JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Kinetic Studies of Cholesterol Oxidation as Inhibited by Stearylamine during Heating

JOHN-TUNG CHIEN, DONG-YONG HUANG, AND BING-HUEI CHEN*

Department of Nutrition and Food Science, Fu Jen University, Taipei, Taiwan 242, Republic of China

The formation of cholesterol oxidation products (COPs) during heating in the presence of stearylamine at 140 °C was analyzed by high-performance liquid chromatography (HPLC) and kinetically studied by use of nonlinear regression models. Results indicated that the COPs concentration increased with increasing heating time, and stearylamine was shown to reduce both oxidation and degradation rates of cholesterol. Without stearylamine, the highest rate constant (per hour) was observed for epoxidation (545.4), followed by free radical chain reaction (251.0), reduction (147.3), dehydration (95.8), triol dehydrogenation (4.7), degradation (0.34), triol formation (0.31), and dehydrogenation (0.13). With stearylamine, the epoxidation and free radical chain reaction rates could be reduced by about 800- and 3.4-fold, respectively, and triol formation during oxidation could be completely inhibited. In addition, the reactions for reduction, dehydration, degradation, and dehydrogenation could proceed slower in the presence of stearylamine. The kinetic model developed in this study can be used to predict the inhibition of COPs formation by stearylamine during heating of cholesterol.

KEYWORDS: Cholesterol oxidation; stearylamine; heating; kinetics; HPLC

INTRODUCTION

Food products such as meats and eggs are rich in cholesterol, which can be susceptible to formation of cholesterol oxidation products (COPs) during processing (*1*). Many reports have shown that most COPs are mutagenic and carcinogenic (*2*, *3*). Thus, the inhibition of COPs formed during heating of cholesterol is important. To date, the oxidation pathways of cholesterol have been well documented (*4*), and the mechanism of cholesterol oxidation is reported to be similar to that of lipid oxidation; that is, it can undergo a series of free radical chain reactions to form COPs and other degradation products (*5*). The authors also pointed out that the cholesterol oxidation can be initiated by the second-order reaction, and the first-order reactions follow afterward (*5*).

The addition of lysine to soy proteins (*6*) or cysteine or alanine to fish oils (*7*, *8*) has been shown to prevent lipid oxidation during thermal processing. Likewise, stearylamine has long been incorporated into cationic liposomes, which can be used to encapsulate various materials as possible delivery vehicles for drugs, enzymes, antibodies and gene (*9*−*11*). The mechanism of primary and secondary amines for exhibiting antioxidative activity in soybean oil has been attributed to the protective effect of oxidized lipid/amine reaction (*12*). Thus, understanding the reactions that lead to inhibition of COPs formation will provide a basis for developing food products with better quality. As there is no information available about the inhibition of COPs as affected by amino-containing compounds, this study was undertaken to determine the kinetics of cholesterol oxidation with or without stearylamine during heating.

MATERIALS AND METHODS

Materials. Stearylamine, cholesterol and COPs standards, 7α- and 7β-hydroxycholesterol, 7-ketocholesterol, 5,6α- and 5,6β-epoxycholesterol, and 5α-cholestane-3β,5,6β-triol were purchased from Sigma Chemical Co. (St. Louis, MO) and Steraloids Inc. (Wilton, NH). These standards were used without further purification. Spraying reagent *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride was also from Sigma. TLC adsorbent silica gel 60 GF254, reagent benzoyl peroxide, and paraffin oil were from Merck Co. (Darmstadt, Germany). The analytical-grade solvents lauryl alcohol, chloroform, benzene, methanol, and ethyl acetate were obtained from Merck Co. Acetone, acetic acid, and sulfuric acid were from Hau-Fong Co. (Taipei, Taiwan). The HPLC-grade solvents *n*-hexane and 2-propanol were from Mallinckrodt Co. (Paris, KY).

Instrumentation. The HPLC instrument consists of a Jasco PU-980 pump (Tokyo, Japan), a Jasco 830 refractive index detector, and a SIC Chromatocoder 12 integrator (System Instruments Co., Tokyo, Japan). A Lichrospher 100 CN column (244 × 4.0 mm i.d.) containing 5-μm packing material was from Merck Co. (Darmstadt, Germany). A CHEN−WIN computer software system (Shuen-Hua Co., Taipei, Taiwan) was used to process data.

Heating of Cholesterol. A mixture containing 0.2 mL of paraffin oil and 0.08 mL of lauryl alcohol was poured into a 100-mL round-bottom flask of a rotary evaporator, which was preheated in a silicon-oil bath (145−150 °C) for 5 min with nitrogen flushed into the flask at the same time. Then 100 mg of cholesterol standard was added alone or mixed with 70 mg of stearylamine to the flask. A thin film of the mixture was heated for 2 min so that the internal temperature could reach 140 °C. This temperature was selected to avoid thermal

* Corresponding author: telephone 886-2-29031111, ext 3626; fax 886-2-29021215; e-mail nutr1007@mails.fju.edu.tw.

10.1021/jf048951+ CCC: $27.50
Published on Web 10/16/2004

degradation of cholesterol during 2-min preheating and facilitate formation of COPs afterward. In the alcoholic system, a significant thermal degradation of cholesterol was found at 150 °C or above. In addition, this temperature can be used for frying of food products (low-temperature frying). Meanwhile, oxygen was pumped through at a rate of 10 mL/min and the heating times, 0, 10, 20, 40, 60, and 120 min, started to count. The dissolved oxygen content was not calculated but was assumed to be constant during heating. This is important to make the kinetic study of cholesterol oxidation much easier as the number of variables can be reduced. After heating, the flask was cooled in dry ice to terminate the reaction.

Purification of Cholesterol and COPs. Twenty milliliters of *n*-hexane-2-propanol (3/2 v/v) was added to the flask and the mixture was vigorously shaken for 3 min to dissolve cholesterol and COPs. The solution was subjected to centrifugation at 26000*g* for 5 min, after which the upper phase was collected and poured into a centrifuged tube. Twelve milliliters of water was added and the mixture was centrifuged again for 5 min. The upper layer was collected and evaporated to dryness for purification. The residue was dissolved in 1 mL of hexane/1,2-dichloroethane (1:1 v/v) and poured into a NH_2 cartridge. Initially 5 mL of hexane was added to remove impurities such as hydrocarbons, cholesterol esters and triglycerides. COPs were next eluted with hexane/1,2-dichloroethane/2-propanol (50/30/15 v/v/v). The eluate was evaporated to dryness at 35 °C, and the residue was dissolved in 1 mL of hexane/2-propanol (95/5 v/v) and filtered through a 0.2-μm membrane filter for HPLC analysis. Duplicate treatments and triplicate analyses were performed, and the data were subjected to nonlinear regression analysis by use of SAS (*13*). A high recovery of 97−100% was obtained when both cholesterol and COPs standards were subjected to the same extraction and purification method.

TLC Analysis of COPs: (a) Preparation of Wurster Dye. The Wurster dye was prepared by a method described by Smith and Hill (*14*). Briefly, 1 g of *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride was dissolved in 100 mL of 50% methanol solution (in water), and the mixture was shaken thoroughly. Glacial acetic acid (1 mL) was added, and the solution was poured into a glass vial and stored at −20 °C until use.

(b) Separation of COPs by TLC. Development of the TLC plate was carried out in a glass tank lined with a filter paper and equilibrated for 30 min with 200 mL of benzene/ethyl acetate (60/40 v/v) (*15*) prior to development. A 10-μL volume of extract was spotted on the glass plate with a micropipet. The chromatogram was developed for a distance of 16 cm at ambient temperature, after which the plate was dried in an oven at 110 °C for 10 min and sprayed with 50% H_2SO_4, and the color development of COPs under UV radiation at 254 nm was observed. Both 7α- and 7β-hydroperoxycholesterol bands were identified and quantified by the Wurster dye method as described in a previous study (*16*).

HPLC Analyses of COPs. An isocratic mobile phase of *n*-hexane/2-propanol (95/5 v/v) with flow rate 1.0 mL/min and refractive index detection (sensitivity 16×10^{-5} RIU) was used (*5*). The injection volume was 100 μL, and two LiChrospher 100 CN columns controlled at 30 °C were used for separation of COPs. The various COPs were identified by comparison of retention times of unknown peaks with reference standards and addition of standards to sample for cochromatography, as well as collection of eluates for TLC analysis.

Because of absence of a suitable internal standard, each COP was quantified by an external calibration method. Eight concentrations of each COP ranging from 10^4 to 10^7 ppm were injected onto an HPLC column, and the calibration curve for each COP standard was obtained by plotting concentration against area. The regression equations and correlation coefficients (r^2) were calculated on a CHEN−WIN computer software system. Each COP was quantified by a method as described by Chien et al. (*5*). The eluates of 7α- and 7β-hydroperoxycholesterol were collected individually and then quantified by the Wurster dye method (*16*).

Kinetic Analyses of COPs. The various concentration changes of cholesterol and COPs during heating of cholesterol were subjected to statistical analysis by a nonlinear regression procedure (*13*). All the rate constants of a nonlinear model were estimated by the least-squares method with a NLIN (nonlinear regression) procedure−Marquardt iterative methods until the convergence of the best-fitted parameters were met. The rate constant (per hour) and correlation coefficient of each kinetic equation of cholesterol oxidation and degradation were determined. The precision of the parameters of the kinetic equations was also assessed.

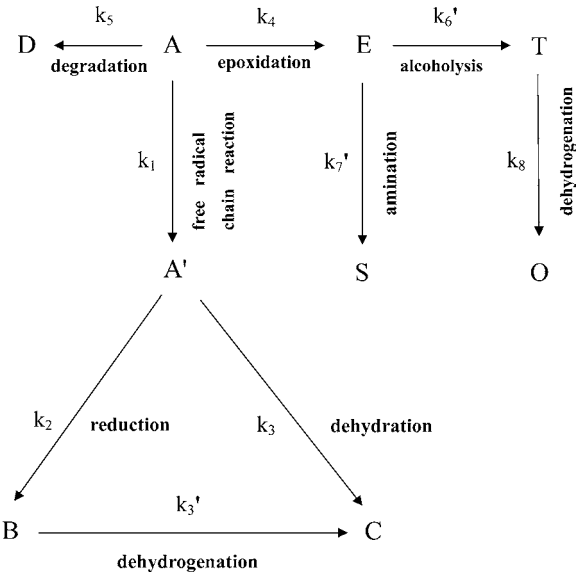

Figure 1. Pathways of cholesterol degradation and oxidation. A = cholesterol; A′ = 7-OOH (7-hydroperoxycholesterol); B = 7-OH (7-hydroxycholesterol); C = 7-Keto (7-ketocholesterol); D = degraded products; E = 5,6-EP (5,6-epoxycholesterol); O = cholestan-3β,5α,6-one; S = 5,6-epoxycholesterol–stearylamine reaction products; T = cholestan-3β,5α,6β-triol; $k_1 \sim k_8$, k_3', k_6', and k_7' = the corresponding rate constants. Thermal degradation, A → D; C-7 oxidation, A → A′ → B or C; epoxidation: A → E → other oxidized products.

RESULTS AND DISCUSSION

Percentage Changes of Cholesterol during Heating. Due to the complexity of food itself, it is difficult to assess the formation mechanism of COPs in real food systems. The formation of COPs during heating of cholesterol at 150 °C has been kinetically studied by nonlinear regression models (*5*). However, the effect of stearylamine on formation or inhibition of COPs remains uncertain. Theoretically, primary and secondary amines are able to react with oxidized lipids to form oxidized lipid/amino acid reaction products, which provide protection against lipid oxidation (*12*, *17*).

Owing to the poor solubility of the mixture of stearylamine, cholesterol, and COPs in water, a solvent system of lauryl alcohol/paraffin oil (2/5 v/v) was used instead. Our preliminary results showed that cholesterol oxidation in this solvent system was too fast to be monitored when temperature reached 160 °C and above. After several studies, the most appropriate temperature for kinetic study of cholesterol was found to be 140 °C. Furthermore, with nitrogen flushing, no significant oxidation of cholesterol in this solvent system was observed during 2-min preheating at 140 °C.

Figure 1 shows the major reaction pathways and rate constants (per hour) of cholesterol oxidation and degradation based on a study by Chien et al. (*5*). Similar to that in an aqueous environment, 5,6α- or 5,6β-epoxycholesterol may undergo alcoholysis to form 5α-cholestane-3β,5,6β-triol (*18*), which can be further dehydrogenated to cholestan-3β,5α,6-one. In the presence of stearylamine, β-hydroxylamine, one of the reaction products between 5,6α- or 5,6β-epoxycholesterol and stearyl-

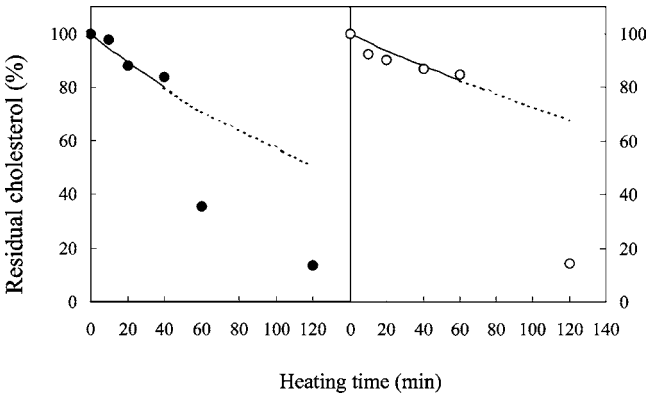

Figure 2. Percentage changes of cholesterol during heating at 140 °C. Mean of experimental data for (●) cholesterol only and the best-fitting line (—) for 0–40 min of heating and (○) cholesterol in the presence of stearylamine and the best-fitting line (—) for 0–60 min of heating. All the standard errors are within ±2.7% of the means ($n = 3$).

amine, is probably formed through amination of epoxides (*19*, *20*). The possible reaction for amination of epoxides is shown below:

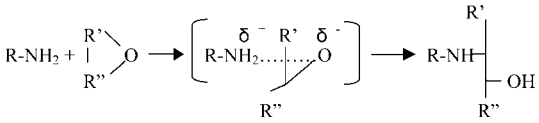

Figure 2 shows the percentage changes of cholesterol during heating at 140 °C for up to 2 h. A loss of cholesterol by 16.2% was found after 40-min heating, and a further decrease of 86.3% occurred after prolonged heating for 2 h. In contrast, in the presence of stearylamine, degradation of cholesterol proceeded more slowly over the 1-h heating period. However, the residual percentage of cholesterol drastically dropped to 14.5% after 2-h heating. This outcome may be attributed to the antioxidative activity of the reaction products of stearylamine with 5,6α- or 5,6*β*-epoxycholesterol. Several authors also demonstrated that some amines may react with alkenals or epoxyalkenals to produce antioxidant compounds (*12*, *17*). In a previous study, Chien et al. (*5*) reported that approximately 33.3% cholesterol remained after dry heating of cholesterol at 150 °C for 30 min. This result showed a higher loss of cholesterol, which may be explained by the heating conditions such as temperature or solvent system.

Figures 3 and **4** show the HPLC chromatograms of COPs formed during heating at 140 °C in the absence and presence of stearylamine, respectively. A total of 7 COPs, including 7α- and 7*β*-hydroperoxycholesterol, 5,6α- and 5,6*β*-epoxycholesterol, 7-ketocholesterol, 7α- and 7*β*-hydroxycholesterol, and 5α-cholestane-3*β*,5,6*β*-triol were adequately resolved within 40 min. Two columns were used and the separation efficiency could be substantially improved. Nevertheless, a prolonged retention time could occur for cholesterol and various COPs. Without stearylamine, the COPs were formed gradually during the initial heating period and sharply increased after 40-min heating, and a plateau was achieved in 2 h. Conversely, with stearylamine, the total percentages of COPs showed a minor increase over a 2-h heating period (**Figure 5**). This phenomenon further demonstrated that stearylamine exerts a strong antioxidative effect on cholesterol oxidation during high-temperature heating. Alaiz et al. (*17*) also reported that *n*-octylamine possesses antioxidative activity when added to soybean oil during heating.

Kinetic Study of Cholesterol Oxidation during Heating. Due to the complexity of COPs formation during heating, only

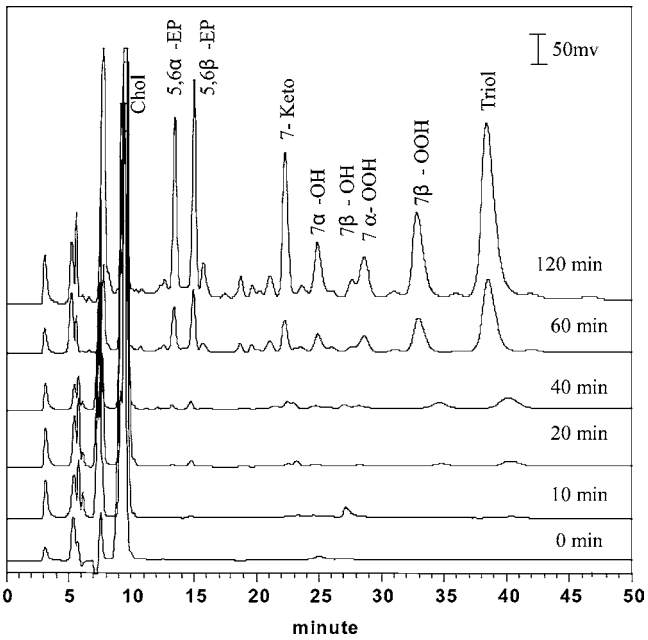

Figure 3. HPLC chromatograms of COPs formation during heating of cholesterol in the absence of stearylamine at 140 °C.

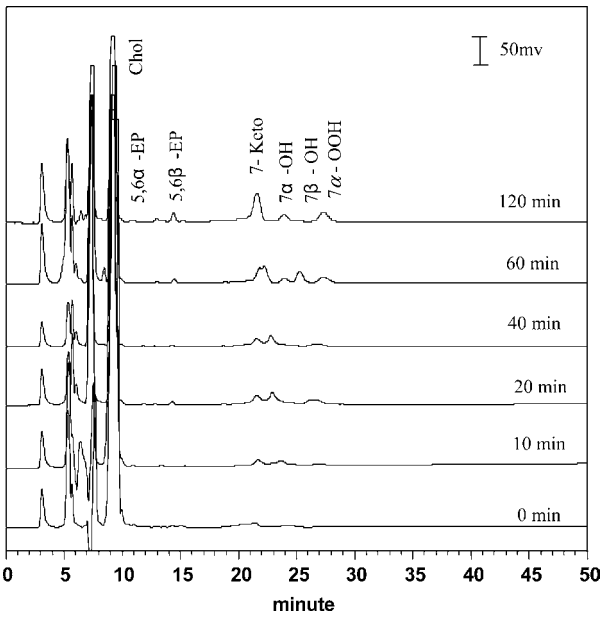

Figure 4. HPLC chromatograms of COPs formation during heating of cholesterol in the presence of stearylamine at 140 °C.

five major COPs, 7-OOH (7α- and 7*β*-hydroperoxycholesterol), 7-ketocholesterol, 7-OH (7α- and 7*β*-hydroxycholesterol), 5,6-EP (5,6α- and 5,6*β*-epoxycholesterol), and triol (5α-cholestane-3*β*,5,6*β*-triol), as well as cholesterol and its degraded products were selected for kinetic study. According to several previous studies (*5*, *21*−*23*), cholesterol heated in the presence of oxygen proceeds in two major pathways—degradation and oxidation. For the oxidative pathway, reactions can be divided into two major routes of C-7 oxidation and epoxidation (**Figure 1**). The oxidation reaction involved the attack of free radicals on cholesterol to result in formation of 7-OOH, which belongs to the second-order pathway, and the rate equation for 7-OOH formation was shown as in a previous study (*5*). According to the C-7 oxidation route in the reaction pathways of cholesterol during heating (**Figure 1**), 7-OOH can be further degraded to 7-OH and 7-keto through the first-order reaction (*5*, *24*). The

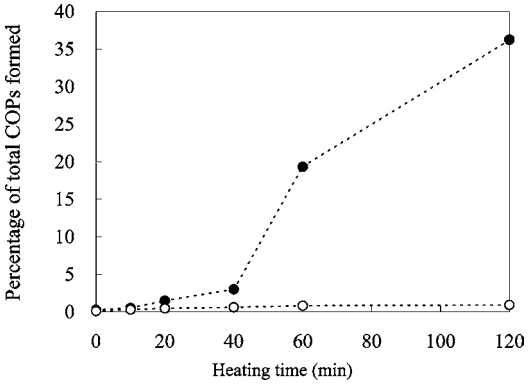

Figure 5. Total percentage of COPs formation during heating of cholesterol at 140 °C. (●) Cholesterol only. All the standard errors are within ±4.8% of the means ($n = 3$). (○) Cholesterol in the presence of stearylamine. All the standard errors are within ±5.9% of the means ($n = 3$).

rate equation (eq 1) for 7-OOH formation was thus modified as follows:

$$\frac{d[A']}{dt} = k_1\left(1 - \frac{[A']}{[A'_{max}]}\right)[A'] - k_2[A'] - k_3[A']$$

$$= (k_1 - k_2 - k_3)[A'] - \frac{k_1}{[A']_{max}}[A']^2 \quad (1)$$

where [A′] is percentage concentration of 7-OOH and $[A']_{max}$ is the maximum attainable concentration of 7-OOH prior to degradation; k_1, k_2, k_3, and t are the reaction rate constants (per hour) and time, respectively. In addition, the rate equations of C-7 oxidation routes B and C were also shown in a previous study (*5*).

In the epoxidation route, the formation of 5,6-EP from cholesterol could be judged from the concentrations of both cholesterol and 7-OOH (*5*, *21*). Thus, the rate equations for both 5,6-EP formation and cholesterol depletion could be respectively written as follows:

$$\frac{d[E]}{dt} = k_4[A][A'] - k_6'[E] \quad (2)$$

$$-\frac{d[A]}{dt} = (k_1 + k_4)[A][A'] + k_5[A] \quad (3)$$

where [A] and [E] are the percentage concentrations of cholesterol and 5,6-EP, respectively. k_4 and k_5 are the reaction rate constants (per hour). According to the study of autoxidation of cholesterol in the suspended oil (*4*), hydrolysis of 5,6-EP leads to formation of triol. Since there is no water present in the solvent system, alcoholysis of 5,6-EP is most likely to occur in our study (*18*). Thus, the rate equation can be written as follows:

$$\frac{d[T]}{dt} = k_6[L]_0[E] - k_8[T] = k_6'[E] - k_8[T] \quad (4)$$

where [T] and $[L]_0$ are the percentage concentrations of triol and of lauryl alcohol at time 0, respectively. k_6, k_6', and k_8 are the reaction rate constants (per hour). Likewise, the formation of reaction products between 5,6-EP and stearylamine could be regarded as a function of concentrations of stearylamine and 5,6-EP. Since the initial concentration of stearylamine at time 0 min is 566 times higher than that of 5,6-EP, the concentration of stearylamine could be considered as a constant to [E] in the equation as shown below:

$$\frac{d[S]}{dt} = k_7[SA]_0[E] = k_7'[E] \quad (5)$$

where [S] is the concentrations of reaction products between 5,6-EP and stearylamine and $[SA]_0$ is the concentration of stearylamine at time 0, respectively. k_7 and k_7' are the reaction rate constants (per hour).

The concentration of 7-OOH formed in the initial 20-min heating was found to be much lower than cholesterol concentration without stearylamine (1400[A′] ≅ [A]) or with stearylamine (88 128[A′] ≅ [A]). The equation of thermal degradation of cholesterol could thus be written as $-d[A]/dt = k_5[A]$. The integration of this equation gave

$$[A]_t = [A]_0 e^{-k_5 t} \quad (6)$$

Equation 1 is in the form of a Bernoulli equation ($n = 2$). By transformation of a variable [A′], the following equation was obtained by integration:

$$[A']_t = \left[\frac{M_2}{M_1} + \left(\frac{1}{[A']_0} - \frac{M_2}{M_1}\right)e^{-M_1 t}\right]^{-1} \quad (7)$$

where $M_1 = k_1 - k_2 - k_3$ and $M_2 = k_1/[A']_{max}$. By substitution of eq 7 into equations for $d[B]/dt$ and $d[C]/dt$, the differential equations for $[B]_t$ and $[C]_t$ could be integrated as shown in a previous study (*5*). Substituting eqs 6 and 7 into eq 2, we could obtain eq 8 by integration:

$$[E]_t = [E]_0 e^{-k_6' t} + k_4[A]_0 e^{-k_6' t}\int_0^t \frac{e^{(k_6' - k_5)s}}{\frac{M_2}{M_1} + \left[\frac{1}{[A']_0} - \frac{M_2}{M_1}\right]e^{-M_1 s}}\,ds \quad (8)$$

Equation 9 and 10 were respectively obtained by integration after substitution of eq 8 into eqs 4 and 5:

$$[T]_t = k_6' k_4[A]_0 e^{-k_8 t}\int_0^t e^{(k_8 - k_6')v}\left(\int_0^v \frac{e^{[k_6' - k_5]s}}{\frac{M_2}{M_1} + \left[\frac{1}{[A']_0} - \frac{M_2}{M_1}\right]e^{-M_1 s}}\,ds\right)dv \quad (9)$$

$$[S]_t = k_7' k_4[A]_0\int_0^t e^{-k_7' r}\int_0^r \frac{e^{(k_7' - k_5)v}}{\frac{M_2}{M_1} + \left[\frac{1}{[A']_0} - \frac{M_2}{M_1}\right]e^{-M_1 v}}\,dv\,dr \quad (10)$$

where $[A]_0$, $[A']_0$, $[B]_0$, $[C]_0$, $[E]_0$, and $[T]_0$ are percentage concentrations of cholesterol, 7-OOH, 7-OH, 7-keto, 5,6-EP, and triol, respectively, at heating time 0 min. Likewise, $[A]_t$, $[A']_t$, $[B]_t$, $[C]_t$, $[E]_t$, $[S]_t$, and $[T]_t$ are percentage concentrations of cholesterol, 7-OOH, 7-OH, 7-keto, 5,6-EP, 5,6-EP−stearylamine reaction products, and triol at heating time t min, and r, s, t, and v are time.

For the above equations, only eq 6 can obtain an exact integration. As the exact integrations for eqs 7−10 are not possible, we can only get a better approximation to the integral term in each equation by using a quadratic polynomial of

Table 1. Rate Constants of Cholesterol Degradation and Oxidation with or without Stearylamine Addition

rate equations[a]	reactions	C[b]		S[b]	
		K (h^{-1})	R^2	K (h^{-1})	R^2
Chol $\xrightarrow{k_1}$ 7-OOH	free radical chain reaction	251.0 ± 0.2	0.96	74.3 ± 0.0	0.77
7-OOH $\xrightarrow{k_2}$ 7-OH	reduction	147.3 ± 0.1	0.96	59.7 ± 0.0	0.77
7-OOH $\xrightarrow{k_3}$ 7-keto	dehydration	95.8 ± 14.6	0.96	4.74 ± 0.0	0.77
7-OH $\xrightarrow{k_3'}$ 7-keto	dehydrogenation	0.13 ± 0.51	0.88	2.49 ± 0.26	0.45
Chol $\xrightarrow{k_4}$ 5,6-EP	epoxidation	545.4 ± 135.2	0.99	0.69 ± 0.25	0.73
Chol $\xrightarrow{k_5}$ D	degradation	0.34 ± 0.05	0.92	0.19 ± 0.02	0.91
5,6-EP $\xrightarrow{k_6'}$ triol	alcoholysis	0.31 ± 0.14	0.99	*c*	*c*
triol $\xrightarrow{k_8}$ O	dehydrogenation	4.70 ± 0.40	0.73	*c*	*c*
5,6-EP $\xrightarrow{k_7'}$ S	amination	*c*	*c*	1.51 ± 0.20	0.73

[a] Chol (cholesterol), 7-OOH (7α- or 7β-hydroperoxycholesterol), 7-OH (7α- or 7β-hydroxycholesterol), 7-keto (7-ketocholesterol), D (degraded products), 5,6-EP (5,6-epoxycholesterol), triol (5α-cholestan-3β,5α,6β-triol), O (cholestan-3β,5α-diol-6-one), S (5,6-epoxycholesterol–stearylamine reaction products), and $k_1 \sim k_5$, k_3', k_6', $k7'$, and k_8 are the rate constants. [b] C, cholesterol only. S, cholesterol in the presence of stearylamine. [c] Not detected.

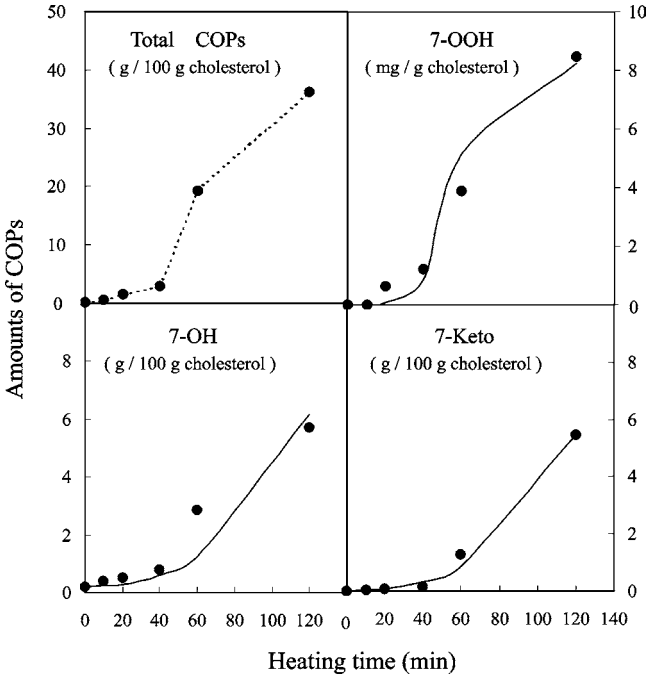

Figure 6. Comparison of predicted lines and experimental data for total COPs and C-7 route formed during heating at 140 °C. Mean of experimental data (●); best-fitting lines (—); data-point-connecting line (···). All the standard errors are within ±4.8% of the means ($n = 3$).

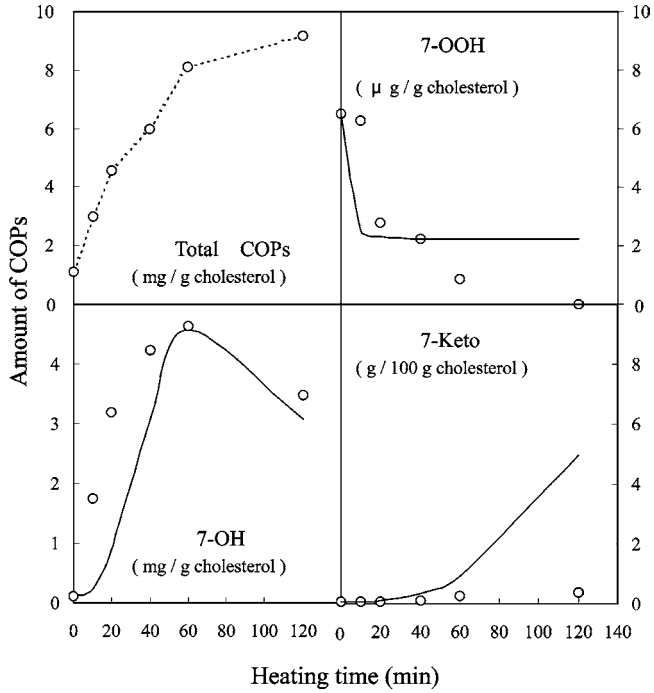

Figure 7. Comparison of predicted lines and experimental data for total COPs and C-7 route formed during heating at 140 °C in the presence of stearylamine. Mean of experimental data (○); best-fitting lines (—); data-point-connecting line (···). All the standard errors are within ±5.9% of the means ($n = 3$).

Taylor's series. The experimental data were thus analyzed in sequence and fitted best to the corresponding nonlinear regression model.

For computing k_5 in eq 6 by use of a linear regression model, the data with or without stearylamine were obtained by subtracting total COPs formed (**Figure 5**) from total loss of cholesterol (**Figure 2**) over a 60- or 40-min heating period, respectively. k_5 values were found to be 0.19 ± 0.02 with stearylamine and 0.34 ± 0.05 without stearylamine, with correlation coefficients (r^2) 0.91 and 0.92, respectively, and the corresponding best-fitting lines are shown in **Figure 2**. The rate constants in eqs 7−10 were estimated by using the least-squares method with a nonlinear Marquardt iterative method until the convergences of best-fitted parameters were met. **Table 1** shows the rate constants of the reaction pathways of cholesterol degradation and oxidation during heating at 140 °C. Without stearylamine, the r^2 values of all the reactions were higher than 0.88, with the exception of dehydrogenation of triol ($r^2 = 0.73$). Likewise, with stearylamine, the r^2 values of all the reactions were higher than 0.73, with the exception of dehydrogenation of 7-OH ($r^2 = 0.45$). Each COP was further plotted on the basis of the corresponding rate equation and the rate constants listed in **Table 1**, which are shown in **Figures 6**−**8**. Without stearylamine, all the curves fit well with the data points (**Figure 6**). However, in the presence of stearylamine, only the curve of 7-OH fit well with the data points (**Figure 7**). Neither curves of 7-OOH nor 7-keto fit well for the heating times 1 and 2 h (**Figure 7**). The above results suggested that eq 6 could only successfully estimate the degradation of cholesterol during the

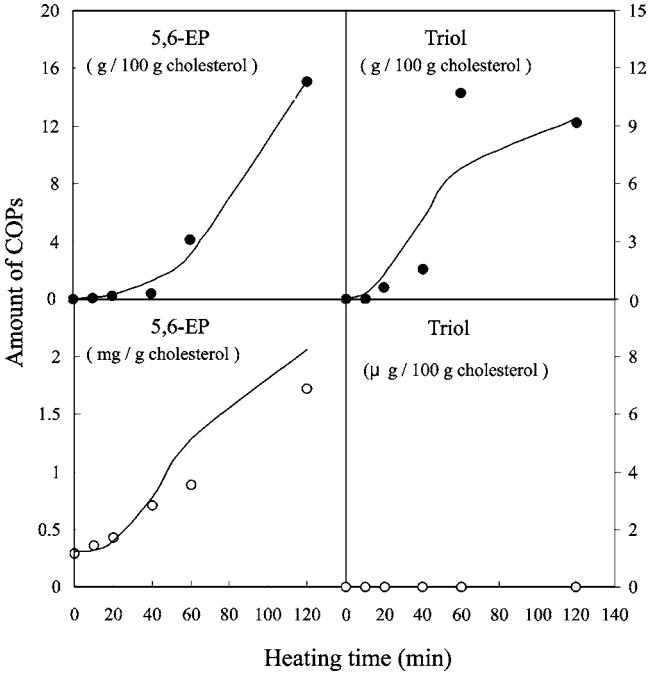

Figure 8. Comparison of predicted lines and experimental data for 5,6-epoxycholesterol and triol formed during heating at 140 °C. Mean of experimental data for cholesterol only (●) and for cholesterol in the presence of stearylamine (○); best-fitting lines (—). All the standard errors are within ±5.4% of the means ($n = 3$).

initial 40-min heating period and eqs 7−10 could be used to predict the concentration changes of COPs during the initiation and propagation periods of oxidation.

Of the various reactions, the r^2 values of the free radical chain reaction and epoxidation were 0.96 and 0.99, respectively, in the absence of stearylamine. It can be inferred that both reactions for formation of 7-OOH and 5,6-epoxide from cholesterol are second-order. For the reduction and dehydration reactions, the r^2 values of both were 0.96. This result also demonstrated that, in the absence of stearylamine, the reactions for formation of 7-OH and 7-keto were first-order. The rate constant for dehydrogenation of 7-OH to 7-keto is too small to be significantly analyzed by using a nonlinear simulation. The formation of triol by alcoholysis can be further inferred from eq 9 with high correlation coefficient (0.99), revealing that this reaction is pseudo-first-order.

It was also observed that the k_4 value is very large without stearylamine addition, implying that the 5,6-EP concentration increased sharply as soon as 7-OOH was formed during the initial period of oxidation (**Table 1**). It can be postulated that most 7-OOH was converted to 5,6-EP during heating, and thus 7-OOH concentrations were maintained at a low level (0.2%) over a 40-min heating period. This is an initial maximum attainable concentration for 7-OOH ($[A']_{max}$ in eq 7). However, in practice it is difficult for 7-OOH to maintain at this level, because it could be further degraded to form 7-OH or 7-keto in the C-7 route and to triol in the epoxidation route. Therefore, the 7-OOH level could be increased to about 0.8% in 2-h heating (**Figure 6**). By comparing the rate constants (per hour) of the major reaction pathways of cholesterol oxidation, it can be found that $k_4 > k_1 > k_2 > k_3 > k_8 > k_5 > k_6' > k_3'$. This order seemed to be different from that by Chien et al. (*5*), who reported that the rate constants of COPs formed during dry heating of cholesterol at 150 °C were $k_1 \approx k_4 \approx k_3 \approx k_2 \approx k_3'$. This difference can be attributed to the fact that, in our study, cholesterol is heated in an alcoholic solvent and a lower temperature is employed.

Table 1 also shows the rate constants of cholesterol degradation and oxidation in the presence of stearylamine during heating. The rate constants (per hour) are in the following order: $k_1 > k_2 > k_3 > k_3' > k_7' > k_4 > k_5$. Compared to the control treatment, all the rate constants decreased substantially, especially for the epoxidation reaction (k_4), which decreased by 800-fold. The incorporation of stearylamine not only reduces the COPs formation but also completely inhibits triol production during heating. However, we have to point out here that we do not have a good explanation for the data points of 7-OOH and 7-keto in **Figure 7** for heating times 1 and 2 h. It may be postulated that, with stearylamine, epoxidation proceeds faster than C-7 oxidation as shown in **Figure 8**. Also, β-hydroxylamine may be formed from the amination reaction between epoxide and stearylamine, which may expedite the consumption of 5,6-EP. The k_2 value was found to be about 12.6 times higher than the k_3 value, implying that dehydration is less susceptible to proceed than reduction. Similar results were reported by Nielsen et al. (*25*), who studied the cholesterol oxidation in a heterogeneous system initiated by water-soluble radicals. Despite the small k_6' value, the level of triol sharply rose after 40-min heating (**Figure 8**). However, the formation of triol in the presence of stearylamine remained extremely slow even up to 2-h heating (**Figure 8**). This is probably because 5,6-epoxides reacted with stearylamine rapidly to form β-hydroxylamine, as reported by Ingold (*19*). In **Figures 6−8**, the curves fit well with the experimental data based on the oxidation of cholesterol during heating. These results suggested that the kinetic models developed in this study can be used to predict the concentration changes of COPs during heating of cholesterol with or without addition of stearylamine.

LITERATURE CITED

(1) Paniangvait, P.; King, A. J.; Jones, A. D.; German, B. G. Cholesterol oxides in foods of animal origin. *J. Food Sci.* **1995**, *60*, 1159−1174.

(2) Ansari, G. A. S.; Walker, R. D.; Smart, V. B.; Smith, L. L. Further investigations of mutagenic cholesterol preparation. *Food Chem. Toxicol.* **1982**, *20*, 35−41.

(3) Watanabe, K.; Nakamura; Hosono, A. Mutagenic activity of heat-induced chilesterol degredation products. *J. Food Sci.* **1988**, *53*(6), 1913−1916.

(4) Maerker, G. Cholesterol autoxidation-current status. *J. Am. Oil Chem. Soc.* **1987**, *64*(3), 388−392.

(5) Chien, J. T.; Wang, H. C.; Chen, B. H. Kinetic model of the cholesterol oxidation during heating. *J. Agric. Food Chem.* **1998**, *46*(7), 2572−2577.

(6) Kritchevsky, D. Dietary protein, lipid metabolism and atherosclerosis. *J. Am. Oil Chem. Soc.* **1978**, *64*, 1167−1171.

(7) Li, Y. J. Processing induced changes in fish lipid with emphasis on cholesterol. *Dissertation Abstr. Int. B: Sci. Eng.* **1992**, *52*(9), 4536−4537.

(8) Nawar, W. W.; Kim, S. K.; Vajdi, M. Measurement of oxidative interactions of cholesterol. *J. Am. Oil Chem. Soc.* **1991**, *68*(7), 496−498.

(9) Yen, G. C.; Kao, H. H. Antioxidative effect of biogenic amine on the peroxidation of linoleic acid. *Biosci., Biotechnol., Biochem.* **1993**, *57*(1), 115−116.

(10) Aoki, H.; Tottroi, T.; Sakurai, F.; Fuji, K.; Miyajima, K. Effects of positive charge density on the liposomal surface on disposition kinetics of liposomes in rats. *Int. J. Pharm.* **1997**, *156*, 163−174.

(11) Kokkona, M.; Kallinteri, P.; Fatouros, D.; Antimisiaris, S. G. Stability of SUV liposomes in the presence of cholate salts and pancreatic lipases: effect of lipid composition. *Eur. J. Pharm. Sci.* **2000**, *9*, 245−252.

(12) Alaiz, M.; Barragán, S. Reaction of a lysyl residue analogue with (*E*)-2-octenal. *Chem. Phys. Lipids* **1995**, *75*(1), 43−49.
(13) SAS. *SAS Procedures and SAS/Graph User's Guide*, version 6; Software Release 8.2 (TS2M0) of the SAS System for Microsoft Windows; SAS Institute, Inc.: Cary, NC, 2003.
(14) Smith, L. L.; Hill, F. L. Detection of sterol hydroperoxides on thin layer chromatoplates by means of the wurster dyes. *J. Chromatogr.* **1972**, *66*, 101−109.
(15) Maerker, G.; Bunick, F. J. Cholesterol oxides I: isolation and determination of some cholesterol oxidation products. *J. Am. Oil Chem. Soc.* **1986**, *63*(6), 767−771.
(16) Chien, J. T.; Lu, Y. F.; Hu, P. C.; Chen, B. H. Cholesterol photooxidation as affected by combination of riboflavin and fatty acids methyl esters. *Food Chem.* **2003**, *81*, 421−431.
(17) Alaiz, M.; Zamora, R.; Hidalgo, F. J. Contribution of the formation of oxidized lipid/amino acid reaction products to the protective role of amino acid in oils and fats. *J. Agric. Food Chem.* **1996**, *44*(7), 1890−1895.
(18) March, J. Chapter 10, Aliphatic nucleophilic substitution: Reaction 0−20, Alcoholysis of epoxides. *Advanced organic chemistry*, 2nd ed.; McGraw-Hill: New York, 1977; pp 360−361.
(19) Ingold, C. K. *Structure and mechanism in organic chemistry*, 2nd ed.; Cornell University Press: Ithaca, NY, 1969; pp 457−463.
(20) March, J. Chapter 10, Aliphatic nucleophilic substitution: Reaction 0−51, Amination of epoxides. *Advanced organic chemistry*, 2nd ed.; McGraw-Hill: New York, 1977; p 381.
(21) Smith, L. L. Cholesterol autoxidation 1981−1986. *Chem. Phys. Lipids* **1987**, *44*, 87−125.
(22) Park, S. W.; Addis, P. B. Identification and quantitation estimation of oxidized cholesterol derivatives in heated tallow. *J. Agric. Food Chem.* **1986**, *34*(4), 653−659.
(23) Park, S. W.; Addis, P. B. Further investigation of oxidized cholesterol derivatives in heated fats. *J. Food Sci.* **1986**, *51*(5), 1380−1381.
(24) Yan, P. S.; White, P. J. Linalyl acetate and methyl silicone effects on cholesterol and triglyceride oxidation in heated lard. *J. Am. Oil Chem. Soc.* **1990**, *68*(10), 763−768.
(25) Nielsen, J. H.; Olsen, C. E.; Skibsted, L. H. Cholesterol oxidation in a heterogeneous system initiated by water-soluble radicals. *Food Chem.* **1996**, *56*(1), 33−37.

Received for review June 28, 2004. Revised manuscript received August 31, 2004. Accepted September 5, 2004. This study was supported by a grant (NSC-90-2313-B-030-008) from the National Science Council, Taiwan, R.O.C.

JF048951+