeable Cryovac E bags (4000 cc/m²/24 h @ 1 atm) [W. R. Grace Co., Duncan, SC, USA (12 × 12 × 1 cm)] and equilibrated at a temperature of 5°C prior to being irradiated.

Irradiation

The samples were irradiated with a ¹³⁷Cs source (Lockheed Nuclear Products, Atlanta, GA, USA) at a dose rate of 0.108 kGy/min. to levels of 0.0, 0.24, 0.47,

0.94, 1.88, 2.81, 5.62 and 9.37 kGy. The dose rate was determined by using reference dosimeters from the National Physical Laboratory (Middlesex, UK). The thickness of the sample packets were held to approximately 1 cm to minimize self absorption. Temperature during radiation was thermostatically controlled at $5^{\circ}\text{C} \pm 1.5^{\circ}\text{C}$ by injection of boil off vapor from liquid nitrogen.

Extraction

Five grams of muscle tissue were weighed into screw-capped centrifuge bottles (130 ml). The following were then added: 50 ml cyclohexane containing 0.01% BHT, and 60 μl δ -tocopherol [(40 $\mu\text{g}/\text{ml}$) internal standard]. The non-polar compounds were extracted by homogenizing the tissue in a Tissumizer (Tekmar Co., Cincinnati, OH, USA). The samples were centrifuged at 1500 g for 10 min at 4°C , supernatant was retained, and the centrifugate homogenized and extracted with an additional 40 ml cyclohexane/BHT solution. The combined supernatants were dried over anhydrous sodium sulfate for approximately 2 h, filtered through pre-washed glass wool into round bottom flasks. The solvent was removed on a rotary evaporator at ambient temperature under vacuum. The samples were quantitatively transferred into 13 ml centrifuge tubes and evaporated under nitrogen in a 28°C water bath. Samples were reconstituted with 0.9 ml isooctane, centrifuged and the supernatant was placed into 2 ml volumetric flasks. The last step was repeated and the contents of the volumetric flask brought to volume with isooctane. Duplicate analyses were always performed.

High performance liquid chromatography (HPLC)

The samples were filtered through a 0.25 μm Arco LC13 filter (Gelman Science, Ann Arbor, MI, USA) prior to HPLC. Samples were injected on to a Chrom-Spher 5 μm Si column [(100 mm \times 3.0 mm i.d.) Chrompack Inc., Raritan, NJ, USA]. The mobile phase was iso-octane/THF (98:2, v/v). At a flow rate of 1.2 ml/min, complete separation occurred within 8 min. Fluorescence was measured with a spectrophotofluorometer [(E_x 292 nm, E_m 324, nm) Perkin-Elmer, Norwalk, CT, USA] equipped with a 20 μl flow cell.

Free water

The procedure of Wierbicki & Deatherage (1958) was employed to measure gross physical changes in the integrity of muscle tissue and native protein. Thin slices from whole intact muscle, ~ 0.6 cm thick, were removed and put into sealed low moisture permeable IKD bags (0.03 gms/100 in²/24 h, International Kenfield Distributing Co., Rosemont, IL, USA), and refrigerated. Prior to measurement, tissue slices were removed from the bags and sampled by preparing round disks with a #8 cork borer. The samples were weighed on tared Whatman #1 filter paper (9 cm) [pre-equilibrated over a saturated KCl solution]. The filter paper and sample were then sandwiched between two plexiglass plates (7" \times 7") and compressed (Carver Laboratory Press, Wabash, IN, USA) with a force of 500 lb/in² for 60 s and removed. The outlines of the area encompassed by the squeezed tissue and the circular front formed by the exudate were both traced and the respective areas determined using a planimeter. Free water was calculated by

subtracting the area of sample from exudate and multiplying by the factor 61.1, as established by Wierbicki & Deatherage (1958). The values were then normalized for sample weight. Each analysis consisted of six replicates for the non-irradiated control and three replicates for each dose level. Planimetry readings were performed in triplicate. Desirable sample weight was ~ 500 mg.

RESULTS AND DISCUSSION

The current study was designed to approximate the irradiation conditions likely to be employed by commercial processors in the USA. Irradiation was carried out in non-evacuated oxygen permeable plastic bags, as meat may not currently be irradiated under anaerobic conditions for fear of possible botulinum outgrowth at a temperature of 5°C. To facilitate comparisons between species, *Longissimus dorsi* muscle was used exclusively for all red meat studies (beef, lamb, pork). Turkey breast and leg muscles were analyzed and considered to be representative of fowl. Samples were irradiated at eight dose levels between 0 and 9.4 kGy to bracket the doses currently permitted in the US or the UK. The maximum dose studied approached 10 kGy (1 Mrad). The Joint FAO/IAEA/WHO Expert Committee World Health Organization (1981) judged that irradiation at this low dose would not result in any special nutritional or microbiological hazards, and toxicological studies did not indicate any evidence of adverse effects. The committee did not endorse higher levels of radiation due to insufficient data at higher doses.

We studied the effects of ionizing radiation on the fat soluble α -tocopherol in meat, in order to gain additional insight into the mechanism of free radical interaction in tissue. Although meat is a minor nutritional source of vitamin E for humans, the vitamin can serve as an extremely sensitive model compound. Knapp & Tappel (1961) determined that tocopherols were the least stable of the fat soluble vitamins, followed by vitamins A, D and K. Vitamin E is a collective term used to describe the four tocopherols and four corresponding tocotrienols. Within this group α -tocopherol possesses over 80% of the biological activity, and is the most sensitive to ionizing radiation.

In nature, tocopherols are produced solely by plants and therefore the presence of these vitamins in animal tissue is totally dependent on ingestion of plant materials. An additional source of vitamin E is animal feeds fortified with free tocopherols or their esters. Tocopheryl esters have been shown to increase stability and retard rancidity of meat (Marusich *et al.*, 1975; Lin *et al.*, 1989).

Tocopherols are present primarily within the cell membranes either free or complexed to lipoproteins as esters. In this study we only studied the effects of ionizing radiation on the free α -tocopherol eliminating the need to saponify and thereby reducing the risk of loss of this labile compound (Nelis *et al.*, 1985). As a result, extraction was relatively simple. Direct extraction of the tissue with cyclohexane, was followed by concentration of the extract and separation and quantitation of α - and δ -tocopherol (internal standard) on a silica HPLC column equipped with a flow cell and a fluorescent detector. Few natural compounds have fluorescent characteristics similar to those of the tocopherols (Duggan *et al.*, 1957). This characteristic reduced the need for extensive cleanup prior to chromatography. In addition to determining the identity of tocopherol by means of their retention times by normal phase HPLC on silica columns, select samples

were additionally chromatographed on reverse phase columns (LC18, Supelco, Bellefont, PA, USA). In some cases co-chromatography with authentic tocopherol was also conducted. These additional chromatographic procedures confirmed the identity of the peaks to be α -tocopherol.

A micro procedure was also developed which permitted the sample size (3 g) as well as the quantity of organic solvents used to be reduced. Tocopherols were concentrated on a silica based solid phase extraction column prior to HPLC. However there was no significant reduction in analysis time. In our estimation, larger samples were deemed preferable in order to obtain greater sample homogeneity. Therefore, the micro procedure was not employed.

A number of studies have been conducted by others to measure the effects of ionizing radiation on vitamin E in meat; however the scope of these investigations was limited. de Groot *et al.* (1972) reported on the total losses of vitamins in chicken as a result of 'combination treatment' which included irradiation, storage and cooking. A single study on the effect of temperature (-180°C to $+60^{\circ}\text{C}$) during irradiation at 50 kGy on minced pork was conducted by Diehl (1981). Roussel (1988) irradiated mechanically deboned meat and reported diminished levels of vitamin E. The effect of gamma radiation on the concentration of free and total tocopherols in chicken was detailed by Lakritz & Thayer (1992). The results of most of the aforementioned studies are difficult to compare because they were conducted under many different experimental conditions. It has been established that factors such as the physical state of the sample, the environment in close contact with the sample, including the nature of the gaseous atmosphere and type of packaging, temperature, dose rate and source type, all may exert a profound impact on the retention of the micronutrients. As a consequence, to assess the utility of the concept of chemiclearance properly and to determine whether species type is a factor, we conducted parallel studies on all the meat under identical conditions.

The relative change in the α -tocopherol concentration for each sample compared to its unirradiated control is given in Table 1. Each value is an average of duplicate samples and is expressed as a percentage of the non-irradiated control. A high degree of variability was found between samples. Sample to sample variation may be a result of variation within a species, sampling techniques, methodology, interstitial fat, a difference in breeds, seasonal variations and animal feeds (Piironen *et al.*, 1985). The influence of feed on tocopherol levels may explain the ~ 10 -fold higher concentration of α -tocopherol detected in the muscle tissue in lamb sample #2. To rule out lab error, the study of lamb #2 was repeated several months later on a portion of the ground tissue which had been set aside and stored at -50°C in a sealed evacuated aluminum foil packet. The samples were irradiated at all eight dose levels, extracted and analyzed. The result of the repeat analysis is indicated in brackets [2], and the α -tocopherol concentration in the control is comparable to the initial determination.

The data in Table were analyzed using the General Linear Models procedure of the SAS Software System (SAS Institute Inc., 1989) and were subjected to an analysis of covariance (ANCOVA) and regression analysis. The data were analyzed to determine the effects of dose levels of gamma radiation on α -tocopherol content within a species and between species. The data was subjected to statistical analysis only for doses between 0.00 and 1.88 kGy, since the effects due to irradiation reached a plateau at higher dose levels (Fig. 1).

TABLE 1
Percentage α -Tocopherol Retained after γ -Radiation

<i>Sample</i>	<i>Pork</i>					<i>Beef</i>					<i>Lamb</i>				
	<i>1</i>	<i>2</i>	<i>3</i>	<i>mean</i>	<i>s.d.</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>mean</i>	<i>s.d.</i>	<i>1</i>	<i>2</i>	<i>[2]</i>	<i>3</i>	<i>mean</i>
<i>conc. ng/g</i>	695	617	711	674	50	829	1075	646	850	215	199	2390	[2278]	255	1281
<i>dose (kGy)</i>															
0	100	100	100	100		100	100	100	100		100	100	100	100	100
0.234	79.7	78.3	80.2	79.2		58.2	67.9	50.4	58.8		86.1	86.7	97.3	66.8	84.2
0.468	54.1	50.3	62.7	55.7		60.5	63.0	49.1	57.5		65.7	77.4	83.3	66.1	73.1
0.937	44.4	32.1	53.9	43.3		50.3	49.5	46.0	48.6		51.2	53.5	61.1	57.5	55.8
1.875	40.6	35.3	49.4	41.9		35.1	50.9	40.5	42.2		39.8	51.0	54.5	52.9	49.6
2.812	42.2	28.0	55.2	41.7		40.5	42.9	37.4	40.3		40.6	43.8	64.3	49.3	49.5
5.624	40.9	31.5	44.8	39.1		24.9	49.7	44.0	39.5		26.6	40.9	48.8	45.1	40.4
9.374	28.0	25.7	49.8	34.5		37.4	55.1	44.9	45.8		29.4	46.7	58.9	41.4	44.1

TABLE 1 — contd

<i>Sample</i>	<i>Turkey breast</i>					<i>Turkey leg</i>				
	<i>1</i>	<i>2</i>	<i>3</i>			<i>1</i>	<i>2</i>	<i>3</i>		
<i>conc. ng/g</i>	50	334	87	<i>mean 157</i>	<i>s.d. 154</i>	542	330	705	<i>mean 526</i>	<i>s.d. 188</i>
<i>dose (kGy)</i>										
0	100	100	100	100		100	100	100	100	
0.234	58.7	61	41.2	53.6		50.7	70.3	72.2	64.4	
0.468	25.4	31.4	13.2	23.3		51.3	47.5	43.6	47.5	
0.937	n.d.	12.8	7.1	6.6		66.1	21.9	13.9	34.0	
1.875	11.5	10.0	4.7	8.7		60.1	17.9	11.9	30.0	
2.812	3.4	7.1	13.8	8.1		64.9	17.6	13.4	32.0	
5.624	20.6	12.2	18.3	17.0		46.3	23.5	22.2	30.7	
9.374	21.2	9.6	26.8	19.2		44.8	28.9	24.3	32.7	

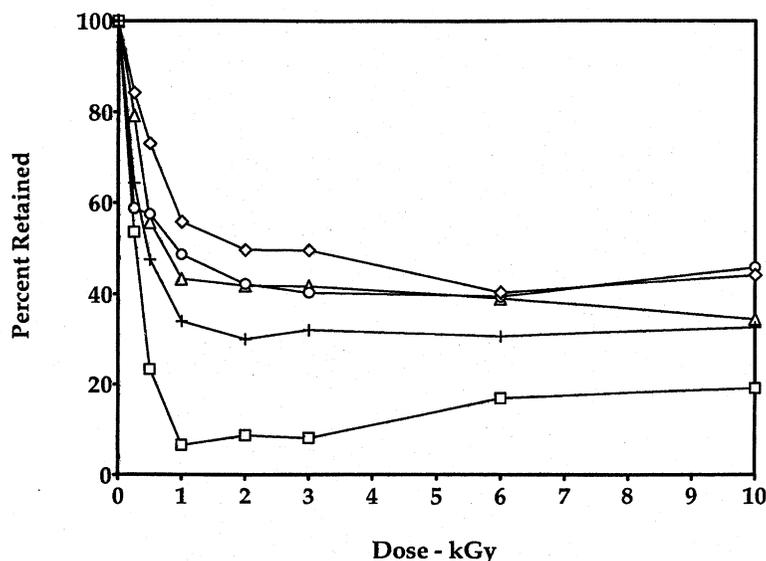


Fig. 1. Percentage of α -Tocopherol Remaining in Muscle Tissue After γ -Radiation at 5°C. ◇ Lamb, ○ Beef, △ Pork, + Turkey Leg, □ Turkey Breast.

Effect of dose. Statistical analysis indicates that dose had a highly significant ($P < 0.01$) negative effect on free tocopherol levels in all types of meat regardless of origin.

Effect of species. The data was then examined to determine possible bias due to varietal variation. ANCOVA indicated that there were no statistically significant differences ($P < 0.05$) in rate of loss of tocopherol when beef, pork, lamb or turkey leg were irradiated. However, at the $P < 0.05$ confidence level, turkey breast muscle was statistically different and was more sensitive to dose than the other meats (Table 2). The higher rate of loss in turkey breast (Table 3 — first order kinetic rate constants) might be correlated to the low lipid content of turkey breast tissue. Proximate analysis indicated that turkey breast contained 40% less lipid than turkey leg, and 68% less than was present in the beef samples. The Bonferroni (Dunn) T -test (Miller, 1981) indicated that the difference in fat content between beef and turkey breast was statistically significant. Merritt & Taub (1983) conducted ESR studies of irradiated chicken, beef and pork fat, and indicated that the fatty acid composition of chicken is significantly different only with regard to linoleic acid. They postulated that linoleic acid would promote the formation of another radical. It is not possible to speculate if this compound influenced loss of tocopherol in this investigation.

Tocopherols are lipophilic and are predominantly located in non-polar sites in tissue. This class of compounds is subjected primarily to direct rather than indirect interactions. The hydroxy radicals and other free radicals produced as a result of the interaction of ionizing radiation with the water in muscle tissue would be expected to react to the greatest extent with the water soluble food components

TABLE 2
Analysis of Covariance (ANCOVA) General Linear Model Procedure. List of Probabilities $> F$. Interaction in Bold Statistically Significant

	<i>Pork</i>	<i>Lamb</i>	<i>Turkey leg</i>	<i>Turkey breast</i>
Beef	0.5643	0.483	0.2264	0.0014
Pork		0.0187	0.0639	0.0001
Lamb			0.6076	0.0097
Turkey leg				0.0334

TABLE 3
Rate Constants for α -Tocopherol Loss due to Gamma Radiation Between 0 and 2 kGy

<i>Species</i>	k_{1s} in KGy^{-1}	<i>SD</i>
Beef	-0.367	(0.078)
Pork	-0.419	(0.090)
Lamb	-0.370	(0.079)
Turkey leg	-0.989	(0.169)
Turkey breast	-1.213	(0.254)

such as carbohydrates, proteins and other polar constituents in food. According to Simic (1983), reaction of electrons would be negligible due to predominant recombination of electrons with positive lipid ions. In addition, some further protection would be afforded tocopherol by the presence of free radical scavengers such as cystine, cysteine, thymine and maleic, orotic and ascorbic acids.

Ionizing radiation induced a rapid loss of free tocopherol (Fig. 1) which occurred primarily between 0.0 and 1.88 kGy, after which it apparently reached equilibrium. This indicates that α -tocopherol was possibly being regenerated at approximately the same rate as it was being destroyed. von Sonntag (1987) states that the rate constant for the bimolecular decay of the tocopherol radical is only $3.5 \times 10^2 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. The long lifetime of the tocopherol radical makes it possible for it to be reduced by other cell components, thus regenerating itself. These factors, may in part, explain the reduction in the rate of loss of tocopherol between 2.8 and 9.4 kGy in all species. Diehl (1981) reported total destruction of tocopherol in pork irradiated with 50 kGy at 20°C.

Physical effect: To measure the effect of ionizing radiation on the water binding capacity and on the possible disruption of muscle tissue, a procedure which determined free water in tissue was employed (Wierbicki & Deatherage, 1958). The study was carried out on all the species except turkey leg, since the non-uniformity of the leg muscle made it impossible to obtain comparable multiple samples. Samples which were analyzed by this procedure were previously irradiated at 0.00, 0.94, 1.88, 2.81, 5.62 and 9.37 kGy. Statistical analysis, Bonferroni (Dunn) *T*-test, indicated ionizing radiation (0–10 kGy) had no effect on the expression of free water on any of the tissues tested. A study conducted on chicken breast tissue by Zabielski *et al.* (1984) detected a 1% increase in free water at 5 kGy and 30°C. However, they utilized a different procedure to determine free water.

CONCLUSIONS

Gamma irradiation of meat at 5°C resulted in a significant decrease in the concentration of α -tocopherol, the most labile of the lipophilic micronutrients. The rate of loss of free α -tocopherol in turkey breast was statistically significantly greater than in the other meats examined. The rates of loss of vitamin E were comparable for all of the red meats (beef, pork, lamb) and for turkey leg. The fact that the red meats reacted in similar fashion with respect to α -tocopherol may be of value with regard to the chemiclearance concept.

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