Kinetic Analysis of Temperature-Programmed Autodestruction of Ethyl Eicosapentaenoate and Ethyl Docosahexaenoate

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On the basis of an autocatalytic and a first order reaction kinetics, a nonisothermal oxidation reaction model was developed for n-3 polysaturated fatty acid (PUFA) under temperature-programmed linear heating conditions. With this model, the activation energy of oxidative reaction can easily be obtained with at least three linear heating conditions. The temperature-programmed oxidation experiments of ethyl eicosapentaenoate and ethyl docosahexaenoate were done under linear heating conditions of 4 to 12 K/h. The activation energies and the frequency factors obtained were in good agreement with those by the isothermal oxidation experiments reported previously.

Key words: temperature-programmed autodestruction; DHA; EPA; kinetic energy

To use the n-3 polysaturated fatty acids (PUFA) as food additives, it is important to understand the oxidation kinetics of PUFA, since PUFA have chemically quite reactive properties.1,2 However, quantitative analyses of the oxidative kinetics of PUFAs are very few compared to food chemical research into the oxidative stability of PUFAs.3,4 Özilgen and Özilgen,5 and Adachi et al.6 have suggested that the entire oxidation period could be expressed by simple equations of the unoxidized substrate concentration; an autocatalytic reaction and a first order reaction. Yoshii et al.,6,8 analyzed the oxidation reaction constants of ethyl eicosapentaenoate at different oxygen levels on the basis of the reaction kinetics proposed by Adachi et al.,6,8 suggesting that the oxidation reaction constants could be expressed by the Langmuir equation, and that the Langmuir constant was independent of temperature.

The oxidative experiments on PUFAs have been usually done under isothermal conditions.4,6,7 To measure the kinetics parameters, such as the activation energy, it is necessary to carry out several calculation steps which might easily lead to calculation errors. The temperature-programmed method (TPM) presented in this study involves a very simple experimental procedure to measure the activation energy of the oxidative reaction.

The TPM has been widely used to analyze chemical reaction kinetics. The kinetic parameters such as the activation energy and the frequency constant can be easily calculated by a few experiments, in which the reaction progresses under a linearly increasing sample temperature with time (linear heating). Niwa et al.9 analyzed the kinetics of the adsorption of ammonia from zeolite by TPM. The activation energy of desorption could be calculated from the relationship between the temperature at the peak maximum of the concentration of the desorbed ammonia in the carrier gas. For studies of drug stability, Zhan et al.10 demonstrated the applicability of the nonisothermal linear heating and cooling method to measure the rate order of the degradation and shelf-life of vitamin C tablets. Yoshii et al.11 applied the TPM to the soy-protein denaturation of defatted soy flour and isolated soy protein of various low moisture contents. The degree of denaturation of the soy protein was expressed as a function of the temperature of the protein. The activation energy of denaturation could be obtained by a graphical fitting method with the experimental denaturation vs. temperature curves under various linear heating conditions.

In this study, the temperature-programmed oxidative experiments of ethyl eicosapentaenoate and ethyl docosahexaenoate were done under various linear heating rates. The theoretical relationships between the unoxidized fraction of PUFA ester and temperature were derived on the basis of the kinetic models proposed by Adachi et al.,6,8 and the experimental findings by Yoshii et al.,6,8 in which the Langmuir constants were independent of temperature. The activation energies of the oxidation reaction could be found from the oxidation curves of PUFA against temperature, and were compared with those of isothermal oxidation to examine the potential use of the TPM in the autodestruction of PUFA esters.

Materials and Methods

Materials. Ethyl eicosapentaenoate (EPA, purity 97%) was a gift from Q.P. Corporation (Tokyo, Japan),

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Abbreviations: TPM, temperature-programmed method; EPA, ethyl eicosapentaenoate; DHA, ethyl docosahexaenoate; PUFA, polyunsaturated fatty acid
and ethyl docosahexaenoate (DHA, purity 95%) was from Harima Chemicals Inc. (Tsukuba, Japan). Other chemicals were of analytical grade.

**Temperature-programmed autoxidation of EPA or DHA.** Temperature-programmed autoxidation of EPA or DHA was investigated using the equipment shown in Fig. 1, which is basically similar to the equipment in previous works. Ten to twelve flat bottomed glass bottles (16 x 30 mm) (9) containing EPA or DHA of 4 μl were connected at a time in a series by Tygon tubes (Weyerhaeuser, OH), and placed in an air bath (3). The gas mixture of oxygen and nitrogen gas (oxygen level of 3-20%) was blown through the tube at a rate of 40 ml/min. The temperature of the air bath was linearly increased with time at the varying rate of 4 to 12 K/h by using a programmable temperature controller (7) (Chino DB-1000, Tokyo). After a prescribed time, the glass bottle was taken out, and the unoxidized amount of EPA or DHA was measured by the procedure described previously.

**Temperature-programmed autoxidation analysis.** The mathematical model of the oxidation of EPA and DHA under linear heating conditions was developed on the basis of the oxidation kinetics proposed by Adachi et al. and Yoshih et al. In isothermal oxidation, the autoxidative reaction progresses autocatalytically in the first half of the autoxidation period, followed by a first order reaction in the latter half, as described by Eq. 1:

\[
dY/dt = -k_1f(Y) \quad (i = a, 1)
\]

where \( f(Y) = Y(1 - Y) \) for \( Y \geq 0.5 \) and \( f(Y) = Y \) for \( Y < 0.5 \), \( Y \) is the oxidized fraction of EPA or DHA, and \( k_1 \) means the respective reaction rate constants of autoxidative \((k_a)\) and first order \((k_1)\) reactions. Yoshih et al. indicated that the reaction rate constant \( k_1 \) could be expressed as functions of the oxygen level \( C_o \) and temperature \( T \) by Eq. 2:

\[
k_1 = k_{o1} \exp \left( -E_1/RT \right) C_o / (K_1 + C_o) \quad (i = a, 1)
\]

where \( E_1(=E_a or E_1) \) is the activation energy, and Langmuir parameters \( K_1(=K_a or K_1) \) are constant and independent of temperature. When the temperature of PUFA, \( T \), is increased at a constant rate \( \alpha \) as a function of time (i.e. \( T = T_o + \alpha t, T_o : \) the initial temperature), Eq. 1 can be formally described in terms of \( T \) as Eq. 3:

\[
dY/dT = (-k_{o1}f(Y)/\alpha) \exp \left( -E_1/RT \right) \quad (i = 1, 1)
\]

where \( k_{o1}(=k_{o1} or k_{o1}) \) is the modified frequency factor equal to \( k_{o1} \) multiplied by \( C_o / (K_1 + C_o) \) in Eq. 2.

In the period of \( Y \leq 0.5 \), where the oxidative reaction progresses autocatalytically, integration of Eq. 3 yields Eq. 4:

\[
\int_{Y_0}^{Y_0} dY/Y(1-Y) = -\int_{T_o}^{T} \exp \left( -E_1/RT \right) dT
\]

Although \( k_{o1} \) is in general dependent on temperature because \( C_o \) is a function of temperature, the variation of \( k_{o1} \) between the initial and the final temperature was less than 5%. Therefore, we assumed that \( k_{o1} \) was constant in deriving Eq. 4. When one can assume that the reaction proceeds slowly at \( T_o \) we can replace \( T_o \) in Eq. 4 with 0, and then obtain Eq. 5:

\[
\ln \left( y/y_0 \right) = \left( -k_{o1}E_1/\alpha R \right) \int_{t}^{0} \exp \left( -E_1/RT \right) du
\]

where \( y = Y(1 - Y), y_0 = Y_0/(1 - Y_0), \) and \( u = E_1/RT \). The partial integration of RHS of Eq. 5 has been proposed to be approximated with the following Eq. 6:

\[
-e^{-u} + \int_{-\infty}^{0} (e^{-u}/u) du = -e^{-u}/u
\]

Substituting Eq. 6 into Eq. 5, we can obtain Eq. 7:

\[
y/y_0 = \exp \left\{ (-k_{o1} R/\alpha E_1) T^2 \exp \left( -E_1/RT \right) \right\}
\]

Substitution of \( y = 1 \) (i.e. \( Y = 0.5 \)) in Eq. 7 and taking a logarithm of the resulting equation yields Eq. 8:

\[
\ln \left( T_w/\alpha \right) = \left( E_1/E_a \right) / T + \ln \left( y/y_0 \right) + \ln \left( E_a/k_{o1} R \right)
\]

where \( T_w \) is the temperature of PUFA ester at \( Y = 0.5 \). Equation 8 indicates that the activation energy \( E_1 \) can be calculated by a linear plot of \( \ln \left( T_w/\alpha \right) \) against \( 1/T_w \) for a different increasing rate of PUFA's temperature.

When the autoxidation proceeded beyond the first half Eq. 3 should be integrated in two regions, using a different equation of \( f(Y) \) for each region; \( f(Y) = Y(1 - Y) \) for \( Y = Y_0 \) to 0.5 and \( f(Y) = Y \) for \( Y = 0.5 \) to \( Y \). The integration of Eq. 3 can be written as Eq. 9:

\[
\int_{Y_0}^{0.5} dY/Y(1-Y) + \int_{0.5}^{Y} dY/Y = -\left\{ k_{o1} \int_{T_o}^{T} \exp \left( -E_1/RT \right) dT + k_{o1} \int_{T_o}^{T} \exp \left( -E_1/RT \right) dT \right\} \left( \alpha \right)
\]

The integration in the second term of RHS of Eq. 9 can be written as Eq. 10:

\[
\int_{T_o}^{T} \exp \left( -E_1/RT \right) dT
\]
\[ \int_{0}^{T} \exp \left( -E_i / RT \right) dT \left[ \exp \left( -E_i / RT \right) dT \right] \]

Using the approximation of Eq. 6, the first integration term of RHS in Eq. 10 can be written as Eq. 11:

\[ \int_{0}^{T} \exp \left( -E_i / RT \right) dT = (RT^2 / E_i) \exp \left( -E_i / RT \right) \]

The substitution of Eq. 11 into Eqs. 10 and 9 yields Eq. 12:

\[ -\ln \left( \frac{Y_0}{1 - Y_0} \right) + \ln \left( \frac{Y}{0.5} \right) = -\frac{k_{10}R}{\alpha E_i T^2} \exp \left( \frac{-E_i}{RT} \right) + \frac{R}{\alpha} \frac{T^2}{E_i} \exp \left( \frac{-E_i}{RT} \right) \]

Finally, substituting \( Y = 0.25 \) and \( T = T_n \) into Eq. 12, and taking logarithms of both sides, we can obtain Eq. 13.

\[ \ln \left( T_n^2 / \alpha \right) = (E_i / R) / T_n + \ln A \]

where \( T_n \) is the temperature of PUFA at \( Y = 0.25 \), and \( A \) is a constant written as Eq. 14:

\[ A = -\ln \left( \{0.5Y_0 / (1 - Y_0)\} + (RT_n^2 / \alpha) (k_{10} / E_i) \exp \left( -E_i / RT_m \right) \right) \]

The activation energy \( E_i \) for the first order oxidative reaction can be evaluated in a similar manner as \( E_{av} \), i.e. by plotting \( \ln \left( T_n^2 / \alpha \right) \) against \( 1 / T_n \).

**Results and Discussion**

The oxidative process under the temperature-programmed conditions of EPA and DHA at the oxygen level of 20% are shown in Figs. 2 and 3, respectively. The rates of temperature increase were 4.0, 6.0, and 11.9 K/h. For the initial 1.5 hours, an induction period was always observed. The change of the unoxidized EPA or DHA against time was similar to that observed under isothermal conditions. The oxidation progressed markedly with the increase of the increment rate of temperature for both EPA and DHA. The calculation of the activation energy of oxidation was done on the basis of these oxidation courses of EPA and DHA, as shown schematically in Fig. 4. To find the temperatures of \( T_m \) and \( T_n \) a smooth curve was drawn, fitting each data point of the unoxidized fraction of PUFA against time suitably by hand. On this curve, temperatures at \( Y = 0.5(T_m) \) and \( Y = 0.25(T_n) \) were measured graphically as illustrated in Fig. 4 by the dotted and chain-dotted lines, respectively. Using \( T_m \) and \( T_n \) obtained at different increasing rates of temperature, a plot of the logarithms of \( T_n^2 / \alpha \) or \( T_m^2 / \alpha \) against \( 1 / T_m \) or \( 1 / T_n \) was obtained. The activation energies \( E_i \) and \( E_i \) were obtained from the respective slope of the plots multiplied by the gas constant \( R \) according to Eqs. 8 and 13. Figures 5 and 6 shows the plots of the logarithms of \( T_n^2 / \alpha \) vs. \( 1 / T_m \) and \( T_n^2 / \alpha \) vs. \( 1 / T_n \) for EPA (Fig. 5) and DHA (Fig. 6), at different oxygen levels. The slopes of the fitting lines to the data are nearly the same for different oxygen levels, though there are some differences for DHA. The activation energies obtained were \( E_i = 65.8 \pm 2.5 \) kJ/mol and \( E_i = 57.2 \pm 2.6 \) kJ/mol for EPA, and \( E_i = 64.8 \pm 2.1 \) kJ/mol and \( E_i = 56.1 \pm 3.7 \) kJ/mol for DHA. The activation energies obtained by the TPM were compared with those measured by the isothermal methods\(^{40} \) in Table 1, in which the activation energy by the TPM was 4 to 7% higher for both EPA and DHA.
Fig. 5. Plots of Logarithm of $\frac{T^2}{\alpha}$ (a) and $\frac{T^2}{\alpha}$ (b) against $1/T$ for EPA at Four Different Oxygen Levels.

Activation energies $E_a$ and $E_i$ were obtained by the slope of the lines multiplied by gas constant.

○, $C_i=20\%$; △, 13.2%; ○, 5.7%; ○, 3.2%.

Fig. 6. Plots of Logarithm of $\frac{T^2}{\alpha}$ (a) and $\frac{T^2}{\alpha}$ (b) against $1/T$ for DHA at Three Different Oxygen Levels.

Activation energies $E_a$ and $E_i$ were obtained by the slope of the lines multiplied by gas constant.

○, $C_i=20.8\%$; △, 13.2%; □, 8.7%.

Table 1. Comparison of Activation Energies Obtained by TPM and Isothermal Method

<table>
<thead>
<tr>
<th>PUFA</th>
<th>TPM (kJ/mol)</th>
<th>Isothermal method (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_a$</td>
<td>$E_i$</td>
</tr>
<tr>
<td>EPA</td>
<td>65.8±2.5</td>
<td>57.2±2.6</td>
</tr>
<tr>
<td>DHA</td>
<td>64.4±2.1</td>
<td>56.1±7</td>
</tr>
</tbody>
</table>

* measured at oxygen level of 20%.

than that by the isothermal method. However, in considering the experimental errors involved, the results by the two methods agree well with each other. The unoxidized fraction of EPA and DHA against time could be simulated by Eqs. 7 and 12, using the activation energies determined in Figs. 5 and 6. The initial value of $Y_0$ (or $y_0$) and the apparent frequency factors ($k_{90}$ and $k_{10}$) in Eqs. 7 and 12 were regarded as fitting parameters. The solid and dotted lines in Figs. 2 and 3 are the calculated results in the range of Y ≥ 0.5 and Y < 0.5, respectively. The course of oxidation of EPA and DHA could be well correlated with Eqs. 7 and 12. The values of $k_{90}$ and $k_{10}$ used for calculations were $2.53 \times 10^{-10}$ and $3.0 \times 10^{-9}$ s⁻¹ for EPA, and $1.20 \times 10^{-10}$ and $1.00 \times 10^{-8}$ s⁻¹ for DHA. These values are comparable to those found by the isothermal method⁰ in which we obtained $k_{90}=1.97 \times 10^{-10}$ and $k_{10}=4.4 \times 10^{-10}$ s⁻¹ for EPA, and $k_{90}=0.93 \times 10^{-10}$ and $k_{10}=1.08 \times 10^{-9}$ s⁻¹ for DHA. As indicated above, the apparent parameters $k_{90}$ and $k_{10}$ cannot be calculated explicitly from the constant terms in Eqs. 7 and 12, since $Y_0$ (or $y_0$) is also an unknown parameter. It should be also noted that the determinations of $T_m$ and $T_a$ at $Y = 0.5$ and $y = 0.25$ are quite arbitrary. The temperature $T_a$ could be determined at any point of $Y$ between 0.5 < $Y$ < 1.0, if the point is out of the induction period. The same is true for $T_m$ ($Y < 0.5$). A few points were selected for $T_m$ and $T_a$ other than $T_m=0.5$ and $T_a=0.25$, and the respective activation energies were calculated by the above method. However, the deviations of the activation energies determined at different $T_m$ and $T_a$ were within a few percent.

The TPM was verified as a useful technique to analyze the kinetic parameters for the autoxidation of PUFA, particularly to determine the activation energy. The oxidation course of PUFA needs at least three different rates of the increasing temperature. However, the calculation procedure to obtain the activation energy is so simple that the calculation errors accompanying by the calculation could be minimized.

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References