

Note

Oxidation of Polyunsaturated Acylglycerol Mixed with Saturated or Unsaturated Acylglycerol

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The oxidation process of a polyunsaturated acylglycerol mixed with a saturated or unsaturated acylglycerol was measured at 65°C and ca. 0% relative humidity. When two polyunsaturated acylglycerols were mixed, their oxidation processes were expressed by the kinetic model in which one acylglycerol acts as a diluent for another acylglycerol, and the oxidation product of the acylglycerol participates in the oxidation of another acylglycerol. However, the oxidation process of 1-monolinolein mixed with a saturated or monounsaturated acylglycerol was not expressed by the model but obeyed the kinetics of an autocatalytic type. The rate constant in the mixed system was greater than the constant which was predicted under the assumption that the saturated or monounsaturated acylglycerol merely acted as a diluent.

Keywords: polyunsaturated acylglycerol, oxidation, kinetics

Lipid is deteriorated through its oxidation, which includes initiation, propagation and termination steps. The oxidation kinetics has usually been studied for each step (Labuza, 1971; Brimberg, 1993; Simic *et al.*, 1992). Özilgen and Özilgen (1990) reported that a kinetic expression of the autocatalytic type in terms of the fraction of unoxidized substrate could describe the entire oxidation process for some lipids. We also showed that the expression was successfully applicable to the entire oxidation processes of the n-6 fatty acids, their esters (Adachi *et al.*, 1995) and their acylglycerols (Minemoto *et al.*, 1999), and to the first half of the oxidation processes of the n-3 fatty acids and their esters (Adachi *et al.*, 1995). The latter half of the processes obeyed simple first-order kinetics (Adachi *et al.*, 1995).

These studies were conducted in a single-component system where the oxidation of a polyunsaturated fatty acid (PUFA), its ester or acylglycerol was observed. However, the edible oil is a mixture of acylglycerols consisting of a variety of saturated and unsaturated fatty acids. Therefore, we measured the oxidation processes of a PUFA or its ester in the presence of a saturated fatty acid or its methyl ester (Ishido *et al.*, 2001), and those of PUFAs in a system where two PUFAs or their esters coexisted (Ishido *et al.*, 2002).

In this study, we measured the oxidation of a polyunsaturated acylglycerol mixed with an unsaturated or saturated acylglycerol and investigated the applicability of our previous kinetic equations to the oxidation processes.

Materials and Methods

Materials 1-Monolinolein (purity, > 98%), trilinolein (> 99%), triarachidonin (> 99%), dilaurin (mixture of 1,2- and 1,3-

isomers, > 99%), 1-monoolein (> 99%), diolein (mixture of isomers, > 99%) and triolein (> 99%) were purchased from Sigma (St. Louis, MO). 1-Monolaurin (> 98%), trilaurin (> 98%) and methyl palmitate (> 95%) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The acylglycerols were stored at -30°C until use and were used without any purification. Other chemicals were purchased from either Wako Pure Chemical Industries (Osaka) or Nacalai Tesque (Kyoto).

Oxidation An acylglycerol (100 mg) was dissolved in 2 ml of hexane or chloroform. Another acylglycerol was also dissolved in the same manner. These solutions were mixed to produce a given molar ratio of one acylglycerol to another, 1 : 1 in the most cases. One hundred microliters of the mixture was pipetted into a flat-bottomed glass cup (1.5 cm i.d. and 3.0 cm height) using a micropipette, and the hexane or chloroform was then removed under reduced pressure. About 10 cups were placed in a desiccator, and the desiccator was stored in the dark in a temperature-controlled chamber at 65°C. The relative humidity in the desiccator was regulated at ca. 0% using phosphorus (V) oxide. At appropriate intervals, a cup was removed from the desiccator, and 0.3 ml of methyl palmitate dissolved in hexane at a concentration of 0.5 µl/ml was added to the cup. Hexane was then removed under reduced pressure. One milliliter of methanol and 0.1 ml of sodium methoxide, dissolved in methanol at a concentration of 0.5 g/l, were added to the cup and shaken to dissolve the acylglycerols. Transesterification was then carried out at 70°C for 30 min; by adding 10 µl of acetic acid to the cup, transesterification was stopped. Methanol was removed under reduced pressure. The remainder was dissolved in 0.5 ml of hexane and used for gas chromatographic analysis.

Analysis The amount of unoxidized acylglycerol was determined using a Shimadzu GC-14A or a GC-7A gas chromatograph (Kyoto) with a hydrogen flame-ionization detector. The

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column was 3.2 mm in diameter and 3.1 m long and was packed with Advance-DS 5% on Shinchrom A (Shinwa Kako, Kyoto) when the sample did not contain 1-monolinolein, diolein or triolein. When the sample contained the oleins, a column packed with therman-3000 50% on Shincarbon A (Shinwa Kako) was used. The analytical conditions were as follows: a column temperature of 180°C, an injection temperature of 230°C, a sample-injection volume of 5 μ l and N₂ as the carrier gas at a flow rate of 50 ml/min.

Results and Discussion

Oxidation processes of polyunsaturated acylglycerols in their mixed system Figures 1 (a) and (b) show the oxidation processes of 1-monolinolein and triarachidonin, and trilinolein and triarachidonin in their equimolar mixed systems at 65°C and *ca.* 0% relative humidity. The closed symbols in the figures indicate the oxidation processes of 1-monolinolein, trilinolein and triarachidonin in their single-component systems. As shown in (a), the oxidation of 1-monolinolein was promoted, while that of triarachidonin was decelerated in their mixed system compared with their oxidation in the single-component system. Similar to the results shown in (a), trilinolein was oxidized faster in the presence of triarachidonin, while the oxidation of triarachidonin was decelerated in the presence of trilinolein ((b)).

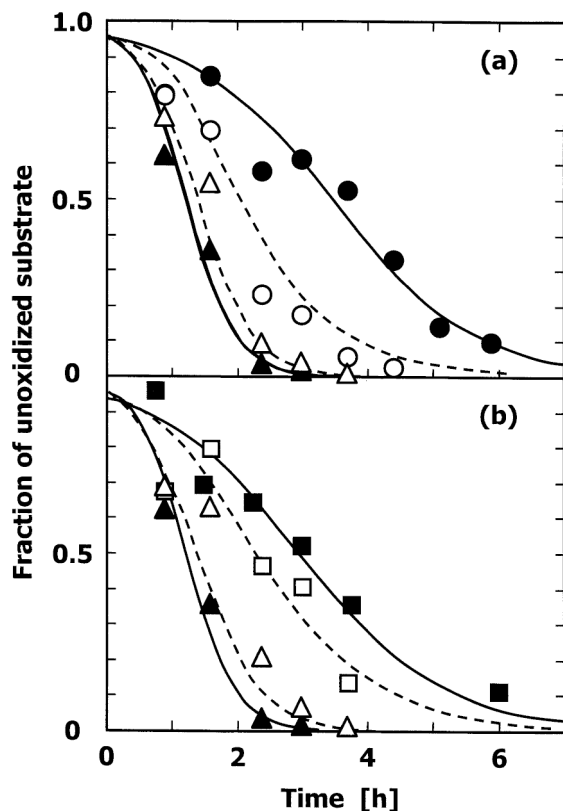


Fig. 1. Oxidation processes of (a) 1-monolinolein and triarachidonin and (b) trilinolein and triarachidonin in their mixed systems at a molar ratio of 1 : 1 and at 65°C and *ca.* 0% relative humidity. The symbols, ●, ■ and ▲, represent the oxidation processes of 1-monolinolein, trilinolein and triarachidonin, respectively, in their single-component systems. The symbols, ○, □ and △, indicate the oxidation processes of 1-monolinolein, trilinolein and triarachidonin, respectively, in the mixed system. The solid curves were calculated using the parameters, k_i° and Y_{i0} , evaluated according to Eq. (5). The broken curves represent the oxidation processes calculated by Eq. (1).

We have proposed the rate equations for the oxidation of two PUFAs, A and B, in their mixed system under the assumption that the oxidation rate of component i ($i=A$ or B) is proportional to both the concentration of the unoxidized substrate i and the sum of the concentrations of oxidized A and B (Ishido *et al.*, 2002):

$$-\frac{dY_A}{dt} = k_A Y_A [(1 - Y_A) + \gamma_{B/A} (1 - Y_B)], \quad (1a)$$

$$-\frac{dY_B}{dt} = k_B Y_B \left[\frac{1}{\gamma_{B/A}} (1 - Y_A) + (1 - Y_B) \right], \quad (1b)$$

where Y_i is the fraction of the unoxidized substrate ($i=A$ or B) and t is the time. The $\gamma_{B/A}$ is the molar ratio of residue B to residue A in the mixture of polyunsaturated acylglycerols and is given by

$$\gamma_{B/A} = \frac{n_B m_B}{n_A m_A}, \quad (2)$$

where n_i is the moles of component i ($=A$ or B), and m_i is the number of PUFA residues of each acylglycerol. The rate constant in the binary system, k_i , is related to the rate constant in a single-component system, k_i° , by Eq. (3) based on the molecular mass, M_i , and the density, ρ_i , of component i .

$$k_i = \frac{n_i M_i / \rho_i}{n_A M_A / \rho_A + n_B M_B / \rho_B} k_i^\circ. \quad (3)$$

The densities of the acylglycerols used in this study were assumed to be 0.9 g/ml because they were not available in the literature. In the single-component system, the oxidation kinetics for n-6 polyunsaturated acylglycerol obeyed a simple autocatalytic-type expression (Minemoto *et al.*, 1999):

$$-\frac{dY_i}{dt} = k_i^\circ Y_i (1 - Y_i). \quad (4)$$

The integration of Eq. (4) under the initial condition of $Y_i = Y_{i0}$ at $t=0$ gives

$$\ln \frac{1 - Y_i}{Y_i} = k_i^\circ t + \ln \frac{1 - Y_{i0}}{Y_{i0}}. \quad (5)$$

According to Eq. (5), the rate constants, k_i° , and the parameters, Y_{i0} , for 1-monolinolein, trilinolein and triarachidonin were evaluated using the experimental data shown by the closed symbols in Fig. 1. The rate constant of each acylglycerol in the mixed system, k_i , was then calculated by substituting the k_i° value and the molecular masses into Eq. (3). The oxidation processes of 1-monolinolein and triarachidonin or of trilinolein and triarachidonin in their equimolar mixture were calculated by numerically and simultaneously solving Eqs. (1a) and (1b) under the initial conditions of $Y_A = Y_{A0}$ (A: 1-monolinolein or trilinolein) and $Y_B = Y_{B0}$ (B: triarachidonin) at $t=0$ by the Runge-Kutta-Gill method, and are shown by the broken curves in Fig. 1. Because Y_{i0} is a parameter reflecting the initial state of an acylglycerol, the value determined in the single-component system was used for the binary system. The calculated curves fairly well expressed the experimental results in the binary systems. This indicates that a component acts as a diluent for another component (Eq. (3)) and that the oxidation product of the component participates in the oxidation of another component (Eq. (1)).

Oxidation of 1-monolinolein mixed with oleoyl or lauroyl acylglycerol Figure 2 shows the oxidation processes of 1-monolinolein mixed with 1-monoolein, diolein or triolein at a molar ratio of 1:1 under the same conditions as in Fig. 1. The closed circles in the figure represent the oxidation process of 1-monolinolein alone. The oxidation of 1-monolinolein mixed with the monounsaturated acylglycerols proceeded more slowly than that of 1-monolinolein in the single-component system. If it is assumed that the oleins are not oxidized but act merely as diluents for 1-monolinolein, the oxidation processes of 1-monoli-

nolein in the mixed systems can be calculated by substituting the k_i value evaluated from Eq. (3) into Eq. (4) or (5) instead of the k_i^o value. The broken curves calculated under the assumptions were far from the experimental results, while the experimentally observed oxidation processes of 1-monolinolein mixed with 1-monoolein, diolein or triolein were almost the same.

The monounsaturated acylglycerols, oleins, themselves were slightly oxidized, and the fractions of unoxidized oleoyl residue were *ca.* 0.8 at 10 h for every olein (data not shown). The extent of oxidation was too small to estimate their k_i^o and Y_{i0} values and

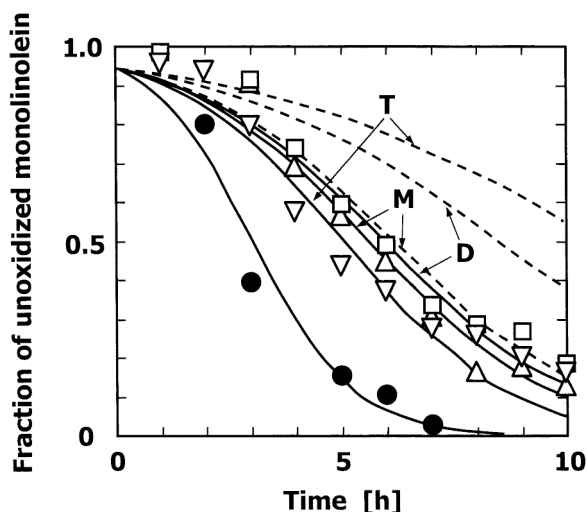


Fig. 2. Oxidation process of 1-monolinolein mixed with (Δ) 1-monoolein, (\square) diolein or (∇) triolein at a molar ratio of 1:1 and at 65°C and *ca.* 0% relative humidity. The closed circles represent the oxidation process of 1-monolinolein alone. The broken curves were calculated under the assumption that each olein acted as a diluent for 1-monolinolein. The solid curves were drawn using the k_{obs} values which were evaluated by fitting the experimental results to Eq. (5). The labels, M, D and T, represent the oxidation processes calculated for 1-monolinolein mixed with 1-monoolein, diolein and triolein, respectively.

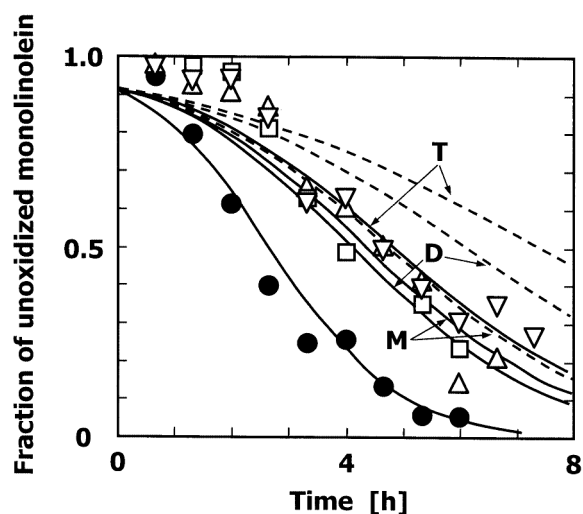


Fig. 3. Oxidation process of 1-monolinolein mixed with (Δ) 1-monolaurin, (\square) dilaurin or (∇) trilaurin at a molar ratio of 1:1 and at 65°C and *ca.* 0% relative humidity. The closed circles are the same as in Fig. 2. The broken and solid curves were also drawn in the same manner as in Fig. 2. The labels, M, D and T, indicate that 1-monolinolein was mixed with 1-monolaurin, dilaurin and trilaurin, respectively.

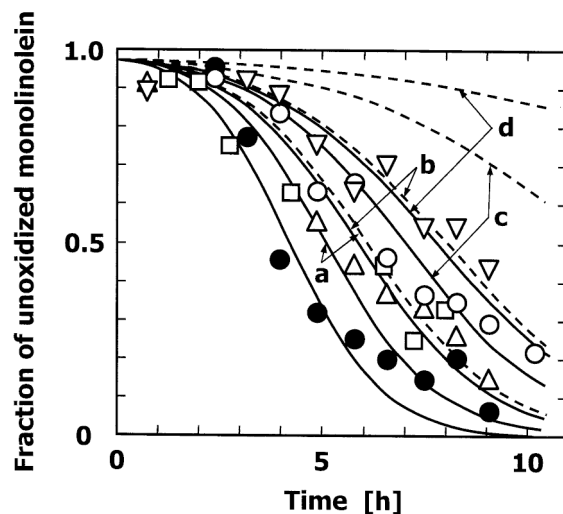


Fig. 4. Oxidation processes at 65°C and *ca.* 0% relative humidity of 1-monolinolein mixed with trilaurin at various molar ratios. The molar ratios of 1-monolinolein to trilaurin were (\square , a) 4, (Δ , b) 2, (\circ , c) 1 and (∇ , d) 0.5. The closed circles represent the oxidation process of 1-monolinolein in its single-component system. The broken and solid curves were drawn in the same manner as in Fig. 2.

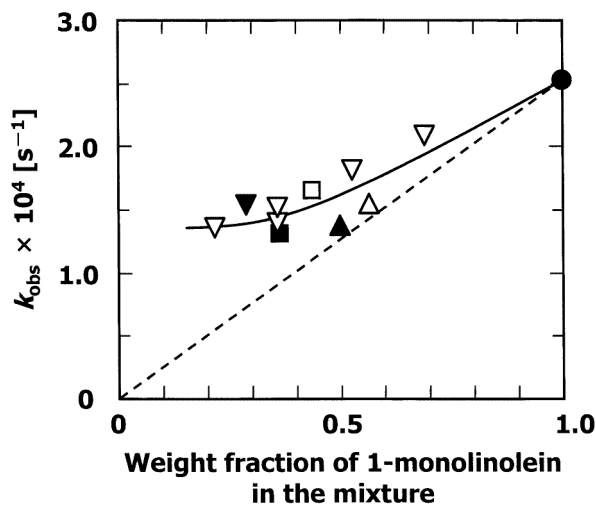


Fig. 5. Relationship between the rate constant, k_{obs} , of 1-monolinolein in a mixed system and its weight fraction in the mixture. The k_{obs} values were evaluated under the assumption that the oxidation process obeyed the autocatalytic kinetics, Eq. (5), even in the mixed system. The closed circle represents the rate constant in the single-component system. The closed symbols, \blacktriangle , \blacksquare and \blacktriangledown , represent the k_{obs} values of 1-monolinolein mixed with 1-monoolein, diolein and triolein, and the open ones, \triangle , \square and ∇ , are the k_{obs} values of 1-monolinolein mixed with 1-monolaurin, dilaurin and trilaurin, respectively. The broken line indicates the relationship in the case where the oleins and laurins act merely as a diluent for 1-monolinolein. The solid curve was drawn empirically.

to calculate the oxidation processes of 1-monolinolein by using Eq. (1). Therefore, the oxidation processes of 1-monolinolein mixed with the oleins were analyzed by Eq. (5) under the assumption that they obeyed the kinetics of the autocatalytic type even in the mixed systems, and the apparent rate constants, k_{obs} , were evaluated. The solid curves in the figure were drawn using the k_{obs} values.

Figure 3 shows the oxidation processes of 1-monolinolein mixed with 1-monolaurin, dilaurin or trilaurin under the same conditions as in Fig. 1. The closed circles in the figure represent the oxidation processes of 1-monolinolein alone, and the k_i^0 and Y_{i0} values were determined from the results according to Eq. (5). 1-Monolinolein mixed with saturated acylglycerols was oxidized more slowly than that in the single-component system. The broken curves in the figure were calculated under the assumption that the laurins acted merely as diluents for 1-monolinolein. They could not express the experimentally observed oxidation processes, and the practical oxidation proceeded faster than the calculated one. The k_{obs} values were evaluated for 1-monolinolein mixed with the laurins according to Eq. (5), and the oxidation processes calculated using the k_{obs} values are shown in the figure by the solid curves.

The effect of the molar ratio of 1-monolinolein to trilaurin on the oxidation of 1-monolinolein was examined (Fig. 4). The oxidation of 1-monolinolein decelerated as its molar ratio in the mixture decreased. The oxidation processes where trilaurin was assumed to act as a diluent are shown by the broken curves; these curves deviated greatly from the experimental results. As shown by the solid curve, the oxidation process of 1-monolinolein mixed with trilaurin at any molar ratio obeyed the autocatalytic kinetics (Eq. (4) or (5)), and the k_{obs} value was evaluated.

As shown above, saturated or monounsaturated acylglycerols did not merely act as diluents for the oxidation of 1-monolinolein mixed with them, although the oxidation process obeyed the autocatalytic kinetics in every case. To examine the relationship

between the k_{obs} value and the weight fraction of 1-monolinolein in the mixture, they are plotted in Fig. 5. When the acylglycerols used are assumed to have the same density and the additivity in volume is also assumed to hold, the weight fraction corresponds to the volume fraction of 1-monolinolein in the mixture. The broken line represents the hypothetical relationship between the k_{obs} value and the weight fraction under the assumption that the saturated and monounsaturated acylglycerols act as diluents. All the symbols lie over the line, indicating that the acylglycerols do not act only as a diluent but also promote the oxidation of 1-monolinolein through some mechanism(s) which remains unclear.

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