

Effect of Emulsifier on the Stability of O/W Emulsion Containing Limonene through Freezing and Thawing

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Abstract

Oil-in-water-type (O/W) emulsions containing flavoring and fragrance ingredients are extensively used in food and cosmetics. This study examined the effects of emulsifiers on the stability of an O/W emulsion containing limonene through freezing and thawing. The destabilization of the emulsion would affect the stability of limonene in the emulsion, and the high hydrophilicity of the emulsifiers contributed to the high stabilities of the emulsion and limonene in the emulsion. Crystallization and the crystal growth of the disperse phase and medium influenced the coalescence of droplets and the destabilization of the emulsion after thawing. No relationship was observed between the changes in the droplet size and the hydrophilic–lipophilic balance numbers of four tested emulsifiers.

Keywords: Emulsifier, Freezing, Limonene, O/W Emulsion, Thawing

1. INTRODUCTION

Limonene is an essential oil component typically present in citrus fruits and has a good aromatic properties¹⁾. It has been used as a flavoring and fragrance ingredient in food and cosmetics. However, because of the presence of double bonds in its molecular structure, limonene gets oxidized, which leads to off-flavor and deterioration of the products (Fig. 1)²⁾. In addition, the usage of limonene is limited to certain products owing to its strong lipophilicity. Therefore, an oil-in-water-type (O/W) emulsion, where oil and liposoluble components are dispersed into water phase, is useful as a utility form of limonene. The emulsion is a thermodynamically metastable disperse system, and it leads to phase separation after creaming, flocculation, and coalescence owing to the high interfacial tension between the aqueous phase and the oil phase³⁾. Hence, it is unsuitable for long-term storage. In addition, it is difficult to use

the emulsion by freezing and storage at low temperature for enhancing the storage stability of the components and the thawing process, as the coalescence of the particles of the oil droplets tends to increase during the process⁴⁾. Therefore, it is important to elucidate the factors that destabilize the emulsion and the mechanism and degree of their contribution from the viewpoint of quality improvement and wider usage of the emulsion products containing flavoring components such as limonene. This study examined the effects of emulsifiers on the stability of an O/W emulsion containing limonene through freezing and thawing. An alkane containing 18 carbon atoms was used as the lipophilic component co-existing in the oil phase because of its simplest molecular structure.

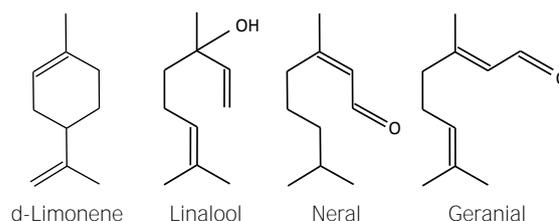


Fig. 1. Molecular structures of limonene and its oxides

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2. MATERIALS AND METHODS

2.1 Materials

d-Limonene, octadecane, sodium dodecylsulfate (SDS), polyoxyethylene sorbitan monooleate (Tween80), polyoxyethylene sorbitan monolaurate (Tween20), and all the other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Decaglycerol monolaurate SY-Glyster[®] ML-750 (ML-750) was supplied by Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka).

2.2 Preparation of emulsion and measurement of median diameter of oil droplets

The oil mixture was prepared by mixing 0.1 mL of limonene and 1.83 mL of octadecane. The mixture was emulsified using 17.37 mL of 1% (w/v) SDS, ML-750, and Tween80 or Tween20 as an emulsifier solution using a membrane emulsification device (DH-01, SPG Technology Co., Ltd., Miyazaki, Japan) containing a Shirasu porous glass membrane filter, whose pore diameter was 0.8 μm , at a flow rate of 0.3 mL/h. The prepared emulsion was stored at 37°C and was periodically sampled. For the freezing and thawing experiments, the emulsion was frozen for 24 h at -80°C or -20°C and further thawed for 10 min at 10°C or 30°C. The particle size distribution and the median diameter of the oil droplets in the emulsion were measured using a laser diffraction particle size analyzer (SALD-2200, Shimadzu Co., Ltd., Kyoto, Japan).

2.3 Measurement of the amount of remaining limonene in the emulsion

The emulsion sampled before and after storage was centrifuged for 10 min at 6,000 rpm, and 100 μL of the oil phase was diluted with 900 μL of methanol. The amount of remaining limonene in 5 μL of the sample was measured by gas chromatography (G-3900, Hitachi High-Tech Science Co., Ltd., Tokyo, Japan) containing an Rtx[®] 2330 capillary column (15 m \times 0.32 mm I.D., 0.2 μm df, Shimadzu GLC Ltd., Tokyo) and a flame ionization detector. Helium and methyl myristate were used as the carrier gas and internal standard, respectively. The flow rate was 1.8 mL/min. The temperatures of the injector and the detector were 220°C and 240°C, respectively. The column temperature was maintained at 30°C for 2 min and raised at 20°C/min and maintained at 180°C for 2 min.

3. RESULTS AND DISCUSSION

3.1 Effect of emulsifier on the stability of the

emulsion containing limonene with slight heating

Four emulsifiers, SDS, ML-750, Tween80, and Tween20, were evaluated for their effects on the stability of the emulsion containing limonene. Figure 2 shows the transient changes in the median diameter of the oil droplets in the emulsions prepared at 37°C using different emulsifiers. No difference was observed among the initial median diameters for all the four emulsions, indicating that the initial emulsifying abilities were similar to each other. The median diameter of the oil droplets in all the emulsions gradually increased for about 20 days and rapidly grew larger thereafter. The increments in the droplet diameter could be because of flocculation and coalescence of the oil droplets in the emulsion, but little difference was observed among the four emulsion stabilities.

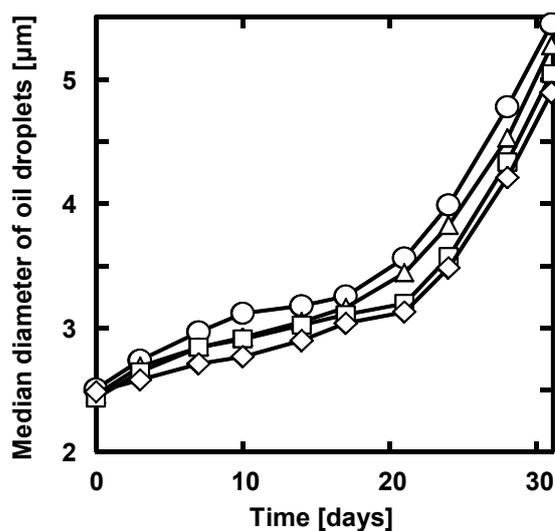


Fig. 2. Transient changes in the median diameter of oil droplets in the emulsions containing (○) SDS, (△) ML-750, (□) Tween80, and (◇) Tween20 at 37°C.

Figure 3 shows the time courses of the remaining fraction of limonene in the four emulsions containing SDS, ML-750, Tween80, and Tween20 at 37°C. The fraction of limonene in each emulsion decreased with time. The dissolution and then the volatilization of limonene appear to be the primary cause of its disappearance. The disappearance kinetics of limonene in the emulsion is expressed by the following Weibull equation, which is flexible and has a potential for describing several deterioration kinetics⁵⁾:

$$Y = \exp [-(kt)^n] \quad \text{Eq. 1}$$

where Y is the fraction of limonene remaining at time t , k is the rate constant, the reverse of which is known as the scale parameter, and n is the shape constant. The kinetic parameters k and n were calculated by best fitting the experimental results of Solver in Microsoft® Excel 2010 and are shown in Table 1. The curves in Figure 3 were drawn on the basis of the equation using the estimated parameters. The k values were high in the order of $\text{ML-750} > \text{Tween80} > \text{SDS} > \text{Tween20}$, but the stability of limonene in the emulsion containing SDS was the lowest of the four samples. The n value for SDS was also the lowest. The Weibull model has the characteristics of a sigmoidal pattern that can be described when $n > 1$, that the model expresses the simple first-order kinetics at $n = 1$, and that Y value steeply decreases during the early stage when $n < 1$. Therefore, this result quantitatively indicates that the disappearance of limonene in the emulsion containing SDS was the fastest.

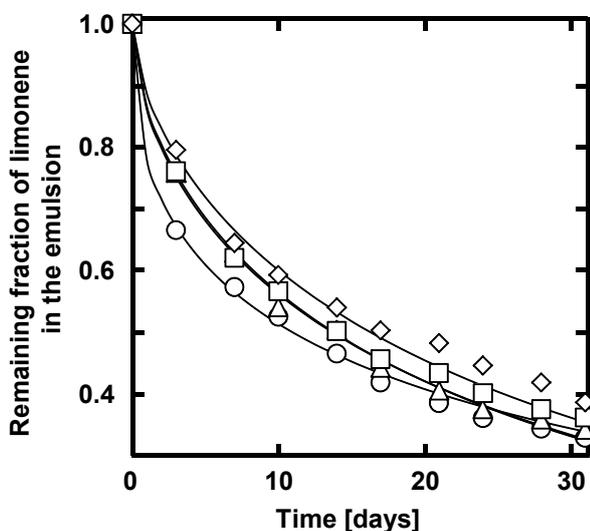


Fig. 3. Transient changes in the remaining fraction of limonene in the emulsions containing (○) SDS, (△) ML-750, (□) Tween80, and (◇) Tween20 at 37°C. The solid curves were drawn using the estimated kinetic parameters of the Weibull model.

Table 1. The parameters k and n of the Weibull

equation for the disappearance of limonene in the emulsions at 37°C

Emulsifier	k [d ⁻¹]	n
SDS	0.0390	0.429
ML-750	0.0417	0.614
Tween80	0.0395	0.600
Tween20	0.0343	0.629

The order of limonene stability was consistent with that of the emulsifying stability among the four emulsions. Destabilization of the emulsion would affect the stability of limonene in the emulsion. The chemical property of the emulsifier is evaluated by the hydrophilic–lipophilic balance (HLB) number, which is calculated according to the Griffin’s equation⁶⁾:

$$HLB = 20M_H/M \quad \text{Eq. 2}$$

where M_H is the total formula mass of the hydrophilic groups in the molecule, and M is the molecular mass. The HLB numbers of SDS, ML-750, Tween80, and Tween20 are 8.3, 14.8, 15.0, and 16.7, respectively. The high hydrophilicity of the emulsifier would contribute to the high stabilities of the O/W emulsion and limonene in the emulsion. SDS is an ionic emulsifier, whereas the other three are nonionic emulsifiers. The surface charge on the oil droplets may lead to the destabilization of the emulsion and the disappearance of limonene. The median diameter in all the emulsions steeply increased when the remaining fraction of limonene became less than 0.5. The creaming possibility of the emulsion increases owing to the high terminal velocity, v_T , of the droplets, which is expressed by the Stokes’ law:

$$v_T = (\rho - \rho_0)gd^2/18\eta \quad \text{Eq. 3}$$

where ρ is the density of the disperse phase, ρ_0 is the disperse medium, g is the gravitational acceleration, d is the diameter of the droplet, and η is the viscosity. The densities of limonene and octadecane are 0.840 and 0.782, respectively. The disappearance of limonene from the disperse phase lowered the density of the disperse phase and enhanced the terminal velocity. Consequently, the creaming process promotes the coalescence of the droplets.

3.2 Relationship between the stability of the

emulsion through freezing and thawing and the emulsifier

Figure 4 shows the changes in the median diameter of the oil droplets in the emulsions containing SDS, ML-750, Tween80, and Tween20 after freezing and thawing at different temperatures. The melting points of limonene and octadecane are -74°C and 28°C , respectively. The change in the diameter was strongly suppressed in each emulsion containing a different emulsifier, when the emulsion was frozen at -80°C and then thawed at 30°C . In this case, both disperse phase components suffered the phase transition during freezing and thawing processes; furthermore, the cooling and heating velocities were the highest. In contrast, the change was high when octadecane, which was a major component in the disperse phase, remained in the solid state. This would indicate that the crystallization and the crystal growth of the disperse phase and medium influenced the coalescence of the droplets and the destabilization of the emulsion after thawing. The droplet diameter in the emulsion containing SDS changed, but no relationship was observed between the changes in the droplet size and the HLB numbers of the four tested emulsifiers. The effect of the emulsifiers on the emulsion stability through freezing and thawing may not be large, though more information regarding the melting points of the emulsifiers is required for clarifying the phenomena.

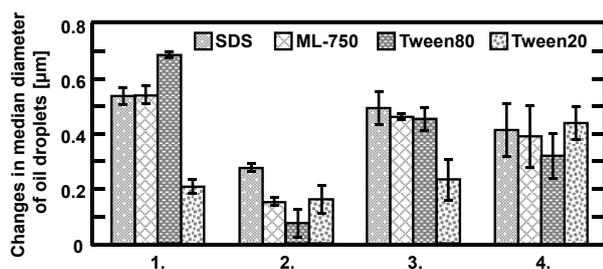


Fig. 4. Changes in the median diameter of the oil droplets in the emulsions containing SDS, ML-750, Tween80, and Tween20 after freezing and thawing at 1) -80°C and 10°C , 2) -80°C and 30°C , 3) -20°C and 10°C , and 4) -20°C and 30°C . The bars indicate 95% confidence intervals.

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REFERENCES

1) M. G. Peixoto, L. M. Costa-Júnior, A. F.

- Blank, A. S. Lima, T. S. A. Menezes, D. A. Santos, P. B. Alves, S. C. H. Cavalcanti, L. Bacci, and M. F. Arrigoni-Blank, *Vet. Parasitol.*, **210**, 118-122 (2015).
- 2) A. Soottitantawat, F. Bigeard, H. Yoshii, T. Furuta, M. Ohkawara, and P. Linko, *Innov. food sci. & emerg. technol.*, **6**, 107-114 (2005).
- 3) W. Kang, L. Guo, H. Fan, L. Meng, and Y. Li, *J. Pet. Sci. Eng.*, **81**, 177-181 (2012).
- 4) B. M. Degner, C. Chung, V. Schlegel, R. Hutkins, and D. J. McClements, *Compr. Rev. Food Sci. Food Saf.*, **13**, 100-113 (2014).
- 5) L. M. Cunha, F. A. R. Oliveira, and J. C. Oliveira, *J. Food Eng.*, **37**, 175-191 (1998).
- 6) W. Griffin, *J. Soc. Cosmet. Chem.*, **1**, 311-326 (1949).