Cholesterol Oxidation in Meat Products during Cooking and Frozen Storage

Jae Eun Pie, Khira Spahis, and Christine Seillan*

Laboratoire de Nutrition et Sécurité Alimentaire, INRA–CRJ, 78352 Jouy-en-Josas Cédex, France

The effect of cooking time and method and freezer storage on the oxidation of cholesterol in muscle food such as beef, veal, and pork was studied by capillary gas chromatography. Cholesterol was oxidized in meat samples during household cooking, and the rate of oxidation differed according to the cooking time and cooking temperature. The range of total amount of oxysterols produced varied from 0.3 to 0.8% cholesterol. There was a greater increase in primary oxysterols (oxidized on carbon 7 or side chain) than in secondary derivatives (cholesterol epoxides and cholestanetriol) during cooking of fresh meat. All oxidized derivatives increased progressively in meat samples stored for 3 months at −20 °C in both raw and cooked meat. The sum of oxysterols doubled in the commercially available precooked frozen meals after 3 months at −20 °C with the secondary oxysterols showing the highest increase. We may assume from our observations that the daily dietary intake of oxysterols derived from meat source must probably include between 0.5 and 1% total cholesterol.

INTRODUCTION

Oxidation derivatives of cholesterol display a wide range of biological activities, such as angiotoxicity [Jacobson et al., 1985; Mattias et al., 1987; reviewed by Hubbard et al. (1989)] or mutagenic potency (Sevanian and Peterson, 1986). Oxysterol formation and presence in foodstuffs have raised concerns and interest (Smith, 1987; Maerker, 1987), and several analytical methods have been developed that allow proper quantification of these compounds (Park and Addis, 1985; Maerker et al., 1988).

Recent studies have demonstrated the presence of cholesterol oxidation derivatives in animal foodstuffs such as egg products (Missler et al., 1985; Morgan and Armstrong, 1987; Nourooz-Zadeh and Appelqvist, 1987), heated tallow (Bascoul et al., 1986; Park and Addis, 1986), dairy products (Nourooz-Zadeh and Appelqvist, 1988; Sander et al., 1989), and meat products (Higley et al., 1986; Park and Addis, 1987; De Vore, 1988). Nevertheless, assessing the overall level of oxidized cholesterol in the daily diet is still difficult, and the effects of food-processing conditions on cholesterol oxidation are not completely known.

As meat products represent an important source of dietary cholesterol, we studied the extent of cholesterol oxidation caused by two household cooking methods. We also studied the effect of storage at −20 °C on oxysterol formation: indeed, we have previously noticed that, after exposure to heat, cholesterol oxidation was more intense in butter stored at −20 °C for 3 or 6 months than in fresh butter (Pie et al., 1990).

The present paper describes the effects of cooking time and temperature and freezer storage on oxysterol formation in muscle food such as beef, veal, and pork.

MATERIALS AND METHODS

Reagents. Cholesterol, cholestanol, cholestanetriol, cholesterol α-epoxide, 20-hydroxycholesterol, 7-ketocholesterol, and 19-hydroxycholesterol were obtained from Sigma Chemical Co. (St. Louis, MO). Cholesterol β-epoxide came from Research Plus (Bayonne, NJ), and 25-hydroxycholesterol and 7α- and 7β-hydroxycholesterol were purchased from Steraloids Inc. (Wilton, NH).

All standards were found to be pure upon purchase, as determined by means of gas chromatography (GC) analysis. All authentic sterols were dissolved in absolute ethanol, stored at −20 °C, and prepared monthly. All solvents were of analytical grade.

Food Samples. All meats and frozen meals were purchased from a local supermarket with no knowledge of their previous history. All meat samples were ground thoroughly by using an electric blender before extraction.

The minced meats were fried individually in a preheated electric skillet at 135 °C for 3 or 10 min per side for beef and 10 min per side for veal and pork. The oven-cooked meats were placed on a cookie sheet and roasted in a preheated oven at 220 °C for 60, 80, and 90 min for beef, pork, and veal, respectively. The frozen meals were heated in boiling water according to the manufacturer's instructions.

Sample Preparation. Ten micrograms of 19-hydroxycholesterol and 500 μg of cholestanol were added to 1 g of meat samples and 5 g of frozen samples as internal standards for GC analysis. The total lipids were extracted by the procedure of Folch et al. (1957) using 20 mL of chloroform/methanol (2/1 v/v) for all meat samples and 100 mL for frozen meal samples, containing 0.002% butylated hydroxytoluene (BHT, Sigma). The extract was decanted and the process repeated twice. The organic layer was evaporated in a rotary evaporator and was then processed as described previously (Pie et al., 1990). Lipid extracts were saponified overnight at room temperature with 20 mL of 1 N KOH in methanol. The unsaponifiable fraction was spotted on a silica thin-layer chromatography (TLC) plate and developed with hexane/ether (70:30 v/v) to separate oxysterols from cholesterol. The extracts of scraped silica gel were derivatized as previously described (Pie et al., 1990).

Gas Chromatography. Cholesterol and oxysterols were quantified by GC analysis on a Girdel 30 gas chromatograph (Suresnes, France) with a flame ionization detector according to the method of Pie et al. (1990).

The GC conditions were as follows: a 30-m fused silica capillary DB5 column (J & W Scientific, Folsom, CA) with a film thickness of 1.0 μm; oven temperature, 280 and 270 °C for oxysterols and cholesterol, respectively; detector at 310 °C; injector at 300 °C; flow rate of helium, 1 mL/min; and pressure 12 psi.

Identification of Substances. Gas chromatography–mass spectrometry (GC–MS) was performed on a Dani 3800 HR-VG 70E-PDP8A digital mass spectrometer as previously described (Pie et al., 1990). The GC conditions were as follows: a CPsil 5CB column (25 m × 0.22 mm i.d., 0.12-μm film thickness; Chrompack, France) temperature programing from 30 to 140 °C in 1 min and to 280 °C at 10 °C/min rise; injector and interface temperature at 280 °C; and flow rate of helium, 1 mL/min.

* To whom correspondence should be addressed.

0021-8561/91/1439-0250$02.50/0 © 1991 American Chemical Society

Table I. Quantification of Oxysterols and Cholesterol in Raw and Cooked Minced Meats[a]

		cholesterol	7α-hydroxy-cholesterol	7β-hydroxy-cholesterol	cholesterol α-epoxide	cholesterol β-epoxide	7-keto-cholesterol	20-hydroxy-cholesterol	25-hydroxy-cholesterol	choles-tanetriol	total amount of oxysterol	total amount of oxysterol/choles-terol, %
beef	raw	0.66 ± 0.02	0.33 ± 0.02	0.34 ± 0.05	0.42 ± 0.01	1.06 ± 0.08	1.12 ± 0.04	0.18 ± 0.03	0.14 ± 0.03	ND	3.40 ± 0.29	0.51
	cooked 3 min)	0.83 ± 0.02	0.51 ± 0.08	0.58 ± 0.08	0.50 ± 0.04	1.29 ± 0.08	1.66 ± 0.05	0.30 ± 0.07	0.23 ± 0.06	ND	5.07 ± 0.32	0.61
	cooked (10 min)	1.00 ± 0.05	0.58 ± 0.06	0.67 ± 0.02	0.55 ± 0.03	1.31 ± 0.11	2.11 ± 0.08	0.30 ± 0.08	0.34 ± 0.05	ND	5.89 ± 0.05	0.59
veal	raw	0.82 ± 0.04	0.18 ± 0.04	0.21 ± 0.04	0.17 ± 0.02	0.47 ± 0.04	0.71 ± 0.05	0.04 ± 0.02	0.05 ± 0.01	ND	1.79 ± 0.12	0.22
	cooked	1.28 ± 0.01	0.61 ± 0.03	0.38 ± 0.04	0.33 ± 0.12	0.84 ± 0.08	1.70 ± 0.09	0.10 ± 0.01	0.19 ± 0.04	0.07 ± 0.01	4.20 ± 0.32	0.33
pork	raw	0.67 ± 0.01	0.19 ± 0.01	0.28 ± 0.01	0.22 ± 0.01	0.35 ± 0.02	0.92 ± 0.03	ND	0.13 ± 0.01	0.04 ± 0.01	2.24 ± 0.21	0.34
	cooked	0.99 ± 0.01	0.64 ± 0.01	0.85 ± 0.01	0.39 ± 0.01	1.01 ± 0.03	2.25 ± 0.20	0.14 ± 0.01	0.38 ± 0.01	0.06 ± 0.01	5.61 ± 0.49	0.57

[a] Results are expressed in milligrams per gram of sample for the cholesterol and in micrograms per gram of sample for the oxysterols. Mean ± SD (n = 3) ND, not detected.

Table II. Effect of Cooking on Oxysterol Concentrations in Minced Meats[a]

		C7	C20-25	epoxide
beef	raw	2.73 ± 0.22	0.45 ± 0.04	2.23 ± 0.13
	cooked	3.30 ± 0.18 (+21%)	0.63 ± 0.22 (+40%)	2.15 ± 0.06 (0%)
veal	raw	1.34 ± 0.18	0.11 ± 0.02	0.77 ± 0.03
	cooked	2.11 ± 0.12 (+57%)	0.23 ± 0.04 (+109%)	0.95 ± 0.19 (+23%)
pork	raw	2.08 ± 0.06	0.19 ± 0.01	0.91 ± 0.01
	cooked	3.77 ± 0.30 (+81%)	0.38 ± 0.01 (+100‰)	1.44 ± 0.08 (+58%)

[a] Results are expressed in parts per thousand of cholesterol. C_7, Sum of 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol. C20-25, sum of 20-hydroxycholesterol and 25-hydroxycholesterol. Epoxide, sum of cholesterol α-epoxide, cholesterol β-epoxide, and cholestanetriol. Numbers in parentheses indicate the percentage of increase observed in the cooked value as compared to the raw value (n = 3).

MS conditions were as follows: electron impact ionization at 70 eV; accelerating voltage, 6 kV; scan speed, 0.5 s/decade; and mass spectra scanning for mass/charge (m/z) 20–700.

Some oxysterols were present in amounts too low to be detected by GC–MS; to characterize these compounds, the following experiment was realized: meat samples were processed under the usual conditions until the TLC step. Silica gel containing the oxysterols was scraped and extracted. The extract was deposited again on a TLC plate and developed in 100 mL of hexane/diethylether/ethyl acetate (50:50:50 v/v/v). Bands that corresponded to migration areas of standards were scraped and extracted separately. The extracts were derivatized and analyzed under the usual GC conditions.

RESULTS AND DISCUSSION

Effect of Cooking on Cholesterol Oxidation in Fresh Meats. We first studied the oxidation of cholesterol during pan cooking of minced meats. The concentration of oxysterols and cholesterol per gram of raw and cooked meat is shown in Table I. The concentration of cholesterol was greater in cooked than in raw meat. The increase in cholesterol correlated well with the observed loss in weight due to water evaporation during cooking (data not shown). The concentration of oxysterols was also increased in cooked meats. However, the ratio of total oxysterols/cholesterol was 15–69% higher in cooked meat than in raw meat, suggesting that oxidation of cholesterol had occurred and that the increase in oxysterol concentration was not only the result of a change in total weight due to water loss.

Initially, oxysterols were found in high amounts of raw beef. This level was slightly affected during cooking. Veal and pork cholesterol were degraded significantly during

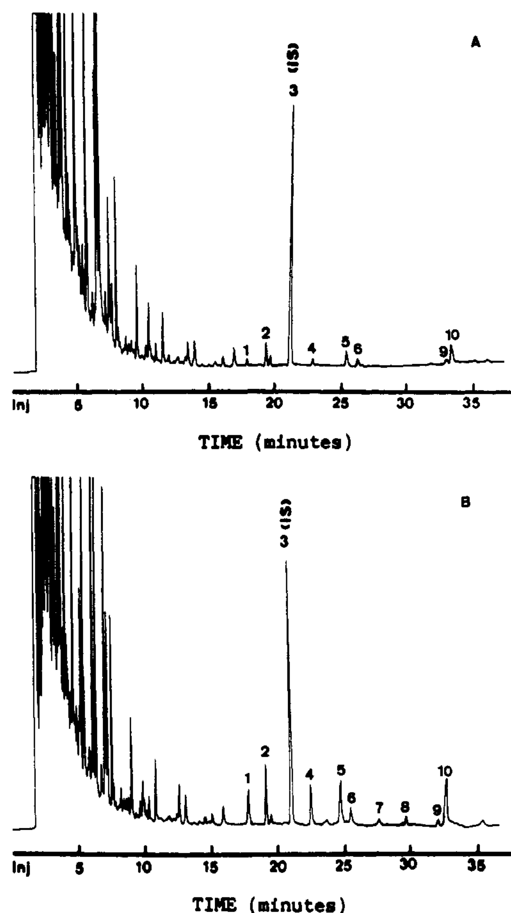

Figure 1. Gas–liquid chromatography profiles of raw veal (A) and cooked veal (B) at 220 °C for 90 min. Peaks as trimethylsilyl ether sterols: 1,7α-hydroxycholesterol; 2, cholesterol; 3, 19-hydroxycholesterol (IS); 4, 7β-hydroxycholesterol; 5, cholesterol β-epoxide; 6, cholesterol α-epoxide; 7, 20-hydroxycholesterol; 8, cholestanetriol; 9, 25-hydroxycholesterol; 10, 7-ketocholesterol. IS, internal standard.

heating, but in both cases the cooking time was longer. The ratios of specific oxysterols to cholesterol were compared before and after cooking (Table II). Primary oxysterols showed the largest increase, whether they were oxidized on the carbon 7 or on the side chain. However, the latter represented only a small proportion of the oxysterols detected. The secondary oxysterols (cholesterol epoxides and cholestanetriol) showed little or no increase, which might be explained by the short heating period.

We determined the oxidation of cholesterol in oven-cooked meats. Figure 1 shows the typical chromatographic profiles obtained for raw and cooked veal. Table III shows

Table III. Effect of Cooking on Oxysterol Formation in Oven-Cooked Meats[a]

		total oxysterol, μg/g	cholesterol, mg/g	total oxysterol/cholesterol, %	C7	C20–25	epoxide
beef	raw	1.26 ± 0.15	0.51 ± 0.01	0.25	1.32 ± 0.18	0.23 ± 0.02	1.10 ± 0.09
	cooked	4.44 ± 0.18	0.68 ± 0.03	0.65	3.21 ± 0.23 (+143%)	0.51 ± 0.05 (+121%)	2.90 ± 0.05 (+163%)
veal	raw	1.81 ± 0.09	0.73 ± 0.01	0.25	1.52 ± 0.11	0.08 ± 0.01	0.89 ± 0.02
	cooked	9.80 ± 0.42	1.27 ± 0.05	0.77	4.97 ± 0.28 (+227%)	0.23 ± 0.01 (+187%)	2.55 ± 0.12 (+186%)
pork	raw	1.74 ± 0.10	0.54 ± 0.01	0.32	0.65 ± 0.01	0.08 ± 0.02	2.48 ± 0.15
	cooked	7.33 ± 0.16	0.96 ± 0.02	0.76	3.08 ± 0.18 (+374%)	0.47 ± 0.09 (+487%)	4.20 ± 0.10 (+69%)

[a] Total oxysterols are determined by addition of each individual oxysterol measured by GC. The values obtained for total oxysterol and cholesterol are expressed per gram of sample. The oxysterol groups are the same as explained in Table II and are expressed in parts per thousand of cholesterol. Numbers in parentheses indicate the percentage of increase observed in the cooked value as compared to the raw value ($n = 3$).

Table IV. Quantification of Oxysterols and Cholesterol in Fresh and Frozen Minced Meats[a]

			total amount of oxysterol, μg/g	cholesterol, mg/g	total amount of oxysterol/cholesterol, %
beef	fresh	raw	3.40 ± 0.29	0.66 ± 0.02	0.51
		cooked	5.07 ± 0.28	0.83 ± 0.02	0.61
	stored at −20 °C for 3 months	raw	3.73 ± 0.32	0.62 ± 0.02	0.61
		cooked	5.96 ± 0.14*	0.79 ± 0.01	0.76
veal	fresh	raw	1.79 ± 0.12	0.82 ± 0.04	0.22
		cooked	4.20 ± 0.32	1.28 ± 0.01	0.33
	stored at −20 °C for 3 months	raw	2.21 ± 0.05**	0.81 ± 0.02	0.27
		cooked	8.00 ± 0.42**	1.29 ± 0.02	0.62
pork	fresh	raw	2.24 ± 0.21	0.67 ± 0.01	0.34
		cooked	5.61 ± 0.49	0.99 ± 0.01	0.57
	stored at −20 °C for 3 months	raw	3.32 ± 0.23**	0.62 ± 0.01	0.53
		cooked	6.79 ± 0.56**	0.92 ± 0.01	0.74

[a] Results are expressed per gram of sample for total oxysterols and cholesterol. Mean ± SD ($n = 3$). *, Significantly different from raw at $P < 0.05$. **, Significantly different from raw at $P < 0.01$.

the values obtained for total oxysterols and cholesterol per gram of sample. Once again, the ratio of total oxysterols/cholesterol was higher in cooked meat than in raw meat. The increase was much greater under these cooking conditions since the ratio of total oxysterols/cholesterol was 140–212% higher in cooked meat than in raw meat. The ratio of each oxysterol to cholesterol had greatly increased, although on average the secondary oxysterols showed less increase than oxysterols oxidized on carbon 7 or the side chain (Table III). Therefore, oven cooking led to significant cholesterol oxidation, while during pan frying, the oxidation was more moderate. Meat cholesterol oxidation had already been described by Park and Addis (1987). This study showed that the total concentration of sterols oxidized on carbon 7 represented 1.74% of the cholesterol in cooked meat samples previously stored at 4 °C for 1 week. 7-Ketocholesterol was the most abundant and represented half of the total oxysterols. De Vore (1988) measured only 7-ketocholesterol and also found important amounts of this derivative comprising up to 0.3% of cholesterol in cooked meats that had previously been stored at 4 °C.

The quantities we detected were on average much lower, since the total oxysterol concentration represented 0.2–0.5% of cholesterol in fresh, raw meat and 0.3–0.8% in cooked meat. This discrepancy might have been due to differences in the nature of samples or their treatment and also differences in the techniques used. Nevertheless, in spite of the lower amounts that we found, we assume that our assay gives a reliable indication of the oxidation of cholesterol, since, when the meats were subjected to more intensely oxidizing conditions, the method enabled us to detect higher oxysterol concentrations.

Effect of Storage at −20 °C. We studied changes in cholesterol and oxysterol concentration in meat samples stored for 3 months in a food freezer. Minced beef, veal,

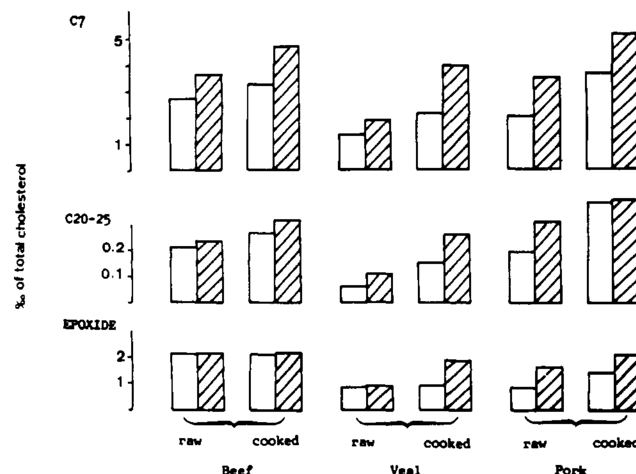

Figure 2. Changes in oxysterol groups as a function of cooking and storage conditions (for 3 months at −20 °C). Oxysterols are expressed in percent of cholesterol, and oxysterol groups are the same as explained in Table II. (Open bars) Fresh meat; (slashed bars) meat stored for 3 months at −20 °C.

and pork were prepared in the same way both before and after storage.

Table IV shows the total amount of oxysterols detected in raw frozen meat at −20 °C, which was slightly higher than that recorded in fresh raw meat. Thus, it appears that oxidation of cholesterol was not totally inhibited by storage at −20 °C. After cooking, the total amount of oxysterols in frozen meat was higher than that detected in fresh meat. Cholesterol values for each meat remained similar before and after freezing.

Figure 2 shows the changes in oxysterol groups as a function of cooking and storage conditions. All oxysterols increased progressively at the end of 3 months of storage at −20 °C, in both raw and cooked meat. These data are

Table V. Quantification of Oxysterols and Cholesterol in Commercially Available Frozen Meals[a]

		total amount of oxysterol, μg	cholesterol, mg	total amount of oxysterol/cholesterol, %
beef	fresh	170.87 ± 5.33	66.89 ± 1.19	0.26
	stored at -20 °C for 3 months	384.24 ± 20.25	66.06 ± 0.69	0.58
veal	fresh	250.87 ± 5.03	145.90 ± 6.55	0.17
	stored at -20 °C for 3 months	728.23 ± 22.70	117.40 ± 3.13	0.62
pork	fresh	190.83 ± 20.51	96.94 ± 2.93	0.20
	stored at -20 °C for 3 months	382.63 ± 5.83	83.16 ± 1.14	0.46

[a] Results are reported by total weight of each frozen meal for total oxysterols and cholesterol. Mean ± SD (n = 3).

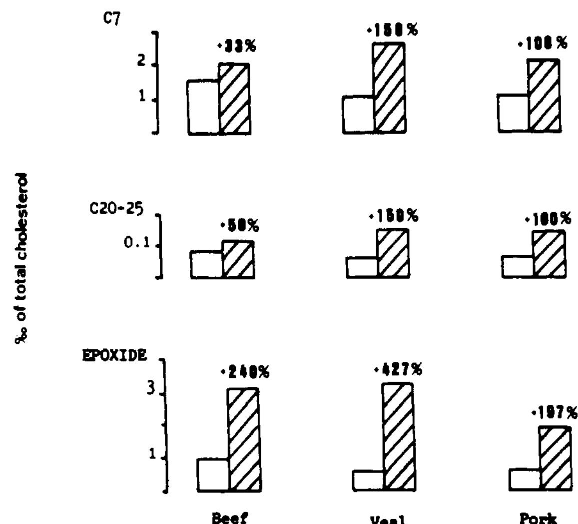

Figure 3. Comparison of quantities of oxysterols from commerically available frozen meals after 3 months at -20 °C. Oxysterols are expressed in percent of cholesterol, and oxysterol groups are the same as explained in Table II. Percentages express the increase noticed in the sample stored at -20 °C as compared to the fresh value. (Open bars) Fresh sample; (slashed bars) sample stored at -20 °C for 3 months.

consistent with our previous findings on butter stored at -20 °C (Pie et al., 1990): when samples of butter were stored at -20 °C for different periods of time, the amounts of each oxysterol increased with the length of storage. It therefore seems that the cholesterol of a frozen foodstuff is more susceptible to oxidation than the cholesterol in a fresh product.

Given these results, we studied the behavior of cholesterol during heating of commercially available precooked frozen meals. In fact, the meat in these foods undergoes initial cooking, is then frozen for variable periods, and is finally heated once again before consumption. We felt it would be interesting to compare the level of oxidation for a 3-month period of freezing.

We selected three commercially available meals which were heated under identical conditions in boiling water. Two determinations were made 3 months apart on the same batch of each meal. The results are given in Table V and Figure 3. The sum of oxysterols was double the original value after 3 months at -20 °C. The amounts of primary oxysterols had greatly increased (Figure 3), but in this case, the secondary derivatives showed the largest increase. Therefore, after a rather short period of storage, the oxidation was much more intense. It is noteworthy that these meals could have been stored for 14 months longer according to the manufacturer's expiration date.

Thus, storage at -20 °C led to a more intense oxidative degradation of cholesterol during heating and particularly in precooked frozen meals in which oxidation was well under way after initial cooking. These data are consistent with previous studies. Keller and Kinsella (1973) have shown that when meat samples were stored at -20 °C from 2 to 16 weeks and then cooked, malondialdehyde concentrations were consistently higher after frozen storage. A recent study has demonstrated that 2-thiobarbituric acid reactive substances (TBA-RS) were increased in raw chicken meat after storage at -20 °C (Pikul et al., 1989).

Increase of H_2O_2 generation after storage of muscle tissues at 4 °C has been demonstrated (Harel and Kanner, 1985a), and lipid peroxidation can result from this generation (Harel and Kanner, 1985b). Although low temperatures can slow down this reaction, Apgar and Hultin (1982) have shown that lipid peroxidation was not totally inhibited in fish muscle microsomes stored at -20 °C. Thus, lipid profile is altered during storage at -20 °C, which cannot fully block peroxidation processes. Similar findings have also been noted during the storage of biological samples like rabbit kidneys (Gower et al., 1989) or human extracellular fluids (Winyard et al., 1989). In the studies cited above, TBA-RS measurement has been used as an indicator of lipid peroxidation. From our results, we can conclude that cholesterol is also affected by these more intense oxidative conditions.

In light of the biological activities of oxysterols, these data raise concerns over the safety of freezer storage, particularly in the case of precooked meals. Indeed, Jacobson et al. (1985) have shown that trace amounts of cholestanetriol could lead to coronary stenosis in White Carneau pigeons. More recently, Kosykh et al. (1989) have shown that feeding rabbits with cholesterol containing 5% (w/w) oxysterols modified significantly very low density lipoprotein secretion by hepatocytes. Whether these experimental data can apply to humans is still questionable, but it is nevertheless important to determine which food-processing conditions can worsen cholesterol oxidation.

Identification of Substances. Some samples were processed and derivatized under the usual conditions and analyzed by mass spectrometry to confirm the presence of oxysterols. Mass spectra of the peaks observed in the samples were compared to mass spectra of trimethylsilyl derivatives of pure compounds. The presence of 7α- and 7β-hydroxycholesterol (m/z 351, 366, 456, 546), 7-ketocholesterol (m/z 367, 382, 457, 472), and cholesterol α- and β-epoxide (m/z 366, 384, 456, 474) could be demonstrated, but other oxysterols were present in amounts too low to be detected by this method. For these compounds, the samples were deposited on a TLC plate and developed as described under Methods. Figure 4 shows the migration profile of standards. Silica gel was scraped as explained in Figure 4, and the extracts were analyzed by GC under the usual conditions. Identifications of substances were deduced from the similarities of the R_f in the TLC step and the retention time in GC. This analysis enabled us to confirm the presence of 20-hydroxycholesterol, 25-hydroxycholesterol, and cholestanetriol in food samples.

Our analysis of meat samples showed that daily cooking conditions lead to cholesterol oxidation. The percentage

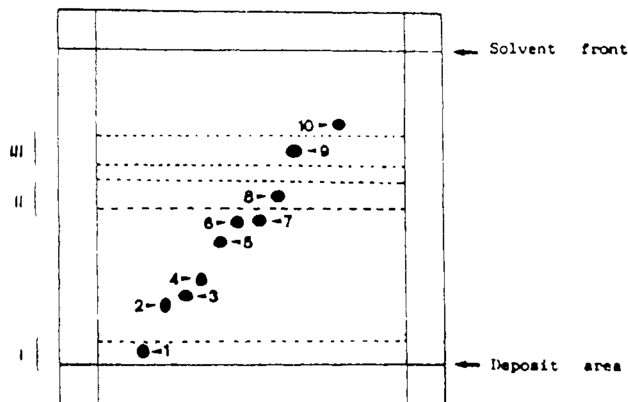

Figure 4. Migration profile of standards developed in 100 mL of hexane/diethyl ether/ethyl acetate (50:50:50 v/v/v). 1, Cholestanetriol; 2, 7α-hydroxycholesterol; 3, 19-hydroxycholesterol; 4, 7β-hydroxycholesterol; 5, 7-ketocholesterol; 6, cholesterol α-epoxide; 7, cholesterol β-epoxide; 8, 25-hydroxycholesterol; 9, 20-hydroxycholesterol; 10, cholesterol. For the food samples, three bands (I–III) of silica were scraped, extracted, derivatized, and analyzed by GC.

of oxysterols to cholesterol varied on average from 0.5 to 0.8%. As meat products represent an important source of dietary cholesterol and undergo intense heating conditions, we may thus deduce from this observation that 0.5–1% of the cholesterol consumed in the diet is in an oxidized form. Nevertheless, in light of our results obtained from frozen foods, it is likely that an increase in the consumption of industrial or frozen foods will increase the levels of oxidized cholesterol in the diet.

ABBREVIATIONS USED

Cholest-5-en-3β-ol, cholesterol; 5α-cholestan-3β-ol, cholestanol; cholest-5-ene-3β,7α-diol, 7α-hydroxycholesterol; cholest-5-ene-3β,7β-diol, 7β-hydroxycholesterol; cholest-5-ene-3β,19-diol, 19-hydroxycholesterol; cholest-5-ene-3β,20-diol, 20-hydroxycholesterol; cholest-5-ene-3β,25-diol, 25-hydroxycholesterol; cholestane-3β,5α,6β-triol, cholestanetriol; 5,6α-epoxy-5α-cholestan-3β-ol, cholesterol α-epoxide; 5,6β-epoxy-5α-cholestan-3β-ol, cholesterol β-epoxide; 3β-hydroxycholest-5-en-7-one, 7-ketocholesterol.

ACKNOWLEDGMENT

We are grateful to Dr. Gaudin (Centre d'Etudes Nucléaires, Saclay, France) for performing the GC–MS analysis and to Mrs. G. Cauneau for careful typing of the revised version of the manuscript.

LITERATURE CITED

Apgar, M. E.; Hultin, H. O. Lipid peroxidation in fish muscle microsomes in the frozen state. *Cryobiology* **1982**, *19*, 154–162.

Bascoul, J.; Domergue, N.; Olle, M.; Crastes de Paulet, A. Autoxidation of cholesterol in tallows heated under deep frying conditions: evaluation of oxysterols by GLC and TLC-FID. *Lipids* **1986**, *21*, 383–387.

De Vore, V. R. TBA values and 7-ketocholesterol in refigerated raw and cooked ground beef. *J. Food Sci.* **1988**, *53*, 1058–1061.

Folch, J.; Lees, M.; Sloan-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509.

Gower, J. D.; Healing, G.; Fuller, B. J.; Simpkin, S.; Green, C. J. Protection against oxidative damage in cold-stored rabbit kidneys by desferrioxamine and indomethacin. *Cryobiology* **1989**, *26*, 309–317.

Harel, S.; Kanner, J. Hydrogen peroxide generation in ground muscle tissues. *J. Agric. Food Chem.* **1985a**, *33*, 1186–1188.

Harel, S.; Kanner, J. Muscle membranal lipid peroxidation initiated by H_2O_2-activated met myoglobin. *J. Agric. Food. Chem.* **1985b**, *33*, 1188–1192.

Higley, N. A.; Taylor, S. L.; Herian, A. M.; Lee, K. Cholesterol oxides in processed meats. *Meat Sci.* **1986**, *16*, 175–188.

Hubbard, R. W.; Ono, Y.; Sanchez, A. Atherogenic effect of oxidized products of cholesterol. *Prog. Food Nutr. Sci.* **1989**, *13*, 17–44.

Jacobson, M. S.; Price, M. G.; Shamoo, A. E.; Heald, F. P. Atherogenesis in White carneau pigeons. Effects of low-level cholestan triol feeding. *Atherosclerosis* **1985**, *57*, 209–217.

Keller, J. D.; Kinsella, J. E. Phospholipid changes and lipid oxidation during cooking and frozen storage of raw ground beef. *J. Food Sci.* **1973**, *38*, 1200–1204.

Kosykh, V. A.; Lankin, V. Z.; Podrez, E. A.; Novikov, D. K.; Volgushev, S. A.; Victorov, A. V.; Repin, V. S.; Smirnov, V. N. Very low density lipoprotein secretion by cultured hepatocytes of rabbits fed purified or autoxidized cholesterol. *Lipids* **1989**, *24*, 109–115.

Maerker, G. Cholesterol autoxidation. Current status. *J. Am. Oil. Chem. Soc.* **1987**, *64*, 388–392.

Maerker, G.; Nungesser, E. H.; Zulak, I. M. HPLC separation and quantitation of cholesterol oxidation products with flame ionization detection. *J. Agric. Food Chem.* **1988**, *36*, 61–63.

Matthias, D.; Becker, C. H.; Gödicke, V.; Schmidt, R.; Ponsold, K. Action of cholestan 3β, 5α, 6β triol on rats with particular reference to the aorta. *Atherosclerosis* **1987**, *63*, 115–124.

Missler, S. R.; Wasilchuk, B. A.; Merritt, C. J. R. Separation and identification of cholesterol oxidation products in dried egg preparations. *J. Food Sci.* **1985**, *50*, 595–598.

Morgan, J. N.; Armstrong, D. J. Formation of cholesterol-5,6-epoxides during spray-drying of egg yolk. *J. Food Sci.* **1987**, *52*, 1224–1227.

Nourooz-Zadeh, J.; Appelqvist, L. A. Cholesterol oxides in swedish foods and food ingredients: fresh eggs and dehydrated egg products. *J. Food Sci.* **1987**, *52*, 57–62.

Nourooz-Zadeh, J.; Appelqvist, L. A. Cholesterol oxides in swedish foods and food ingredients: milk powder products. *J. Food Sci.* **1988**, *53*, 74–79.

Park, S. W.; Addis, P. B. Capillary column gas-liquid chromatographic resolution of oxidized cholesterol derivatives. *Anal. Biochem.* **1985**, *149*, 275–283.

Park, S. W.; Addis, P. B. Identification and quantitative estimation of oxidized cholesterol derivatives in health tallow. *J. Agric. Food Chem.* **1986**, *34*, 653–659.

Park, S. W.; Addis, P. B. Cholesterol oxidation products in some muscle foods. *J. Food Sci.* **1987**, *52*, 1500–1503.

Pie, J. E.; Spahis, K.; Seillan, C. Evaluation of oxidative degradation of cholesterol in food and food ingredients: identification and quantification of cholesterol oxides. *J. Agric. Food Chem.* **1990**, *38*, 973–979.

Pikul, J.; Leszczynski, D. E.; Kummerow, F. A. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *J. Agric. Food Chem.* **1989**, *37*, 1309–1313.

Sander, B. D.; Smith, D. E.; Addis, P. B.; Park, S. W. Effects of prolonged and adverse storage conditions on levels of cholesterol oxidation products in dairy products. *J. Food Sci.* **1989**, *54*, 874–879.

Sevenian, A.; Peterson, A. R. The cytotoxic and mutagenic properties of cholesterol oxidation products. *Food Chem. Toxicol.* **1986**, *24*, 1103–1110.

Smith, L. L. Cholesterol autoxidation, 1981–1986. *Chem. Phys. Lipids* **1987**, *44*, 87–125.

Winyard, P. G.; Arundell, L. A.; Blake, D. R. Lipoprotein oxidation and induction of ferroxidase activity in stored human extracellular fluids. *Free Radical Res. Commun.* **1989**, *5*, 227–235.

Received for review March 6, 1990. Revised manuscript received August 13, 1990. Accepted August 31, 1990.

Registry No. Cholesterol, 57-88-5; 7α-hydroxycholesterol, 566-26-7; 7β-hydroxycholesterol, 566-27-8; 7-ketocholesterol, 566-28-9; cholesterol α-epoxide, 1250-95-9; cholesterol β-epoxide, 4025-59-6; 20-hydroxycholesterol, 29913-75-5; 25-hydroxycholesterol, 2140-46-7; cholestanetriol, 72879-16-4.