Effects of Frying and Storage on Cholesterol Oxidation in Minced Meat Products[1]

B. Larkeson, P.C. Dutta*, and I. Hansson

Department of Food Science, Swedish University of Agricultural Sciences, Uppsala, Sweden

ABSTRACT: The presence of cholesterol oxidation products (COP) in the diet is a health concern for their various known adverse effects. It is important that the generation of COP be assessed during different stages of production, handling, and storage of meats and meat products so that relevant measures can be taken to minimize the production of COP. In a preliminary study, we investigated the content of COP in the lipids of raw meatballs (50% pork + 50% beef), prefried meatballs (50% pork + 50% beef), raw hamburger (100% beef), and prefried burger (50% pork + 50% beef). Six of the common COP, *viz.* 7α- and 7β-hydroxycholesterol, 7-ketocholesterol, 5α,6α-epoxycholestanol, 5β,6β-epoxycholestanol, and cholestanetriol, were analyzed by gas chromatography (GC) and GC–mass spectroscopy. The total content of these COP was in the range of 7 to 10 μg/g lipids in raw meatballs, prefried meat balls, and raw hamburger, after frying these samples for consumption. The prefried hamburger had *ca.* 8 μg/g lipids of the total COP before frying, and this amount increased to 29 μg/g lipids after frying. During the storage of this fried sample, the total COP increased to 42 and 50 μg/g lipids, after 1 and 2 wk of storage, respectively. The results of this study show that freshly prepared meat products are a minor source of COP in the diet. However, if semiprepared frozen meat products are fried once and then stored for future consumption, the levels of COP can increase considerably, and this may be of concern for certain groups of consumers.

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KEY WORDS: Cholesterol oxides, COP, frying lipids, meat products, storage.

Cholesterol (cholest-5-en-3β-ol), a 27-carbon steroid alcohol that is abundant in animal tissues, is readily susceptible to oxidation (1). More than 70 oxidation products of cholesterol (COP) have been identified so far (1–5); however, only eight of these compounds are generally reported in foods (6–8). These are 5-cholesten-3β,7α-diol (7α-OH), 5-cholesten-3β, 7β-diol (7β-OH), 5-cholesten-3β-ol-7-one (7-keto), 5-cholestan-5α,6α-epoxy-3β-ol (5α,6α-epoxy), 5-cholestan-5β,6β-epoxy-3β-ol (5β,6β-epoxy), 5-cholesten-3β,20α-diol (20α-OH), 5-cholesten-3β,25-diol (25-OH), and cholestane-3β,5α,6β-triol (cholestanetriol) (8). Some of these COP can be produced both enzymatically and by autoxidation (9,10).

The presence of COP in the diet is a health concern because of their various known adverse effects (4,5,9–13). Cholesterol is one of the lesser components in meat lipids. However, meat products can be one of the important sources of COP in the diet (6–8). The consequences of cholesterol oxidation on meat quality have been reviewed recently (14). The levels of COP in meat and meat products can be found in a recent review articles (6–8). However, published results of analyses of COP in meat and meat products may not be comparable due to variations in methodology (8). In a study on the development of COP in raw and cooked beef round steak (15), the contents of COP at day 0 were quite low in both raw and cooked samples of beef, 1.4 and 3.1 ppm, respectively. However, after storage of the meats at 4°C for 4 d, the levels increased considerably, particularly in cooked beef, where the levels increased from 3.1 to 17.3 ppm, in contrast to raw meat, in which the level content increased from 1.4 to 5.9 ppm in sample. The effects of cooking and storage on the production of COP recently were studied in buffalo meat and mutton (16,17), and cooking method was shown to influence the generation of COP. Also it was demonstrated that frozen storage of cooked meat did not prevent the development of COP in those meats (16,17). In a study on the effect of different cooking methods on lipid and protein components in hamburgers it was demonstrated that level of 7-ketocholesterol was in the range of 16 to 25 ppm in lipids, and the combination of roasting and microwave heating caused more oxidation than other treatments like roasting, barbecue, boiling, and pan-frying (18). The authors pointed out that storage conditions in the supermarket where refrigerated meat is subjected to continuous lighting can be a major factor in the development of lipid oxidation in raw ground meat (18). In a recent article (19), where four COP were determined, the levels were found to be very low or below detection level in 14 samples of Salame Milano, a typical Italian fermented pork product, (with a few exceptions), but the 11 samples of Mortadella, another typical Italian minced and cooked pork product, analyzed generally contained 2–6 ppm 5α,6α-epoxy in the samples; the other COP were below detection levels with a few exceptions (19).

The generation of COP in industrially prepared food prod-

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*To whom correspondence should be addressed at the Department of Food Science, SLU, Box 7051, SE-750 07 Uppsala, Sweden.
E-mail: Paresh.Dutta@lmv.slu.se

ucts is influenced by many factors, e.g., contact with air/oxygen, temperature treatment during processing, exposure to light, presence of metal ions, amount of antioxidants, techniques of packaging, and storage conditions (8,18,20). To minimize the production of the COP in foods, it is important that the generation of COP be assessed during different stages of production, handling, and storage. In a preliminary study, we investigated the content of COP in the lipids of raw meatballs, prefried meatballs, raw hamburger, and prefried hamburger. Also the effects of frying (cooking) and storage on the production of COP in the fried samples were investigated.

EXPERIMENTAL PROCEDURES

Samples. Raw and prefried meatballs (50% pork + 50% beef) were purchased from SCAN-FARMEK (Uppsala, Sweden). The shelf life of prefried meat balls in a gas-tight package is generally 25 d in supermarkets. Raw hamburger (100% beef) was purchased from Scanfood (Skara, Sweden), and prefried frozen burgers (50% pork + 50% beef) were purchased directly from a supermarket in Uppsala, Sweden. The shelf life of frozen burger in a supermarket is 1 yr at –18°C. The samples used in this experiment were within the recommended periods for consumption. The food samples were stored immediately at –80°C (0 time samples) and at –20°C until further analysis. The samples were thawed at 4°C overnight prior to extraction of 0 time samples and to frying in a frying pan without frying fat at *ca.* 150–160°C. After frying, the samples were divided into almost equal portions, and a portion of the sample was used for lipid analyses. Other portions were stored for up to 2 wk at 4°C for subsequent analysis.

Analytical. Moisture content was determined by drying the samples at 102°C until constant weight, following the AOAC method 950.46B (21). Extraction of lipids was done following the method of Hara and Radin (22) with slight modification. In brief, 20–25 g of each meat sample was homogenized 3 × 30 s, in a 250-mL Erlenmeyer flask with 200 mL of hexane/isopropanol, 3:2, vol/vol) using an Ultra-Turrax T25 homogenizer (Jankel & Kunkel GmbH, Staufen, Germany). The homogenates were filtered, and the filtrates were collected in a separatory funnel. The pooled filtrate was mixed with 85 mL of 6.67% anhydrous Na_2SO_4 in water, and the upper phase was collected and and dried with anhydrous sodium sulfate by filtering through a Whatman no. 2 filter paper (Maidstone, England). The filtrate was collected in a 250-mL volumetric flask and filled up with hexane. Lipid content of each solution was estimated by weighing on a Mettler UMT2 microbalance (Mettler-Toledo AG, Greifensee, Switzerland). All extracted samples were stored at –20°C until further analysis. Peroxide value (PV) was determined by International Dairy Federation standard method (23). Methods of analysis of fatty acid methyl esters from total lipids by capillary column gas chromatography (GC) is described elsewhere (24). Preparation of trimethylsilylether (TMS-ether) derivatives of cholesterol and cholesterol oxides and quantification of cholesterol in total lipids were done according to the method described previously (24). Standard samples of cholesterol oxides (COP) were purchased from Steraloids (Wilton, NH). All other chemicals and solvents were of analytical grade and were purchased from Merck (Darmstadt, Germany) unless otherwise stated.

Saponification for COP analysis. For COP, *ca.* 0.1–0.2 g of lipids were mixed well with 5 mL of 2 M potassium hydroxide in ethanol in a glass tube and left overnight (about 18 h) in the dark at room temperature (25). After addition of 10 mL of dichloromethane and 10 mL of water, the tube was shaken vigorously. The water phase was removed and the organic phase was washed once with 0.5 M KOH in water, and then repeatedly washed with 10 mL water until the solution became clear. The solvent was dried under nitrogen, and the unsaponifiables were dissolved in 1 mL hexane/diethyl ether (75:25, vol/vol). The COP fraction was enriched further by solid phase extraction (SPE) as described below.

Enrichment of COP from total unsaponifiables by SPE. The enrichment of COP was done following the method described previously (26) after slight modification. In brief, a 0.5-g silica cartridge (International Sorbent Technology Ltd., Mid Glamorgan, United Kingdom) was used for this purpose. The column was washed with 3 mL of hexane. The total unsaponifiables prepared earlier by cold saponification were dissolved in 1 mL hexane/diethyl ether (75:25, vol/vol), and were charged to the column. The tube was washed with an additional 2 mL hexane/diethyl ether (75:25, vol/vol), and the unsaponifiables were eluted through the column dropwise. Thereafter, the column was eluted with 3 mL hexane/diethyl ether (60:40, vol/vol), and the eluates were discarded. The COP and the remaining cholesterol were eluted with 4 mL acetone. The acetone was dried under nitrogen, and the residue was derivatized to TMS-ether derivatives as described below for subsequent analyses by GC and GC–mass spectrometry (MS).

Analysis of COP by GC. Quantification of COP as TMS-ether derivatives was carried out by GC using a Varian 3700 instrument (Palo Alto, CA) fitted with a falling needle injector and flame-ionization detector. A capillary column CP-Sil 8 CB-MS, 30 m × 0.25 mm, 0.50 μm film thickness (Chrompack International, Middelburg, The Netherlands) was used for the separation of the COP. The column temperature was 265°C, and detector temperature was 290°C. Helium was used as carrier gas at a pressure 17 psig and as make-up gas at a flow rate of 30 mL/min. An HP 3396 A integrator (Hewlett-Packard Company, Avondale, PA) was used to quantify the peak areas. Identification was carried out by using the TMS-ether derivatives of COP standards by GC and GC–MS as described below. Quantification was accomplished by using 19-hydroxycholesterol as internal standard. No detector response factors or factors to correct the recovery of COP during sample preparation were used to calculate the amounts of COP. Minimal level of quantification was 0.1 μg/g lipids. The mean values of duplicate analyses of the COP are presented in the results.

GC–MS. GC–MS analyses were performed on a HP5890

series II gas chromatograph (Hewlett-Packard) coupled to a TRIO-1000 mass spectrometer with an LAB-BASE™ data system, version R2.10 (Fisons Instruments, VG MASSLAB, Manchester, England). The TMS-ether derivatives of the COP were separated on a fused-silica capillary column DB-5MS 30 m × 0.25 mm × 0.50 μm (J&W Scientific, Folsom, CA), using helium as carrier gas at an inlet pressure of 15 psi. The injector temperature was 230°C, the samples were injected in a splitless mode, and purge delay time was 0.6 min. A temperature-programmed oven was set at 85°C for 1 min and then raised to 270°C at a rate of 25°C/min and then held at this temperature for 30 min before finally being raised to 290°C at 1°/min and held at this temperature for an additional 15 min. The full scan mass spectra were acquired at an electron energy of 70 eV, and the ion source temperature was at 200°C.

RESULTS AND DISCUSSION

Contents of moisture, lipids, and cholesterol, and PV, and composition of the major fatty acids at time 0 in various meat products are presented in Table 1. The content of lipids in the prefried meatballs (11.3%) was considerably higher compared with raw meatballs (6.6%). In contrast, raw meatballs contained more cholesterol than prefried meatballs (5895 vs. 2985 ppm). Both lipids (12.6%) and cholesterol (2269 ppm) contents were relatively lower in the prefried burger compared with the raw sample of hamburger (17% and 2711 ppm). The PV is quite low in all the samples, and no drastic increases of these values were observed after frying and after 2 wk of storage (results not shown here). The content of lipids in lean meats and the cholesterol content in the lipids of beef and pork were within the range of published reports (8,27). The major fatty acids were palmitic, stearic, and oleic. The percentages of different fatty acids also concur with those in published reports on beef and pork lipids (27). In the lipids of prefried burger, the content of linoleic acid was considerably higher (*ca.* 7%), compared with other samples ranging from 1 to 5% .

The levels of COP in the lipids of raw and prefried meatballs after frying and 1 or 2 wk of storage are presented in Tables 2 and 3. Six of the common polar COP generally encounterd in meats (8) are reported here. Raw meatballs had lower levels of COP at 0 time, after frying, and during storage, compared with prefried meatballs. The increase in the levels of COP during storage was more pronounced in the prefried meatballs; the level of COP increased from 5.5 μg/g lipids at day 0 to 16.1 μg/g lipids after 2 wk of storage. No literature data are available on such particular meat products, however, the levels can be compared with other meat products as discussed below.

The levels of COP in raw hamburger and prefried burger are presented in Tables 4 and 5. It should be reiterated that, in contrast to the meatball samples, these two samples were not from the same source (see Experimental Procedures section). Total COP in raw hamburger was 5.5, 6.7, 6.9, and 7.2 μg/g lipids at time 0, after frying, and after storage of 1 and 2 wk, respectively (Table 4). These levels and the distribution of COP are comparable to those in raw meat balls (Table 2) where epimers of 7-OH-cholesterol, 7-ketocholesterol, and epimers of epoxycholestanol dominated. Only small amounts of cholestanetriol were present after frying and storage (Table 4). No significant increase in COP was observed in this hamburger sample after frying and storage at 4°C up to 2 wk.

At time zero, the levels of COP in the prefried burger sample were higher compared with other samples (Table 5). There was a considerable increase of the COP in this sample throughout the experiment, from 8.4 μg/g lipids at time 0 to 29.4 μg/g lipids after frying. The levels rose to 41.9 and 49.5 μg/g lipids after 1 and 2 wk of storage, respectively. Published reports on the content of COP in hamburger are limited (8,18,28).

In a previous study, Higley *et al.* (28) reported levels of 7β-OH (5.6 ppm) and cholestanetriol (1298 ppm) in a sample of raw hamburger which was analyzed by high-performance liquid chromatography. This exceptionally high level of cholestanetriol, however, was not confirmed by MS. De Vore (29) determined 7-keto in both raw and cooked ground beef patties during storage for up to 4 d at 4°C. It was shown that the cooked sample had level of 7-keto that was almost 10 times higher at 2 d (27.8 μg/g cooked patties) and 4 d (48.5 μg/g cooked patties), compared with the raw counterpart which had 2.4 and 4.2 μg/g sample at 2 and 4 d of storage, respectively (29). In another study on raw beef and cooked beef round steak held at 4°C for 4 d, it was demonstrated that the levels of several COP increased after cooking and during storage for 4 d; the total COP of raw beef was 1.4 ppm at day 0 and this increased to 5.9 ppm after 4 d of storage at 4°C (15). In contrast, the cooked sample had 3.1 ppm COP at day 0, and

TABLE 1
Content of Moisture, Total Lipids, Cholesterol, and Peroxide Value (PV), and Composition of the Major Fatty Acids in the Time 0 Samples of Meat Products (means of duplicate analyses)[a]

Sample	Moisture (%)	Lipids (%)	Cholesterol (μg/g lipids)	PV (meq/kg lipids)	Fatty acids (%) 14:0	16:0	16:1	18:0	18:1	18:2	18:3	Others
RMB	61	6.6	5895	0.07	2.4	28.2	3.2	13.5	43.7	5.2	0.4	3.5
PMB	54	11.3	2895	0.13	2.9	30.2	3.4	12.5	42.7	4.6	0.3	3.4
RH	57	17.0	2710	0.04	3.6	28.4	4.8	13.3	40.5	1.0	0.2	8.2
PB	59	12.6	2270	0.13	2.6	28.8	2.9	13.3	42.1	6.7	0.5	3.4

[a]RMB, raw meaballs; PMB, prefried meatballs; RH, raw hamburger, PB, = prefried burger.

TABLE 2
Content of Cholesterol Oxides (μg/g lipids) in the Lipids of RMB at Time 0, After Frying for Consumption, and After Storage of 1 and 2 wk of the Fried Sample at 4°C (means of duplicate analyses)[a]

Sample	7α-OH	7β-OH	7-Keto	5α,6α-epoxy	5β,6β-epoxy	Cholestanetriol	Total
Time 0	0.9	0.8	0.8	0.2	0.6	Trace	3.3
Fried	2.8	3.6	1.0	1.4	1.6	Trace	10.4
1 wk	0.9	3.9	1.6	2.0	2.6	Trace	11.0
2 wk	1.9	4.3	1.8	0.9	1.5	0.4	10.8

[a]7α-OH, 5-cholesten-3β,7α-diol; 7β-OH, 5-cholesten-3β,7β-diol; 7-keto, 5-cholesten-3β-ol-7-one; 5α,6α-epoxy, 5-cholestan-5α,6α-epoxy-3β-ol; 5β,6β-epoxy, 5-cholestan-5β,6β-epoxy-3β-ol; cholestanetriol, cholestane 3β,5α,6β-triol. Time 0, samples before frying for consumption; trace, less than 0.1 μg/g lipids. For other abbreviation see Table 1.

TABLE 3
Content of Cholesterol Oxides (μg/g lipids) in the Lipids of PMB at Time 0, After Frying for Consumption, and After Storage of 1 and 2 wk of the Fried Sample at 4°C (means of duplicate analyses)[a]

Sample	7α-OH	7β-OH	7-Keto	5α,6α-epoxy	5β,6β-epoxy	Cholestanetriol	Total
Time 0	0.9	2.2	1.3	0.5	0.6	Trace	5.5
Fried	2.3	2.8	1.6	0.4	1.0	0.1	8.2
1 wk	1.8	5.0	2.9	1.9	2.6	0.1	14.3
2 wk	2.0	6.0	2.0	2.5	3.4	0.2	16.1

[a]For abbreviations, see Tables 1 and 2.

TABLE 4
Content of Cholesterol Oxides (μg/g lipids) in the Lipids of RH at Time 0, After Frying for Consumption, and After Storage of 1 and 2 wk of the Fried Sample at 4°C (means of duplicate analyses)[a]

Sample	7α-OH	7β-OH	7-Keto	5α,6α-epoxy	5β,6β-epoxy	Cholestanetriol	Total
Time 0	0.7	1.4	1.0	0.8	1.6	Trace	5.5
Fried	1.7	2.1	0.8	0.7	1.0	0.4	6.7
1 wk	0.7	2.5	1.4	0.8	0.9	0.6	6.9
2 wk	1.8	1.5	0.9	0.8	1.4	0.8	7.2

[a]For abbreviations, see Tables 1 and 2.

TABLE 5
Content of Cholesterol Oxides (μg/g lipids) in the Lipids of PB at Time 0, After Frying for Consumption, and After Storage of 1 and 2 wk of the Fried Sample at 4°C (means of duplicate analyses)[a]

Sample	7α-OH	7β-OH	7-Keto	5α,6α-epoxy	5β,6β-epoxy	Cholestanetriol	Total
Time 0	1.2	1.9	2.7	1.0	1.6	Trace	8.4
Fried	5.3	6.1	10.1	1.9	5.4	0.6	29.4
1 wk	8.3	9.4	11.6	2.4	9.8	0.4	41.9
2 wk	9.9	8.9	15.7	3.7	10.3	1.0	49.5

[a]For abbreviations, see Tables 1 and 2.

this level increased to 17.3 ppm after 4 d of storage at 4°C (15). In that study it was also demonstrated that cholesterol oxidation in the cooked veal could be controlled by feeding animals with vitamin E. Vitamin E-supplemented veal did not show increased level of COP after the cooked sample had been stored for 4 days at 4°C, in contrast to the unsupplemented veal (15). A similar result was demonstrated by supplementation of pig diets with α-tocopherol acetate, which prevented cholesterol oxidation in raw meat; but COP content increased during storage of cooked meat (30).

In a recent study (18), the level of 7-keto alone was investigated in hamburger prepared by different cooking methods. The range of 7-keto was from 16 to 25 ppm in the lipids after cooking by different cooking methods. No considerable differences were observed among different cooking methods. Rodriguez-Estrada *et al.* (18) mentioned that the raw sample was already in an advanced stage of oxidation, containing the highest level of 7-keto compared with samples cooked by various methods. Among the parameters mentioned that influence the quality and oxidative stability of meat are animal breed, age, type of feed, fat/muscle ratio, holding period, storage conditions, and packaging, in particular, the high surface/volume ratio of ground meat. In addition, refrigerated raw meat samples bought from a supermarket in that study were subjected to exposure to light, which might be the key factor in the development of cholesterol oxidation (18). The prefried burger studied in this experiment, however, had an additional hardpaper package to minimize direct exposure to

light during storage in the supermarket. Post-slaughter handling of meats and handling during preparation of the ground meat also contribute to the higher oxidation of meat samples (18,30). In addition, the considerably higher levels of COP in the sample of prefried burger can be related to the higher content of linoleic acid (18:2) compared with other samples, since degree of unsaturation of lipids influences cholesterol oxidation (31). Other factors, such as higher levels of metal ions, prooxidants, and low levels of antioxidants may have contributed to the observed higher COP content in the prefried burger sample.

COP have been shown to have a wide variety of biological effects *in vitro* and *in vivo*, and may contribute to human diseases and cholesterol metabolism (4,5,9–13). The only two known reports on the absorption of COP in human subjects have shown that COP from foods are absorbed within a few hours of consumption (32,33). A growing number of reports suggest that COP play an important role in the development of atherosclerosis in human (34–36). Low density lipoprotein is enriched in COP and lipid peroxides, part of which may be of dietary origin (34). Recently it was demonstrated that atherosclerotic lesions in rabbits fed 25 mg COP/d for 12 wk increased 100% compared with rabbits not fed the extra COP (35). According to Staprans *et al.* (35) dietary oxidized cholesterol may contribute to atherogenesis, and dietary modification that reduces the intake of dietary oxidized cholesterol may have a role in the prevention and treatment of atherosclerosis. Staprans *et al.* (35) cautioned that, with the popularity of fried foods and the widespread fast-food industry, oxidized fats including oxidized cholesterol are common in the Western diet and may contribute to heart disease.

The results of the present study show that freshly prepared and consumed fried meat products are not rich in COP. However, half-prepared (prefried) ground meat products purchased from supermarkets, when fried for consumption, can contain higher levels of COP. Thus, additional care is needed to minimize lipid oxidation in industrially prepared minced meat products from the slaughterhouse to supermarket level. Although the sensory quality still might be acceptable, prefried meat products stored for consumption at a later time can contain substantially higher levels of COP. Whereas this may not be considered as a significant health risk, some segment of the population in the Western society may be subjected to higher intakes of COP.

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