

Impact of Fat and Water Crystallization on the Stability of Hydrogenated Palm Oil-in-Water Emulsions Stabilized by a Nonionic Surfactant

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The influence of (0–40 wt %) sucrose and (0 and 150 mmol/kg) sodium chloride on the physical properties of 20 wt % hydrogenated palm oil-in-water emulsions stabilized by 2 wt % Tween 20 after crystallization of the oil phase only or both the oil and water phases has been examined. Emulsion stability was assessed by differential scanning calorimetry measurements of fat destabilization after cooling–heating cycles and by measurements of mean particle size, percent destabilized fat, and percent free oil obtained from gravitational separation after isothermal storage (at –40 to +37 °C). At storage temperatures where the oil phase was partially crystalline and the water was completely liquid, the emulsions were unstable to droplet coalescence and oiling off because of *partial coalescence*. Both NaCl and sucrose increased the extent of partial coalescence in the emulsions. At storage temperatures where both oil and water crystallized, the emulsions were completely destabilized. The stability of the emulsions to freezing and thawing could be improved somewhat by adding sucrose (>20 wt %). Emulsions stabilized by whey proteins were shown to have better freeze–thaw stability than those stabilized by Tween 20, especially in the presence of sucrose. These results may help formulate food emulsions with improved freeze–thaw stability.

KEYWORDS: Emulsions; freeze–thaw stability; partial coalescence; Tween 20; sucrose; NaCl

INTRODUCTION

The stability and physicochemical properties of emulsions are strongly influenced by the physical state of both the dispersed and continuous phases (1–5). Crystallization of the fat phase within the droplets and/or of the aqueous phase surrounding the droplets can have a pronounced influence on the texture, mouth feel, stability, and appearance of many oil-in-water food emulsions (6). In some food emulsions crystallization is detrimental to quality because it causes product instability. For example, fat crystallization in dressings and dairy creams can promote droplet aggregation due to partial coalescence (7), whereas water crystallization has been shown to lead to extensive droplet flocculation, coalescence, and oiling off in many types of oil-in-water emulsions (8–10). On the other hand, crystallization is beneficial in other food products because it leads to desirable product attributes or improved long-term stability. Freezing of the water phase of oil-in-water emulsions may extend their shelf life because ice crystallization can retard

undesirable microbial growth, biochemical processes, and chemical reactions. Partial crystallization of the fat phase of oil-in-water emulsions is an essential step in the production of many types of food products because it promotes droplet aggregation through partial coalescence, e.g., butter, margarine, whipped toppings, and ice cream (11–13). The practical importance of phase transitions in oil-in-water emulsions has therefore meant that a great deal of research has been directed toward understanding the factors that influence fat and water crystallization in emulsions and in understanding the relationship between phase transitions and bulk physicochemical properties of emulsions.

One of the most important factors that influence the stability of emulsions to fat and water crystallization is emulsifier type, since this influences the susceptibility of the interfacial membrane surrounding the droplets to penetration by crystals and rupture. For example, oil-in-water emulsions stabilized by milk proteins are much more stable to fat and water crystallization than those stabilized by small molecule surfactants (5, 9, 10). If nonionic surfactants are to be used to stabilize emulsions that are to be cooled to a temperature where the fat phase and/or the water phase crystallizes, then it is necessary to develop strategies to prevent emulsion destabilization.

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Sugars and salts are ingredients that are widely used in food products to modify their flavor, texture, appearance, and stability. For example, relatively high sugar concentrations are present in products such as ice cream, fruit pie fillings, fillings for baked goods, and icings. Recent studies have shown that oil droplets stabilized by nonionic surfactants have an appreciable electrical charge, which has been attributed to preferential adsorption of either H_3O^+ (low pH) or OH^- (high pH) from the surrounding water (14). One may therefore expect that NaCl would adversely affect emulsion stability by screening the electrostatic repulsion between the droplets (1). Sugars are widely used in food products as cryoprotectants, which have mainly been attributed to freeze concentration effects and their ability to modify ice crystal formation and structure (6, 15, 16). The objective of this study was to systematically examine the influence of sodium chloride and sucrose on the stability of Tween 20 stabilized emulsions when oil crystallization only or both oil and water crystallization occurred. The results of the present study should lead to an improved understanding of the factors that determine the stability of oil-in-water emulsions to chilling and freezing, which may help in the more rational design of food products.

MATERIALS AND METHODS

Materials. Hydrogenated palm oil (Cebes 27–50) with a Wiley melting point of 33–35 °C was donated by Aarhus Inc. (NJ). Tween 20 (poly(oxyethylene) sorbitan monolaurate, lot no. 69H0143), sodium azide (NaN_3), sucrose, and sodium chloride (NaCl) were purchased from Sigma Chemical Co. (St. Louis, MO).

Emulsion Preparation. An aqueous nonionic surfactant solution was prepared by dispersing 6 wt % Tween 20 in 5 mM phosphate buffer (pH 7.0) and stirring with a magnetic stirrer for more than 2 h to ensure complete dissolution and mixing. Coarse hydrogenated palm oil-in-water emulsions (40 wt % oil; 4 wt % Tween 20) were prepared by blending the oil and emulsifier solution together using a high-speed blender (Model 33BL79, Warring Inc., New Hartford, CT) for 1 min. These emulsions were passed 3 times through a high-pressure valve homogenizer (Rannie High Pressure, APV-Gaulin, Model Mini-Lab 8.30H, Wilmington, MA) at 3000 psi to reduce the mean droplet diameter (d_{32}) to $0.3 \pm 0.05 \mu\text{m}$. Sodium azide (0.02 wt %) was then added to the emulsions to prevent microbial growth. Emulsions were diluted with phosphate buffer (5 mM, pH 7) containing sodium chloride or sucrose to obtain emulsions with a final composition of 20 wt % hydrogenated palm oil, 2 wt % Tween 20, 0 or 150 mmol/kg NaCl, and 0, 10, 20, 30, or 40 wt % sucrose. All of these procedures were carried out at a temperature between 40 and 50 °C to prohibit crystallization of the hydrogenated palm oil.

Differential Scanning Calorimetry Measurements. Samples (15 mg) were placed in aluminum pans, which were then sealed. The thermal behavior of the samples was then characterized using differential scanning calorimetry (DSC; Q1000, TA Instruments, DE). An empty aluminum pan (ME-00026763, Mettler Toledo, OH) was used as the reference pan. A preliminary experiment was carried out to establish the temperature at which the oil and water crystallized under the conditions used in our experiments, i.e., scan rate, starting temperature, and sample size. We found that bulk oil crystallized around 21 °C, and pure water crystallized around -17 °C during cooling at a scan rate of 1.5 °C/min. These values were then used to design the subsequent DSC experiments.

Two temperature cycling programs were used to investigate the influence of oil crystallization alone and combined oil and water crystallization on the stability of the emulsions:

Oil Crystallization Only. The sample was kept at 40 °C for 5 min, then cooled to -10 °C at 1.5 °C/min to ensure complete oil crystallization (but no water crystallization), and then heated at the same rate to 40 °C. This cooling–heating cycle was repeated 3 times.

Oil and Water Crystallization. The sample was kept at 40 °C for 5 min, then cooled to -40 °C at 1.5 °C/min to ensure complete oil

and water crystallization, and then heated at the same rate to 40 °C. This cooling–heating cycle was repeated 3 times.

Measurements were carried out at least in duplicate. The enthalpy changes due to crystallization of emulsified oil and “destabilized” oil in the samples were determined from the measured peak areas in the DSC thermograms as described by previous workers (3, 4). Briefly, the emulsified oil was designated as that fraction of the oil that crystallized at a temperature well below that observed for bulk oil, whereas the destabilized oil was designated as that fraction of the oil that crystallized at a temperature approximately equal to that of bulk oil. Thus, the percentage of destabilized fat in the emulsions was defined by the following equation:

$$\%DF = 100 \times \Delta H_{DF}/\Delta H_{TF} \quad (1)$$

where ΔH_{DF} and ΔH_{TF} are the enthalpy changes per unit mass of oil associated with crystallization of the destabilized fat and for the total fat in the emulsion (in small droplets + destabilized fat), respectively.

The DSC technique was also used to determine the amount of unfrozen water in the emulsions. The enthalpy change under the large exothermic water crystallization peak observed in a DSC thermogram was measured for a known mass of emulsion and for a known mass of pure water. The amount of freezable water in the emulsion was then calculated:

$$\%FW = 100 \times \Delta H_E/\Delta H_W \quad (2)$$

where ΔH_E and ΔH_W are the enthalpy changes per unit mass of water for samples of emulsion and pure water, respectively. The percentage of unfrozen water (UW) within the emulsion was therefore as follows: $\%UW = 100 - \%FW$. The percentage of unfrozen aqueous phase (UAP) in the emulsions was calculated from this value and the known composition of the emulsions:

$$\%UAP = 100 \times (\%UW/100 \times \%W + \%E + \%S + \%NaCl)/(\%AP) \quad (3)$$

where %W, %E, %S, %NaCl, and %AP are the weight percentages of water, emulsifier, sugar, salt, and aqueous phase in the emulsions: $\%AP = \%W + \%E + \%S + \%NaCl$.

Effect of Storage Temperature on Emulsion Properties. Emulsions were stored in glass test tubes for 24 h, either at -40 and -20 °C in a temperature-controlled refrigerator or at -2.5, 0, 2.5, 5.0, 7.5, 10, 15, and 37 °C in a water bath (Model 9200, Polyscience, IL). The particle size, gravitational separation, oiling off and microstructure were then measured.

Gravitational Separation. Emulsions (10 g) were poured into glass tubes (100 mm height, 16 mm internal diameter), which were then capped and stored at different temperatures (-40 to +37 °C) for 24 h as described above. These emulsions were then kept at 37 °C for 24 h (to melt any crystalline fat) before the heights of any separated layers were measured. The liquid oil had a lower density than the surrounding aqueous phase and therefore tended to move upward due to gravity. After frozen storage, many of the emulsions separated into a transparent aqueous layer at the bottom and a transparent oil layer at the top. After storage at some higher temperatures, some of the emulsions separated into a turbid layer at the bottom and a transparent oil layer at the top. The thickness of each of the layers formed was determined manually using a ruler.

$$\text{oiling off (\%)} = 100 \times H_0/H_{\text{total}} \quad (4)$$

where H_0 is the height of the oil layer measured in the emulsion samples and H_{total} is the total height of the oil layer in a completely destabilized system (which was established by putting the same amount of nonemulsified oil on top of an aqueous solution).

Percentage of Free Oil. The method of determining the amount of free oil present in the emulsions after storage was adapted from that described by Palanuwech and co-workers (17). In summary, the amount of free oil in the emulsion samples was determined by adding 6 g of soybean oil containing Oil Red O (10^{-5} wt %) to 15 g of emulsion. The resulting systems were then mixed and centrifuged, and a sample

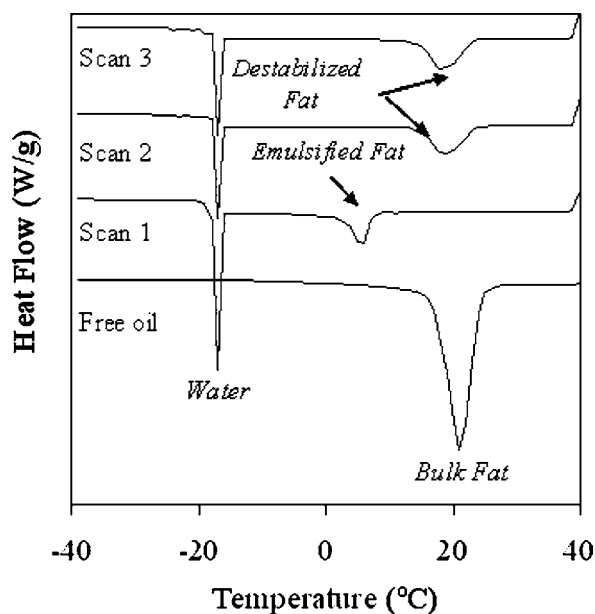


Figure 1. Examples of DSC cooling scans obtained for 20 wt % hydrogenated palm oil-in-water emulsions (no salt or sucrose) after 1, 2, and 3 cycles from +40 to -40 °C. The curves have been off-set on the y-axis to enhance clarity.

of the oil layer containing the dye was collected from the top of the emulsions. The absorbance of the oil layer was then measured at 520 nm using an UV-visible spectrophotometer (Perkin-Elmer, Lambda 3, Norwalk, CT) with pure soybean oil as a blank. The concentration of free oil (wt %) in the oil layer was then obtained from a regression equation prepared using emulsions of known free oil concentration.

Particle Size Measurement. The particle size distribution of the emulsions was measured using a laser diffraction particle size analyzer (Masterizer S, Malvern Instruments, Ltd., Southborough, MA). This instrument measures the angular dependence of the intensity of light scattered from a stirred dilute emulsion, and the particle size distribution is calculated using Mie theory. To avoid multiple scattering effects, the emulsions were diluted with buffer prior to making the light scattering measurements. The emulsions were stirred continuously throughout the measurements to ensure the samples were homogeneous. Dilution and stirring were likely to disrupt any flocculated droplets or breakup any free oil into droplets; hence, particle size data on highly flocculated or coalesced samples should be treated with caution. Particle sizes were reported as volume-weighted mean diameters, d_{43} ($=\sum n_i d_i^4 / \sum n_i d_i^3$), or surface-weighted mean diameters, d_{32} ($=\sum n_i d_i^3 / \sum n_i d_i^2$), where n_i is the number of particles with diameter d_i .

Statistics. Each of the measurements described above was carried out using at least replication with two subsamples per replication, and the results are reported as the mean and standard deviation. The data were subjected to analysis of variance (ANOVA) and least significant difference tests (LSD) to determine the significant differences between treatments.

RESULTS AND DISCUSSION

DSC Study of Emulsion Stability. Differential scanning calorimetry was used to measure the enthalpy changes resulting from controlled cooling of Tween 20 stabilized oil-in-water emulsions containing different concentrations of sucrose and NaCl. Enthalpy changes for an emulsion containing no sucrose and no NaCl that was subjected to successive cooling-heating cycles (+40 to -40 °C) are shown in **Figure 1**. During the first cooling scan, emulsified fat crystallized at ~ 6 °C, whereas bulk fat crystallized at ~ 21 °C under the same conditions. The crystallization temperature of emulsified fat is much lower than that of bulk fat because the probability of finding at least one catalytic impurity within an oil droplet decreases dramatically

Table 1. Fat Destabilization in 20 wt % Hydrogenated Palm Oil-in-Water Emulsions Stabilized with Tween 20 Measured by DSC after 3 Cycles from +40 to -10 °C and from +40 to -40 °C in the Absence (0 mmol/kg NaCl) and Presence of Salt (150 mmol/kg NaCl)^a

sucrose (%)	+40 to -10 °C		+40 to -40 °C	
	0 mmol/kg NaCl	150 mmol/kg NaCl	0 mmol/kg NaCl	150 mmol/kg NaCl
0	32.2 \pm 2.4 b	20.4 \pm 8.7 c	100 \pm 0 a	100 \pm 0 a
20	47.0 \pm 7.7 a,b	45.9 \pm 4.9 b	100 \pm 0 a	94.8 \pm 2.0 a
40	93.3 \pm 1.7 a	94.8 \pm 2.6 a	98.4 \pm 0.4 b	98.8 \pm 0.1 a

^a Different letters (a–c) within a column indicate the data are significantly different ($p < 0.05$).

once the droplet diameter falls below a critical level (6). Consequently, in bulk oils crystallization tends to occur via a heterogeneous nucleation mechanism promoted by the presence of impurities distributed throughout the oil, whereas in finely emulsified oils crystallization tends to occur via a homogeneous nucleation mechanism. When the same emulsion was cooled a second or third time the crystallization temperature of the emulsified oil (~ 20 °C) was close to that of the bulk oil (~ 21 °C) (**Figure 1**). This suggested that extensive droplet coalescence and possibly oiling-off had occurred in the emulsions after cooling to -40 °C the first time, since the droplet diameter must have increased above the critical value where the nucleation switches from homogeneous to heterogeneous. **Figure 1** also shows that the water in the aqueous phase surrounding the oil droplets in the emulsions crystallized at around -17 °C (which is much lower than the equilibrium melting point of water because of supercooling effects). Thus, both the oil and the water phases crystallized in the emulsions cooled to -40 °C in the DSC instrument. The stability of the emulsions to cooling and heating in the DSC was characterized in terms of the percentage of destabilized fat (%DF) in the emulsion before each cooling cycle (eq 1).

The DSC technique described above was used to examine the influence of sucrose and salt on the stability of 20 wt % hydrogenated palm oil-in-water emulsions stabilized by 2 wt % Tween 20 to (i) oil crystallization only (+40 to -10 °C) and (ii) oil crystallization followed by water crystallization (+40 to -40 °C).

Oil Crystallization Only. In the absence of sucrose and salt, the DSC experiments (+40 to -10 °C) indicated that an appreciable amount of fat destabilization (20–30%) occurred during the second and third cooling cycles (**Table 1**). These results suggest that substantial droplet coalescence occurred when the droplets were cooled to a temperature where the oil droplets were partially crystalline. This phenomenon can be attributed to partial coalescence of the oil droplets, where a crystal from one droplet penetrates into a liquid region of another droplet leading to clump formation (1, 6). There was a progressive increase in the amount of destabilized fat in the emulsions when the sucrose concentration in the aqueous phase was increased from 0 to 40 wt % in both the absence and presence of 150 mmol/kg NaCl (**Table 1**). Indeed, over 90% of the fat in the emulsions was destabilized when the emulsions were cooled to -10 °C in the presence of 40 wt % sucrose.

Oil and Water Crystallization. We now examine the influence of sucrose and salt on the stability of emulsions at temperatures where both the oil and (freezable) water were crystallized (**Table 1**). In the absence of salt, the oil droplets in the emulsions were completely destabilized after the emulsions had been cooled to -40 °C at all the sucrose concentrations studied; i.e., $\sim 100\%$

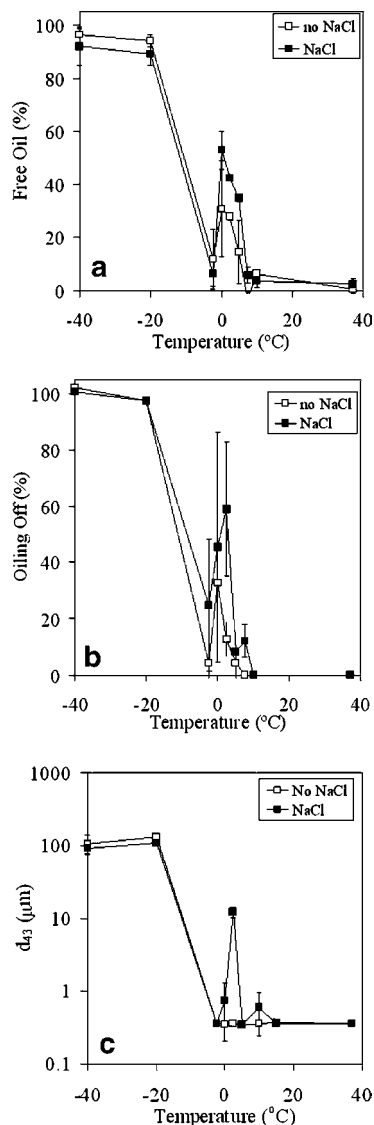


Figure 2. Influence of salt (0 or 150 mmol/kg NaCl) on the percentage of (a) the percentage of free oil, (b) the percentage of oiling off obtained from gravitational separation, and (c) the mean particle diameter after storage at different temperatures.

fat destabilization was observed on the second and third cooling cycles. In the presence of salt (150 mmol/kg NaCl), the oil droplets were also completely destabilized after three cycles (Table 1). These measurements clearly indicate that neither sucrose (0–40 wt %) nor salt (0 or 150 mmol/kg NaCl) can substantially improve the freeze–thaw stability of oil droplets stabilized by Tween 20.

Isothermal Storage Studies of Emulsion Stability. DSC results were confirmed by measurements of emulsion stability made after isothermal storage at various holding temperatures (Figures 2 and 3). Measurements of the % free oil (Figure 2a), the % oiling off obtained from gravitational separation (Figure 2b), and the mean particle diameter (Figure 2c) of the emulsions after storage indicated that they were stable to droplet aggregation at temperatures above the crystallization temperature of the oil (>10 °C). In other words, the emulsions were stable to droplet aggregation at temperatures where the oil droplets remained completely liquid. On the other hand, when the emulsions were stored at holding temperatures where the oil droplets were partially crystalline but the aqueous phase remained liquid (0–10 °C), appreciable droplet aggregation was

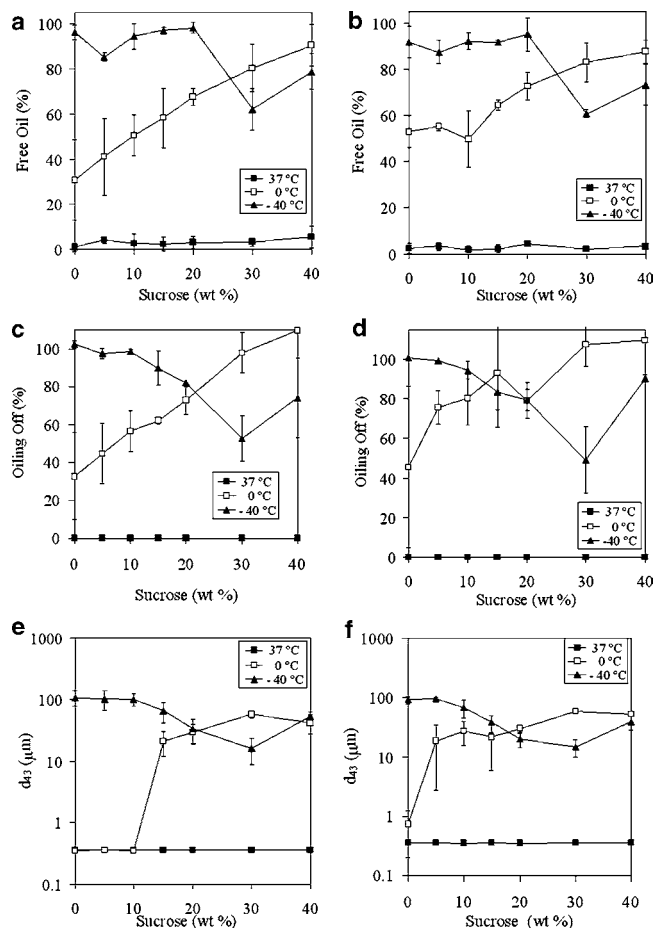


Figure 3. Influence of sucrose concentration on the percentage of free oil with (a) 0 and (b) 150 mmol/kg NaCl, the percentage of oiling off with (c) 0 and (d) 150 mmol/kg NaCl, and the mean particle diameter with (e) 0 and (f) 150 mmol/kg NaCl after storage at different temperatures.

observed, as demonstrated by an increase in the % free oil (Figure 2a), the % oiling off obtained from gravitational separation (Figure 2b), and the mean particle diameter (Figure 2c). This type of emulsion instability can be attributed to partial coalescence of the partly crystalline oil droplets (1, 6, 9, 10). When the emulsions were held at a temperature where the oil droplets were almost completely solidified but the aqueous phase remained liquid (i.e., -2.5 °C), the stability of the emulsions to droplet aggregation was much greater than at temperatures where the oil droplets were only partially solidified (0 °C). This phenomenon can be attributed to the fact that fully solidified fats cannot undergo substantial partial coalescence because a crystal from one droplet cannot then penetrate into another droplet (1, 6). When the emulsions were held at temperatures where the freezable water in the aqueous phase solidified (i.e., <-2.5 °C), the oil droplets were almost completely destabilized.

We then examined the influence of sucrose and salt concentrations on the stability of the emulsions to droplet aggregation at selected temperatures (Figure 3). On the basis of the measurements of emulsion stability versus storage temperature (Figure 2), we selected three holding temperatures to examine the effects of sucrose and salt: -40 °C (freezable water fully solidified, oil fully solidified); 0 °C (oil partially solidified, water liquid); 37 °C (oil liquid, water liquid). Measurements of the % free oil (Figure 3a,b), the % oiling off obtained from gravitational separation (Figure 3c,d), and the mean particle

Table 2. Influence of Sucrose (0–40 wt %) and Salt (0 and 150 mmol/kg NaCl) on the Percentage of Unfrozen Aqueous Phase in 20 wt % Hydrogenated Palm Oil-in-Water Emulsions Stabilized with Tween 20^a

sucrose (%)	unfrozen aqueous phase (%)	
	0 mmol/kg NaCl	150 mmol/kg NaCl
0	4.05 ± 0.72 c	14.10 ± 0.52 c
20	45.33 ± 0.12 b	48.03 ± 0.23 b
40	93.34 ± 1.27 a	83.7 ± 2.1 a

^a Different letters (a–c) within a column indicate the data are significantly different ($p < 0.05$).

diameter (Figure 3e,f) of the emulsions after storage indicated that sucrose had a much bigger impact on the emulsion stability than NaCl.

The emulsions stored at 37 °C were stable to droplet aggregation at all sucrose and NaCl concentrations, as indicated by the % free oil (Figure 3a,b) and the % oiling off obtained from gravitational separation (Figure 3c,d), remaining close to zero, and the mean particle diameter (Figure 3e,f), remaining close to the original value ($d_{43} = 0.4 \pm 0.1 \mu\text{m}$). As mentioned earlier, this suggested that Tween 20 stabilized emulsions containing droplets that were entirely liquid were stable to droplet aggregation under the conditions used in this study. On the other hand, appreciable emulsion instability occurred when the emulsions were stored at a temperature where the droplets were partially crystalline but the aqueous phase remained liquid (i.e., 0 °C). The extent of droplet destabilization increased progressively as the sucrose concentration was increased from 0 to 40 wt %, as shown by increases in the % free oil (Figure 3a,b), the % oiling off obtained from gravitational separation (Figure 3c,d), and the mean particle diameter (Figure 3e,f). The presence of salt in the emulsions appeared to make little difference to their stability at this holding temperature. When the emulsions were stored at a temperature where both the oil and freezable water solidified (–40 °C), the emulsions were highly unstable to droplet aggregation at all sucrose and salt concentrations.

Nevertheless, there appeared to be a slight, but significant ($p < 0.05$), improvement in emulsion stability when the sucrose concentration exceeded about 30 wt %. This slight improvement may be attributed to the fact that the amount of unfrozen water in the aqueous phase increased as the sucrose concentration increased (Table 2); therefore the emulsion droplets were not forced so closely together in the frozen sample when sucrose was present. Nevertheless, it appears that sucrose alone cannot prevent extensive droplet coalescence in oil-in-water emulsions stabilized by this nonionic surfactant. Overall, the isothermal storage measurements of emulsion instability confirmed the DSC measurements discussed in the previous section.

Comparison of WPI and Tween 20 Stabilized Emulsions.

In this section, we compare our measurements of the influence of sucrose and NaCl on the freeze–thaw stability of Tween 20 stabilized emulsions to similar measurements reported in an earlier study for whey protein isolate (WPI) stabilized emulsions (9, 10).

The impact of emulsifier type (WPI vs Tween 20) on the influence of storage temperature on the percentage of destabilized fat for 20 wt % oil-in-water emulsions containing no NaCl and no sucrose is shown in Figure 4. In contrast to the Tween-stabilized emulsion, the WPI stabilized emulsion did not show extensive fat destabilization at temperatures where the oil phase was only partially crystalline (0–10 °C). This was primarily

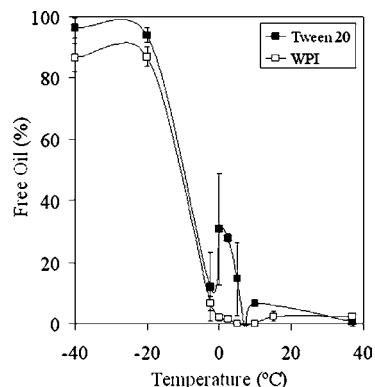


Figure 4. Impact of emulsifier type (WPI vs Tween 20) on the influence of storage temperature on the percentage of destabilized fat for 20 wt % oil-in-water emulsions containing no NaCl and no sucrose.

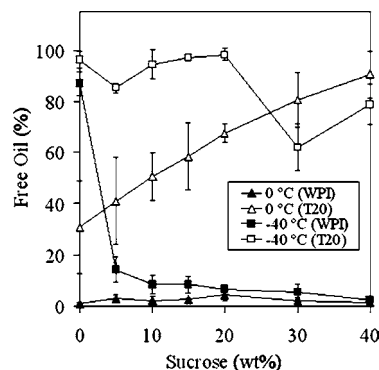


Figure 5. Impact of emulsifier type (WPI vs Tween 20) on the influence of sucrose concentration on the percentage of free oil for 20 wt % oil-in-water emulsions containing no NaCl.

attributed to the ability of the protein to form a relatively thick membrane around the oil droplets, which prevented fat crystals from one droplet penetrating into the liquid region of another droplet, thereby inhibiting partial coalescence (9, 10). Nevertheless, both emulsions were highly unstable to droplet coalescence and oiling off after they were stored at –40 °C, which indicated that neither the protein nor the nonionic surfactant could prevent droplet coalescence when the oil and water phases crystallized. It should be noted that extensive droplet flocculation (but not coalescence) was observed in WPI stabilized emulsions around 0–10 °C when 150 mmol/kg NaCl was added to the emulsions, which was attributed to electrostatic screening (10).

We then examined the influence of the sucrose concentration on the stability of the emulsions to droplet coalescence and oiling off in the absence of NaCl (Figure 5). After storage at 37 °C all of the emulsions were stable to droplet aggregation (data not shown). After storage at 0 °C, the WPI stabilized emulsions were stable to droplet aggregation at all sucrose concentrations, but the Tween stabilized emulsions became progressively unstable to partial coalescence as the sucrose concentration was increased. After storage at –40 °C, the stability of the WPI stabilized emulsions to droplet aggregation increased dramatically when the sucrose concentration was increased from 0 to 5 wt %, but the stability of the Tween stabilized emulsions only increased slightly when the sucrose concentration exceeded 20 wt %. These results clearly show that the stability of oil-in-water emulsions to chilling and freezing depends strongly on emulsifier type, as does the cryo-protection effects of sucrose.

Origin of Observed Effects. One of the most interesting findings of this study was the different behavior of sucrose in

emulsions stabilized by different types of emulsifiers. In Tween 20 stabilized emulsions, sucrose promoted partial coalescence at temperatures where the oil droplets were partly crystalline, but only slightly improved the stability of droplets to aggregation at temperatures where the water phase crystallized. On the other hand, in WPI stabilized emulsions, sucrose did not promote partial coalescence and greatly improved the stability of the droplets to aggregation when the water phase crystallized.

A number of physicochemical phenomena might explain the promotion of partial coalescence by sucrose in Tween stabilized emulsions. First, the presence of sucrose in the aqueous phase increases the short-range attraction between oil droplets by a mechanism similar to depletion flocculation, i.e., exclusion of sucrose molecules from a thin region surrounding the droplets leading to the generation of an osmotic attractive force. Consequently, the oil droplets could come into closer contact during a collision, which may have enabled fat crystals from one droplet to penetrate into another droplet. Second, high concentrations of sucrose may have altered the balance of the intermolecular forces acting at the oil–fat crystal, oil–water, and fat crystal–water interfaces (6). For example, it is known that sucrose increases the surface tension at air–water and oil–water interfaces (CRC, *Handbook of Chemistry and Physics*). This could have changed the contact angle of the fat crystals at the droplet surfaces, enabling them to penetrate further into the aqueous phase and promoting partial coalescence. Presumably, these two effects outweighed the ability of sucrose to increase the solution viscosity and therefore slow droplet–droplet collisions.

A number of possible physicochemical mechanisms may also account for the ability of sucrose to improve the stability of emulsions to droplet aggregation when the aqueous phase froze:

The addition of sucrose increased the fraction of unfrozen water present in the emulsions (**Table 2**), thereby increasing the volume of aqueous phase available for the emulsion droplets to occupy and reducing the tendency for emulsion droplets to be forced together when the water freezes (6, 15). In addition, an increase in the amount of unfrozen water would have reduced any freeze concentration effects, e.g., pH or ionic strength changes.

The sucrose molecules and unfrozen water could form hydrogen bonds with the adsorbed emulsifier molecules, thereby preventing them from becoming dehydrated and reducing the tendency for emulsifier molecules adsorbed onto different droplets from associating with each other (18, 19).

The presence of sucrose in the aqueous phase of the emulsions may have altered the kinetics and mechanisms of both ice crystal nucleation and growth, thereby altering the size and morphology of the water crystals formed in the frozen emulsions (15, 20). As a consequence, the ice crystals formed in the presence of sucrose may have been less likely to disrupt the interfacial membranes surrounding the oil droplets.

In the case of the protein-stabilized emulsions, the presence of sucrose may have increased the conformational stability of the adsorbed globular proteins, thereby preventing their cold denaturation during cooling and freezing (16, 19).

The results suggest that interfacial membranes stabilized by proteins are more resistant to rupture during freezing and thawing of emulsions than those stabilized by a nonionic surfactant.

CONCLUSIONS

This study has shown that when Tween 20 stabilized emulsions are stored at temperatures where the oil phase is partly

crystalline appreciable fat destabilization occurs. This phenomenon has been attributed to partial coalescence because the thin interfacial layers surrounding the oil droplets cannot prevent the penetration of a fat crystal from one droplet into a liquid region of another droplet. The addition of sucrose to the emulsions actually increased the amount of droplet aggregation in the emulsions, which was attributed to the ability of the sucrose to increase the attraction between the droplets or alter the morphology of the fat crystals. At storage temperatures where both oil and water crystallized, the emulsions were completely destabilized. The stability of the emulsions to freezing and thawing could be improved slightly by adding sucrose (>20 wt %). Emulsions stabilized by whey proteins were shown to have better freeze–thaw stability than those stabilized by Tween 20, especially in the presence of sucrose. These results may help formulate food emulsions with improved freeze–thaw stability.

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