Quantitation of Cholesterol Oxidation Products in Unirradiated and Irradiated Meats

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A method to detect 7-ketocholesterol, cholesterol-5β,6β-epoxide, cholesterol-5α,6α-epoxide, 4-cholesten-3-one, 4,6-cholestadien-3-one and 4-cholestene-3,6-dione in unirradiated and irradiated beef, pork and veal was developed by use of chloroform-methanol-water extraction, solid-phase extraction, column separation, thin-layer chromatography and gas chromatography. This method recovered 78–88% of the cholesterol oxidation products and detected the cholesterol oxidation products at 10 ppb or higher. Irradiation of the meats to a dose of 10 kGy increased these compounds, except 4,6-cholestadien-3-one for all three types of meat, over unirradiated, and except cholesterol-5α,6α-epoxide and 4-cholesten-3-one for the pork. All the cholesterol oxidation products in the unirradiated meats increased during storage at 0–4°C for 2 wk with some exceptions for the pork. The increases of cholesterol oxidation products in stored irradiated meats were greater than those in the unirradiated.

KEY WORDS: Beef, cholesterol, irradiation, oxidation, pork, veal.

The susceptibility of cholesterol to oxidation is well known and has been studied in various aspects over half a century. A great number of oxidation products derived from cholesterol have been identified. The most common cholesterol oxidation products encountered in nature are 7-ketocholesterol (3β-hydroxycholest-5-en-7-one), 7β-hydroxycholesterol (cholest-5-ene-3β,7β-diol), 7α-hydroxycholesterol (cholest-5-ene-3β,7α-diol), cholesterol-5β,6β-epoxide (5,6β-epoxy-5α-cholestan-3β-ol; β-epoxide), cholesterol-5α,6α-epoxide (5,6α-epoxy-5α-cholestan-3β-ol; α-epoxide) and 3β,5,6β-trihydroxycholesterol (5α-cholestane-3β,5,6β-triol; triol) (1). Other cholesterol oxidation products, generated in lesser amounts, are 4-cholesten-3-one, 4,6-cholestadien-3-one and 4-cholestene-3,6-dione (2).

As part of a search for a diagnostic test capable of detecting whether meats have been previously irradiated, the oxidation products derived from cholesterol have been investigated in our laboratory. In recent studies with model systems, γ-radiation generated different cholesterol oxidation product ratios than did autoxidation. In irradiated aqueous sodium stearate dispersions of cholesterol, the ratio of 7-ketocholesterol to α-epoxide plus β-epoxide was approximately 1 or below, while the ratio in autoxidation of similar dispersions was normally six or greater (3). In a subsequent study with cholesterol in aqueous suspensions of liposomes, the ratio of those compounds was less than one for irradiated samples, much lower than the ratio of ten commonly produced by autoxidation (4). More recently, A-ring cholesterol derivatives such as 4-cholesten-3-one, 4,6-cholestadien-3-one and 4-cholestene-3,6-dione were found to be generated from cholesterol in liposomes by the exposure to γ-radiation (5).

Methods to detect cholesterol oxidation products in meat systems have been reported (6–14). Some of these methods have limited sensitivity and are inadequate for measurement of some of the compounds of interest. To our knowledge, the presence of A-ring oxidation products of cholesterol in meat or poultry has not been reported, although their presence in biological tissue (15), low density lipoproteins (16) and various mammalian organ tissues (17) is known. These 3-keto derivatives of cholesterol are less polar (by thin-layer chromatography) than the parent sterol and hence are more difficult to separate from the neutral lipids than is cholesterol and/or cholesterol derivatives with hydroxy groups.

There was a need to determine whether the findings from model systems were applicable to actual meat systems. Because of the complex composition of animal tissue and the extremely low concentration of cholesterol oxidation products in this tissue, it was important to develop methodology consistently capable of detecting endogenous cholesterol oxidation products as well as measuring the changes of the concentration of those compounds by autoxidation and/or irradiation.

The objectives of the present study were to develop methodology capable of measuring the cholesterol oxidation products, especially A-ring oxidation products, present in meat at concentrations as low as 10 ppb and to detect the differences in those compounds between raw and irradiated beef, pork and veal. An additional goal was to measure changes of the oxides in the meats during refrigerated storage.

EXPERIMENTAL PROCEDURES

Materials and reagents. Cholesterol (5-cholesten-3β-ol), α-epoxide (5,6α-epoxy-5α-cholestan-3β-ol) and 6-ketocholestanol (3β-hydroxy-5α-cholestan-6-one) were purchased from Sigma Chemical Co. (St. Louis, MO); β-epoxide (5,6β-epoxy-5α-cholestan-3β-ol) and 7-ketocholestanol (3β-hydroxy-5α-cholestan-7-one), 5-cholesten-3-one, 4,6-cholestadien-3-one and 4-cholestene-3,6-dione were purchased from Research Plus, Inc. (Bayonne, NJ); 7-ketocholesterol (3β-hydroxycholest-5-en-7-one) and desmosterol (5,24-cholestadien-3β-ol) were purchased from Steraloids Inc. (Wilton, NH); and 4-cholesten-3-one was purchased from Aldrich Chemical Co. (Milwaukee, WI). Solid-phase extraction (SPE) columns (B&J SPE Inert Silica 500 mg) were purchased from Baxter Diagnostic Inc. (McGaw Park, IL) and thin-layer chromatography (TLC) plates (scored silica gel GHL, 250 μm) from Analtech, Inc. (Newark, DE). Beef (eye round steak), pork (loin center-cut chops, boneless) and veal (loin chops) were purchased from a local retail store. Water used was double-deionized and glass-distilled. All solvents used were "distilled-in-glass" grade, and chemicals were reagent-grade quality.

Sample preparation and irradiation. Visible fat was removed from meat. The meat was sliced (about 2.5 mm thick) and put into an oxygen-permeable (2500 mL/100 in^2/24 h) meat and poultry bag (Mobile Chemical Co., Macedon, NY), which was then fastened with rubber bands. The bag with the meat was irradiated in a Cs-137 γ-radiation source at 0–4°C to a dose of 10 kGy (0.114 kGy/min). The irradiated or unirradiated (control) meat

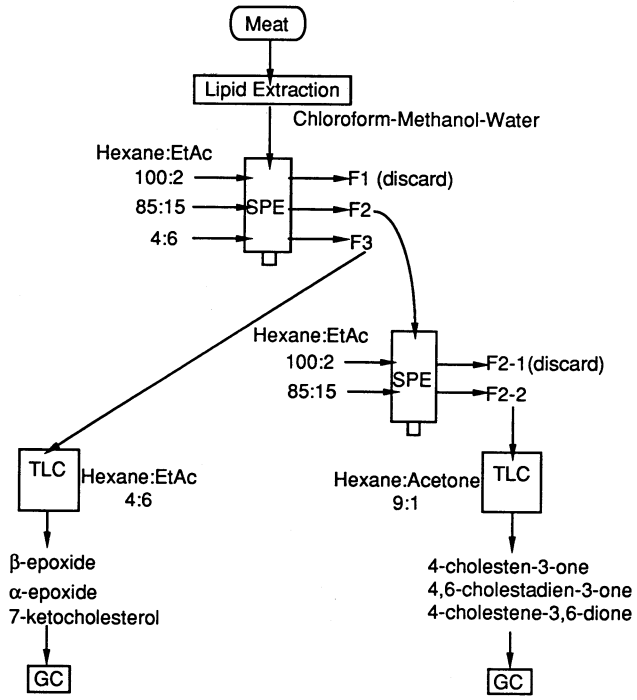

FIG. 1. Isolation and quantitation scheme of cholesterol oxidation products in meats. Abbreviations; SPE, solid-phase extraction; TLC, thin-layer chromatography; GC, gas chromatography.

was cut into smaller pieces. Half of the samples were stored for 2 wk at 0–4°C. The dose rate was established by using National Physical Laboratory (Middlesex, United Kingdom) dosimeters. Variations in dose were minimized by the use of small samples placed in a uniform portion of the radiation field. Samples were maintained at the desired temperature during irradiation by the injection of liquid nitrogen into the irradiation chamber.

Fat extraction. The overall procedure for isolation and quantitation of cholesterol oxidation products in meats is depicted in Figure 1. A modification of the Bligh and Dyer method (18) was used for fat extraction. Thirty mL chloroform and 30 mL methanol were added to 10 g of meat sample in a 125-mL Erlenmeyer flask. The content was homogenized with a Tekmar SDT-1810 motor and SDT-182EN shaft (Tekmar Co., Cincinnati, OH) for 1 min. The homogenate was filtered through Whatman No. 2 filter paper (7 cm) (Whatman International Ltd., Maidstone, England) on a Buchner funnel and vacuum flask connected to mild vacuum. The filtrate was transferred to a 100-mL graduated cylinder. The homogenizer and the sample container were washed twice with 20 mL and then with 10 mL chloroform:methanol (1:1), and the wash was filtered through the filtering system with the sample residue. The filtrate was added to the first filtrate in the graduated cylinder. Twenty mL of 0.88% KCl solution was added to the solvent extract in the cylinder, and the content was vigorously shaken. The extract was allowed to settle and to separate into two phases. The upper layer was removed by aspiration and collected in a 200-mL centrifuge bottle. Thirty mL of 0.88% KCl solution and 15 mL methanol were added to the lower layer. After being shaken vigorously, the content was settled to separate the phases, and the upper layer was removed and added to the first upper layer in the centrifuge bottle. Fifteen mL chloroform was added to the collected upper layer in the centrifuge bottle, and the content was shaken and centrifuged at 3000 rpm for 10 min. The upper layer was removed and discarded. The lower layer in the centrifuge bottle was combined with the chloroform extract in the graduated cylinder from the procedure above. After mixing, half of the chloroform extract was transferred to a 125-mL Erlenmeyer flask together with 15 g Na_2SO_4 (anhyd.). The extract was swirled and filtered through a Whatman No. 2 folded filter paper. The filtrate was collected in a 50-mL test tube provided with a Teflon cap. The Erlenmeyer flask was rinsed twice with 10 mL and then with 5 mL chloroform, and the rinse was filtered. The filtering system was washed with 10 mL chloroform. The combined filtrate in the test tube was evaporated to dryness under a nitrogen stream.

SPE column separation. An SPE column was prewashed with 5 mL hexane, 5 mL ethyl acetate and then 5 mL hexane consecutively just before sample application. The dry fat sample was dissolved in 2 mL hexane:ethyl acetate (100:2), the sample solution was applied to a prewashed SPE column, and sufficient nitrogen pressure was applied for the solvent to elute at a rate of about 2–3 drops per s. The sample container was washed twice with 2 mL hexane:ethyl acetate (100:2), and the wash solvent was applied to the column. The eluate was discarded. Five mL hexane:ethyl acetate (85:15) was applied to the SPE column, and the eluate was collected and named fraction 2. Five mL hexane:ethyl acetate (4:6) was applied to the SPE column, and the eluate was collected and named fraction 3. Fractions 2 and 3 were dried under nitrogen stream. The dry fraction 2 was dissolved in 2 mL hexane:ethyl acetate (100:2), the solution was added to a fresh, prewashed SPE column, and the separation was repeated (see Fig. 1). The first eluate was discarded. Elution with 5 mL hexane:ethyl acetate (85:15) gave fraction 2-2. The solvent of fraction 2-2 was evaporated to dryness under nitrogen. The dry fraction 2-2 and fraction 3 were dissolved in 500 μL and 250 μL chloroform, respectively, for the following TLC experiment.

TLC separation. For fraction 2-2, half of the sample was applied on the middle section of a scored 20 × 20 cm TLC plate with a Camag Linomat IV TLC applicator (Camag, Muttenz, Switzerland) at a speed of 4 s/μL. After the sample on the TLC plate was dried under a mild nitrogen stream, the plate was developed with hexane: acetone (9:1) for 40 min. The edges of the plate, on which a mixture of standards had been spotted, were snapped off, sprayed with 50% H_2SO_4 and then charred on a hot plate. The silica band containing 4-cholesten-3-one, 4,6-cholestadien-3-one and 4-cholestene-3,6-dione (about 4 cm) was scraped into a 20-mL test tube. The silica was extracted three times with 5 mL acetone each time: the content was vortexed for 30 s, followed by centrifuging at 2200 rpm for 2 min. The supernatant was filtered through a Nylon 66 membrane (13 mm, 0.2 μ) (Analtech, Inc.) contained in a 13-mm syringe holder (Nucleopore Corp., Pleasanton, CA), which was connected to a 50-mL Luer lock syringe. The filtrate was pooled in a conical test tube. 7-Ketocholestanol (about 4 μg in ethyl acetate) was added as internal standard, and the solvent was evaporated under nitrogen. The

residue was reconstituted in 200 μL ethyl acetate for gas-chromatographic analysis.

TLC separation of fraction 3 was conducted in the same manner as that of fraction 2-2 except for the following: the application speed was 1.6 s/μL and the developing solvent was hexane:ethyl acetate (4:6). The TLC silica band for β-epoxide, α-epoxide, α-epoxide, 7-ketocholesterol, 7-ketocholestanol and 6-ketocholestanol (about 3 cm) was scraped. The sample was extracted in the same manner as for fraction 2-2. Desmosterol (about 4 μg in ethyl acetate) was added as an internal standard, and the solvent was evaporated. The residue was dissolved in 200 μL ethyl acetate.

Gas-chromatography (GC) analysis. Samples were analyzed on a Varian 3600 Gas Chromatograph (Varian Associates, Sugar Land, TX) equipped with a flame-ionization detector (FID) and an on-column capillary injector as described previously (14) with the following modifications. The column was a 0.25 mm i.d. × 30 m 5% phenylsilicone column with 0.25 μm film thickness (DB-5, J&W Scientific, Folsom, CA). The initial column temperature of 100°C was held for 3 min, then programmed at 30°C/min to 260°C and 0.6°C/min to 277°C, then 30°C/min to 310°C where it was held for 10 min. The initial injector temperature of 70°C was held for 0.5 min, then programmed at 50°C/min to 275°C where it was held for 10 min. The detector temperature was 325°C. One μL of the sample was injected.

Recovery of cholesterol oxidation products. After beef was cut as described above, 10 g of the beef was weighed in a 125-mL Erlenmeyer flask. About 10 μg of each of β-epoxide, α-epoxide and 7-ketocholesterol, and about 20 μg of each of 4-cholesten-3-one, 4,6-cholestadien-3-one and 4-cholestene-3,6-dione, dissolved at about 1 μg/μL in ethyl acetate, were added to the beef and mixed well. The recovery of the spiked compounds through the fat extraction, SPE separation, TLC and GC procedures described above was determined by comparison with nonspiked samples (controls).

Statistical analysis. All the experiments were in triplicate unless otherwise stated. Data were analyzed by *t*-test ($\alpha = 0.05$) comparing the values of (0 day-unirradiated) *vs.* (0 day-irradiated); (0 day-unirradiated) *vs.* (2 week-unirradiated); (2 week-unirradiated) *vs.* (2 week-irradiated); and (0 day-irradiated) *vs.* (2 week-irradiated) for each cholesterol oxidation product in each type of meat by means of SAS (19).

RESULTS AND DISCUSSION

Isolation and measurement of cholesterol oxidation products from meat. Most of the published methods to measure cholesterol oxidation products present in meats basically follow the steps (i) extraction of lipids from meat muscle, (ii) removal of other lipid materials from cholesterol and cholesterol oxidation products, (iii) clean-up of the sample and derivatization of cholesterol and its oxides and finally (iv) quantitation by GC or high-performance liquid chromatography (HPLC).

The overall scheme of the method carried out in the present study to detect the cholesterol oxidation products is shown in Figure 1. The method of Folch *et al.* (20), which has been used by others (6,8–11,13) in the isolation of cholesterol oxidation products, requires small meat samples (0.1–1 g) so that the cholesterol oxidation products present in meats at low levels might be lost in the isolation procedure. Cholesterol oxidation products in 10 g of meat were measured at concentrations of less than 1 ppm by using the dry-column procedure (14). This method, however, proved to be physically cumbersome in our laboratory. The number of samples that could be analyzed simultaneously was limited. In the present study, a modified Bligh and Dyer method (18) was used to extract lipids from meat (10 g) samples. The chloroform layer was washed twice with a methanol-water mixture to remove polar materials, and the water-methanol layer was also washed with chloroform to increase the recovery level of the compounds.

One of the most commonly used methods to recover cholesterol and cholesterol oxidation products from lipid extracts has been cold saponification. Saponification has been criticized because of the artifactual formation of oxidation products (2). Enolizable ketocholesterols are believed to be especially sensitive to oxidation in alkali, yielding α-hydroperoxyketocholesterols or α-diketocholesterols. Thus, 4-cholesten-3-one, one of the compounds of interest in the present study, can be converted to 4-hydroxy-4,6-cholestadien-3-one and 4-cholestene-3,6-dione in alkali (2). Higley *et al.* (7) observed that 7-ketocholesterol was also degraded to a large extent by saponification and determined that the loss of this compound was 20%, even if a milder saponification was practiced. The saponification process requires many washing steps to remove large amounts of saponifiables, resulting in the loss of the non-saponifiable compounds present in minute amounts. Higley *et al.* (7) reported that only about 60% of the cholesterol oxidation products in meats were recovered after saponification. In our preliminary experiment, a mixture of 4-cholesten-3-one, 4,6-cholestadien-3-one and 4-cholestene-3,6-dione, at about 10 μg each, was subjected to saponification according to the method of Park and Addis (21). This was followed by TLC and GC as described above. Recoveries were less than 70%.

Prepacked SPE columns were used in the present study. The SPE glass columns used avoided sample contamination that resulted from use of plastic columns. Cholesterol A-ring derivatives (4-cholesten-3-one, 4,6-cholestadien-3-one and 4-cholestene-3,6-dione) are less polar than cholesterol, and they often elute from the SPE columns together with neutral lipids, which are abundant in meat. Co-elution was avoided by use of a low polarity solvent mixture (hexane:ethyl acetate 100:2). Large amounts of triglycerides were thus removed without loss of the A-ring derivatives. Subsequent TLC development with hexane:acetone (9:1) of fraction 2-2 well resolved the compounds between triglycerides and cholesterol. Fraction 3 after elution from the SPE column was considered clean, but to obtain a better base line on GC, TLC was also employed. The solvent mixture used for this separation was hexane:ethyl acetate (4:6), which replaced benzene:ethyl acetate (6:4) previously used in our laboratory (14). The hard analytical (250 μm) TLC plates used in the present study for preparative purposes increased recovery over the use of preparative (500 μm) plates used previously (14). For extraction of the cholesterol compounds from the scraped silica bands, acetone was superior to ethyl acetate.

The GC analysis method reported previously (14) was modified slightly. The temperatures of the on-column in-

jector and the column were lowered slightly to avoid potential degradation of some of the oxides. Because of the possible production of 6-ketocholestanol by irradiation (22), the internal standard for the GC analysis of epoxides and 7-ketocholesterol was replaced with desmosterol. 7-Ketocholestanol was used as the GC internal standard for analyzing the A-ring derivatives.

The recovery of the spiked compounds from 10 g of beef is shown in Table 1. The recovery was high, ranging from 78 to 88%, although the spiked levels of the compounds (about 1–2 ppm) were considerably below those of other studies, *e.g.*, 20–100 ppm (6). The internal standard in the present study was added just prior to GC analysis. In contrast, many reported results showed almost 100% recovery of the cholesterol oxidation products by basing calculations on the addition of internal standard to the starting materials and assuming that the standard was recovered 100%. It is concluded that the method developed in the present study is satisfactory for measuring the cholesterol oxidation products present in meat at low levels.

Cholesterol oxidation products in unirradiated and irradiated meats. As briefly mentioned earlier, the work previously performed in our laboratory revealed that the ratios of 7-ketocholesterol to α- and β-epoxides resulting from irradiation of cholesterol in aqueous sodium stearate dispersions were less than unity (3), whereas autoxidation, although at higher temperatures, gave ratios between six and ten (23). When cholesterol in liposomes was exposed to γ-radiation, the ratios of 7-ketocholesterol to total 5,6-epoxides were also less than one, much lower than the ratio of ten caused by autoxidation (4). The present study attempted to determine whether these unusual ratios are also applicable to actual meat samples.

A-ring oxidation products of cholesterol have not attracted much attention, although these compounds have been known to occur in mammalian tissue. Recently, it was found that these compounds are produced in significant quantities during the irradiation of cholesterol in liposomes (5), suggesting that these compounds could be the indicators of irradiation treatment of meats.

The data from the current study with meats are shown in Table 2. Most of the six cholesterol oxidation products of interest in the present study were detected in fresh beef, pork and veal purchased in a local store. Among these

TABLE 1

Recovery of Added Cholesterol Oxidation Products from Beef Muscle Tissue

	Spiked amount (μg/10 g)	Detected amount[a] (μg)		Recovery[a] (%)
		Control[b]	Spiked	
β-Epoxide	9.4	1.08 ± 0.14	8.69 ± 0.23	81.0 ± 1.0
α-Epoxide	13.4	0.47 ± 0.06	11.1 ± 0.45	79.4 ± 3.1
7-Ketocholesterol	12.2	1.75 ± 0.30	12.0 ± 0.41	84.4 ± 2.6
4-Cholesten-3-one	21.6	0.41 ± 0.03	19.6 ± 0.26	88.7 ± 1.3
4,6-Cholestadien-3-one	20.6	0.36 ± 0.06	18.6 ± 0.49	88.5 ± 2.2
4-Cholestene-3,6-dione	18.6	trace[c]	14.6 ± 0.51	78.3 ± 2.8

[a]Value ± SD; triplicate.
[b]Beef before addition of oxides.
[c]< 0.05 μg/10 g sample.

TABLE 2

Content of Cholesterol Oxidation Products in Unirradiated and Irradiated (10 kGy) Meats (trace: <0.05 μg/10 g sample)

Type	Value ± SD; triplicate μg/10 g sample					
	β-Epoxide	α-Epoxide	7-Ketocholesterol	4-Cholesten-3-one	4,6-Cholestadien-3-one	4-Cholestene-3,6-dione
Beef—0 d						
Unirradiated	1.30 ± 0.14	0.48 ± 0.04	2.13 ± 0.40	0.36 ± 0.01	0.19 ± 0.02[a]	0.09 ± 0.02
Irradiated	3.22 ± 0.42	1.15 ± 0.14	6.62 ± 0.88	0.67 ± 0.03	0.23 ± 0.03[a]	0.77 ± 0.04
Beef—2 wk						
Unirradiated	6.01 ± 0.61	1.82 ± 0.16	15.2 ± 1.55	1.69 ± 0.21	0.44 ± 0.05[b]	0.65 ± 0.11
Irradiated	16.6 ± 3.50	5.77 ± 1.29	44.3 ± 9.19	2.52 ± 0.19	0.43 ± 0.04[b]	1.34 ± 0.22
Pork—0 d						
Unirradiated	0.28 ± 0.05	0.19 ± 0.06[c]	0.26 ± 0.05	3.97 ± 0.73[d,e]	0.59 ± 0.07[d,e]	trace
Irradiated	0.86 ± 0.18	0.45 ± 0.15[c]	1.18 ± 0.24	3.84 ± 0.48[d,f]	0.61 ± 0.04[h]	trace
Pork—2 wk						
Unirradiated	0.60 ± 0.04	0.44 ± 0.02	0.51 ± 0.04	4.55 ± 0.73[e,g]	0.58 ± 0.09[i]	trace
Irradiated	4.76 ± 0.66	1.68 ± 0.28	10.8 ± 2.19	4.79 ± 0.57[f,g]	0.39 ± 0.03	trace
Veal—0 d						
Unirradiated	1.39 ± 0.17	0.66 ± 0.04[j]	2.28 ± 0.31[k]	0.32 ± 0.17	0.58 ± 0.08[l]	trace
Irradiated	2.73 ± 1.14	1.14 ± 0.16	4.44 ± 0.20	0.70 ± 0.08	0.57 ± 0.05[l,m]	0.27 ± 0.06
Veal—2 wk						
Unirradiated	2.39 ± 0.33	0.70 ± 0.12[j]	3.16 ± 0.70[k]	1.26 ± 0.23	0.77 ± 0.04[n]	trace
Irradiated	8.50 ± 0.36	3.04 ± 0.20	18.3 ± 0.85	2.89 ± 0.04	0.76 ± 0.11[m,n]	0.49 ± 0.09

[a–n]Same letters noting no significant difference with *t*-test ($\alpha = 0.05$) comparing the values among a single meat type of (0 day-unirradiated) *vs.* (0 day-irradiated); (0 day-unirradiated) *vs.* (2 week-unirradiated); (2 week-unirradiated) *vs.* (2 week-irradiated); and (0 day-irradiated) *vs.* (2 week-irradiated); no notation means significant difference.

compounds, 7-ketocholesterol and β-epoxide were the dominant cholesterol oxidation products in each type of fresh meat. α-Epoxide was detected below 1 μg in each of 10 g fresh meat. The pork contained much lower amounts of 7-ketocholesterol and epoxides than did the other meats. Each of the A-ring cholesterol derivatives was present at less than 1 μg/10 g fresh sample except for the pork, in which about 4 μg of 4-cholesten-3-one per 10 g was detected. Among the A-ring compounds, 4-cholesten-3-one was present in the largest and 4-cholestene-3,6-dione in the smallest amounts.

When the meats were exposed to γ-radiation (10 kGy) at 0–4°C, the cholesterol oxides generally increased. Beef showed the highest increases of these compounds: 7-ketocholesterol, β-epoxide and α-epoxide in beef increased by more than 3, 2 and 2 times, respectively. A-ring derivatives in beef also increased with the exception of 4,6-cholestadien-3-one. Cholesterol oxidation products in irradiated pork and veal were also increased over unirradiated, with the exception of 4,6-cholestadien-3-one for both meats and α-epoxide and 4-cholesten-3-one for pork.

Storage of unirradiated beef at 0–4°C for 2 wk raised the cholesterol oxide content considerably (Table 2). Unirradiated pork and veal during storage also showed an increase in most of the cholesterol oxidation products, although the increases were smaller than those of beef.

Irradiation of the meats followed by 2-wk storage at 0–4°C raised the cholesterol oxidation product level considerably over the unirradiated, stored samples with exception of 4,6-cholestadien-3-one.

In model systems, the cholesterol oxide distribution patterns produced by γ-radiation have been reported to be different from those generated by autoxidation (4,5). Similar differences were not observed between irradiated and unirradiated, fresh or stored meats. This dissimilarity of behavior of cholesterol in model membranes and in meat is probably attributable to the complexity of the meat system. The energy from the low dose γ-radiation might be distributed to other compounds besides cholesterol, such as proteins and other lipids in meats, resulting in a decreased effect on cholesterol, which is present in a relatively small amount compared to other components. Also, numerous cholesterol oxidation products are already present in different patterns in meats prior to irradiation, which may cause the resulting cholesterol oxide ratios to be different than those seen in irradiated model systems. Natural antioxidants present in meats might also participate in the oxidation mechanism, providing additional complex results.

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