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Effect of Droplet Size on the Oxidative Stability of Soybean Oil TAG and Fish Oil TAG in Oil-in-Water Emulsion

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Abstract: The effect of droplet size on the oxidative stability of triacylglycerol (TAG)-in water emulsion was examined. Microchannel (MC) emulsification was used to make monodispersed emulsion of soybean oil TAG and fish oil TAG with different droplet size. The main polyunsaturated fatty acids (PUFA) of soybean oil TAG and fish oil TAG were linoleic acid (LA; 53%) and docosahexaenoic acid (DHA; 37.3%), respectively. Oxidation was induced by the addition of 2,2'-azobis(2-amidinopropane)-dihydrochloride (AAPH) or ferrous ion. The oxidative stability was followed by the decrease in the oxygen consumption in the solution, peroxide formation, and decrease in the unoxidized PUFA during oxidation, indicating that the oxidative stability of fish oil TAG increased with decreasing the droplet size, while the reverse effect of the droplet size was observed on the oxidation of soybean oil TAG. The decrease in the droplet size induces the increase in the droplet interface, from which the oxidation proceeds to the oil droplet interior. DHA in fish oil TAG would take highly protective interface against oxidative attack of free radicals and metal ions, whereas LA in soybean oil TAG would be more easily oxidized at the interface because of its less protective conformation. The reverse effect of the droplet size on fish oil TAG and soybean oil TAG could be explained by the different interface conformation of both TAG.

Key words: microchannel, emulsion, lipid oxidation, droplet size, emulsifier

1 INTRODUCTION

In a food emulsion system, various molecules become distributed according to their polarity and surface activity between different phases, which include the oil phase, the water phase and the interfacial region. Lipid oxidation in such systems is an interfacial phenomenon that is greatly influenced by the nature of interface. Since the lipid oxidation generally proceeds from the interface to the interior of the oil droplet in oil-in water emulsions, the oxidation of lipids at the interface is an important factor predicting the oxidative stability of lipids in the emulsion.

The lipid oxidation at the interface is affected by many factors such as the size and concentration of the emulsion droplets, the thickness, electrical charge, the packing degree of emulsifier and lipid at the interface, and composition of the interface, and the extent of droplet-droplet interactions. In particular, the oxidative stability of lipids in emulsion is strongly affected by altering the droplet size and the packing of the emulsifier and lipid molecules at the interface[1].

When the concentration of lipid and emulsifier is the same, the area of interface increases with decreasing droplet size. The opportunity for the attack by oxidation inducer such as free radicals and metal ions on lipids at the interface increases with increasing area of interface, therefore, the oxidative stability of lipids in emulsion generally decreases with decreasing droplet size. However, this relationship may not be always true and will be strongly affected by the protective ability of the interface against oxidation. The protective level of polyunsaturated fatty acids (PUFA) at the emulsion interface against oxidation can be predicted from that in an aqueous micells, because main part of micells is interfacial region composed from lipid, emulsifier, and water, but micells have little droplet interior.

Our previous study[2] indicated that docosahexaenoic

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acid (DHA) is oxidatively more stable than linoleic acid (LA) in an aqueous micells, although DHA is chemically more susceptible to oxidation than LA. At the interfacial region, main part of micells, DHA itself - or DHA and emulsifier - would take a more protective conformation against oxidative attack of free radicals and/or oxygen as compared with LA[3], therefore, the oxidative stability of DHA was higher than that of LA in micells. On the other hand, DHA is more rapidly oxidized than LA at the droplet interior (oil phase) in oil-in-water emulsion, because the oxidation behavior of DHA and LA in the droplet interior is the same as that in bulk phase.

Since the aqueous structure of interface in emulsion is almost the same as that of micelles[1], DHA at the interface will be very protective against oxidative attack of free radicals and/or oxygen as well as that in micells[3]. Thus, in the oxidation of DHA in emulsion, there may be a possibility that the larger the area of the interface and the smaller the interior oil phase, the more markedly the effects of the interface on the lipid oxidation will be recognized. Therefore, DHA may become more oxidatively stable with decreasing droplet size. This assumption is against the general concept that the oxidative stability of lipids in emulsion decreases with decreasing droplet size.

In this study, we compared the effect of lipid droplet size on the oxidative stability of triacylglycerol (TAG) containing LA or DHA in oil-in water emulsion system. For the comparison, we prepared monodisperse emulsions with microchannel (MC) apparatus because we can interpret experimental results more simply than for that of polydisperse emulsions. MC emulsification enables formulation of monodisperse emulsions with coefficients of variation below 5% from a channel array with a slit-like terrace micro-fabricated on a silicon plate[4]. The resultant droplet size ranging between 3 and 90 μm[5,6] is primarily controlled by the MC geometry[7]. The droplet size is driven by interfacial tension and the dominant force on a micrometer scale. The droplet formation requires no mechanical stress at very low energy input[8], suggesting the less oxidation of TAG during emulsion preparation.

2 EXPERIMENTAL

2.1 Sample preparation

Soybean oil was obtained from Nacalai Tesque Co., Kyoto, Japan. Fish oil was kindly donated from Maruha Co., Tsukuba, Japan. The oil was passed through a column packed with a 1:1 *n*-hexane slurry mixture (wt/wt) of activated carbon and Celite 545 to remove tocopherols[9]. The column chromatographic separation was done more than two times and confirmed that the oil contained no tocopherol. The tocopherol content was measured by HPLC equipped with a fluorescence detector[10]. The recovered oil (*ca*. 30 g) was refined on a silicic acid column (50 cm × 4 cm i.d.) (Silicagel 60, Merck, Darmstadt, Germany) by eluting with *n*-hexane (200 mL) and a mixture of diethyl ether/*n*-hexane solution (2:98 (200 mL), 10:90 (1200 mL), and 20:80 (200 mL), v/v). TAG fraction eluted with diethyl ether/*n*-hexane (10:90) was used for the present study as substrate for oxidation. The each purified oil sample gave only a single spot corresponding to TAG on the TLC with normal-phase silica plates (Merck) developed with diethyl ether/*n*-hexane/acetic acid (40:60:1, v/v/v). The detection of the spot on TLC was done by spraying with 50% aqueous H_2SO_4 and heating on a hot plate to clear the organic material. Triolein (Merck) was used as standard TAG. The peroxide value of each sample was less than 1.0 meq/kg as determined by the AOCS Official Method[11]. D-α-Tocopherol was obtained from Kanto Kagaku, Tokyo, Japan. The recovered oil was refined on a silicic acid column eluting with *n*-hexane and diethyl ether/*n*-hexane solution just before use. The fraction eluted with diethyl ether/*n*-hexane (10:90 and 20:80, vol/vol) was used as oil sample for oxidation. The purified oil sample contained no tocopherol as determined by HPLC[10] and gave only a single spot corresponding to TAG on the thin layer-chromatogram with normal-phase silica plates (Merck, Darmstadt, Germany) developed with diethyl ether/*n*-hexane/acetic acid (40:60:1, v/v/v).

2.2 Fatty acid composition

The fatty acid composition of the fish oil TAG was determined by gas chromatography (GC) after conversion of fatty acyl groups in the TAG to their methyl esters. Fatty acid methyl esters (FAME) were prepared according to the method by Prevot & Mordret[12]. Briefly, to an aliquot of TAG (*ca*. 20 mg) 1 mL n-hexane and 0.2 mL 2N NaOH in methanol were added, vortexed and incubated at 50℃ for 30 min. And then, 0.2 mL 2N HCl in methanol solution was added to the solution followed by gentle mixing. The upper hexane layer containing FAME was recovered and subjected to GC analysis. GC analysis was performed on a Shimadzu GC-14B (Shimadzu Seisakusho, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column [Omegawax 320 (30 m × 0.32 mm i.d.); Supelco, Bellefonte, PA, USA]. The injection port and flame ionization detector were operated at 250℃ and 260℃, respectively, the column temperature being held at 200℃.

2.3 Emulsifier

In the present study, we used nonionic emulsifiers, sucrose fatty acid ester, L-1695, and polyglycerol fatty acid esters, L-7D, L-10D, and L-20D, which are most common food emulsifiers in Japan. These emulsifiers were kindly donated by Mitsubishi Food Co., Tokyo, Japan. HLB of L-1695 was 16. Total fatty acids esterified to L-1695 was 15 wt%, which composed of 95 wt% lauric acid (12:0). On the

other hand, those esterified to L-7D, L-10D, and L-20D were 5.8 wt%, 8.3 wt%, and 16.7 wt%, respectively. Main fatty acid in L-7D, L-10D, and L-20D was also lauric acid (99.6 wt%). The % of esterification represents the ratio of the number of fatty acid esterified to that of available hydroxyl groups for esterification in each emulsifier. The amount of fatty acid esterified was estimated by the determination of saponification value. In case of the polyglycerol ester, that was calculated, considering the amount of free polyglycerol. The free polyglycerol content was determined by HPLC equipped with a combination of a reversed phase column (YMC-AM312; YMC, Kyoto, Japan) and a gel permeation column (Asahipak GS310Q; Asahi Kasei Co., Tokyo, Japan). The HPLC analysis was done isocratically using a mixture of methanol/water (30:70, v/v) as a mobile phase at a constant flow rate of 0.7 mL/min. Peaks were monitored by reflactive index detector.

2.4 Preparation and oxidation of emulsion

An emulsifier was mixed with Milli-Q water by stirring and sonicating with a Sonifier 250D (Branson, Atsugi, Kanagawa, Japan). Monodisperse O/W emulsion was prepared according to the method as reported by Kawakatsu *et al*.[4] Emulsification instrument consists of a module, silicon straight-through MC plates (MS309, MS307, M337-7; EP TEC Co., Hitachi, Japan), a syringe pump (Model 11; Harvard Apparatus Inc., Boston, MA, USA). The module was initially filled with continuous emulsifier solution phase. The silicon plate was fixed in the module after 20 min of ultrasonic degassing in the continuous phase. The pressurized to-be-dispersed phase reached the back of the silicon plate in the module and then was pushed out into the continuous phase via the through-holes to form emulsion droplets. The emulsification behavior was observed using a CCD camera attached to a microscope (Fig. 1). The formulated emulsion was recovered by continuous-phase flow in the 1.0-mm-height space between the top of the silicon plate and the glass plate. Emulsion was also obtained by sonicating the mixture of the TAG and emulsifier solution. The oxidation was initiated by adding an 2,2′-azobis(2-amidinopropane)-dihydrochloride (AAPH) aqueous solution or $FeSO_4$ solution to the emulsion. The AAPH or $FeSO_4$ was added by dissolving them with Milli-Q water. The final concentrations of the TAG, emulsifier, AAPH, and Fe^{2+} in a reaction solution were 0.5 wt%, 0.2 wt%, 3.0 mM, and 20 μM, respectively. Oxidation was done in the dark at 37℃.

2.5 Droplet size measurement

Emulsion droplet size and size distribution were determined with a Shimadzu SALD-200V laser scattering device (Shimadzu Seisakusho). An emulsion was diluted more than 100 times with water before it was transferred into the chamber of the instrument. All particle size measurements were carried out 10 min after emulsification. The relative standard deviation (RSD) is represented by the equation, RSD=SD/Dav × 100, where Dav is the average diameter and SD is the standard deviation of the diameter.

2.6 Analysis of aqueous oxidation

Oxidation was followed by the analysis of the decrease in oxygen concentration in the solution or in the unoxidized PUFA (LA for soybean oil TAG and DHA for fish oil TAG) in the TAG molecule during oxidation. For continuously monitoring oxygen uptake by the oxidation of lipids in the solution, a model 5300 biological oxygen monitor (Yellow Springs Instrument, Yellow Springs, OH) was used. As soon as the AAPH solution had been added to the substrate solution, the reaction vessel was charged with 3 mL of the reaction solution and the concentration of dissolved oxygen in the solution was measured. The decrease in LA or DHA was also monitored by GC method. After a timed period of incubation, the oxidized TAG in the emulsion (1 mL) was extracted with chloroform-methanol (2:1, v/v). The extract was dried over anhydrous sodium sulfate, concentrated *in vacuo*, and transmethylated as described above. The decrease (%) in the unoxidized LA or DHA by

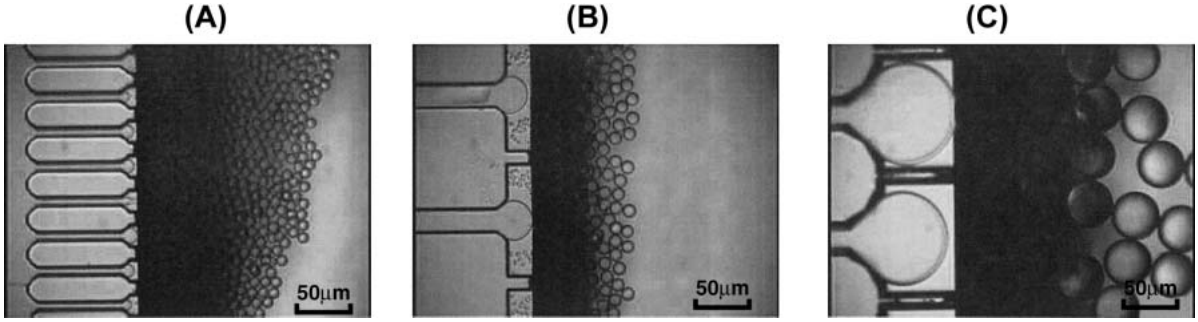

Fig. 1 Microscope Photographs of Microchannel Emulsification.
(A), channel: MS309; TAG: fish oil TAG; emulsifier: sucrose ester (L1695);
(B), channel: MS307; TAG: soybean oil TAG; emulsifier: polyglycerol ester (L-7D);
(C), channel: MS337-7; TAG: fish oil TAG; emulsifier: polyglycerol ester (L-7D).

oxidation was calculated from the changes in the ratio of GC peak area of LA or DHA to that of methyl stearate, which would not be oxidized under the present oxidation conditions.

An emulsion solution (100 μL) was taken at certain time intervals through the oxidation period and peroxide formation was evaluated by the thiocyanate method[13]. Briefly, 100 mL aliquot of the solution was mixed with 4.7 mL of 75% ethanol solution (4.7 mL). 30% ammonium thiocyanate solution (100 μL) and 20 mM ferrous chloride solution (100 μL) were added. The reaction mixture was mixed thoroughly and allowed to stand for 3 min at room temperature in the dark. After incubation, absorbance of the sample solution was measured at 500 nm using spectrophotometer (Nihon Bunko, Japan). Increased absorbance is indicated increased peroxide formation.

Oxidation was done more than three times for each emulsion sample. For each determination there was a slight difference in the oxidation rate, but the order of the oxidative stability of different emulsion samples used in the present study was unchanged.

3 RESULTS

The main PUFA of soybean oil TAG and fish oil TAG were LA (18:2n-6) and DHA (22:6n-3), respectively (Table 1). Palmitic acid (16:0) and oleic acid (18:1n-9) were major fatty acids in both TAG. Microscopic observations of the emulsification process (Fig. 1) revealed that uniformly sized droplets were stably formed through MC-holes. Table 2 shows the droplet diameter distributions of the emulsions formulated using the asymmetric straight-through MC, indicating the formation of monodisperse emulsions with different droplet size. The average droplet diameter of the emulsion formulating with M337-7 was the highest, being followed by those with M307 and M309, respectively. There was a little difference in the average diameter between soybean oil TAG and fish oil TAG when they were dispersed with the same MC.

The droplet size affected the oxidative stability of the TAG in emulsion (Figs 2-4). Figure 2 shows the oxidative stability of TAG (0.5 wt%) in emulsion dispersed with sucrose ester, L1695 (0.2 wt%), under the presence of 3.0 mM of AAPH as an oxidation inducer. The oxidative stability was evaluated by the decrease in the oxygen concentration (A) and in the increase in the peroxide formation (B). Both analytical methods clearly indicated that soybean oil

Table 1 Fatty Acid Composition of TAG.

Fatty acid (mol%)	Soybean oil TAG	Fish oil TAG
14:0	0.1	3.2
16:0	10.1	13.9
18:0	2.9	2.8
16:1n-7	0.1	5.0
18:1n-7	1.6	2.1
18:1n-9	22.2	14.1
20:1n-9	0.2	1.9
16:2n-4	ND	1.9
18:2n-6	53.0	0.9
18:3n-3	5.6	0.5
20:4n-6	ND	2.9
20:5n-3	ND	3.6
22:5n-3	ND	1.6
22:5n-6	ND	2.3
22:6n-3	ND	37.3

[a]ND: Not detected.

Table 2 Droplet Size of Emulsions Prepared by Mixing TAG (0.5 wt%) and Emulsifier (0.2 wt%) with Microchannel Emulsification.

Emulsifier	Microchannel	Diameter (μm) (Mean$\pm$SD)	
		Soybean oil TAG	Fish oil TAG
L1695	MS309	6.59$\pm$0.07 (1.03)[a]	7.39$\pm$0.07 (0.93)[a]
L1695	MS337-7	39.46$\pm$0.07 (0.17)[a]	39.11$\pm$0.06 (0.16)[a]
L7D	MS309	6.43$\pm$0.07 (1.01)[a]	6.92$\pm$0.07 (0.97)[a]
L7D	MS307	10.47$\pm$0.07 (0.62)[a]	10.50$\pm$0.07 (0.62)[a]
L7D	M337-7	37.50$\pm$0.07 (1.89)[a]	37.24$\pm$0.07 (0.18)[a]

[a]Relative standard deviation (RSD), %.

TAG dispersed with MS309 (mean droplet diameter: 6.585 μm) was more rapidly oxidized than the TAG dispersed with MS337-7 (mean droplet diameter: 39.457 μm). In contrast, the oxidative stability of fish oil TAG dispersed with MS309 (mean droplet diameter: 7.385 μm) was higher than that of the TAG dispersed with MS337-7 (mean droplet diameter: 39.106 μm).

The reverse effect of the droplet size on the oxidative stability of soybean oil TAG and fish oil TAG was also observed in the emulsion dispersed with polyglycerol ester, L7D (Fig. 3). Judging from the decrease rate in the oxygen concentration (Fig. 3 (A)), the oxidative stability of soybean oil TAG decreased with decreasing the droplet size (Table 2), while the stability of fish oil TAG increased with decreasing the droplet size (Table 2). The same effect of droplet size was confirmed by the analysis of peroxide formation (Fig. 3 (B)). Further, the effect of droplet size on the TAG oxidation was analyzed in the monodipersed emulsion formulated with two different MS, where the oxidation was induced by ferrous ion and the oxidative stability was estimated by the decrease in the unoxidized LA and DHA for soybean oil TAG and fish oil TAG, respectively (Fig. 4). The

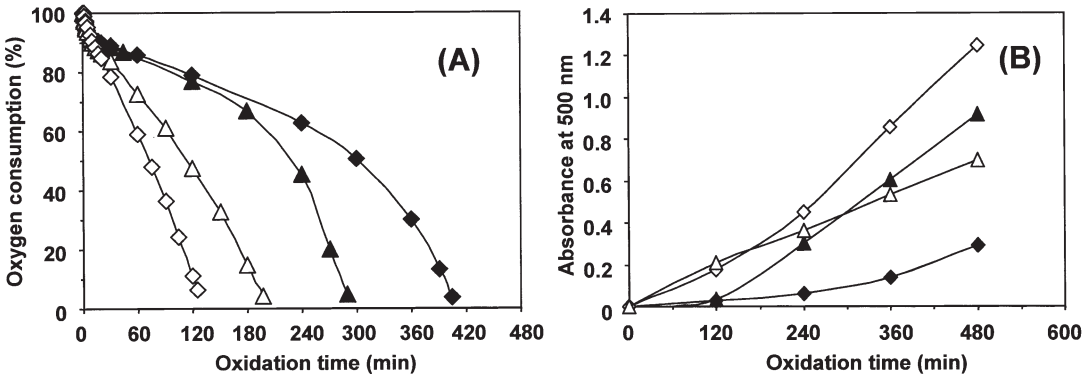

Fig. 2 Oxidative Stability of TAG Dispersed with Sugar Ester (L1695) in Emulsion.
The concentrations of TAG and emulsifier were 0.5 wt% and 0.2 wt%, respectively. Oxidation was induced by 3.0 mM of AAPH. Oxidative stability was measured by oxygen consumption (A) and peroxide formation (B). Emulsion was prepared with different microchannels, MS309 (soybean oil TAG: solid triangle and fish oil TAG: open triangle), and MS337-7 (soybean oil TAG: solid diamond and fish oil TAG: open diamond).

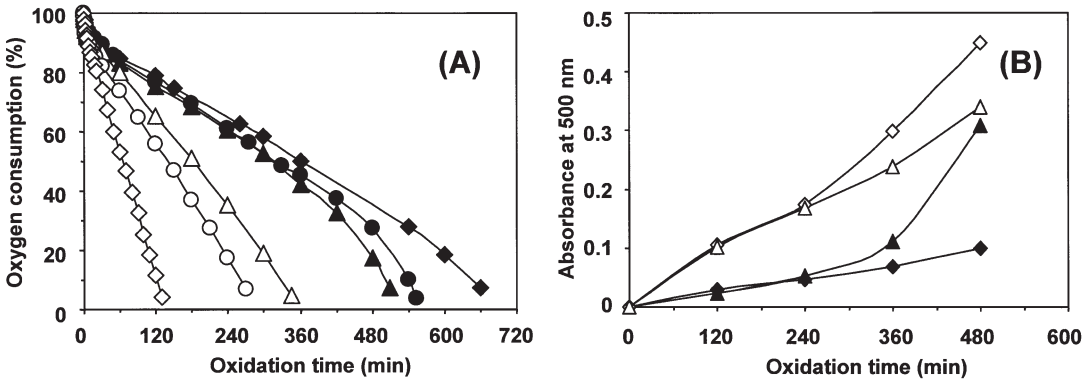

Fig. 3 Oxidative Stability of TAG Dispersed with Polyglycerol Ester (L7D) in Emulsion.
The concentrations of TAG and emulsifier were 0.5 wt% and 0.2 wt%, respectively. Oxidation was induced by 3.0 mM of AAPH. Oxidative stability was measured by oxygen consumption (A) and peroxide formation (B). Emulsion was prepared with different microchannels, MS309 (soybean oil TAG: solid triangle and fish oil TAG: open triangle), MS307 (soybean oil TAG: solid circle and fish oil TAG: open circle), and MS337-7 (soybean oil TAG: solid diamond and fish oil TAG: open diamond).

decrease rate of unoxidized LA in the emulsion with smaller droplet size (6.428 μm) was higher than that with larger droplet size (37.499 μm) in soybean oil TAG (Fig. 4 (A)). On the contrary, the effect of the droplet size was the reverse in the decrease rate of unoxidized DHA during the oxidation of fish oil TAG in the monodispersed emulsion (Fig. 4 (B)).

Figure 5 shows the oxidative stability of soybean oil TAG and fish oil TAG dispersed with polyglycerol esters, L7D, L10D, and L20D. Both TAG were emulsified by sonicating, being resulted in the less droplet diameters, but the larger RSD, as compared with those produced by MS method (Table 2 and 3). The oxidative stability of fish oil TAG was higher than that of soybean oil TAG in all emusions prepared with three kinds of emulsifiers (Fig. 5), though soybean oil TAG was oxidatively more stable than fish oil TAG in emulsions produced by MS method (Fig. 2-4). There was a little difference in the droplet sizes of emulsions dispersed with different kinds of polyglycerol esters (Table 3). When the oxidative stability of each TAG dispersed with three kinds of polyglycerol esters was compared, the stability increased with decreasing their % of esterification, namely, 5.8 wt%, 8.3 wt%, and 16.7 wt% for L-7D, L-10D, and L-20D, respectively.

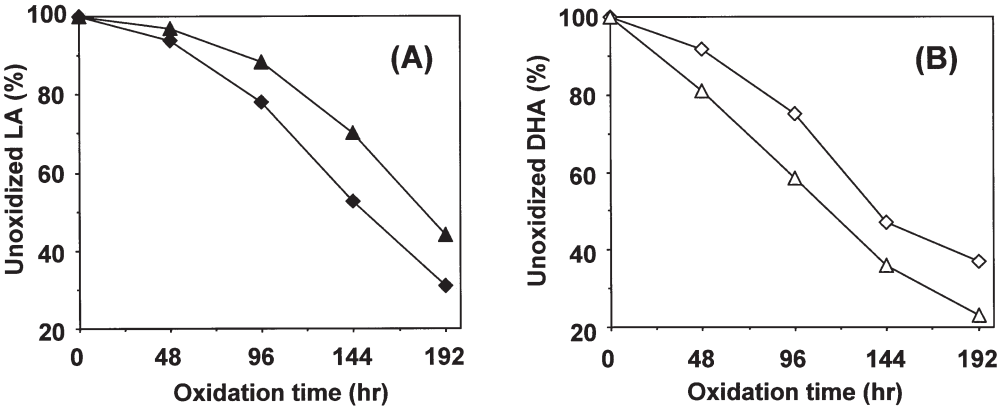

Fig. 4 Oxidative Stability of Soybean Oil TAG (A) and Fish Oil TAG (B) Dispersed with Polyglycerol Ester (L7D) in Emulsion.
The concentrations of TAG and emulsifier were 0.5 wt% and 0.2 wt%, respectively. Oxidation was induced by 20 μM of Fe^{2+}. Oxidative stability was estimated by the decrease in linoleate (for soybean oil TAG) and DHA (for fish oil TAG) during oxidation. Emulsion was prepared with different microchannels, MS309 (soybean oil TAG: solid triangle and fish oil TAG: open triangle) and MS337-7 (soybean oil TAG: solid diamond and fish oil TAG: open diamond).

Table 3 Droplet Size of Emulsions Prepared by Mixing Soybean Oil TAG (0.5 wt%) and Emulsifier (0.2 wt%) with Sonication.

Emulsifier	Diameter (μm) (Mean$\pm$SD)	
	Soybean oil TAG	Fish oil TAG
L7D	1.38$\pm$0.17 (12.65)[a]	1.12$\pm$0.07 (6.18)[a]
L10D	1.18$\pm$0.16 (13.17)[a]	1.12$\pm$0.12 (10.35)[a]
L20D	1.19$\pm$0.15 (12.39)[a]	1.15$\pm$0.16 (13.97)[a]

[a]Relative standard deviation (RSD), %.

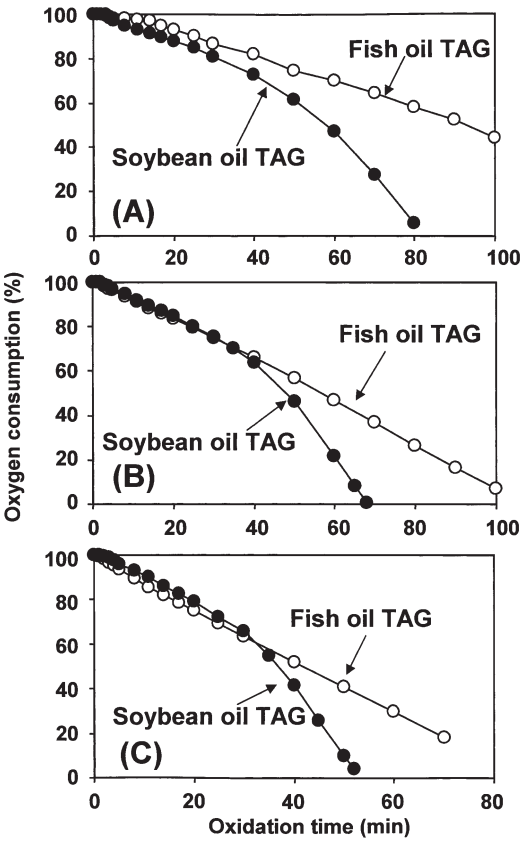

Fig. 5 Oxidative Stability of Soybean Oil TAG (solid circle) and Fish Oil TAG (open circle) Dispersed with L7D (A), L10D (B), and L20D (C) in an Emulsion.
The concentrations of TAG and emulsifier were 0.5 wt% and 0.2 wt%, respectively. Oxidation was induced by 3.0 mM of AAPH. Emulsion was prepared by sonicating TAG in an emulsifier solution.

4 DISCUSSION

The lipid oxidation generally proceeds from the interface to the oil droplet interior in oil-in-water emulsions, therefore, the susceptibility of PUFA to oxidation at the interface is most important factor affecting the oxidative stability of lipids in emulsion. The different effect of droplet size on the oxidative stability of both soybean oil TAG and fish oil TAG found in the present study (Fig. 2-4) could be explained by the different protective property at the interface against oxidative attack of free radicals or metal ions in both emulsion systems. For the explanation, the results[2,3] obtained in the oxidation of LA and DHA in aqueous micells would be useful, because the interface structure in emulsion is almost the same as structure of micelles[1,3]. Main parts of micells are interfacial region composed from lipid, emulsifier, and water, in which the polar head-groups of acyl moiety and emulsifier lie in the aqueous phase, and non-polar tails of both molecules are located in the interior[1,3].

In aqueous micelles, DHA or DHA ester is oxidatively more stable than LA or LA ester, although DHA is chemically more susceptible to oxidation than LA[2,14]. This unusual order of oxidative stability is closely related to molecular dynamics of PUFA in aqueous micelles. Kato *et al.*[15] have reported that *n*-3 double bond of DHA and EPA reacted with *N*-bromosuccinimide to convert to the corresponding bromohydrin with 87% and 89% selectivity, respectively. They demonstrated that this high selectivity at *n*-3 position is due to the preventive configurations of DHA and EPA in an aqueous medium against attack by bromohydrin to their double bonds.

The conformation and kinetics of a molecule or part of a molecule are reflected in NMR relaxation times, i.e., spin-lattice relaxation (T_1) and spin-spin relaxation (T_2) time. When comparing the protons in LA and DHA forming micelle with those of PUFA in chloroform solution, T_2 give important information for the difference in the molecular conformation of each PUFA between chloroform solution and aqueous micelles[16]. There is little difference between the T_2 of corresponding protons on PUFA in the chloroform solution, but those of methyl protons of DHA in micelles had a much larger value than those of LA. Olefin protons and bisallylic protons showed a similar tendency as for methyl protons. For the proton on the carboxyl terminal, LA had a slightly longer T_2 than DHA[16]. The mobility of the hydrophobic part of the DHA molecule is thus considered higher than that of LA when forming micelles. DHA molecules in micelles is may be packed more loosely than in LA, and the hydrophobic moiety of DHA may move more freely in micelles. This flexibility may allow water molecules to permeate DHA micelles. The penetration of water molecule to acyl moieties in micelles inhibits the hydrogen abstraction from bis-allylic positions of DHA by free radicals. On the other hand, the abstraction of hydrogen from LA will occur easily due to no protection by water molecule.

In an aqueous micells, fatty acyl molecules and emulsifier molecules arrange themselves so that the polar head-groups are located at the surface and non-polar tails are located in the interior, therefore, the interaction of emulsifier and lipid at the micelles also affects the oxidative stability of lipids. When sodium salts of LA and DHA were used as substrates, the oxidative stability of DHA was markedly increased by addition of Tween 20 (polyoxyethylenesorbitan monolaurate) as an emulsifier[17]. In contrast, the stability of LA with Tween 20 was slightly less than that of LA without Tween 20. This specific effect of Tween 20 to protect DHA against oxidation in aqueous micelles is associated with its different interaction with

two acids.

DHA in fish oil TAG, a main target molecule of oxidation, would be also protected by penetrated water and emulsifier at the interface against oxidative attack of free radicals and metal ions as found in the case of DHA in an aqueous micells, while LA rich in soybean oil TAG would very easily oxidized at the interface because of the less protective conformation of LA. The area of the interface increased with decreasing droplet size because the concentrations of both TAG and emulsifiers used in the present study were the same. It is generally accepted that the opportunity for the attack by oxidation inducer such as free radicals and metal ions on lipids at the interface increases with increasing area of interface. Thus, the oxidative stability of TAG in emulsion should decrease with decreasing droplet size. However, the opposite result was obtained in the oxidation of fish oil TAG in emulsions. As shown in Fig. 2-4 and Table 2, the oxidative stability of fish oil TAG increased with decreasing the droplet size, while the stability of soybean oil TAG decreased with decreasing droplet size. The specific oxidative stability found in fish oil TAG could be explained by the highly protective interface produced from DHA in the fish oil TAG in emulsion.

NMR analysis and molecular dynamics simulation of phosphatidylcholine (PC) containing DHA in liposomes indicates the wide variety of DHA conformation - including back-bended, helical and angle-iron conformations-occurring in liposome systems[18-21]. This variety in the DHA chain conformation gives looser packing of the lipid chains[22,23]. The looser packing of the membrane at the lipid-water interface brings about the high water permeability[20]. Molecular dynamics simulation also indicates the remarkable overlapping of water molecules with double bond regions of the DHA chain. The presence of water molecules near a DHA molecule will lower the density of the bis-allylic hydrogen and reduce the chain-carrying reaction of lipid peroxidation. The higher water permeability of DHA and its specific conformation found in PC in liposome support the assumption in this study that DHA in fish oil TAG would be protective against oxidative attack of free radicals and metal ions.

With decreasing the droplet size, the oxidative stability of fish oil TAG increased, while that of soybean oil TAG decreased (Fig. 3), being resulted in shortening the difference in the oxidative stability of both TAG. However, there was no reversal in the relative oxidative stability of both TAG in the oxidation of monodispersed emulsion produced with MC. On the other hand, when the emulsion was prepared by sonicating to produce droplet with a diameter around 1 μm (Table 3), the stability of fish oil TAG became higher than that of soybean oil TAG (Fig. 5).

The typical miceller size is around 10 nm, while that of emulsion droplet size varies from 0.1 to 50 μm. As described above, miceller structure of DHA is protective against oxidation. However, the presence of droplet interior in emulsion results in the decrease in the oxidative stability of DHA because DHA is very easily oxidized in the droplet interior. Thus, the stability of DHA in the emulsion decreases with increasing the droplet interior. On the contrary, LA is oxidatively much more stable than DHA in the droplet interior and the relative oxidative stability of LA to DHA in emulsion increases with increasing the droplet interior part, namely the droplet size.

Monodispersed and size-controlled emulsions with droplet diameters of 3-90 μm can be successfully produced by MC emulsification[5,6]. The droplet formation mechanism for MC emulsification has been proposed in which a dispersed oil phase is cut off spontaneously into the spherical droplet by interfacial tention[8]. The energy input for MC emulsification is very low as compared to the conventional emulsification technique such as sonicating because droplet formation from MC is based on spontaneous transformation. TAG is, therefore, less oxidized during MC emulsification as compared with other mechanical emulsification. The mild emulsification with MC method may produce preventive oil droplet against oxidation.

As shown in Table 2, monodispersed emulsions of fish oil TAG with three kinds of droplet size, 6.92±0.07 μm, 10.50±0.07 μm, and 37.24±0.07 μm, could be produced using L7D as an emulsifier by different silicon straight-through MC plates, MS309, MS307, and M337-7, respectively. On the other hand, fish oil TAG emulsion with less droplet size (1.12±0.07 μm) was prepared by sonicating. The present study demonstrated that the oxidative stability of fish oil TAG increased with decreasing droplet size (Fig. 2-4). However, when the oxidative stability of fish oil TAG emulsion with the least droplet size (1.12±0.07 μm) prepared by sonicating was compared with those of the emulsions with the bigger droplet sizes (6.92±0.07 μm and 10.5±0.07 μm) prepared by MS method, the former was oxidized more rapidly than the latter. The analysis of oxygen consumption during oxidation of fish oil TAG dispersed with MC method (Fig. 3 (A)) showed that the time over 50% oxygen consumption were more than 60 min, 120 min, and 180 min, for 37.24±0.07, 10.50±0.07, and 6.92±0.07 μm droplet size, respectively. On the other hand, in the fish oil TAG emulsion dispersed by sonicating with the same emulsifier (L7D) (Fig. 5 (A)), the time over 50% oxygen consumption was more than 90 min, although the droplet size (1.12±0.07 μm) was less than those obtained by MC method (Fig. 3 (A)). The higher oxidative stability of the emulsion prepared by MS method would be due to the relatively less oxidation of fish oil TAG during MS emulsification as compared with that during sonicating.

We reported the effect of commercial emulsifiers, sucrose fatty acid esters and polyglycerol fatty acid esters, on the oxidation of soybean oil TAG-in-water emulsion[24]. Both emulsifiers influenced the oxidative stability of soy-

bean oil TAG in the emulsion, while they had little effect on the oxidation of the TAG in bulk phase. When the TAG was dispersed with sucrose esters having the same fatty acid composition, the oxidative stability increased with increasing their HLB or decreasing their % of esterification[24]. However, polyglycerols used in the previous study[24] were mixture of those having different degrees of polymerization, they had a broad distribution in their chain length. Therefore, the number of hydroxyl groups for esterification per one polyglycerol molecule also varied widely, while sucrose has only eight available hydroxyl groups for esterification. Furthermore, polyglycerol ester contained unreacted polyglycerol. The complexity of the composition of polyglycerol esters made it difficult to explain the relationship between the nature of polyglycerol ester and its effect on the oxidative stability of soybean oil TAG.

In the present study, we used deca-glycerol esters, L7D, L10D, and L20D. They have the same polyglycerol bone having the same fatty acid, lauric acid (99.6 wt%), but have different ratio (%) of the number of fatty acid esterified to that of available hydroxyl groups for esterification. The comparison of the oxidative stability of soybean oil TAG and fish oil TAG emulsified with three kinds of polyglycerol esters (Fig. 5 (A), (B), and (C)) showed the stability increased with decreasing the % of esterification. This relationship was the same as that found in the oxidation of soybean oil TAG emulsified with sucrose esters[24]. There was a little difference in the droplet size between emulsions of soybean oil TAG and fish oil TAG dispersed with three kinds of polyglycerol esters (Table 3). Therefore, the different effect of the polyglycerol ester on the oxidative stability of both TAG is expected to be derived from the difference in the conformation of emulsifier and each TAG molecule at the interface.

DHA and EPA are rich in fish oils and have beneficial health and physiological effects. The functions of these PUFA have attracted consumer attention and fish oil is used in functional foods and nutraceuticals. In the course of the application of the fish oil, lipid peroxidation has received considerable attention because lipid oxidation products cause undesirable flavors and lower the nutritional quality and safety of lipid-containing foods. Many foods are complex, multi-component, and heterogeneous systems, in which lipids are present with various types of other components in aqueous medium. It follows that the lipid oxidation in aqueous solutions is very important if we are to fully understand the factors that affect lipid oxidation in foods. The present study will provide useful information for protecting lipid oxidation, especially fish oil oxidation, in food emulsion systems.

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