Autoxidation Kinetics for Fatty Acids and Their Esters

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ABSTRACT: The autoxidation kinetics for n-3 and n-6 polyunsaturated fatty acids and their esters, which are collectively referred to as polyunsaturated fatty acids (PUFA), were investigated. Changes in the amounts of unreacted n-6 PUFA during the entire period of autoxidation could be expressed by $dY/dt = -k_1 Y(1 - Y)$, where Y was the fraction of unreacted PUFA, t was the time, and k_1 was the rate constant. For n-3 PUFA, autoxidation had to be separated into two parts. The first half of autoxidation ($Y \geq 0.5$) was expressed by the same equation as above, while the latter half ($Y < 0.5$) relates to $dY/dt = -k_2 Y$, where k_2 was the rate constant. The apparent activation energies and the frequency factors of k_1 and k_2 were evaluated. The apparent activation energies were in a range of 50 to 60 kJ/mol for both k_1 and k_2. The frequency factor became large as the number of double bonds of PUFA increased.

JAOCS 72, 547–551 (1995).

KEY WORDS: Autoxidation, ethyl docosahexaenoate, ethyl eicosapentaenoate, kinetics, n-3 unsaturated fatty acid, n-6 unsaturated fatty acid.

Autoxidation of lipids has been the subject of considerable research. Kinetics of chemical reactions involved in rancidity development have also been studied. Labuza (1) reviewed the kinetics of lipid oxidation in foods, and Brimberg (2) discussed the autoxidation mechanism based on kinetics. Autoxidation is a complicated process that proceeds through initiation, propagation, and termination steps, for which the kinetics have usually been considered individually (1–3).

If the entire autoxidative process could be expressed by an equation and if that equation was a function of only the amounts of unreacted substrate and oxygen, it would be convenient to predict the autoxidative process under all conditions and to quantitatively compare the susceptibilities of various lipids to autoxidation. Özilgen and Özilgen (4) have reported on an equation to describe the entire oxidative processes of lipids.

We showed that the autoxidation of linoleic acid, methyl linoleate, and ethyl eicosapentaenoate can be retarded by encapsulating them into powdery matrixes of saccharides and proteins (5). The extent of retardation depended largely on the combination of the fatty acid and entrapping agent. Elucidation of the retardation mechanism calls for knowledge of both the autoxidation kinetics and the diffusivity of oxygen through the matrix. The latter has been reported (6). In this study, the autoxidation kinetics of some polyunsaturated fatty acids (PUFA) and their esters, which are collectively abbreviated as PUFA, were investigated. Simple kinetic expressions, which can describe the entire autoxidative process, are proposed, and the parameters involved in the expressions have been experimentally determined.

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EXPERIMENTAL PROCEDURES

Materials. Linoleic acid (purity, >90%), methyl linoleate (>95%), and methyl palmitate (>95%) were obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Ethyl linoleate (>99%), propyl linoleate (99%), ethyl γ-linolenate (>99%), ethyl arachidonate (>99%), α-linolenic acid (>98%), methyl α-linolenate (>99%), and ethyl α-linolenate (>98%) were purchased from Sigma Chemical (St. Louis, MO). Ethyl eicosapentaenoate (>95%) and ethyl docosahexaenoate (>95%) were supplied by Maruha Corporation (Tokyo, Japan). These were stored at −20 or −80°C (for ethyl eicosapentaenoate and ethyl docosahexaenoate) and used as received. These PUFA were guaranteed by the suppliers to be free from metals.

Trimethylsilyldiazomethane (10% wt/vol in hexane) for methylation of linoleic acid and α-linolenic acid was a product of Chisso Co. (Tokyo, Japan). It was transferred to a benzene solution of the same concentration before use. Sodium thiosulfate solution (0.01 mol/L) for determining the peroxide value (POV) was purchased from Nacalai Tesque (Kyoto, Japan). Other chemicals were of analytical grade.

Autoxidation. PUFA was mixed with an equal weight of methyl palmitate. Methyl palmitate was used as an internal standard when unreacted substrate was anayzed by gas chromatography. Five microliters of the mixture was placed by means of a micropipette on the center of the bottom of a flat-bottomed glass cup (1.5 cm i.d. and 3.0 cm height). These operations were carried out under nitrogen for ethyl eicosapentaenoate and ethyl docosahexaenoate. About 15 cups prepared as described above were placed in a desiccator, in which a beaker, filled with a saturated salt solution, was placed to regulate relative himidity (RH). Sodium chloride was used (about 75% RH) unless otherwise specified. The

desiccator was stored in the dark in a temperature-controlled chamber. Periodically, a cup was taken, and the amount of unreacted substrate was analyzed by gas chromatography.

Analysis. Unoxidized substrate was determined with a Shimadzu GC-14A gas chromatograph (Kyoto, Japan) equipped with a hydrogen flame-ionization detector. The column was 3 mm in diameter and 3 m long with Advance-DS 5% packing (Shimadzu) for separation on 80–100 mesh Shinchrom A. The analytical conditions were: a column temperature of 180°C, except for ethyl eicosapentaenoate and ethyl docosahexaenoate, an injection temperature of 230°C, and N_2 gas as the carrier gas at a flow rate of 50 mL/min. In the analyses of ethyl eicosapentaenoate and ethyl docosahexaenoate, the column temperature was programmed as follows: 180°C for the first 7 min, then raised at a rate of 20°C/min for 2 min, and held at 220°C until the end of the analysis. Linoleic acid and α-linolenic acid were methylated prior to analysis. For some samples, triplicate analyses were carried out. Because variation coefficients were less than 0.3% and a rate constant was evaluated from a linear regression of three to ten data points, the analysis was done one time for most samples. The POV was determined by iodometric titration (7).

RESULTS AND DISCUSSION

Autoxidation processes. Figure 1 shows the autoxidative processes of ethyl linoleate, ethyl γ-linolenate, ethyl arachidonate, ethyl α-linolenate, ethyl eicosapentaenoate, and ethyl docosahexaenoate at 50°C and 74.5% RH in air. Autoxidation proceeded rapidly in all samples after an induction period. The n-3 PUFA were more liable to autoxidation than n-6 PUFA. In the n-3 and n-6 groups, autoxidation proceeded more rapidly as the degree of substrate unsaturation increased, as reported previously (8,9). The autoxidation of other PUFA was also measured under the same conditions. Linoleic acid and α-linolenic acid were more rapidly autoxidized than their esters. This is consistent with the findings of Miyashita and Takagi (8).

Kinetic expression of autoxidation. The kinetic expression of PUFA autoxidation was considered. Bolland (10) proposed the following rate equation for the autoxidation of ethyl linoleate:

$$\text{rate} = k_\alpha \frac{C_X}{K + C_X} C_{RH} C_{ROOH} \quad [1]$$

where C_{RH}, C_{ROOH}, and C_X are the concentrations of unreacted ethyl linoleate, hydroperoxide, and oxygen, respectively; k_α is the rate constant; and K is the saturation constant. If we assume that C_{ROOH} is proportional to the consumption of substrate, then:

$$\frac{dY}{dt} = -\frac{k_X C_X}{K + C_X} Y(1 - Y) \quad [2]$$

where Y is the fraction of unoxidized substrate, and t is the time. Equation 2 is autocatalytic in terms of Y. This conforms with the observation that lipid oxidation is often autocatalytic (11,12). When C_X is always in equilibrium with the partial pressure of oxygen in atmosphere, Equation 2 can be simplified as follows:

$$dY/dt = -k_1 Y(1 - Y) \quad [3]$$

where

$$k_1 = k_X C_X/(K + C_X) \quad [4]$$

Equation 3 is the same in form as the equation that was applied to lipid oxidation by Özilgen and Özilgen (4). The integration of Equation 3 under the condition of $Y = Y_0$ at $t = 0$ gives:

$$\ln \frac{1 - Y}{Y} = k_1 t + \ln \frac{1 - Y_0}{Y_0} \quad [5]$$

The applicability of Equation 5 to autoxidative processes of PUFA is shown in Figure 2. Equation 5 satisfactorily repre-

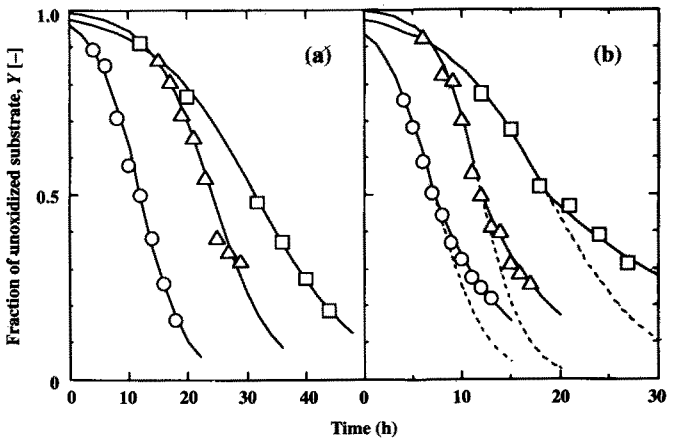

FIG. 1. Changes in the amount of unoxidized substrate during autoxidation at 50°C and 75% relative humidity in air: (a) □, ethyl linoleate; △, ethyl γ-linolenate; ○, ethyl arachidonate. (b) □, Ethyl α-linolenate; △, ethyl eicosapentaenoate; ○, ethyl docosahexaenoate.

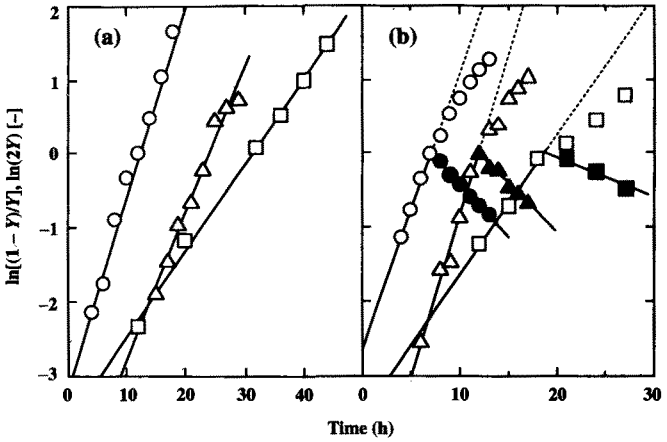

FIG. 2. The applicability of Equations 5 and 7 to changes in the amount of unoxidized substrate during autoxidation. The conditions and keys are the same as those in the legend to Figure 1. The closed symbols represent ln (2 *Y*).

sented the changes in the amount of unoxidized substrate for the entire autoxidation period for n-6 PUFA (Fig. 2a). The variation coefficients of slope and intercept of a linear regression were both less than 5% for each PUFA. The equation was also applicable to the first half ($Y \geq 0.5$) of the autoxidation of n-3 PUFA. However, in the range of $Y < 0.5$, the experimental points deviated from the lines (Fig. 2b), and it seemed unreasonable to represent the experimental points with an equation.

To describe the changes in the latter half of n-3 PUFA autoxidation as simply as possible, first-order kinetics were assumed:

$$dY/dt = -k_2 Y \ (Y < 0.5) \qquad [6]$$

where k_2 was the rate constant. Integrating Equation 6 when $Y = 0.5$ at $t = t_{0.5}$, we obtained:

$$\ln(2Y) = -k_2(t - t_{0.5}) \qquad [7]$$

where $t_{0.5}$ was the time when Y reached 0.5, and was obtained from Equation 5 as follows:

$$t_{0.5} = \frac{1}{k_1} \ln \frac{Y_0}{1 - Y_0} \qquad [8]$$

For the autoxidation of n-3 PUFA, logarithms of $2Y$ were plotted against time in Figure 2b, as shown by the closed symbols. The plots could be connected by a line for each of ethyl α-linolenate, ethyl eicosapentaenoate, and ethyl docosahexaenoate. The line was drawn to pass through the point $Y = 0.5$ and $t = t_{0.5}$, which was evaluated from Equation 8.

The kinetic expressions described above were not always based on the autoxidation mechanism of each PUFA. However, they were conveniently used to represent the autoxidative processes. Equation 3 was useful for n-6 PUFA tested in this study, except for autoxidation of ethyl arachidonate at 70 and 80°C. At these temperatures, the plots of $\ln[(1 - Y)/Y]$ against t were slightly convex. For n-3 PUFA, the kinetic expressions of their autoxidation should be separately treated. Equation 3 was applicable to the first half of n-3 PUFA autoxidation and Equation 6 to the latter half. The solid curves in Figure 1 were calculated with the parameters obtained here. The broken curves in Figure 1b represent Equation 3 for $Y < 0.5$.

The effect of temperature on the rate constants. Figure 3 illustrates the plots of Equation 5 for linoleic acid, and of Equations 5 and 7 for α-linolenic acid, for their autoxidation obtained at different temperatures. The RH was regulated with a saturated solution of NaCl, and was about 75%, although it depended slightly on temperature (13). Autoxidation of linoleic acid could be expressed by only Equation 5 at all temperatures tested. On the other hand, autoxidation of α-linolenic acid required separation into the two parts. From the slopes of these lines, we evaluated the rate constants k_1 and k_2.

The dependency of the rate constants k_1 and k_2 on temperature was analyzed according to the Arrhenius equation:

$$k_i = k_{i0} \exp(-E_i/RT) \qquad (i = 1 \text{ and } 2) \qquad [9]$$

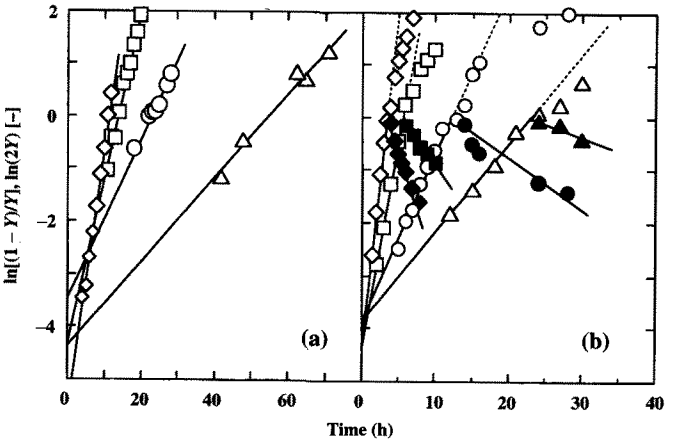

FIG. 3. Dependence of autoxidation processes of (a) linoleic acid and (b) α-linolenic acid on temperature under air. The relative humidity was regulated at about 75% with a saturated solution of NaCl. Temperature: △, 37°C; ○, 50°C; □, 60°C; ◇, 70°C. The closed symbols represent ln (2Y).

where k_{i0} is the frequency factor, E_i is the apparent activation energy, R is the gas constant, and T is the absolute temperature. Figure 4 shows the Arrhenius plots of k_1 and k_2 for some PUFA. The frequency factors and the apparent activation energies were evaluated from the plots and are listed in Table 1 together with those of other PUFA. The apparent activation energies for k_1 and k_2 were in a range of 50 to 60 kJ/mol. There was a tendency for the frequency factor to become large as the number of double bonds increased.

Effect of ester type on the rate constants. PUFA esters reportedly oxidize more slowly than acids (8). The k_1 for esters of linoleic acid and the k_1 and k_2 for esters of α-linolenic acid were obtained at 50°C in air. The rate constants of the acids were higher than those of their esters, as shown in Figure 5.

The effect of the RH on the rate constant. RH often affects lipid oxidation in foods (1,14). The autoxidation of ethyl docosahexaenoate at 50°C was followed at 31.5% RH ($MgCl_2 \cdot 2H_2O$), 67.5% ($NaNO_3$), 74.5% (NaCl), and 100%,

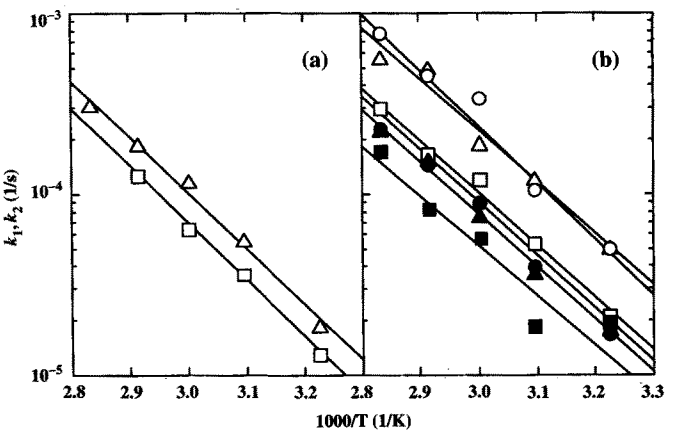

FIG. 4. Arrhenius plots for the rate constants k_1 and k_2. The open and closed symbols represent k_1 and k_2, respectively: (a) □, methyl linoleate; △, ethyl γ-linolenate; (b) □ and ■, ethyl α-linolenate; △ and ▲, ethyl eicosapentaenoate; ○ and ●, ethyl docosahexaenoate.

TABLE 1
The Frequency Factors (k_{10} and k_{20}) and Apparent Activation Energies (E_1 and E_2) of the Rate Constants k_1 and k_2 for Autoxidation of Polyunsaturated Fatty Acids (PUFA) or Their Esters Under Air at 75% Relative Humidity[a]

PUFA	ln k_{10}	E_1 [kJ/mol]	ln k_{20}	E_2 [kJ/mol]
Linoleic acid	8.78 ± 1.55	50.1 ± 4.2	—	—
Methyl linoleate	12.1 ± 0.3	60.0 ± 0.7	—	—
Ethyl γ-linoleate	12.1 ± 1.4	59.0 ± 3.9	—	—
α-Linoleic acid	11.6 ± 2.7	55.8 ± 7.3	9.99 ± 6.84	54.6 ± 18.5
Ethyl α-linolenate	10.8 ± 1.4	55.4 ± 3.9	9.05 ± 7.62	52.4 ± 15.8
Ethyl eicosapentaenoate	11.0 ± 3.4	53.7 ± 9.2	10.6 ± 0.9	55.6 ± 2.5
Ethyl docosahexaenoate	13.2 ± 4.3	59.7 ± 12.0	10.9 ± 0.9	56.0 ± 2.4

[a]Values are mean ± 95% confidence level, k_{10} and k_{20} are in a unit of s^{-1}.

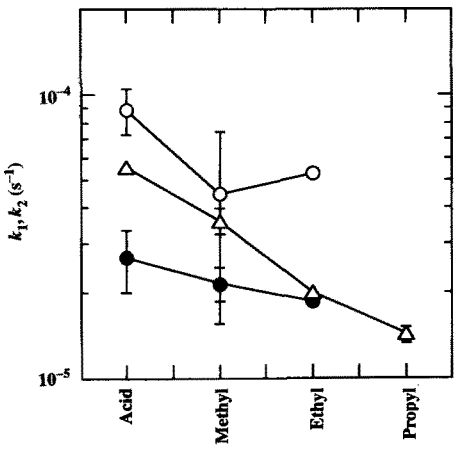

FIG. 5. The effect of ester types of linoleic and α-linolenic acids on the rate constants k_1 and k_2 at 50°C. The error bars indicate 95% confidence levels. Symbols: △, k_1 of linoleate; ○, k_1 of α-linolenate; ●, k_2 of α-linolenate.

and the k_1 and k_2 values were estimated (Fig. 6). The fact that the rate constants did not significantly depend on RH may be because our experimental systems consisted of only PUFA while foods include many ingredients.

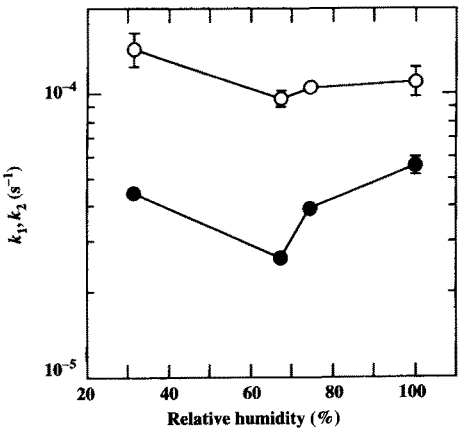

FIG. 6. The effect of the relative humidity on the rate constants of ethyl docosahexaenoate at 50°C. The error bars indicate 95% confidence levels. Symbols: ○ and ● reprsent k_1 and k_2, respectively.

The relationship between the parameter Y_0 and POV. It seems that Y_0 is a parameter that reflects the initial state of the PUFA, although it has been introduced to solve Equation 3. If so, there should be a relationship between Y_0 and POV. A mixture of equal amounts of linoleic acid and methyl palmitate was placed at ambient temperature to prepare samples with various POV values. A portion of the mixture was occasionally taken and used for autoxidation experiments. The initial POV (POV_0) of each sample was titrated. The changes in the amount of unoxidized linoleic acid at 50°C are shown in Figure 7, according to Equation 5 for samples with different POV_0. For all samples, the plots gave lines with almost the same slope. This means that the rate constant k_1 is independent of POV_0. The samples with lower POV_0 values resulted in higher Y_0 values. From the nature of Equation 5, the Y_0 value determines the period of induction. Because the quantity $1 - Y_0$ may correspond to the fraction of oxidized substrate, the quantity was plotted against POV_0 in Figure 8. In the range where POV_0 was less than 90 meq/kg, the Y_0 value could be correlated with POV_0, as shown by the solid line in Figure 8, although the correlation coefficient (0.57) was not high. Thus, measuring POV_0 of the

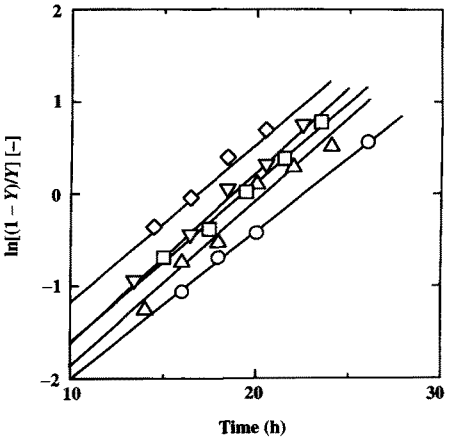

FIG. 7. Changes in the amount of unoxidized linoleic acid during the autoxidation at 37°C of fatty acids with different initial peroxide values. Initial peroxide value: ○, 8.08 meq/kg; △, 32.3; □, 55.6; ▽, 82.5; ◇, 111.

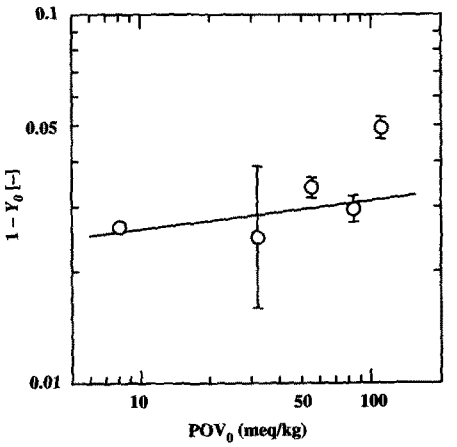

FIG. 8. The relationship between the parameter Y_0 and the initial peroxide value (POV_0) for linoleic acid. The error bars indicate 95% confidence levels.

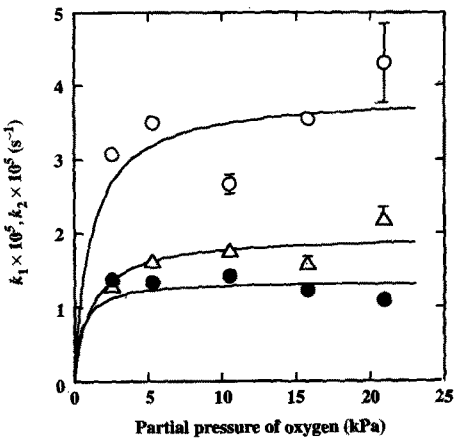

FIG. 9. The dependencies of rate constants k_1 and k_2 at 37°C on the partial pressure of oxygen p_X. The error bars indicate 95% confidence levels. Symbols: △ represents the k_1 of linoleic acid; ○ and ● are the k_1 and k_2 of ethyl docosahexaenoate, respectively.

sample enables autoxidation to be predicted at any temperature, because k_{10} and E_1 have already been evaluated. This may be applicable to other PUFA if a relationship between the Y_0 and POV_0 is found.

The effect of partial oxygen pressure on the rate constant. All the results shown above were obtained in air. Lipid autoxidation depends on the partial pressure of oxygen p_X (1,11). Autoxidation of linoleic acid and ethyl docosahexaenoate were observed at 37°C and at different p_X. The p_X was varied by mixing air with nitrogen gas at an appropriate ratio. The regulation of p_X below 2.5 kPa was difficult with our flow meters. Figure 9 shows the dependence of the k_1 and k_2 on p_X. Both k_1 and k_2 depended weakly on p_X and were smaller at lower p_X. Equation 4 predicts that k_1 is a function of the Langmuir–Hinshelwood type isotherm on p_X, when the absorption coefficient of oxygen to a PUFA is constant. The parameters k_{1X} and K_1, which are the k_X and K values for k_1, were evaluated for linoleic acid and ethyl docosahexaenoate. For the k_2 of ethyl docosahexaenoate, we postulated the same type of equation as Equation 4. The parameters evaluated were $k_{1X} = 1.97 \times 10^{-5}$ s^{-1} and $K_1 = 1.1$ kPa for linoleic acid, and $k_{1X} = 3.87 \times 10^{-5}$ s^{-1}, $K_1 = 1.1$ kPa, $k_{2X} = 1.35 \times 10^{-5}$ s^{-1} and $K_2 = 0.5$ kPa for ethyl docosahexaenoate. The curves in Figure 9 were calculated by using these parameters. Because the dependencies of k_1 and k_2 on p_X were weak, and values at low p_X were not obtained, the accuracy of K_1 and K_2, which is the K value for k_2, may not be high. However, the autoxidation of the PUFA proceeds rapidly even at low p_X.

In this study, we investigated the kinetics of PUFA autoxidation. Kinetic expressions have been presented for n-3 and n-6 PUFA, and the parameters included in the expressions were also evaluated. Autoxidation of PUFA or their glycerides brings unfavorable changes. Some attempts have been made to retard it, for example by adding antioxidants and intercepting oxygen by packaging. To examine if these efforts are effective, determining the kinetics of autoxidation are important. The results presented here may provide useful information for such efforts.

REFERENCES

1. Labuza, T.P., *Crit. Rev. Food Technol. 2*:355 (1971).
2. Brimberg, U.I., *J. Am. Oil Chem. Soc. 70*:249 (1993).
3. Simic, M.G., S.V. Jovanovic and E. Niki, in *Lipid Oxidation in Foods,* ACS Symposium Series 500, edited by A.J. St. Angelo, Washington, D.C., 1992, p. 14.
4. Özilgen, S., and M. Özilgen, *J. Food Sci. 55*:498 (1990).
5. Imagi, J., K. Muraya, D. Yamashita, S. Adachi and R. Matsuno, *Biosci. Biotech. Biochem. 56*:1235 (1992).
6. Adachi, S., K. Muraya, T. Ishiguro and R. Matsuno, *J. Pack. Sci. Technol. 3*:93 (1994).
7. Fukuzawa, K., and J. Terao, in *Experimental Procedures on Lipid Peroxidation* (in Japanese), Hirokawa Publishing, Tokyo, 1990, p. 55.
8. Miyashita, K., and T. Takagi, *J. Am. Oil Chem. Soc. 63*:1380 (1986).
9. Cho, S.-Y., K. Miyashita, T. Miyazawa, K. Fujimoto and T. Kaneda, *Ibid. 64*:876 (1987).
10. Bolland, J.L., *Quart. Revs. 3*:1 (1949).
11. Karel, M., in *Concentration and Drying of Foods,* edited by D. MacCarthy, Elsevier Science Publishing, New York, 1986, p. 37.
12. Yamamoto, Y., E. Niki and Y. Kamiya, *Bull. Chem. Soc. Japan 55*:1548 (1982).
13. Winston, P.W., and D.H. Bates, *Ecology 41*:232 (1960).
14. Nelson, K.A., and T.P. Labuza, in *Lipid Oxidation in Foods,* ACS Symposium Series 500, edited by A.J. St. Angelo, Washington, D.C., 1992, p. 93.

[Received July 7, 1994; accepted February 22, 1995]