



Incorporation of soybean oil improves the dilutability of essential oil microemulsions

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ABSTRACT

Plant essential oils (EOs) have strong antimicrobial and antioxidant activities. However, their water insolubility and volatility limit their practical application. Microemulsions are thermodynamically stable delivery systems for hydrophobic bioactive compounds but can be destabilized after dilution by the polar phase. In the present study, soybean oil (SBO) was studied for the impacts on formation and dilutability of EO microemulsions comprised of polysorbate 80 (Tween™ 80) as a surfactant and equal mass of water and propylene glycol as the polar phase. The oil phase contained EO (cinnamon bark oil, eugenol, or thymol) and SBO at 1:0, 2:1 or 4:1 mass ratios. Pseudo-ternary phase diagrams were constructed after titrating the polar phase into Tween™ 80–oil mixture at 1:1 to 9:1 mass ratios. Blending SBO with EO expanded the regimes of microemulsions and reduced the droplet dimensions that were stable over 90 days. Viscosity and electrical conductivity data indicated the transition from W/O to O/W microemulsions as the content of polar phase increased from 10% to 90% w/w. The enhanced dilutability of microemulsions after blending with SBO can broaden the application of EOs.

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1. Introduction

Essential oils (EOs) are secondary metabolites from plants and are known for their strong flavor and antioxidant and antimicrobial properties (Burt, 2004; Kim, Marshall, & Wei, 1995). Many EOs or their components (EOCs), such as cinnamon oil, eugenol (major EOC in clove bud oil), and thyme oil, have been approved by the United States Food and Drug Administration as generally recognized as safe (GRAS) food additives (FDA, 2013). EOs have gained growing attention in recent years for use as natural preservatives to improve the microbiological quality and safety of food products. However, direct application of EOs in food systems is limited by the poor water solubility and the volatile nature of these bioactive compounds.

Various colloidal systems can be used to incorporate hydrophobic compounds in aqueous systems, including emulsions and microemulsions. Microemulsions are thermodynamically stable and isotropic mixtures of water, oil, and surfactants, and co-surfactants are used to enhance the loading of dispersed phase (Danielsson & Lindman, 1981). The dimension of dispersed phase is typically below 100 nm that corresponds to the transparent appearance of microemulsions (Moulik & Paul, 1998). Microemulsions are usually formed by spontaneous self-assembly of components under appropriate compositions and are

easily prepared under mild mechanical mixing (Garti, Yaghmur, Leser, Clement, & Watzke, 2001). With these unique features, microemulsions have been studied to dissolve hydrophobic compounds (Weiss & McClements, 2000) for food (Garti, Clement, Fanun, & Leser, 2000; Rao & McClements, 2011), pharmaceutical (Gupta et al., 2005), cosmetic (Boonme, 2007; Linn & West, 1989), and oil recovery applications (Santanna et al., 2009). Recent studies have shown the potential application of EO microemulsions as antimicrobial preservatives to improve the microbiological safety (Zhang, Critzer, Michael Davidson, & Zhong, 2014) and quality (Wang et al., 2014) of fresh produce and as antioxidants to improve food quality (Hamed, Sadek, & Edris, 2011).

However, many microemulsion systems are formed at limited combinations of their constituents. Ideally, microemulsions can be prepared with surfactant and a high level of oil as stock solutions and the dilution with water to any oil concentration before application still produces transparent and stable microemulsions. These fully dilutable microemulsions can reduce the cost of transportation and storage which are important for practical food applications.

Garti et al. (2000) studied a class of microemulsions consisting of sucrose monostearate as a surfactant, medium-chain triglycerides (MCTs) as the oil phase, and alcohols such as pentanol as co-surfactants. They demonstrated that the maximum water content was only 15.3% w/w when the mass ratio of pentanol:MCTs was 1:2 and increasing the mass ratios to 1:1 and 3:1 increased the maximum water content to 21.5 and 27.5% w/w, respectively. The water dilutability of these microemulsion systems was thus limited. Rao and McClements (2011) found that the turbidity increased when a microemulsion system composed of lemon

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oil and polyoxyethylene-20-sorbitan monooleate (Tween™ 80) was diluted with water and elaborated the limitation in practical application. The addition of propylene glycol (PG) has been reported to significantly enlarge the monophasic (1-phase) microemulsion region of *R*(+)-limonene/ethanol/polyoxyethylene (10) oleyl alcohol (Brij 96v) mixture after dilution with a polar phase of water and PG at 1:0, 2:1, and 1:1 mass ratios. In addition, organic acids such as acetic acid and propionic acid (Spernath, Aserin, & Garti, 2006), temperature, and chemistry of surfactants and oils (Paul & Mitra, 2005; Warisnoicharoen, Lansley, & Lawrence, 2000) affect the dilutability of microemulsions greatly. Currently, there are scarce alcohol-free food grade EO microemulsions that are fully dilutable.

To reduce the loss of volatile EOs during shelf storage, blending long-chain triacylglycerols such as those of soybean oil (SBO) with EOs (Kim, Wu, Kubota, & Kobayashi, 1995) is a potential strategy. The possibility of forming microemulsions with SBO and EO mixture as the oil phase has not yet been studied. The dilutability of these systems is also to be characterized.

Therefore, the first objective of this study was to prepare fully-dilutable microemulsions of EOs/EOCs including cinnamon bark oil (CBO), eugenol, and thymol. The second objective was to study the influence of SBO on the phase behavior of microemulsions. Pseudo-ternary phase diagrams were used to present 1-phase region when mixtures with different mass ratios of surfactant and oil phase were diluted by a polar phase with equal mass of water and PG. The third objective was to partially characterize physical properties of microemulsions including the dimension of oil droplets, viscosity, stability, and electrical conductivity.

2. Materials and methods

2.1. Materials

Thymol (99% purity) was from Acros Organics (Morris Plains, NJ). Eugenol and CBO were purchased from Sigma-Aldrich Corp. (St. Louis, MO). PG, SBO, and Tween™ 80 were procured from Thermo Fisher Scientific Inc. (Waltham, MA).

2.2. Preparation of microemulsions by the polar phase titration method

Surfactant (Tween™ 80) and oil phase were mixed at a mass ratio from 1:1 to 9:1, with the oil phase comprised of EO/EOC (CBO, eugenol or thymol) and SBO at a mass ratio of 1:0, 2:1, or 4:1. The polar phase with equal mass of water and PG was added into the oil/surfactant mixture to obtain a final concentration from 10 to 90% w/w. After magnetic stirring till no visual change in turbidity, the mixtures were placed on the bench for at least 24 h equilibrium at room temperature (21 °C) and the sample appearance was recorded by photographing. Transparent samples after incubation and confirmation by cross-polarized light microscopy as being isotropic were treated as microemulsions. The compositions corresponding to microemulsion formations were plotted in pseudo-ternary phase diagrams. Microemulsions remaining transparent and isotropic after diluting the oil/surfactant mixture with 90% w/w of polar phase were considered as fully-dilutable. Additionally, surfactant efficiency (Q_m , Eq. (1)) was calculated as a parameter indicating the dilution properties (Mehta, Kaur, Mutneja, & Bhasin, 2009).

$$Q_m = [W]/([W] + [S]) \quad (1)$$

where $[W]$ and $[S]$ are the respective mass concentrations of polar phase and surfactant at the failure point upon dilution, i.e., when the system becomes turbid or phase-separated (Mehta et al., 2009). A larger magnitude of Q_m indicates the better capability of the mixture to contain a larger amount of polar phase as microemulsions. The highest Q_m in the present study was 90%, for the fully-dilutable microemulsions that remained stable after dilution with 90% of the polar phase.

2.3. Droplet dimension measured by dynamic light scattering (DLS)

To characterize the stability of microemulsions, hydrodynamic diameters (D_h) of microemulsions were measured during a 90-day storage at 21 °C. The DLS instrument was a Delsa Nano analyzer (Beckman Coulter, Atlanta, GA) with a scattering angle of 165°. All samples were diluted with deionized water to the instrument sensitivity range, and experiments were repeated for triplicate samples, each tested twice.

2.4. Electrical conductivity of microemulsions

Electrical conductivity was measured using a YSI 3200 conductivity meter (Yellow Spring, OH) for the oil phase composed of EO/EOC and SBO at mass ratios of 1:0, 2:1 and 4:1. Changes in conductivity were tested as the polar phase with equal mass of water and PG was titrated from 10 to 90% w/w into the Tween™ 80 and oil phase mixture at a 9:1 mass ratio. This mass ratio was chosen because it allowed the incorporation of the highest amount (≥ 80) of polar phase for each EO/EOC. At least 30 g of a sample was used in each measurement, and each sample was tested in triplicate.

2.5. Viscosity of microemulsions

As in the electrical conductivity measurements, the viscosities of microemulsions prepared at a Tween™ 80:oil mass ratio of 9:1 were measured using an AR2000 rheometer (TA Instruments, Inc., New Castle, DE). About 14 mL of each sample was loaded into the concentric cylinder geometry at the Searle setup, where the outer diameter of bob was 28 mm and the cup inner diameter was 30 mm. Stress sweep tests were conducted at 25 °C at a stress range of 1–100 Pa. Each test was repeated at least once.

2.6. Statistical analysis

The D_h data of microemulsions were analyzed by one way ANOVA Tukey test using SPSS 20 software (IBM, Armonk, NY) at a 5% significance level.

3. Results and discussion

3.1. Microemulsion formation and effects of dilution

3.1.1. Effect of polar phase composition

To test the effect of PG on the formation of microemulsions, mixtures of Tween™ 80 and thymol at mass ratios of 4:1, 5:1, and 6:1, and polar phase consisting of water only or water–PG mixtures at mass ratios of 1:1 and 2:1 were investigated. As shown in Fig. 1, when the polar phase was water or water–PG at a mass ratio of 2:1, none of the mixtures was transparent at all three Tween™ 80:thymol mass ratios. Conversely, equal mass of water and PG enabled the formation of microemulsions at Tween™ 80:thymol mass ratio of 6:1. Thus, increasing PG content in the polar phase facilitated the formation of microemulsion of thymol. Equal mass of water and PG was chosen as the polar phase to further prepare fully-dilutable microemulsions of EOs/EOCs.

3.1.2. Partial phase diagrams for microemulsion regimes

To prepare fully-dilutable microemulsions and study the effect of SBO, the polar phase with equal mass of water and PG was titrated at 10–90% w/w into the mixture of Tween™ 80 and oil phase with various mass ratios of EO/EOC:SBO. An example is shown in Fig. 2 for the oil phase with thymol and SBO at mass ratios of 1:0 and 4:1, which was mixed with Tween™ 80 at a Tween™ 80:oil mass ratio from 4:1 to 9:1. Without SBO (Fig. 2 left), samples with a Tween™ 80:oil mass ratio from 4:1 to 8:1 became turbid when diluted with a sufficient amount of the polar phase. In contrast, at a thymol:SBO mass ratio of

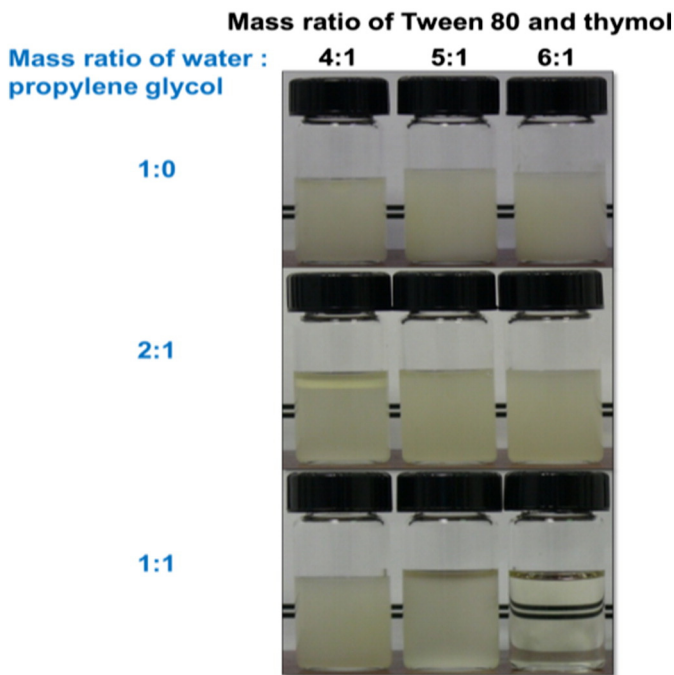


Fig. 1. Appearance of mixtures containing an oil phase of thymol, 60% w/w polar phase with water–propylene glycol at mass ratios of 1:0 (top), 2:1 (middle) or 1:1 (bottom), and Tween™ 80 used at different mass ratios to thymol.

4:1, fully-dilutable microemulsions were observed at a Tween™ 80:oil mass ratio from 7:1 to 9:1 (Fig. 2 right).

Pseudo-ternary phase diagrams are shown in Fig. 3 for formulations corresponding to the formation of microemulsions. Without SBO, microemulsions of CBO, thymol, and eugenol were fully-dilutable at the surfactant:oil mass ratio of $\geq 4:1$, $9:1$, and $\geq 3:1$, respectively. At the 4:1 mass ratio of EO/EOC:SBO, fully-dilutable microemulsions of CBO, thymol, and eugenol were observed at surfactant:oil mass ratios of $\geq 4:1$, $\geq 7:1$, and $\geq 2:1$, respectively. At the EO/EOC:SBO mass ratio

of 2:1, fully-dilutable microemulsions were only observed for thymol at a surfactant:oil mass ratio of $\geq 7:1$, while microemulsions of CBO and eugenol can only be prepared with up to 80% polar phase at a surfactant:oil mass ratio of $\geq 8:1$ and $\geq 3:1$, respectively. Therefore, under the studied conditions, fully-dilutable microemulsions of CBO, thymol and eugenol can be successfully prepared by titrating the polar phase with equal mass of water and PG into the oil–surfactant mixture. Furthermore, the use of SBO at 4:1 mass ratio of EO:SBO reduced the amount of surfactants needed to formulate fully-dilutable microemulsions of thymol and eugenol, while this improvement was only observed for microemulsions with thymol at the EO:SBO mass ratio of 2:1. For dilution lines that did not reach 90% mass of polar phase, the addition of SBO increased the maximum amount of polar phase in microemulsions (Q_m), further discussed below. Overall, microemulsion dilutability can be enhanced after blending a certain amount of SBO, but the extent of enhancement is a function of EO chemistry.

3.1.3. Maximum amount of polar phase incorporated in microemulsions (Q_m)

The Q_m is additionally plotted in Fig. 4 as an indicator of the maximum amount of polar phase that can be added in microemulsions at different mass ratios of surfactant and oil (Eq. (1)). For the convenience of description, microemulsions with the oil phase consisting of 1:0, 2:1 and 4:1 mass ratios of CBO and SBO were abbreviated as C1:0, C2:1 and C4:1, respectively, and the abbreviation letter was substituted for E and T for respective microemulsions containing eugenol and thymol. Q_m of C4:1 was much higher than that of C1:0 and C2:1 at a surfactant and oil phase mass ratio from 1:1 to 3:1. At a surfactant:oil phase mass ratio from 4:1 to 9:1, Q_m of C2:1 was much lower than that of C1:0 and C4:1.

For microemulsions of eugenol, Q_m of E4:1 (fully-dilutable) was much higher than that of E2:1 and E1:0 at 2:1 mass ratio of Tween™ 80:oil, while at 3:1 to 9:1 mass ratios of surfactant and oil, Q_m of E2:1 was the lower than those of fully-dilutable E1:0 and E4:1. However, for microemulsions of thymol, Q_m of T2:1 was much higher than T1:0 and T4:1 at a Tween™ 80 and oil phase mass ratio from 2:1 to 5:1. Therefore, the structure of EOs/EOCs and their compatibility with SBO impact the dilutability of surfactant/oil mixtures.

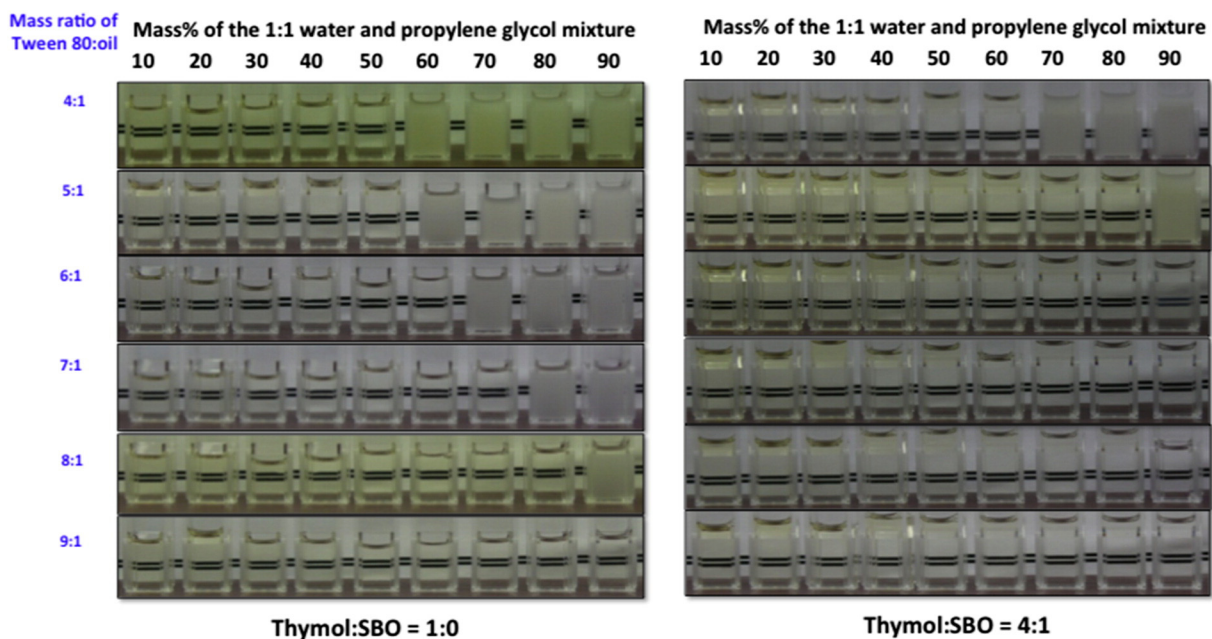


Fig. 2. Appearance of mixtures containing a polar phase with equal mass of water and propylene glycol, an oil phase with thymol and soybean oil (SBO) at a mass ratio of 1:0 (left) or 4:1 (right), and Tween™ 80 used at different mass ratios to the oil phase.

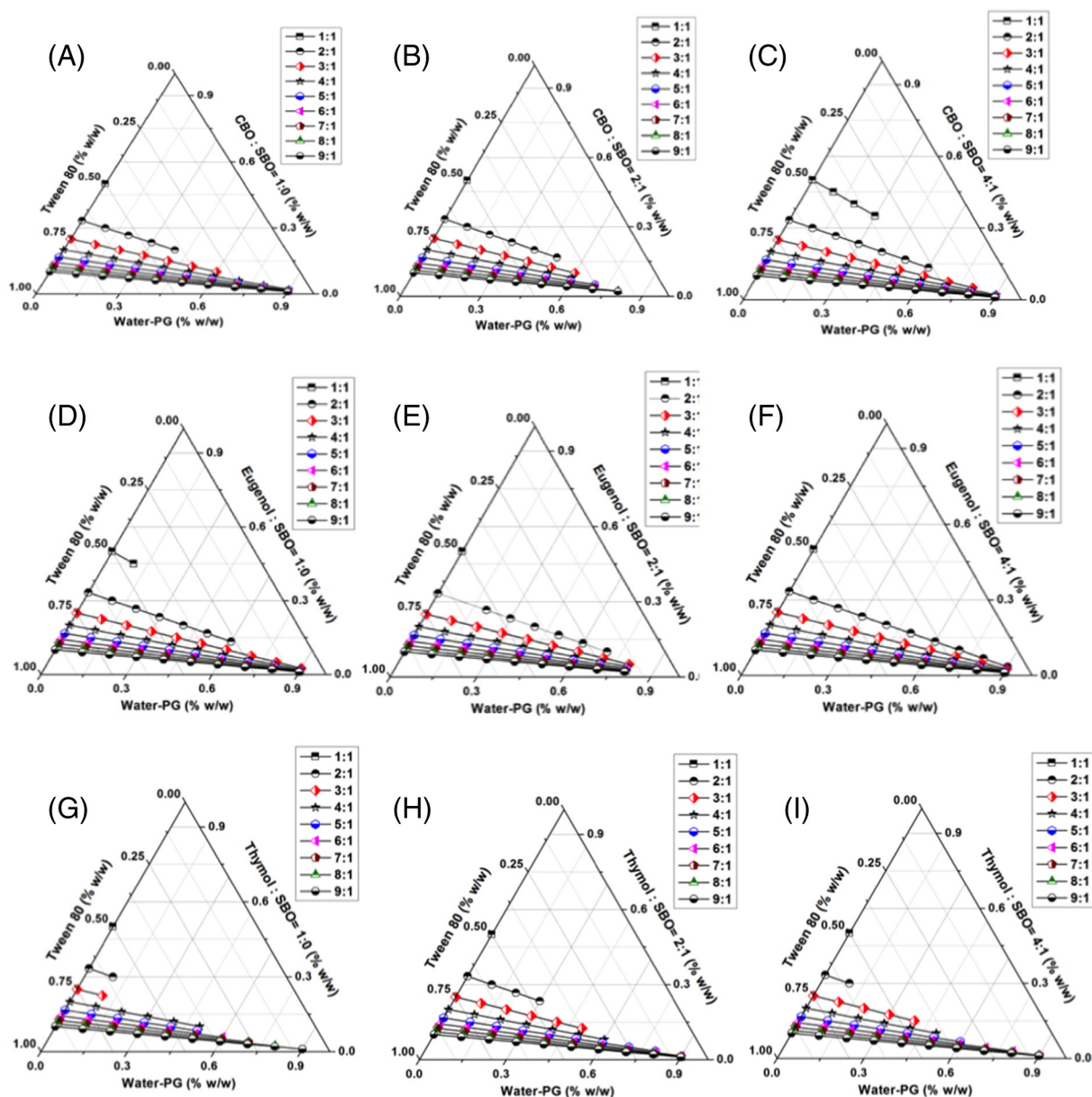


Fig. 3. Pseudo-ternary phase diagrams at 21 °C showing formulations corresponding to transparent microemulsions with the oil phase prepared with essential oil and soybean oil (SBO) at 1:0, 2:1, and 4:1 mass ratios and the polar phase formulated with equal mass of water and propylene glycol (PG).

3.1.4. Possible effects of SBO on microemulsion formation

The phase behavior of a surfactant–oil–water mixture is influenced by factors such as temperature, and chemistry and quantity of surfactant, oil, and co-surfactant (Aboofazeli, Patel, Thomas, & Lawrence, 1995; Paul & Mitra, 2005; Warisnoichareon et al., 2000). Interpretation of the enhanced dilutability of microemulsions after adding SBO may not be straightforward. The compatibility of surfactant and lipid structures is important to the formation of microemulsions. Warisnoichareon et al. (2000) found that surfactants with a longer unsaturated alkyl chain ($C_{18:1}E_{10}$) dissolved a larger amount of SBO (molecular volume = 1592 \AA^3) than oils with shorter-chain glycerides, such as Miglyol 812 (molecular volume = 1019 \AA^3) and tributyrin (molecular volume = 486 \AA^3). From this perspective, the addition of SBO increases the overall chain length of oil phase and therefore the compatibility with the oleate moiety of TweenTM 80 (Bayrak, 2004; Digout, Bren, Palepu, & Moulik, 2001). This increased compatibility agrees with the improvement in dilutability of surfactant and oil mixtures after incorporation of SBO (Figs. 3 and 4).

Different trends in the phase behaviors after the addition of SBO (Fig. 3) also emphasize the significant impact of EOC structures. This can be interpreted by their difference in hydrophobicity. SBO contains long chain triacylglycerols and is more hydrophobic than EO/EOC used in this study. Thymol is more hydrophobic than eugenol and cinnamaldehyde, the major component in CBO (Chen, Davidson, & Zhong, 2014). The good compatibility between thymol and SBO may have resulted in the favored formation and dilutability of microemulsions after the addition of a larger amount of SBO (Figs. 3 and 4). The opposite can be the case for more polar CBO and eugenol that are less compatible with SBO, resulting in the reduced dilutability at an excess amount of SBO (at a surfactant:oil mass ratio of 2:1).

3.1.5. Microemulsion structure studied by polarized light microscopy

No liquid crystalline region along the dilution lines in Fig. 3 was observed based on cross-polarized light microscopy in the present study (results not shown). Mixtures with a large amount of surfactants typically demonstrate an anisotropic liquid crystalline phase (Borkovec,

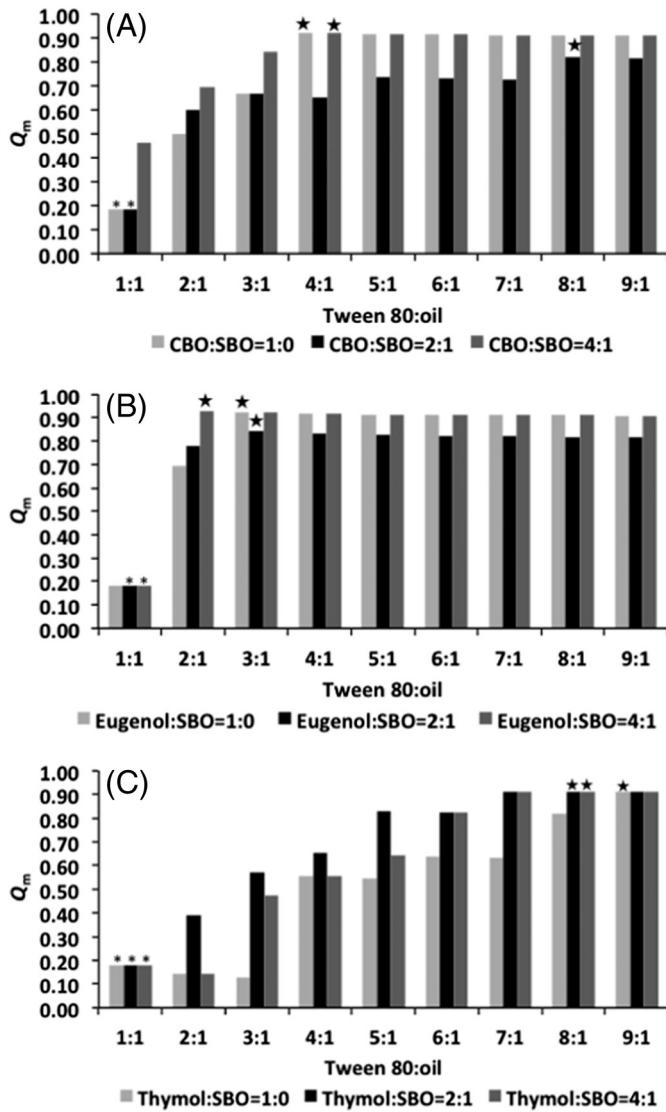


Fig. 4. Surfactant efficiency (Q_m) of TweenTM 80 used to formulate microemulsions with the oil phase being (A) cinnamon bark oil (CBO), (B) eugenol, or (C) thymol blended with soybean oil (SBO) at EO:SBO mass ratios of 1:0, 2:1, and 4:1. Asterisks above bars indicate that the sample was turbid at 10% of polar phase or Q_m was smaller than the value in the figure. The highest Q_m of each EO at a specific EO:SBO mass ratio is marked with a "★".

Eicke, Hammerich, & Das Gupta, 1988) due to the ordering of surfactant molecules (Tiddy, 1980). For instance, a liquid crystalline region of TweenTM 80 was observed at a concentration between 50% and 62% (Friberg, Huang, & Aikens, 1997). The reason for the absence of liquid crystalline region in the present study may be the polar phase being equal mass mixture of water and PG, as reported for the significant decrease of the liquid crystalline phase region after adding PG into the water phase (Edris & Malone, 2011; Yagmur, Aserin, Antalek, & Garti, 2003). The decreased polarity after mixing water with a fully miscible compound such as PG lowers the interfacial tension between oil and polar phases and reduces the attraction between head groups of surfactants, which can result in the diminished liquid crystalline region in the polar phase (Backlund, Bergenstahl, Molander, & Wärnheim, 1989; Wärnheim & Jönsson, 1988). Additionally, the polarity decrease in the polar phase enhances the penetration of polar organic compounds such as EOCs into the interfacial region, which increases the disordering of surfactant tails and thus reduces the liquid crystal formation (Friberg, Liang, Liang, Greene, & Van Gilder, 1986).

3.2. Hydrodynamic diameter (D_h) and storage stability of microemulsions

The dimension of microemulsions with the highest Q_m for each EO at each EO:SBO mass ratio (Fig. 4) was measured over 90 days during ambient storage to study the stability of microemulsions. As shown in Fig. 5A, the D_h of T2:1 sample was about 14 nm, and those of T1:0 and T4:1 samples were similar ($p > 0.05$), about 15 nm. For eugenol treatments (Fig. 5B), the D_h of E1:0, E2:1, and E4:1 samples was about 22, 12 and 15 nm, respectively. Microemulsions with CBO had D_h of about 22, 12, and 14 nm for C1:0, C2:1, and C4:1, respectively (Fig. 5C). The results showed that all samples had stable D_h within 90 days, which indicated that microemulsions were stable. Overall, microemulsions with a larger amount of SBO had a significantly smaller D_h ($p < 0.05$). Jumaa

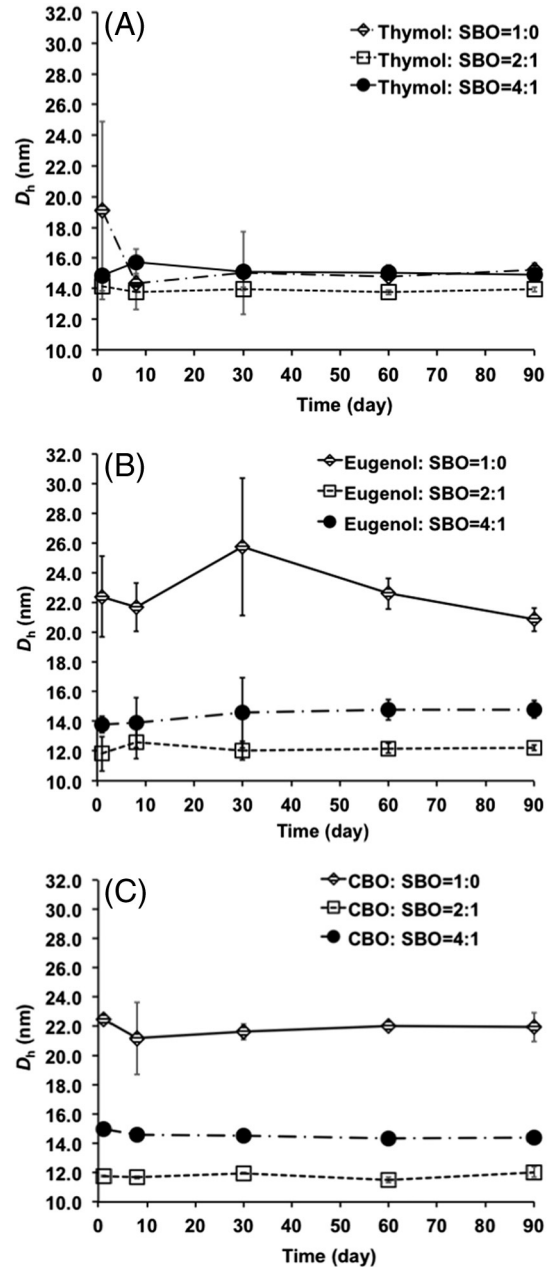


Fig. 5. Average hydrodynamic diameters (D_h) of microemulsions with the highest Q_m for each EO at a specific EO:SBO mass ratio (labeled with "★" in Fig. 4) during storage at 21 °C. Microemulsions were formulated with the oil phase prepared with (A) thymol, (B) eugenol, or (C) cinnamon bark oil (CBO) blended with soybean oil (SBO) at 1:0, 2:1, and 4:1 mass ratios and the polar phase with equal mass of water and propylene glycol. Error bars are standard deviations from six measurements.

and Müller (1998) also reported that castor oil emulsions with a higher amount of MCTs in the oil phase had smaller particles. As discussed previously, the addition of SBO increases the hydrophobicity of oil phase and the compatibility with Tween™ 80, which can lower the interfacial tension and reduce the droplet dimension (Bayrak, 2004; Digout et al., 2001).

3.3. Electrical conductivity of microemulsions after titration by the polar phase

Electrical conductivity was measured in the present study because it indicates the composition, e.g., water content of the continuous phase in microemulsions and unveils structural changes upon dilution by the polar phase (Chatenay, Urbach, Cazabat, & Langevin, 1985; Clause, Peyrelasse, Heil, Boned, & Lagourette, 1981; Eicke, Borkovec, & Das-Gupta, 1989; Lagourette, Peyrelasse, Boned, & Clause, 1979). Fig. 6 shows the results from microemulsions with a Tween™ 80:oil phase mass ratio of 9:1 during dilution with the polar phase. At a low content of the polar phase, the conductivity was low, which can be due to the existence of a large amount of non-ionic Tween™ 80, water-binding effect of PG molecules (Yaghmur, Aserin, Tiunova, & Garti, 2002), and a small quantity of free water molecules. The electrical conductivity increased with an increase in the polar phase content until about 70% w/w, followed by the reductions.

The rate of conductivity increase appeared to be higher at a polar phase content of 30–50% w/w. Conductivity is low at a low water content, and its sharp increase during titration with the polar phase indicates the percolation transition (Eicke et al., 1989). The conductivity data suggest the occurrence of percolation at about 30% w/w of polar phase for most microemulsions in the present study and the transition of W/O microemulsions to bicontinuous structures at about 50% w/w polar phase. After further dilution to more than 70% of the continuous phase, the bicontinuous structures transit to O/W microemulsions with dispersed lipid droplets, corresponding to the reduction of conductivity. Similar results were reported by Clause et al. (1981) for a microemulsion system consisting of water, sodium dodecyl sulfate, 2-methyl-2-butanol and benzene that showed the transition from W/O to bicontinuous microemulsions at a water volume fraction of 0.41 and to O/W microemulsions at a water volume fraction of 0.60 that had the maximal conductivity. Another recent study reported such transitions when the water phase increased to 35% and 65% in a microemulsion system consisting of glycerol monolaurate, propionic acid, Tween™ 80, and water (Zhang, Taxipalati, Que, & Feng, 2013).

3.4. Viscosity of microemulsions

Viscosity is another important parameter to study the structure of microemulsions. Viscosity can be used to study phase inversion from W/O to O/W microemulsions during titration with water (Paul & Moulik, 1997) and can be correlated to interactions between droplets (Saidi, Mathew, Peyrelasse, & Boned, 1990). In this study, viscosities were measured for microemulsions prepared with CBO, thymol and eugenol at a Tween™ 80:oil phase mass ratio of 9:1. All microemulsions demonstrated a linear correlation between shear stress and shear rate (not shown) and an intercept of zero and were therefore Newtonian fluids. The Newtonian viscosities of microemulsions with the polar phase content increasing from 10% to 90% w/v are plotted in Fig. 7. Microemulsions of thymol showed a monotonic decrease in viscosity as the polar phase content increased, and the viscosity decrease was more dramatic when the polar phase content was over 50%. Conversely, microemulsions of CBO and eugenol showed the highest viscosity at an intermediate concentration of the polar phase that was about 50% w/w for most microemulsions, followed by sharp decreases in viscosity at higher polar phase contents.

The presence of a viscosity maximum during titration is an indication of the formation of bicontinuous structures (Georges & Chen,

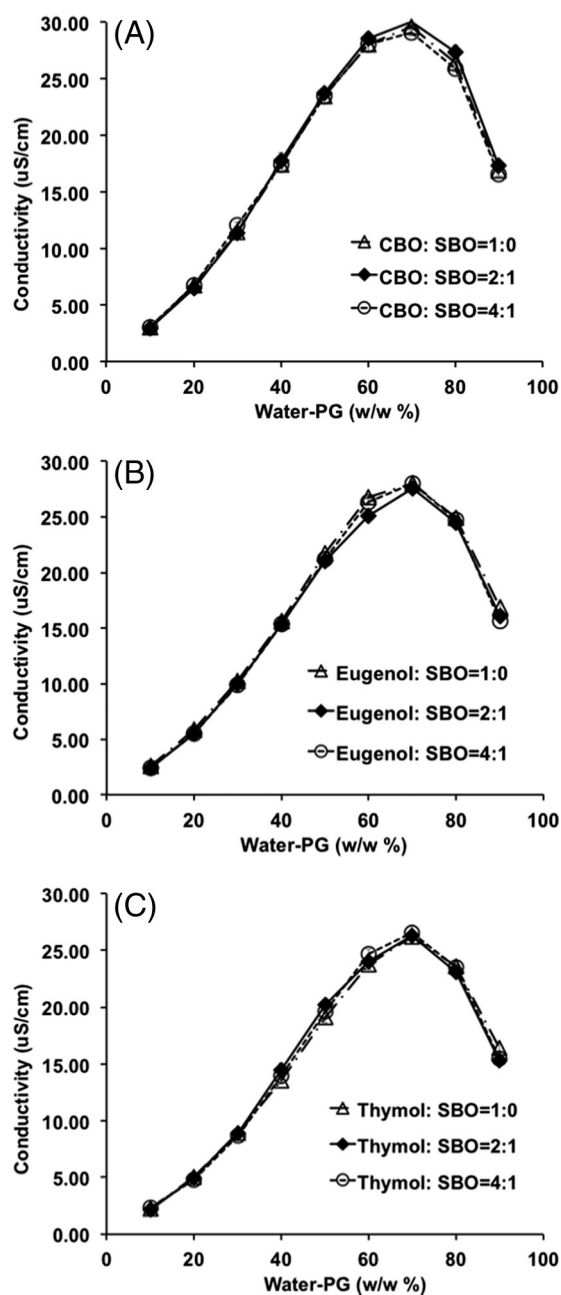


Fig. 6. Electrical conductivity of microemulsions at 21 °C prepared by diluting a mixture of Tween™ 80 and oil phase at 9:1 mass ratio with a polar phase with equal mass of water and PG. The oil phase was formulated with (A) cinnamon bark oil (CBO), (B) eugenol, or (C) thymol blended with soybean oil (SBO) at EO:SBO mass ratios of 1:0, 2:1, and 4:1. Error bars are standard deviations from three measurements.

1986), and the subsequent decrease in viscosity after further addition of polar phase is due to the decrease of particle size (Yaghmur et al., 2003) and transition to O/W microemulsions. The increase of viscosity before reaching 50% w/w polar phase can be caused by the strengthened interaction between dispersed domains of the polar phase (Garti et al., 2001). The bicontinuous structures at ~50% w/w polar phase had the dispersed phase being completely connected, and the further increase of polar phase content led to the decreased continuity of structures and thus viscosity. Upon titration to above 70% w/w of polar phase, the rate of viscosity decrease became smaller, which indicates the complete transition from bicontinuous to O/W microemulsions (Garti et al., 2001). The viscosity data (Fig. 7) therefore generally agreed with conductivity results (Fig. 6). Changes in viscosity along a dilution

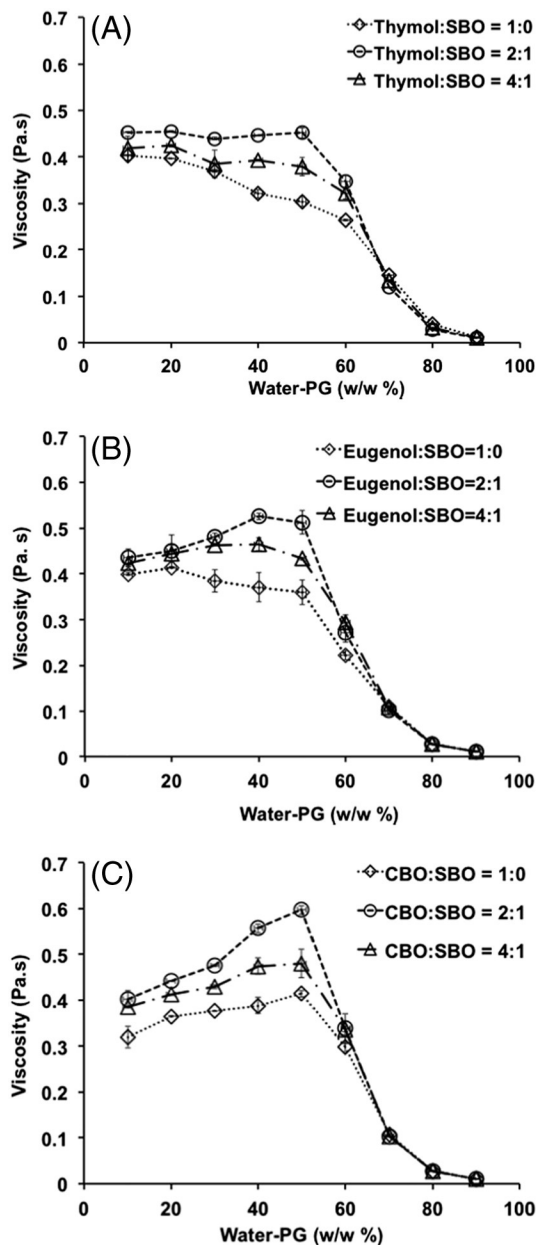


Fig. 7. Newtonian viscosities of transparent microemulsions at 20 °C. Microemulsions were formulated with the oil phase prepared with (A) thymol, (B) eugenol, or (C) cinnamon bark oil (CBO) and soybean oil (SBO) at EO:SBO mass ratios of 1:0, 2:1, and 4:1 and the polar phase with equal mass of water and propylene glycol (PG). Error bars are standard deviations from at least duplicate measurements.

line in the present study were similar to the above five-component microemulsion system studied by Garti et al. (2001). The authors also observed the maximum viscosity at a polar phase content of 45–50% as the content of polar phase gradually increased, followed by a sharp decrease of viscosity with further dilution.

Furthermore, the extent of viscosity decrease above 50% w/w of polar phase followed the EO/EOC:SBO mass ratio order of 2:1, 4:1, and 1:0 (Fig. 7). This can result from the smaller droplets at a higher SBO content (Fig. 5) because smaller droplets at the same volume fraction of the dispersed phase have a higher effective volume fraction and therefore a higher viscosity (McClements, 2005). Additionally, when the polar phase was lower than 70% w/w, microemulsions with a higher content of SBO were more viscous (Fig. 7), because of the higher viscosity of SBO than EO (data not shown).

4. Conclusions

In the present study, fully-dilutable microemulsions were successfully prepared by simple mixing of EO with and without SBO, Tween™ 80, and polar phase with equal mass of water and PG. A large isotropic area was found and no liquid crystalline region was detected due to incorporation of PG in the polar phase. Most importantly, incorporating a certain amount of SBO in the oil phase reduced particle dimension and enhanced the dilutability of microemulsions of EOs, which can broaden the application of microemulsions. Progressive transitions of microemulsions from W/O to bicontinuous to O/W structures were implied based on viscosity and electrical conductivity data. Microemulsions prepared in our study were stable over 90 days at room temperature based on particle size measurement. The stable fully-dilutable microemulsion systems exhibited potential to deliver hydrophobic EOs for various applications.

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