The in vivo measures of protein quality indicated that storage had no significant effect on soy-based I formula. The in vitro measures indicated smaller changes in DBL and browning activity than were seen with the milk-based formula. Several factors may have contributed to this finding: (1) the storage conditions chosen did not give a maximum reaction; (2) corn syrup solids served as the carbohydrate source instead of the more reactive simple sugars; (3) the methods used were not sensitive enough to detect any difference.

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# Identification and Quantitative Estimation of Oxidized Cholesterol Derivatives in Heated Tallow

S. Won Park[1] and Paul B. Addis*

Oxidized cholesterol derivatives (OCDs) in tallow were isolated by saponification overnight at room temperature and quantified by a newly developed capillary gas chromatography method that includes trimethylsilylation. 7-Ketocholesterol and cholesterol $\alpha$-epoxide added to tallow survived saponification as well as the other OCDs tested, displaying recoveries of at least 95%. Continuous heating of tallow at 155 °C resulted in the formation of at least four OCDs as detected by capillary GC. OCDs were identified by capillary GC–mass spectrometry as 7$\alpha$-hydroxy-, 7$\beta$-hydroxy-, and 7-ketocholesterol and $\alpha$-epoxide. 7-Ketocholesterol was the predominant species formed. Its net formation was proportional to heating time, reaching up to ca. 10% of the initial content of cholesterol (in unheated tallow) after 376 h of heating. Capillary GC–MS also revealed the apparent formation of cholesterol $\beta$-epoxide in heated tallow.

Deep fat frying is a common method of food preparation in restaurants and in processing plants. During frying, the heating medium may experience abusive conditions due to repeated exposure to oxygen at elevated temperatures. The changes occurring in heated oils have been studied quite thoroughly with respect to fatty acids. The thermal oxidation and polymerization of fatty acids, including toxicological implications and loss of nutritional value have been reported (Kaunitz, 1967; Perkins, 1967; Chang et al., 1978; Thompson and Aust, 1983). However, concerning the changes induced in sterols, minor components of frying oils, only limited studies have been made (Larsen and Morris, 1943; Ryan et al., 1981).

In light of the potential deleterious impact on human health of cholesterol oxides, including interruption of sterol metabolism, cytotoxicity, atherogenicity, mutagenicity, and carcinogenicity (Bischoff, 1969; Black and Douglas, 1972; Kandutsch et al., 1978; Peng et al., 1978, 1979, 1982; Smith et al., 1979; Imai et al., 1976, 1980; Chan and Chan, 1980; Ansari et al., 1982; Addis et al., 1983), there is an urgent need to investigate thermally induced changes in sterols of fats and oils.

The present study was initiated to investigate whether cholesterol in tallow will undergo oxidation when it is heated at elevated temperatures similar to deep fat frying and specifically concerned the disappearance of cholesterol and appearance of oxidized cholesterol derivatives (OCDs). Findings were confirmed by combined capillary GC–mass

Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108.
[1] Present address: Food Protein R&D Center, Texas A&M University, College Station, TX 77843.

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spectrometry (MS). In order to quantify the amount of OCDs formed and remaining cholesterol in the heated tallow, the recoveries of OCDs and cholesterol were also studied.

## MATERIALS AND METHODS

**Reagents.** The source, purity, and systematic and trivial names of the authentic sterols used in this study were given elsewhere (Park and Addis, 1985a). All standard sterols were dissolved into ethyl acetate and kept at −20 °C.

Ethyl acetate was HPLC grade (J. T. Baker, Phillipsburg, NJ). Triolein, linolenic acid, and Sylon BTZ were from Supelco, Inc. (Bellefonte, PA). All other reagents were ACS grade: diethyl ether (Columbus Chemical Industries, Inc., Paris, KY), potassium hydroxide (MCB Manufacturing Chemists, Inc., Cincinnati, OH), and methanol and anhydrous sodium sulfate (Spectrum Chemical Manufacturing Corp., Gardena, CA).

**Heating of Tallow.** Refined edible beef fat was obtained from a commercial supplier in the form of deodorized tallow. Solid tallow was melted in a beaker by heating it in a drying oven set at 65 ± 2 °C. After mixing well to ensure homogeneity, about 10 mL of melted tallow was transferred into test tubes (20 × 150 mm).

Heating of tallow was conducted continuously at 190 ± 2.5 °C in a Hi-Temp bath (Model 160, Fisher Scientific Co., Pittsburgh, PA) for up to 250 h and at 155 °C with a Sunbeam Crocker cooker fryer (Sunbeam Appliance Co., Chicago, IL) for up to 400 h. Mineral oil from Fisher Scientific Co. was used as a heating medium. Temperature was monitored by placing a thermocouple into a tube containing tallow. Three test tubes were removed from each oil bath daily. After cooling, test tubes were screw-capped tightly and kept at −20 °C until analyzed.

**Cold Saponification and Extraction.** Cholesterol and its oxidized derivatives were extracted from saponified tallow. After adding 40 μg of 5α-cholestane as an internal standard to 0.20 ± 0.003 g of tallow in a test tube (20 × 150 mm), tallow was melted into 5 mL of methanol by heating and shaking in a hot water bath. After cooling but before solidification of the dispersed tallow particles out of methanol, 5 mL of 2 N KOH in methanol was added and the mixture was shaken vigorously until it became free of dispersed fat particles. Saponification was conducted at room temperature overnight according to Chicoye et al. (1968) and Smith et al. (1981). A 10-mL portion of distilled water was added to the saponified mixture, which was transferred to another test tube (25 × 200 mm) with a Teflon-lined screw cap. Nonsaponifiables were extracted three times, each time with 10 mL of diethyl ether. Pooled diethyl ether extracts were washed twice with 5 mL of 0.5 N KOH and three times with 5 mL of distilled water, followed by drying over anhydrous sodium sulfate. The dried extracts were filtered with Whatman No. 1 filter paper, which was reextracted with another 10 mL of diethyl ether and filtered again. The combined filtrates were concentrated to ca. 1 mL in a rotary vacuum evaporator (Buchi Rotavapor R., Switzerland) and freed of solvent on a nitrogen flash evaporator (Organomation Associates, South Berlin, MA) after befing transferred to a small vial.

**Quantification of Sterols by Capillary GC.** The conditions for GC resolution of common major OCDs were reported elsewhere (Park and Addis, 1985a). Briefly, a Varian Vista 6000 GC equiped with flame ionization detector was interfaced to a Spectra-Physics SP4100 computing integrator. A fused silica capillary column DB-1 (15 m × 0.25 mm i.d. × 0.1 μm thick; J & W Scientific, Inc., Rancho Cordova, CA) was used. Temperature was programmed from 180 to 250 °C at a 3°/min rise. The recorder chart speed was 0.5 cm/min. The dried ether extracts of nonsaponifiables were redissolved into 100 μL of pyridine and mixed well with 50 μL of Sylon BTZ for derivatization of sterols into corresponding trimethylsilyl ether sterols. The reaction was run at room temperature for at least 30 min. About 0.8 μL of reaction mixture was directly injected into the capillary column at a split ratio of 100:1.

**Recovery of Sterols.** Recovery studies on sterols, through the saponification and extraction procedures described above, were performed separately for OCDs and cholesterol as follows. For OCDs, a mixture of OCDs (20 μg each) with 40 μg of 5α-cholestane was added to 0.2 g of unheated tallow, all of which went through the entire procedure described earlier; for cholesterol, a mixture of 40 μg of 5α-cholestane with either 150 or 300 μg of cholesterol was added to a mixture of ca. 150 mg of triolein with ca. 50 mg of linolenic acid that substituted for the unheated tallow.

In order to assess the effect of the soaps formed in the saponified media on the recoveries of sterols, the above recovery experiments were repeated without incorporating unheated tallow for OCDs and the triolein/linolenic acid mixture for cholesterol.

Another aliquot of both mixtures used for the recovery study of OCDs and cholesterol was used to calibrate the instrument for calculating the amount of sterols recovered. To estimate the actual amount of each sterol recovered, the same experiment was repeated by adding 5α-cholestane just prior to capillary GC analysis, by which the possible loss of sterols during the workup was not accounted for.

**Gas Chromatography–Mass Spectrometry.** A Hewlett-Packard 5790A GC equipped with 5970A Mass Selective Detector was employed. GC–MS analyses were performed on the same capillary column used for quantification of sterols according to the method of Park and Addis (1985a) with modifications: splitless injection; ion source, 200 °C; electron multiplier, 2200 V; carrier, helium at 4 psi. With a Hewlett-Packard 9825A data processing system and a 9876A printer, mass spectra of trimethylsilyl ether sterols scanned within mass range $m/e$ 100–670 were recorded. Background subtraction and renormalization to the most intense peak were also executed.

## RESULTS

**Recovery and Precision.** The reliability of quantitative data on OCDs and cholesterol prepared through cold saponification and liquid extraction of the nonsaponifiables was checked by studies on the recovery of each OCD and cholesterol (Table I). The recovery studies for cholesterol were conducted without any OCDs in the mixture, because saponification is often criticized as the cause of artifactual formation of oxidation products (Smith, 1981). Linolenic acid was incorporated in the workup mixture because it was postulated that a polyunsaturated fatty acid (PUFA) free radical, if formed during cholesterol isolation, could render cholesterol a free radical by abstracting a hydrogen. However, no detectable peaks were observed through saponification without refluxing for the cholesterol oxidation products of interest in this study; the only sterol peak observed corresponded to cholesterol. This result together with many negative findings of any OCD in many food samples (to be published later) implies that no detectable amount of artifactual oxidation products is produced during the sample preparation procedures employed in this study for GC analysis. The same result also obviated the necessity of adding antioxidants to samples before cold

**Table I. Percentage Recoveries of Cholesterol and Its Oxides, Comparing Recoveries Both with and without Tallow and Correcting for Losses of Sterols by Internal Standard**

| | cholesterol and its oxides | | | | | | |
|---|---|---|---|---|---|---|---|
| conditions | cholesterol[a] | 7α-hydroxy-cholesterol | cholesterol α-epoxide | 7β-hydroxy-cholesterol | cholestanetriol | 7-keto-cholesterol | 25-hydroxy-cholesterol |
| IS init[b] | | | | | | | |
| without tallow | 100.5[d] | 95.4 | 102.5 | 98.8 | 97.3 | 96.7 | 97.0 |
| | 0.6[e] | 1.9 | 0.6 | 1.0 | 1.4 | 0.6 | 0.7 |
| with tallow | 99.0 | 96.9 | 101.2 | 99.5 | 84.7 | 96.4 | 95.3 |
| | 0.5 | 5.7 | 1.6 | 0.3 | 0.7 | 0.9 | 0.9 |
| IS added at GC[c] | | | | | | | |
| without tallow | 85.5 | 94.3 | 87.5 | 88.4 | 85.6 | 85.8 | 91.7 |
| | 6.0 | 2.1 | 4.6 | 4.7 | 3.7 | 3.8 | 6.2 |
| with tallow | 81.5 | 81.7 | 84.4 | 87.7 | 72.3 | 79.4 | 84.4 |
| | 1.4 | 5.9 | 1.6 | 2.1 | 1.3 | 1.9 | 1.8 |

[a] From a mixture of triolein and linolenic acid. [b] Internal standard (IS) added at the initial step in the purification procedure. [c] Internal standard added immediately prior to GC. [d,e] Mean ± SD ($n$ = 3).

saponification, or of handling samples under a nitrogen stream.

Table I also reveals the actual amount of sterols recovered through the sample preparation procedures used in this study. The actual amount recovered ultimately in ether extract was approximately 80–85% for cholesterol and 80–90% for OCDs, depending on the presence of soap formed in the saponification mixture. Cholestanetriol was the only one that showed a significant decrease in recovery when tallow was present in the mixture to be saponified. The reduction of extraction efficiency due to soap formation would be expected to be greater in cholestanetriol than in the other relatively less polar sterols. Considering that the ultimate amount of sterols recovered is usually no less than 80%, the sample preparation through the procedures described in this study can be regarded as very acceptable.

When an internal standard was used from the beginning of sample preparation, the recovery was close to 100% without regard to the presence of soap formed in the saponified mixture, except for cholestanetriol. The recovery of cholestanetriol was reduced to about 85% when it was extracted from tallow, compared with about 97% recovery when there was no tallow in the mixture to be saponified. Since an internal standard was added from the very beginning of sample preparation in the following analysis, no correction was made for the quantitative values of OCDs obtained from GC throughout the studies, except for cholestanetriol whose quantified value was to be corrected for its 85% recovery, if any, in the heated tallow samples.

The standard deviation in recovery data of each sterol was less than 2% variation with the exception of 7α-hydroxycholesterol, which suffered from as much as 6% variation when recovered from a spiked tallow sample. This was due to the emergence of a small peak between cholesterol and 7α-hydroxycholesterol, whose base line was fused more with 7α-hydroxycholesterol, interrupting the precise integration of peak area. In terms of precision, the procedures employed herein for quantification can be regarded as very reproducible when the internal standard is used from the beginning.

**OCDs in Heated Tallow.** The content of cholesterol in unheated tallow samples was 705 ± 12 ppm ($n$ = 6). This value was much lower than the 1400 ppm reported by Ryan and Gray (1984). The cholesterol content in tallow is considered to vary, depending on the source of beef fat from which commercial products are made. The cholesterol content of tallow heated at 155 °C seemed to be unchanged during the early period of heating (Figure 1). This may indicate the induction period expected for

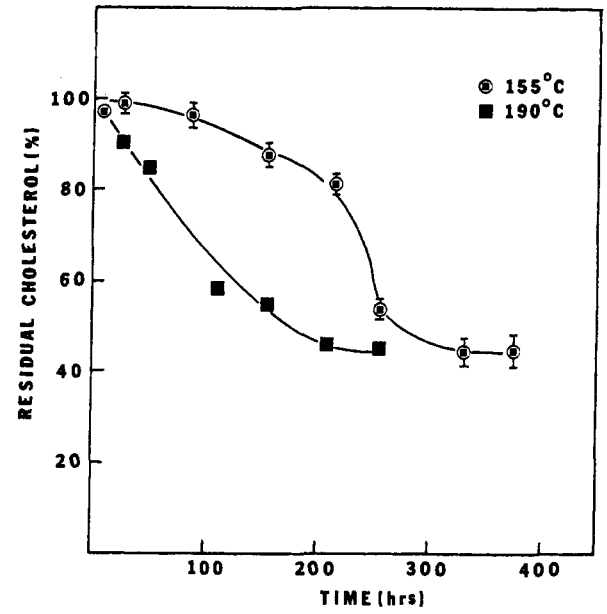



**Figure 1.** Changes in cholesterol content of tallow heated at 155 and 190 °C, respectively. Each point at 155 °C is the mean of three samples ($n$ = 3); each point at 190 °C is a single determination. Initial content of cholesterol at 0 h was 705 ± 12 ppm ($n$ = 6).

autoxidation reactions of cholesterol, similar to that of PUFA. However, this delayed period was not observed at 190 °C where the disappearance of cholesterol proceeded faster than at 155 °C. The loss of cholesterol seemed to arrive at a final state after a certain period of heating. After about 300 h at 155 °C and 200 h at 190 °C, cholesterol content seemed to be suspended at about 40–45% of initial content.

Prolonged heating of tallow at 155 °C produced four peaks whose retention indices were corresponding to those of 7α-hydroxycholesterol, α-epoxide, 7β-hydroxycholesterol, and 7-ketocholesterol, respectively (all were trimethylsilyl ether sterols). The same four peaks for the following were detected at the same retention sites throughout the heating. A chromatogram is illustrated in Figure 2 with the tallow heated for 376 h. Two samples of tallow heated at 155 °C for 156 and 376 h were subjected to mass spectrometric analysis for further identification of four of the peaks that emerged in the steroid region (a, c, d, and e in Figure 2). Mass spectra of those four peaks turned out to be identical with those of authentic 7α-hydroxycholesterol, α-epoxide, and 7β-hydroxy- and 7-ketocholesterol, respectively, as the corresponding mono- and bis(trimethylsilyl) ether sterols (Figure 3; mass spec-

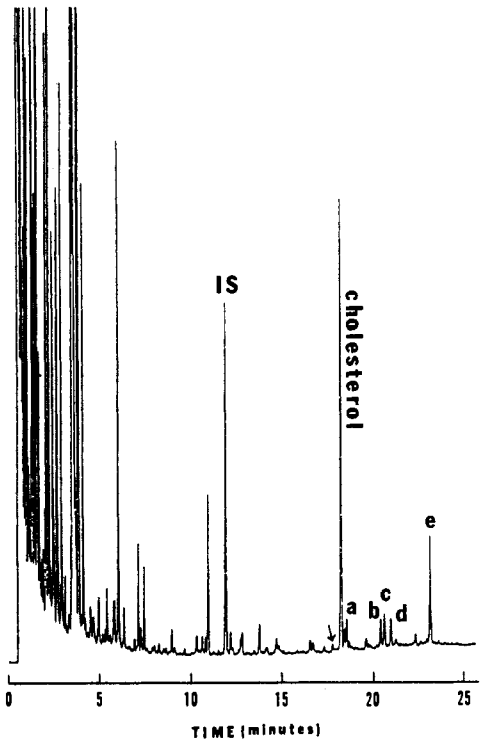



**Figure 2.** Capillary column gas chromatographic profiles of the nonsaponifiables of heated tallow (155 °C, 376 h) after trimethylsilylation. Gas chromatography was carried out on a fused silica capillary column, DB-1 (15 m × 0.25 mm i.d., 0.1-μm film thickness). Temperature was programmed at 3 °C/min from 180 to 250 °C. IS represents 5α-cholestane used for internal standard. Peaks a, c, d, and e were identified as 7α-hydroxycholesterol, cholesterol α-epoxide, and 7β-hydroxy- and 7-ketocholesterol, respectively.

trum of peak a was almost same as that of peak d and thus not presented here).

For peaks a and d, suspected as 7α-hydroxy- and 7β-hydroxycholesterol, respectively, the base peak was observed at $m/e$ 456 with little left at $m/e$ 546 and 366, corresponding to M (molecular ion) and M − 180 as bis(trimethylsilyl) ether sterols, as reported earlier (Brooks et al., 1973; Park and Addis, 1985a). The mass spectrum of peak c revealed some fairly intense peaks at $m/e$ 474, 384 and 366, which are characteristic of α-epoxide as mono(trimethylsilyl) ether (Gray et al., 1971; Park and Addis, 1985a). For peak e suspected as 7-ketocholesterol, the peak at $m/e$ 129 rather than at $m/e$ 472 turned out to be the molecular ion unlike the previous reports (Brooks et al., 1968; Park and Addis, 1985a). However, the sample mass spectrum was almost identical with that of authentic 7-ketocholesterol as mono(trimethylsilyl) ether, which was run under the same conditions. Therefore, they were quantified as 7α-hydroxy-, 7β-hydroxy- and 7-ketocholesterol and α-epoxide. For tallow heated at 155 °C, oxidation products started being detected in the 36-h sample with two small peaks emerging as 7β-hydroxy- and 7-ketocholesterol whereas no detectable oxides had existed at either 12 or 24 h (Figure 4). Formation of 7-ketocholesterol was almost linear with heating time, reaching up to ca. 10% of initial cholesterol content at 376-h heating. The other products of cholesterol oxidation at C-7, i.e., epimeric 7-hydroxycholesterols, did not increase in proportion to the heating time and remained at the 1–2% level throughout the heating period. α-Epoxide was formed in larger quantities than epimeric 7-hydroxycholesterols but did not increase much and remained at about the 4% level.

At 190 °C, OCDs were formed but sporadically with respect to time, species, and consistency (data not presented). Even 7-ketocholesterol formation was not proportional to the heating time, reaching around 3% after 216-h heating. The others such as epimeric 7-hydroxycholesterols and α-epoxide were noted at a level of approximately 1%. Therefore, it was thought that at an extremely elevated temperature there may be either less chance for cholesterol oxidation to occur or more probability for OCDs to breakdown quickly right after formation, leaving very little in the way of residual OCDs.

The peak labeled as b in Figure 2 was also constantly detected right before α-epoxide throughout the heating period, having about the same area counts as those of α-epoxide. The mass spectrum of that peak after trimethylsilylation (Figure 5) revealed its similarity to that of β-epoxide published by Aringer and Eneroth (1974). The mass spectrum was also almost the same as that of its stereoisomer, α-epoxide (Figure 3) just as the mass spectrum of 7β-hydroxycholesterol is almost same as that of its stereoisomer 7α-hydroxycholesterol (Park and Addis, 1985b). The peaks at $m/e$ 474 and 384, which correspond to M and M − 90, were clearly detected, there being no significant peak above $m/e$ 474. Although the retention time of authentic β-epoxide was not checked on the GC, the good agreement between the elution order of peak b followed by α-epoxide in this study and that of Brooks et al. (1983) and Missler et al. (1985) who showed β-epoxide preceded the α isomer is supportive of the identity of peak b as the β isomer. Therefore, the aforementioned mass spectrometric properties together with the elution order of peak b suggest that it was β-epoxide.

## DISCUSSION

**Recovery of 7-Ketocholesterol and α-Epoxide.** Through saponification the recovery of two OCDs, 7-ketocholesterol and α-epoxide, was of special interest in this study due to their structural features. The instability of 7-ketocholesterol in hot alkaline medium such as during hot saponification was mentioned early by Bergstrom and Wintersteiner (1941). Chicoye et al. (1968) reported numerous degradation products produced, including cholesta-3,5-dien-7-one as the major product, when 7-ketocholesterol was heated with a KOH solution and also demonstrated that 7-ketocholesterol survived well in cold alkaline medium. However, unfortunately they did not present any quantitative information for its recovery. Our study clearly established its recovery as high, comparable to other OCDs in quantitative terms. Minimizing the structural alteration of 7-ketocholesterol also minimizes the chance of making possible erroneous identification—namely, the claim that cholesta-3,5-dien-7-one is originally present as an OCD when, in fact, it is an artifact arising from the thermal dehydration of 7-ketocholesterol (more on this later).

The stability of α-epoxide is also of interest because it can be converted to cholestanetriol by hydrolysis of the epoxide ring. The recovery of α-epoxide was studied by Tsai et al. (1980) who recommended no use of alkaline treatment due to the apprehension of ring opening in any quantification procedure for OCDs, based on their poor recovery (25%) of α-epoxide after saponification according to the standard AOCS method. However, the data in Table I revealed that the recovery of α-epoxide was not inferior to that of other sterols, indicating no significant ring-opening reaction occurred during cold saponification. The good recovery of α-epoxide in this study was also due to the use of more polar solvent for extraction of sterol oxides, i.e. diethyl ether in addition to the absence of heat

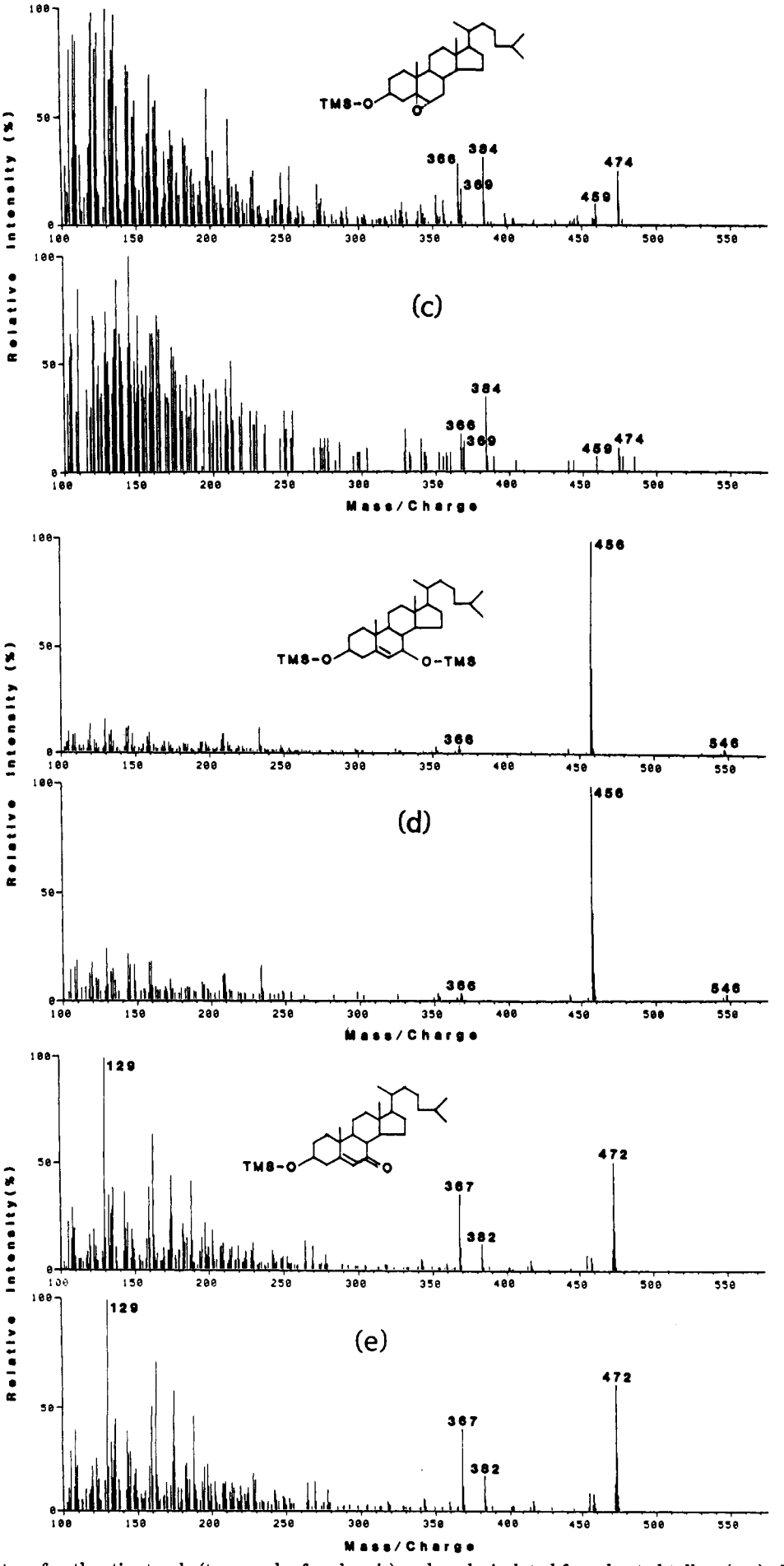



**Figure 3.** Mass spectra of authentic sterols (top graph of each pair) and peaks isolated from heated tallow (c–e). The 155 °C 376-h sample was subjected to 70 eV at 200 °C as trimethylsilyl ether derivatives. Mass was scanned between $m/e$ 100 and 670.

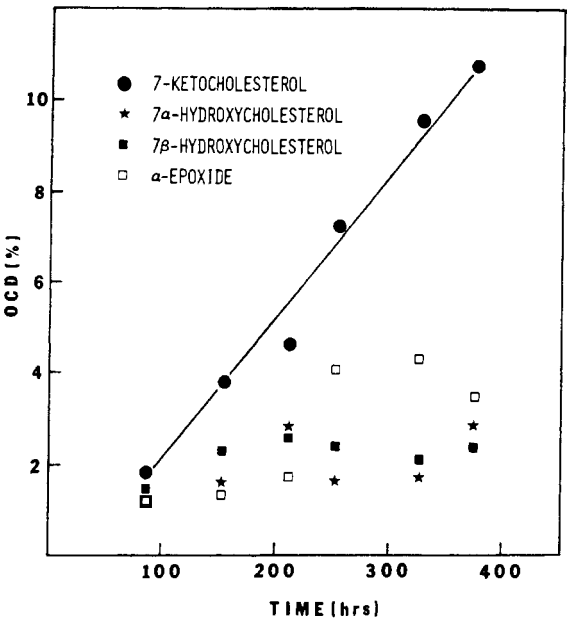



**Figure 4.** Occurrence of OCDs in tallow heated at 155 °C. The percent of OCDs was normalized to initial content of cholesterol, 705 ± 12 ppm. Each point represents the mean of three samples ($n$ = 3).

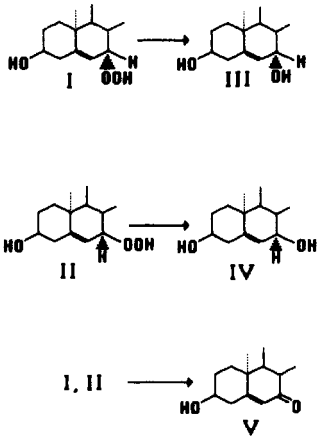
during saponification. When hexane was employed as in Tsai et al. (1980), poor recovery of more polar sterol oxides including α-epoxide resulted (Tsai, 1984).

Other evidence for the stability of α-epoxide stems from the findings that no cholestanetriol, but only α-epoxide, was detected in heated tallow samples, all of which were saponified at room temperature. Nevertheless, the possibility of α-epoxide losses due to the nucleophilicity of a highly strained three-membered ring is not totally excluded. Cholestanetriol was reported together with α-epoxide from colloidal or aqueous dispersions of cholesterol, suggesting that it resulted from hydration of α-epoxide (Kimura et al., 1979; Ansari and Smith, 1979). However, both studies reported the formation of cholestanetriol at very negligible quantities compared to α-epoxide. Consequently, the loss of α-epoxide through saponification, if it occurs at all, is likely negligible when it is performed without the use of heat. These findings, together with the recovery data for α-epoxide in this study, do not agree with those of Tsai et al. (1980).

**Occurrence and Significance of OCDs.** In light of the reported tendency of cholesterol to undergo autoxidation, the oxidation of cholesterol in tallow should be expected. Ryan et al. (1981) reported for the first time the presence of a few cholesterol oxidation products in heated tallow, including the epimeric 7-hydroxycholesterols and cholesta-3,5-dien-7-one, based on TLC analysis. However, quantitative data were not presented, nor was any further confirmation of identities given.

In our study, four OCDs including epimeric 7-hydroxycholesterols and 7-ketocholesterol and α-epoxide were quantified and further identified by combined capillary GC–MS. However, 25-hydroxycholesterol and cholestanetriol, known as the most toxic among OCDs (Taylor et al., 1979), were not detected in heated tallow. Cholesta-3,5-dien-7-one was not detected either. Sometimes, a small peak that is slightly greater than background noise was noticed at the expected retention site of this compound (↓ in Figure 2). Instead, 7-ketocholestrol was the predominant species among OCDs formed in tallow. Thus, it is very possible that what Ryan et al. (1981) recognized as cholesta-3,5-dien-7-one was in fact the dehydration product of 7-ketocholesterol, an artifact formed by the hot saponification they used. The finding of cholesta-3,5-dien-7-one in two other earlier studies, i.e. in chicken embryo (Pennock et al., 1962) and in anhydrous milk fat (Flanagan et al., 1975), also should be suspected as an artifact because hot saponification was used. This is further supported by the fact that there was no 7-ketocholesterol in any of these reports, that cholesta-3,5-dien-7-one is rarely reported as an autoxidation product of cholesterol (Smith, 1981), and that it was present in negligible amounts in a model system (Kimura et al., 1979).

The decomposition of 7α-hydroperoxide (I) and/or 7β-hydroperoxide (II) results in the formation of 7α-hydroxy-




(III), 7β-hydroxy- (IV), and 7-ketocholesterol (V) according to Teng et al. (1973) and Smith et al. (1973). Therefore, it is anticipated that at elevated temperature, in the absence of water (in heated tallow), the decomposition of 7-hydroperoxides is likely to prefer the formation of 7-ketocholesterol through dehydration to that of epimeric 7-hydroxycholesterols.

7-Ketocholesterol was formed more extensively than α-epoxide. The summation of C-7 OCDs exceeded that

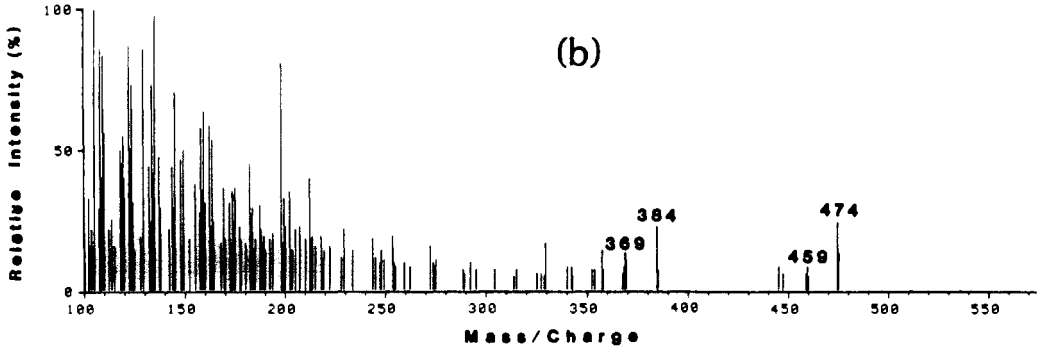



**Figure 5.** Mass spectrum of the trimethylsilyl ether derivative of peak b isolated (see Figure 2 and text).

of epimeric epoxides, suggesting that oxidation products at the allylic position will be formed more abundantly than those at the double bond itself. This is in good agreement with the findings of more 7-ketocholesterol than epimeric epoxides from the model systems of aqueous or colloidal dispersions of cholesterol (Smith and Kulig, 1976; Ansari and Smith, 1979; Kimura et al., 1979). This is probably because epoxidation is a secondary process that is dependent upon the presence of cholesterol 7-hydroperoxides (Smith et al., 1978; Smith, 1981).

In light of potential adverse effects of those oxidation products found in heated tallow, it is perhaps significant from the public health point of view that oxidation products formed in frying media may migrate into finished products.

The presence of OCDs in heated tallow supports our earlier finding of 7-ketocholesterol and 7β-hydroxycholesterol in french-fried potatoes (Park and Addis, 1985b). The formation of 7-ketocholesterol, proportional to heating time at 155 °C, suggests that it will follow zero-order reaction kinetics between certain temperature ranges. This supports the initiation of kinetic research on the formation of 7-ketocholesterol when animal fat is subjected to high temperature in air, together with research on effective measures of suppressing its formation.

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

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