Investigation on the Uniformity and Stability of Sunflower Oil/Water Emulsions Prepared by a Shirasu Porous Glass Membrane

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Shirasu porous glass (SPG) membrane emulsification has many advantages over traditional emulsification methods of stirring, ultrasoubd, and homogenization. The main advantages are much improved uniform size distribution of the droplets and lower mechanical shear stress. This paper examines the uniformity and stability of sunflower oil emulsions prepared by SPG membrane. The effects of emulsifiers on the uniformity and stability of emulsions were investigated. Results showed that emulsions stabilized by Tween 20 are generally more uniform and stable compared to those stabilized by Tween 60. Emulsions prepared by membrane emulsification showed excellent uniformity and stability with no droplet size change after 6-months storage, while those prepared by the homogenizer have a much broad size distribution and are unstable after 7 days of storage with significant further broadening of the droplet size distribution by coalescence and Oswald ripening. Uniform-sized sunflower O/W emulsions prepared by the SPG membrane also have good thermal stability when subjected to either freezing or heating. The mechanical stability of sunflower O/W emulsions prepared by the SPG membrane can be further improved by adding thickeners to the aqueous phase. Therefore, SPG membrane emulsification is a promising method for preparing uniform and stable emulsions, which will have good potential applications in the food industry, in terms of improving shelf life and product quality.

Introduction

Oil/water (O/W) emulsions have important applications in the food industry. Examples include soft drinks, milk, cream, salad dressing, butter, and margarine, to name a few.[1,2] In general, high-quality products of food emulsions should have the characteristics of consistent functional properties, sufficiently long shelf life, and thermal and chilling−freezing stability. During the period of manufacture, transportation, storage, and utilization, food emulsions often experience different environmental conditions, such as shaking, heating, freezing, and ionic strength and pH change.[3,4] Food emulsions tend to flocculate or coalesce under these physiochemical processes, and this will lead to the loss of textural and sensorial property, deterioration of quality, and shortening of shelf life. Therefore, it is necessary to prepare stable emulsions for application in the food industry. Controlling structural stability of food emulsions is also important for the delivery of health and functional actives of foods such as flavor, antioxidants, and bioactive lipids.[5,6]

There are different methods for making O/W emulsions, such as mechanical stirring, homogenization, and ultrasonic emulsification.[7] Emulsions prepared by these methods normally have a broad size distribution and are thermodynamically unstable, due to the tendency of spontaneous interface energy reduction and droplet coalescence. [8–10] Emulsions prepared by traditional methods do not meet the stability requirement of food products, and stabilizers are often added. Therefore, due to the importance of emulsion stability for application in the food industry, it is necessary to develop a method to prepare stable O/W emulsions. Membrane emulsification is a newly developing method for preparing monodispersed emulsions.[11] The droplet size and distribution are well controlled and scale to the pore size of the membrane. Shirasu porous glass (SPG) membrane is a special porous glass membrane, obtained by phase separation of a primary $CaO-Al_2O_3-B_2O_3-SiO_2$.[12] Its unique structure gives a narrow pore-size distribution, which is ideal for preparing emulsions with narrow droplet size distribution. Studies have been reported in using SPG membranes for producing uniform-sized O/W emulsions.[13] Most of the studies have investigated the effect of process conditions on the size and uniformity of the droplets. In the first part of this study, we examine the effect of emulsifiers on the size distribution of sunflower oil/water emulsions prepared by SPG membrane. Emulsifiers not only have important influence on the emulsification process but also affect the emulsion stability. The most commonly used emulsifiers in the food industry are amphiphilic proteins, polysaccharides, phospholipids, and small-molecule surfactants.[2,14,15] Many emulsifiers are unsuitable for preparing food emulsions because they are unstable during thermal processing. For example, globular proteins will denature when they undergo temperatures higher than the thermal denaturation temperature. For small-molecule surfactants, however, some nonionic surfactants have a cloud point, and the emulsions stabilized by this kind of emulsifier have a phase inversion temperature (PIT). In principle, emulsions stored at temperatures of 10−20 °C below the phase inversion temperature are more stable than at other temperatures.[1] As a result, we can choose suitable nonionic surfactants as emulsifiers according to the PIT and storage temperatures.

In spite of considerable amount of work carried out in the field of SPG membrane emulsification in the past decade,[16] little work has been done on emulsion stability in food industry. In the second part of the study, we investigate the uniformity and stability of sunflower emulsions prepared by the SPG membrane method. Tween 20 and Tween 60 have been chosen as emulsifiers because of their comparatively high PIT, which would improve the thermal stability of prepared emulsions. The effect of these two emulsifiers on the stability of SPG

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membrane-produced emulsions has been studied. It is demonstrated that the SPG membrane method combined with nonionic emulsifiers significantly improves the stability of prepared emulsions following heating, chilling, and freezing.

Experiments

Materials. Sunflower oil was kindly provided by Unilever Corporate Research. Tween 60 (polyoxyethylene (20) sorbitan monostearate) was purchased from Wako Chemicals and the cmc is ~27 mg·L^{-1}. Tween 20 (polyoxyethylene (20) sorbitan monolaurate) was purchased from SERVA Eletrophoresis GmbH and its cmc is ~ 60 mg·L^{-1}. Alginate and gelatin were purchased from Acros Organics. PVA was kindly provided by Kuraray Co. Ltd.

Preparation of Sunflower Oil Emulsion by SPG Membrane Emulsification. The SPG membrane (SPG Technology Co.) we employed is a tubular one with the size of o.d. 10 mm × 0.8 mm × 13 mm; it is fixed inside a module that is connected with an oil tank (Teflon tank, 10 mL) where the dispersed phase (sunflower oil) is stored. When the compressed nitrogen (ranging from 3.3 to 4.8 kPa) pressed on the oil tank, the dispersed phase was extruded into continuous phase (aqueous phase) through the pores of the membrane wall and formed uniform droplets. To prevent the droplets from aggregation, the continuous phase was stirred gently with a magnet bar in a beaker with a speed below 200 rpm. The experimental setup is referred in ref 17. In this study, the pore size of the SPG membrane is 7.0 μm.

The emulsion was prepared at room temperature, and the emulsifiers used were Tween 60 and Tween 20. The volume fraction of the oil phase was 20%. The transmembrane pressure was maintained at critical pressure ranging from 3.3 to 4.8 kPa. The experimental critical pressure was defined as that at which the emulsion droplets began to emerge from the end of the membrane pores. To improve the mechanical stability of droplets, alginate, gelatin, or PVA was chosen as an additive of water phase.

When the emulsion was prepared freshly, droplet size distribution was measured immediately. Then it was stored at room temperature for ~200 days (more than six months). The room temperature changed from 15 to 25 °C due to seasonal alternation (from spring to summer). During the storage period, the droplet size distributions were measured frequently, and the emulsions, which kept narrow size distribution after long-time storage, were chosen for further investigation of thermal stability by heating, chilling, and freezing treatments.

Preparation of Sunflower Oil Emulsions by Homogenizer. A homogenizer (T18-IKA Works) was employed to produce emulsions for comparison. It works under ordinary atmosphere and generates emulsion by the high shear force induced by the high-speed rotation of the rotor. In this paper, after predispersion (magnetic stirrer, 900 rpm, 10 min), 48 mL (8 mL oil + 40 mL aqueous) of a comparison emulsion was prepared under a rotary speed of 6000 rpm for 45 s.

Optical Micrograph of Emulsions. The visual morphology of a droplet was observed by an optical microscope (wv-CP230, Panasonic Co., Ltd.), and the pictures were captured by a CCD coupled with the microscope.

Determination of Mean Droplet Size and Droplet Size Distribution. Droplet size distribution of the prepared emulsion was measured by a laser particle analyzer (Mastersizer 2000). In order to prevent aggregation or destruction of the emulsion, an appropriate stirring speed was needed in practice. This stirring speed was determined by comparing the results from a laser particle analyzer and optical observation. When their results were consistent, the stirring speed was fixed in our experiments. It was confirmed that without the addition of thickening agent into emulsion the optimized speed was 150 rpm; when the thickening agent was added, a stirring speed of 2500 rpm was preferable.

The mean droplet diameter was expressed as the volume mean diameter, $d_{4,3}$.

$$d_{4,3} = \sum n_i d_i^4 \Big/ \sum n_i d_i^3 \tag{1}$$

where, n_i is the number of particles with diameter d_i.

The spread of the droplet size distribution was characterized by the span value defined as

$$\text{span} = (d_{90} - d_{10})/d_{50} \tag{2}$$

where d_{x0} is the diameter corresponding to $x0$ vol % on a relative cumulative droplet size distribution curve in volume and the uniformity is a measure of absolute deviation from the median.

The uniformity of the emulsion is characterized by a parameter defined as

$$U = \frac{1}{\bar{d}} \frac{\sum_i N_i d_i^3 |\bar{d} - d_i|}{\sum_i N_i d_i^3} \tag{3}$$

where $\bar{d}$ is median diameter.

Results and Discussion

Effect of Emulsifier Type and Concentrations on the Uniformity of Emulsions. Tween 20 and Tween 60 were used as emulsifiers in this study because they are nontoxic, nonirritating, and safely used in food and cosmetic emulsions. As nonionic surface-active agents, the cloud point of Tween 20 is 95 °C and Tween 60 is 76 °C, which were determined by the method in ref 18. Therefore, it is expected that Tween 20-emulsified emulsions could maintain good stability when stored below 75−85 °C while Tween 60-emulsified emulsions could be stable when stored at slightly lower temperature.

Different concentrations of Tween in the water phase at 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 wt % were used to prepare sunflower oil emulsions. The volume fraction of the dispersed oil phase is 20%. The optical micrographs of emulsions prepared with different Tween 60 concentrations are shown in Figure 1, and the corresponding particle size distribution is shown in Figure 2.

The uniformity of prepared emulsions improved with the increase of Tween 60 concentration in the aqueous phase. The possible reason was that the emulsifier content in the water phase was not enough to protect droplet stability to result in coalescence of droplets when the concentration of Tween 60 in the aqueous phase was lower than 1.0 wt %. When the concentration of Tween 60 reached to 1.5 wt %, droplets could be well protected, and therefore, the coalescence between droplets was avoided. However, when the amount of Tween 60 reached to 2.0, 3.0, and 4.0 wt %, there were many small particles detected as shown in Figure 3.

Tween 20 concentrations in the aqueous phase at 0.5, 1.0, 1.5, and 2.0 wt % were chosen to prepare sunflower oil O/W emulsions. Tween 20 concentrations higher than 2.0 wt % were not chosen because of the results obtained from the above experiments with Tween 60. The optical micrographs of emulsions with different amounts of Tween 20 are shown in Figure 4, and the corresponding particle size distributions are shown in Figure 5. There was no obvious change in the

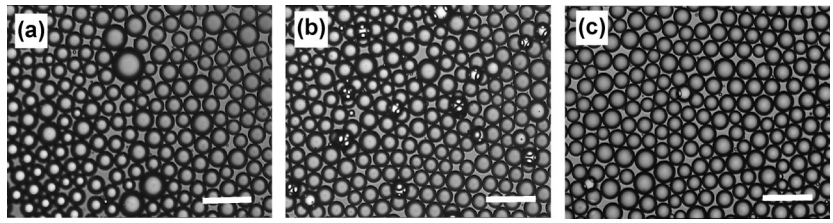

Figure 1. Optical micrographs of sunflower oil O/W emulsions prepared with different aqueous concentrations of Tween 60 at (a) 0.5, (b) 1.0, and (c) 1.5 wt %. Scale bar = 100μm.

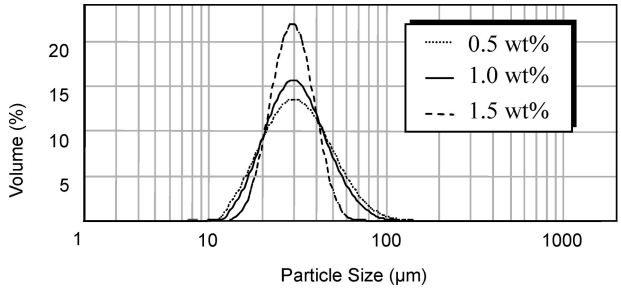

Figure 2. Size distribution of O/W emulsions prepared with different Tween 60 emulsifier concentrations in aqueous phase.

uniformity of the emulsions when the Tween 20 concentration in the aqueous phase increased.

It was evident that the emulsification ability between Tween 60 and Tween 20 was different as shown in Figure 2 and Figure 5. Tween 20 has 10 CH_2 in the hydrophobic group, while Tween 60 has 16 CH_2 in the hydrophobic group. It is possible that Tween 20 formed more compact adsorption on an oil droplet, resulting in more uniform size distribution even at a low concentration at 0.5 wt %, when compared with a higher concentration of Tween 60 at 1.5 wt %.

Storage Stability of Emulsions Prepared by Homogenizer and SPG Membrane. The uniformity and storage stability of emulsions prepared by homogenizer in this study were compared with those of emulsions prepared by SPG membrane. The recipe chosen for the emulsion is as follows: Tween 20 concentration in aqueous phase 1.0 wt % and volume fraction of dispersed oil phase 20%. The optical micrographs of freshly made emulsions by homogenizer and SPG membrane are shown in Figure 6 for comparison. The emulsion prepared by homogenizer has a much broader size distribution. The size distribution of freshly made and stored emulsions by both homogenizer and SPG membrane is shown in Figure 7. The size distribution of the homogenizer emulsion significantly broadened after only seven days of storage, suggesting either coalescence or ripening took place. Such change in the size distribution did not occur for the SPG membrane emulsion even after six-months storage.[19] Therefore, the emulsion prepared by the SPG membrane is much more stable than the emulsion prepared by the homogenizer.

To summarize, for emulsions prepared by the SPG membrane and Tween 20, the span and uniformity values were 0.674 and 0.209 with a Tween 20 concentration of 1.0 wt %. After the emulsions were stored at ambient temperature for six months, the measured values of span and uniformity were 0.678 and 0.208, indicating the emulsions were stable during storage. While for the emulsion prepared by the homogenizer and Tween 20, the span and uniformity values were 1.391 and 0.432 with the same Tween 20 concentration. After only seven days' storage, the measured values of span and uniformity were 2.818 and 0.857, indicating significant coalescence happened in homogenized emulsion.

Effect of Thickening Agent in Water Phase on Mechanical Stability. Emulsion with good mechanical stability is often required in the food industry. Comparing the optical observation with the laser particle analyzer, it was found this property was easily destroyed during measurement when the stirring speed of the analyzer was over 150 rpm, as seen in Figure 8a; more particles bigger than 100 μm were observed due to the droplets that were destroyed and aggregated, resulting from the shear force caused at the measurement stirring speed of 2500 rpm. In this study, the three substances alginate, gelatin, and PVA were used as thickening agents to improve the mechanical stability. The results at different addition concentrations of 0.10, 0.25, and 0.50 wt % are shown in Figure 8b−d. Clearly, alginate with a concentration of ~0.10 wt % could maintain the initial uniformity even under a measurement stirring speed of 2500 rpm, but the emulsion became too thick to be dispersed with the increase of concentration. Addition of gelatin can improve the mechanical stability; the concentration did not effect the size distribution under measurement stirring speed of 2500 rpm, but it needs preservation additives. When PVA was used, the emulsion not only maintained good uniformity but also good storage stability without preservation additives. PVA concentration evidently did not influence the mechanical stability. So PVA is shown to be a better thickener to improve the mechanical stability of sunflower oil/water emulsions.

Thermal Stability of the Prepared Emulsions. Many food emulsions often undergo thermal processing during their production, storage, or utilization, e.g., pasteurization, sterilization, or cooking.[3] It is required that the microstructure of prepared emulsions be capable of withstanding the thermal processes without breaking down by droplet flocculation or coalescence. In this part of the study, sunflower O/W emulsion samples are subjected to a thermal process that was more severe than the storage trial at room temperature. Samples prepared by the SPG membrane are compared with those prepared by homogenization. The aqueous concentration of emulsifiers was at 1.5 wt % for Tween 60 and 1.0 wt % for Tween 20. Freshly made samples were placed at preset isothermal temperatures (30−90 °C) for 30 min. The size distribution of the emulsion before and after heat treatment has been analyzed using Mastersizer 2000. The results are shown in Figure 9.

Figure 9 indicates that emulsions prepared by homogenization are generally thermally unstable with the mean diameter of the droplets increased with increasing temperature. This is because the much broader size distribution of emulsions by homogenization have a higher tendency toward Ostwald ripening. Increasing temperature enhances Ostwald ripening. At higher temperatures, the coalescence of droplets can also occur. Broader size distribution generally promotes droplet coalescence and aggregation. Thus, emulsions prepared by SPG membrane with uniform size distribution are much more stable when subjected to thermal treatment. The mean droplet sizes of SPG membrane-prepared emulsions hardly change when the samples are heated to 80 °C. Above 80 °C, the SPG emulsions stabilized by Tween

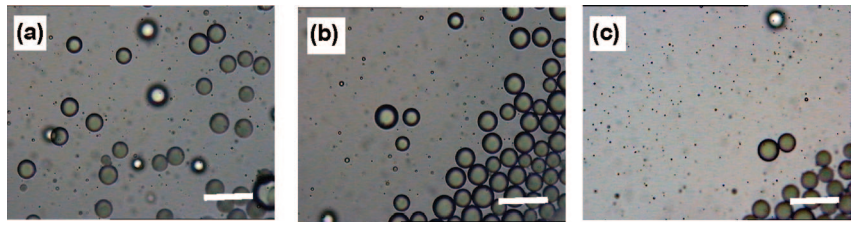

Figure 3. Optical micrograph of O/W emulsions prepared with Tween 60 emulsifier concentration of (a) 2.0, (b) 3.0, and (c) 4.0 wt %. Scale bar, 100 μm.

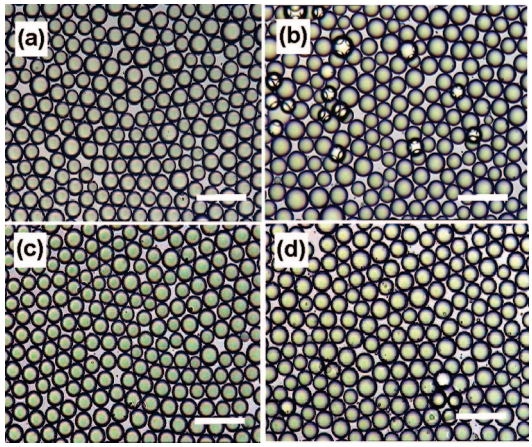

Figure 4. Optical micrographs of O/W emulsions prepared with different Tween 20 emulsifier concentrations: (a) 0.5, (b) 1.0, (c) 1.5, and (d) 2.0 wt %. Scale bar, 100 μm.

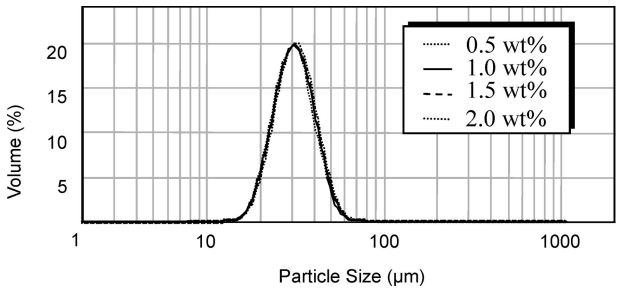

Figure 5. Size distribution of O/W emulsions prepared with different Tween 20 emulsifier concentrations.

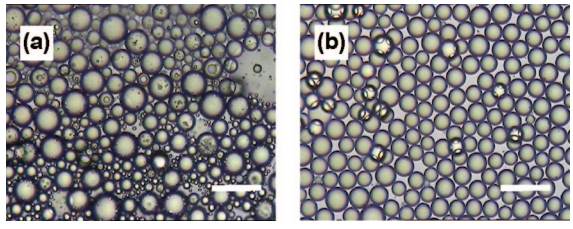

Figure 6. Optical micrographs of freshly made sunflower O/W emulsions by homogenizer (a) and SPG membrane (b). Scale bar, 100 μm.

20 are still stable, but those stabilized by Tween 60 become thermally unstable with mean droplet size increased. For Tween 60, this is the temperature above the cloudy point and droplet coalescence was observed. It was clear that different emulsifiers had different thermal stabilities. The uniformity of emulsions prepared by the SPG membrane emulsification technique can maintain the thermal stability up to the cloudy temperature of the emulsifier.

Chilling and Freezing Stability. Food emulsions are chilled or frozen during storage and then warmed prior to use, for example, dairy products, desserts, sauces, and ice cream.[3] Cold

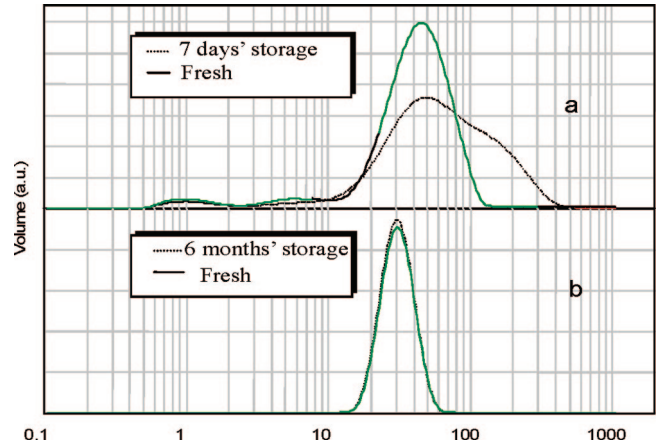

Figure 7. Particle size distribution of freshly made emulsions and aged emulsions. Effect of preparation methods: (a) homogenizer; (b) SPG membrane.

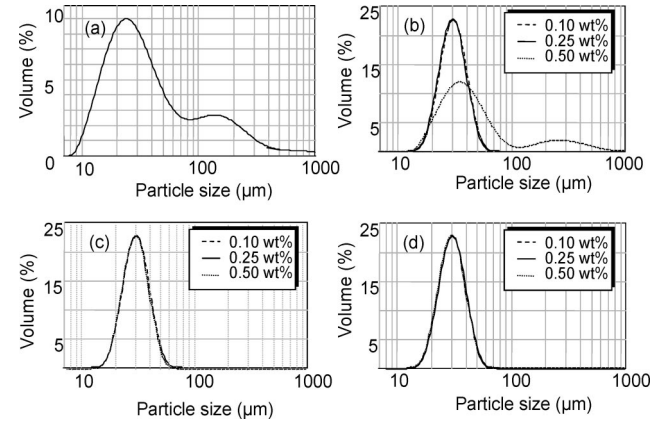

Figure 8. The particle size distribution with various additives at different concentrations: (a) blank, (b) alginate, (c) gelatin, and (d) PVA.

storage is often used to protect product quality by retarding microbial growth and undesirable chemical reactions, such as lipid oxidation. However, many oil-in-water emulsions become physically unstable when they are chilled or frozen and rapidly break down after reheating. Therefore, it is important to improve the stability of food emulsions under chilling, freezing, and thawing conditions.

A variety of different physicochemical processes may occur when an oil-in-water emulsion is cooled, which will promote emulsion instability. The main processes include fat crystallization, ice formation, freeze concentration, and interfacial phase transition. Several mechanisms may also interact to affect the instability of emulsions during freezing.[20,21] At present, there is still a lack of understanding of which mechanism is relatively important. It has been suggested that freezing causes droplet interfaces to rupture, causing some oil-to-oil contact.

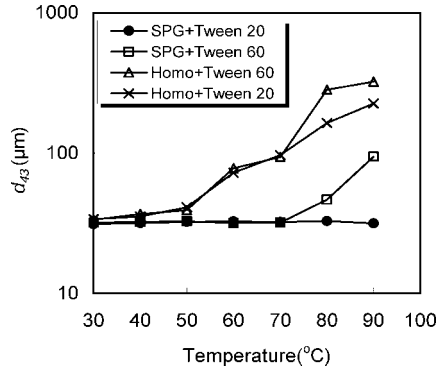

Figure 9. Effect of isothermal heat treatment on the mean particle diameter (d_{43}) of sunflower O/W emulsions prepared by SPG membrane and homogenizer. Concentrations of emulsifiers in the aqueous phase were 1.5 wt % for Tween 60 and 1.0 wt % for Tween 20.

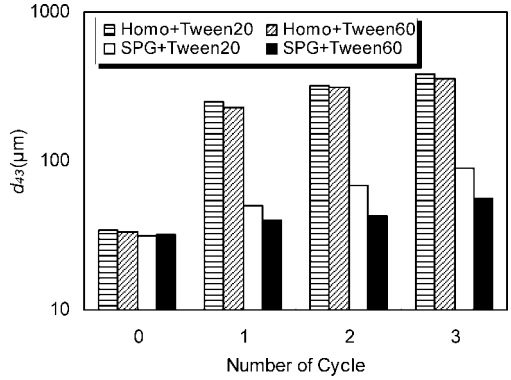

Figure 10. Dependence of mean particle diameter (d_{43}) of emulsions prepared by SPG and homogenizer on numbers of freeze−thaw cycles.

In this part of the study, emulsion samples prepared by the SPG membrane and homogenizer were subjected to freeze−thaw cycles that involved storage at −10 °C for 22 h, followed by thawing at 30 °C for 2 h. This freeze−thaw cycle was repeated three times. The size distribution of the emulsion samples was measured after each cycle. The results are shown in Figure 10.

The results indicate that the SPG membrane-prepared emulsions with a good uniform size distribution significantly improved the stability of sunflower oil-in-water emulsions when subjected to chilling and freezing cycles. According to the study by Ghosh and Coupland,[22] lipid composition influences the stability of emulsions when subjected to the freezing and thawing process. They found that emulsions with droplets remaining at liquid state were more stable than the emulsions whose droplets were in the crystalline state. Under the pressure of ice, liquid droplets can deform to some extent under the freezing stress of ice while crystalline droplets had to withstand the freezing stress and squeezing the surfactant out of the intervening gap. The melting point of sunflower oil is −17 °C. When SPG membrane-prepared sunflower O/W emulsions were cooled to −10 °C, the crystalline droplets experienced more homogeneous stress around them because of the good uniformity. Every droplet had the same tendency of deformation under the ice pressure. The droplet structure is more stable. For emulsions prepared by homogenization, droplets were not uniform. When they were subjected to the ice pressure, smaller droplets could penetrate into bigger droplets and disrupt their interfacial membranes. As a result, in the process of thawing, the uniform-sized emulsions could retain stability while non-uniform-sized droplets tended to coalesce by interfacial rupture.

Figure 10 also indicates that the emulsions stabilized by Tween 60 were more stable than those stabilized by Tween 20. The reason was probably that Tween 60 are longer chained molecules than Tween 20. It is expected that longer chained large molecules can not be easily squeezed out of the interface by stresses like ice pressure.

Conclusions

Sunflower oil emulsions prepared by SPG membrane exhibit much improved uniformity and stability compared to emulsions prepared by homogenization. Sunflower O/W emulsions prepared by SPG membrane are stable after six-months storage, while those prepared by homogenizer exhibited significant size broadening by coalescence and ripening after 7 days of storage. Uniform-sized sunflower O/W emulsions prepared by SPG membrane also have good thermal stability when subjected to either freezing or heating. Emulsions stabilized by Tween 20 are generally more uniform and stable compared to those stabilized by Tween 60. The mechanical stability of sunflower O/W emulsions can be further improved by adding thickeners to the aqueous phase. Future study will be carried out to investigate the influence of other environmental stresses, such as pH and ionic strength on the uniformity and stability of O/W emulsions prepared by the SPG membrane. Nevertheless, SPG membrane emulsification is a promising process for producing uniform-sized emulsions and offers great potential for improving the shelf life and quality of fabricated food products.

Acknowledgment

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