Autoxidation Kinetic Analysis of Docosahexaenoic Acid Ethyl Ester and Docosahexaenoic Triglyceride with Oxygen Sensor

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Received September 11, 2001; Accepted November 19, 2001

The application of ω-3 polyunsaturated fatty acids (PUFAs) as food additives is restricted by their chemically quite reactive properties. However, quantitative analyses of the oxidative kinetics of PUFAs are very few compared to other studies on food chemistry.

In this study, the autoxidation kinetics of ethyl docosahexaenoate (DHAEE), docosahexaenoic triglyceride (DHA oil), and emulsified DHA oil were investigated with an oxygen sensor. The autocatalytic reaction rate constants for DHAEE, DHA oil, and the emulsified DHA oil with 20% (w/v) GA, 20% SSPS, or 20% SSPS containing 5% soy protein were obtained at 35, 50, and 70°C. A plot of the natural logarithm of the frequency factor, ln k0, vs. the activation energy, Ea, demonstrated that ln k0 against Ea fitted well with a single straight line both for the data from this study and for other reported results. This implies that the chemical compensation relationship holds between k0 and Ea for PUFA and emulsified DHA oil.

Key words: autoxidation; ethyl docosahexaenoate; kinetic energy; DHA oil

ω-3 Polyunsaturated fatty acids (PUFAs) have important physiological functions. The beneficial health effects of ω-3 PUFA have been attributed to their ability to lower serum triacylglycerol and cholesterol. Sargent in his review considered the importance of ω-6 and ω-3 polyunsaturated fatty acids as essential constituents of human diets and emphasized the role of oily fish as a dietary source of the long-chain ω-3 polyunsaturates, docosahexaenoic acid and eicosapentaenoic acid. However, they are highly unsaturated and therefore quite sensitive to oxidation damage. Consequently, to use ω-3 PUFAs as food additives, it is important to understand their oxidation kinetics. Oils rich in PUFAs are prone to oxidative deterioration and easily produce off-flavors and off-odors. The oxidation of PUFAs proceeds via a free-radical chain mechanism involving initiation, propagation, and termination steps. Adachi et al. have suggested that the autoxidation kinetics for ω-3 PUFA during the entire period could be expressed by dY/dt = −k1Y(1 − Y) for Y > 0.5, the first half period and dY/dt = −k2Y, for Y < 0.5, the latter half period where Y was the fraction of unreacted PUFA, t was time, and k1 and k2 were rate constants. Yoshii et al. have investigated the oxidation reaction constants of EPA at different oxygen levels and suggested that the oxidation reaction constants could be expressed by the Langmuir-type equation and that the Langmuir constant was independent of temperature.

Miyashita et al. have studied the oxidative stability of emulsified PUFAs and unsaturated phosphatidylcholine in an aqueous phase, and found that the stability increases with the increasing degree of unsaturation, although, in the air, the more highly polyunsaturated FAs were more easily oxidized. Song et al. have shown that DHA-containing oil in the form of phospholipids was more resistant to oxidative degradation of DHA than that in the form of triacylglycerols and ethyl esters in a bulk phase. The oxidation reactions are influenced by several factors such as fatty acid composition, storage conditions, and the physical state of the oil (bulk oil/emulsion). The emulsion state influences the oxidation of oil since antioxidants or pro-oxidants have different affinities for oil and water phases.

In this study, the autoxidation kinetics of ethyl docosahexaenoate (DHAEE), docosahexaenoic triglyceride (DHA oil), and emulsified DHA oil, were investigated by measuring the oxygen concentration in the headspace of a reaction vessel with an oxygen sensor. The emulsifiers used for DHA oil were gum...
arabic (GA) or soybean water-soluble polysaccharides (SSPS). A mathematical model of oxygen concentration (oxygen pressure) changes was proposed by the autoxidation kinetic assumption of PUFAs. The oxidative reaction constants of DHA in the oil and emulsion solution were obtained on the mathematical model. The kinetic parameters of DHA oxidation such as the activation energy and frequency factors were obtained.

Materials and Methods

Materials. DHAEE and DHA oil were from Maruha Corp. DHA oil contained about 47% DHA and 5% EPA. Ethyl docosahexaenoate (DHA, 92% purity) was from Harima Chemicals, Inc. All other chemicals were of analytical grade. Gum arabic was from Nakalai Tesque (Kyoto, Japan). Soybean water-soluble polysaccharides (SSPS) was Soyafive EN-100 from Fuji Oil Co. Ltd.

Measurement of DHAEE, DHA oil, and the emulsified DHA oil with an oxygen sensor. The oxidation rates of DHAEE, DHA oil, and DHA oil emulsion with GA, SSPS, and SP were measured with the oxygen sensor. The emulsion solution of DHA oil with GA or SSPS was homogenized with a Polytron homogenizer at 8000 rpm for 1 min. Triglycerides of docosahexaenoic acid (DHA oil) and ethyl docosahexaenoate (DHAEE) were investigated in the oil and the emulsion solution with 20% gum arabic (GA), 20% soybean water-soluble polysaccharides (SSPS), or 20% SSPS with 5% soy protein (SP). The volume ratio of DHA oil to the emulsifier solution used was 1/5. Figure 1 shows the experimental apparatus to measure the autoxidation of DHA with the oxygen sensor. The reaction vessel (internal diameter: 25 mm, vessel height: 135 mm) was maintained at a constant temperature with a water bath (Eyela, NTB-211, Tokyo). Three ml of DHAEE, DHA oil, or the emulsified DHA oil was put into the reaction vessel, followed by stirring the sample at 400 rpm with a magnetic stirrer (Vari-Mag, Telemode 20 P, Osaka). The oxygen concentration was measured by a fluorescence analyzer (FO-900, ASR, Tokyo), which connected via a RS-232C communication interface to the computer (Fujitsu, FM-V, Tokyo). The auto-catalytic reaction rate constants for DHAEE, DHA oil, and the emulsified DHA oil with 20% GA, 20% SSPS, or 20% SSPS containing 5% soy protein were obtained at 35, 50, and 70°C.

Analysis of autoxidation of DHA oil and the emulsified DHA oil with oxygen sensor. The initial change of oxygen concentration in the reaction vessel was considered with the autoxidation kinetic model. The autoxidative reaction was assumed to progress autocatalytically as follows:

$$\text{DHA} + \text{O}_2 \rightarrow \text{Products} \quad (1)$$

In this oxidation reaction, the oxygen concentration in the closed reaction vessel was expressed with the function of the unoxidized fraction of DHA according to Eq. 2:

$$P_{O_2}^0 - P_{O_2} = \frac{RT}{V} DHA_0 (1 - Y) \quad (2)$$

where $Y$ is the unoxidized fraction of DHA, $DHA_0$ is the initial DHA concentration, $V$ is the closed vessel volume, $T$ is the temperature, and $R$ is the gas constant. On the basis of the oxidative reaction scheme suggested by Yoshii et al., the Langmuir-type autoxidation kinetic equation can be described as Eq. 3:

$$-\frac{dY}{dt} = \frac{k_a P_{O_2}}{K_a + P_{O_2}} Y (1 - Y) \quad (3)$$

where $k_a$ is the apparent reaction rate constant at $Y>0.5$, and $K_a$ is the Langmuir parameter for oxygen (in the reaction system of DHA, the $K_a$ used was 1.1 kPa according to Adachi et al.\(^9\)). From Eqs. 2 and 3, the dimensionless oxygen concentration, $\lambda$, given by the divided value of the oxygen pressure $P_{O_2}$ by the initial oxygen pressure $P_{O_2}^0$, could be described by Eq. 4:

$$-\frac{d\lambda}{dt} = \frac{k_a \lambda}{K_a^* + \lambda} (1 - \lambda) \left( 1 - \frac{\alpha}{\lambda} \right) \quad (4)$$

where $K_a^*$ is $K_a P_{O_2}$ and $\alpha = V/R \cdot DHA_0$. This equation is based on the assumption that the rate-limiting step of PUFAs oxidation is the oxidation reaction. The integration of Eq. 4 gives Eq. 5.
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\[ F(t) = -(1 + K_a) \ln(1 - \lambda) - \frac{K_a(T/\alpha) \ln \lambda}{1 - (T/\alpha)} + \frac{(1 + K_a - (T/\alpha)) \ln (\alpha - 1 + T/\alpha + \lambda)}{1 - (T/\alpha)} = F_0 - k_a t \]

where \( F_0 \) is the initial value of \( F(t) \) at time zero. Equation 5 indicates that the reaction rate constant, \( k_a \), can be obtained from a linear plot of the right-hand side of Eq. 5 against time, \( t \).

**Results and Discussion**

To check the kinetic analysis method of dimensionless oxygen concentration, \( \lambda \), the oxidation of DHAEE was investigated with the oxygen sensor. The oxidation processes of DHAEE at 308 K, 323 K, and 343 K are shown in Fig. 2 by monitoring the oxygen concentration with the oxygen sensor. The oxygen concentration in the reaction vessel decreased with time. The calculated values of the right-hand side value, \( F \) of Eq. 5, \( F(t) \) were plotted against time in Fig. 3. To analyze the oxidation rate in the first half period, \( Y \geq 0.5 \), the data were correlated with straight lines. The experimental data in the initial period fitted well with the autoxidation reaction model, and the autoxidation rate constant \( k_a \) could be obtained with Eq. 5. The Arrhenius plot of the autoxidation rate constant, which was obtained with the slope of the straight line, was also plotted in Fig. 3. This plot yielded the activation energy of 50 kJ/mol and the frequency factor, \( \ln k_0 \) of 12. These values were comparable with the values of 59.7 kJ/mol and 13.2 reported by Adachi et al.\(^4\).

The oxidation processes of DHA oil at 308 K, 323 K, and 343 K are shown in Fig. 4 and the plot of the right-hand side value, \( F(t) \) of Eq. 5 for Fig. 4 is shown in Fig. 5. The induction period at 308 K was observed clearly in the oxidation of DHA oil. The straight lines for the three-oxidation temperatures were obtained for the autoxidation of DHA oil.

The oxidation reactions are influenced by several factors such as fatty acid composition, storage conditions, and physical state of the oil (bulk oil/emulsion). The emulsion state influences oil oxidation since antioxidants as proteins in GA or SSPS or pro-oxidants as water-soluble polysaccharides in GA or SSPS show different affinities for the oil and water phases. In the oxidation of the emulsified DHA oil with 20 w/v% GA, 20% SSPS, or 20% SSPS and 5% SP, the oxidation processes at 323 K were measured with the oxygen sensor. The oxygen concentrations were plotted with time in Fig. 6. The oxidation of DHA oil in the oil, the emulsion solution (5:1, 5% GA or SSPS solution/DHA oil) and the emulsion...
solution (5:1, 20% GA or SSPS solution with 5% SP / DHA oil) were markedly different. The oxidation stability of the emulsion with GA was less than that with SSPS. The addition of soy protein to the emulsion had an antioxidant effect in the emulsion. Duh et al. investigated the oxidative stability of PUFA and soybean oil in an aqueous solution with emulsifiers and indicated that the characteristic oxidative stability of PUFA in an aqueous phase can be strongly influenced by the nature of the emulsifier. Miyashita studied the antioxidant activity of soy proteins in DHA oil-in-water emulsion systems and indicated that the oxidation stability could be improved by adding soy protein. The autoxidation rate constants for the emulsified DHA oil were obtained by correlating the initial \( F(t) \) value of Eq. 5 with the straight lines as shown in Fig. 7.

The dependency of the autoxidation rate constant, \( k_o \) on the temperature was analyzed with the Arrhenius equation as shown in Fig. 8. The activation energies and the frequency factors for DHA oil, and the emulsified DHA oil with 20% (w/v) GA, 20% SSPS, or 20% SSPS containing 5% SP are shown in Table 1. The activation energy obtained for the DHA oil was 62 kJ/mol. The autocatalytic activation energy of DHA oil with POV measurement has been reported to be 59 kJ/mol. The activation energies of DHA oil-in-water with 20% SSPS and 20% SSPS containing 5% SP were about one third lower than that of the DHA oil. On the other hand, the natural log of the frequency factors were 15 for DHA oil, −3 for DHA oil-in-water emulsion with 20% SSPS, and −2 for DHA oil-in-water with 20% SSPS with 5% SP.

In Fig. 9, the natural log of the frequency factors, ln \( k_o \) for DHAEE, DHA oil, and the emulsified...
Table 1. Activation Energies and Frequency Factors for The Autoxidation Kinetics of DHAEE, DHA Oil, and Emulsified DHA Oil

<table>
<thead>
<tr>
<th>Sample</th>
<th>$E_a$ (kJ/mol)</th>
<th>$\ln k_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHAEE</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>DHA oil</td>
<td>62</td>
<td>15</td>
</tr>
<tr>
<td>Emulsified DHA oil with 20% GA</td>
<td>33</td>
<td>3.2</td>
</tr>
<tr>
<td>Emulsified DHA oil with 20% SSPS</td>
<td>19</td>
<td>-3</td>
</tr>
<tr>
<td>Emulsified DHA oil with 20% SSPS and 5% SP</td>
<td>24</td>
<td>-2</td>
</tr>
</tbody>
</table>

These results suggested that the oxidation reaction of DHA oil had the same mechanism for the DHA oil and the emulsified DHA oil in water. The exact mechanism of PUFA oxidation in the emulsion system remains unknown. However, this relationship of the chemical compensation suggested that the emulsified DHA oil with GA or SSPS was efficient in protecting DHA oxidation in water. This protection mechanism of the oxidation might affect the activation energy of DHA oxidation in the emulsion system. The role of the emulsion system in DHA oxidation is now under investigation.

References