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Abstract

The objective of this study was to determine the effect of electron-beam irradiation on the oxidation of cholesterol in raw and cooked chicken meats with different packaging and storage times. Patties were prepared with skinless chicken breasts and legs. Half of the patties were used for raw meat study and the other half for cooked meat work. For cooked samples, patties were cooked in an electric oven to an internal temperature of 70 C. Raw and cooked meat patties were either aerobically or vacuum-packaged before irradiation. Irradiated patties were stored at 4 C up to 2 wk, and the amounts of cholesterol oxides in the patties were analyzed at 0, 7, and 14 d of storage. In raw chicken meat with vacuum packaging, 7β-hydroxycholesterol and β-epoxide were the only two cholesterol oxides present in significant amounts. In raw chicken meat with aerobic packaging, 7α-hydroxycholesterol and 7-keiocholesterol, which were not detected in vacuum-packaged raw chicken meat, were found. 7β-Hydroxycholesterol in raw chicken meat was increased by irradiation and storage time, regardless of packaging. The kinds of cholesterol oxides found in cooked meat were basically the same as those found in raw chicken, but the levels in cooked meats at all storage time were higher than those of the raw meats. With vacuum packaging, irradiation had no consistent effect on the amount of β-epoxide, 7α-hydroxycholesterol, or 7-ketocholesterol, but storage significantly influenced the amount of 7-ketocholesterol, 7β-hydroxycholesterol, and total cholesterol oxides in cooked chicken meat. With aerobic packaging, irradiation significantly increased the formation of 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol in cooked meat stored for 0 and 7 d. After 14 d of storage, however, irradiation had minor effects on the formation of cholesterol oxides in aerobically packaged cooked chicken.

Keywords

chicken meat, cholesterol oxide, irradiation, packaging, storage time

Disciplines

Agriculture | Animal Sciences | Food Biotechnology | Poultry or Avian Science

Comments

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Formation of Cholesterol Oxides in Irradiated Raw and Cooked Chicken Meat During Storage

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ABSTRACT The objective of this study was to determine the effect of electron-beam irradiation on the oxidation of cholesterol in raw and cooked chicken meats with different packaging and storage times. Patties were prepared with skinless chicken breasts and legs. Half of the patties were used for raw meat study and the other half for cooked meat work. For cooked samples, patties were cooked in an electric oven to an internal temperature of 70 C. Raw and cooked meat patties were either aerobically or vacuum-packaged before irradiation. Irradiated patties were stored at 4 C up to 2 wk, and the amounts of cholesterol oxides in the patties were analyzed at 0, 7, and 14 d of storage. In raw chicken meat with vacuum packaging, 7β-hydroxycholesterol and β-epoxide were the only two cholesterol oxides present in significant amounts. In raw chicken meat with aerobic packaging, 7α-hydroxycholesterol and 7-ketocholesterol, which were not detected in vacuum-packaged raw chicken meat, were found. 7β-Hydroxycholesterol in raw chicken meat was increased by irradiation and storage time, regardless of packaging. The kinds of cholesterol oxides found in cooked meat were basically the same as those found in raw chicken, but the levels in cooked meats at all storage time were higher than those of the raw meats. With vacuum packaging, irradiation had no consistent effect on the amount of β-epoxide, 7α-hydroxycholesterol, or 7-ketocholesterol, but storage significantly influenced the amount of 7-ketocholesterol, 7β-hydroxycholesterol, and total cholesterol oxides in cooked chicken meat. With aerobic packaging, irradiation significantly increased the formation of 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol in cooked meat stored for 0 and 7 d. After 14 d of storage, however, irradiation had minor effects on the formation of cholesterol oxides in aerobically packaged cooked chicken.

(*Key words*: chicken meat, cholesterol oxide, irradiation, packaging, storage time)

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INTRODUCTION

Cholesterol oxides have been reported to have a wide range of adverse biological effects on animals, such as atherogenesis, cytotoxicity, mutagenesis, and carcinogenesis (Guardiola et al., 1996). The oxidation of cholesterol occurs easily in various foods, including meat and poultry, and their products as cholesterol oxidation occurs through a chemical process similar to that of unsaturated fatty acid oxidation. In addition, oxidation of cholesterol in foods is affected by the environment surrounding cholesterol, especially nearby polyunsaturated lipids (Gray et al., 1996). Accordingly, prolonged storage, application of heat, and exposure to light or irradiation promote the oxidation of cholesterol (Paniangvait et al., 1995). With growing concerns about food safety, the use of irradiation has been well accepted as one of the best methods for the production of safe meat and poultry (Lee et al., 1996). However, major concerns with irradiating meat and poultry are quality changes such as the occurrence of off-flavor and the acceleration of lipid oxidation.

Ionizing radiation has been used in food processing to control microbial growth but has been known to generate hydroxyl radicals in aqueous or oil emulsion systems (O'Connell and Garner, 1983). Lebovics et al. (1992) found that ionizing radiation induces similar chemical reactions to cholesterol autoxidation, and the quantity of oxidation products formed by the irradiation is dose-dependent. Katusin-Razem et al. (1992) reported that radiation-induced oxidative chemical changes in egg products are dose-dependent, and the presence of oxygen has a significant effect on the rate of oxidation. Ahn et al. (1998) found that packaging and storage conditions of raw meat after irradiation are more important factors than irradiation in lipid oxidation of cooked meat. Li et al. (1996) found that freeze-dried chicken meat powder stored in contact with air contains cholesterol oxides and the

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Abbreviation Key: kGy = kiloGray.

amount of total cholesterol oxides formed in meat powder are significantly affected by the composition of lipids and increases with storage time.

The objective of this study was to investigate the effect of electron-beam irradiation on the oxidation of cholesterol in raw and cooked chicken meats with different packaging and storage times.

MATERIALS AND METHODS

Chemicals and Reagents

Cholesterol oxides standards were purchased from Sigma;[2] bis-(trimethylsiyl) trifluoroacetamide + 1% trimethylchlorosilane and pyridine were from Supelco;[3] celite 545, calcium phosphate, and solvents (hexane, ethyl acetate, ethyl ether, and acetone, and methanol, HPLC grades) were from Fisher Scientific;[4] and silicic acid was from Aldrich.[5]

Sample Preparation

Chickens (total of 18 birds) were procured from a local store, and breast and leg meats were harvested. Breast and leg meats from six birds were pooled and used as a replicate, and three replicates were prepared. Ground, fresh chicken meats without skin were made into patties (~100 g) and packaged in air with polyethylene or under vacuum with polyvinylidienechloride film.[6] Half of the patties were used for the study of raw meat, and the other half were used for studying cooked meat.

For the cooked samples, patties were cooked in an electric oven until an internal temperature of 70 C was reached. Patties were either aerobically packaged in oxygen-permeable bags or vacuum-packaged in polyvinylidienechloride bags 3 h after cooking. After packaging, the samples were irradiated, on both sides of packages, by electron-beam with a Samsung electrostatic type linear accelerator (Model 2LV4)[7] at Central Lab of Samsung Heavy Industry Co., Inc. Cellulose triacetate (FTR 125 film) was used as a dosimeter.[8]

The raw and cooked meats were stored for 14 d at 4 C, and the amounts of cholesterol oxides were determined at 0, 7, and 14 d of storage.

Cholesterol Oxides Analysis

A solid-phase column preparation for the separation of cholesterol oxides (Zubillaga and Maerker, 1991; Park and Addis, 1985) was made by mixing silicic acid, celite 545, and $CaHPO_4 \cdot 2H_2O$ (10:9:1, wt/wt/wt) with 30 mL chloroform and then packing in a glass column (12 mm × 30 cm). A prepared column was prewashed with 5 mL hexane, 5 mL ethyl acetate, and then 5 mL hexane, consecutively, just before sample application. Total lipids were extracted by the method of Folch et al. (1957). The 0.2-g lipid sample was dissolved in 2 mL hexane:ethyl acetate (100:2, vol/vol), and then the sample solution was applied to a prewashed column. The sample container was washed twice with 2 mL hexane:ethyl acetate, and the wash solvent was applied to the column. Neutral lipid and cholesterol (phospholipids) were removed by adding 50 mL solvent I ($CHCl_3$:CH_3OH = 2:1, vol:vol) and 60 mL solvent II (hexane:ethyl acetate = 4:1, vol/vol). Forty milliliters of solvent III (acetone:ethylacetate:methanol = 50:50:5, vol/vol/vol) was used to elute at 1 mL/min and to collect cholesterol oxides. The collected solution was dried on a 50 C hot plate with nitrogen gas flushing. The dried extracts were derivatized by heating at 80 C for 1 h in the presence of 200 μL pyridine and 100 μL sylon bis-(trimethylsiyl)trifluoroacetamide + 1 % trimethylchlorosilane.

Cholesterol oxides were analyzed with a gas chromatograph (HP 5890 plus)[9] equipped with an on-column capillary injector and a flame ionization detector. We used a gas chromatography column of 0.32 mm i.d. × 30 m with 0.32-μm film thickness (Supelcowax-10 column).[3] A splitless inlet was used to inject samples (0.5 μL) onto the capillary column, and a ramped oven temperature was used. The initial oven temperature of 70 C was held for 0.5 min and then increased to 275 C at 40 C/min and held at 275 C for 0.5 min. The temperature increased again to 280 C at 2 C/min. Temperatures of the inlet and detector were 260 and 300 C, respectively. Helium was the carrier gas at constant pressure of 14.0 psi. Flame ionization detector air, H_2, and make-up gas (He) flows were 300 mL/min, 30 mL/min, and 28 mL/min, respectively. The area of each peak (pA*s) was integrated with the ChemStation software,[9] and the amounts of cholesterol oxides were calculated using an internal standard (5α-cholestane).

Statistical Analysis

The effects of irradiation dose on the formation of cholesterol oxides in raw and cooked meats were analyzed independently by SAS® software (SAS Institute, 1988). The statistical analysis was by ANOVA, and Duncan's multiple range test ($P < 0.05$) was used to compare differences among mean values. Mean values and standard deviations were reported.

RESULTS AND DISCUSSION

In the absence of oxygen (vacuum packaging), 7β-hydroxycholesterol was the only cholesterol oxide present in significant amounts in raw chicken meat after 0 and 7 d of storage at 4 C. After 14 d of storage, another cholesterol oxide, β-epoxide, was found in raw meat. The amount of

[2]Sigma, St. Louis, MO 63178.
[3]Supelco, Bellefonte, PA 16823-0048.
[4]Fisher Scientific, Pittsburgh, PA 15205-9913.
[5]Aldrich, Milwaukee, WI 53233-2641.
[6]Cryovac, Duncan, SC 29334.
[7]Samsung Heavy Industry, Inc., Taejon, Korea, 305-600.
[8]Fuji Photo Film Co., Tokyo, Japan, 106-8620.
[9]Hewlett Packard Co., Wilmington, DE 16808-1610.

TABLE 1. Effect of irradiation and storage time on the amount of cholesterol oxides in vacuum-packaged raw chicken meat[1]

	0-d storage at 4 C			7-d storage at 4 C			14-d storage at 4 C		
Cholesterol oxides	0 kGy[2]	1 kGy	2 kGy	0 kGy	1 kGy	2 kGy	0 kGy	1 kGy	2 kGy
	(μg cholesterol oxides/g fat)								
β-epoxide	Trace[3]	Trace	Trace	Trace	Trace	Trace	2.49 ± 0.2[a]	3.01 ± 0.1[a]	2.80 ± 0.4[a]
7β-hydroxycholesterol	3.47 ± 0.3[b]	4.05 ± 0.2[b]	10.72 ± 1.3[a]	8.59 ± 0.4[c]	14.33 ± 1.1[b]	18.01 ± 0.9[a]	12.66 ± 1.0[c]	18.28 ± 0.4[b]	24.46 ± 0.5[a]
Total	3.47 ± 0.3[b]	4.05 ± 0.2[b]	10.72 ± 1.4[a]	8.59 ± 0.4[c]	14.33 ± 1.1[b]	18.01 ± 0.9[a]	15.15 ± 0.9[c]	21.29 ± 0.4[b]	27.26 ± 0.5[a]

[a–c]Different letters within a row of the same storage time are different ($P < 0.05$).

[1]Mean value ± standard deviation; n = 3.

[2]Absorbed irradiation dose (average); kGy = kiloGray.

[3]<0.5 μg/g.

7β-hydroxycholesterol significantly increased with storage time. Irradiated chicken meat had more 7β-hydroxycholesterol than the control at all storage times except at Day 0 with 1 kGy irradiation. The amount of β-epoxide in raw chicken meat was not influenced by irradiation (Table 1).

With aerobic packaging, 7α-hydroxycholesterol and 7-ketocholesterol, which are not detected in vacuum-packaged raw chicken meat, were found. Also, significantly more 7β-hydroxycholesterol and total cholesterol were found in aerobically packaged than in vacuum-packaged meat. Irradiation significantly increased the formation of cholesterol oxides, especially 7-ketocholesterol, in raw chicken meat, and the total cholesterol oxides increased with storage time. β-Epoxide was found in chicken meat after 7 d of storage in aerobic packages, but the amount was not influenced by irradiation. The effect of irradiation on the formation of 7α-hydroxycholesterol in raw chicken meat was inconsistent (Table 2). Zubillaga and Maerker (1991) reported that 7-ketocholesterol was the principal product and constituted well over 50% of the mixture in meat and chicken during storage, whereas 7β-hydroxycholsterol and 7-ketocholsterol were the major cholesterol oxides in fish (Ohshima et al., 1993). In this study, 7α-hydroxycholesterol was the major oxidation product. Cholesterol oxide content increased significantly ($P < 0.05$) with the level of irradiation, regardless of packaging type. Vacuum packaging prevented formation of 7α-hydroxycholesterol and 7-ketocholesterol during irradiation. The hydroperoxides of polyunsaturated fatty acids formed during lipid oxidation may be necessary to initiate cholesterol oxidation, which may be the reason that vacuum packaging lowered the content of cholesterol oxides significantly ($P < 0.05$) as shown in this study. Hwang and Maerker (1993) reported that γ-irradiation increased 7-ketocholesterol and α- and β-epoxides in beef and pork packaged in an oxygen-permeable bag. They also showed that the amounts of cholesterol oxides increased considerably during storage in irradiated and nonirradiated meats, but irradiated meats had higher cholesterol oxides than did nonirradiated meats.

The kinds of cholesterol oxides found in cooked meat were basically the same as that found in raw chicken (Tables 3 and 4), but the levels in cooked meats at all storage times were higher than those of the raw meats (Tables 1 to 4). Monahan et al. (1992) demonstrated that the rate of cholesterol oxidation in pork was greatly accelerated during storage after cooking. As with raw meat, 7β-hydroxycholesterol was the major cholesterol oxide in cooked meat, and the amount was influenced by irradiation. With vacuum packaging, irradiation had no consistent effect on the amounts of β-epoxide, 7α-hydroxycholesterol, or 7-ketocholesterol, but storage significantly influenced the amount of 7-ketocholesterol, 7β-hydroxycholesterol, and total cholesterol oxides in vacuum-packaged, cooked chicken meat (Table 3).

With aerobic packaging (Table 4), irradiation significantly increased the formation of 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol in cooked meat stored for 0 and 7 d. After 14 d of storage, however,

TABLE 2. Effect of irradiation and storage time on the production of cholesterol oxides in aerobically packaged raw chicken meat[1]

	0-d storage at 4 C			7-d storage at 4 C			14-d storage at 4 C		
Cholesterol oxides	0 kGy[2]	1 kGy	2 kGy	0 kGy	1 kGy	2 kGy	0 kGy	1 kGy	2 kGy
	(μg cholesterol oxides/g fat)								
β-epoxide	Trace[3]	Trace	Trace	1.24 ± 0.2[a]	0.98 ± 0.2[a]	1.09 ± 0.1[a]	4.18 ± 0.2[a]	2.42 ± 0.6[b]	2.22 ± 0.7[b]
7α-hydroxycholesterol	0.88 ± 0.2[b]	0.83 ± 0.1[b]	1.05 ± 0.1[a]	4.75 ± 0.2[a]	2.57 ± 0.1[c]	3.01 ± 0.2[b]	3.70 ± 0.2[a]	3.01 ± 0.1[b]	2.91 ± 0.1[b]
7β-hydroxycholesterol	14.24 ± 1.6[b]	16.23 ± 1.3[b]	20.35 ± 0.5[a]	12.50 ± 0.7[c]	19.07 ± 0.2[b]	27.21 ± 1.9[a]	34.50 ± 1.0[c]	69.03 ± 1.6[b]	78.84 ± 0.6[a]
7-ketocholesterol	Trace[b]	2.78 ± 0.2[a]	2.78 ± 0.1[a]	Trace[c]	2.86 ± 0.1[b]	3.30 ± 0.2[a]	Trace[b]	5.82 ± 0.2[a]	5.33 ± 0.7[a]
Total	15.12 ± 1.7[c]	19.84 ± 1.5[b]	24.05 ± 0.5[a]	18.49 ± 0.5[c]	25.48 ± 0.5[b]	34.61 ± 2.2[a]	42.38 ± 1.3[c]	80.28 ± 0.9[b]	89.31 ± 1.9[a]

[a–c]Different letters within a row of the same storage time are different ($P < 0.05$).

[1]Mean value ± standard deviation; n = 3.

[2]Absorbed irradiation dose (average); kGy = kiloGray.

[3]< 0.5 μg/g.

TABLE 3. Effect of irradiation and storage time on the production of cholesterol oxides in vacuum-packaged cooked chicken meat[1]

	0-d storage at 4 C			7-d storage at 4 C			14-d storage at 4 C		
Cholesterol oxides	0 kGy[2]	1 kGy	2 kGy	0 kGy	1 kGy	2 kGy	0 kGy	1 kGy	2 kGy
	(μg cholesterol oxides/g fat)								
β-epoxide	0.87 ± 0.3^{b}	1.34 ± 0.2^{a}	1.59 ± 0.2^{a}	1.42 ± 0.1^{a}	1.35 ± 0.2^{a}	1.68 ± 0.3^{a}	1.62 ± 0.3^{b}	3.92 ± 0.5^{a}	3.89 ± 0.7^{a}
7α-hydroxycholesterol	1.44 ± 0.1^{c}	1.91 ± 0.2^{b}	2.29 ± 0.1^{a}	2.98 ± 0.1^{a}	2.38 ± 0.2^{b}	2.64 ± 0.1^{b}	1.74 ± 0.1^{a}	1.55 ± 0.4^{a}	2.15 ± 0.3^{a}
7β-hydroxycholesterol	10.10 ± 0.4^{c}	12.52 ± 1.2^{b}	15.64 ± 0.2^{a}	19.23 ± 0.5^{c}	23.05 ± 0.7^{b}	25.25 ± 1.7^{a}	18.73 ± 0.6^{c}	38.43 ± 0.9^{b}	53.88 ± 1.2^{a}
7-ketocholesterol	2.89 ± 0.1^{a}	2.86 ± 0.1^{a}	2.99 ± 0.1^{a}	3.57 ± 0.1^{a}	3.11 ± 0.1^{c}	3.37 ± 0.1^{b}	7.38 ± 0.4^{b}	8.47 ± 0.2^{a}	8.04 ± 0.3^{a}
Total	15.30 ± 0.9^{c}	18.63 ± 1.7^{b}	22.51 ± 0.2^{a}	27.20 ± 0.3^{c}	29.89 ± 0.4^{b}	32.94 ± 2.1^{a}	29.47 ± 0.2^{c}	52.37 ± 1.6^{b}	67.96 ± 0.6^{a}

$^{a–c}$Different letters within a row of the same storage time are different ($P < 0.05$).

[1]Mean value ± standard deviation; n = 3.

[2]Absorbed irradiation does (average); kGy = kiloGray.

TABLE 4. Effect of irradiation and storage time on the production of cholesterol oxides in aerobically packaged, cooked chicken meat[1]

	0-d storage at 4 C			7-d storage at 4 C			14-d storage at 4 C		
Cholesterol oxides	0 kGy[2]	1 kGy	2 kGy	0 kGy	1 kGy	2 kGy	0 kGy	1 kGy	2 kGy
	(μg cholesterol oxides/g fat)								
β-epoxide	1.0 ± 0.1^{a}	1.1 ± 0.2^{a}	1.1 ± 0.2^{a}	0.9 ± 0.1^{b}	1.6 ± 0.2^{a}	1.6 ± 0.1^{a}	3.3 ± 0.2^{a}	3.6 ± 0.8^{a}	3.6 ± 0.9^{a}
7α-hydroxycholesterol	4.5 ± 0.2^{b}	6.6 ± 0.4^{ab}	8.1 ± 2.6^{a}	10.2 ± 0.2^{c}	21.5 ± 0.8^{a}	20.0 ± 0.8^{b}	32.9 ± 1.5^{a}	33.8 ± 0.5^{a}	26.1 ± 0.7^{b}
7β-hydroxycholesterol	18.3 ± 1.8^{c}	47.2 ± 0.7^{b}	61.7 ± 8.8^{a}	23.7 ± 0.3^{c}	72.4 ± 1.2^{b}	135.2 ± 3.6^{a}	141.3 ± 6.2^{b}	147.6 ± 1.3^{b}	174.2 ± 6.6^{a}
7-ketocholesterol	6.8 ± 0.1^{b}	9.3 ± 0.5^{ab}	9.6 ± 2.3^{a}	9.1 ± 0.4^{c}	21.9 ± 0.5^{a}	20.4 ± 1.0^{b}	18.1 ± 0.7^{c}	29.9 ± 1.0^{b}	39.7 ± 1.2^{a}
Total	31.2 ± 2.0^{c}	64.8 ± 0.5^{b}	81.0 ± 13.8^{a}	44.5 ± 0.8^{c}	117.9 ± 2.4^{b}	177.8 ± 4.7^{a}	196.3 ± 7.2^{c}	215.2 ± 1.4^{b}	244.2 ± 4.0^{a}

$^{a–c}$Different letters within a row of the same storage time are different ($P < 0.05$).

[1]Mean value ± standard deviation; n = 3.

[2]Absorbed irradiation dose (average); kGy = kiloGray.

irradiation had minor effects on the formation of cholesterol oxides in cooked chicken that was aerobically packaged. The oxidation of cholesterol during storage of cooked chicken meat that was aerobically packaged was much faster than that in vacuum-packaged; 7β-hydroxycholesterol was the major cholesterol oxide in aerobically and vacuum-packaged cooked meat. This study showed that oxidative changes of cholesterol in cooked meat during storage is faster than that of the raw meat and suggested that cooked meat should be vacuum-packaged as soon as possible after cooking to reduce oxidative changes in cholesterol during storage.

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