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# Thermal Analysis of Autoxidation of Ethyl Esters of n-3 and n-6 Fatty Acids

Shuji ADACHI, Tatsuji ISHIGURO and Ryuichi MATSUNO

*Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan*

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**The autoxidation of the ethyl esters of n-3 and n-6 fatty acids, abbreviated as PUFAs (polyunsaturated fatty acids), was followed by analysis using thermogravimetry and calorimetry as well as by gas chromatography. The thermogravimetric measurements indicated that the stoichiometric coefficient between n-6 PUFA and oxygen was 1 during the entire autoxidation, and that the coefficient between n-3 PUFA and oxygen was dependent on the unoxidized substrate fraction. For n-3 PUFAs, the coefficient was 1 when the fraction was greater than 0.5, and the coefficient became larger as the autoxidation proceeded (i.e., the unoxidized fraction was less than 0.5). Calorimetric measurements also gave the same results. The heat of the autoxidative reaction based on moles of oxygen consumed was almost constant for both the n-3 and n-6 PUFAs, and it was not dependent on the unoxidized substrate fraction.**

Keywords: autoxidation, polyunsaturated fatty acid, thermogravimetry, calorimetry

The autoxidation of lipids is responsible for quality deterioration in stored foods (Kanner, 1992), and is a complicated process proceeding through initiation, propagation and termination steps. The kinetics of autoxidation have usually been considered for the individual steps (Labuza, 1971; Brimberg, 1993; Simic *et al*., 1992).

An equation which can describe the entire autoxidative process has, however, been reported (Özilgen & Özilgen, 1990). We have also reported equations to describe the autoxidative processes of n-3 and n-6 polyunsaturated fatty acids and their esters (Adachi *et al*., 1995). Here, polyunsaturated fatty acids and their esters are referred to as polyunsaturated fatty acids (PUFAs). For n-6 PUFAs, the entire autoxidation process can be expressed by an equation similar to that previously reported (Özilgen and Özilgen, 1990), as a function only of the fraction of unoxidized substrate $Y$ under a constant oxygen pressure. On the other hand, the autoxidation of n-3 PUFAs required separation into two parts. The first half of the autoxidation ($Y \geq 0.5$) was expressed by the same equation used for the n-6 PUFAs, while the latter half of the autoxidation ($Y<0.5$) was expressed by another equation, which was also a function of only $Y$.

PUFAs increase in weight as they are oxidized, and the weight gain can be followed using thermogravimetry (Nakamura, 1988). The autoxidation of PUFAs is exothermic. Thus, the weight gain and heat of reaction generated during the autoxidation of n-3 and n-6 PUFAs were measured by thermogravimetry and calorimetry, respectively, and the amount of unonidized PUFA was also followed by gas chromatographic analysis. Based on these measurements, the reasons for the differences in the kinetic expressions for the n-3 and n-6 PUFAs are discussed.

## Materials and Methods

*Materials* Ethyl $\gamma$-linolenate (purity, >99%) and ethyl arachidonate (>99%) were purchased from Sigma Chemicals, St. Louis, MO. Ethyl eicosapentaenoate (>95%) and ethyl docosahexaenoate (>95%) were supplied by Maruha Corporation, Tokyo, Japan. Methyl palmitate (>95%), which is stable against autoxidation and was used as an internal standard in gas chromatographic determination of the fraction of unoxidized substrate, was obtained from Tokyo Kasei Kogyo, Tokyo. They were stored at −20°C or −80°C (for ethyl eicosapentaenoate and ethyl docosahexaenoate), and used as received. The PUFAs were guaranteed to be free of metals by the suppliers. The PUFAs were mixed with an equal weight of methyl palmitate, and the mixtures were used in the thermogravimetric, calorimetric and gas chromatographic measurements of autoxidation. After preparation of the mixture, the measurements were carried out as soon as possible to prevent changes in the quality of the mixtures during storage.

*Gas chromatographic monitoring of the autoxidative process* The autoxidative processes of the PUFAs were monitored as described elsewhere (Adachi *et al*., 1995). The mixture of PUFA and methyl palmitate (5 $\mu$l) was placed by means of a micropipette on the center of a flat-bottomed glass cup. The cups were placed in a desiccator along with a beaker filled with a saturated NaCl solution to regulate the relative humidity at about 75% (Winston and Bates, 1960). The desiccator was stored in the dark in a temperature-controlled chamber, and the amount of unoxidized PUFA was periodically analyzed by gas chromatography.

A gas chromatograph (Shimadzu Seisakusho, Kyoto, Japan, GC-14A) equipped with a hydrogen flame ionization detector was used. The column was 3 mm in diameter and 3 m long, with Advance-DS 5% packing (Shimadzu Seisakusho) for separation on 80–100 mesh Shinchrom A. The analytical conditions were the same as those described elsewhere (Adachi *et al*., 1995).

*Thermogravimetric monitoring of the autoxidative process* PUFA increases its weight due to the addition of oxygen during autoxidation. The weight changes in samples were measured with a thermogravimeter (TGA-50H, Shimadzu Seisakusho). About 5 mg of the mixture was put in a 6 mm$\phi\times$2.5 mm platinum cell. The mixture was heated in increments of 10°C/min from room temperature to a given temperature (50, 60, 70 or 80°C), and then kept at that temperature under flowing air at a rate of 20 ml/min until the PUFA was almost completely oxidized. Since there is no significant effect of the relative humidity on the autoxidation of PUFAs (Adachi *et al.*, 1995), the relative humidity of the air was not regulated during the thermogravimetric and calorimetric measurements. The period of measurement depended on the PUFA tested and on the temperature. The change in weight of the sample was recorded on a floppy disk using a personal computer (PC-9801E, Nippon Electric Company, Tokyo) connected to the thermogravimeter.

*Calorimetric monitoring of the autoxidative process* About 5 mg of the mixture, measured precisely in each experiment, was put into an aluminum cell, the dimensions of which were the same as those of the platinum cell used for the thermogravimetry. The heat of reaction generated during the autoxidation of PUFA was measured using a differential scanning calorimeter (DSC-50, Shimadzu Seisakusho) operated under isothermal conditions. The differential scanning calorimeter used was a heat-flux type, and aluminum oxide of the same weight as the sample was put into the reference cell. The conditions for measurement were the same as those for the thermogravimetric analysis.

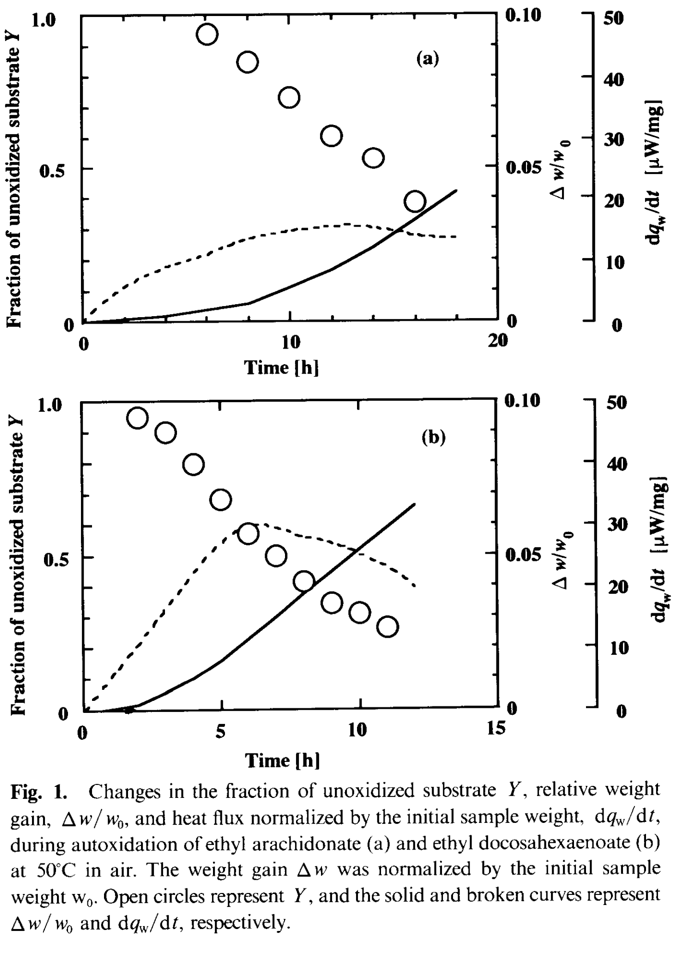

**Fig. 1.** Changes in the fraction of unoxidized substrate $Y$, relative weight gain, $\Delta w/w_0$, and heat flux normalized by the initial sample weight, $dq_w/dt$, during autoxidation of ethyl arachidonate (a) and ethyl docosahexaenoate (b) at 50°C in air. The weight gain $\Delta w$ was normalized by the initial sample weight $w_0$. Open circles represent $Y$, and the solid and broken curves represent $\Delta w/w_0$ and $dq_w/dt$, respectively.

## Results and Discussion

*Autoxidative process* Figures 1 (a) and (b) show the changes in the fraction of unoxidized substrate , in relative weight gain, $\Delta w/w_0$, and in the heat of the autoxidative reaction normalized by the initial sample weight, $dq_w/dt$, during the autoxidation of ethyl arachidonate and ethyl docosahexaenoate, respectively, at 50°C. As shown by the open circles, the autoxidation proceeded rapidly after an initial induction period. As PUFA is autoxidized, its weight gradually increased, and the heat of the autoxidative reaction (heat flux), $dq_w/dt$, also increased. The heat reached a maximum when the $Y$ value was nearly 0.5, and then gradually reduced.

The same measurements as shown in Fig. 1 were carried out at different temperatures and for other PUFAs, and similar tendencies were observed in all samples.

*Decrease in weight due to evaporation* The autoxidation of PUFAs was measured at relatively high temperatures. At such temperatures, evaporation of the sample would occur during measurement, and thus the observed $\Delta w/w_0$ would be smaller than the intrinsic $\Delta w/w_0$ value. Therefore, we observed the weight changes of samples with various $Y$ values under a nitrogen atmosphere. The samples were prepared by holding the mixture of PUFA and methyl palmitate at 50°C for different periods.

Figure 2 shows the weight change for the mixture of ethyl docosahexaenoate and methyl palmitate at 80°C under a nitrogen atmosphere. A weight loss, ascribable to evaporation, occurred in all samples. There was not a significant difference in the rate of weight loss for the samples with $Y$ values larger than 0.7, but those with $Y$ values of less than 0.7 were liable to evaporate. Similar tendencies were observed for other PUFAs.

During autoxidation, the $Y$ value changed with time. We could not express the rate of weight loss as a function of $Y$, so it was difficult to precisely correct the $\Delta w/w_0$ observed during autoxidation. However, ignoring the weight loss during measurement would result in a large error in the weight gain, $\Delta w/w_0$, due to autoxidation. Thus, the $\Delta w/w_0$

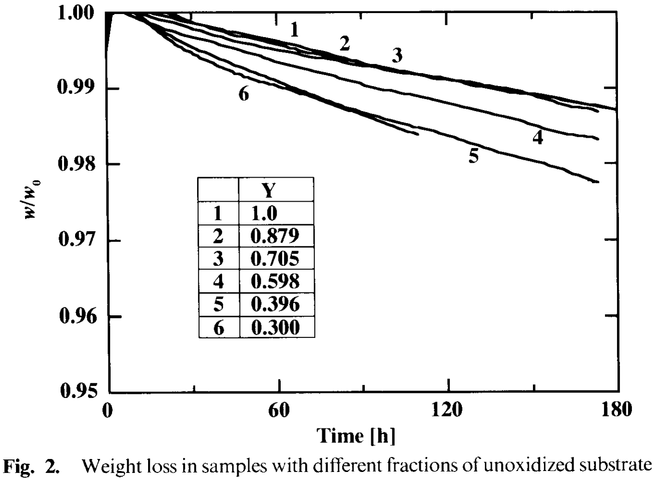

**Fig. 2.** Weight loss in samples with different fractions of unoxidized substrate $Y$ at 80°C under a nitrogen atmosphere. The samples were prepared by holding mixtures of the same weights of ethyl docosahexaenoate and methyl palmitate at 50°C for various times.

value at any time was roughly corrected by adding the $1-(w/w_0)_{ev}$ value at that time, where $(w/w_0)_{ev}$ is the relative weight observed under a nitrogen atmosphere for the sample with $Y=1.0$. Hereafter, the $\Delta w/w_0$ values corrected by this method will be shown.

*Weight gain during autoxidation* If we assume that the weight gain, $\Delta w$, is ascribable to the addition of oxygen to PUFA and that no degradation of PUFA or its oxidized product occurs, we can estimate the moles of oxygen reacted, $n_X$, from the $\Delta w$ using the following equation:

$$n_X = \Delta w / M_X, \tag{1}$$

where $M_X$ is the molecular weight of oxygen. The initial weight of PUFA is $w_0/2$ because a mixture of PUFA and methyl palmitate of the same weight was used. This value can be converted to an initial moles of unoxidized PUFA $n_{F0}$ by dividing it by the molecular weight of PUFA $M_F$. So, when the fraction of unoxidized PUFA is $Y$, the moles of unoxidized PUFA $n_F$ is given by

$$n_F = n_{F0} Y = \frac{w_0}{2M_F} Y. \tag{2}$$

From Eqs. (1) and (2), we obtain the following equation:

$$\frac{d(\Delta w/w_0)}{dY} = \frac{M_X}{2M_F} \cdot \frac{dn_X}{dn_F}. \tag{3}$$

This equation indicates that the slope of a curve which connects $\Delta w/w_0$ and $Y$ gives the stoichiometric coefficient between oxygen and PUFA, $\gamma = dn_X/dn_F$.

Figure 3 (a) shows the relationship between $\Delta w/w_0$ and $Y$ for ethyl arachidonate, which is an n-6 PUFA at various temperatures. The line in the figure represents the relationship when the coefficient $\gamma$ is 1. The plots are scattered along this line, indicating that the stoichiometric coefficient is independent of the fraction of unoxidized ethyl arachidonate $Y$ and is 1 throughout the entire autoxidation process.

Figure 3 (b) illustrates the relationships for ethyl docosahexaenoate, which is an n-3 PUFA. The line in this figure also represents the relationship at $\gamma=1$. The plots in the range of $Y$ greater than 0.5 lay along the line. However, when $Y$ was less than 0.5, the plots deviated from the line. The slope of the curve smoothly connecting the plots became larger as the $Y$ value decreased. The $\gamma$ value calculated from the slope changed from 1 to about 5 with decreasing $Y$ value.

*Heat of autoxidative reaction* Heat flux normalized by the initial sample weight, $dq_w/dt$, is related to the real heat flux, $dq/dt$, by

$$dq_w/dt = (1/w_0)(dq/dt), \tag{4}$$

where $t$ is the time. On the other hand, the rate of change in unoxidized PUFA, $dn_F/dt$, is related to $dY/dt$ from Eq. (2) by

$$\frac{dn_F}{dt} = \frac{w_0}{2M_F} \cdot \frac{dY}{dt}. \tag{5}$$

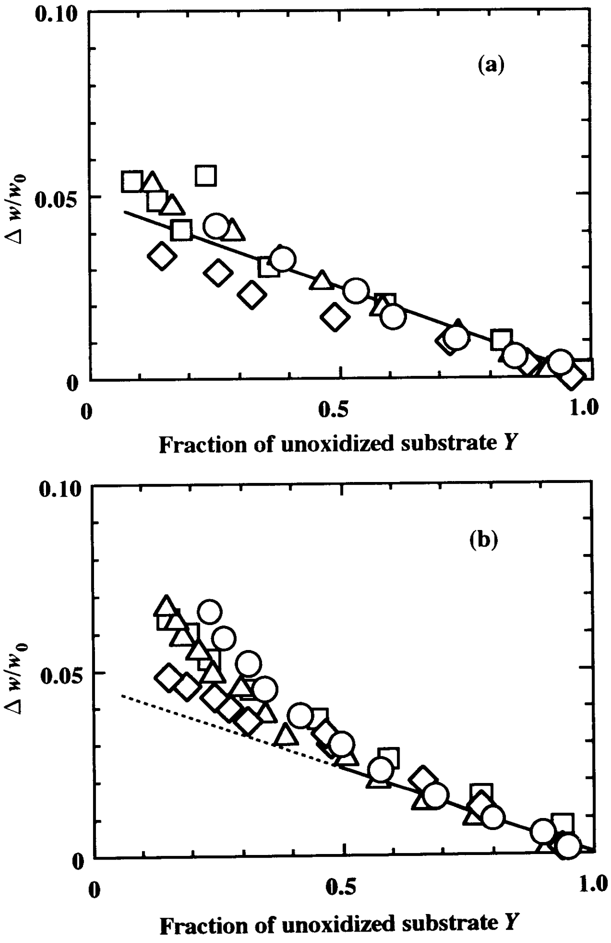

**Fig. 3.** Relationships between the relative weight gain, $\Delta w/w_0$, and the fraction of unoxidized substrate $Y$ for ethyl arachidonate (a) and ethyl docosahexaenoate (b) at various temperatures. The symbols: ○, 50°C; △, 60°C; □, 70°C; ◇, 80°C.

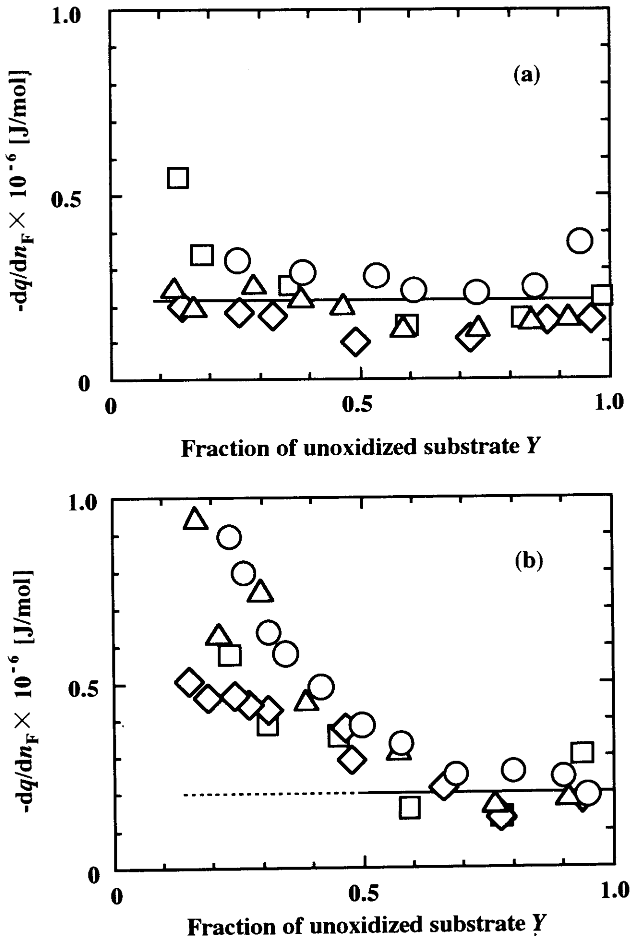

**Fig. 4.** Heat of autoxidative reaction per mole of substrate consumed, $-dq/dn_F$, as a function of the fraction of unoxidized substrate $Y$ for ethyl arachidonate (a) and ethyl docosahexaenoate (b). Symbols are the same as those in Fig. 3.

The d$Y$/d$t$ value can be evaluated as follows: the $Y$ values are plotted versus $t$, and the plots are connected by a smooth curve. Graphical differentiation with respect to time gives the d$Y$/d$t$ value. From Eqs. (4) and (5), we can estimate the heat of the autoxidative reaction based on reacted PUFA,d$q$/d$n_F$, using the following equation:

$$\frac{dq}{dn_F}=\frac{dq/dt}{dn_F/dt}=2M_F\frac{dq_W/dt}{dY/dt}. \qquad (6)$$

The d$q$/d$n_F$ values during autoxidation at various temperatures were evaluated for each PUFA according to the procedures described above. Figures 4 (a) and (b) show the relationships between $-$d$q$/d$n_F$ and $Y$ for ethyl arachidonate and ethyl docosahexaenoate, respectively. Figure 2 suggested that evaporation of the sample occurred during the measurements. Since evaporation consumes latent heat, we should add the latent heat to d$q$/d$t$ to obtain the intrinsic heat of the autoxidative reaction from d$q$/d$t$. However, we had no information on the latent heat, and furthermore evaporation was dependent on $Y$. Therefore, no correction for d$q$/d$t$ was made. Thus, the intrinsic $-$d$q$/d$n_F$ values should be greater than those shown here.

For ethyl arachidonate, the $-$d$q$/d$n_F$ value was unchanged and was about $2\times10^5$ J/mol throughout the autoxidation process as shown in Fig. 4 (a). On the other hand, the $-$d$q$/d$n_F$ value for ethyl docosahexaenoate was dependent on $Y$. During the early stage of autoxidation ($Y\geq0.5$), the $-$d$q$/d$n_F$ value was almost constant and was about $2\times10^5$ J/mol, which was consistent with that for ethyl arachidonate. However, the $-$d$q$/d$n_F$ value increased as the autoxidation proceeded in the region of $Y$ less than 0.5.

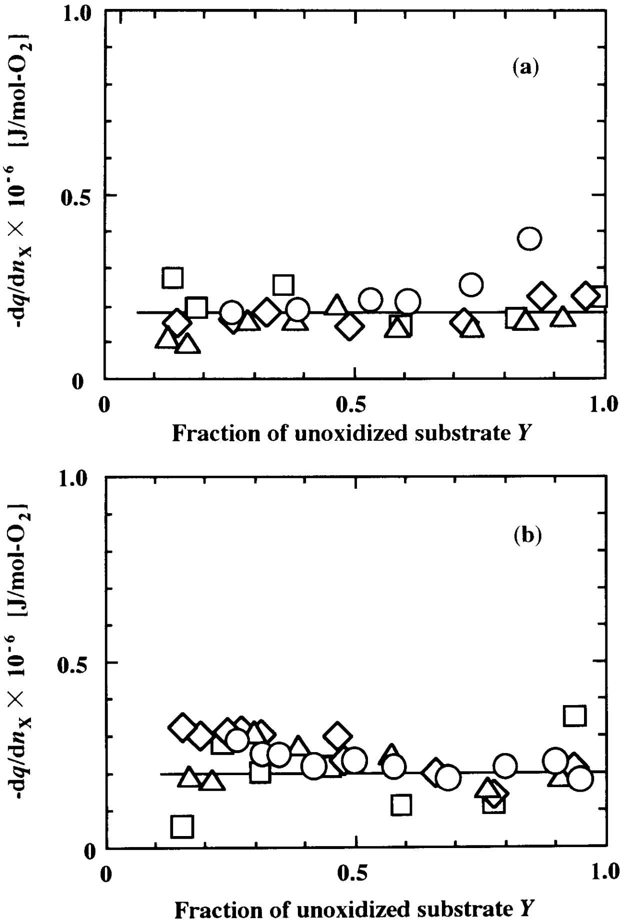

**Fig. 5.** Heat of autoxidative reaction per mole of oxygen reacted, $-$d$q$/d$n_X$, as a function of the fraction of unoxidized substrate $Y$ for ethyl arachidonate (a) and ethyl docosahexaenoate (b). Symbols are the same as those in Fig. 3.

The $-$d$q$/d$n_F$ values were converted to the heats of the autoxidative reactions based on moles of oxygen reacted, $-$d$q$/d$n_X$ using the following equation:

$$\frac{dq}{dn_X}=\frac{dq/dn_F}{dn_X/dn_F}. \qquad (7)$$

The stoichiometric coefficient, $\gamma$=d$n_X$/d$n_F$, was estimated by graphically differentiating the curve connecting the plots of $\Delta w/w_0$ versus $Y$ according to Eq. (3). The estimation of $\gamma$ was separately performed for each PUFA and at each temperature.

Figures 5 (a) and (b) show plots of $-$d$q$/d$n_X$ versus $Y$ for ethyl arachidonate and ethyl docosahexaenoate, respectively. The $-$d$q$/d$n_X$ value was unchanged throughout the entire autoxidation process for both PUFAs, and was about $2\times10^5$ J/mol-O$_2$. This meant that the heat generated during the consumption of one mole of oxygen did not depend on the autoxidation level of any PUFA.

The results for ethyl $\gamma$-linolenate and ethyl eicosahentaenoate were also similar to those for ethyl arachidonate and ethyl docosahexaenoate, respectively, although these are not shown. Therefore, we infer that the results observed here are common for all n-3 and n-6 PUFAs, although further investigations for other PUFAs are required to confirm this inference. Also, we propose that the differences in the rate equations for n-3 and n-6 PUFAs are ascribable to the differences in the stoichiometric coefficients between oxygen and each PUFA. The latter is phenomenological, and is not based on the autoxidation mechanism of each PUFA. However, the results obtained here would provide useful information for consideration of the mechanism.

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