Kinetic Model of the Cholesterol Oxidation during Heating

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The formation of cholesterol oxidation products (COPs) during heating of cholesterol at 150 °C for up to 30 min was kinetically studied using nonlinear regression models. The various COPs were analyzed by TLC and HPLC. Results showed that the COPs concentration increased with increasing heating time, and the cholesterol loss reached 64.8% after 30 min of heating. In the early stage of oxidation, the highest rate constant (h^{-1}) was observed for 7-hydroperoxycholesterol (7-OOH) formation, followed by epoxidation, dehydration, reduction, and dehydrogenation. The reactions for formation of 7-OOH (7α-OOH and 7β-OOH) and 5,6-epoxycholesterol (5,6α-EP and 5,6β-EP) fit the second-order, while the other reactions fit the first-order. The correlation coefficients (r^2) for the reactions ranged from 0.79 to 0.99. The kinetic model developed in this study can be used to predict the concentration changes of COPs during the heating of cholesterol.

Keywords: *Cholesterol oxidation; heating; kinetic model; HPLC analysis*

INTRODUCTION

Cholesterol is widely distributed in various kinds of foods, of which meat and egg products contain the most (Osada et al., 1993a; Paniangvait et al., 1995). Due to the presence of one double bond, cholesterol is susceptible to oxidation during heating and illumination (Pie et al., 1990; Kim and Nawar, 1991; Osada et al., 1993b). Many studies have shown that most cholesterol oxidation products (COPs) can be mutagenic and carcinogenic (Kandutsch et al., 1978; Bowden et al., 1979; Peng et al., 1979; Taylor et al., 1979; Imai et al., 1980; Ansari et al., 1982; Watanabe et al., 1988). Thus, it is necessary to study the mechanism of COPs formation during heating or illumination.

The oxidation pathways of cholesterol have been well documented (Smith, 1981; Maerker, 1987), and the mechanism of cholesterol oxidation is reported to be similar to that of lipid oxidation because both can undergo a series of free radical chain reactions to form peroxides and other degradation products (Smith, 1981). It has been established that the initiation period of lipid oxidation belongs to the first-order reaction, and the second-order reaction follows afterward (Bateman et al., 1953). The kinetic models of lipid oxidation have been thoroughly reviewed by Labuza (1971) and were applied to study the oxidation of polyunsaturated fatty acids (Adachi et al., 1995), the oxidation of lipids in dehydrated foods (Kacyn et al., 1983), and the oxidation of lipids in foods under various processing conditions (Ozilgen and Ozilgen, 1990). However, the kinetic study of cholesterol oxidation is seldom conducted. Yan and White (1990) studied the kinetics of cholesterol oxidation during the heating of lard and found that the reactions for the formation of 7-hydroxycholesterol (7-OH), 7-ketocholesterol (7-keto) and 5,6-epoxycholesterol (5,6-EP) fit the first-order. In contrast, in another study Park and Addis (1986) reported that the reaction for the formation of 7-keto during heating fits the zero-order. As these results are contradictory and no general mathematic models of cholesterol oxidation have been proposed, it is necessary to develop the rate laws for the elementary reaction steps. Also, the reaction orders of COPs formation from cholesterol during heating have to be examined. Finally, the precision of the kinetic equations of cholesterol oxidation through nonlinear regression analysis needs to be evaluated.

MATERIALS AND METHODS

Materials. Cholesterol and COPs standards, 7-ketocholesterol (7-keto), 7α-hydroxycholesterol (7α-OH), 7β-hydroxycholesterol (7β-OH), 5,6α-epoxycholesterol (5,6α-EP), and 5,6β-epoxycholesterol (5,6β-EP) were purchased from Sigma Chemical Co. (St. Louis, MO) and Steraloids Inc. (Wilton, NH). Spraying reagent, *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride, was also from Sigma. TLC adsorbent silica gel 60 GF254 and reagent benzoyl peroxide were from Merck Co. (Darmstadt, Germany). The analytical grade solvents, chloroform, benzene, methanol, and ethyl acetate, were 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 (250 × 4.6 mm i.d.) containing 5-μm packing material was from Merck Co. A CHEN-WIN computer software system (Shuen-Hua Co., Taipei, Taiwan) was used to process data.

Methods. *I. Heating of Cholesterol.* Approximately 50 mg of cholesterol standard was dissolved in 4 mL of chloroform, and the solution was placed in a 100-mL round-bottom flask. After evaporation of solvent with a rotary evaporator, a thin film of ~77 cm^2 was formed on the inner surface of the bottom of the flask. After flushing with nitrogen, the flask was preheated to 150 °C in an oil bath (200 °C) for 38 s. The flask was then removed and placed in another oil bath set at 150 °C, and oxygen was pumped through at a rate of 10 mL/min. The heating time, 0, 1.5, 3.0, 6.0, 10, 15, and 30 min, started to count as soon as oxygen was pumped. After heating, the flask was cooled in dry ice to terminate the reaction. Four milliliters of chloroform was added to the flask to dissolve cholesterol and COPs, and the solution was poured into a small

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glass vial. After evaporation of solvent with nitrogen gas, the residue was dissolved in 1 mL of chloroform for TLC and in 1 mL of hexane/2-propanol (95:5, v/v) for HPLC. For HPLC analysis the solution was filtered through a 0.2-μm membrane filter. Duplicate treatments and triplicate HPLC analyses were conducted, and mean values were determined. The data were subjected to nonlinear regression analysis (SAS, 1990).

II. TLC Analysis of COPs. 1. Preparation of TLC Plate. An appropriate amount of silica gel was dissolved in water (1:2, v/v) to form a slurry mixture, which was then spread uniformly on the glass plate (20 × 20 cm) as a thin film with a thickness of 250 μm. The plate was dried in the air at ambient temperature and activated at 110 °C for 2 h in an oven prior to TLC analysis.

2. Preparation of Wurster Dye. The Wurster dye was prepared using a method described by Smith and Hill (1972). One gram of *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride was dissolved in 100 mL of 50% methanolic solution (in H_2O), and the mixture was shaken thoroughly. One milliliter of acetic acid was added and mixed thoroughly, and the solution was poured into a glass bottle and stored at −20 °C until use.

3. 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) (Maerker and Bunick, 1986) prior to development. A 10-μL volume of extract was spotted on the glass plate using a micropipet. The chromatogram was developed over a distance of 15 cm at ambient temperature, after which the plate was dried and the color development of COPs under UV radiation at 254 nm was observed. Both the 7α-OOH and 7β-OOH bands were then sprayed with the Wurster dye, while the other COPs bands were sprayed with 50% H_2SO_4. The COPs bands were further heated at 110 °C in an oven for 10 min for color development as reported by Chen et al. (1994).

III. HPLC Analysis of COPs. A mobile phase of *n*-hexane/2-propanol (95:5, v/v) with a flow rate of 1.0 mL/min and sensitivity of 16×10^{-5} RIU was used (Chen and Chen, 1994). The injection volume was 20 μL and the column temperature 30 °C. The various COPs were identified by (1) comparison of retention times of unknown peaks with those of reference standards, (2) addition of standards to sample for cochromatography, and (3) collection of eluates for TLC analysis.

Each COP was quantified using an external calibration method. Eight concentrations of each COP ranging from 10^4 to 10^7 ppm were injected onto the HPLC, 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 using CHEN-WIN computer software system. Each COP was quantified using a method described by Chen et al. (1994). Due to the absence of standards of cholesterol hydroperoxides, the quantification of 7-OOH (7α-OOH and 7β-OOH) was carried out using a method similar to that described by Smith and Hill (1972). Benzoyl peroxide was used instead of 7-OOH because both could react with the Wurster dye to form the same red compound with maximum absorption (λ_{max}) at 554 nm (Smith and Hill, 1972). The eluates of 7α-OOH and 7β-OOH were collected individually and then reacted with the Wurster dye, and the absorbance of each solution was measured at 554 nm. The result was then compared with that of the standard curve, which was prepared using four concentrations of benzoyl peroxide and one concentration of the Wurster dye.

Kinetic Analysis of COPs. The various concentration changes of cholesterol and COPs during heating of cholesterol thin film at 150 °C were subjected to statistical analysis using a nonlinear regression procedure (SAS, 1990), and the parameters of a nonlinear model by using the least-squares method were estimated. The rate constant and correlation coefficient of each kinetic equation of cholesterol oxidation were determined from six data points. The precision of the parameters of the kinetic equations was also assessed.

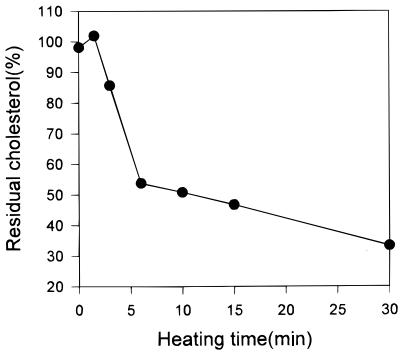

Figure 1. Percentage changes of cholesterol during heating at 150 °C for up to 30 min.

RESULTS AND DISCUSSION

Percentage Changes of Cholesterol during Heating. Cholesterol (T_m = 148.5 °C) is reported to be more stable in solid form than in liquid form, and the latter is more susceptible to oxidation than the former (Nawar et al., 1991; Kim and Nawar, 1993). In addition, cholesterol is reported to be more susceptible to degradation during heating at 200−300 °C (Park and Addis, 1986). Results of our preliminary studies showed that cholesterol oxidation is too fast when accomplished at 200 °C and above. After various studies, the most appropriate temperature for kinetic study was found to be 150 °C. Also, this temperature is similar to that used for low-temperature frying of foods such as instant noodle. Thus, in this study the heating temperature 150 °C was used for the reaction of cholesterol oxidation to proceed. Figure 1 shows the percentage changes of cholesterol during heating at 150 °C for up to 30 min. Although the percentage of residual cholesterol rose slightly after 1.5 min, the change was not significant ($p > 0.05$) and represents the induction period of oxidation. During 1.5−6 min, the cholesterol percentage decreased sharply and then decreased gradually during prolonged heating from 6 to 30 min. The residual percentage of cholesterol was 33.3% after 30 min of heating. In a similar study, Osada et al. (1993b) studied cholesterol stability during heating at 150 °C for 24 h in an electric oven and found that ∼35% cholesterol remained. This result indicated that differences in heating conditions may accelerate or delay the oxidation rate of cholesterol. In the current study, a flask containing cholesterol spread over the surface as a thin film was preheated to 150 °C in an oil bath (200 °C) for 38 s prior to being placed in another oil bath set at 150 °C. However, the preheating treatment resulted in the degradation of 1.87% cholesterol. Thus, the initial percentage of cholesterol was counted as 98.13%.

HPLC Analysis of COPs. Figure 2 shows the HPLC chromatograms of COPs during heating of cholesterol for up to 30 min. Cholesterol and seven COPs, 5,6α-EP, 5,6β-EP, 7-keto, 7α-OOH, 7β-OOH, 7α-OH, and 7β-OH, were identified. The identification of 7α-OOH and 7β-OOH was based on the color development on the TLC plates after spraying with the Wurster dye. According to a paper by Smith and Hill (1972), both 7α-OOH and 7β-OOH could become red in the presence of *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride. In addition, the R_f values for 7α-OOH and 7β-OOH were 0.35 and 0.39, respectively, when a mobile phase of benzene/ethyl acetate (60:40, v/v) was employed. In a similar study Maerker and Bunick (1986) also used the same

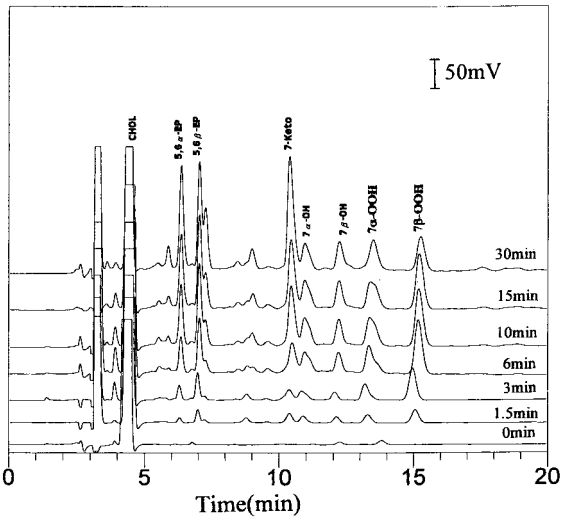

Figure 2. HPLC chromatograms of COPs during heating of cholesterol at 150 °C for up to 30 min.

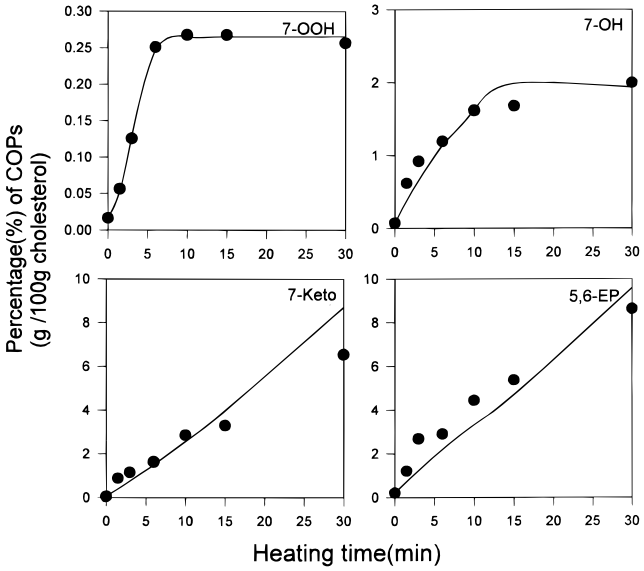

Figure 3. Percentage of COPs formed during heating of cholesterol at 150 °C for up to 30 min: ●, mean of experimental data; —, the best fitting line.

mobile phase to separate 7α-OOH and 7β-OOH with the R_f values 0.37 and 0.40, respectively.

Figure 3 shows the percentages of the major COPs formed during heating at 150 °C for up to 30 min. The percentages of 7-OOH (7α-OOH and 7β-OOH) increased sharply during the initial heating period and then reached a plateau after heating for 6 min or more. Likewise, the percentage of 7-OH (7α-OH and 7β-OH) also increased sharply during the first 10 min of heating and reached a plateau thereafter. Unlike 7-OOH and 7-OH, the percentages of both 7-keto and 5,6-EP (5,6α-EP and 5,6β-EP) increased with increasing heating time over a 30-min period. The formation of 7-keto can be attributed to dehydration of 7-OOH or dehydrogenation of 7-OH under dry and oxygen-rich conditions (Teng et al., 1973; Kim and Nawar, 1993; Nielsen et al., 1996). For 5,6-EP formation, it has been reported that the amount of 7-OOH formed during heating should be enough for the reaction of epoxidation to proceed (Smith, 1981, 1987; Maerker and Bunick, 1986). In this study, epoxide formation was observed during the initial period of heating, probably because the cholesterol thin film

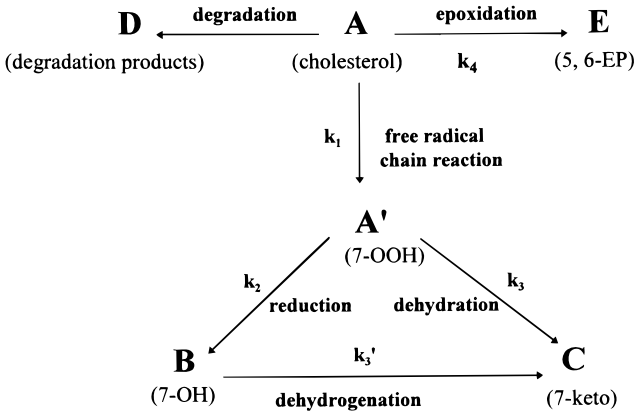

Figure 4. Major pathways of cholesterol oxidation: A, cholesterol; A′, 7-hydroperoxycholesterol (7-OOH); B, 7-hydroxycholesterol (7-OH); C, 7-ketocholesterol (7-keto); E, 5,6-epoxycholesterol (5,6-EP); D, degradation products; k_1−k_4, k_3', rate constants of the reactions of cholesterol oxidation.

was preheated and the heating time required for reaching 150 °C is very short. Thus, the 7-OOH was formed in adequate amounts for the reaction of epoxide formation to proceed at the beginning of heating. In another study, Maerker and Bunick (1986) also demonstrated that the 5,6-EP concentration increased along with increasing 7-OOH concentration during heating of cholesterol at 80 °C in an aqueous dispersion at pH 8 for 24 h. However, in our study the amount of 7-OOH increased only up to 10 min and then reached a steady state. This result implied that differences in heating treatment of cholesterol may greatly affect the formation and degradation rates of 7-OOH. The 5,6-EP was formed in the greatest amount, followed by 7-keto, 7-OH, and 7-OOH.

Kinetic Study of Cholesterol Oxidation during Heating. Due to the complexity of COPs formation during heating, only four major COPs, 7-OOH (7α-OOH and 7β-OOH), 7-OH (7α-OH and 7β-OH), 7-keto, and 5,6-EP (5,6α-EP and 5,6β-EP), were selected for kinetic study. Figure 4 shows the major pathways of cholesterol oxidation. The reaction can be divided into two major routes of C-7 oxidation and epoxidation. The former (C-7) implies that cholesterol oxidation is initiated by hydrogen abstraction, predominantly at C-7. The C-7 oxidation route includes (1) formation of 7-OOH (A′) from cholesterol (A) through free radical chain reaction (A → A′), (2) formation of 7-OH (B) from 7-OOH through reduction (A′ → B), (3) formation of 7-keto (C) from 7-OOH through dehydration (A′ → C), and (4) formation of 7-keto (C) from 7-OH through dehydrogenation (B → C). The epoxidation route includes the formation of 5,6-EP (E) from cholesterol through 7-OOH (A + A′ → E). In addition, cholesterol may also be degraded to form other products (D) such as short-chain hydrocarbons, aldehydes, ketones, or alcohols. However, the degradation reaction was not kinetically studied because these degraded compounds were not identified. Also, it can make the kinetic study even more complex.

Because the mechanism of cholesterol oxidation is similar to that of lipid oxidation, the kinetic reaction of the latter can be applied to study the kinetic equation of the former. It has been reported that the reaction of lipid oxidation belongs to the first-order if the concentration of hydroperoxides formed is <0.02 M during the induction period (Bateman et al., 1953). In contrast,

the reaction belongs to the second-order if the hydroperoxide concentration is >0.02 M (Bateman et al., 1953; Labuza, 1971; Kacyn et al., 1983; Weist and Karel, 1992). In addition, the oxygen concentration should also be considered because it can affect the reaction rate (Labuza, 1971). In the current study, oxygen was continuously provided during heating of cholesterol thin film at 150 °C so that the induction period can be shortened. Therefore, in the kinetic study of cholesterol oxidation the oxygen concentration can be regarded as a constant and can be ignored. On the basis of the studies of Labuza (1971) and Ozilgen and Ozilgen (1990), the initiation and propagation reactions dominate in the early stage of lipid oxidation, and the probability for the termination reaction to occur is low. Once free radicals ($R^{\bullet}$) are produced, it is very likely that the peroxy free radicals ($ROO^{\bullet}$) can be formed afterward in the presence of abundant oxygen. This implies that in the early stage of oxidation the subsequent hydroperoxide (ROOH) formation can be assumed to occur between the peroxy free radicals and the predominant substrate (RH) because the latter is present at a greater amount than the former. Thus, the following rate equation for 7-OOH formation in the early stage of cholesterol oxidation can be proposed:

$$dC_{A'}/dt = k_i C_{A'} C_A \quad (1)$$

In eq 1 $C_{A'}$ is the concentration of 7-OOH, C_A is concentration of unreacted cholesterol, k_i is the reaction rate constant, t is the time.

For convenience in calculation, the fraction of conversion based on the initial concentration of cholesterol is used. Equation 1 is in the same form as the equation applied to study kinetics of lipid oxidation by Ozilgen and Ozilgen (1990), who used the empirical approach of the Logistic Equation. Therefore, the oxidative degradation of lipids belongs to the second-order, and this result can be used to derive the rate equation for 7-OOH formation. The following equation was obtained:

$$\frac{d[A']}{dt} = k_1[A']\left(1 - \frac{[A']}{[A'_{max}]}\right) \quad (2)$$

In eq 2 [A′] is the percentage concentration of 7-OOH, $[A'_{max}]$ is the maximum attainable concentration of 7-OOH prior to degradation, k_1 is the reaction rate constant, and t is the time.

The high value of the rate constant k_1 in eq 2 indicated that a large amount of free radicals might be involved in the propagation step and eventually would result in a higher A'_{max} concentration.

According to the reaction pathways of cholesterol oxidation (Figure 4), 7-OOH can be further degraded to 7-OH and 7-keto through the first-order reaction (Yan and White, 1990). The rate equation for 7-OOH formation was thus modified as follows:

$$\frac{d[A']}{dt} = k_1\left(1 - \frac{[A']}{[A'_{max}]}\right)[A'] - k_2[A'] - k_3[A'] \quad (3)$$

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

k_2 and k_3 are the reaction rate constants.

In addition, the reaction for formation of 7-keto from 7-OH through dehydrogenation was assessed to be first-order according to a paper by Nielsen et al. (1996). Thus, the rate equations of C-7 oxidation routes B and C could be written as

$$d[B]/dt = k_2[A'] - k_3'[B] \quad (4)$$

$$d[C]/dt = k_3[A'] + k3'[B] \quad (5)$$

where [B] is the percentage concentration of 7-OH and [C] is the percentage concentration of 7-keto.

For formation of 5,6-EP from cholesterol, the concentrations of both cholesterol and 7-OOH should be considered (Smith, 1987). Thus, the rate equations for both 5,6-EP formation and concentration decrease of cholesterol (because of epoxidation and degradation) could be respectively written as follows:

$$d[E]/dt = k_4[A]\,[A'] \quad (6)$$

$$-d[A]/dt = k_4[A]\,[A'] + k_5[A] \quad (7)$$

In eqs 6 and 7 [A] is the cholesterol concentration, [E] is the percentage concentration of 5,6-EP, and k_4 and k_5 are reaction rate constants.

As the concentration of 7-OOH formed in the early stage of oxidation was found to be much lower than cholesterol concentration ($1300[A'] \cong [A]$), the first term ($k_4[A][A']$) in eq 7 is relatively small when compared to the second term ($k_5[A]$). Furthermore, by comparing the percentages of 5,6-EP formed (8.6% at heating time of 30 min) and cholesterol degraded (64.8% at heating time of 30 min), it can be found that cholesterol degradation is the main reaction to proceed. Thus, eq 7 could be simplified as follows:

$$-d[A]/dt = k5[A] \quad (8)$$

The integration of eq 8 gave

$$[A] = [A_0]e^{-k_5 t} \quad (9)$$

Equation 3 is in the form of a Bernoulli equation ($n = 2$). By transformation of a variable [A′], the following eq 10 was obtained by integration.

I. C-7 Oxidation Route

$$[A'] = \left[\frac{M_2}{M_1} + \left(\frac{1}{[A'_0]} - \frac{M_2}{M_1}\right)e^{-M_1 t}\right]^{-1} \quad (10)$$

$M_1 = k_1 - k_2 - k_3$ and $M_2 = k_1/[A'_{max}]$.

Substituting eq 10 into eqs 4 and 5, eqs 11 and 12 could be integrated as follows:

$$[B] = [B_0]e^{-k_3' t} + e^{-k_3' t}\int_0^t \frac{k_2 e^{k_3' s}}{\frac{M_2}{M_1} + \left(\frac{1}{[A'_0]} - \frac{M_2}{M_1}\right)e^{-M_1 s}}ds \quad (11)$$

$$[C] = [C_0] + \int_0^t \frac{k_3}{\frac{M_2}{M_1} + \left(\frac{1}{[A'_0]} - \frac{M_2}{M_1}\right)e^{-M_1 s}}ds + \int_0^t k_3'\left[[B_0]e^{-k_3' s} + e^{-k_3' s}\int_0^s \frac{k_2 e^{k_3' \tau}}{\frac{M_2}{M_1} + \left(\frac{1}{[A_0']} - \frac{M_2}{M_1}\right)e^{-M_1 t}}d\tau\right]ds \quad (12)$$

Table 1. Rate Constants of the Major Pathways of Cholesterol Oxidation

rate eq[a]	reaction	k (h^{-1})	r^2
Chol $\xrightarrow{k_1}$ 7-OOH (7α-OOH and 7β-OOH)	free radical chain reaction	1587 ± 1[b]	0.99
7-OOH $\xrightarrow{k_2}$ 7-OH (7α-OH and 7β-OH)	reduction	781 ± 107	0.91
7-OOH $\xrightarrow{k_3}$ 7-keto	dehydration	805 ± 2	0.79
7-OH $\xrightarrow{k_3'}$ 7-keto	dehydrogenation	3 ± 2	0.91
Chol $\xrightarrow{k_4}$ 5,6-EP (5,6α- and 5,6β-EP)	epoxidation	1357 ± 358	0.88

[a] Chol, cholesterol; 7-OOH, 7-hydroperoxycholesterol; 7-OH, 7-hydroxycholesterol; 7-keto, 7-ketocholesterol; 5,6-EP, 5,6-expoxycholesterol. [b] Mean ± standard deviation.

II. Epoxidation Route

$$[E] = [E_0] + k_4[A_0]\int_0^t \frac{e^{-k_5 t}}{\frac{M_2}{M_1} + \left[\frac{1}{[A'_0]} - \frac{M_2}{M_1}\right]e^{-M_1 t}}dt \quad (13)$$

In eq 13 $M_1 = k_1 - k_2 - k_3$, $M_2 = k_1/[A'_{max}]$, $[A_0]$ is the percentage concentration of cholesterol at heating time of 0 min, $[A'_0]$ is the percentage concentration of 7-OOH at heating time of 0 min, $[B_0]$ is the percentage concentration of 7-OH at heating time of 0 min, $[C_0]$ is the percentage concentration of 7-keto at heating time of 0 min, $[E_0]$ is the percentage concentration of 5,6-EP at heating time of 0 min, and s and t are the time.

Equation 13 was obtained by integration after substitution of eqs 9 and 10 into eq 6. As the exact integrations for eqs 11−13 are not possible, we can only get a better approximation to the integral term in each equation by using a quadratic polynomial of Taylor's series. The experimental data were thus analyzed in sequence and were fit best to the corresponding nonlinear regression model according to the C-7 oxidation and epoxidation pathways.

Table 1 shows the rate constants of the reaction pathways of cholesterol oxidation. The data were obtained from eqs 10−13 with a nonlinear regression model. With the exception of the dehydration reaction, the correlation coefficients (r^2) of all the other reactions were >0.88. This result implied that the above equations can be used to predict the concentration changes of COPs during the initial and propagation period of oxidation. Equations 9−13 were also shown to be the major kinetic equations of cholesterol oxidation during heating in the presence of oxygen. Of the various reactions, the correlation coefficient of the free radical chain reaction (eq 10) was 0.99. As this equation is derived from lipid oxidation, it can be inferred that the reaction for formation of 7-OOH from cholesterol fit the second-order. For the reduction and dehydrogenation reactions, the correlation coefficients of eqs 11 and 12 were the same (0.91). This result also demonstrated that the reactions for formation of 7-OH and 7-keto and for dehydrogenation of 7-OH fit the first-order. The formation of 5,6-EP in the presence of cholesterol and 7-OOH can be further demonstrated from eq 13 with a correlation coefficient of 0.88, and the reaction fits the second-order. Similar results were observed by Yan and White (1990), who studied cholesterol oxidation in lard containing two levels of added cholesterol during heating at 180 °C for 240 and 160 h.

From Table 1 it can be found that the k_1 value is very large, showing that the 7-OH concentration increased very rapidly during the initial period of oxidation. Compared to the sum of k_2 and k_3, the k_1 value is slightly higher. This phenomenon indicated that most 7-OOH was converted to 7-OH or 7-keto as soon as it were formed, and the 7-OOH concentration was thus maintained at low level (0.27%) after the heating time reached 6 min and above. Before 6 min, the 7-OOH concentration was maintained at an even lower level, and the reaction rate was mainly dependent upon the first term ($(k_1 - k_2 - k_3)[A']$) in eq 3. However, at a slightly higher concentration (after 6 min) the effect of the second term $[-k_1([A']^2/[A'_{max}])]$ in eq 3 would be greater, and the reaction rate could be almost equal to zero after 7-OOH concentration reached a certain level. According to the theory of free radical chain reaction, there is a maximum attainable concentration for 7-OOH. From the nonlinear regression analysis in this study, the highest attainable level of 7-OOH ($A'_{max} = 7.9\%$) was formed during heating. However, in practice it is impossible for 7-OOH to reach this level because it can be further degraded to form 7-OH and 7-keto. As the k_3 value was shown to be higher than the k_2 value, it can be inferred that dehydration is more susceptible to proceeding than reduction under dry condition (Park and Addis, 1986). Also, the formation rate of 7-keto was found to increase with increasing 7-OH concentration. Similar results were reported by Nielsen et al. (1996), who studied the cholesterol oxidation in a heterogeneous system initiated by water-soluble radicals and found that 7-keto was the major COP formed through a two-step radical reaction by dehydrogenation of 7-OH. Despite the small k_3' value, the formation curve of 7-OH was thus affected and the percentage concentration did not increase after the heating time reached 10 min (Figure 3). In contrast, the 7-keto concentration increased almost linearly with heating time over a 30-min period. This result often leads to an incorrect conclusion that the formation rate of 7-keto belongs to the zero-order, which was previously reported by Park and Addis (1986).

By comparing the rate constants of the major reaction pathways of cholesterol oxidation, it can be found that $k_1 > k_4 > k_3 > k_2 > k_3'$. This result seemed to be quite different from that of Yan and White (1990), who reported that the rate constants for 7-keto formation were 0.8×10^{-3} and 1.0×1.0^{-2} h^{-1} during heating of lard with two levels of cholesterol added. This difference can be attributed to the fact that the cholesterol concentration used by the authors (Yan and White, 1990) was much lower than that used in the current study and the data were analyzed by a linear regression model.

Due to the complexity of food itself, it is difficult to study cholesterol oxidation in real food systems. Thus, to do a neat and clean study of the oxidation, having only cholesterol present in the system makes it a much easier study. The results observed in this study may not be identical to those in real food systems because of numerous factors such as the presence of water, protein, fat, carbohydrate, and other components in foods. For instance, Kim and Nawar (1991) demonstrated that the stability of cholesterol in complex mixtures is influenced by interaction among lipid components and/or their decomposition products. Yan and White (1990) also concluded that the accumulation of COPs in a high-temperature, cholesterol-rich food system is a dynamic one, depending upon the amount of cholesterol present, the treatment of oil (intermittent or continuous heating), and the severity of the heat treatment. Finally, in the current study the amount of cholesterol lost is not equal to the amount of COPs formed, probably because cholesterol may also be degraded to other compounds that were not measured.

In conclusion, the major reaction pathways during heating of cholesterol include free radical chain reaction, reduction, dehydration, dehydrogenation, and epoxidation, and the correlation coefficients for the reactions are 0.99, 0.91, 0.79, 0.91, and 0.88, respectively, by nonlinear regression analyses. The reactions for 7-OOH and 5,6-EP formation fit the second-order, while all of the other reactions fit the first-order. The kinetic model developed in this study can be used to predict the concentration changes of COPs during initiation and propagation periods of oxidation of cholesterol during heating.

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