Influence of Surfactant Type and Thermal Cycling on Formation and Stability of Flavor Oil Emulsions Fabricated by Spontaneous Emulsification

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Abstract

Food-grade emulsions can be fabricated using simple and inexpensive low-energy homogenization methods. In this study, we examined the influence of surfactant type (Tween 40, 60, and 80), oil phase composition (limonene-to-medium chain triglyceride ratio), and temperature (25 to 95 °C) on the formation and stability of flavor oil-in-water emulsions (10 wt% oil, 15 wt% surfactant, pH 3) fabricated using spontaneous emulsification. Transparent emulsion-based delivery systems containing ultrafine droplets ($d < 40$ nm) could be formed at room temperature at certain limonene contents for all three surfactants. When these emulsions were heated and then cooled, appreciable droplet growth occurred at lower limonene levels ($< 60\%$ limonene) leading to cloudiness, but ultrafine droplets were still present at higher limonene concentrations (80% limonene) leading to optical clarity. These results were attributed to the influence of oil phase composition and surfactant type on the phase inversion behavior of the surfactant-oil-water systems.

37 **1. Introduction**

38 Emulsion-based delivery systems are widely used in the food industry to encapsulate
39 lipophilic ingredients, such as ω -3 rich oils, oil-soluble vitamins, nutraceuticals, flavor oils, and
40 essential oils (Cerqueira et al., 2014; Ezhilarasi, Karthik, Chhanwal, & Anandharamakrishnan,
41 2013; Silva, Cerqueira, & Vicente, 2012). Oil-in-water emulsions are thermodynamically
42 unstable systems that contain emulsifier-coated lipid droplets dispersed within an aqueous
43 medium (McClements, 2010). The physicochemical, sensory, and functional properties of oil-in-
44 water emulsions can be tailored by controlling the dimensions and surface characteristics of the
45 lipid droplets they contain. In certain food and beverage applications there are advantages to
46 using emulsions containing ultrafine lipid droplets (diameter < 50 nm) since they tend to be
47 optically transparent, have good stability to aggregation and gravitational separation, and may
48 enhance the activity of encapsulated ingredients (McClements, 2013; McClements & Rao, 2011).
49 Ultrafine emulsions can be produced using high-energy methods such as high-pressure valve
50 homogenizers, microfluidizers, or sonicators, provided that system composition and
51 homogenization conditions are optimized. The main drawback of these methods is that specially
52 designed mechanical devices are required (“homogenizers”) that may be expensive to purchase
53 and operate. Ultrafine emulsions can also be fabricated using low-energy methods that do not
54 require any sophisticated equipment, such as spontaneous emulsification (SE), emulsion
55 inversion point (EIP), and phase inversion temperature (PIT) methods (Perazzo, Preziosi, &
56 Guido, 2015; Solans & Solé, 2012). These methods rely on the spontaneous formation of
57 ultrafine oil droplets in certain surfactant-oil-water mixtures when system composition and/or
58 environmental conditions are altered in a specific manner. The major advantages of these low-
59 energy methods over the more commonly used high-energy methods are: (i) they do not require
60 any specially designed homogenizers; (ii) they are simple and inexpensive to implement; (iii)
61 they can often produce smaller oil droplets; and (iv) they are more energy efficient (Anton &
62 Vandamme, 2011; McClements & Rao, 2011). Conversely, the major disadvantages of low-
63 energy methods are: (i) they typically require high amounts of surfactant; (ii) they are limited in
64 the range of oil and surfactant types that can be used; and, (iii) the droplets produced are often
65 unstable to coalescence at elevated temperatures.

The spontaneous emulsification and phase inversion temperature methods are two of the most popular low-energy approaches for producing ultrafine emulsions (Anton, Benoit, & Saulnier, 2008; Anton & Vandamme, 2009). The SE method involves titrating a mixture of oil and surfactant into water, while the PIT method involves heating a mixture of surfactant, oil, and water above the phase inversion temperature and then cooling rapidly. Despite these differences in implementation, it has been proposed that these two methods generate ultrafine oil droplets through a similar physicochemical mechanism based on movement of surfactant molecules from the oil phase to the water phase (Anton & Vandamme, 2009). The SE approach has been used to encapsulate various kinds of lipophilic active agents within ultrafine lipid droplets, including antimicrobial essential oils (Chang, McLandsborough, & McClements, 2013), capsaicin (Choi, Kim, Cho, Hwang, & Kim, 2009), flavor oils (Chang & McClements, 2014), resveratrol (Davidov-Pardo & McClements, 2015), vitamin D (Guttoff, Saberi, & McClements, 2015), vitamin E (Saberi, Fang, & McClements, 2013b, 2013c), and ω -3 oils (Gulotta, Saberi, Nicoli, & McClements, 2014; Walker, Decker, & McClements, 2015). As mentioned earlier, one of the major potential limitations of emulsions produced by this method is the tendency for droplet coalescence to occur when they are heated above a certain temperature (Saberi, Fang, & McClements, 2013a). The origin of this effect is associated with changes in the molecular characteristics of non-ionic surfactant molecules with temperature (Anton & Vandamme, 2009; Israelachvili, 2011). At relatively low temperatures, the head groups of these molecules are highly hydrated, which makes them predominantly water-soluble and favors the formation of oil-in-water emulsions. Conversely, at relatively high temperatures, the head groups are less hydrated, which makes them more oil-soluble and favors the formation of water-in-oil emulsions. At the PIT, the surfactants are approximately equally soluble in both oil and water phases, and do not stabilize oil-in-water or water-in-oil emulsions very well. Consequently, oil-in-water emulsions are highly prone to droplet coalescence when their temperature is raised to a region just below the PIT (the so-called “droplet coalescence zone” or DCZ) (Kabalnov & Wennerstrom, 1996). The increase in droplet size that occurs when emulsions are exposed to these temperatures may be undesirable since it leads to changes in their optical properties or stability, *e.g.*, cloudiness, creaming, or oiling off.

An understanding of the thermal stability of emulsions is important for many of their practical applications because commercial products are often exposed to different temperatures

during their production, storage, or utilization. Previous studies have shown that the droplet size in emulsions produced by the SE method may increase, decrease or remain the same when exposed to a heating-cooling cycle (Anton & Vandamme, 2009; Saberi et al., 2013a; Saberi, Fang, & McClements, 2015b). The behavior of a given system depends on its composition, the temperatures employed (compared to the PIT), and the cooling rate used (Anton & Vandamme, 2009; Izquierdo et al., 2002; Izquierdo et al., 2004; Kunieda, Fukui, Uchiyama, & Solans, 1996; Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). A number of recent studies have examined the impact of temperature on the properties of emulsions containing Tween-coated oil droplets prepared using low-energy methods (Hategekimana, Chamba, Shoemaker, Majeed, & Zhong, 2015; Mashhadi, Javadian, Tyagi, Agarwal, & Gupta, 2016; Prasert & Gohtani, 2016; Saberi et al., 2013a; Saberi, Fang, & McClements, 2015a; Saberi et al., 2015b). These studies show that the formation and stability of these emulsions depends on surfactant type, aqueous phase composition, and oil phase composition.

The main objective of the current study was therefore to examine the influence of system composition on the thermal stability of emulsions formed using a commonly used flavor oil (limonene). In particular, we examined the influence of surfactant type (Tween 40, 60, 80) and oil phase composition (limonene-to-MCT ratio) on the thermal stability of the emulsions. Here MCT refers to medium chain triglycerides, which are commonly used in the development of food-grade delivery systems. This information will be useful for identifying suitable conditions for forming flavor oil emulsions that are stable when exposed to temperature abuse during storage.

2. Materials and methods

2.1. Materials

Medium chain triglyceride (MCT) oil (MIGLYOL[®] 812) was purchased from Warner Graham Company (Sasol Germany GmbH). Limonene, non-ionic surfactants (Tween 40, Tween 60, and Tween 80), citric acid, and sodium benzoate were purchased from Sigma–Aldrich Co. (St. Louis, MO). Double distilled water was used in the preparation of all solutions and nanoemulsions. All concentrations are expressed as a mass percentage (wt/wt%).

2.2. Emulsion preparation

Emulsion formation was carried out using the spontaneous emulsification method explained in our previous study (Sabeti et al., 2013c). In brief, SE was performed by addition of an organic phase to an aqueous phase that was being continuously stirred using a magnetic stirrer. The composition of the buffer solution was designed to imitate the aqueous phase of many commercial beverage products (citric acid, pH 3.0). Unless otherwise stated, the experiments were carried out using standardized conditions: (i) *composition* - 10 wt% total oil, 15 wt% surfactant, and 75 wt% aqueous phase; (ii) *stirring* - magnetic stirrer speed of 600 rpm; (iii) *preparation temperature* - 45 °C. In these samples, the oil (10 g) and surfactant (15 g) were first mixed together and then the mixture was slowly poured into 75 g of aqueous phase over a 15 min period with continuous stirring.

2.3. Particle size measurements

Particle size distributions were measured using a dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). This instrument determines the particle size from intensity-time fluctuations of a laser beam (633 nm) scattered from a dilute emulsion. Each individual measurement was an average of 13 runs. To avoid multiple scattering effects, samples were diluted before the particle size measurements using acidic buffer solution (pH 3.0). The mean particle diameter (Z-average) was calculated from the particle size distribution. All measurements were conducted at ambient temperature.

2.4. Turbidity measurements

The influence of thermal treatment on the turbidity (absorbance at 600 nm) of the samples was determined using a UV–visible spectrophotometer with temperature scanning capabilities (Evolution Array, Thermo Scientific). Temperature scanning measurements were carried out by measuring the turbidity (τ) of the emulsions as the temperature increased from 25 to 95 °C at 1 °C per minute, and then decreased back to 20 and/or 25 °C at different cooling rates.

2.5. Statistical analysis

All experiments were carried out at least twice using freshly prepared samples. Turbidity versus temperature profiles are only shown for one of the samples to enhance the clarity of

presentation, but both samples behaved very similarly with less than $\pm 5\%$ standard deviation in the turbidity values. The results for the impact of limonene concentration and surfactant type are reported as the calculated mean and standard deviation.

3. Results and Discussion

3.1. Influence of oil composition and surfactant type on formation

Initially, we examined the influence of oil phase composition and surfactant type on the formation of emulsions by spontaneous emulsification. Emulsions were prepared containing different limonene levels in the oil phase (50 to 80%) using three non-ionic surfactants (Tween 40, 60, and 80). These limonene levels were used because previous studies have reported that only large droplets can be produced (i) if the limonene content is lower due to inefficient emulsion formation or (ii) if the limonene content is higher due to droplet growth caused by Ostwald ripening (Li, Zhang, Yuan, Liang, & Vriesekoop, 2013; Rao & McClements, 2012a; Rao & McClements, 2012b). All three surfactants could produce emulsions containing ultrafine oil droplets ($d < 60$ nm) with the smallest droplets (24-30 nm) being produced when there was 60 to 70% of limonene in the oil phase. Tween 40 was able to produce small droplets over a wider range of limonene levels than the other surfactants. The nanoemulsions with small droplet diameters ($d < 50$ nm) typically had narrow monomodal particle size distributions, with low polydispersity indices (PDI from 0.1 to 0.2). The turbidity measurements supported the light scattering measurements: those emulsions that contained small droplets tended to have lower turbidities. This effect can be attributed to the fact that the light scattering efficiency of emulsion droplets increases as their dimensions increase in this particle size range (McClements, 2002). In general, the light scattering efficiency of oil droplets first increases with increasing droplet size, but then reaches a maximum value (around the wavelength of light), and then decreases with a further increase in droplet size.

The formation of a bicontinuous microemulsion at certain surfactant-oil-water ratios is believed to be a precursor for the formation of oil-in-water emulsions containing ultrafine droplets (Komaiko & McClements, 2016; Wang, Mutch, Eastoe, Heenan, & Dong, 2008; Wang

et al., 2009). The formation of small droplets by the spontaneous emulsification method may therefore be due to the creation of a bicontinuous microemulsion at the boundary between the oil and aqueous phases when the surfactant-oil mixture is added to the aqueous phase (Yang, Jiang, Guan, Qiu, & Lu, 2014). This microemulsion is a thermodynamically stable isotropic liquid that contains some small lipid domains surrounded by surfactant molecules (Lee, Morrison, Frethem, Zasadzinski, & McCormick, 2014). When the microemulsion breaks down, ultrafine oil droplets may be generated as these small lipid domains are released into the surrounding water phase. The formation and break down of these lipid domains will depend on the molecular characteristics of the oil and surfactant molecules, as this will determine their structural organization in surfactant-oil-water mixtures. Tween surfactants all have polar head groups that consist of polyoxyethylene chains attached to sorbitan, but they differ in the nature of their non-polar tail groups: palmitic, stearic, and oleic acid for Tween 40, 60, and 80 respectively. These differences in non-polar tails will change the organization of the surfactant and oil molecules within the bicontinuous microemulsions, which will influence structural and physicochemical properties, such as lipid domain size, rheology, and dispersibility. Consequently, surfactant type may influence the size of the lipid domains formed as well as their subsequent breakdown in the presence of water (Lee et al., 2014).

3.2. Influence of oil composition and surfactant type on thermal stability

The emulsions prepared by spontaneous emulsification were subjected to a heating-cooling cycle (25 to 95 to 25 °C at 1 °C/min), which led to an appreciable change in their particle sizes and turbidities. After heating, the emulsions containing the lowest limonene levels (50 and 60%) contained relatively large droplets (Fig. 1b), became highly turbid (Fig. 2b) and appeared visibly cloudy (data not shown), which can be attributed to the greater light scattering efficiency of larger particles. Conversely, the emulsions with the highest limonene level (80%) contained relatively small droplets ($d < 25$ nm) and appeared optically transparent after heating, because the droplet size was much smaller than the wavelength of light (around 380-780 nm). The relationship between emulsion turbidity and mean droplet size is shown in Figure 3, which shows that the droplet diameter must be less than about 40 or 50 nm to obtain low turbidities ($\tau < 0.5 \text{ cm}^{-1}$ for 10% oil). Interestingly, the limonene levels where the smallest droplets were formed before heating (60 to 70%) were different from those where they were formed after heating

(80%). This difference may have been because the emulsions tested before heating were formed by the spontaneous emulsification method, whereas those tested after heating would have been formed by the phase inversion temperature method.

These results suggest that flavor oil emulsions can be formed with high optical clarity and small droplet sizes by all surfactants used after a heating/cooling cycle. An interesting question that arises from this study is: are the colloidal dispersions formed nanoemulsions or microemulsions? Both types of colloidal dispersions contain small particles comprised of an outer hydrophilic shell of surfactant head groups and an inner hydrophobic core of oil molecules and surfactant tail groups (Anton & Vandamme, 2011; McClements, 2012). In addition, both types of colloidal dispersion may be optically transparent because of the small size of the droplets relative to the wavelength of light. Consequently, it is difficult to distinguish between them in terms of their particle size or overall appearance. However, they can be distinguished from each other in terms of their stability characteristics: microemulsions are thermodynamically stable, whereas nanoemulsions are not (McClements, 2012). Thus, the particle size should always be the same for a given system composition and temperature for a microemulsion at equilibrium. We therefore calculated a relative particle size: $RPS = 100 \times D_{\text{after}}/D_{\text{before}}$, where D_{after} and D_{before} are the mean droplet diameters of the emulsions after and before heating, respectively. For a microemulsion, this value should be equal to 100% since the particle size should be the same before and after heating. However, we observed that the relative particle size was much greater than 100% for emulsions containing low limonene concentrations (*e.g.*, $RPS = 270\text{--}1800$ for 50% limonene), but much lower than 100% for emulsions containing high limonene levels (*e.g.*, $RPS = 40$ to 82% for 80% limonene) (Fig. 4). These results suggest that nanoemulsions were formed rather than microemulsions. However, there is the possibility that the systems had not reached thermodynamic equilibrium.

3.3. Influence of oil composition and surfactant type on turbidity-temperature profile

To obtain further insight into the influence of temperature on the formation of ultrafine oil droplets we measured the turbidity *versus* temperature profiles of the emulsions using a UV-visible spectrophotometer (Figure 5). Only the results for the emulsions stabilized by Tween 40 are shown for the sake of concision however, similar trends were observed for the other

surfactants. At the lowest limonene level (50%), the emulsions initially appeared optically clear because they contained ultrafine droplets, which highlighted the effectiveness of the spontaneous emulsification method at generating small droplets. Upon heating, the turbidity increased steeply around 70 °C, which suggested that there was an increase in particle size due to droplet fusion in the droplet coalescence zone (DCZ). Upon further heating, there was a steep decrease in turbidity around 90 °C, which suggested that the PIT had been reached and a bicontinuous microemulsion containing small oil and water domains that did not scatter light strongly was formed. Upon cooling, the emulsions remained transparent until they reached a temperature of 85 °C, after which there was a steep and irreversible increase in turbidity. Presumably, this was due to extensive droplet coalescence within the temperature range just below the PIT. A similar kind of behavior was observed for the system containing 60% limonene, but the increase in turbidity associated with droplet instability in the DCZ was smaller, and the PIT occurred at a lower value (85 °C). Upon heating the system containing 70% limonene, there was a much lower increase in turbidity associated with droplet instability in the DCZ, followed by a region (77 to 85 °C) where the system was transparent (PIT), and then there was a large increase in turbidity at temperatures greater than 85 °C. This large increase in turbidity can be associated with the formation of a water-in-oil emulsion containing relatively large water droplets due to phase inversion. Upon cooling, the turbidity initially decreased as the water-in-oil emulsion changed to a bicontinuous microemulsion, and then increased slightly, which suggested that some droplet growth still occurred in the DCZ. The system containing 80% limonene showed quite different behavior during heating/cooling. The turbidity remained low from 25 to 77 °C, and then increased steeply presumably due to the formation of a water-in-oil emulsion. Upon cooling the reverse behavior was observed: the emulsion went from highly turbid at high temperatures to transparent below a critical temperature around 65 °C, and then remained transparent from 65 to 25 °C. These results suggest that droplet growth did not occur appreciably in the DCZ during the heating or cooling stages for this system.

Overall, these results suggest that increasing the limonene concentration in the oil phase decreased the phase inversion temperature and suppressed the level of droplet growth in the droplet coalescence zone. As a result, it was possible to form small droplets using the spontaneous emulsification method and these droplets remained stable to coalescence when they

were heated up to over 80 °C. Even when these emulsions were heated to temperatures sufficiently high to promote phase inversion, small droplets were again generated when they were cooled back to ambient temperature. This effect suggests that a bicontinuous microemulsion was formed at the PIT that easily broke down into a nanoemulsion upon cooling (Wang et al., 2008). A possible explanation for the fact that we did not observe rapid droplet growth just below the PIT in the system containing the highest limonene levels may have been due to the nature of the surfactant head groups. The non-ionic surfactants will tend to be more hydrated at lower temperatures, which may mean there is a stronger steric repulsion between surfactant-coated droplets. This steric repulsion may be sufficiently large to inhibit droplet coalescence even at temperatures just below the PIT.

4. Conclusions

In this study, we have investigated the combined influence of oil phase composition and surfactant type on the formation and thermal stability of flavor oil-in-water emulsions. The research has shown that relatively small droplets could be produced by spontaneous emulsification for all three surfactants at intermediate limonene levels (60 to 70% in the oil phase). Emulsions fabricated using Tween 40 had very small oil droplets ($d < 30$ nm) across the whole range of limonene levels studied (50 to 80%), and were therefore optically transparent. As expected the turbidity of the emulsions increased with increasing oil droplet size, which can be attributed to increased light scattering by larger particles. The turbidity of the emulsions exhibited a complex dependence on temperature with regions of clarity and opacity depending on droplet coalescence (DCZ), microemulsion formation (PIT), and phase inversion (O/W to microemulsion to W/O). Emulsions that were stable to heating up to relatively high temperatures could be formed under optimized oil phase composition and surfactant type. These emulsions may be useful for commercial applications where the product must remain stable to droplet aggregation induced by temperature changes during production, transport, and storage. Nevertheless, the main drawback of the low-energy methods for fabricating ultrafine emulsions is the fact that high surfactant levels are required. Indeed, a surfactant-to-oil ratio of 1.5 was required to fabricate the flavor oil emulsions used in this study. This level is acceptable in commercial products that only require very low droplet concentrations, such as many soft drinks

and flavored waters, but not in products that require relatively high droplet concentrations, such as dressings, dips, or creams.

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6. References

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Figure Captions

Figure 1a. Dependence of mean particle diameter on surfactant type and limonene level in the oil phase for oil-in-water emulsions produced by spontaneous emulsification prior to heating: 10% oil phase (limonene+MCT); 15% surfactant; 75% water (citrate, pH 3).

Figure 1b. Dependence of mean particle diameter on surfactant type and limonene level in the oil phase for oil-in-water emulsions produced by spontaneous emulsification after heating/cooling: 10% oil phase (limonene+MCT); 15% surfactant; 75% water (citrate, pH 3).

Figure 2a. Dependence of turbidity on surfactant type and limonene level in the oil phase for oil-in-water emulsions produced by spontaneous emulsification prior to heating: 10% oil phase (limonene+MCT); 15% surfactant; 75% water (citrate, pH 3).

Figure 2b. Dependence of turbidity on surfactant type and limonene level in the oil phase for oil-in-water emulsions produced by spontaneous emulsification after heating: 10% oil phase (limonene+MCT); 15% surfactant; 75% water (citrate, pH 3).

Figure 3. Dependence of turbidity on mean droplet diameter for oil-in-water emulsions produced by spontaneous emulsification: 10% oil phase (limonene+MCT); 15% surfactant (Tween 40, 60 or 80); 75% water (citrate, pH 3). The line is included to guide the eye.

Figure 4. Dependence of relative particle size ($RPS = 100 \times D_{\text{after}}/D_{\text{before}}$) on limonene content for oil-in-water emulsions produced by spontaneous emulsification: 10% oil phase (limonene+MCT); 15% surfactant; 75% water (citrate, pH 3).

Figure 5. Dependence of turbidity-temperature profile on limonene level in the oil phase for oil-in-water emulsions produced by spontaneous emulsification after heating: 10% oil phase (limonene+MCT); 15% surfactant (Tween 40); 75% water (citrate, pH 3).

Figure 1a.

Figure 1b.

Figure 2a.

Figure 2b.

Figure 3.

Figure 4.

Figure 5.

Influence of Surfactant Type and Thermal Cycling on Formation and Stability of Flavor Oil Emulsions Fabricated by Spontaneous Emulsification, by Amir Hossein Saberi, Yuan Fang, and David Julian McClements *

Flavor oil emulsions fabricated by spontaneous emulsification have different droplet sizes and appearances depending on oil phase composition and surfactant type.