Original paper

Kinetics of Oxidation of Different Depths of Methyl Linoleate in Bulk Phase

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Bulk methyl linoleate was oxidized at 65℃ and essentially 0% relative humidity to quantitatively evaluate the effect of the depth of the layer (i.e., the amount) of methyl linoleate on its oxidation kinetics. Oxidation was retarded when the thickness was greater than 1 mm or the volume was greater than 10 μL, and barely occurred at thicknesses greater than 5 mm. Oxidation behavior was also evaluated under a limited range of oxygen partial pressures (0.2 − 10 kPa). The saturation constant of oxygen for oxidation was estimated to be 1.23 kPa, which is significantly lower than the partial pressure of oxygen in air at atmospheric pressure.

Keywords: depth, kinetics, methyl linoleate, oxidation, saturation constant

Introduction

Oxidation, which includes initiation, propagation, and termination steps, is the most common process by which oil deteriorates (Labuza, 1973). It has been reported that many factors such as temperature, humidity, and the presence of prooxidants or antioxidants affect the progress of oxidation during oil processing, storage, and cooking (Frankel, 1980; Aladedunye *et al.*, 2012; Kadowaki *et al.*, 2012a; Kadowaki *et al.*, 2012b; Roman *et al.*, 2013; Kim *et al.*, 2014). During storage, oils are usually in the bulk phase and thus there is an interface between the oil and air through which oxygen diffuses into the oil. In gas-liquid reactions, the reaction is generally affected by the diffusion of gas into the liquid, and by the reaction rate of the gas with the liquid (Levenspiel, 1962). When the specific interfacial area between the gas and liquid is sufficiently small, diffusion of the gas into the liquid is limited, thereby retarding the reaction. This is applicable to the oxidation of bulk oil because the specific interfacial area between air and oil stored in the bulk phase is usually small; thus, diffusion of oxygen into the oil is limited. However, there are few quantitative studies regarding the oxidation behavior of bulk oil.

The stoichiometric coefficient of oxygen to unsaturated fatty acid is equal to or greater than unity (Adachi *et al.*, 1995a). Therefore, oxidation is also retarded when the amount (i.e., partial pressure) of oxygen is limited. There are several reports regarding the effect of the partial pressure or concentration of oxygen on the oxidation kinetics of oil (Adachi *et al.*, 1995b; Yoshii *et al.*, 1999). However, current quantitative studies on the oxidation of oil remain inadequate.

In this study, the oxidation of different amounts of methyl linoleate as a model oil was kinetically analyzed. The effect of the partial pressure of oxygen on the oxidation of methyl linoleate was also evaluated.

Materials and Methods

Materials Methyl linoleate (purity: >95%) and methyl myristate (>98%) were purchased from Tokyo Chemical Industry (Tokyo, Japan).

Oxidation of different depths or amounts of methyl linoleate To evaluate the effect of the depth of methyl linoleate on oxidation, different amounts of methyl linoleate (*i.e.*, 0.147, 0.299, 0.736, 1.47, or 2.94 g) were placed in flat-bottomed glass cups (1.5 cm I.D. x 3.0 cm); these amounts corresponded to oil depths of 1.0, 2.0, 5.0, 10, and 20 mm, respectively. Approximately 90 sets of samples were placed in a plastic container (300 mm long x 150 mm wide x 150 mm high). Air was then flowed through the container at 15 mL/min after passing through silica gel to conduct the oxidation

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reaction. The plastic container was stored at 65℃ in an oven (DN-400, Yamato Scientific, Tokyo, Japan).

Three samples were used to monitor the change in the weight of methyl linoleate. At appropriate intervals, these samples were removed from the container, weighed, and replaced in the container. Concurrently, a sample for gas chromatographic (GC) analysis was also removed, and the substrate in the cup was mixed well. Approximately $0.12 - 0.14$ g of accurately weighed substrate was transferred to another cup containing a given amount (*ca.* $4 - 5$ g) of 0.1 mol/L methyl myristate in n-hexane as the internal standard for GC analysis.

A small sample of methyl linoleate was also oxidized. Methyl linoleate (2.208 g) was dissolved in 50 mL of n-hexane, and a sample of the solution (20, 40, 100, or 200 μL) was placed in a flat-bottomed glass cup. The n-hexane was then removed under reduced pressure, resulting in 1, 2, 5, and 10 μL, respectively, of methyl linoleate in the cups. Oxidation was then performed using the method described above. At appropriate intervals, a cup was removed from the container, and the amount of unoxidized methyl linoleate in the sample was determined by GC.

Effects of partial pressure of oxygen on oxidation Methyl linoleate (2 μL) was placed in a flat-bottomed cup, which was then loaded into a 50 mL screw-cap bottle. Approximately 10 bottles were placed in a desiccator, which was then evacuated and filled with nitrogen-air mixed gas. The partial pressures of oxygen in the mixed gas ranged from 0.2 to 10 kPa. The evacuation and filling operations were performed twice to ensure sufficient replacement of air with the mixed gas. The bottles were then immediately capped and placed in an oven at 65℃ to perform the oxidation. Periodically, a bottle was removed and its content of unoxidized methyl linoleate was measured by GC.

Gas chromatographic analysis The amount of unoxidized methyl linoleate was determined by GC (GC-2014A, Shimadzu, Kyoto, Japan) using a DB-1ht column (0.25 mm I.D. × 30 m, Agilent Technologies, CA, USA) and a flame ionization detector. The injector, column, and detector were held at 230, 205, and 240℃, respectively.

Results and Discussion

Effect of depth or amount of methyl linoleate on oxidation Figure 1 shows the time courses of the oxidation of different depths or amounts of methyl linoleate. For $1 - 10$ μL of methyl linoleate, the oxidation proceeded quickly and was almost complete within 20 h, except for the 10 μL sample of methyl linoleate. The induction periods for oxidation were approximately $4 - 6$ h for $1 - 5$ μL of methyl linoleate. Ten microliters of the substrate required 24 h for complete oxidation. These results indicate that the effect of the diffusion of oxygen on the oxidation of methyl linoleate became significant when 10 μL or more of the substrate was used under these experimental conditions.

When a greater amount of methyl linoleate was exposed to

oxidation, *i.e.*, the depth of methyl linoleate was increased from 1 to 20 mm, oxidation was significantly retarded. The induction periods in these cases were longer than 8 h. In addition, no significant decrease in the fraction of unoxidized methyl linoleate was observed during the duration of the test when the depths were 10 and 20 mm, indicating that most of the oxygen supplied through the oil-air interface was consumed at or near the interface.

Figure 2 shows the time courses of oxidation with increased relative weight, $\Delta w / w_0$, of different depths of methyl linoleate, where Δw is the increase in the weight of the substrate and w_0 is the initial weight of the substrate. After the induction period, the relative weights of the samples with depths of $1 - 5$ mm began to
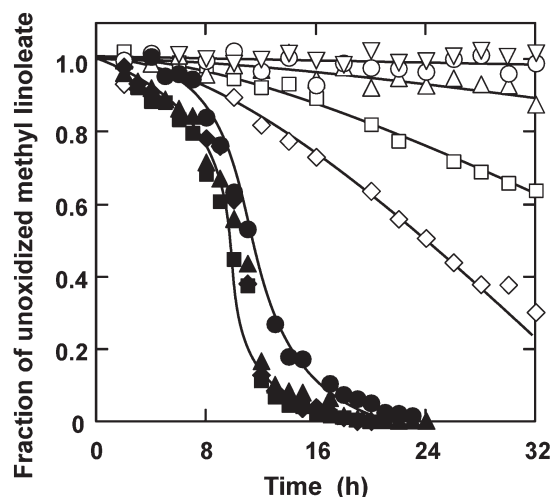

Fig. 1. Time courses of the fraction of unoxidized methyl linoleate at various thicknesses (amounts) at 65℃. Open symbols ◇, □, △, ○, and ▽ represent depths of 1, 2, 5, 10, and 20 mm, respectively. Closed symbols ◆, ■, ▲, and ● represent 1, 2, 5, and 10 μL of methyl linoleate, respectively. The curves are empirically drawn.
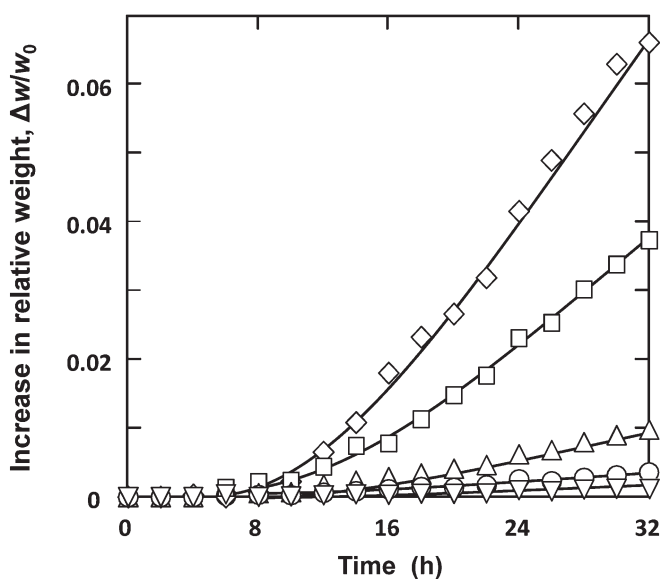

Fig. 2. Increase of the relative weight of methyl linoleate with depth during oxidation at 65℃; w_0 and Δw indicate the initial weight and increase in the weight of methyl linoleate, respectively. The symbols are the same as in Fig. 1.

increase. In contrast, only a slight increase was observed for the samples with depths of 10 and 20 mm. These results correlate well with those in Fig. 1, indicating that measurement of the weight change can be used to effectively estimate the induction period (Usuki, 1983).

The fraction of unoxidized methyl linoleate for different depths of sample was plotted against the increase in the relative weight of the substrate (Fig. 3). The fractions in all cases linearly decreased with increasing relative weight. It was previously reported that the stoichiometric coefficient of oxygen and methyl linoleate was unity for the entire period of substrate oxidation (Adachi *et al.*, 1995a). The straight line in Fig. 3 represents the calculated result based on this stoichiometry. There was a small difference between the observed and calculated results, with the observed increase in the relative weight being slightly smaller than the calculated increase at the same fraction of unoxidized methyl linoleate. One reason for this discrepancy may be that evaporation of volatile compounds such as aldehydes, hydrocarbons, epoxides, alcohols and ketones that formed during oxidation contributed to the weight loss (Berdeaux *et al.*, 2012).

In order to estimate the effect of the diffusion of oxygen on oxidation, the oxidation rate, v, at fractions of unoxidized substrate, Y, of 0.8 and 0.6 for methyl linoleate depths of $1-5$ mm were compared to the oxidation rate without limited diffusion of oxygen ($1-5$ μL), v_0 (Fig. 4). The v and v_0 values were calculated from the slope of the tangent that contacts the smooth curves at $Y = 0.8$ and 0.6 in Fig. 1 by graphical differentiation. The rates at $Y = 0.6$ and 0.8 for the 2- and 5-mm-thick substrates, respectively, were roughly estimated by extrapolating the smooth curves. Using this approach, the average v_0 value and the standard deviation for $1-5$ μL of methyl linoleate were calculated to be 0.078 ± 0.024

h^{-1} and 0.115 ± 0.004 h^{-1} for $Y = 0.8$ and 0.6, respectively. For 10- and 20-mm-thick methyl linoleate samples, a negligible decrease in the unoxidized fraction was observed. Therefore, the oxidation rates could not be evaluated. The relative oxidation rate, v/v_0, was plotted against the depth of methyl linoleate for $1-5$ mm thick substrates. The results indicate that depth influenced the oxidation rate, and that the relative rate decreased with increasing depth. At a depth of 5 mm, the relative rate was 0.14, and even at a depth of 1 mm, the relative rate was approximately 0.3; that is, the oxidation rate of 1-mm-deep methyl linoleate was approximately one-third of that of substrates without limited diffusion of oxygen. Thus, only 1 mm of depth significantly affected oxidation rate.

By assuming that methyl linoleate did not undergo convection during the experiment, the effect of the diffusion of oxygen on the oxidation rate of the substrate could be analyzed based on a reaction-diffusion model (Levenspiel, 1962). In this model, the ratio of the diffusion-controlled reaction rate to that free from the effect of diffusion, which corresponds to the effectiveness factor for gas or liquid–solid reactions, can be expressed as a function of the Thiele modulus. In the present system, the modulus, φ, can be defined as follows:

$$\phi = L \sqrt{k / D} \qquad \cdots\cdots\text{Eq. 1}$$

where L is the depth of the substrate, k is the rate constant for oxidation under conditions of sufficient oxygen, and D is the diffusion coefficient of oxygen in the substrate. The line in Fig. 4 was extrapolated, as shown by the dashed line; the φ value at the intersection of the extrapolated line and a ratio of 1, ϕ_e, was evaluated to be 0.11. At φ values larger than 0.11, the diffusion of oxygen affects the oxidation rate of the substrate. The oxidation of n-6 unsaturated fatty acids and their esters can be expressed by the following equation, which is based on the autocatalytic model (Adachi *et al.*, 1995b):

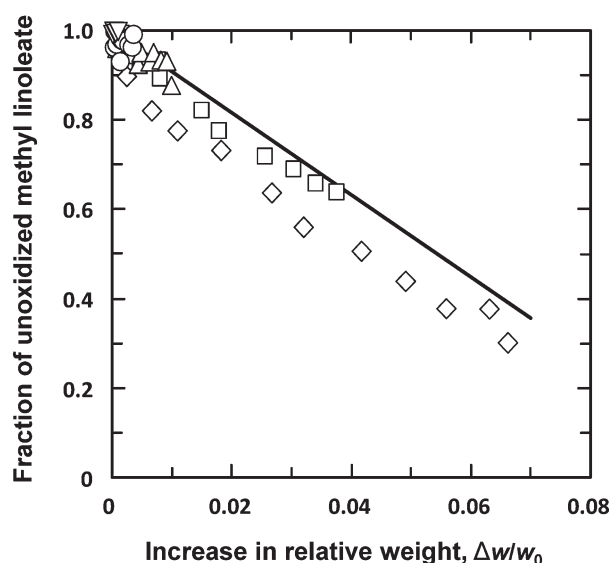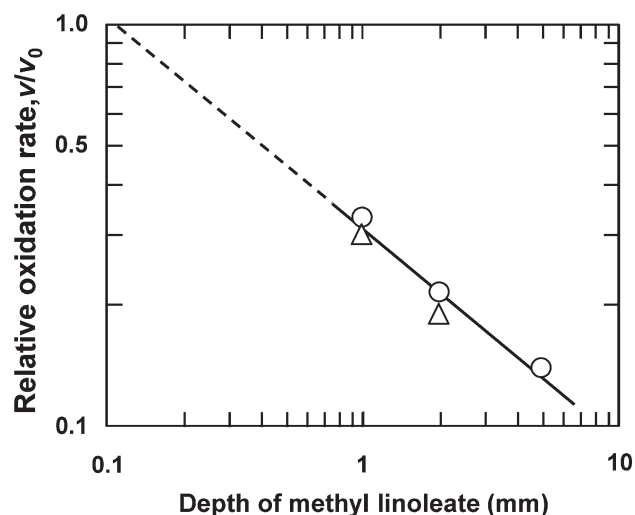$$\ln \frac{1-Y}{Y} = kt + \ln \frac{1-Y_0}{Y_0} \qquad \cdots\cdots\text{Eq. 2}$$

Fig. 3. Relationship between the fraction of unoxidized methyl linoleate and the increase in the relative weight of the substrate. The straight line represents the calculated result when an equimolar amount of oxygen reacts with methyl linoleate during its oxidation. The symbols are the same as in Fig. 1.

Fig. 4. Relationships between the relative rates, v/v_0, at $Y = (\bigcirc)$ 0.8 and $(\triangle)$ 0.6 and the depth of methyl linoleate.

where t is the time elapsed, k is the rate constant for the oxidation, and Y_0 is Y at $t = 0$. Based on Eq. (2), the rate constant k could be obtained by plotting $\ln(1 - Y)/Y$ versus t. Accordingly, the k values for 1, 2, and 5 μL of methyl linoleate were evaluated, with the average value ($0.505 \pm 0.019 \text{ h}^{-1}$) regarded as the k value. The D value was roughly estimated to be $1.2 \times 10^{-9} \text{ m}^2/\text{s}$ by the Wilke-Chang method (Wilke and Chang, 1955). From the ϕ_c, k, and D values, it can be estimated that the diffusion of oxygen affects the oxidation rate at a substrate depth of 0.33 mm or more. The k and D values at any temperature can be evaluated according to our previous study (Adachi *et al.*, 1995b) and the Wilke-Chang method, respectively; thus, the minimum depth at which the diffusion of oxygen begins to affect the oxidation rate of methyl linoleate can be determined. The ϕ_c value would be applicable to the oxidation of other lipids with oxidation kinetics that can be expressed via an autocatalytic rate equation.

Oxidation of methyl linoleate at different partial pressures of oxygen   As described above, oxidation was increasingly retarded with increasing thickness of methyl linoleate. There are several factors that affect the oxidation of methyl linoleate, including the partial pressure of oxygen; therefore, we investigated the effect of the partial pressure of oxygen on the oxidation rate constant.

Figure 5 shows the time courses of the fraction of unoxidized methyl linoleate at different partial pressures of oxygen, P_{O2}. The induction period increased with decreasing partial pressure. When the oxidation was performed in air ($P_{O2} = 21$ kPa) or at a P_{O2} of 5 kPa, the induction period was around 6 h, but extended to around 9 h at a P_{O2} of 0.2 kPa. Based on Eq. (2), the rate constants, k, were obtained by plotting $\ln(1 - Y)/Y$ versus t.

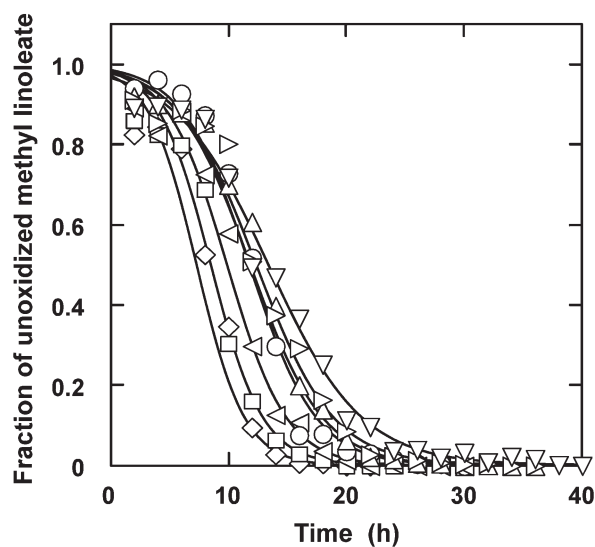The rate constant was ca. 0.5 h^{-1} when the oxidation was performed in air ($P_{O2} = 21$ kPa); it gradually decreased with decreasing partial pressure of oxygen, and sharply decreased at partial pressures lower than 5 kPa (Fig. 6). From these results, the saturation constant of oxygen required for the oxidation can be calculated based on the Langmuir-Hinshelwood-type equation (Adachi *et al.*, 1995b; Yoshii *et al.*, 1999):

$$k = \frac{k_{max} P_{O2}}{K_x + P_{O2}} \qquad \cdots\cdots\text{Eq. 3}$$

where K_X is the saturation constant and k_{max} is the maximum rate constant. The saturation and maximum rate constants were estimated to be 1.23 kPa and 0.515 h^{-1}, respectively, although the experimental and calculated results differed at low P_{O2} values. This difference can be ascribed to difficulties in the complete replacement of gases at a specific composition.

In conclusion, the depth of methyl linoleate greatly affected its oxidation. When the sample was deeper than 1 mm, or the amount of methyl linoleate was greater than 10 μL, the diffusion of oxygen became a rate-limiting factor and oxidation was retarded. Therefore, a small amount of methyl linoleate (<5 μL) should be used to evaluate the oxidation kinetics without limited diffusion of oxygen at 65℃. The saturation constant of oxygen for oxidation was also evaluated and was much lower than the partial pressure of oxygen in air at atmospheric pressure. Therefore, oxidation was only slightly affected by the partial pressure at pressures higher than 5 kPa.

Fig. 5. Oxidation of methyl linoleate at different partial pressures of oxygen at 65℃. Symbols $\triangledown$, $\triangleright$, $\triangle$, $\bigcirc$, $\triangleleft$, $\square$, and $\diamond$ represent oxygen partial pressures of 0.2, 0.5, 1, 2, 5, 10, and 21 kPa, respectively. The curves were calculated using the estimated k and Y_0 values.
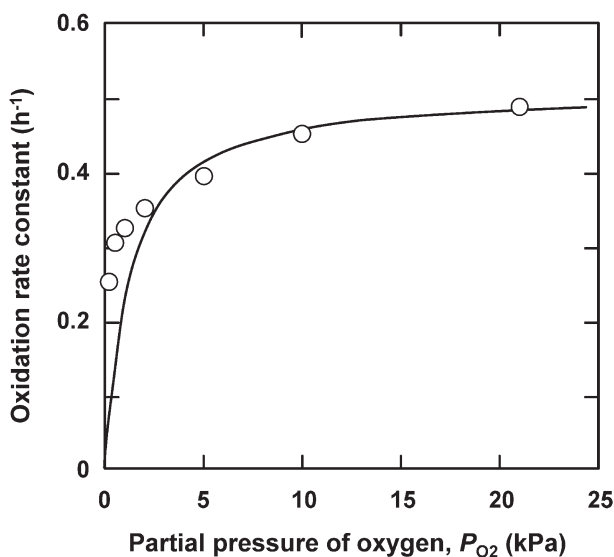

Fig. 6. Dependence of the oxidation rate constant on the partial pressure of oxygen at 65℃.

References

Adachi, S., Ishiguro, T., and Matsuno, R. (1995a). Thermal analysis of autoxidation of ethyl esters of n-3 and n-6 fatty acids. *Food Sci. Technol. Int.*, **1**, 1-4.

Adachi, S., Ishiguro, T., and Matsuno, R. (1995b). Autoxidation kinetics for fatty acids and their esters. *J. Am. Oil Chem. Soc.*, **72**, 547-551.

Aladedunye, F., Catel, Y., and Przybylski R. (2012). Novel caffeic acid amide antioxidants: Synthesis, radical scavenging activity and performance under storage and frying conditions. *Food Chem.*, **130**, 945-952.

Berdeaux, O., Fontagné, S., Sémon, E., Velasco, J., Sébédio, J.L., and Dobarganes, C. (2012). A detailed identification study on high-temperature degradation products of oleic and linoleic acid methyl esters by GC-MS and GC-FTIR. *Chem. Phys. Lipids*, **165**, 338-347.

Frankel, E.N. (1980). Lipid oxidation. *Progr. Lipid Res.*, **19**, 1-22.

Kadowaki, A., Iwamoto, S., and Yamauchi, R. (2012a). The antioxidative effect of fullerenes during the peroxidation of methyl linoleate in toluene. *Biosci. Biotechnol. Biochem.*, **76**, 212-214.

Kadowaki, A., Iwamoto, S., and Yamauchi, R. (2012b). Reaction products of [60]fullerene during the autoxidation of methyl linoleate in bulk phase. *Chem. Phys. Lipids*, **165**, 178-185.

Kim, J.Y., Kim, M.-J., and Lee, J. (2014). Role of moisture on the lipid oxidation determined by D_2O in a linoleic acid model system. *Food Chem.*, **146**, 134-140.

Labuza, T.P. (1973). Kinetics of lipid oxidation in foods. *Crit. Rev. Food Technol.*, **2**, 355-404.

Levenspiel, O. (1962). Fluid-fluid reactions and solid-catalyzed reactions. In "Chemical Reaction Engineering." John Wiley & Sons, Chapters 13 and 14, NJ, USA.

Roman, O., Heyd, B., Broyart, B., Castillo, R., and Maillard, M. (2013). Oxidative reactivity of unsaturated fatty acids from sunflower, high oleic sunflower and rapeseed oils subjected to heat treatment, under controlled conditions. *LWT - Food Sci. Technol.*, **52**, 49-59.

Usuki, R. (1983). Measurements of lipid peroxides. In "Experimental Techniques for Lipid Peroxides (in Japanese)." ed. Kaneda, T. and Ueda, N., Ishiyaku Shuppan, pp. 42-43.

Wilke, C.R. and Chang P. (1955). Correlation of diffusion coefficients in dilute solutions. *AIChE J.*, **1**, 264-270.

Yoshii, H., Furuta, T., Itoh, T., Yoshimura, K., Misawa, Y., Hata, N., Kobayashi, H., Linko, Y.Y., and Linko, P. (1999). Kinetic analysis of the autoxidation of eicosapentaenoate at different oxygen levels. *Biosci. Biotechnol. Biochem.*, **63**, 662-665.