The Effects of Ethanol Content and Emulsifying Agent Concentration on the Stability of Vegetable Oil–Ethanol Emulsions

Qingyi Xu[a,b], **Mitsutoshi Nakajima**[a,b,*], **Hiroshi Nabetani**[a],
Satoshi Iwamoto[a], **and Xinqi Liu**[a]

[a]National Food Research Institute, Tsukuba, Ibaraki 305-8642, Japan, and [b]Institute of Agricultural and Forestry Engineering, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

ABSTRACT: In vegetable oil–ethanol emulsions ethanol is the polar phase and vegetable oil is the nonpolar phase. The primary advantage of vegetable oil–ethanol emulsions over conventional water–oil emulsions is that they enable the incorporation of water- and oil-insoluble or poorly soluble functional compounds and/or drugs into emulsions. A number of nonionic surfactants were used to select appropriate stabilizers for stable vegetable oil–ethanol emulsions. We found decaglycerol mono-oleate (MO750) to be the best stabilizer for ethanol-in-oil (E/O) emulsions. The effects of ethanol content and of emulsifying agent concentration on the stability of vegetable oil–ethanol emulsions were examined with MO750. After emulsification, two turbid layers formed simultaneously when ethanol content exceeded 20 wt%. The top layers (oil-in-ethanol emulsions; O/E emulsions) were very unstable, whereas the stability of the bottom layers (E/O emulsions) depended on the ethanol content. The stability of E/O emulsions is closely related to the effective concentration of MO750 aggregates, which play an important role in the film thickness stability of interfacial films formed by surfactant aggregates. Instability of E/O emulsion at 5 wt% MO750 is probably due to the polydispersity (i.e., nonuniform size and shape) of MO750 aggregates at high MO750 concentration. E/O emulsions prepared with 0.1, 0.5, and 1 wt% MO750 were stable, suggesting that the interfacial films formed were effective in protecting the droplets against coalescence and Ostwald ripening.

Paper no. J9879 in *JAOCS 78,* 1185–1190 (December 2001).

KEY WORDS: Aggregate, emulsifying agent, interfacial film, stability, vegetable oil–ethanol emulsion.

Research into substitutes for conventional petroleum-based fuels has led to the development of vegetable oil–alcohol microemulsions to be used as biodiesel (1). A vegetable oil–ethanol microemulsion for possible biofuel utilization was developed using monoolein as the surfactant; its structure was clarified with a dynamic light-scattering spectrophotometer and small-angle X-ray scattering (SAXS) in our laboratory (2). Besides use as biodiesel, the vegetable oil–ethanol system holds promise as an emulsion system to overcome the solubility limitation of conventional water–oil emulsion systems (i.e., water-in-oil or oil-in-water emulsions or their double emulsions). Although much attention has been paid to vegetable oil–alcohol microemulsions, few studies have been conducted on vegetable oil–alcohol emulsions. The study of such an emulsion system may make possible the incorporation of water- and oil-insoluble or poorly soluble functional compounds or anticancer drugs into emulsions. Our current work is directed toward nonionic surfactant (decaglycerol mono-oleate; MO750)-stabilized vegetable oil–ethanol emulsions.

MO750 completely dissolves and disperses in 95% ethanol but exhibits poor monomer solubility in oil. It exists as a spherical structure with a radius of gyration of about 7 Å in 95% ethanol and forms aggregates with a radius of gyration of nearly 90 Å in oil (3). In a vegetable oil–ethanol system, the nonionic surfactant MO750 has only a limited effect on the interfacial tension between the two phases (3). Consequently, it appears that the surface-active stabilization-driving mechanism (i.e., the Gibbs-Marangoni effect) and the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (4) cannot be used to explain the stability of vegetable oil–ethanol emulsions. We consider the stabilization of a vegetable oil–ethanol emulsion to be closely related to the monomer solubility and dispersion behavior of MO750 in the ethanol and the oil phases, and especially to the presence of aggregates of MO750 in the oil phase after emulsification. A possible stabilization mechanism based on the stratification theory (5–9) and the diffusion redistribution concept (4,10) was proposed for E/O emulsions stabilized by MO750 (3). The mechanism involves (i) the diffusion of MO750 from the ethanol phase to the oil phase in the nonpre-equilibrated condition, resulting in the concentration of MO750 at the ethanol/oil interface and the formation of MO750 aggregates at the interface of the two phases; and (ii) the formation of ordered layers of MO750 aggregates inside the interfacial film, possibly contributing to the long-term stability of E/O emulsions.

The results of selection of suitable emulsifying agents for the preparation of stable model vegetable oil–ethanol emulsions [i.e., ethanol-in-oil (E/O) emulsions or oil-in-ethanol (O/E) emulsions] are presented. We discuss the influence of ethanol content on the stability of vegetable oil–ethanol emulsions stabilized by MO750 and the effect of MO750 concentration on E/O emulsion stability. Discussion of the stabilization mechanism related to the buildup of MO750 aggregate layers inside the interfacial film is presented.

EXPERIMENTAL PROCEDURES

Materials. High-oleic sunflower oil (oleic acid content >90%) was supplied by Nippon Lever Co. Ltd. (Tokyo, Japan).

---

*To whom correspondence should be addressed at National Food Research Institute, 2-1-12, Kannondai, Tsukuba, Ibaraki 305-8642, Japan.
E-mail: mnaka@nfri.affrc.go.jp

TABLE 1
Effect of Emulsifier Type on the Stability of Oil-in-Ethanol (O/E) Emulsions

Number	Emulsifiers[a]	HLB[b]	Emulsion stability (d)
1	Monoolein		<1
2	Sorbitan monolaurate (Span 20)	8.6	<1
3	Polyoxyethylene(20) sorbitan trioleate (Tween 85)	11.0	<1
4	Polyoxyethylene(20) sorbitan monooleate (Tween 80)	15.0	<1
5	Polyoxyethylene(20) sorbitan monostearate (Tween 60)	14.9	<1
6	Polyoxyethylene(20) sorbitan monopalmitate (Tween 40)	15.6	<1
7	Decaglycerol mono-caprylate (MCA750)	16.0	<1
8	Tetraglycerol mono-laurate (ML310)	10.3	<1
9	Hexaglycerol mono-laurate (ML500)	13.5	<1
10	Decaglycerol mono-laurate (ML750)	14.8	<1
11	Tetraglycerol mono-oleate (MO310)	8.8	<1
12	Hexaglycerol mono-oleate (MO500)	11.6	<1
13	Decaglycerol mono-oleate (MO750)	12.9	<1
14	Lecithin (from soybean)		1–2
15	Lecithin (from egg yolk)		1–2

[a] Emulsifiers Nos. 1 and 7–13 were kindly provided by Taiyo Kagaku Co., Ltd. (Yokkaichi, Japan) and Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan), respectively. Nos. 2–6, 14, and 15 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).
[b] HLB is the hydrophile–lipophile balance of an emulsifier. Monoolein, lecithin, and lipophobic emulsifiers were used. All the samples were prepared with an ethanol-to-oil ratio of 8:2 w/w and an emulsifier concentration of 5 wt%.

Ethanol, containing by volume 95% ethanol and 5% water, was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The emulsifying agents used in this study are shown in Tables 1 and 2.

Preparing emulsions. Our aim was to produce an emulsion system with an average diameter of several micrometers; therefore, considering the properties of the system, all the emulsions were prepared with a low-shear homogenizer (Polytron® PT3000; Kinematica AG, Lucerne, Switzerland) at 3,000 rpm for 10 min. Because the volume of the sample significantly influences the emulsification effectiveness, as confirmed by our preliminary experiments and reported by Pearce and Kinsella (11), the volume of samples was kept constant at 30 mL. The space between the neck of the vial and the rotating shaft was sealed with Parafilm to prevent evaporation of ethanol during homogenization. Samples were prepared in duplicate for screening tests and in triplicate for other measurements. After emulsification, samples were kept at room temperature. Emulsion stability was judged by the time taken for complete phase separation.

Measuring droplet size and size distribution. We used a laser diffraction particle size analyzer for highly turbid samples (SALD-200V-ER-HC; Shimadzu Co., Kyoto, Japan) to measure droplet size and size distribution of the emulsions. To prevent multiple scattering effects, samples were placed into the narrow space between two specially made glass

TABLE 2
Effect of Emulsifier Type on the Stability of Ethanol-in-Oil (E/O) Emulsions

Number	Emulsifiers[a]	HLB[b]	Emulsion stability (d)
1	Monoolein		<1
2	Sorbitan trioleate (Span 85)	2.0	<1
3	Sorbitan monooleate (Span 80)	4.3	1–2
4	Sorbitan monostearate (Span 60)	6.5	1–2
5	Sorbitan monopalmitate (Span 40)	7.7	1–2
6	Decaglycerol deca-oleate (DAO750)	3.0	<1
7	Tetraglycerol mono-oleate (MO310)	8.8	~20
8	Hexaglycerol mono-oleate (MO500)	11.6	~40
9	Decaglycerol mono-oleate (MO750)	12.9	~150
10	Tetraglycerol penta-oleate (PO310)	3.0	<1
11	Hexaglycerol penta-oleate (PO500)	4.9	<1
12	Lecithin (from soybean)		1–2
13	Lecithin (from egg yolk)		1–2

[a] Emulsifiers Nos. 1 and 6–11 were kindly provided by Taiyo Kagaku Co., Ltd. and Sakamoto Yakuhin Kogyo Co., Ltd., respectively. Nos. 2–5, 12, and 13 were purchased from Wako Pure Chemical Industries, Ltd. See Table 1 for supplier locations.
[b] HLB is the hydrophile–lipophile balance of an emulsifier. Monoolein, lecithin, polyglycerol esters of mono-oleate, and lipophilic emulsifiers were used. All samples were prepared with an oil-to-ethanol ratio of 8: 2 w/w and an emulsifier concentration of 5 wt%.

slides. This obviated the need for dilution, with its attendant negative effects. Measurements were repeated at least three times per sample, and mean values were calculated.

Measuring viscosity. Viscosity was measured on a microviscosimeter from Haake (Karlsruhe, Germany). Measurements were repeated five times, and mean values were calculated.

RESULTS AND DISCUSSION

Selecting appropriate emulsifying agents. The emulsifying agents listed in Table 1 were used in the screening test for O/E emulsions. All the emulsions obtained were very unstable. Complete phase separations were observed within 1 or 2 d. Consequently, attempts to develop stable O/E emulsions failed. The screening test results of E/O emulsions are presented in Table 2; all the emulsifiers studied showed poor stabilizing properties except for polyglycerol mono-oleates. Of these, MO750 was the best stabilizer for E/O emulsions. Therefore, further studies were carried out with MO750.

Effect of ethanol content on emulsion stability. Ethanol content affected the formation and stability of vegetable oil–ethanol emulsions (Fig. 1). When ethanol content exceeded 20 wt%, two turbid layers coexisted in the sample vials after homogenization. From the densities of oil and ethanol (Table 3), it was obvious that the top layer was oil droplets dispersed in ethanol (i.e., O/E emulsion) and the bottom layer was ethanol droplets dispersed in oil (i.e., E/O emulsion). The phenomenon of two turbid emulsified layers coexisting in a sample vial supports an idea proposed by Davies and Riedal and affirmed by Ivanov and Kralchevsky (10). They suggested that both types of emulsions are formed during homogenization, but only the emulsion with a lower coalescence rate survives. The turbidity of the top layer increased with ethanol content. Top layers were very unstable, becoming clear within 1 d. In contrast, the emulsified bottom layers were more stable, and their stabilities depended on ethanol content. That is, for an ethanol content of 80 wt%, the stability of the E/O emulsion was <1 d. The remaining ethanol contents (%) tested had the following stabilities (d): 70, <1; 60, <1; 50, <2; 40, <4; 30, <4; 20, <150; and 10, >365. [All samples were prepared with 1 wt% MO750 (in total sample). MO750 was dissolved in ethanol and then mixed with oil with a homogenizer. After emulsification, samples were kept at room temperature.]

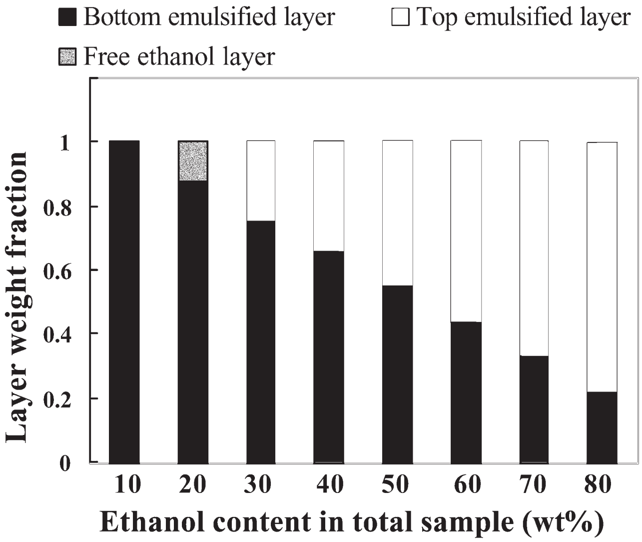

FIG. 1. Layers formed 1 h after homogenization in samples containing 1 wt% MO750 (decaglycerol mono-oleate), prepared with different ethanol contents.

TABLE 3
Characteristic Parameters of the Phases (at 25°C)

Phase	Density (g/cm^3)	Viscosity (mPa·s)
95% Ethanol	0.81	1.27^a
Oil	0.91	59

^{a}From Reference 20.

For an ethanol content of 20 wt%, only one turbid layer (i.e., E/O emulsion) was observed immediately after homogenization. However, an apparent free-ethanol phase appeared on the top of the emulsified layer within 1 h. For an ethanol content less than 10 wt%, only one stable turbid emulsified layer (E/O emulsion) was formed. Complete phase separation was observed after more than 1 yr at room temperature (in a range of 0 to 40°C) and after about 3 mon at 45°C. Smaller droplet size and higher emulsion viscosity were observed at lower temperatures, suggesting that higher stability could be achieved at a lower storage temperature.

As we indicated earlier, the surface-active stabilization-driving mechanism (i.e., the Gibbs-Marangoni effect) and the DLVO theory do not explain the stability of vegetable oil–ethanol emulsions. The stabilization of vegetable oil–ethanol emulsions is likely related to the stratification of MO750 aggregates in the interfacial film. Stratification takes place when the continuous phase contains small colloidal particles such as micelles or latex spheres (4). For the top emulsified layers (O/E emulsions), no aggregates of MO750 formed in the ethanol phase (the continuous phase), since MO750 was completely dissolved and dispersed in the ethanol phase as a result of its high solubility in the solvent. This was confirmed in our previous study of the dispersion behavior of MO750 in ethanol with SAXS (3). Therefore, one might surmise that the interfacial film formed without aggregate layers was very weak and that the droplets would coalesce rapidly, which was confirmed by microscopic examination. The instability of the O/E emulsions is also due to low viscosity of the continuous (ethanol) phase (Table 3). Accordingly, a stable O/E emulsion is difficult to prepare.

In contrast, the greater stability observed in the bottom-emulsified layers (the E/O emulsions) is probably a result of the distribution of MO750 between the two layers of the samples. Since all the samples were prepared with a constant MO750 concentration of 1 wt% (in total sample), the lower the ethanol content, the less MO750 would be in the top layer, and the more MO750 would be in the bottom layer after emulsification. Therefore, the concentration of MO750 in bottom layers would increase with the decrease in ethanol content. In consequence, more MO750 would concentrate and form aggregates at the interface of the two phases, thus minimizing the unfavorable contact area between the hydrophilic groups of the surfactant and oil and resulting in an increase in the effective aggregate concentration. As pointed out by Nikolov *et al.* (13), film particle structuring is greatly influenced by the effective micellar concentration. Thus, an increase in effective concentration of MO750 aggregates as the ethanol content decreases may enhance the buildup of ordered aggregate layers, strengthening the interfacial films. High viscosity of the continuous phase (the oil phase) (Table 3) will also contribute to the stability of E/O emulsions, lowering the mobility of the droplets and thus preventing coalescence. Accordingly, the tendency toward O/E or E/O emulsion depends on the properties of the oil and ethanol, the solubility and the dispersion behavior of an emulsifying agent in the two phases, and the ethanol content. The higher stability of E/O emulsion compared to O/E emulsion may be attributed to the layering of MO750 aggregates inside the interfacial film and the high viscosity of the continuous phase (Table 3), vital to keeping the droplets from coalescence.

Studies of the effect of ethanol content on the stability of oil-in-water emulsions stabilized by sodium caseinate have shown that the presence of a low content of ethanol (~15%) can enhance emulsion stability. This has been attributed to the interfacial tension reduction effect of ethanol. In contrast, the presence of a high content of ethanol (exceeding 30%) can result in an unstable emulsion owing to protein precipitation. The addition of alcohol also reduces steric stabilization and electrostatic stabilization (14). The instability at high ethanol content of vegetable oil–ethanol emulsions stabilized by a nonionic surfactant is apparently caused by a different mechanism. The high ethanol content obstructs formation of aggregates of the nonionic surfactant and hence the layering of MO750 aggregates, resulting in the formation of a weak interfacial film. Knowledge of the effect of the polar phase content on effective aggregate concentration is necessary to the formulation of ethanol-containing emulsions that are stabilized by small-molecule surfactants. Furthermore, it may be possible to predict the stability of this kind of emulsion by measuring the dispersion behavior and/or the solubility of an emulsifying agent in the two phases of an emulsion.

Effect of MO750 concentration on the properties of E/O emulsions. The effect of MO750 concentration on emulsion characteristics was investigated by analyzing the apparent emulsion viscosity, emulsion droplet size, and size distribution. The viscosities of the emulsions showed a slight increase with MO750 concentration. For an MO750 concentration of 0.1 wt% at 25°C (5 wt% alcohol), the viscosity was 39.6 mPA·s; likewise, the following wt% concentrations produced the corresponding viscosities: 0.5, 40.5; 1, 42.6; and 5, 46.2. Although emulsifying agents can also stabilize emulsions by increasing the viscosities of emulsions and thus restricting the mobility of the emulsion droplets and preventing coalescence, these viscosity measurements imply that MO750 mainly stabilizes an E/O emulsion by forming a rigid interfacial film, preventing droplet coalescence.

The emulsions with 0.1, 0.5, and 1 wt% MO750 have monomodal distributions and volume mean diameters of 2.4, 2.2 and 2.7 μm, respectively (Fig. 2). This suggests that the characteristics of E/O emulsions are not greatly influenced by MO750 concentration within a range of 0.1 to 1 wt% under our experimental conditions. However, the emulsion prepared with 5 wt% MO750 had a bimodal distribution, with a main peak of large droplets, giving a bigger volume mean diameter of 6.1 μm (Fig. 2). Complete phase separation occurred within 1 d. Surfactants, amphiphilic molecules, can form micelles and lyotropic liquid crystalline by self-association, depending on the surfactant concentration. The surfactant concentration can influence the size and the shape of micelles, resulting in changes in interfacial film characteristics and thus in emulsion stability (12,15,16). Investigations of the effects of surfactant concentration on the film thickness stability of films formed by micellar layers (12,13) have shown that micellar layering is very weak at low micellar concentrations, while micellar layering is reduced due to micellar polydispersity at high micellar concentrations. This leads to lower thickness stability at both low and high micellar concentrations. It has also been reported that micellar polydispersity increases with increasing micellar volume; the degree of polydispersity is high when it approaches the micellar sharp transition (sphere to rod). Thus, the instability of the E/O emulsion at 5 wt% MO750 (Fig. 2) appears to be a consequence of the polydispersity of MO750 aggregates at high MO750 concentration. Our investigation (3) of the dispersion behavior of MO750 in oil at various MO750 concentrations has shown that it forms aggregates with a radius of gyration of about 90 Å in oil as well as a higher-order structure that gradually becomes apparent as the MO750 concentration increases (within a range of 0.1 to 2 wt%). The presence of the higher-ordered structure of MO750

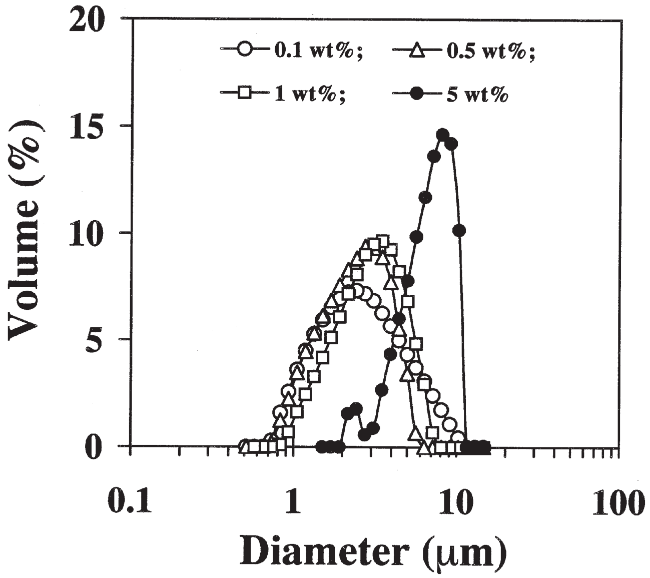

FIG. 2. Comparison of droplet size distributions of emulsions with different MO750 concentrations (5 wt% ethanol). Measurements were carried out immediately after emulsions had been prepared. For abbreviation see Figure 1.

may cause structural irregularity inside the interfacial film, leading to markedly reduced film stability. The instability of the E/O emulsion containing 5 wt% MO750 is probably the result of the combined effect of the polydispersity of MO750 aggregates and the coexistence of the aggregates with the higher-ordered self-assembly of MO750.

We also monitored the droplet size distribution of emulsions with 0.1, 0.5, and 1 wt% MO750 over 20 d of storage. All the emulsions were stable; there was no shifting of the peak to larger volume mean diameters and no obvious change in the width of the size distributions over the study period (Fig. 3). Although the interfacial film formed by micellar layers at low micellar concentration was very weak (9,13), reduction of MO750 concentration to 0.1 wt% did not decrease the emulsion stability, indicating that the interfacial films formed could provide sufficient steric hindrance to prevent droplet coalescence.

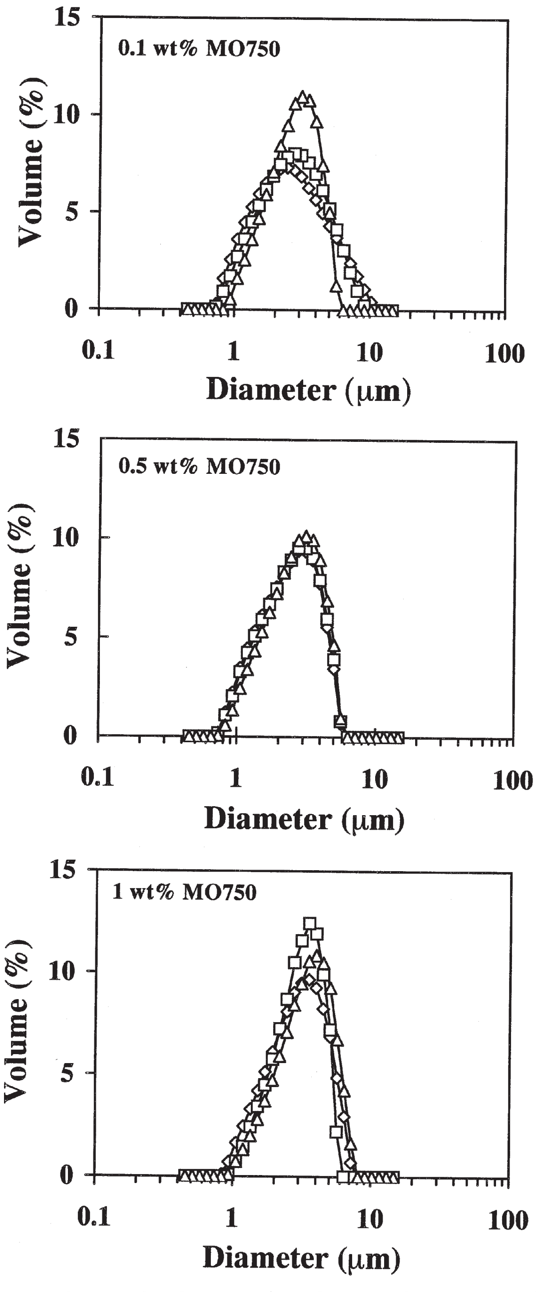

FIG. 3. Droplet size distributions of emulsions with 0.1, 0.5, and 1 wt% MO750 (5 wt% ethanol). Measurements were carried out after preparation (◊), after 10 d (□), and after 20 d (△). For abbreviation see Figure 1.

In the case of ethanol-containing oil-in-water emulsions stabilized by milk protein, Ostwald ripening was reported as another factor in emulsion instability, due to the diffusion of oil molecules from the droplet phase to the ethanol-containing aqueous phase (14,17). Since Ostwald ripening is a result of the Gibbs effect, that is, the solubility of the droplet phase material increases with the decrease in droplet size, the rate of Ostwald ripening increases with the decrease in droplet size and decreases with increase in droplet size. Therefore, its influence is dominant in emulsions with a smaller droplet size, becoming less important in emulsions with a larger droplet size. Even though Ostwald ripening is significant when the interfacial film formed is weak (17), it can be reduced by increasing the rigidness of the protein interfacial layer through enzymatic cross-linking of milk protein (18,19). Thus, a higher stability with respect to coalescence and Ostwald ripening can be achieved. Ostwald ripening can be characterized by the gradual shifting of the monomodal distribution to higher average droplet sizes, which differs from coalescence. The latter tends to give shifting bimodal distributions (19). Obvious Ostwald ripening was not observed in the E/O emulsions with 0.1, 0.5, and 1 wt% MO750, as the monomodal distributions did not shift to large mean droplet sizes (Fig. 3). This suggests that the interfacial films formed were strong enough to protect the ethanol droplets against Ostwald ripening. Another possible reason for no detectable Ostwald ripening in the emulsions is probably the larger droplet size studied in the present work (about one order of magnitude larger than that in the literature), resulting in slower Ostwald ripening.

ACKNOWLEDGMENT

This work was supported financially by research grant from Bio-oriented technology research advancement institute of Japan (BRAIN).

REFERENCES

1. Knothe, G., R.O. Dunn, and M.O. Bagby, Biodiesel: The Use of Vegetable Oils and Their Derivatives as Alternative Diesel Fuels, in *Fuels and Chemicals from Biomass*, American Chemical Society Symposium Series No. 666, edited by B.C. Sara and J. Woodward, American Chemical Society, Washington, DC, 1997, pp. 196–199.
2. Nabetani, H., T. Yoshizawa, M. Nakajima, S. Ichikawa, and Y. Sano, Characteristics of Ethanol–Vegetable Oil Microemulsion for Biofuel Utilization, in *Proceedings of the Third International Soybean Processing and Utilization Conference*, The Japanese Society for Food Science and Technology, Korin Publishing Co., Tokyo, 2000, pp. 686–688.
3. Xu, Q.Y., M. Nakajima, H. Nabetani, S. Ichikawa, and X. Liu, Effect of the Dispersion Behavior of a Nonionic Surfactant on Surface Activity and Emulsion Stability, *J. Colloid Interface Sci. 242*:443–449 (2001).
4. Borwankar, R.P., B. Campbell, C. Oleksiak, T. Gurkov, D.T. Wasan, and W. Xu, Food Dispersion Stability, in *Food Emul-*

sions and Foams, edited by E. Dickinson and J.M. Rodríguez Patino, RS·C, London, 1999, pp. 45–60.

5. Nikolov, A.D., and D.T. Wasan, Ordered Micelle Structure in Thin Films Formed from Anionic Surfactant Solutions (I. Experimental), *J. Colloid Interface Sci. 133*:1–12 (1989).
6. Nikolov, A.D., and D.T. Wasan, Ordered Micelle Structure in Thin Films Formed from Anionic Surfactant Solutions (II. Model Development), *Ibid. 133*:13–22 (1989).
7. Kralchevsky, P.A., A.D. Nikolov, D.T. Wasan, and I.B. Ivanov, Formation and Expansion of Dark Spots in Stratifying Foam Film, *Langmuir 6*:1180–1189 (1990).
8. Nikolov, A.D., and D.T. Wasan, A Novel Method for Studying the Dynamic Behavior of Both Plane-Parallel and Curved Thin Liquid Films, *Colloids Surf. A 123–124*:375–381 (1997).
9. Nikolov, A.D., D.T. Wasan, N.D. Denkov, P.A. Kralchevsky, and I.B. Ivanov, Drainage of Foam Films in the Presence of Nonionic Micelles, *Prog. Colloids Polym. Sci. 82*:87–98 (1990).
10. Ivanov, I.B., and P.A. Kralchevsky, Stability of Emulsions Under Equilibrium and Dynamic Conditions, *Colloids Surf. A 128*:155–175 (1997).
11. Pearce, K.N., and J.E. Kinsella, Emulsifying Properties of Proteins: Evaluation of a Turbidimetric Technique, *J. Agric. Food Chem. 26*:716–723 (1978).
12. Nikolov, A.D., and D.T. Wasan, Effects of Film Size and Micellar Polydispersity on Film Stratification, *Colloids Surf. A 128*: 243–253 (1997).
13. Nikolov, A.D., D.T. Wasan, and S.E. Friberg, Effect of Surfactant Concentration on Dispersion Stability as Probed by the Film Thickness Stability Mechanism, *Ibid. 118*:221–243 (1996).
14. Dickinson, E., and M. Golding, Influence of Alcohol on Stability of Oil-in-Water Emulsions Containing Sodium Caseinate, *J. Colloid Interface Sci. 197*:133–141 (1998).
15. Chidambaram, N., and D. Burgess, Effect of Cationic Surfactant on Transport of Surface-Active and Non-Surface-Active Model Drugs and Emulsion Stability in Triphasic Systems, *AAPS PharmSci. 2(3)*:article 28 (2000).
16. Chidambaram, N., and D. Burgess, Effect of Nonionic Surfactant on Transport of Surface-Active and Non-Surface-Active Model Drugs and Emulsion Stability in Triphasic Systems, *Ibid. 2(3)*:article 30 (2000).
17. Agboola, S.O., and D.G., Dalgleish, Effects of pH and Ethanol on the Kinetics of Destabilization of Oil-in-Water Emulsions Containing Milk Proteins, *J. Sci. Food Agric. 72*:448–454 (1996).
18. Færgemand, M., J. Otte, and K.B. Qvist, Emulsifying Proprties of Milk Proteins Cross-Linked with Microbial Transglutaminase, *Int. Dairy J. 8*:715–723 (1998).
19. Dickinson, E., C. Ritzoulis, Y. Yamamoto, and H. Logan, Ostwald Ripening of Protein-Stabilized Emulsions: Effect of Transglutaminase Crosslinking, *Colloids Surf. 12*:139–146 (1999).
20. *Kagaku Binran II* (Chemistry Handbook: in Japanese), 3rd edn., edited by The Chemical Society of Japan, Maruzen Co., Ltd., Tokyo, 1984, p. 52.

[Received January 29, 2001; accepted October 4, 2001]