

Water-Retaining Ability of Diacylglycerol

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ABSTRACT: The water-in-oil emulsification characteristics and the adsorption properties of DAG at the oil/water interface were investigated for DAG having different FA compositions. The water-retaining ability of DAG is dependent on the FA composition but is not dependent on the interfacial tension at the oil/water interface in a simple way. The water-retaining ability is very different between uni-chain DAG (two FA have the same chain length) and complex-chain DAG (one FA is oleic acid and the other has a shorter alkyl chain). Uni-chain DAG, having long FA chains ($R = C_{12}$ or $C_{18:1}$) have the ability to emulsify water at the volume fraction of 80% ($\Phi 80\%$), but uni-chain DAG with short or medium chain-length FA ($R = C_3, C_4, C_6, C_8$) show little ability to retain water. For complex-chain DAG, all the DAG studied here ($R_1 = C_{18:1}, R_2 = C_2-C_{12}$) have the ability to emulsify water at $\Phi 80\%$. The stability of the emulsions, however, varies with the chain length of the R_2 FA (R_2 stability order: $C_2, C_3 > C_{18:1}, C_{10} > C_8 > C_4, C_6$). The relationship between the water-retaining ability and the molecular structure of DAG is discussed from the viewpoint of intra- and intermolecular interactions between the FA chains.

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KEY WORDS: DAG, diacylglycerol, fatty acid, oil/water interface, structure, w/o emulsion.

DAG is a minor ingredient of edible fats and oils or a by-product during the manufacture of MAG. Recently, it has been found that “DAG oil,” which consists of 80% DAG or more, has novel nutritional functions compared with conventional TAG oil (1–7), such as suppressing the postprandial increase in serum TAG and lowering the accumulation of fat. Owing to its potential as a “healthy” cooking oil, DAG oil has received a great deal of attention in the food industry.

It is important to understand the physicochemical properties of DAG for the practical application as a cooking oil. In particular, studying the emulsification characteristics provides useful information when incorporating DAG oil into emulsion foods, such as mayonnaise, dressings, and margarines. However, only a small number of works have been published concerning the emulsification properties of DAG (8,9).

In this paper, the emulsification properties of DAG, particularly the ability to form high water content water-in-oil (W/O) emulsions (water-retaining ability), were investigated. The water-retaining ability was evaluated for DAG having different FA compositions in order to establish the relationship between this ability and the molecular structure of DAG.

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EXPERIMENTAL PROCEDURES

Materials. (i) *Uni-chain DAG.* Diolein ($C_{18:1}$ - $C_{18:1}$ -DAG) and dilaurin (C_{12} - C_{12} -DAG) were prepared by esterifying glycerol with refined FA (purity >99%; Tokyo Kasei Inc., Tokyo, Japan) using 1,3-specific lipase (10). The esterification reaction was carried out with 200 g of FA, glycerol (half the molar amount of FA), and Lipozyme RM IM (10 wt% of FA, *Rhizomucor miehei* lipase immobilized on anion exchange resins; Novozymes Industry, Bagsvaerd, Denmark) in a 500-mL four-necked round-bottomed flask at 40°C for diolein and at 60°C for dilaurin. During the reaction, pressure was kept at 0.4 kPa using a vacuum pump, and the mixture was agitated at 400 rpm by an impeller. The acid value of the reaction mixture was monitored by AOCS Official Method Ca 5a-40 (11) at appropriate times. When the acid value decreased to 10% of the initial value, the reaction mixture was separated from immobilized lipase by filtration, and the free glycerol and FFA of the reaction mixture were removed by distillation. Short and middle chain-length DAG (C_3, C_4, C_6 , or C_8) samples were prepared by the esterification of glycerol with FA chlorides (Tokyo Kasei Inc.). Glycerol (10 g, super grade; Tokyo Kasei Inc.) was dissolved in 100 g of pyridine; FA chloride (twice the molar amount of glycerol) was gradually added to the pyridine solution with agitation in an ice bath. After standing for 12 h at room temperature, pyridine was removed by distillation. The reaction mixture was dissolved in 100 mL of diethyl ether and was washed with water, 0.1 N hydrochloric acid solution, 5% sodium hydrogen carbonate solution, and water. A small amount of anhydrous sodium sulfate was then added to the ether solution to remove water, and ether was removed by distillation after filtration.

(ii) *Complex-chain DAG.* Complex-chain DAG ($R_1 = C_{18:1}, R_2 = C_2, C_3, C_4, C_6, C_8, C_{10},$ or C_{12}) samples were prepared with refined monoolein and FA chlorides (Tokyo Kasei Inc.) by the same method as above except for using the same molar amount of FA chloride as monoolein.

All the DAG samples were purified by silica gel column chromatography. The total content of MAG and TAG in the purified DAG samples was less than 0.1%. The DAG samples were then heated at 100°C for 8 h under vacuum (40 Pa) so that the ratio of the geometric isomers reached the thermodynamic equilibrium rate (1,3-DAG/1,2-DAG \approx 65:35). In this paper, the effects of FA compositions (chain lengths) on the emulsification properties were investigated. For this purpose, we classify the FA in DAG molecules by chain length as follows: short chain (C_2 - C_4), middle chain (C_6 - C_{10}), and long chain ($\geq C_{12}$).

Dioleylglycerylether (dioleyl GE) was provided by the Kao Performance Chemicals Research Laboratories (Wakayama, Japan). *n*-Decane (super grade) was purchased from Wako Pure Chemical Industries Inc. (Tokyo, Japan) and used as received. Deionized distilled water was used throughout the experiments.

Melting point. The m.p. was defined as the endothermal peak temperature measured using DSC. DAG samples were cooled to -30°C and held at that temperature for 5 min. Then the temperature was increased at $2^{\circ}\text{C}/\text{min}$.

Interfacial analysis. (i) *Interfacial tension.* The interfacial tension between DAG or the DAG/*n*-decane solution and water was measured by the drop volume method at 25 or 35°C . The exact volume of the sample drop that detaches from the capillary was measured accurately. The interfacial tension γ was obtained from the following equation:

$$\gamma = Vg\Delta\rho F/2\pi r \quad [1]$$

where V is the critical volume that detaches from the capillary, g is the gravitational constant, $\Delta\rho$ is the difference of the densities of two adjacent liquid phases, F is the correction factor, and r is the radius of the capillary. Measurements were conducted with a Lauda drop volume tensiometer (TVT1, standard mode; syringe volume: 5.0 mL; capillary radius: 1.700 mm; drop creation rate: 0.5 s/ μL ; Lauda Dr. R. Wobster GmbH & Co. KG, Lauda-Königshofen, Germany) (12).

(ii) *Interfacial concentration/occupied area per molecule.* The interfacial concentration and occupied area of DAG molecules at the oil/water interface are important parameters in discussing the emulsification properties of DAG. However, it is difficult to obtain the parameters directly for DAG/water systems. Therefore, we obtained the parameters of DAG at the *n*-decane/water interface (DAG is regarded as an emulsifier) and used the values as indications of the emulsification characteristics of DAG.

The interfacial concentration of DAG at the oil/water interface was evaluated from the interfacial tension of the DAG/*n*-decane solutions and water at 25 or 35°C . Measurements were made for DAG/*n*-decane solutions at various DAG concentrations. The saturated adsorption (Γ_{∞}) of DAG at the *n*-decane/water interface was calculated from the steepest inclination of the interfacial tension concentration curve (13), using Gibbs' adsorption equation (Eq. 2),

$$\Gamma = -\frac{C}{RT} \cdot \frac{\delta\gamma}{\delta C} = -\frac{1}{RT} \cdot \frac{\delta\gamma}{\delta \ln C} = -\frac{1}{2.3RT} \cdot \frac{\delta\gamma}{\delta \log C} \quad [2]$$

where Γ is the surface adsorption concentration, C is the concentration of DAG in *n*-decane, R is the gas constant, T is temperature (K), and γ is the interfacial tension. The occupied area per molecule was calculated from the saturated adsorption concentration.

Water-retaining ability. The water-retaining ability of DAG was evaluated for the W/O-type emulsions of the DAG/water system at the water volume fraction of 80%

($\Phi 80\%$). A W/O emulsion of $\Phi 80\%$ was prepared as follows: 1 mL of DAG oil was injected into the chamber of an ultra disperser (Yamato Scientific Co., Tokyo, Japan), and 4 mL of distilled water was added gradually and continuously during the rotation of a propeller at maximum speed for 5 min.

The water-retaining ability was evaluated from two different viewpoints: water-holding capacity and emulsion stability. For the DAG/water systems studied here, DAG itself worked as an emulsifier. If the potential as emulsifier was not sufficient, the W/O emulsion exhibited a phase transition and formed an oil-in-water emulsion during the addition of water. Water-holding capacity was evaluated with respect to whether the system had the ability to emulsify water at $\Phi 80\%$. If a phase transition occurred during the emulsification process, the volume fraction of water at the phase transition was indicated.

The stability of the emulsion was evaluated for samples with $\Phi 80\%$. The emulsion sample (4 mL) was placed on a 100-mesh sieve, which was placed in a beaker. The amount of water separated from the emulsion sample at 25°C was measured as a function of time. The stability of the W/O emulsion was defined as the emulsion preservation ratio, i.e., the volume of water that separated from the emulsion divided by the total volume of water contained in the emulsion sample.

RESULTS AND DISCUSSION

Water-retaining ability. Table 1 shows the m.p., O/W interfacial tension, and water-retaining ability of DAG samples. The O/W interfacial tension was dependent on the FA composition. However, the water-retaining ability was not simply dependent on the composition.

The water-holding capacity showed different trends between uni-chain and complex-chain DAG. If the chain lengths of two FA for uni-chain DAG were C_{12} and above, the DAG emulsified a given amount of water of $\Phi 80\%$. If the chain lengths were C_8 or shorter, the maximal volume fraction of water emulsified by the DAG was less than $\Phi 60\%$.

For the complex-chain DAG, all the samples showed the ability to emulsify water at $\Phi 80\%$.

The results for dioleyl GE, which has a molecular structure similar to that of uni-chain DAG of $C_{18:1}$, are also listed in Table 1. Dioleyl GE exhibited the ability to emulsify water at a relatively high volume, but a phase transition was observed at $\Phi 80\%$.

Figure 1 shows the W/O emulsion stability (emulsion preservation ratio) as a function of time for seven of the DAG samples in Table 1 that exhibited a water-holding capacity of $\Phi 80\%$. The stabilities shown in Table 1 are the preservation ratio at 10 min (Fig. 1). The effect of FA composition in DAG on the emulsion stability was rather complicated. The highest stability was obtained for the $C_2 R_2$ complex-chain DAG. The stability dropped with an increase in the R_2 chain length, exhibited the poorest stability for the $C_6 R_2$ complex-chain DAG, and then rose with the R_2 chain length.

TABLE 1
Oil/Water Interfacial Tension and Water-Retaining Ability of DAG

			m.p. (°C)	Interfacial tension (mN/m)	Water-retaining ability	
					Water-holding capacity ^a	W/O emulsion stability ^b (%)
Complex-chain DAG						
C ₂	—C _{18:1}	DAG	— ^c	4.0	Φ80% ≤	100
C ₃	—C _{18:1}	DAG	−4.4	7.9	Φ80% ≤	98
C ₄	—C _{18:1}	DAG	−2.4	10.4	Φ80% ≤	41
C ₆	—C _{18:1}	DAG	0.3	13.3	Φ80% ≤	25
C ₈	—C _{18:1}	DAG	8.7	14.9	Φ80% ≤	77
C ₁₀	—C _{18:1}	DAG	18.5	16.0	Φ80% ≤	89
C ₁₂	—C _{18:1}	DAG	26.6	17.0 ^d	Φ80% ≤	—
Uni-chain DAG						
C ₃	—C ₃	DAG	—	6.4	PT ^e at Φ50%	—
C ₄	—C ₄	DAG	—	8.3	PT at Φ50%	—
C ₆	—C ₆	DAG	—	12.4	PT at Φ55%	—
C ₈	—C ₈	DAG	7.8	14.2	PT at Φ60%	—
C ₁₂	—C ₁₂	DAG	53.5	—	Φ80% ≤ ^f	—
C _{18:1}	—C _{18:1}	DAG	18.6	17.2	Φ80% ≤	90
Glycerol ether (GE)						
C _{18:1}	—C _{18:1}	GE	7.8	22.4	PT at Φ80%	—

^aWater-in-oil (W/O) emulsification ability at a water volume fraction of 80% (Φ80%).

^bThe emulsion preservation ratio at $t = 10$ min in Figure 1.

^c—, not measured.

^dMeasured under a supercooled state.

^ePT, phase transition.

^fMeasured at 60°C.

Interfacial concentration/occupied area per molecule. The relationship between interfacial tension at the *n*-decane/water interface and the DAG concentration in *n*-decane phases is shown in Figure 2, and the saturated adsorption and the occupied area of DAG calculated using Equation 2 are shown in Table 2. The occupied areas of uni-chain DAG of C_{18:1} and C₁₂ obtained in this study are very close to the literature data: 1,3-dilaurin, 85.6 Å²; 1,2-dilaurin, 82.2 Å²; and 1,3-diolein, 116.2 Å², obtained by similar interfacial tension measurements using the Wilhelmy method (13).

The occupied areas of DAG at the interface were basically increased with the chain length of FA for both complex-chain DAG and uni-chain DAG (see also Fig. 3). If we compare the occupied areas between complex-chain DAG and uni-chain DAG at similar M.W., uni-chain DAG tended to exhibit larger areas than complex-chain DAG.

Since DAG have a “hydrophilic” polar group within the molecular structure, DAG themselves exhibit surface activity and could have the ability to form W/O emulsions without using additional emulsifiers. Previous studies showed the emulsification potential of DAG having two long unsaturated FA chains. Researchers found (8,9) that DAG containing long-chain unsaturated FA (C₁₈) could form emulsions with extremely high water content (Φ85%) without an emulsifier. In this study, we found that DAG having at least one long-chain FA (≥C₁₂) in the molecule can form a W/O emulsion at a high water volume (Φ80%). The water-holding capability of DAG observed here is not considered to be due to the formation of self-assembled structures such as a hexagonal II phase or a lamellar phase, deduced from a polarized light microscopy analysis (data not shown).

In general, high emulsifying capability and stability are obtained when the emulsifier (DAG itself in this study) adsorbs densely at the oil/water interface and forms a closely packed interfacial film *via* strong lateral intermolecular forces (van der Waals forces) (14,15). The closely packed interfacial film has a high elasticity, which is one of the most desirable properties to improve the emulsion stability.

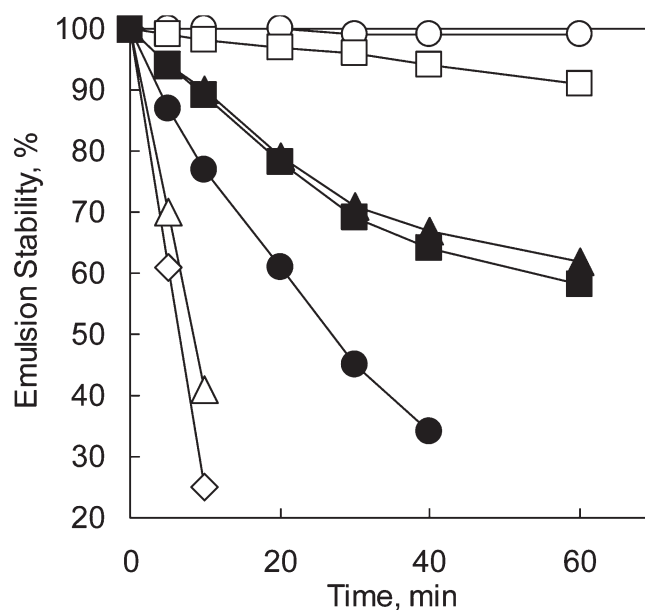


FIG. 1. Water-in-oil emulsion stability (emulsion preservation ratio) of complex-chain DAG and uni-chain DAG of C_{18:1} as a function of time. C₂-C_{18:1} DAG, ○; C₃-C_{18:1} DAG, □; C₄-C_{18:1} DAG, △; C₆-C_{18:1} DAG, ◇; C₈-C_{18:1} DAG, ●; C₁₀-C_{18:1} DAG, ■; C_{18:1}-C_{18:1} DAG, ▲.

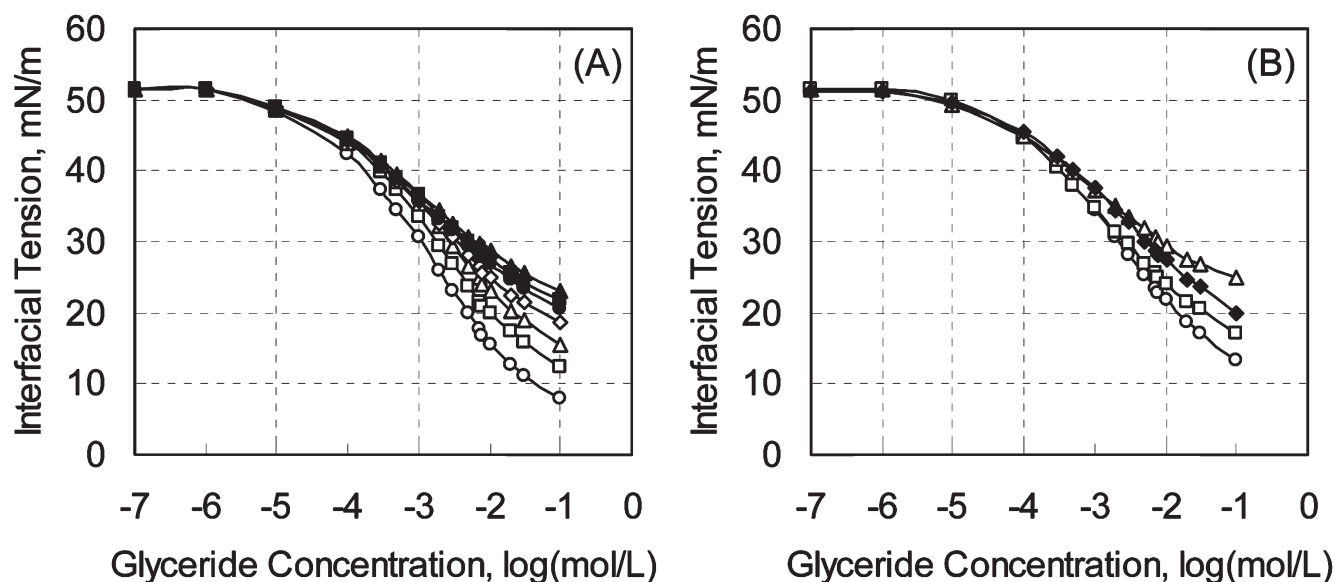


FIG. 2. Relationship between interfacial tension at the *n*-decane/water interface and the DAG concentration in *n*-decane phases of (A) complex-chain DAG, and (B) uni-chain DAG at 25°C. (A) C₂-C_{18:1} DAG, ○; C₃-C_{18:1} DAG, □; C₄-C_{18:1} DAG, △; C₆-C_{18:1} DAG, ◇; C₈-C_{18:1} DAG, ●; C₁₀-C_{18:1} DAG, ■; C₁₂-C_{18:1} DAG, ▲. (B) C₆-C₆ DAG, ○; C₈-C₈ DAG, □; C_{18:1}-C_{18:1} DAG, △; C₁₂-C₁₂ DAG (at 35°C), ◆.

It is possible that the geometrical isomers of DAG (1,2-DAG and 1,3-DAG) may contribute unequally to the effects of FA composition on the packing structure of interfacial films. However, separating the contribution of each isomer to the observed properties is difficult. The ratios of the isomers were almost the same for all the samples studied here, so the contribution of the effects of different geometric isomers on the observed water-retaining ability was estimated to be fairly similar between samples. Therefore, we focused our attention on the chain length of FA in DAG.

The water-holding capacity of uni-chain DAG is generally lower than that for complex-chain DAG, but it rises with the

alkyl chain length (Table 1). This is reasonable, because DAG molecules having short and middle chain-length FA cannot form a closely packed, ordered structure at the oil/water interface owing to the low van der Waals attraction between alkyl chains. The packing density of molecules at the interface increases with the chain length, which results in the high water-holding capacity for the C₁₂ and C_{18:1} uni-chain DAG. Results of the uni-chain DAG can be interpreted as simply a function of the chain length of FA in the molecules.

All the complex-chain DAG exhibit water-holding capacity at Φ80%. But the stability of the emulsion is not simply dependent on the length of the FA chain. This suggests that

TABLE 2
Value of the Interfacial Concentration (Γ_{∞}) and the Molecular Area (\AA^2) on the Adsorption of DAG, MAG, and GE at *n*-Decane/Water Interface at 25°C

			M.W. (g)	$\Gamma_{\infty} (\times 10^{-10} \text{ mol/cm}^2)$	Occupied area per molecule (\AA^2)
Complex-chain DAG					
C ₂	-C _{18:1}	DAG	398.6	2.74	60.7
C ₃	-C _{18:1}	DAG	412.6	2.58	64.3
C ₄	-C _{18:1}	DAG	426.6	2.19	75.7
C ₆	-C _{18:1}	DAG	454.7	1.98	83.8
C ₈	-C _{18:1}	DAG	482.7	1.70	97.6
C ₁₀	-C _{18:1}	DAG	510.8	1.63	101.7
C ₁₂	-C _{18:1}	DAG	538.9	1.61	102.9
Uni-chain DAG					
C ₆	-C ₆	DAG	288.4	2.26 [2.15 ^a]	73.4 [77.3 ^a]
C ₈	-C ₈	DAG	344.5	1.98 [1.94 ^a]	83.8 [85.8 ^a]
C ₁₂	-C ₁₂	DAG	456.7	— [1.82 ^a]	— [91.4 ^a]
C _{18:1}	-C _{18:1}	DAG	621.0	1.49	111.4
MAG					
C _{18:1}		MAG	356.6	3.88	42.8
Glycerol ether					
C _{18:1}	-C _{18:1}	GE	589.0	1.58	105.2

^aMeasured at 35°C. For abbreviation see Table 1.

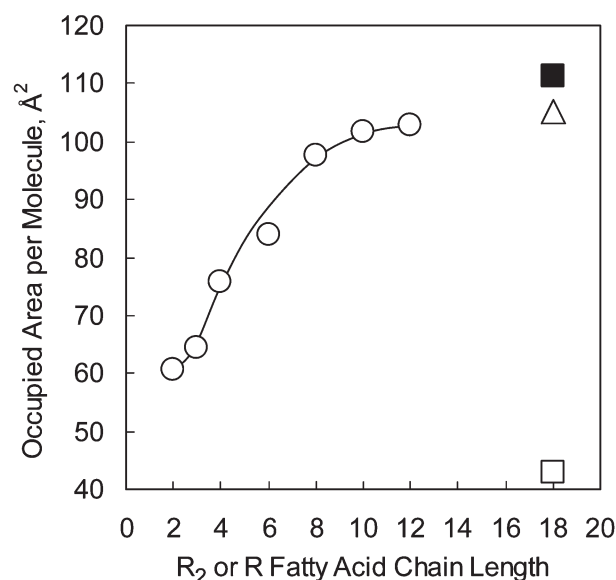


FIG. 3. The occupied area per molecule at the *n*-decane/water interface for complex-chain DAG, uni-chain DAG with C_{18:1}, MAG, and glyceryl ether (GE). Complex-chain DAG, ○; uni-chain DAG with C_{18:1}, ■; monoolein, □; dioleoyl GE, △.

the combination of the length of two FA chains in the molecule might play a role in the emulsion stability. This issue can be considered from the viewpoint of intramolecular interaction (the interaction between two acid chains in the molecules) and from intermolecular interaction.

If the chain lengths of the two FA chains in the DAG molecules are very different ($R_2 = C_2-C_6$ may be illustrative), intramolecular interaction is small and the two alkyl chains may not be ordered in the molecules. In this situation, increasing the chain length of R_2 shifts the molecules to less ability to pack into an ordered structure (i.e., a larger effective volume of R_2 prevents the molecules from ordering into closely packed structures at the interface) (16). Therefore, the molecular packing density and resulting emulsion stability are highest for C_2 complex-chain DAG (smallest effective volume of R_2). Increasing the chain length results in a drop in the emulsion stability.

If the R_2 chain length in complex-chain DAG is C_8 or longer, emulsion stability again increases. This occurs presumably because the two acid chains are relatively aligned owing to the increase in intramolecular attractions. Of course, this results in an increase in intermolecular interaction between adjacent molecules.

The effects of the structure of the head group on the emulsification properties can be illustrated by comparing the results of uni-chain DAG of C_{18:1} and dioleoyl GE. These two molecules have the same FA composition, and the occupied area is slightly smaller for dioleoyl GE than that of uni-chain DAG of C_{18:1}. This agrees with the behavior reported in the literature, where the replacement of the ester linkage of glycerolipids with the ether linkage causes the increase in the density of molecules in the liquid crystalline phase (17,18). However, the emulsification capability is better for uni-chain DAG

of C_{18:1}, which is inconsistent with the explanation given above. This suggests that the difference in the emulsification capability is possibly due to the effect of the head group. It is known that the polarity of the ether linkage is lower than that of the ester linkage owing to the lack of a carbonyl group and results in the low ability to form hydrogen bonds (19). Therefore, the lower water-retaining capability of dioleoyl GE compared with uni-chain DAG containing C_{18:1} is presumably due to the decline in hydrogen bonding.

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