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

Tiezheng Ma, Takashi Kobayashi and Shuji Adachi*

Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University (Sakyo-ku, Kyoto 606-8502, JAPAN)

Abstract: Methyl linoleate and α-linolenate were used as representative n-6 and n-3 polyunsaturated fatty acid esters, respectively, to examine the effect of oil droplet size on autoxidative stability in oil-in-water systems. The emulsions, which were prepared via membrane emulsification and had a mean oil droplet size of approximately 1–30 μm, and had a stable size during the autoxidation of each substrate at 55℃. 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.

Key words: autoxidation, emulsion, oil droplet size, polyunsaturated fatty acid

1 INTRODUCTION

Polyunsaturated fatty acids reduce susceptibility to mental illness and protect against heart disease by decreasing serum triacylglycerol and cholesterol1–3. However, their chemical instability makes them susceptible to autoxidation and other chemical changes that lead to deterioration and rancidity in natural and processed foods4, 5. Some products of lipid autoxidation have adverse health effects6. Although the anti-oxidative method, which is used extensively in the food industry, involves the addition of antioxidants6, 7, some food additives are recognized as unsafe ingredients and are subject to food safety regulations7. Natural and processed lipids are frequently used as emulsions in food products. When an oil phase of an oil-in-water (O/W) emulsion contains unsaturated fatty acid, the fatty acid is oxidized using oxygen supplied from the aqueous phase through the oil-water interface. When the specific surface area of the oil droplet is large and a sufficient amount of oxygen is supplied through the interface, the oxidation reaction itself is expected to 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. Thus, lipid oxidation in O/W emulsion systems depends on oil droplet size. Ghotani et al.8 and Lethuaut et al.9 have reported that the autoxidation of docosahexaenoic acid and sunflower seed oil, respectively, are accelerated in emulsions with smaller oil droplets. Conversely, decreases in oil droplet size reportedly decelerate lipid oxidation10, 11. No significant effect of oil droplet size has also reported12–15. The inconsistency in conclusions regarding the dependence of susceptibility to autoxidation on oil droplet size may be explained by differences in susceptibility to autoxidation of the lipid used as the oil phase, the range of tested oil droplet sizes, temperature, and the method used to assess lipid oxidation.

In this context, we measured the autoxidation processes of methyl linoleate and methyl α-linolenate, which are n-6 and n-3 unsaturated fatty acids, 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 analyzed kinetically to estimate the rate constants, and the effect of oil droplet size on the rate constants was determined.

2 EXPERIMENTAL

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).
Goettingen, Germany. 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).

2.2 Preparation of O/W emulsion

The aqueous phase of emulsion contained 0.15% (w/v) ML-750 and 1 mmol·L⁻¹ 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.

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.

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 Varimag 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 15,000 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.

3 RESULTS AND DISCUSSION

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

Figure 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 polyunsaturated fatty acids and their esters can be expressed with the following kinetic equation:

\[ \frac{dY}{dt} = -k_1Y(1-Y) \]  

(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. (1) can be integrated to give

\[ \ln \left[ \frac{(1-Y)/Y}{1-Y_0/Y_0} \right] = k_1t + \ln \left( \frac{(1-Y_0)/Y_0} \right) \]  

(2)

where \( Y_0 \) is a parameter reflecting the initial state of the substrate, and \( k_1 \) is the autoxidation rate constant. The applicability of eq. (2) to the autoxidation processes of methyl linoleate was examined by plotting \( \ln \left[ \frac{(1-Y)/Y} \right] \) versus \( t \) as shown in Fig. 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. 2a were calculated using the kinetic parameters \( k_1 \) and \( Y_0 \) estimated from the lines in Fig. 2b.

Figure 3a shows the change in the fraction of unoxidized methyl α-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, the rate constants for the first and last halves of the autoxidation were evaluated for each emulsion.

The autoxidation of methyl α-linolenate could be expressed by eq. (1). However, the equation was not applicable to the last half of the autoxidation process. According to our previous work, the autoxidation in the last half period was empirically expressed by

\[ \frac{dY}{dt} = -k_2 Y \]  

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

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

where \( t_{0.5} \) is the time when \( Y \) reaches 0.5. This value can be obtained from eq. (2) as follows:

\[ t_{0.5} = \frac{1}{k_1} \ln \left[ \frac{Y_0}{1 - Y_0} \right] \]  

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

### 3.3 Effect of oil droplet size on autoxidation rate constants

Figure 4 shows the oil droplet size dependencies of the rate constants for the autoxidation of methyl linoleate and methyl α-linolenate. During the entire autoxidation period of methyl linoleate and in the first half of methyl α-linolenate autoxidation, the rate constants did not depend on oil droplet size. Conversely, the rate constant for the last half of the methyl α-linolenate autoxidation was smaller for emulsions with larger oil droplet sizes. Roozen et al. and Dimakou et al. have 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. Therefore, in the case of n-6 polyunsaturated fatty acids,
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. 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 interface becomes significant. This effect explains why oil droplet size affected the autoxidation rate only in the last half of the process.

4 CONCLUSION

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

ACKNOWLEDGEMENTS

This study was performed during the project of the Creation of Innovative Technology for Marine Products Industry, Program for Revitalization Promotion, Japan Science and Technology Agency. T. M. expresses sincere gratitude for a Monbukagakusho (Ministry of Education, Culture, Sports, Science and Technology) scholarship from the Japanese government.

References

