Cholesterol Oxides in Swedish Food and Food Ingredients: Lard and Bacon

Jaffar Nourooz-Zadeh and **Lars-Åke Appelqvist**
Department of Food Hygiene, Swedish University of Agricultural Sciences, P.O. Box 7009, S-750 07 Uppsala, Sweden

Lard samples, manufactured from adipose tissue, were found to contain traces and/or quantifiable concentrations of certain cholesterol oxides, viz., 5α-, 6α-epoxycholestanol, 7-ketocholesterol, 7α-hydroxycholesterol, 20 α-hydroxycholesterol and 25-hydroxycholesterol at the detection limit 0.1 ppm. The influence of steam refining and chemical refining on the cholesterol oxide levels in a few of the crude lard samples was investigated. The analyses showed that these processes had no appreciable effect on the concentrations of cholesterol oxides, compared with the controls. Analysis of cold-stored consumer packages of lard (2-mo old up to 18-mo old samples) indicated that there were no differences in the pattern of cholesterol oxides. On the other hand, storage of refined superior and regular grade lard samples at 50°C for up to 18 days caused only a slight increase in the levels of the isomeric 5,6-epoxycholestanols. Raw bacon, bacon fried at 170°C for 10 and 20 min and bacon dripping were analyzed. The analyses showed that quantifiable levels of 5α-,6α-epoxycholestanol, C-7 oxidation derivatives of cholesterol and 25-hydroxycholesterol could be detected only in the fried rind. A substantial amount (about 0.5 ppm) of a cholesterol metabolite was detected in the lard samples. Its identification as 7α-hydroxycholest-4-en-3-one was based on the comparison of RRT value and mass spectral information, as TMS-ether, to that of synthetic 7α-hydroxycholest-4-en-3-one.

Cholesterol is an unstable compound that undergoes autoxidation in the presence of molecular oxygen and light, through a free-radical reaction predominantly at position C-7. Ultimately, an array of autoxidation products is formed. The full or partial structures of some 70-80 of the autoxidation products have been reported (1). Several of these compounds have been shown to be toxic both in vitro and in vivo (2-5). Generally, the undesirable biological effects of the cholesterol oxides lead to two classes of disorders, viz., atherosclerosis and cancer.

Other effects of the cholesterol oxides indirectly related to cytotoxicity are: (a) inhibition of 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase (6), a rate-limiting enzyme in cholesterol biosynthesis from other lipids. The resulting reduction of cellular cholesterol content causes defective membrane formation and membrane malfunction (3); (b) membrane alteration caused by the incorporation of the cholesterol oxides (instead of cholesterol) into the cellular membrane could, by virtue of their analogous structure, affect the membrane's barrier properties and lead to cell death (7,8). The dead cells could in turn be the primary area for lipid infiltration, leading to atherosclerosis; (c) exogenous cholesterol oxides are resorbed in mammals and transported by the very low and low density lipoproteins, VLDL and LDL, respectively. The VLDL is metabolized to LDL, which is then absorbed by the vascular endothelium, and the cholesterol oxides would be incorporated into the vascular tissue (9,10). Further, it has been demonstrated that the feeding of certain cholesterol oxides, viz., 25-hydroxycholesterol and cholestane-triol to rabbits causes injuries to the intima of the aorta and other arteries, as revealed by scanning electron microscopy (11,12).

Another possible deleterious effect is the risk associated with the intake of 5α-,6α-epoxycholestanol. The latter is the sole autoxidation product of cholesterol, among the oxides analyzed here, that is known to possess carcinogenic properties when administered subcutaneously in mice in the absence of oil vehicle (13). However, no information is available concerning the carcinogenicity of the 5β-,6β-epoxycholestanol isomer. The mutagenic properties of the isomeric 5,6-epoxycholestanols have been implicated in a few publications (14-16). A comprehensive review of the aforementioned undesirable deleterious biological effects of the cholesterol oxides has been published by Peng and Taylor (17).

Only one published work has dealt with the presence of cholesterol oxides in pork fat (18). Williams and Pearson isolated and tentatively identified 7-ketocholesterol from the unsaponifiable fraction of pork fat. There are no reports in the literature on the formation of cholesterol oxides during bacon frying.

The purpose of the present investigation was to obtain quantitative data on the concentrations of all biologically important cholesterol oxides in unrefined and refined lard, cold-stored consumer blocks of lard and refined lard stored at 50°C, and in raw bacon, bacon fried under controlled conditions and the drippings recovered from the frying experiments.

MATERIALS AND METHODS

Reagents. Commercially available cholesterol oxides were purchased from Sigma Chemical Co., St. Louis, Missouri or Steraloids Inc., Wilton, New Hampshire. The 5,6β-epoxy-5β-cholestan-3β-ol was a gift from Peter Eneroth, and the 7α-hydroxycholest-4-en-3-one was donated by Ingmar Björkhem, both of the Karolinska Institute at Huddinge Hospital, Stockholm, Sweden. PA grade solvents were purchased from E. Merck, Darmstadt, Federal Republic of Germany, and the 1,2-dichloroethane was obtained from Fischer Scientific, Fairlawn, New Jersey. Tri-Sil was purchased from Pierce Chemicals, Rockford, Illinois. Sep-Pak silica cartridges were obtained from Waters Associates, Milford, Massachusetts, and the Lipidex-5000 was purchased from Packard Instrument Inc., Downers Grove, Illinois.

Food samples. Lard was obtained immediately after manufacture from two plants: (1) Konvex, Klippan, Sweden, and (2) Andelsflott, Göteborg, Sweden. The bacon was supplied by Scan Co., Ängelholm, Sweden, from pigs slaughtered at Tomelilla abattoir; accurate background information regarding the feeding system used in the production of these samples had been kept. Consumer packages of lard were purchased from local groceries or supermarkets. All regular lard samples were

kept in a dark room at 4°C while raw bacon, fried bacon and the bacon's dripping samples were kept at -20°C until analysis.

Bacon frying. Temperature, frying time and use of a shallow frying pan with a thermostat were chosen to match as closely as possible ordinary bacon frying conditions in an everyday household kitchen. The temperature was set and the frying pan allowed to stand for about 10 min to achieve the desired temperature before starting the experiment. A consumer package of bacon was opened, and the rashers were placed in the frying pan. After the desired frying time, the fried bacon was covered with aluminum foil and placed in a plastic box. The dripping was transferred to a glass tube with Teflon cap. The frying pan was cleaned and allowed to stand for about 5 min before the next experiment. The frying experiment was performed at 170°C for 10 and 20 min in duplicate. The samples were kept in an icebox during transport and later stored at -20°C.

Preparation of triethylaminohydroxypropyl-Lipidex (TEAP-Lipidex). The TEAP-Lipidex is an anion exchange derivative of Lipidex-5000. It is used mainly to remove the phospholipids and free fatty acids from the bulk of cholesterol oxides. The gel was prepared from the reaction of Lipidex-5000 with triethylamine, essentially as described by Nourooz-Zadeh and Appelqvist (19). The ion exchange capacity of the gel was 0.7 meq/g.

Column packing. The procedures of column packing are described in detail in a previous paper (19).

Lipid extraction. (a) Bacon: Before weighing, the subcutaneous and intramuscular adipose tissues were removed from the lean and minced separately. A 30-g sample of the lean and a 10-g sample of the rind were put into separate centrifuge tubes, and 30 ml hexane/isopropanol (2:3, v/v) (HIP) was added. The samples were homogenized for 3 min using an Ultra Turrax homogenizer, centrifuged at 4000 rpm for 5 min and the supernatants transferred to separator funnels. The residue was homogenized with another 30 ml HIP and centrifuged at 4000 rpm for 5 min. The supernatants were pooled and 40 ml 0.47 M Na_2SO_4 were added. The mixture was agitated and allowed to settle. The upper layer was transferred to an evaporation flask and the solvent was removed in *vacuo* at 35°C. About 200 mg of the lipid extract was transferred to a glass-stoppered test tube and kept at 4°C until analysis. (b) Lard: The sample was melted on a water bath at 60°C and a 500-mg portion was transferred to a glass-stoppered test tube.

Analytical procedure. The cholesterol oxides were isolated using three concentration steps, essentially as described by Nourooz-Zadeh and Appelqvist (20). Briefly, the sample was dissolved in one ml hexane/1,2-dichloroethane (9:1, v/v) (H/DC) and applied on a prewashed Sep-Pak silica cartridge. The column was washed with 8 ml H/DC (9:1) to remove the bulk of the triacylglycerols (TG). The remaining TG together with polar lipids were eluted by washing the column with 12 ml methanol/1,2-dichloroethane (1:1, v/v). The solvent was removed in a rotary evaporator at 35°C and the residue dissolved in DC and transferred to a glass-stoppered test tube. The solvent was removed under a stream of nitrogen, after which the sample was redissolved in 0.3 ml H/DC (9:1) and applied onto the Lipidex-5000 column. The different lipid classes were eluted according to Nourooz-Zadeh and Appelqvist (19). After the cholesterol oxide fraction had been dried in *vacuo*, it was transferred to a glass-stoppered test tube and the solvent was removed under a stream of nitrogen. The sample was dissolved in 0.1 H/DC (3:7), applied onto the TEAP-Lipidex column, and the cholesterol oxide fractions were collected.

Thin layer chromatography. The cholesterol oxide fractions were applied as spots on a HPTLC plate (10 × 10 cm, 0.2 mm, UV-254, E. Merck, Darmstadt, F.G.R.) using an automated applicator, Linomat III. For reference purposes a mixture of cholesterol oxides was applied on one side of the plate and the plate was developed in diethyl ether/cyclohexane (9:1, v/v). The chromatoplate was dried at room temperature and viewed with UV light to locate the 7-ketocholesterol. The other cholesterol oxide spots were visualized by spraying the plate with 50% H_2SO_4, followed by heating at 110°C for about 3 min.

Saponification and derivatization. The cholestanetriol fraction was saponified according to Nourooz-Zadeh and Appelqvist (19) to remove the mono- and diacylglycerols. Regarding the other cholesterol oxide fractions, cholestane (about 0.2 μg) was added as an internal standard and the samples were transferred to glass-stoppered test tubes. The solvent was removed under a stream of nitrogen and 100 μl Tri-Sil were added. The samples were kept at 60°C for 30 min, the solvent removed under nitrogen and the residue dissolved in 100 μl hexane. One to two μl was injected into the GLC.

GLC and GC/MS. The TMS derivatives of the cholesterol oxides were separated on a crosslinked methyl silicone column (Ultra Hp, 25m × 0.33 mm, film thickness 0.35 μm, or a 25m × 0.25mm, film thickness 0.25μ Quadrex Corp.) using a Varian 3700 equipped with a falling-needle injector system and a flame ionization detector. Helium was used as carrier gas. Operating conditions were: column temperature 270°C, detector temperature 310°C. The peaks were recorded using a Hp 3390 A Integrator. The cholesterol oxides were tentatively identified by comparing the RRT values of the components in the TEAP-Lipidex fractions with those of the eight synthetic cholesterol oxides analyzed in the present study. The quantification was based on peak area measurement, and the data are mean values of duplicate analyses (Sep-Pak silica, Lipidex-5000, TEAP-Lipidex and GLC).

The assignment of the TMS derivatives was confirmed using a 5970 A HP mass selective detector equipped with capillary direct interface or a Finnigan 4000 GC-MS MAT. The GC-MS instruments were equipped with the falling-needle injector system and a chemically bonded methyl silicone column (25 m × 0.2 mm, film thickness 0.25 μm, Quadrex Corp.). The operating conditions were essentially those described previously by Nourooz-Zadeh and Appelqvist (19,20).

RESULTS

Although in Sweden lard is extracted from bones and adipose tissues, only the latter was analyzed in the present study. Lard is manufactured from adipose tissue as follows. The tissues are minced and then heated to 95°C. The mixture is transferred to a decantation centrifuge and the protein-rich tissue material is removed. The emulsion is transferred to a separator, water is removed and the lard is placed in a storage tank.

TABLE 1

Content of Cholestrol Oxides in Lard

Company[a]	Lot	Quality	Cholesterol oxides[b], ppm				
			5α-,6α-epoxy	7-keto	7α-hydroxy	20α-hydroxy	25-hydroxy
A	1	Unrefined	0.3	TR[c]	TR	ND[d]	ND
A	1	Refined	0.3	TR	TR	ND	ND
A	2	Unrefined	ND	0.2	TR	TR	TR
A	2	Refined	ND	0.2	TR	TR	TR
B	3	Unrefined	TR	TR	ND	0.3	TR
B	3	Refined and deodorized[e]	TR	TR	TR	0.2	ND
B	3	Refined and deodorized[f]	TR	TR	TR	TR	ND
B	4	Unrefined	ND	0.2	TR	TR	TR
B	5	Unrefined	ND	0.3	TR	0.3	0.2
B	6	Unrefined	ND	0.3	TR	0.3	0.2
B	7	Unrefined	ND	0.3	TR	TR	TR

[a]A, The Andelsflott plant, Göteborg, Sweden; B, The Konvex plant, Kävlinge, Sweden.
[b]The 5β-,6β-epoxycholestanol, 7β-hydroxycholesterol and the cholestane-triol were not detected, detection limit 0.1 ppm.
[c]Traces (<0.1 ppm).
[d]Not detected.
[e]Refined, bleached with Tonsil LFF80 1% and deodorized in a pilot plant at the Margarine Company, Helsingborg, Sweden.
[f]Refined, bleached with Tonsil LFF80 1% and deodorized in a pilot plant at the Karlshamns AB, Karlshamn, Sweden.

TABLE 2

Content of Cholesterol in Consumer Packages of Lard[a]

Age	Cholesterol oxides[b,c], ppm				
mo	5α-,6α-epoxy	7-keto	7α-hydroxy	20α-hydroxy	25-hydroxy
2	0.4	0.3	0.2	0.3	0.2
6	0.2	0.3	TR[d]	0.5	0.2
6	0.2	TR	TR	0.2	TR
18	0.2	0.3	0.3	0.4	TR

[a]A, The lard samples were manufactured at the Andelsflott plant, Göteborg, Sweden.
[b]The 5β-,6β-epoxycholestanol, 7β-hydroxycholesterol were not detected, detection limit 0.1 ppm.
[c]Cholestane-triol was not analyzed because of the low level of the 5β-,6b-epoxycholestanol.
[d]Traces (<0.1 ppm).

In the present study, three series of lard samples were investigated regarding cholesterol oxide content. In the first series, the effects of physical refining (steam refining) and chemical refining (neutralization, bleaching and deodorization) on cholesterol oxide concentration were studied. Seven unrefined lard samples (Table 1, lots 1-7) were obtained from the two above-mentioned plants (see Materials and Methods).

The analytical results presented in Table 1 showed that the unrefined lard samples from Company A (lots 1 and 2) contained traces and/or quantifiable concentrations of some of the predominant oxidation products of cholesterol, viz, 5α-,6α-epoxycholestanol, 7-ketocholesterol and 7α-hydroxycholesterol, at the detection limit 0.1 ppm. Obviously, lot 1 had a slightly higher level of the 5α-,6α-epoxycholestanol than lot 2. Analysis of the two steam-refined lard samples (lots 1 and 2) indicated that this processing technique does not affect the concentrations of cholesterol oxides. Analysis of the unrefined lard samples from Company B (lots 3-7) showed that these samples had detectable levels of the above-mentioned cholesterol oxides. In some cases, side-chain derivatives of cholesterol, viz, 20α-hydroxycholesterol and 25-hydroxycholesterol were observed. Lot 3 was chemically refined under conditions similar to those used in margarine manufacturing, as follows. The lard was refined, bleached with Tonsil LFF80 1% and deodorized at the Margarine Company, Helsingborg, and at Karlshamns AB. Analysis of the chemically refined lard sample (lot 3) showed that no remarkable change had occurred in the concentration of the cholesterol oxides compared to the control. The GC analysis of the lard samples in Table 1 indicated the presence of an unknown compound at the same elution region as the 5α-,6α-epoxycholestanol peak.

In the second series, four consumer packages of lard (250 g) were investigated. According to the label, their age varied from 2 to 18 mo. The analytical results (Table 2) showed that the samples contained quantifiable levels of

TABLE 3

Peroxide Value (PV), Anisidine Value (AV) and Cholesterol Oxides in Chemically Refined Lard Stored at 50°C

Quality	Storage time (days)	PV[a]	AV[b]	Cholesterol oxides[c], ppm				
				5α-,6α-epoxy	5β-,6β-epoxy	7-keto	7α-hydroxy	7β-hydroxy
Superior	0	0.0	0.1	0.2	ND[d]	0.2	TR[e]	ND
Superior	8	2.3	0.6	0.2	ND	TR	TR	ND
Superior	14	4.1	1.5	0.5	MS+[f]	TR	TR	ND
Superior	18	6.1	2.1	NQ[g]	MS+	ND	TR	ND
Regular	0	0.0	0.6	0.2	ND	ND	TR	ND
Regular	8	2.6	1.4	TR	ND	ND	TR	ND
Regular	14	5.5	2.5	0.3	MS+	TR	TR	ND
Regular	18	6.6	3.2	NQ	MS+	ND	TR	ND

[a]According to *Standard Methods for the Analysis of Oil, Fats and Derivatives*, 17th edn., edited by C. Paquot and A. Hautfenne, Blackwell Sci. Pub., Oxford, 1987, IUPAC Method 2:501.
[b]According to *Ibid.*, IUPAC Method 2:504.
[c]The 20α-hydroxycholesterol, 25α-hydroxycholesterol and cholestane-triol were not detected, detection limit 0.1 ppm.
[d]Not detected.
[e]Traces (<0.1 ppm).
[f]Only detected by GC-MS analysis as the 5β-,6β-epoxy peak was completely overlapped by an unknown compound.
[g]Not quantifiable because of increased amounts of interfering compound or compounds.

some of the cholesterol oxides. No obvious difference in the levels of cholesterol oxides were observed, despite the cold storage for up to 18 mo. The lard packages represented different batches, and the variation in the individual levels and/or total cholesterol oxide content could reflect different initial cholesterol oxide levels rather than the progress of cholesterol autoxidation during storage. The aforementioned "unknown" compound was also observed in the chromatograms of the samples presented in Table 2. The statement on "absence" of the 5β-,6β-epoxycholestanol in series 1 and 2 is based on combined GC-MS analysis. In the third series, crude lard classified as "superior grade" and "regular grade," on the basis of peroxide value and anisidine value, was obtained from Ellco Food (Klippan, Sweden) immediately after manufacture. Lard (a 5-kg sample) was refined in a pilot plant under conditions similar to those used during margarine manufacturing at Karlshamns AB (Karlshamn, Sweden) as follows. The lard was bleached with Tonsil LFF80 1% and deodorized. The purpose of this experiment was to study the influence of lard quality on the formation of the primary and secondary oxidation products of the triacylglycerol fatty acids (peroxide value and anisidine value) and on the levels of cholesterol oxides during storage at 50°C for up to 18 days.

Thirty-gram samples of the refined lard were transferred to 50-ml plastic vials and kept at 50°C for up to 18 days, in order to obtain a high peroxide value (PV = 6). The peroxide value (PV) and anisidine value (AV) at various storage times are presented in Table 3.

Analysis of the controls, a superior quality and a regular quality lard stored at -70°C indicated that the samples contained detectable levels of 7-ketocholesterol and 5α-, 6α-epoxycholestanol and 7α-hydroxycholesterol (Table 3). After a storage period of eight days, the lard samples reached PV 2.3 and 2.6 for superior and regular quality, respectively. The eight-day-old samples showed no remarkable differences in the concentrations of cholesterol oxides, compared with the controls. After storage of the samples for 14 days at 50°C (PV 4.1 and 5.5), only a slight increase in the concentration of 5α-,6α-epoxycholestanol was observed, compared with the eight-day-old samples. Further storage for 18 days caused no significant change in the pattern of cholesterol oxides. No quantitative data are reported concerning the level of 5α-,6α-epoxycholestanol in the 18-day-old samples, as the 5α-,6α-epoxycholestanol peak was partially overlapped by an unknown small peak. However, the presence of 5α-,6α-epoxycholestanol in the 18-day-old sample was confirmed by combined GC-MS. Furthermore, the GC-MS analysis indicated that the 14- and 18-day-old samples also had detectable levels of the 5β-,6β-epoxycholestanol, but no quantitative data can be presented. The reason was that the 5β-,6β-epoxycholestanol peak was completely overlapped by an unknown compound (the structure elucidation will be discussed later). The spectra of the isomeric 5, 6-epoxycholestanols were identical to those reported by Nourooz-Zadeh and Appelqvist (19).

Raw lean and raw rind samples of specially produced bacon contained no detectable levels of any of the eight oxidation products of cholesterol at the detection limit 0.1 ppm in the lipids (Table 4). Analysis of the rind taken after frying bacon for 10 min at 170°C showed that there were quantifiable concentrations of the 5α-,6α-epoxycholestanol, 7-ketocholesterol, 7α-hydroxycholesterol and 25-hydroxycholesterol at the above mentioned detection limit. The concentration of the cholesterol oxides was slightly increased when the frying time was extended to 20 min. On the other hand, no detectable levels of any of the oxides were noticed in the lean or drippings collected from the frying experiments. The cholestane-triol fractions were not analyzed because of the low concentration of the 5α-,6α-epoxycholestanol in these samples.

Adipose tissue contains 70% more cholesterol than lean does on a weight basis (21). Most of the tissue lipids, mainly triacylglycerols, are eliminated during frying. The absence of cholesterol oxides in the dripping may be a reflection of its low cholesterol content. However, the lev-

TABLE 4

Content of Cholesterol Oxides in Raw and Fried Bacon

Sample	Cholesterol oxides[a,b]				
	5α-,6α-epoxy	7-keto	7α-hydroxy	7β-hydroxy	25-hydroxy
Raw rind	ND[c]	ND	ND	ND	ND
Raw lean	ND	ND	ND	ND	ND
Rind fried 10 min at 170°C	0.2	ND	0.2	0.2	ND
Lean fried 20 min at 170°C	ND	ND	ND	ND	ND
Recovered fat	ND	ND	ND	ND	ND
Rind fried 10 min at 200°C	0.2	0.2	0.3	0.3	0.5
Lean fried 20 min at 200°C	ND	ND	ND	ND	ND
Recovered fat	ND	ND	ND	ND	ND

[a]The 5β-,6β-epoxycholestanol, 20α-hydroxycholesterol and the cholestane-triol were not detected, detection limit 0.1 ppm in the lipids.
[b]Cholestane-triol was not analyzed because of the low level of the 5α-,6α-epoxycholestanol.
[c]Not detected.

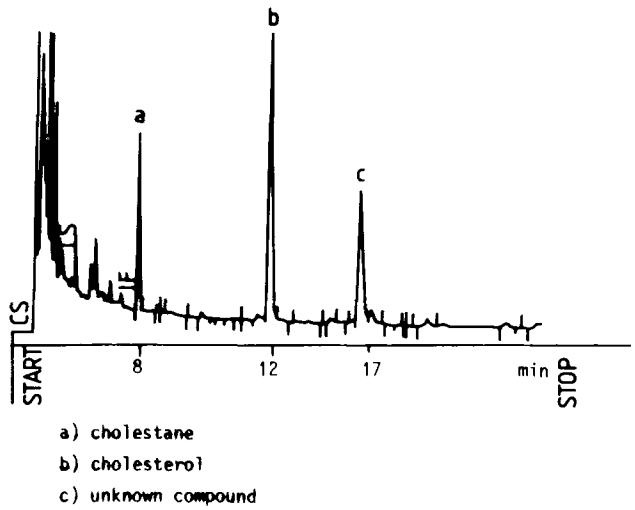

FIG. 1. Gas chromatogram of TMS derivatives of cholesterol oxides in TEAP-fraction number 2 Nourooz-Zadeh and Appelquist (19) from lard. Peak a, cholestane; peak b, cholesterol; peak c, unknown compound.

els of the cholesterol oxides—if these are generated during frying—are probably below the detection limit in the present study, 0.1 ppm in the lipids.

The unknown peak marked (c) in Figure 1 was a constant feature of the chromatograms of all lard samples. The compound was eluted just before the 5β-,6β-epoxycholestanol peak and at the same RRT value as the TMS-ether of 5β-,6β-epoxycholestanol. The mass spectrum of the interfering compound revealed the presence of several ions which were characteristic of some of the oxidation derivatives of cholesterol (Fig. 2a). The peak at m/e 472 in the mass spectrum suggests a compound with the molecular weight 472. This is equivalent to the elemental composition $C_{27}H_{47}O_2$. The peak at m/e 382 (M-90) indicated the presence of one hydroxyl group, while the second oxygen must be presented in a carbonyl moiety. Furthermore, the mass spectrum of the unknown compound contained several molecule fragments which are characteristic of 3β-hydroxycholest-5-en-7-one (22), but it was less polar than the latter. The aforementioned mass spectrometric information, together with the elution order, suggests that the carbonyl moiety is present at position C-3 of the tetracyclic steroidal structure. Hence, the mass spectrum of the unknown compound was mostly like that of the TMS-ether derivative of 7α-hydroxycholest-4-en-3-one, reported by Aringer and Nordström (23). A comparison of the higher portion of the mass spectrum in Figure 2b with that of the TMS derivative of a synthetic 7α-hydroxycholest-4-en-3-one showed that they were identical. Thus, the mass spectrometric data, together with the excellent agreement in the RRT values, confirm that the compound is 7α-hydroxycholest-4-en-3-one.

DISCUSSION

The analytical results in Tables 1 and 2 and the figures concerning the controls on Table 3 showed the presence of certain cholesterol oxides which, presumably, are of endogenous origin. In the present study considerable attention was devoted to finding the suspected carcinogenic compound 5α-,6α-epoxycholestanol in some of the crude lard samples. Because lard constitutes the bulk of the lipids in infant formula, it is of great importance to minimize the level of the biologically active cholesterol oxides in lard. The finding of 5α-,6α-epoxycholestanol cannot be accounted for, but it may be due to different animal feeding systems, breed and/or physiological conditions of the pigs at slaughter.

In this study, an effort was made to investigate the possible connection between the animal feeding systems and the detection of 5α-,6α-epoxycholestanol. In Sweden, pigs are generally fed a fishmeal-supplemented diet up to a short period before slaughter. Therefore, the fatty acid patterns of lots 1 and 3 (Table 1), and of the controls (Table 3) were analyzed. Attention was directed to detecting the long-chain fatty acids C22:5 and C22:6 because they reflect the presence of fishmeal in the diet. Capillary GLC analysis showed that the three samples

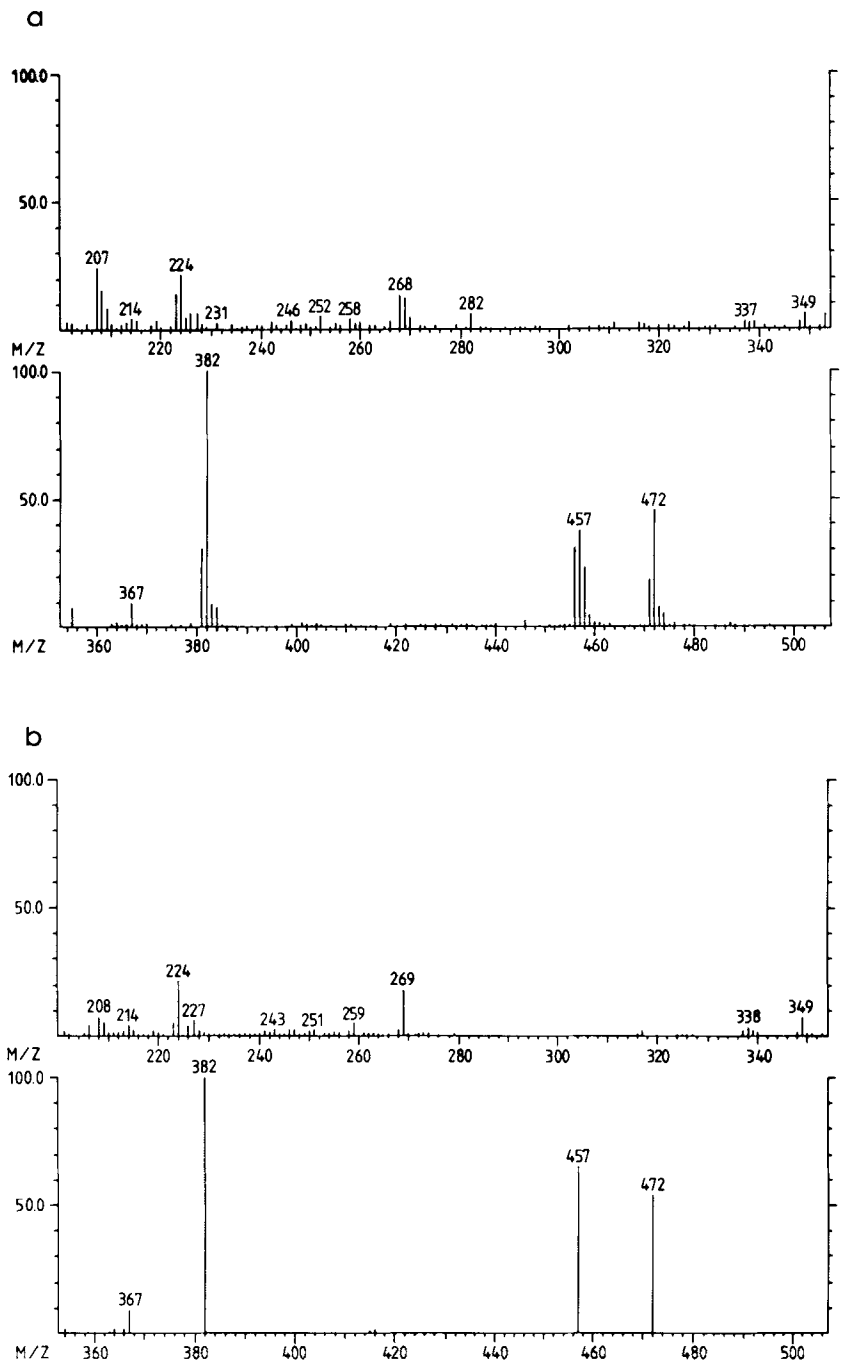

FIG. 2. Mass spectra of TMS derivatives of (a) unknown compound isolated from lard; (b) synthetic 7α-hydroxycholest-4-en-3-one.

containing 5α-,6α-epoxycholestanol also had about 0.1 and 0.3% of C22:5 and C22:6, respectively. Hence, it is conceivable that even a small intake of fishmeal rich in polyunsaturated fatty acids could enhance in vivo cholesterol peroxidation. Whether collection, transport and processing of the adipose tissue also have an effect on the concentration of cholesterol oxides in lard remains to be established.

Quite substantial amounts (about 0.5 ppm) of 7α-hydroxycholest-4-en-3-one were detected in all the lard samples analyzed. The 7α-hydroxycholest-4-en-3-one, a minor product of the autoxidation of cholesterol (1), is also formed via an enzymatic pathway during the degradation of cholesterol to bile acids (24). The present study is the first to report the presence of this cholesterol metabolite in porcine adipose tissues. Further studies are required to elucidate the accumulation of 7α-hydroxycholest-4-en-3-one in fat depots in other animals and, in particular, the extent of accumulation in adipose tissue of pigs of various ages and physiological conditions.

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REFERENCES

1. Smith, L.L., in *Cholesterol Autoxidation*, pp 13-47 Plenum Press, N.Y., 1981.
2. Peng, S.K., C.B. Taylor, P. Tham and B. Mikkelson, *Arch. Path. Lab. Med. 102:*57 (1978).
3. Taylor, C.B., S.K. Peng, N.T. Werthessen, P. Tham and K.T. Lee, *Am. J. Clin. Nutr. 32:*1033 (1979).
4. Baranowski, A., C.W.M. Adams, O.B. Bayliss High and D.B. Bowyer, *Atherosclerosis 41:*225 (1982).
5. Matthias, D., C.H. Becker, W. Gödicke, R. Schmidt and K. Ponsold, *Ibid. 63:*115 (1987).
6. Kandutsch, A.A., pp 589-597 in *Autoxidation in Food and Biological Systems*, edited by M.G. Simic and M. Karel, Plenum Press, London, U.K., 1980.
7. Herian, A.M., M.K. Noreen and L. Ken, *J. Food Prot. 48:*1050 (1985).
8. Kummerow, F.A., *Annals N.Y. Acad. Sci. 414:*29 (1983).
9. Peng, S.K., C.B. Taylor, E.H. Mosbach, W.Y. Haung, J. Hill and B. Mikkelson, *Atherosclerosis 41:*395 (1982).
10. Peng, S.K., G.A. Phillips, G.Z. Xia and R.J. Morin, *Ibid. 64:*1 (1987).
11. Peng, S.K., C.B. Taylor, J.C. Hill and R.J. Morin, *Ibid. 54:*121 (1985).
12. Jacobson, M.S., M.G. Price, A.E. Shamoo and F.P. Heald, *Ibid. 57:*209 (1985).
13. Bischoff, F., *Adv. Lipid Res. 7:*165 (1969).
14. Sevanian, A., and A.R. Peterson, *Proc. Natl. Acad. Sci. USA 81:*4198 (1984).
15. Sevanian, A., and A.R. Peterson, *Food Chem. Toxic. 24:*1103 (1986).
16. Raaphorst, G.P., E.J. Azzam, R. Langolis and J.E. Van Lier, *Biochem. Pharmac. 36:*2369 (1987).
17. Peng, S.K. and C.B. Taylor, *World Rev. Nutr. Diet. 44:*117 (1984).
18. Williams, L.D., and A.M. Pearson, *J. Agric. Food Chem. 13:* (1965).
19. Nourooz-Zadeh, J., and L.Å. Appelqvist, *J. Food Sci. 52:*57 (1987).
20. Nourooz-Zadeh, J., and L.Å. Appelqvist, *Ibid. 53:*74 (1988).
21. Hood, R.L., *CSIRO Food Res. 47:*44 (1987).
22. Park, S.W., and P.B. Addis, *Anal. Biochem. 149:*275 (1985).
23. Aringer, L., and L. Nordström, *Biochem. Mass Spect. 8:*183 (1981).
24. Björkhem, I., *Euro. J. Biochem. 8:*337 (1969).

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