Oxidation Kinetics of Methyl Linoleate and α-Linolenate in Bulk and Oil-in-water Emulsion Systems

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2014
CONTENTS

General Introduction ...........................................................................................................1

I.1 Oxidation of Unsaturated Fatty Acids in Food Systems .............................................1
I.2 Oxidation of Unsaturated Fatty Acids in Bulk Phase .................................................3
I.3 Oxidation of Unsaturated Fatty Acids in Emulsion .....................................................4
I.4 Aims of This Thesis ........................................................................................................6

Chapter 1

Kinetics of Oxidation of Different Depths of Methyl Linoleate in Bulk Phase .............7

1.1 Introduction .....................................................................................................................7
1.2 Materials and Methods .................................................................................................8
1.3 Results and Discussion .................................................................................................9
1.4 Conclusion .....................................................................................................................17

Chapter 2

Oxidation of Methyl Linoleate Mixed with Methyl Octanoate, Laurate or Palmitate .........................................................................................................................18

2.1 Introduction .....................................................................................................................18
2.2 Materials and Methods .................................................................................................18
2.3 Results and Discussion .................................................................................................21
2.4 Conclusion .....................................................................................................................27
Chapter 3
Effects of Various Emulsification Methods on the Oxidation of Methyl Linoleate

3.1 Introduction ..............................................................................................................29
3.2 Materials and Methods .........................................................................................29
3.3 Results and Discussion .........................................................................................32
3.4 Conclusion ...............................................................................................................37

Chapter 4
Effect of Droplet Size on Autoxidation Rates of Methyl Linoleate and α-Linolenate
in an Oil-in-water Emulsion .........................................................................................38
4.1 Introduction ..............................................................................................................38
4.2 Materials and Methods .........................................................................................38
4.3 Results and Discussion .........................................................................................41
4.4 Conclusion ...............................................................................................................47

Concluding Remarks ..................................................................................................49

References ...................................................................................................................51

Acknowledgements .....................................................................................................59

List of Publications .....................................................................................................61
General Introduction

I.1 Oxidation of Unsaturated Fatty Acids in Food Systems

Polyunsaturated fatty acids (PUFAs), such as linoleic, α-linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids, would have many biological functions. It was reported that PUFAs reduce the susceptibility to mental illness or Alzheimer’s disease, and protect against heart disease by decreasing serum triacylglycerol and cholesterol (Krutulyte et al., 2008; Ritter-Gooder et al., 2008; Siró et al., 2008; Ramsden et al., 2013). However, their chemical instability makes them susceptible to oxidation and other chemical changes that lead to deterioration and rancidity in natural and processed foods (Coupland et al., 1996; Furuta et al., 2005). The lipid oxidation, which occurs during processing, storage and cooking, is always of great concern to the food industry because it leads to the generation of undesirable rancidity and potentially toxic reaction products (Frankel, 1980; Guillén and Goicoechea, 2008; Uriarte and Guillén, 2010). Oxidation of lipids results in the formation of hydroperoxide, which is the primary product of the oxidation and may have physiological toxicity. Nutritional values of foods would be damaged by a wide range of volatile and non-volatile secondary products formed from the hydroperoxides (Frankel, 1980; Simic et al., 1992; Halliwell et al., 1995). Therefore, food manufactures need to develop methods for preventing, or at least retarding the oxidation in food products.

Progression of the oxidation, which is affected by many factors, such as temperature, humidity, and the presence of a prooxidant or antioxidant, is considered to be very complicated (Labuza, 1973; Frankel, 1980; Simic et al., 1992; Park et al., 2005; Aladedunye et al., 2012; Kadowaki et al., 2012a; Kadowaki et al., 2012b; Roman et al.,
The oxidation process, including initiation, propagation and termination steps, is interpreted as a free radical chain mechanism (Frenkel et al., 1991; Simic et al., 1992). At the initiation step, unsaturated fatty acids (LH) form alkyl radicals (L·) in the presence of initiators (In·), including endogenous and exogenous species (Frankel, 1980).

\[ \text{In} \cdot + \text{LH} \rightarrow \text{InH} + \text{L} \cdot \]

In the presence of light, unsaturated lipids also form hydroperoxides by reacting with singlet oxygen which is produced by sensitized photooxidation (Gollnick, 1978). Because of high reactivity of peroxyl radicals with the allylic or bisallylic bonds as well as high reactivity of resulting fatty acid radicals with oxygen, the peroxidation of unsaturated fatty acids is mainly a chain reaction. By the radical chain mechanism, hydroperoxides (LOOH) is prone to be formed as the primary product of the oxidation at the propagation step (Simic et al., 1992).

\[ \text{L} \cdot + \text{O}_2 \rightarrow \text{LOO} \cdot \]

\[ \text{LOO} \cdot + \text{LH} \rightarrow \text{LOOH} + \text{L} \cdot \]

This radical chain mechanism terminates through the following reactions (McClements and Decker, 2000).

\[ \text{LOO} \cdot + \text{LOO} \cdot \rightarrow \text{LOOL} + \text{O}_2 \]

\[ \text{LOO} \cdot + \text{L} \cdot \rightarrow \text{LOOL} \]

\[ \text{L} \cdot + \text{L} \cdot \rightarrow \text{LL} \]

By controlling a certain step, usually the initiation step or one of the early propagation steps, it is possible to effectively retard the lipid oxidation (McClements and Decker, 2000).

The most commonly used method for retarding lipid oxidation in foods is the addition
of antioxidants (Giroux et al., 2007; Huber et al., 2009). Unfortunately, some of the food additives, especially the chemically synthesized antioxidants, are recognized as unsafe ingredients in spite of their relatively higher efficiency. In addition, it is difficult to approve new antioxidants due to economic barriers and food safety regulations. Thus, food manufacturers are prompted to use existing antioxidants more efficiently (Chaiyasit et al., 2007). Combination of different kinds of the antioxidants would be better for lipid antioxidation (Choe and Min, 2006). Furthermore, undesirable lipid oxidation is also retarded by the control of oxygen concentration in food. The concentration of oxygen can be reduced by packing foods under vacuum or nitrogen. The rancidity potential can also be reduced by partial hydrogenation of lipids to convert the unsaturated lipid constituents into the saturated ones. However, it would lead to the formation of trans fatty acids, which is atherogenic by increasing low density lipoprotein and decreasing high density lipoprotein (Duxbury, 2005).

I.2 Oxidation of Unsaturated Fatty Acids in Bulk Phase

When oils are stored in a bulk phase, there is an interface between oil and air through which oxygen diffuses into oil. In a gas-liquid reaction, diffusion of the gas into the liquid generally affects the reaction rate of the gas with the liquid (Levenspiel, 1962). During the oxidation reaction, the stoichiometric coefficient of oxygen to PUFA would be equal to or greater than unity (Adachi et al., 1995a). Thus, at low oxygen concentrations, the rate-limiting step for lipid oxidation is the diffusion of oxygen. At the higher concentrations, the rate of oxygen diffusion is much higher than that of lipid oxidation, thus oxidation itself becomes the rate-limiting step (Coupland et al., 1996). Therefore, the reaction rate of oxidation can be limited by the supply of oxygen in some
cases (Karel, 1986).

Moreover, the reaction rate of lipid oxidation depends on the degree of unsaturation and the concentration of fatty acids (Chen et al., 2001; Achir et al., 2006; Marmesat et al., 2012; Richaud et al., 2012; Maire et al., 2013). It was reported that the presence of saturated fatty acid or its methyl ester also retarded the oxidation of an unsaturated substrate because the saturated component acts as a diluent to decrease the concentration of the unsaturated substrate (Ishido et al., 2001; Ishido et al., 2002a; Ishido et al., 2002b). Methyl esters of fatty acids, which are more prone to evaporate, are often used in the model oxidation systems for kinetic analysis. In these model systems, there is a possibility that the concentration of the coexisting methyl esters changes as the reaction processes by the evaporation, especially at relatively high temperature. Thus, the oxidation behavior of unsaturated substrate would also be affected.

I.3 Oxidation of Unsaturated Fatty Acids in Emulsion

A system that consists of oil droplets dispersed in an aqueous phase is called an oil-in-water (O/W) emulsion. To prepare O/W emulsions that are kinetically stable for a reasonable period of time, chemical substances known as emulsifiers must be added prior to emulsification. Emulsifiers, which are surface active molecules that adsorb on the surface of the freshly formed droplets during emulsification, would greatly impact the lipid oxidation rate in the oil phase of emulsion by affecting the electrical charge of droplet, or the physical- or chemical-barrier effect (Chen and Nawar, 1991; Mei et al., 1998; Mancuso et al., 1999; McClements and Decker, 2000; Silvestre et al., 2000; Boon et al., 2008; Taherian et al., 2011; Berton-Carabin et al., 2013). As mentioned before, the
presence of a saturated fatty acid affects the oxidation of the mixed unsaturated substrate. This dilution effect also occurred in an O/W emulsion system where droplets of the unsaturated lipid are covered with an emulsifier, the hydrophobic moiety of which was a saturated acyl group (Imai et al., 2008).

There are various emulsification methods for preparing emulsions. High-speed stirring, high-pressure homogenization, ultrasonic homogenization and membrane filtration are commonly used. In some methods, the emulsification process subjects the oil to mechanical stress, which causes deterioration of the oil (Kuhn et al., 2012). However, some results have shown that oil deterioration is suppressed in emulsions (Atarés et al., 2012; Poliseli-Scopel et al., 2012; Rodriguez-Turienzo et al., 2012; Kim et al., 2013). Therefore, there are no definite conclusions regarding the loss of oil quality, including its oxidation, during emulsification. This uncertainty arises because the oxidation of lipid is evaluated by using food-based materials containing various components, especially the emulsifiers.

As a critical physical characteristic of the droplets in O/W emulsions, the size of oil droplet is very considerable, especially for the possibility of its effect on oxidation rate of the unsaturated fatty acid which would be contained in the oil phase. These unsaturated fatty acids can be oxidized by oxygen supplied from the aqueous phase through the O/W interface. When the specific surface area of oil droplet becomes large, and sufficient amount of oxygen is supplied through the interface, the oxidation reaction itself would be the rate limiting step. However, the effect of the mass transfer of oxygen through the interface on the oxidation rate of the fatty acid can be significant when the oil droplet size becomes large. Thus, lipid oxidation in O/W emulsion systems may depend on oil droplet size. It was reported that the oxidation of docosahexaenoic acid
and sunflower seed oil is accelerated in emulsions with smaller oil droplets (Gohtani et al., 1999; Lethuaut et al., 2002). Conversely, decreases in oil droplet size reportedly decelerate lipid oxidation (Nakaya et al., 2005; Imai et al., 2008; Ries et al., 2010). In addition, no significant effect of oil droplet size on the oxidation rate has also been reported (Roozen et al., 1994; Osbern et al., 2004; Dimakou et al., 2007; Sun et al., 2009). The inconsistency in conclusions regarding the dependence of susceptibility to oxidation on oil droplet size may be explained by differences in susceptibility to oxidation of the lipid used as the oil phase, the range of tested oil droplet sizes, temperature, and the method used to assess lipid oxidation.

I.4 Aims of This Thesis

In this study, methyl linoleate as a representative PUFA was used for kinetic analysis of oxidation with different depths or amounts in bulk phase to elucidate the impact of oxygen diffusion. Effect of the partial pressure of oxygen on the oxidation was also evaluated. In addition, the oxidation process of methyl linoleate was evaluated in the presence of saturated fatty acid methyl esters in the consideration of the volatility of the methyl esters.

Different methods were applied for the emulsification of methyl linoleate without an emulsifier to estimate the effects of the emulsification method on lipid deterioration. Furthermore, in order to evaluate the effect of droplet size on the oxidation of PUFAs in O/W emulsion system, the oxidation processes of methyl linoleate and methyl α-linolenate were evaluated when they were emulsified in various oil droplet sizes on a micrometer scale.
Chapter 1

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

1.1 Introduction

When the specific interface area between the gas and liquid is sufficiently small in gas-liquid reaction, 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 of air and oil stored in bulk phase is usually small; thus, diffusion of oxygen into oil would be limited. However, there are few quantitative studies regarding the oxidation behavior of bulk oil from the viewpoint of the effect of oxygen diffusion on the oxidation reaction rate.

The stoichiometric coefficient of oxygen to PUFA is equal to or greater than unity for the oxidation (Adachi et al., 1995a). Therefore, the oxidation is also retarded when the amount or partial pressure of oxygen is limited. There are some reports for the effect of the partial pressure or the concentration of oxygen on the oxidation kinetics (Adachi et al., 1995b; Yoshii et al., 1999). However, the quantitative studies of the oxidation still remain insufficient.

In this chapter, 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.
1.2 Materials and Methods

1.2.1 Materials

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

1.2.2 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. × 3.0 cm); these samples corresponded to oil depths of 1.0, 2.0, 5.0, 10 and 20 mm, respectively. Approximately 90 sets of the samples were placed in a plastic container (300 mm length × 150 mm width × 150 mm height). Air was then flowed through the container at 15 mL/min after passing through silica gel for conducting the oxidation reaction. The plastic container was stored at 65°C 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 well mixed. Approximately 0.12–0.14 g of accurately weighed substrate was transferred into another cup containing a given amount (ca. 4–5 g) of 0.1 mol/L methyl myristate in hexane as the internal standard for GC analysis.

A small-amount sample of methyl linoleate was also oxidized. Methyl linoleate (2.208 g) was dissolved in 50 mL of hexane, and a sample of the solution (20, 40, 100 or 200 μL) was placed in a flat-bottomed glass cup. 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 by 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.

1.2.3 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°C to perform the oxidation. Periodically, a bottle was removed and its contents of unoxidized methyl linoleate were measured by GC.

1.2.4 Gas chromatographic analysis

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

1.3 Results and Discussion

1.3.1 Effect of depth or amount of methyl linoleate on oxidation

Figure 1-1 shows the time courses for the oxidation of different depths or amounts of methyl linoleate. For 1–10 µL of methyl linoleate, the oxidation proceeded quickly and
Fig. 1-1. Time courses of the fraction of unoxidized methyl linoleate at various depths (amounts) at 65°C. 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.

was almost complete within 20 h except for 10 μL of methyl linoleate. The induction periods for the oxidation were around 4–6 h for 1–5 μL of methyl linoleate, while 10 μL 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 the investigated conditions.

When a greater amount of methyl linoleate was exposed to oxidation, i.e., the depths of methyl linoleate were increased from 1 to 20 mm, oxidation was significantly retarded. The induction periods in these cases were longer than 8 h. In addition, a significant decrease in the fraction of the unoxidized methyl linoleate was not observed during the test duration when the depths were 10 and 20 mm, which indicates that most of the oxygen supplied through the oil and air interface was consumed at or near the
Fig. 1-2. Increase of the relative weight of methyl linoleate with depth during oxidation at 65°C; \( w_0 \) and \( \Delta w \) indicate the initial weight and increase in the weight of methyl linoleate, respectively. The symbols are the same as in Fig. 1-1.

Figure 1-2 shows the time courses of the oxidation with increasing relative weight, \( \Delta w/w_0 \), of different depths of methyl linoleate, where \( \Delta w \) 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 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. 1-1, indicating that the measurement of the weight change can also be used to effectively estimate the induction period (Usuki, 1983).

The fractions of unoxidized methyl linoleate at different depths of the original sample were plotted against the increase in the relative weight of the substrate (Fig. 1-3). The
Fig. 1-3. Relationship between the fraction of unoxidized methyl linoleate and 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. 1-1.

fractions in all the cases linearly decreased with increasing relative weight. It was 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. 1-3 represents the calculated result based on this stoichiometry. There was a small difference between the observed and calculated results: The observed increase in the relative weight was 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 the oxidation, the
oxidation rates, $v$, at fractions of the unoxidized substrate, $Y$, of 0.8 and 0.6 for methyl linoleate depths of 1–5 mm were compared to those without limited diffusion of oxygen (1–5 μL), $v_0$ (Fig. 1-4). 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. 1-1 by graphical differentiation. The rates at $Y = 0.6$ and 0.8 for the 2- and 5-mm-deep substrates, respectively, were roughly estimated by extrapolating the smoothly drawn curves; from these results, the average $v_0$ values and standard deviations for 1–5 μL of methyl linoleate were calculated to be $0.078 \pm 0.024 \text{ h}^{-1}$ and $0.115 \pm 0.004 \text{ h}^{-1}$ for $Y = 0.8$ and 0.6, respectively. For 10- and 20-mm-deep 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 substrate depths. The results indicate that the depth influenced the oxidation rate and 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 around 0.3; that is, the oxidation rate of 1-mm-deep methyl linoleate was approximately one-third of that for substrates without limited diffusion of oxygen. Thus, only 1 mm of depth significantly affected the oxidation behavior.

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, $\phi$, can be defined as follows:
\[ \phi = L \sqrt{\frac{k}{D}} \]  

(1-1)

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. 1-4 was extrapolated, as shown by the dashed line; the \( \phi \) value at the intersection of the extrapolated line and a ratio of 1, \( \phi_k \), was evaluated to be 0.11. At \( \phi \) values larger than 0.11, the diffusion of oxygen affects the oxidation rate of the substrate. The oxidation of n-6 PUFAs and their esters can be expressed by the following equation, which is based on the autocatalytic model (Adachi et al., 1995b):

\[ \ln \frac{1-Y}{Y} = kt + \ln \frac{1-Y_0}{Y_0} \]  

(1-2)

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. (1-2), the rate constant \( k \) could be obtained by plotting \( \ln (1-Y)/Y \) versus \( t \).

Fig. 1-4. Relationships between the relative rates, \( v/v_0 \), at \( Y = (\bigcirc) 0.8 \) and (\( \triangleright \) 0.6 and the depth of methyl linoleate.
Accordingly, the $k$ values for 1, 2, and 5 μL of methyl linoleate were evaluated, and the average value ($0.505 \pm 0.019$ h$^{-1}$) is regarded as the $k$ value. The $D$ value was roughly estimated to be $1.2 \times 10^{-9}$ m$^2$/s from the Wilke-Chang method (Wilke and Chang, 1955). From the $\phi$, $k$, 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 $\phi$ value would be applicable to the oxidation of other lipids with oxidation kinetics that can be expressed via an autocatalytic rate equation.

1.3.2 Oxidation of methyl linoleate at different partial pressures of oxygen

As described above, oxidation was increasingly retarded with increasing depths of methyl linoleate. There are a few 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 1-5 shows the time courses of the fraction of unoxidized methyl linoleate at different partial pressures of oxygen, $P_{O_2}$. The induction period increased with decreasing partial pressure. When the oxidation was performed in air ($P_{O_2} = 21$ kPa) or at a $P_{O_2}$ of 5 kPa, the induction period was around 6 h, but extended to around 9 h at a $P_{O_2}$ of 0.2 kPa. Based on Eq. (1-2), 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; it gradually decreased with decreasing partial pressure of oxygen, and sharply decreased
Fig. 1-5. Oxidation of methyl linoleate at different partial pressures of oxygen at 65°C. Symbols ▼, ▲, △, ○, ▽, □ and ◇ 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. 1-6. Dependence of the oxidation rate constant on the partial pressure of oxygen at 65°C.
at partial pressures lower than 5 kPa (Fig. 1-6). 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):

\[ k = \frac{k_{\text{max}} P_{O2}}{K_X + P_{O2}} \]  \hspace{1cm} (1-3)

where \( K_X \) is the saturation constant and \( k_{\text{max}} \) 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 would be ascribed to difficulties in the complete replacement of gases at a specific composition.

### 1.4 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, the small amount of methyl linoleate (< 5 μL) should be used to evaluate the oxidation kinetics without limited diffusion of oxygen at 65°C. The saturation constant of oxygen for the oxidation was also evaluated and was much lower than the partial pressure of oxygen in air at atmospheric pressure. Therefore, the oxidation was only slightly affected by the partial pressure higher than 5 kPa.
Chapter 2

Oxidation of Methyl Linoleate Mixed with Methyl Octanoate, Laurate or Palmitate

2.1 Introduction

In the previous study on the kinetics of the oxidation at 65°C of linoleic acid or methyl linoleate mixed with one of lauric, myristic and palmitic acids or their methyl esters, no volatility of the fatty acids and their methyl esters was assumed (Ishido et al., 2001). However, there is a possibility that the unsaturated lipid and the coexisting saturated one are volatilized at 65°C. Especially, methyl esters of fatty acids are prone to evaporation.

In this chapter, the oxidation process of methyl linoleate was re-evaluated in the presence of methyl octanoate, laurate or palmitate in consideration of the volatility of the methyl esters.

2.2 Materials and Methods

2.2.1 Materials

Methyl linoleate (purity, > 95%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Methyl octanoate (> 98%), methyl laurate (> 98%), and methyl palmitate (> 95%) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2.2 Oxidation of methyl linoleate

For the oxidation of methyl linoleate in a single-component system, 2 g of methyl linoleate was dissolved in 10 mL of methanol. Fifty microliters of the solution was
placed using a micropipette in a flat-bottomed glass cup (1.5 cm i.d. and 3.0 cm height); then the methanol was removed under reduced pressure. About 80 cups were placed in a plastic container. Air was allowed to flow at 5 mL/min (0.03 cm/min) into the container after passing through a column packed with silica gel. The rate of oxygen supplied by the air flow was sufficiently higher than that of oxygen consumed by the oxidation of methyl linoleate. The desiccator was stored in a DN-400 oven (Yamato Scientific, Tokyo, Japan) at 65°C. Periodically, a cup was removed, and 1 mL of 0.01 mol/L methyl myristate was added to the cup as the internal standard in gas chromatographic analysis. Unoxidized methyl linoleate was determined by subjecting 1 μL of the mixture to a Shimadzu GC-2014AFsc gas chromatograph (Kyoto, Japan) equipped with a hydrogen flame-ionization detector and a DB-1 column (J&W Scientific, Santa Clara, CA, USA). The inner diameter and the length of the column were 0.25 mm and 30 m, respectively. The analytical conditions were a column temperature of 230°C, injection temperature of 260°C, detector temperature of 280°C and helium as the carrier gas at a flow rate of 168 mL/min. The ratio of the amount of methyl linoleate to that of methyl myristate was obtained from the areas under their peaks. The fraction of the unoxidized substrate was calculated from the ratio.

Methyl linoleate was mixed with methyl octanoate, methyl laurate or methyl palmitate to produce a weight of 2 g at a molar ratio of 1:1 in 10 mL of methanol in order to measure the oxidation processes of methyl linoleate in a mixed system. The oxidation of methyl linoleate mixed with methyl laurate at molar ratios of 1:3 and 3:1 was also measured. Other procedures were the same as those above-mentioned for the oxidation in the single-component system.
2.2.3 Additivity in volume of fatty acid esters

In order to measure the density of methyl octanoate, methyl laurate or methyl palmitate, 0.5 g of the methyl ester was put into a 10-mL graduated cylinder, which had been calibrated using distilled water. The cylinder was immersed in a water-bath kept at 25°C for methyl octanoate and laurate or 35°C for methyl palmitate for 10 min, and then the volume was read. A further 0.5 g of the methyl ester was added to the cylinder. The same operations were repeated until 2.5 g of the methyl ester was added.

One gram of methyl linoleate had been put in the graduated cylinder, and 0.5 g of methyl octanoate, methyl laurate or methyl palmitate was added to the cylinder. The mixture was mixed magnetically at 25°C or 35°C for 10 min, and its volume was read. At every 0.5 g of methyl ester added, the volume was read until 2.5 g of the methyl ester was added.

2.2.4 Volatility of fatty acid ester

Methyl octanoate (16.96 mg), methyl laurate (17.40 mg), methyl palmitate (21.05 mg) or methyl linoleate (22.13 mg) was put in a 6 mmφ × 2.5 mm platinum cell, and the weight change was measured at 65°C under flowing nitrogen gas at a rate of 20 mL/min using a TGA-50H thermogravimeter (Shimadzu, Kyoto, Japan).

Methyl linoleate and methyl laurate were mixed to produce the molar ratio of 1:3, 1:1 or 3:1, and the mixture was put in the glass cups. The cups were held in the plastic container at 65°C under flowing nitrogen gas at 5 mL/min. A cup was removed and the amount of remaining methyl laurate was determined by gas chromatography as above-mentioned. It was also confirmed by determining the amount of unoxidized methyl linoleate that no oxidation of methyl linoleate occurred.
2.3 Results and Discussion

2.3.1 Oxidation of methyl linoleate

Figure 2-1 shows the oxidation processes of methyl linoleate mixed with methyl octanoate, laurate or palmitate at the molar ratio of 1:1 as well as the oxidation process of methyl linoleate alone. Methyl linoleate without any additive was most rapidly oxidized. The oxidation of methyl linoleate mixed with methyl octanoate was slightly retarded compared to that of methyl linoleate alone. The reason for the slight difference in the oxidation process between methyl linoleate alone and methyl linoleate mixed with methyl octanolate will be discussed later based on the volatility of methyl

![Oxidation processes at 65°C of methyl linoleate mixed with methyl octanoate, laurate and palmitate at the molar ratio of 1:1. The solid curves were calculated for the oxidation of methyl linoleate mixed with the methyl fatty acid esters in consideration of both the dilution of methyl linoleate with the esters and the evaporation of the esters. The closed circle and dotted curve indicate the observed and calculated oxidation processes, respectively, of methyl linoleate alone.](image_url)

Fig. 2-1. Oxidation processes at 65°C of methyl linoleate mixed with methyl octanoate, methyl laurate and methyl palmitate at the molar ratio of 1:1. The solid curves were calculated for the oxidation of methyl linoleate mixed with the methyl fatty acid esters in consideration of both the dilution of methyl linoleate with the esters and the evaporation of the esters. The closed circle and dotted curve indicate the observed and calculated oxidation processes, respectively, of methyl linoleate alone.
Fig. 2-2. Oxidation processes at 65°C of methyl linoleate mixed with methyl laurate at the molar ratios of (△) 1:3, (◯) 1:1 and (◇) 3:1. The data for the ratio of 1:1 are the same as those in Fig. 2-1. The dotted curve is the oxidation process calculated for methyl linoleate alone. The solid curves were calculated in consideration of both the dilution of methyl linoleate with methyl laurate and the evaporation of the methyl laurate, while the broken curves were calculated taking only the dilution of methyl linoleate by the methyl laurate into consideration.

The addition of methyl laurate or palmitate to methyl linoleate largely retarded the oxidation of methyl linoleate. Because the molar volume is larger in the order of methyl palmitate, laurate and octanoate, the concentration of methyl linoleate was lower in its equimolar mixtures with methyl palmitate, laurate and octanoate in this order. The lower concentration of methyl linoleate would retard its oxidation more.

The oxidation processes of methyl linoleate mixed with methyl laurate at the molar ratios of 1:3 and 3:1 were also measured (Fig. 2-2). The oxidation process of methyl linoleate mixed with methyl laurate at the molar ratio of 1:1, which has been shown in Fig. 2-1, is also shown in Fig. 2-2 in order to facilitate understanding the effect of the
molar ratio on the oxidation of methyl linoleate. Methyl linoleate was more slowly oxidized at the higher molar ratio of methyl laurate to methyl linoleate. Because the concentration of methyl linoleate in its mixture with methyl laurate was lower at the higher molar ratio, the slower oxidation of methyl linoleate at the higher molar ratio would be ascribed to the dilution effect by methyl laurate (Ishido et al., 2001; Ishido et al., 2002a; Ishido et al., 2002b).

2.3.2 Additivity in volume

The densities of methyl linoleate, octanoate and laurate at 25°C were estimated to be 835.3, 879.2 and 861.2 g/L, respectively, in their single-component systems. The density of methyl palmitate at 35°C was also estimated to be 839.8 g/L. The volume, \( V \), of methyl linoleate mixed with methyl octanoate, laurate or palmitate is calculated by

![Graph showing additivity in volume between methyl linoleate and methyl octanoate, methyl laurate, and methyl palmitate.](image)

**Fig. 2-3.** Additivity in volume between methyl linoleate and (▲) methyl octanoate, (○) methyl laurate and (◇) methyl palmitate.
Eq. (2-1) if the additivity in the volume holds.

\[ V = \frac{w_{\text{ML}}}{\rho_{\text{ML}}} + \frac{w_{\text{MS}}}{\rho_{\text{MS}}} \]  

(2-1)

where \( w \) and \( \rho \) are the weight and density, and the subscripts, ML and MS, indicate methyl linoleate and methyl ester of saturated fatty acid (methyl octanoate, laurate or palmitate). The densities of methyl linoleate, octanoate, laurate and palmitate at 65°C were assumed to be 801.9, 844.0, 826.8 and 806.2 g/L, respectively, with the help of the temperature dependence on density of some methyl esters of fatty acids.

The observed volumes of methyl linoleate mixed with methyl esters of saturated fatty acids, \( V_{\text{obs}} \), are plotted against the volumes calculated by Eq. (2-1), \( V_{\text{cal}} \), in Fig. 2-3. The plots lie on a diagonal line, indicating that the additivity in volume holds.

![Fig. 2-4. Evaporation at 65°C of (> methyl octanoate, (O) methyl laurate and (◇) methyl palmitate in their single-component system, and that of methyl laurate mixed with methyl linoleate at the molar ratios of (▲) 1:3 (methyl linoleate:methyl laurate), (●) 1:1 and (◆) 3:1.](image-url)
2.3.3 Volatility of fatty acid ester

No decrease in weight was observed for methyl linoleate at 65°C under flowing
nitrogen gas. The decrease in methyl octanoate, laurate or palmitate at 65°C was
observed as a function of time. The fractions of evaporated methyl ester, which is
calculated by \((n_{MS0} - n_{MS})/n_{MS0}\) where \(n_{MS0}\) and \(n_{MS}\) are the initial moles of methyl ester
and that at any time \(t\), are plotted against \(t\) in double logarithmic chart (Fig. 2-4). The
plots for methyl ester alone lie on a line with a slope of unity, indicating that the fraction
is proportional to the time. Because the fractions of methyl laurate mixed with methyl
linoleate at various molar ratios also lie on the line for methyl laurate alone and the
molar ratio of methyl laurate to methyl linoleate did not affect the evaporation, it would
appear that methyl laurate was evaporated independently of the presence of methyl
linoleate. Therefore, the amount of remaining methyl ester, \(n_{MS}\), is expressed as the
function of \(t\) by the following equation:

\[
n_{MS} = n_{MS0}(1 - k_{ev}t)
\]

where \(k_{ev}\) is the rate constant for the evaporation, and the \(k_{ev}\) values were evaluated to be
0.685, 0.0279 and 0.00117 h\(^{-1}\) for methyl octanoate, laurate and palmitate, respectively.

2.3.4 Oxidation kinetics for methyl linoleate in the mixed system

The whole process for the oxidation of an n-6 fatty acid or its ester, such as methyl
linoleate, could be expressed by the kinetic equation of the autocatalytic type for both
single-component and mixed system (Ishido et al., 2001; Özilgen et al., 1990; Adachi et
al., 1995b).

\[
\frac{dC_{ML}}{dt} = -k_{ox}C_{ML}(C_{MLt} - C_{ML})
\]

where \(C_{ML}\) is the concentration of unoxidized methyl linoleate, \(C_{MLt}\) is the total
concentration of unoxidized and oxidized substrates, and $k_{ox}$ is the rate constant.

For the oxidation of methyl linoleate alone, Eq. (2-3) can be rewritten as follows:

$$\frac{dY}{dt} = -k_Y Y(1-Y)$$  \hspace{1cm} (2-4)

where $Y = \frac{C_{ML}}{C_{MLt}}$ is the fraction of unoxidized substrate, and $k_Y$ is the rate constant and is related to $k_{ox}$ by Eq. (2-5).

$$k_Y = k_{ox}C_{MLt}$$  \hspace{1cm} (2-5)

Equation (2-4) can be solved under the initial condition of $Y = Y_0$ at $t = 0$ as follows:

$$Y = \frac{1}{1 + \exp \{k_Y t + \ln[(1-Y_0)/Y_0]\}}$$  \hspace{1cm} (2-6)

where $Y_0$ is the parameter corresponding to the initial fraction of unoxidized methyl linoleate. The $k_Y$ and $Y_0$ values, by which the calculated $Y$ values were best-fitted to the experimental ones, were estimated to be 0.435 h$^{-1}$ and 0.981, respectively, using the Solver of Microsoft® Excel 2007. The calculated process is given by the dotted curve in both Figs. 2-1 and 2-2.

For the oxidation of methyl linoleate mixed with methyl octanoate, laurate or palmitate, Eq. (2-3) can be rewritten as follows:

$$\frac{dn_{ML}}{dt} = -\frac{k_{ox}n_{ML}(n_{ML} - n_{MLt})}{V}$$  \hspace{1cm} (2-7)

where $n_{ML}$ and $n_{MLt}$ are the moles of unoxidized methyl linoleate and the total moles of unoxidized and oxidized substrates. As mentioned above, the volume, $V$, changes with time because of the evaporation of methyl octanoate, laurate or palmitate and is calculated by the following equations:

$$V = \frac{n_{MLt}M_{ML}}{\rho_{ML}} + \frac{n_{MS0}(1-k_{ev}t)M_{MS}}{\rho_{MS}} \hspace{1cm} (t < t_{ev})$$  \hspace{1cm} (2-8a)

$$= \frac{n_{MLt}M_{ML}}{\rho_{ML}} \hspace{1cm} (t \geq t_{ev})$$  \hspace{1cm} (2-8b)

where $M$ is the molecular mass, and $t_{ev}$ is the time when all of the methyl ester of
saturated fatty acid is evaporated and is calculated by

\[ t_{ev} = \frac{1}{k_{ev}} \]  

(2-9)

The oxidation processes of methyl linoleate mixed with methyl octanoate, laurate or palmitate were calculated by solving Eqs. (2-7) and (2-8) simultaneously as shown by the solid curves in Figs. 2-1 and 2-2. The curves coincided with the experimental results.

Because methyl octanoate was evaporated in the early stage of the process \( t_{ev} = 1.46 \) h), methyl linoleate mixed with methyl octanoate was oxidized similarly to the substrate alone (Fig. 2-1). The dilution effect by methyl palmitate was significant because of the low volatility of methyl palmitate and the oxidation of methyl linoleate was largely retarded.

Under the assumption of no volatilization of methyl laurate, the oxidation processes of methyl linoleate mixed with methyl laurate at various molar ratios were calculated and are shown by the broken curves in Fig. 2-2. The difference between the solid and broken curves was small at the molar ratios of 3:1 and 1:1, while the difference was large at the ratio of 1:3. The broken curve for the ratio of 1:3 was far from the experimental results. This fact indicates that the evaporation of methyl ester of a saturated fatty acid should be considered to exactly predict the oxidation process of an unsaturated fatty acid mixed with the ester.

2.4 Conclusion

The evaporation of the mixed methyl esters of saturated fatty acids affected the oxidation rate of methyl linoleate. Methyl linoleate was oxidized more rapidly when it was mixed with equimolar amount of methyl ester of saturated fatty acid with higher
volatility. When methyl linoleate was mixed with methyl laurate at lower molar ratio, its oxidation was more retarded. The oxidation processes could be well calculated by the autocatalytic kinetic equation in consideration of the independent evaporation of the mixed fatty acid esters.
Chapter 3

Effects of Various Emulsification Methods on the Oxidation of Methyl Linoleate

3.1 Introduction

Emulsions are widely used in many foods, and there are various methods for preparing emulsions, such as high-speed stirring, high-pressure homogenization, ultrasonic homogenization, and membrane emulsification. As described in General Introduction, mechanical stress during the emulsification would cause deterioration of oil.

In this chapter, the four emulsification methods were applied to the mixtures of water and methyl linoleate without an emulsifier to estimate the effects of the emulsification method on lipid oxidation. After the emulsification, the extent of the oxidation of bulk methyl linoleate was determined to evaluate the influence of mechanical stress applied during emulsification on the oxidation rate constant and induction period.

3.2 Materials and Methods

3.2.1 Materials

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

3.2.2 Emulsification of methyl linoleate by different methods

Emulsification was performed as follows. Methyl linoleate was added to distilled water at a final concentration of 10% (w/v), and the mixture was vortexed for 10 s using...
a test-tube mixer (TM-351, Iwaki Glass, Tokyo, Japan). The mixture was then rapidly subjected to one of the four emulsification methods (high-pressure homogenization, high-speed stirring, ultrasonic homogenization, and membrane emulsification).

Homogenization by high-speed stirring was carried out using a polytron homogenizer (PT20SK, Kinematica, Luzern, Switzerland). First, 40 mL of the mixture of water and methyl linoleate were transferred to a 50-mL beaker, in which the stirring was performed. The stirring speeds were $3 \times 10^3$, $8 \times 10^3$ and $1.5 \times 10^4$ rpm, with a stirring time of 2.0 min. The effects of the stirring time were also evaluated by setting the time at 0.50, 2.0 and 8.0 min, with a stirring speed of $8 \times 10^3$ rpm.

Ultrasonic homogenization was performed using an ultrasonic homogenizer (Nissei US-300T, Nihon Seiki, Tokyo, Japan) with an ultrasonic tip diameter of 20 mm. The ultrasonic tip was inserted into the 40 mL of the mixture of water and methyl linoleate. The intensity of ultrasonic wave was controlled by adjusting the output dial at the levels 1.0, 5.0 and 9.0, with a treatment time of 2.0 min, to investigate the influence of the intensity. The effects of the treatment time (0.50, 2.0 and 8.0 min) were also examined, with an output level of 5.0.

For high-pressure homogenization, the mixture of water and methyl linoleate was homogenized using a high-pressure homogenizer (Nanomizer PEL-20, Yoshida Kikai, Aichi, Japan). The homogenization was performed at 40, 81 and 152 MPa, and the number of passes through the homogenizer was two. The effects of the number of passes were also evaluated for 1, 2, 4 and 8 passes at 81 MPa.

Membrane emulsification was performed as follows. The mixture of water and methyl linoleate was transferred to a 50-mL plastic syringe connected to a membrane filter (DISMIC-25CS cellulose acetate membrane filter, pore size 0.80 μm, or mixed
cellulose ester membrane filter, pore size 0.80 μm; Toyo Roshi, Tokyo, Japan). The syringe interior was pressurized with nitrogen at 0.20 MPa. The number of passes through the membrane filter was 1, 2 or 4.

3.2.3 Effects of the amount of a membrane on the oxidation

A cellulose acetate membrane was taken from a DISMIC cellulose acetate membrane filter. The cellulose acetate membrane (2.5–40 mg) or mixed cellulose ester membrane (2.5–40 mg) was immersed in 4 g of methyl linoleate placed in a 50-mL amber vial. The vial was filled with nitrogen and then kept at 4°C in dark for 18 h, with occasional stirring.

3.2.4 Oxidation of methyl linoleate

The mixture, which was subjected to each emulsification method, was then separated into oil and aqueous phases. The oil phase was collected and centrifuged at $1.5 \times 10^4$ rpm for 10 min using a high-speed microcentrifuge (MC-150, Tomy, Tokyo, Japan). The oxidation of methyl linoleate was performed as follows. The oil phase (440 mg) was dissolved in 25 mL of methanol. Then, 100 μL of the solution were placed in a flat-bottomed glass cup (1.5 cm I.D. × 3.0 cm); 120–240 samples were prepared. Methanol was then removed under reduced pressure. The cups were placed in a plastic container (300 mm width × 150 mm height × 150 mm depth), with a dry-air flow of 5 mL/min after passing through silica gel. The plastic container was stored at 55°C in an oven (DN-400, Yamato Scientific, Tokyo, Japan).

The cups were periodically removed from the container, and 1.0 mL of a methanol solution of methyl myristate (1.455 g/L), as the internal standard for gas chromatographic analysis, was added to the cup. Unoxidized methyl linoleate was
determined using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a DB-1ht column (0.25 mm I.D. × 30 m, Agilent Technologies, CA, USA). The temperatures of the injector, column, and detector were 230, 205 and 240°C, respectively.

3.3 Results and Discussion

3.3.1 Oxidation of emulsified methyl linoleate

Figures 3-1, 3-2, 3-3 and 3-4 show the time course characteristics of the changes in the unoxidized fractions of methyl linoleate emulsified by different methods. In high-speed stirring emulsification, the oxidation of methyl linoleate with different stirring speeds showed almost the same behavior as that of methyl linoleate without

Fig. 3-1. Oxidation of methyl linoleate treated by high-speed stirring. The treatment was performed for 0.50–8.0 min at $8 \times 10^3$ rpm: (□) 0.50, (△) 2.0 and (◇) 8.0 min. Symbol ○ represents the result obtained by performing oxidation without emulsification; all the curves are the calculated results.
Fig. 3-2. Oxidation of methyl linoleate treated by ultrasonic homogenization. The treatment was carried out at output levels of 1.0–9.0 for 2.0 min: (□) 1.0, (△) 5.0 and (◇) 9.0. Symbol ● is the same as in Fig. 3-1; all curves are the calculated results.

Fig. 3-3. Oxidation of methyl linoleate treated by high-pressure homogenization. The treatment was performed one to four times at 81 MPa: (□) 1, (△) 2, (◇) 3 and (○) 4 times. Symbol ● is the same as in Fig. 3-1; all the curves are the calculated results.
Fig. 3-4. Oxidation of methyl linoleate treated by emulsification method of membrane filtration. The treatment was performed by using a cellulose acetate membrane with one to four passes through the membrane: (□) 1, (△) 2 and (◇) 4 passes. Symbol ● is the same as in Fig. 3-1; all the curves are the calculated results.

emulsification (Fig. 3-1). Other emulsification methods, i.e., ultrasonic emulsification and high-pressure homogenization, showed that the effects of power of ultrasonic wave and number of passes on oxidation were minor (Figs. 3-2 and 3-3). Although the rise of temperature would occur during the emulsification, its effects were not also significant. Therefore, the mechanical stress on methyl linoleate during emulsification would not affect the stability of methyl linoleate.

In contrast, membrane emulsification affected the oxidation behavior of methyl linoleate when a cellulose acetate membrane was used (Fig. 3-4). The induction period for the oxidation significantly increased on increasing the number of passes of the emulsion through the membrane filter. These results indicate that some radicals, which had been initially present in methyl linoleate, were adsorbed on the membrane, delaying
the oxidation.

The oxidation of n-6 PUFAs and their esters, including methyl linoleate, can be expressed by an autocatalytic kinetic equation (Adachi et al., 1995b):

\[
\ln \frac{1-Y}{Y} = k \cdot t + \ln \frac{1-Y_0}{Y_0}\]

(3-1)

where \(Y\) is the fraction of unoxidized methyl linoleate, \(t\) is the time, \(k\) is the oxidation rate constant, and \(Y_0\) is \(Y\) at \(t = 0\), which reflects the initial state of methyl linoleate. The \(Y_0\) value decreases on increasing the initial concentration of radicals in methyl linoleate. Based on Eq. (3-1), the \(k\) and \(Y_0\) values can be estimated from the slope and intercept, respectively, of the line obtained by plotting \(\ln (1-Y)/Y\) vs. \(t\). The calculated results in Figs. 3-1, 3-2, 3-3 and 3-4 coincide well with the experimental results, indicating that the autocatalytic oxidation model represents the entire oxidation process of methyl linoleate, even after emulsification.

Table 3-1 shows the relative oxidation rate constants, \(k/k^*\), and \((1-Y_0^*)/(1-Y_0)\) values under various emulsifying conditions, where \(k^*\) and \(Y_0^*\) are the rate constant and the parameter, respectively, for the oxidation of methyl linoleate without emulsification. None of the emulsification conditions such as the number of homogenizations, homogenization pressure, and stirring time had any significant effect on \(k/k^*\) and \((1-Y_0^*)/(1-Y_0)\) during high-pressure homogenization, high-speed stirring, and ultrasonic emulsification. These results show that the mechanical stress during these emulsification procedures did not change the initial state of methyl linoleate under the tested conditions.

Moreover, \(k/k^*\) did not change after membrane emulsification, even on increasing the number of passes through the membrane. However, the value for \((1-Y_0^*)/(1-Y_0)\) clearly increased on increasing the number of passes when the cellulose acetate membrane was
Table 3-1. Dependences of the relative oxidation rate constant and the parameter, \((1-Y_0^*)/(1-Y_0)\), on the emulsifying conditions.

<table>
<thead>
<tr>
<th>Emulsification condition</th>
<th>Relative rate constant, (k/k^*)</th>
<th>((1-Y_0^*)/(1-Y_0))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stirring time (min)</td>
<td>0.50 0.98 1.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0  1.00 1.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.0  1.01 0.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0  1.02 1.04</td>
<td></td>
</tr>
<tr>
<td>Stirring speed×10^-3 (rpm)</td>
<td>8.0  0.99 1.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.0 0.99 1.10</td>
<td></td>
</tr>
<tr>
<td>Time of ultrasonic treatment (min)</td>
<td>0.50 0.96 1.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0  0.99 1.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.0  0.98 1.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0  0.99 0.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0  0.98 0.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.0  0.98 1.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1   1.05 0.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2   1.07 1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4   1.06 1.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8   1.06 1.10</td>
<td></td>
</tr>
<tr>
<td>Number of homogenization</td>
<td>40  1.03 0.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>81  1.03 1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>152 1.02 1.10</td>
<td></td>
</tr>
<tr>
<td>Pressure of homogenization (MPa)</td>
<td>1   1.03 1.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2   1.08 2.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4   1.06 3.71</td>
<td></td>
</tr>
<tr>
<td>Number of passes through a cellulose acetate filter</td>
<td>1   1.03 1.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2   1.01 0.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4   1.03 0.90</td>
<td></td>
</tr>
<tr>
<td>Number of the passes through a mixed cellulose ester filter</td>
<td>0.63 0.96 0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 0.98 0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10  0.92 1.68</td>
<td></td>
</tr>
<tr>
<td>Concentration of a cellulose acetate membrane (mg-membrane/g-oil)</td>
<td>0.63 0.98 1.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 0.99 0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10  0.99 0.89</td>
<td></td>
</tr>
<tr>
<td>Concentration of a mixed cellulose ester membrane (mg-membrane/g-oil)</td>
<td>0.63 0.98 1.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 0.99 0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10  0.99 0.89</td>
<td></td>
</tr>
</tbody>
</table>
used. A possible reason for this increase is the adsorption of the radicals initially present in methyl linoleate on the membrane. However, the value of \((1-Y_0^*)/(1-Y_0)\) did not depend much on the number of passes when a mixed cellulose ester membrane was used. Although the reason for this observation is not clear at present, the chemical structures of the membranes probably affect the adsorption behavior.

3.3.2 Effects of the weight ratio of membrane to methyl linoleate on the oxidation

Significant changes in \((1-Y_0^*)/(1-Y_0)\) were not observed when the concentration of the cellulose acetate membrane was \(\leq 2.5 \text{ mg/g-oil}\), or when a mixed cellulose ester membrane was used. However, the value of \((1-Y_0^*)/(1-Y_0)\) increased when the cellulose acetate membrane concentration was 10 mg/g-oil. These results show that a cellulose acetate membrane concentration of at least 2.5–10 mg/g-oil is needed for the adsorption of the radicals in methyl linoleate.

3.4 Conclusion

The mechanical stress caused during emulsification did not affect the oxidation behavior of methyl linoleate. The relative oxidation rate constant was almost constant, regardless of the type of emulsification method. However, the induction period and the value of \((1-Y_0^*)/(1-Y_0)\) increased in the presence of a cellulose acetate membrane. This increase was probably caused by the adsorption of radicals initially present in methyl linoleate on the membrane.
Chapter 4

Effect of Droplet Size on Autoxidation Rates of Methyl Linoleate and α-Linolenate in an Oil-in-water Emulsion

4.1 Introduction

Lipid autoxidation in O/W emulsion systems depends on their oil droplet sizes as described in General Introduction; there are many contradictory reports whether the lipid autoxidation rate would be affected by the droplet size.

In this chapter, we measured the autoxidation processes of methyl linoleate and methyl α-linolenate, which are n-6 and n-3 PUFAs, respectively, that differ greatly in their susceptibility to autoxidation in O/W emulsions with various oil droplet sizes on a micrometer scale. The processes were kinetically analyzed to estimate the rate constants, and the effect of oil droplet size on the rate constants was determined.

4.2 Materials and Methods

4.2.1 Materials

Methyl linoleate (purity, > 95%) and methyl α-linolenate (> 98%) were purchased from Tokyo Chemical Industry (Tokyo, Japan), and methyl palmitate (> 95%) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Decaglycerol monolaurate (ML-750, Lot. 11010) was obtained from Sakamoto Yakuhin Kogyo (Osaka, Japan). Advantec Dismic-25cs cellulose acetate membrane filters (pore sizes of 0.45 and 0.80 μm) were purchased from Toyo Roshi (Tokyo, Japan), and membrane filters with a pore size of 5 μm were from Sartorius Stedim Biotech (Goettingen, Germany). Borosilicate glass filters (type 3G3 with pore sizes of 20–30 μm and 3G1
with pore sizes of 100‒120 μm) were purchased from Asahi Glass (Tokyo, Japan).

4.2.2 Preparation of O/W emulsion

The aqueous phase of emulsion contained 0.15% (w/v) ML-750 and 1 mmol/L sodium azide as the emulsifier and the preservative, respectively, in distilled water. Methyl linoleate or methyl α-linolenate (1%, w/v) was added to the aqueous phase, and the mixture was vortex-mixed using a test tube mixer.

The coarse emulsion then underwent membrane emulsification. Emulsions of small oil droplet size (≤ 10 μm) were prepared by passage through a cellulose acetate membrane filter, and those of large oil droplet size (> 10 μm) were prepared by passage through a borosilicate glass filter. The coarse emulsion was transferred into a plastic syringe (50 mL) connected to a membrane filter on the bottom to produce emulsions with small oil droplet sizes. The syringe was pressurized with nitrogen gas at 0.25, 0.20 or 0.12 MPa. Membrane emulsification was repeated twice to obtain stably monodispersed emulsions. For the preparation of stably monodispersed emulsions with large oil droplet size, the coarse emulsion was passed through a 3G3 or 3G1 borosilicate glass filter. When type 3G3 was used, the membrane emulsification was repeated twice.

4.2.3 Measurement of oil droplet size

A Shimadzu laser diffraction particle size analyzer (SALD-2100, Kyoto, Japan) was used to measure the oil droplet size of the emulsion. The oil droplet size of the methyl linoleate emulsion was measured immediately after preparation and then monitored every 4 h until autoxidation was complete. Measurement of the oil droplet size of the methyl α-linolenate emulsion was performed just after preparation and after complete autoxidation.
4.2.4 Autoxidation in O/W emulsion

For the autoxidation experiment, 1.2 mL of emulsion was sealed in a sample vial (20 mL) with adequate air (18.8 mL). The sample vials were placed on a Variomag magnetic stirrer (Telesystem 60.07, Oberschleißheim, Germany) with gentle stirring at 55°C in a DN-400 oven (Yamato Scientific, Tokyo, Japan). Periodically, a vial was removed from the oven and 500 μL of the emulsion was removed and thoroughly blended with 1200 μL of the extraction solvent, which was composed of chloroform and methanol in a 2:1 ratio (v/v). The mixture was then centrifuged at $1.5 \times 10^4$ rpm for 5 min using a Tomy high-speed microcentrifuge (MC-150, Tokyo, Japan). A portion of the lower phase (400 μL), which was composed mainly of chloroform, was mixed with 400 μL of 0.02 mol/L methyl palmitate solution in methanol as the internal standard for gas chromatographic analysis.

Unoxidized methyl linoleate or methyl α-linolenate was measured by analyzing 1 μL of the mixture using a Shimadzu GC-2014AFsc gas chromatograph (Kyoto, Japan) equipped with a hydrogen flame ionization detector and a J&W Scientific DB-1ht column (Santa Clara, USA). The inner diameter and the length of the column were 0.25 mm and 30 m, respectively. The temperatures for the column, injector, and detector were 205°C, 230°C and 240°C, respectively. Helium was used as the carrier gas at a flow rate of 139 mL/min. The fractions of unoxidized methyl linoleate and methyl α-linolenate were calculated from the ratio of their areas under the peak to that of methyl palmitate.
4.3 Results and Discussion

4.3.1 Oil droplet size of emulsion

The change in the droplet size of methyl linoleate in O/W emulsion during autoxidation at 55°C is shown in Fig. 4-1. The mean oil droplet sizes (Sauter diameter) of emulsions, which were measured immediately after their preparation, were 1.5, 2.6, 6.5, 20 and 29 μm for emulsions prepared using cellulose acetate membranes with pore sizes of 0.45, 0.80 and 5.0 μm and borosilicate glass filters of types 3G3 and 3G1, respectively. During the autoxidation processes, no significant change in oil droplet size was observed for emulsions prepared using any of the filters.

The mean droplet sizes of methyl α-linolenate emulsions measured just after their preparation are shown in Fig. 4-1. ◇, □ and △ represent the mean oil droplet sizes in emulsions prepared using cellulose acetate membranes with pore sizes of 0.45, 0.80 and 5.0 μm, respectively. ○ and ▲ indicate the mean oil droplet sizes in emulsions prepared using borosilicate glass filters of type 3G3 and 3G1, respectively.

Fig. 4-1. Change in droplet size of methyl linoleate in O/W emulsions during autoxidation at 55°C. ◇, □ and △ represent the mean oil droplet sizes in emulsions prepared using cellulose acetate membranes with pore sizes of 0.45, 0.80 and 5.0 μm, respectively. ○ and ▲ indicate the mean oil droplet sizes in emulsions prepared using borosilicate glass filters of type 3G3 and 3G1, respectively.
preparation were 1.4, 2.8, 7.4, 31 and 33 μm for emulsions prepared using cellulose acetate membranes with pore sizes of 0.45, 0.80 and 5.0 μm and borosilicate glass filters of types 3G3 and 3G1, respectively. The oil droplet sizes prepared using cellulose acetate membranes were almost the same as those obtained using methyl linoleate. Because the emulsions prepared using the borosilicate glass filters had almost the same oil droplet size, only the emulsion prepared using type 3G3 was used in the autoxidation experiment that follows. When autoxidation was complete, the oil droplet sizes of the emulsions, which were prepared using cellulose acetate membranes with pore sizes of 0.45, 0.80 and 5.0 and the 3G3 borosilicate glass filter, were 1.1, 2.1, 6.4 and 26 μm, respectively. Thus, all of the methyl α-linolenate emulsions were also stable during autoxidation.

4.3.2 Autoxidation of methyl linoleate and methyl α-linolenate in O/W emulsion

Figure 4-2a shows the change in the residual fraction of methyl linoleate during its autoxidation at 55°C in O/W emulsions with 5 oil droplet sizes. The autoxidation of methyl linoleate showed almost the same behavior, indicating that oil droplet size did not affect the autoxidation of methyl linoleate. This observation was confirmed by evaluating the autoxidation rate constant for each emulsion. The entire autoxidation process of n-6 PUFA's and their esters can be expressed with the following kinetic equation (Adachi et al., 1995b; Minemoto et al., 1999):

\[
\frac{dY}{dt} = -k_1 Y(1-Y) \tag{4-1}
\]

where \( Y \) is the fraction of the unoxidized substrate, \( t \) is the time, and \( k_1 \) is the autoxidation rate constant. Under the conditions of \( Y = Y_0 \) at \( t = 0 \), Eq. (4-1) can be integrated to give
Fig. 4-2. Autoxidation of methyl linoleate at 55°C in O/W emulsion. (a) Change in the fraction of unoxidized methyl linoleate during its autoxidation. (b) Estimation of the rate constant based on Eq. (4-2). The symbols are the same as those in Fig. 4-1. The curves in Fig. 4-2a were drawn using the kinetic parameters $k_1$ and $Y_0$ estimated from the lines in Fig. 4-2b.
Fig. 4-3. Autoxidation of methyl \( \alpha \)-linolenate at 55°C in O/W emulsion. (a) Change in the fraction of unoxidized methyl \( \alpha \)-linolenate during its autoxidation. (b) Estimation of the rate constants for the first and last halves of the autoxidation was based on Eqs. (4-2) and (4-4), respectively. The symbols are the same as those in Fig. 4-1.
\[
\ln \frac{1-Y}{Y} = k_1 t + \ln \frac{1-Y_0}{Y_0}
\]  \hspace{1cm} (4-2)

where \(Y_0\) reflects the initial state of the substrate, and \(k_1\) is the autoxidation rate constant. The applicability of Eq. (4-2) to the autoxidation processes of methyl linoleate was examined by plotting \(\ln \left[(1-Y)/Y\right]\) versus \(t\) as shown in Fig. 4-2b. The plots gave straight lines for each oil droplet size. Therefore, \(k_1\) and \(Y_0\) could be evaluated from the slope and the intercept of the line, respectively. The curves in Fig. 4-2a, which were calculated using Eq. (4-1) and estimated \(k_1\) and \(Y_0\) values, coincided well with the experimental results.

Figure 4-3a shows the change in the fraction of unoxidized methyl \(\alpha\)-linolenate during autoxidation at 55°C in O/W emulsions with 4 oil-droplet sizes. The first half of the autoxidation process did not depend on oil droplet size \((Y \geq 0.5)\), whereas the autoxidation progressed more slowly in the emulsion with larger oil droplet size in the last half \((Y < 0.5)\). Based on our previous study (Adachi et al., 1995b), the rate constants for the first and last halves of the autoxidation were evaluated for each emulsion.

The autoxidation in the first half of the autoxidation process of methyl \(\alpha\)-linolenate could be expressed by Eq. (4-1), and that in the last half could be empirically expressed by

\[
\frac{dY}{dt} = -k_2 Y
\]  \hspace{1cm} (4-3)

where \(k_2\) is the rate constant. Integrating Eq. (4-3) with \(Y = 0.5\) at \(t = t_{0.5}\) gives the following equation:

\[
\ln 2Y = -k_2 (t - t_{0.5})
\]  \hspace{1cm} (4-4)

where \(t_{0.5}\) is the time when \(Y\) reaches 0.5. This value can be obtained from Eq. (4-2) as follows:
\[ t_{0.5} = \frac{1}{k_1} \ln \frac{Y_0}{1-Y_0} \]  

(4-5)

For the autoxidation of methyl \( \alpha \)-linolenate, \( \ln [(1-Y)/Y] \) in the first half and \( \ln 2Y \) in the last half of the process were plotted against time, \( t \), as shown in Fig. 4-3b. The plots gave straight lines for each emulsion. The curves in Fig. 4-3a were calculated using Eqs. (4-1) and (4-3) and estimated \( k_1, Y_0 \) and \( k_2 \) values.

4.3.3 Effect of oil droplet size on autoxidation rate constants

Figure 4-4 shows the oil droplet size dependencies of the rate constants for the autoxidation of methyl linoleate and methyl \( \alpha \)-linolenate. During the entire autoxidation period of methyl linoleate and in the first half of methyl \( \alpha \)-linolenate autoxidation, the rate constants did not depend on oil droplet size. Conversely, the rate constant for the

![Graph showing oil droplet size dependencies of the rate constants for the autoxidation of methyl linoleate and methyl \( \alpha \)-linolenate.](image)

**Fig. 4-4.** Dependence of rate constants on oil droplet size during the autoxidation of methyl linoleate and \( \alpha \)-linolenate at 55°C. \( \diamond \), \( \square \) and \( \triangledown \) represent the rate constants \( k_1 \) for the autoxidation of methyl linoleate and \( k_1 \) and \( k_2 \) for the autoxidation of methyl \( \alpha \)-linolenate, respectively.
last half of the methyl α-linolenate autoxidation was smaller for emulsions with larger oil droplet sizes. It has been reported that the autoxidation of linoleic acid and sunflower oil, respectively, containing linoleoyl residues did not depend on oil droplet size in O/W emulsion systems, findings similar to our results (Roozen et al., 1994; Dimakou et al., 2007). Therefore, in the case of n-6 PUFA and their esters, oil droplet size apparently did not affect the autoxidation rate in O/W emulsions with oil droplet sizes on the micrometer scale.

The rate constant for the autoxidation of methyl α-linolenate was independent of oil droplet size during the first half of the autoxidation, but it decreased with increasing oil droplet size during the last half. The stoichiometric coefficient of oxygen during the autoxidation of methyl α-linolenate was unity during the first half of the process, but it gradually became larger during the last half as autoxidation proceeded (Adachi et al., 1995a). These results indicate that more oxygen is necessary to oxidize methyl α-linolenate during the last half of autoxidation and that the effect of the mass transfer of oxygen through the oil-water phase on the autoxidation becomes significant. This effect explains why oil droplet size affected the autoxidation rate only in the last half of the process.

### 4.4 Conclusion

The effect of oil droplet size on the autoxidation of methyl linoleate and methyl α-linolenate in O/W emulsions was investigated at 55°C in the range of 1–30 μm. Oil droplet size did not affect the rate constant throughout the entire autoxidation process of methyl linoleate or during the first half of methyl α-linolenate autoxidation. However, during the last half of the autoxidation of methyl α-linolenate, the rate constant was
smaller for the emulsions with larger oil droplet size. The slower autoxidation for the larger oil droplet size can be ascribed to the consumption of more oxygen in the last half period, during which the effect of the mass transfer of oxygen through the oil-water interface becomes significant.
Concluding Remarks

Chapter 1

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

Chapter 2

The oxidation process of methyl linoleate mixed with methyl octanoate, laurate or palmitate at the molar ratio of 1:1 was measured at 65°C. Methyl linoleate with methyl octanoate was oxidized most rapidly, and the oxidation of the substrates mixed with methyl laurate and palmitate followed. Methyl linoleate was mixed with methyl laurate at the molar ratios of 1:3, 1:1 and 3:1, and its oxidation processes were measured. Methyl linoleate mixed at the lower ratio (1:3) was the most retarded. The oxidation processes were well simulated based on the kinetic equation of the autocatalytic type in consideration of the independent evaporation of methyl octanoate, laurate or palmitate.

Chapter 3

Effects of mechanical stress during emulsification on the oxidation of methyl
linoleate were investigated by four methods (high-speed stirring, ultrasonic homogenization, high-pressure homogenization and membrane emulsification). The oxidation rates and induction periods were almost constant, regardless of the emulsification method, except for membrane emulsification. When membrane emulsification was performed using a cellulose acetate membrane, the induction period was prolonged.

Chapter 4

Methyl linoleate and α-linolenate were used as representative n-6 and n-3 PUFA esters, respectively, to examine the effect of oil droplet size on autoxidative stability in O/W emulsion systems. The emulsions, which were prepared via membrane emulsification and had a mean oil droplet size of approximately 1–30 μm, had a stable size during the autoxidation of each substrate at 55°C. The autoxidation of methyl linoleate did not depend on oil droplet size during the entire process, and that of methyl α-linolenate was independent of oil droplet size during the first half of the autoxidation process. However, the autoxidation rate of methyl α-linolenate proceeded faster in the emulsion with smaller oil droplet size during the last half of the autoxidation process.
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Acknowledgements

This three years and a half of study at Kyoto University is one of the most valuable experiences in my life. Studying abroad changed my outlook on life and gave me more confidence in the future. I want to take this opportunity to convey my sincere appreciations to those people who helped me and shaped my experience for the better.

First and the most, I would like to express my deepest gratitude and highest respect to my supervisor, Professor Shuji Adachi, for his patient guidance and direction with professional insight and experience. It would be an impossible mission for me to finish this thesis without his kind helps, great supports and inspiring advices. I would also like to appreciate his gracious tolerance of my persistence.

I would like to express my cordial acknowledgement to Associate Professor Kyuya Nakagawa for his helpful discussions, warm encouragements and valuable advices.

My sincere gratitude is due to Assistant Professor Takashi Kobayashi, for his supportive assistances and valuable discussions on my experiments. I could not finish my research paper writing work on time without his careful guidance and responsible supervision.

I would like to express my particularly grateful to Rumiko Kamiya, for her valuable works and special cares for me and other foreign students in this laboratory. With her help and support, the daily laboratory life goes more smoothly and efficiently.

I want to appreciate the great cares and kind helps I have received from all the members of the Bioengineering Laboratory. I am deeply grateful to Rongchun Wang and Tai-Ying Chiou for their instant helps and encouraging supports with endless patience, especially in the first days when I just came to Japan. My sincerely thanks go to the members of emulsions and enzymes research group, Kyohei Sasaki, Akiko
Minami, Tomoko Takahashi, Naho Mizuno and Akane Ogino, for their valuable discussions and generous helps, particularly for their precious supports and guidance on instruments and technologies. I want to thank the foreign students, Tangkhavanich Boonnakhom, Incharoensakdi Chaiyapat, Daming Gao, Intira Koomyart and Teeraya Jarunlumlert, for their kind helps and caring supports with my study and life throughout those memorable days. I would also like to give thanks to Takenobu Ogawa for his helpful advices and suggestions of the laboratory seminar and scientific meeting presentation. I want to express my thanks to Utano Aimoto, Satoshi Koizumi, Yukie Ohishi, Yuki Sha, Masashi Yoshino, Takao Roppongi, Hironori Nagamizu and Yayoi Miyagawa, for their patient assistances and kind supports, especially for their helps on the Japanese language modifications of my laboratory seminar resumes and oral presentation materials. My thankfulness also goes to Keisuke Nakata, Ayako Hasegawa, Yayoi Tomita, Kenichiro Takeda, Kaori Koyama, Kazutaka Katsuki, Soma Fukuzawa and Risako Yamamoto, who were nice, helpful and encouraging to me.

I gratefully acknowledge the Monbukagakusho scholarship from the Japanese Government for financial support of my doctoral study and contribution to my life in Japan.

Finally, I would like to express my sincere appreciate to my parents, for their fully trust, supports and encouragements throughout the entire period of my study and my life. No matter what I do and where I go, my parents always stand beside me, providing their incessant helps, loves and cares.
List of Publications


