

Effect of Gamma Radiation on Total Tocopherols in Fresh Chicken Breast Muscle

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ABSTRACT

Chicken breasts were irradiated in air with a ^{137}Cs source at 0.0, 1.0, 3.0, 5.6 and 10.0 kGy at 0–2°C. The fresh muscle tissue was saponified and the total tocopherols were isolated and quantitated using normal phase high performance liquid chromatography with a fluorescence detector. Gamma irradiation of the chicken resulted in a decrease in alpha tocopherol with increasing dose. At 3 kGy and 2°C, the radiation level approved by the FDA to process poultry, there was a 6% reduction in the alpha tocopherol level. No significant changes were observed for gamma tocopherol.

INTRODUCTION

Information on the effect of low dose ionizing radiation on the micronutrient levels in meat is sparse. The vast majority of studies conducted on the effects of radiation on foods were conducted at high doses of 10–300 kGy (Kraybill, 1962; Vakil *et al.*, 1973; Diehl, 1979). While there are no significant changes to the macronutrients at low levels of radiation (Diehl, 1990), thiamine and tocopherol (both micronutrients) were found to be sensitive to radiation. As part of a comprehensive investigation to determine the effects of low dose gamma radiation (<10 kGy) on the micronutrients and pathogens present in poultry and meat, studies were

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‡ Mention of a brand or firm name does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

initiated on the fate of the vitamins. This study concerns the effects of low dose radiation on α - and γ -tocopherols (vitamin E) in meat. Since tocopherol is the most labile of the fat soluble vitamins (Knapp & Tappel, 1961), vitamin E would be the most sensitive indicator of effects on this class of compounds.

Very few studies have measured the effects of ionizing radiation on vitamin E in muscle tissue at levels below 10 kGy. DeGroot *et al.* (1972) measured vitamin E in cooked chicken, Roussel (1988) worked on deboned dehydrated meat, and Lakritz and Thayer (1992) measured the effect on free tocopherols in fresh chicken. In this study we have examined the effect of gamma radiation on the total tocopherols (esterified, bound and unesterified) in fresh chicken breast muscle. The information from this and similar studies on the effects of low dose irradiation are timely. With the passage of legislation by the FDA (Federal Register, 1990) permitting the use of ionizing radiation to control food-borne pathogens in poultry, and with the apparent growing concern by consumers of the presence of salmonella and other microflora in chickens, the use of low dose radiation to process poultry and poultry parts commercially is receiving increased consideration.

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). D- δ -tocopherol (8-methyltolcol) 90%, obtained from Sigma Chemical Co. (St Louis, MO), was purified on a semi-preparative Porasil HPLC column (Waters Inc., Milford, MA). Butylated hydroxytoluene (BHT) [99%] was purchased from Aldrich Chemical Co. (Milwaukee, WI) and was recrystallized from methanol. Ethanol was obtained from Pharmco Prod. Inc. (Weston, MO). Tetrahydrofuran, cyclohexane, and isooctane (2,2,4 trimethylpentane) [HPLC spectral grade] were from EM Science (Gibbstown, NJ). The water was deionized and double distilled in glass.

Materials

Thirteen fresh, intact, whole young frying chickens were purchased for this study over a 4 month period. To determine the effects of ionizing radiation on tocopherol in muscle tissue, skin and all visible adipose

tissue were removed from the chicken breasts. Muscle tissue is not homogeneous, and since the sample size was minimal (7 g) per assay, to accurately study the effects of ionizing radiation on replicate samples at five different dose levels necessitated a uniform substrate for the entire study. The chicken breasts were ground through a 4 mm plate and mixed. The procedure was performed in a glove bag under nitrogen to reduce possible autoxidation of the labile tocopherols.

Irradiation

The ground samples were divided and sealed into five oxygen permeable (2500 ml/100 in²/24 h) meat and poultry bags (Mobil Chemical Co., Macedon, NY). The samples were irradiated with a ¹³⁷Cs source (dose rate of 0.118 kGy/min) to levels of 0.0, 1.0, 3.0, 5.6, and 10.0 kGy. The dosimetry and dose distribution for this radiation source were described by Shieh *et al.* (1985). The dose rate was established by using reference dosimeters from the National Physical Laboratory standard dosimeters (Middlesex, UK). Variations in absorbed dose were minimized by using thin samples which were placed only within the uniform portion of the radiation field. The sample temperature during the radiation was maintained between 0 and 2°C by injection of the gas phase from liquid nitrogen into the chamber.

Saponification

The saponification and extraction procedure described in the methods section is the procedure published in *Methods for the Determination of Vitamins in Food—Recommended by COST 91* (Brubacker *et al.*, 1985) with the following exceptions: (1) Cyclohexane was substituted for diethyl ether as the extraction solvent in order to avoid potential oxidation of tocopherols by peroxides. (2) The alcohol to water (sample H₂O+aq. KOH) ratio was increased from 3/1 to 8/1 in order to ensure solubilization and saponification of the lipid. (3) An ethanol+0.1% BHT solution was substituted for the recommended methanol/ascorbic acid.

Muscle tissue (7 g) was weighed into a two-necked 250 ml round bottomed flask. The following were then added: 280 µl (12 µg) of delta tocopherol (internal standard), 85 ml ethanol containing 0.1% BHT, and a magnetic stirring bar. A reflux condenser was connected to one of the ground glass joints and an adapter containing a nitrogen line inserted into the other. A stream of dry nitrogen was bubbled through the contents of the flask for 5 min in order to expel air; this was followed by the addition of 7 ml of aqueous KOH (50%). The sample was lowered into a

thermostatically controlled 90°C water bath for 25 min and stirred under N₂. The flask and contents were then rapidly cooled in an ice water bath under a blanket of nitrogen. Alkaline saponification resulted in complete digestion of the tissue.

Extraction

The contents of the flask were transferred to a graduated cylinder and the total volume recorded. A 30 ml aliquot and 30 ml of water (degassed) were transferred to a 250 ml separatory funnel containing 50 ml cyclohexane. The separatory funnel was shaken gently for 30 s and the phases allowed to separate. After phase separation, the upper organic phase was reserved and the aqueous phase was twice reextracted. The combined cyclohexane extracts were washed with water (degassed) until no longer alkaline to litmus paper. The extract was dried over anhydrous sodium sulfate, filtered through glass wool, transferred to a round bottom flask and evaporated under vacuum on a rotary evaporator at ambient temperature. The residue was quantitatively transferred with isooctane into a centrifuge tube and the solvent removed under a stream of nitrogen in a 28°C water bath. The sample was reconstituted to 2 ml with isooctane and filtered [0.45 μm ARCO LC 13 (Gelman Science, Ann Arbor, MI)] into an auto-sampling vial and sealed.

High performance liquid chromatography (HPLC)

The HPLC instrumentation used to separate and quantitate the tocopherols consisted of an Autochrom M500 pump (Milford, MA), a model AS-100 refrigerated autosampler (Bio Rad, Richmond, CA), and a Perkin-Elmer spectrophotofluorometer [MPF-44E] (Norwalk, CT) equipped with a xenon lamp and a 20 μl flow cell. The fluorometer settings were: E_x 292 nm, E_m 324 nm, cut-off filter 310 nm, and slits 5 nm (emission and excitation). The data were acquired using a SP 4290 integrator (Spectro Physics, San Jose, CA).

Separation of the tocopherol isomers was accomplished using a 100 mm × 3.0 mm i.d. ChromSpher 5 μm Si column (Chrompak Inc., Raritan, NJ). A guard column (10 mm × 2.2 mm) packed with similar material (Si, 35 μm), was also used. The mobile phase was isooctane-tetrahydrofuran (98:2, v/v) degassed by filtering through a 0.45 μm filter (Supelco Inc, Bellefonte, PA) under vacuum. The flow rate was 0.8 ml/min. The column and solvents were at ambient temperature. Under these conditions, analysis of the tocopherols was completed within 8 min (Fig. 1).

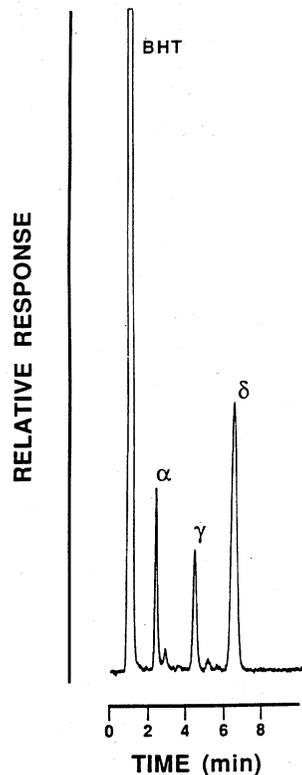


Fig. 1. HPLC chromatogram of chicken breast extract irradiated at 10.0 kGy. Chromatographic conditions: column: ChromSpher Si 5 μ m, 100 \times 3.0 mm i.d.; eluent: isooctane/tetrahydrofuran (98:2, v/v); flow rate: 0.8 ml/min; detection: fluorescence; E_x 292 nm, E_m 324 nm; peak identification: butylated hydroxytoluene (BHT), α -, γ -, δ -tocopherols. Internal standard (δ -tocopherol) and BHT are both additives.

RESULTS AND DISCUSSION

The current FDA statute (Federal Register, 1990) specifies that meat must be processed under aerobic conditions; therefore the samples were irradiated in air permeable bags in spite of the fact that additional autoxidative losses might be incurred. The significance of the dose levels selected (0, 1.0, 3.0, 5.6, 10.0 kGy) to conduct this study were: 0.0, control; 1.0 kGy, dose limit for irradiation of *Trichinella spiralis* in pork (Federal Register, 1986); 3.0 kGy, dose level approved for chicken and poultry; 5.6 kGy midpoint (logarithmic); 10.0 kGy, highest level deemed to introduce no special nutritional or microbial problems by the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) in 1981.

The predominant form of vitamin E in animal tissue is as the free unesterified α -tocopherol, located mainly within the lipid fraction of the cell membrane, in concentrations of <1 mg/100 g (McLaughlin & Weihrauch, 1979). Tocopherols are also found complexed to lipoproteins and may be present as esters. Tocopherol esters are more stable to oxidation than the free phenols, and as a consequence are routinely incorporated into animal feeds. The improvement in the oxidative stability of dark and white broiler flesh during refrigerator and frozen storage has been shown to be correlated with the inclusion of the tocopherol into the feed (Lin *et al.*, 1989).

Numerous procedures to isolate and quantitate tocopherols in agricultural products and processed foods have been published (Ball, 1988). For the determination of tocopherols in animal tissue the tocopherols have either been extracted as the free form by homogenization of the tissue directly in a solvent, or the tissue has been subjected to saponification. Alkaline saponification results in the digestion of muscle tissue and hydrolysis of the esters, and permits the fluorometric estimation of the esters as the free tocopherol. The relative fluorescence of the acetate esters is 1/10 the intensity of the free tocopherols (Barnes & Taylor, 1980).

A previous study by Lakritz and Thayer (1992) measured the effect of radiation of fresh diced chicken on the destruction of free tocopherols. Direct HPLC analysis of solvent extracted tissue was used owing to reports that nearly all of the tocopherols exist in the free form in muscle tissue (McLaughlin & Weihrauch, 1979), and that the use of hot alkaline saponification could result in a substantial loss of the tocopherols since they are exceedingly labile under those conditions (Nelis *et al.*, 1985). The levels of the free tocopherols found in that study were low in comparison to the levels reported by others who measured total (free+esterified/bound) tocopherol via procedures employing saponification.

This study reports on the effect of gamma radiation on both the esterified and unesterified tocopherols in ground, low fat, chicken breasts. The saponification and extraction procedures were a modification of those described by Brubacker *et al.* (1985). Preliminary experiments showed that extraction ($3 \times$) of chicken samples (spiked with α -, γ -, δ -tocopherol) with equal volumes of cyclohexane, cyclohexane/ethyl acetate (85:15), or peroxide free diethyl ether resulted in comparable recoveries of the tocopherols. As stated in the original procedure, it was imperative to extract the samples immediately upon cooling. Initial studies indicated that a delay of 2 h and 4 h after alkaline saponification resulted in the approximate loss of 10% and 30% respectively of α tocopherol, even when stored under nitrogen at 4°C. Employing the saponification procedure rather than direct analysis of unesterified tocopherol resulted in a three-

fold increase in the levels of tocopherol measured from chicken breast muscle.

HPLC of the saponified extracts on a silica column with a spectrophotometric detector resulted in separation of the tocopherols (Fig. 1). The concentration of the tocopherols was determined by comparing peak areas of the fluorescence of the unknown to the fluorescence of the standards determined on the same day. A linear relationship between peak area and concentration of tocopherols existed within the concentration range studied [0–300 ng]. A total of 13 samples, obtained from supermarkets over a 4 month period, were irradiated and assayed. Delta tocopherol was used as the internal standard (IS). A previous study (Lakritz & Thayer, 1992) determined that δ -tocopherol was not present in meat nor was an artifact of irradiation. The IS was added after irradiation and prior to saponification. Samples were always irradiated, saponified, and extracted on the same day. HPLC was usually carried out on the following day. Losses of the IS were factored in to compensate for losses due to manipulation, e.g. transfer. Losses of α -, and δ -tocopherol due to saponification could not be compensated for by use of the IS since δ -tocopherol was far more stable. Recovery studies where breast muscle was spiked with α - and γ -tocopherol resulted in mean recoveries of 108% and 105% respectively.

The relative change in the concentration of α -tocopherol and γ -tocopherol (Table 1) to the unirradiated controls are given for each of the dose levels. Each of these values represent an average of two aliquots chromatographed twice ($n=4$). A high degree of variability was found between samples and within samples. Sample to sample variations may in part be a result of variation between chickens, breeders, and possible

TABLE 1
Percentage of Alpha and Gamma Tocopherol Remaining after Irradiation at Specified Dose Levels, Relative to Unirradiated Controls

<i>Dose (kGy)</i>	<i>Alpha Tocopherol</i>			<i>Gamma Tocopherol</i>		
	<i>Mean (%)</i>	<i>SD^a</i>	<i>N^b</i>	<i>Mean (%)</i>	<i>SD</i>	<i>N</i>
1.0	100	19	10	104	18	8
3.0	94	14	13	102	14	9
5.6	87	15	10	95	16	5
10.0	81	13	13	95	16	10

^a Standard deviation.

^b Number of samples.

seasonal variation (Piironen *et al.*, 1985). Losses as a result of alkaline saponification, autoxidation, sampling error and conducting assays in the low nanogram range may be additional factors.

The data for α tocopherol (Table 1) were analyzed using the General Linear Models procedure for SAS (SAS Institute Inc., 1987). The data were analyzed after eliminating the error between the experiments and looking at differences among doses and among various experiments. Under this type of analysis (ANOVA), both irradiation dose and experiment were found to have highly significant ($p < 0.01$) effects. Statistical analysis excluding variability between experiments demonstrated evidence of a significant decrease in α -tocopherol with increasing dose.

Regression equation:

$$Y = 100 - 2 \times \text{Dose} \quad r = -0.981$$

s.e. intercept = 4.0
s.e. slope = 0.65

The data collected on γ -tocopherol were subjected to the same statistical analysis. Under that protocol, the effect of dose was not found to be significantly ($p < 0.05$) different from zero. γ -Tocopherol has been found to be more stable than α -tocopherol to the effects of ionizing radiation in food (Lakritz & Thayer, 1992) and to autoxidation. α -Tocopherol is the most labile to oxidation (Knapp & Tappel, 1961). Therefore it is not unexpected that the effects of radiation on γ -tocopherol in muscle tissue were less pronounced than on the alpha isomer.

Very few other studies have measured the effects of low dose radiation on the tocopherols, individually or the combination of both isomers, under realistic processing conditions. A 1985 study (Anon.) described the results obtained from the irradiation of mechanically deboned poultry using an electron beam accelerator. The losses of tocopherol in mechanically deboned chicken were: 27%, 67%, 60%, at 1, 3, 5 kGy respectively, while irradiation of deboned turkey incurred lower losses (17–33%). Roussel (1988) in a paper on the irradiation of mechanically deboned meat indicated that the vitamin E content was diminished. A study by DeGroot *et al.* (1972) on the effect of ^{60}Co on the vitamin content of chickens which had been irradiated, then stored for 4–7 days, and finally steam cooked (1 h, 100°C) reported that in one set of experiments there was a 38% reduction at 3 kGy and none at 6 kGy; while in another set there was a 55% loss at both levels. DeGroot *et al.* (1972) noted that there was considerable variation in the vitamin content between batches and treatment within the same batch. Due to the possible synergistic effects resulting from cooking and storage (Diehl, 1981) it would have been difficult to assess the reduction in tocopherol due solely to ionizing radiation. Lakritz and

Thayer (1992) reported the loss of unesterified tocopherols in chicken breast muscle at 3.0 kGy to be 15% and 30% for the gamma and alpha isomers respectively. A comparison of the results between that study and this one would appear to indicate that the unesterified tocopherols may be more sensitive to irradiation than the tocopheryl esters. The free hydroxyl group in the unesterified tocopherol has been shown to be very susceptible to ionizing radiation. Radiation studies on model systems have demonstrated that the phenolic hydrogen atom at the C-6 position on tocopherol can be abstracted by free radicals resulting in the formation of a phenoxyl type radical (von Sonntag, 1987). The very high reaction rate constants ($\approx 10^7 \text{M}^{-1}\text{s}^{-1}$) of the phenoxyl radical in various environments (lipophilic, polar) have been studied by Simic (1980) and Simic and Hunter (1984) using pulse radiolysis. Tocophoryl esters in comparison are exceedingly unreactive.

All of the other studies on the effects of radiation on tocopherols have been conducted at high dose levels or on model systems. It has been shown (Diehl, 1979) that in a food matrix many compounds are afforded a higher degree of protection and are more stable than when irradiated solely in a simple model system.

This study demonstrates that there is only a 6% loss in the tocopherols, the most labile of the fat soluble vitamins, when chicken breast muscle is irradiated at 3 kGy (level approved by the FDA). The minimal loss of tocopherols is significant in that it indicates that there would also only be a negligible reduction in the levels of the other more stable fat soluble vitamins (A, D, K). In addition, the shelf stability of poultry to autoxidation should not be compromised by processing with gamma radiation since the process resulted in a minor reduction in the total tocopherols.

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