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ORIGINAL PAPER

Factors Affecting Initial Retention of a Microencapsulated Sunflower Seed Oil/Milk Fat Fraction Blend

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Abstract To study the effect of emulsion stability, particle size, emulsifier, and crystalline fat in the oil phase on initial retention of a low-trans fat encapsulated in a trehalose matrix, six emulsions were prepared. The six emulsions were formulated with 20 wt% trehalose solution as the aqueous phase, a lipid phase either with no crystalline fat, sunflower seed oil (SFO), or with a crystalline phase, a 40% SFO in high-melting fraction of milk fat (HMF) blend, and sodium caseinate (NaCas), a 50 wt% blend of the palmitic sucrose esters (SE) P-170 and P-1670, or a 50 wt% blend of NaCas/SE as stabilizers. Particle size did not change or it changed only slightly during the freeze thaw or freeze drying process when the fat phase was SFO. However, when a crystalline phase was present, the volume-weighted mean diameter ($D_{4,3}$) increased dramatically for SE and NaCas/SE stabilizers. Encapsulation properties were determined by the counteracting effects of particle size and distribution, the presence of crystalline material in the droplets and interactions between interface components, fat phase and trehalose. In addition, retention was less related to emulsion stability. The emulsions selected for this study were stable for at least 30 h which was enough for obtaining a high degree of encapsulation.

Keywords Core composition · Crystallization · Droplet size · Emulsion stability · Emulsifiers · Encapsulation efficiency · High melting fraction of milk fat · Sunflower seed oil

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Introduction

Microencapsulation is a technique whereby liquid droplets or solid particles are packed into continuous individual shells. The shells or walls are designed to protect the encapsulated material from evaporation, oxidation and chemical reaction [1]. A storage life of 12–24 months at ambient temperature can be achieved when the milk fat is converted to a powder [2]. Consumer concerns associated with the atherogenic effect of *trans* fatty acids limit the future of the hydrogenation process as a way of modifying the solid-to-liquid ratio in vegetable oils/fats. As an alternative to hydrogenated vegetable oils, modification of high melting point stearins by blending with vegetable oils is becoming important, since shortenings with appropriate physicochemical properties and good nutritional characteristics that

are free of *trans* fatty acids and rich in PUFA can be obtained. Thus, it is of interest to encapsulate a low-*trans* fat such as sunflower seed oil blended with a high-melting fraction of milk fat.

There are several methods of encapsulating sensitive materials. Spray drying is the most common method as the cost of drying is 30–50 times less than freeze-drying. Spray-drying provides a very large surface area which enhances oxidation, if the wall material is not thick or dense enough to provide a good oxygen barrier. Freeze-drying occurs at a low temperature from the frozen state avoiding any water phase reactions and most oxidation because of the vacuum. Thus, it is the preferred method for the flavor industry. The efficiency of encapsulation in both, freeze and spray-drying is comparable.

The choice of encapsulant, core material and emulsifiers is critical as they will influence emulsion stability before drying. Superior emulsifying capacity and oil retention have been reported for some materials such as sugars, starches and whey proteins [3–6]. Trehalose has performed well for retaining lipid materials in freeze-drying. Sodium caseinate (NaCas) has been reported to be the most effective emulsion stabilizer for fats [7]. It is also of interest to study the effects of sucrose esters (SE) on oil retention since in addition to their major function of producing and stabilizing emulsions, SE contribute to numerous other functional roles such as texturizers and film former. Physical properties of powder such as flowability, mechanical stability, stickiness and lumpiness were directly related to emulsion stability; the more stable the emulsion the more free-flowing was the powder [2]. The stability of an emulsion was also reported to be strongly affected by the presence of crystalline fat in the oil phase [8]. Emulsions containing oil phases with different melting points will therefore probably give different degrees of fat encapsulation [5].

The aim of this work was to study the effect of emulsion stability, particle size, emulsifier, and crystalline fat in the oil phase on initial retention of a low-*trans* fat encapsulated in a trehalose matrix.

Materials and Methods

Starting Materials

α,α-Trehalose dihydrate [α-D-glucopyranosyl-(1-1)-α-D-glucopyranoside] from *Saccharomyces cerevisiae*, 99%, obtained from Sigma (Sigma-Aldrich, St Louis, MO, USA) was used without any further purification. HPLC water was used for all experimental work. The fat phase was commercial sunflower oil (SFO) or a blend of 40 wt% SFO in high-melting fraction of milk fat (HMF). The HMF was obtained by fractionation of anhydrous milk fat (AMF) with ethyl acetate (3:1). After 2 h at 5 °C, solids were separated by filtration and the solvent was evaporated. Dropping point (the temperature at which a solid fat just begins to flow under controlled conditions) of HMF and the 40 wt% SFO-in-HMF blend was determined with the Mettler FP 80 dropping point apparatus (Mettler Instruments A.G., Greifensee-Zurich, Switzerland), using a heating rate of 1 °C/min. Mettler dropping point of HMF and the 40 wt% SFO-in-HMF blend were 49.5 and 46.5 °C, respectively. Palmitic SE (P-170) with hydrophilic/lipophilic balance (HLB) = 1 and Palmitic SE (P-1670) with HLB = 16 were supplied by Mitsubishi-Kasei Food Corp. (Tokyo, Japan). The SE had Mettler dropping points of 58.0 and 44.0 °C, respectively. Monoester content of P-170 was 1 wt%, with di-, tri-, and polyesters comprising 99 wt%. P-1670 had 80% monoester and 20% di-, tri-, and polyester. NaCas was obtained from ICN (ICN Biomedical, Inc., Aurora, OH, USA) and used without any further purification.

Emulsion Preparation

Six emulsions were prepared. In all cases aqueous phase was a 20 wt/v% solution of trehalose, a known cryoprotectant. Two fat phases were used: with no crystalline material, SFO, and with crystalline material, a 40 wt% SFO-in-HMF blend. Three different stabilizers were formulated in both cases: SE, NaCas, and a 50 wt% blend of SE and NaCas. For SE emulsions, 0.250 g of P-1670 were dissolved in 100 mL aqueous phase while 0.250 g of P-170 were dissolved in 4.000 g of fat phase (SFO or the 40 wt% SFO-in-HMF blend). SE concentration was within the ones usually employed in foods for these esters. We selected SE with extreme HLB values (1 and 16) because it is often the case in food products, and in confectionery too, that a combination of two emulsifiers in a recipe formula containing two distinct phases will result in the longer lasting and more uniform product. In these cases, combinations of low- and high-HLB emulsifiers give the best results [9]. For NaCas emulsions 0.500 g of NaCas were used while for SE/NaCas emulsions 0.125 and 0.250 g of P-1670 and NaCas, respectively, were dissolved in 100 mL aqueous phase, and 0.125 g of P-170 were added to the 4.000 g of fat phase. Fat and aqueous phases were mixed using an Ultra-Turrax T25 high speed blender (S25-20NK-19G dispersing tool, IKA Labortechnik, Janke & Kunkel, GmbH & Co., Staufen, Germany), operated at 20,000 rpm for 1 min. The resultant pre-emulsions were further passed through a two-stage valve high pressure homogenizer (Stansted Fluid Power Ltd, Essex, UK) at pressures of 40 and 4 MPa for the first and second stage, respectively. The homogenization time was sufficient to achieve four passes

through the valves. Emulsions were kept at 60 °C during preparation. Then, they were cooled quiescently to ambient temperature (22.5 °C). Subsequently they were analyzed for particle size distribution, solid fat content (SFC) and stability in quiescent conditions. Experiments were done in duplicate.

Emulsion Stability

The emulsion stability was analyzed using a vertical scan analyzer (Quick Scan; Beckman Coulter, Fullerton, CA, USA) which was described elsewhere [10]. The reading head is composed of a pulsed near-IR light source (λ = 850 nm) and two synchronous detectors. The transmission detector receives the light, which goes through the sample (0°), while the back-scattering detector receives the light back-scattered by the sample (135°). The samples were put in a cylindrical glass measurement cell and the backscattering (BS) and transmission (T) profiles as a function of the sample height (total height = 70 mm) were studied in quiescent conditions at 22.5 °C. In this way, the physical evolution of this process is followed without disturbing the original system and with good accuracy and reproducibility [11]. The Quick Scan head acquired transmission (T) and BS data every 40 µm. Measurements were performed immediately after preparation of the emulsions and for 195 h. Clarification kinetics was followed by measuring the ratio in percentage of serum height to total sample height at 5% transmission as a function of time.

$$h_t(\%) = \frac{h_w}{h_{total}} \times 100, \quad (1)$$

where h_w is the serum height, h_{total} is the total tube length, and h_t is the ratio at quiescent storage time t. As tube section is constant, height ratio can be easily related to volume ratio.

Height of cream layer can be calculated as follows:

$$h_c(\%) = 100 - h_t(\%). \quad (2)$$

Global stability was followed by measuring the BS mean values (BS_{av}) as a function of storage time in the middle zone of the tube. The optimum zone was the one where no significant transmitted light was detected, that is 20–27 mm for all samples but SE/NaCas/SFO-in-HMF emulsion which was measured in the 30–40 mm zone.

Particle Size Analysis

The particle size distribution of emulsions was determined immediately after emulsion preparation, after freeze-thaw treatment and in emulsions reconstituted from powders by light scattering using a Beckman Coulter Particle Analyzer model LS^{TM} 230 (Beckman Coulter, Fullerton, CA, USA), which uses the Fraunhofer method. Samples with NaCas were also diluted (1:1 v/v) with 50 mM Tris/HCl buffer pH 8.0 containing 1.0% SDS. Measurement in the presence of SDS allowed the evaluation of the individual droplet size without flocculation [12, 13]. Determinations were conducted in duplicate and values of the standard deviations were less than 0.2. Calculation from 0.3 to 200 µm was expressed as differential volume. Distribution width (W) was expressed as:

$$W = [d(v, 0.9) - d(v, 0.1)], \quad (3)$$

where $d(v, 0.9)$ and $d(v, 0.1)$ are the 90 and 10% volume percentiles of the size distribution. The v in the expression refers to the volume distribution. Destabilization index (DI) induced by freeze-thaw or freeze-dried treatments was calculated as:

$$DI = \frac{D_{4,3T} - D_{4,3in}}{D_{4,3in}}, \quad (4)$$

$D_{4,3T}$ is the volume-weighted mean diameter ($D_{4,3}$) of emulsion after freeze-thaw or freeze-dried treatments and $D_{4,3in}$ is the volume-weighted mean diameter of initial emulsions. $D_{4,3}$ parameter, obtained from droplet size distribution expressed as differential volume was used following an approach reported in the literature [12–14]. According to these authors, $D_{4,3}$ is more sensitive to fat droplet aggregation (coalescence and/or flocculation) than Sauter mean diameter ($D_{3,2}$). Moreover, the particle size data were also reported as the volume percentage of particles exceeding 1 µm in diameter ($\%V_{d>1}$) [15].

Powder Production

Emulsions were frozen with liquid nitrogen (–190 °C) and stored over night at –80 °C before freeze-drying to allow the highest amount of freezable water to crystallize. An Heto-Holten A/S, cooling trap model CT 110 freeze-dryer (Heto Lab Equipment, Allerød, Denmark) was operated at –110 °C and at a chamber pressure of 4.10^{-4} mbar.

Solid Fat Content of Fat Phase and Powders

Equilibrium SFC determination of the 40% SFO in HMF blend was carried out by pulsed nuclear magnetic resonance in a Minispec mq20 NMR analyzer (Bruker, Karlsruhe, Germany). Samples were tempered according to the AOCS temperature treatment [16] to ensure full crystallization. Samples were run in triplicate and the values were averaged. Results are shown in Fig. 1. The actual SFC of

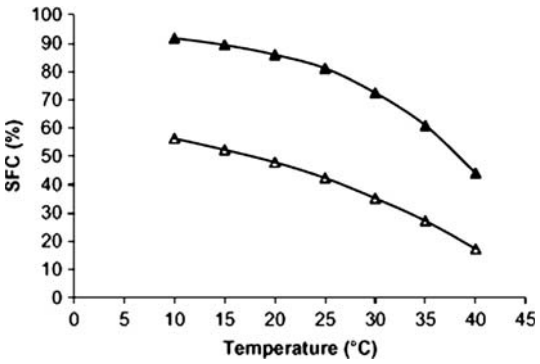

Fig. 1 Solid fat content vs. temperature for the high-melting fraction of milk fat (HMF, *dark filled triangles*) and the 40% sunflower oil (SFO, *open triangles*). Standard deviations were within the symbols size

emulsions was measured immediately after preparation and for 195 h. The SFC of freeze dried powders was also measured at ambient temperature (22.5 °C). Results are the average of three runs.

Extractable and Encapsulated Fat

The dried emulsions were broken into a powder by use of a mortar and pestle and subsequently 6 g powder was dispersed with 45 mL hexane (HPLC grade) and shaken to remove nonencapsulated fat. The soluble fraction was filtered and the solvent was evaporated, leaving the fat which then was weighted to determine the initial efficiency of encapsulation. Then, the powder, free of extractable fat, was mixed with 45 mL water and 45 mL ethanol. In addition, 3.75 mL of ammonium was added to samples with NaCas. The resulting solution was extracted with 40 mL sulfuric ether. The clear organic phase was collected and this extract containing the encapsulated fat was then dried and weighed. Initial efficiency of encapsulation was calculated as a percentage of the total fat in the dry powder.

Statistical Analysis

Significant differences between means were determined by the Student's *t*-test. An α level of 0.05 was used for significance.

Results and Discussion

Initial Emulsions

Figure 2 shows the particle size distribution for the initial emulsions ($t = 0$), the emulsions after freeze-thaw treatment and the emulsions reconstituted from freeze-dried powders for SE, NaCas, and SE/NaCas-stabilizers with SFO (a, b, and c) or the 40 wt% SFO-in-HMF blend (d, e, and f) as the fat phase. $D_{4,3}$, $\%V_{d>1}$, and W for emulsions in Fig. 2 are reported in Table 1. SFO emulsions showed trimodal distributions with a main peak around 0.5 μm regardless of the emulsifying agent selected. When crystalline material was present, size distributions were bimodal. There were no significant differences in $D_{4,3}$ between emulsions formulated with SFO or the blend with crystalline material as the fat phase when the emulsifier was NaCas ($p < 0.05$). For the SE or NaCas/SE-stabilized emulsions, $D_{4,3}$ significantly increased for the 40 wt% SFO-in-HMF blend, as fat phase compared with SFO emulsions ($p < 0.05$). In agreement with $D_{4,3}$ behavior, W did not change significantly and $\%V_{d>1}$ increased only a 7% for the presence of crystalline material when emulsion was stabilized by NaCas. For SE and SE/NaCas-stabilized emulsions, W was greater and $\%V_{d>1}$ dramatically increased when there was crystalline material ($p < 0.05$).

Figure 3 shows the BS profiles as a function of the sample height (total height = 70 mm) for emulsions in Fig. 2. The initial mean value of back scattering along the entire tube ($BS_{av\ 0}$, from BS profile at $t = 0$) for SE, NaCas, and SE/NaCas-stabilized emulsions were 59.96, 59.81, and 58.51%, respectively, when the fat phase was SFO. For the 40 wt% SFO-in-HMF blend as fat phase, $BS_{av\ 0}$ were 56.32, 56.58, and 54.10% for SE, NaCas, and SE/NaCas-stabilized emulsions, respectively. $BS_{av\ 0}$ had the lowest value for the 40 wt% SFO-in-HMF blend emulsion formulated with both emulsifiers in agreement with a greater $D_{4,3}$ value for this emulsion (Table 1). Palazolo et al. reported a similar relationship between $BS_{av\ 0}$ and $D_{4,3}$ values in whey proteins [17]. BS is a parameter directly dependent on the particle's mean diameter and on the particle volume fraction (ϕ), i.e. $BS = f(D,\phi)$ [11]. At $t = 0$, the distribution of particles is homogeneous along the entire tube and all emulsions have the same volume fraction. Therefore, $BS_{av\ 0}$ depends predominantly of mean particle diameter.

Clarification Kinetics

Emulsion stability was evaluated from the T profiles by determining the radio in percentage of serum height to total sample height ($h_t\%$) at different times. In Fig. 4, $h_t\%$ is plotted for initial emulsions to 195 h. NaCas emulsion with SFO as fat phase was very stable. No water phase was quantified during storage. SE emulsions, both with and without crystalline material in the fat phase, exhibited good stability as evidenced by the slow increase in $h_t\%$. It is well known that adding small molecule surfactants to protein stabilized emulsions can displace protein from the interface. Palanuwech and Coupland [8] reported a dramatic

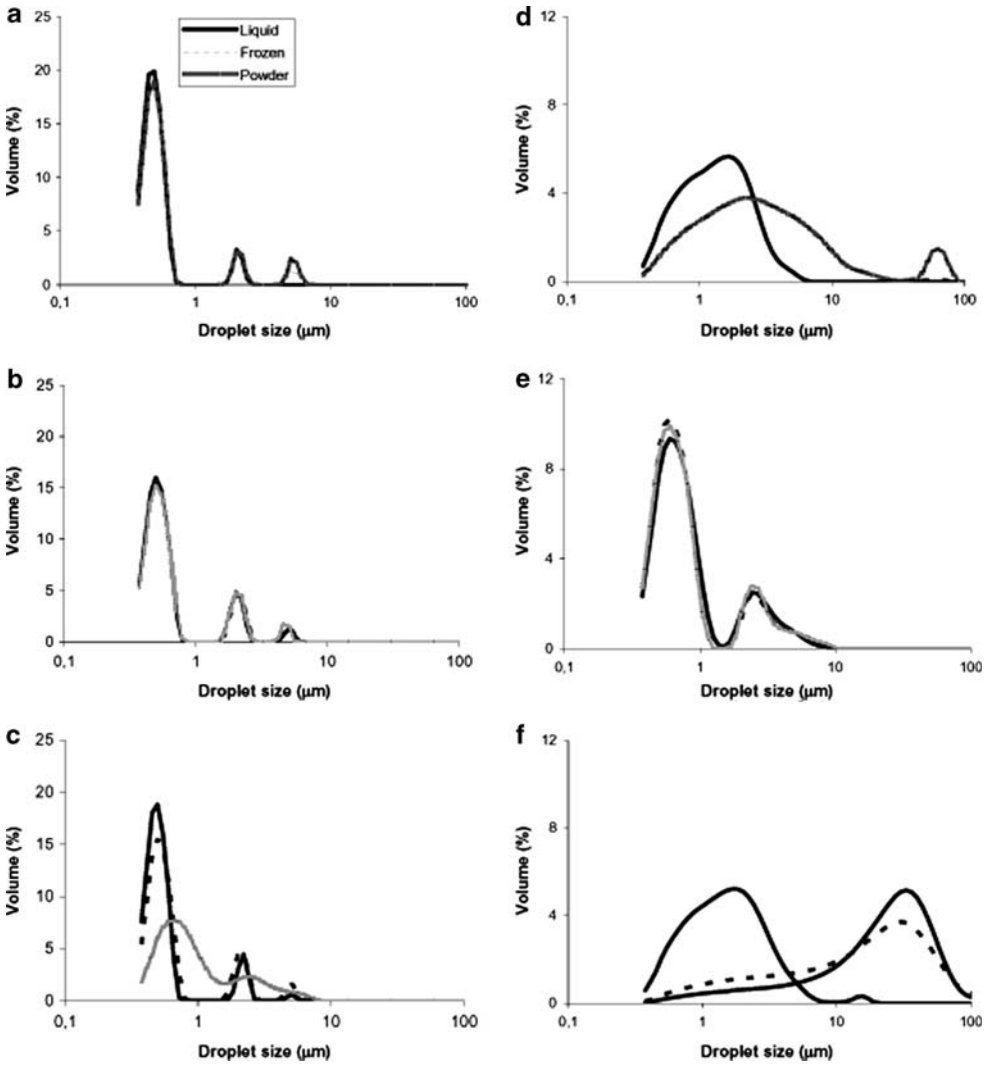

Fig. 2 Particle size distribution of initial (*solid line*), freeze thawed (*dotted line*), and reconstituted (*plotted line*) emulsions. SFO as fat phase: **a** SE, **b** NaCas, and **c** SE/NaCas stabilizers; the 40 wt. % SFO-in-HMF as fat phase **d** SE, **e** NaCas, and **f** SE/NaCas stabilizers. *SE* sucrose esters, *SFO* sunflower seed oil, *NaCas* sodium caseinate

Table 1 Volume-weighted mean diameter ($D_{4,3}$), volume percentage of particles exceeding 1 μm in diameter ($\%V_{d>1}$), and width of the distribution (W) of emulsions, emulsions after freeze thaw treatment, and emulsions reconstituted from dehydrated powders

Sample	Emulsions			After freezing			Dehydrated powders		
	$D_{4,3}$	$\%V_{d>1}$	W	$D_{4,3}$	$\%V_{d>1}$	W	$D_{4,3}$	$\%V_{d>1}$	W
SFO/SE	0.64^{a}	7.7^{b}	0.26^{d}	0.81^{a}	11.3^{c}	1.57^{e}	0.82^{a}	11.5^{c}	1.58^{e}
SFO/NaCas	1.10^{a}	17.8^{b}	1.92^{c}	0.98^{a}	19.4^{b}	1.84^{c}	0.98^{a}	19.8^{b}	1.82^{c}
SFO/SE/NaCas	0.76^{a}	12.0^{c}	1.66^{f}	0.98^{a}	19.4^{d}	1.84^{f}	1.38^{b}	39.1^{e}	2.60^{f}
40% blend/SE	1.60^{a}	67.4^{c}	2.25^{e}	4.02^{b}	83.2^{d}	8.26^{f}	4.11^{b}	83.7^{d}	7.86^{f}
40% blend/NaCas	1.25^{a}	24.8^{b}	2.53^{c}	1.24^{a}	22.9^{b}	2.49^{c}	1.25^{a}	25.3^{b}	2.50^{c}
40% blend/SE/NaCas	2.00^{a}	72.1^{d}	2.93^{f}	24.27^{b}	95.0^{e}	53.26^{g}	28.12^{c}	97.5^{e}	52.23^{g}

Values without same superscript alphabets in the same row are significantly different ($p < 0.05$)

reduction in stability when Tween 20 was added to sodium caseinate stabilized emulsions in a molar ratio greater than 25. In agreement with these results, formulation with 50 wt% blend of SE and NaCas stabilizers was not effective, since surface activity diminished as evidenced by emulsion destabilization (Fig. 4). The high gravitational destabilization rate found was independent of the selected fat phase.

It was reported that creaming rate is strongly affected by floc size and structure, a phenomenon mainly determined

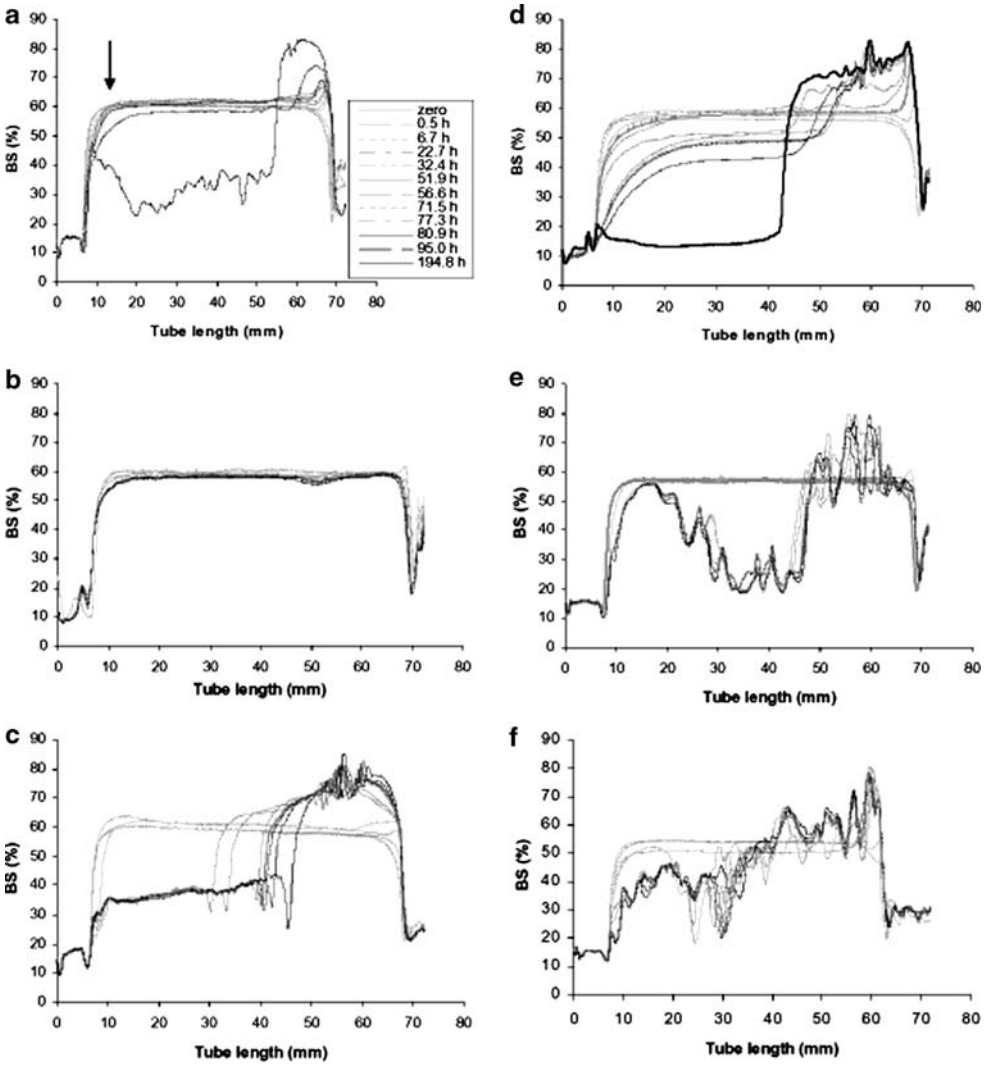

Fig. 3 Changes in backscattering (BS) profiles with storage time (*arrow* denotes time) in quiescent conditions for emulsions with SFO as fat phase: **a** SE, **b** NaCas, and **c** SE/NaCas stabilizers; and for the 40 wt% SFO-in-HMF blend as fat phase: **d** SE, **e** NaCas, and **f** SE/NaCas stabilizers. Tube length 70 mm. Abbreviations as in Fig. 2

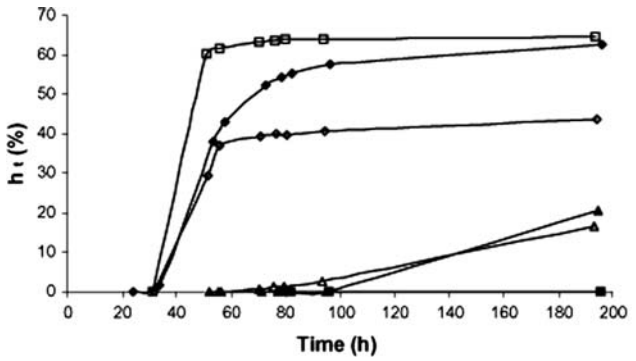

Fig. 4 Ratio in percentage of serum height to total sample height (h_t%) from transmission (T) profiles at different times (initial emulsions to 195 h) for emulsions with SFO as fat phase: SE, *dark filled triangle*; NaCas, *dark filled square*; and SE/NaCas, *dark filled diamond*, stabilizers; the 40 wt% SFO-in-HMF blend as fat phase: SE, *open triangle*; NaCas, *open square*; and SE/NaCas, *open diamond*, stabilizers. Tube length 70 mm. Abbreviations as in Fig. 1

by the nature of colloidal interactions between droplets [18]. Although phase separation rates were not significantly different between SE/NaCas emulsions with and without crystalline material, as can be noticed from the slopes in Fig. 4, a loosely packed cream layer for the fat phase with crystalline material had formed. When the 40 wt% SFO-in-HMF blend destabilized, a cream layer, which represented 56.30 ± 0.90% of total volume (Eq. 2), was formed after 195 h under quiescent conditions. When SFO was the core material, a cream layer represented a 47.25 ± 0.72% (Eq. 2), packing more tightly. This difference was not great enough to modify the gravitational destabilization rate.

The SFC values measured immediately after emulsions reached 22.5 °C for NaCas, NaCas/SE, and SE stabilizers with SFO as fat phase were 0.52 ± 0.16, 0.64 ± 0.25, 0.61 ± 0.19%, respectively. The SFC values for emulsions with the 40 wt% SFO-in-HMF blend as fat phase were

1.22 ± 0.17, 2.00 ± 0.26, and 1.60 ± 0.23%, respectively. No significant differences were found after 195 h of storage at 22.5 °C. Since fat phase was totally crystallized in the droplets immediately after reaching 22.5 °C, changes in stability during storage due to the presence of crystalline material may be caused by fat polymorphic transitions or re-crystallization. The SFC of the fat phase with crystalline material was not great enough to destabilize the SE emulsion. It was reported that addition of hydrophobic surfactants (sucrose polyesters) to the palm oil fraction of oil-in-water emulsions prior to homogenization accelerated the nucleation rate, but reduced the crystal growth rate. These additives enhanced the formation of tiny crystals and impeded morphological changes retarding the crystallization-induced destabilization [19]. In agreement with these results SE/40 wt% SFO-in-HMF blend emulsion was stable. Although NaCas showed a greater emulsifying capacity when the fat phase was SFO, presence of crystalline material in the fat phase caused great instability after 36 h of storage most likely due to changes in protein structure related to interactions between crystals and emulsifier at the interface. As evidenced by the rapid increase in h_t%, the gravitational destabilization rate was the highest for NaCas/40 wt% SFO-in-HMF blend emulsion (Fig. 4).

Global Stability

To study the global stability of emulsions the BS profiles were analyzed at different storage times. These profiles constitute the macroscopic fingerprint of the emulsion sample at a given time [11]. The creaming destabilization process describes the upward movement of droplets because the droplets have a lower density than the surrounding liquid. This destabilization mechanism may be noticed from the decrease in BS mean value (BS_{av}) at the bottom of the tube and a concomitant increase of BS_{av} in the upper zone attributed to the formation of a cream layer [11, 17]. Coalescence is the process whereby two or more liquid droplets merge together to form a single larger droplet. This process may induce a decrease in BS_{av} as a consequence of an increment in the mean diameter particle [11, 13]. On the other hand, when serum phases have a high clarification degree, the BS_{av} increases because of the predominance of glass/suspension interface reflections [11]. Therefore, BS_{av} variation as a function of time was analyzed in the middle zone of the tube (20–27 mm zone for all emulsions and 30–40 mm in SE/NaCas/SFO-in-HMF emulsion) where no significantly transmitted light was detected.

The SE/SFO emulsion showed no changes in BS profiles until 36 h of storage. Then, destabilization started with creaming as shown by a slow shift in the front of the BS profile. This was more evident in the 94 h BS profile; BS_{av} in the 20–30 mm-zone diminished while BS increased in the upper zone of the tube (Fig. 3). However, the clarification degree was still low after 94 h since the serum phase was still optically opaque and no light reached the transmission detector ($h_t\% \approx 0$, Fig. 4). The properties of the emulsions changed markedly at 195 h. The BS profile exhibited an irregular shape and a great decrease of BS_{av} in the zone 20–30 mm (59.98 ~ 25.74), probably attributed not only to the creaming but to the coalescence process. The presence of coalesced droplets may accelerate the gravitational separation, in concordance with the clarification degree in Fig. 4 ($h_t\% \approx 20$). When there was a crystalline phase (SFO-in-HMF as fat phase), the SE emulsion started to destabilize by creaming after 30 h of storage (Fig. 3). No light reached the transmission detector until 75 h. For this period, the serum phase remained optically opaque (Fig. 4). The droplet migration in this emulsion was faster than in the emulsion with SFO in the fat phase, probably attributable to its greater $D_{4,3}$ value (Table 1). At 195 h a high level of destabilization was observed ($BS_{av} < 15\%$ $h_t\% \approx 20$).

The NaCas/SFO emulsion was the most stable. No significantly changes were found in BS (Fig. 3) or h_t% profiles (Fig. 4) during 195 h. The presence of crystalline phase in oil droplets significantly changed the behavior of the system. For NaCas/SFO-in-HMF emulsion destabilization by creaming started slowly after 20 h of storage. The BS profile of 50 h showed a dramatic change in stability, with an important decrease of BS_{av} (56.62 ~ 44.80). Most likely, droplets aggregated and the emulsion was destabilized by partial coalescence [8]. Moreover, the presence of highly coalesced droplets may contribute to the irregular shape of BS profiles at $t > 50$ h. These droplets migrated from the bottom to the top of the tube as may be noticed from the BS profile, as an increment of BS in the upper zone of the tube (Fig. 3). As previously mentioned, although NaCas emulsions showed similar initial $D_{4,3}$ values (Table 1, $p < 0.05$), the presence of HMF in the fat phase favored destabilization.

The SE/NaCas/SFO emulsion was stable up to 33 h of storage. Then, it destabilized by creaming as may be noticed by a decrease in the BS_{av} at the bottom of the tube (59.58 $\rightarrow$ 38.43) and a concomitant increase in BS at the top of the tube. At $t > 33$ h, the clarification degree was very high ($h_t\% > 30$, Fig. 4). With SFO-in-HMF fat phase, destabilization by creaming started after 30 h of storage (Fig. 3). Then, BS profiles had irregular shapes as for NaCas stabilized-emulsion which may be attributed to aggregate formation due to partial coalescence destabilization. These aggregates accelerate the droplet migration to the top of the tube (Fig. 2). Transmitted light reached the detector after 30 h in agreement with the high degree of clarification shown in Fig. 4.

Particle Size Distribution of Freeze Thawed and Reconstituted Emulsions

When the fat phase was SFO, $D_{4,3}$ did not change significantly after freeze thawing or freeze-drying for SE or NaCas-stabilized emulsions (Table 1). The DI was 0.27 and 0.00, respectively, for both processes. For SE/NaCas-stabilized emulsion $D_{4,3}$ significantly increased after freeze drying. The DI was 0.81. *W* did not change after both processes but in the case of the SE-stabilized emulsion it showed a significant change. When the fat phase contained crystalline material, as it did for the 40 wt. % SFO-in-HMF blend, $D_{4,3}$ and *W* showed different behaviors which depended upon the emulsifier selected. For NaCas-stabilized emulsions there were no significant differences after freeze thawing or freeze drying. The DI was 0.00 in both processes. Thanasukarn et al. [20] reported that even relatively small amounts of sucrose (>5 wt%) added to the continuous phase of oil-in-water emulsions greatly improved their freeze-thaw stability. According to Thanasukarn et al. [20], sucrose increased the fraction of unfrozen water present in the emulsions preventing droplets from being together. In addition, sucrose may protect proteins from dehydration, reduce the tendency of associating with each other and increase the conformational stability. Sucrose may also alter ice crystal nucleation and growth. The ice crystals formed could be less likely to disrupt the interfacial membranes surrounding the oil droplets. In agreement with their results, trehalose prevented oil particle growth in a NaCas/40 wt% SFO-in-HMF blend emulsion. For SE and SE/NaCas-stabilized emulsions, however, significant differences in $D_{4,3}$ and *W* were found after freeze thawing and freeze drying (Table 1). DI were 1.52 and 1.57 (SE-stabilized emulsions), and 11.13 and 13.05 (SE/NaCas-stabilized emulsions). $\%V_{d>1}$ dramatically increased for these emulsions when both the oil and water phases crystallized.

The SFC of the powders at ambient temperature (22.5 °C) were 74.89 ± 0.26, 74.92 ± 0.21, and 75.01 ± 0.14%, for SE, NaCas, and SE/NaCas stabilizers with SFO as fat phase, respectively, and 82.79 ± 0.31, 82.25 ± 0.27, and 83.76 ± 0.15%, for the 40 wt% SFO-in-HMF fat phase, respectively, which means that the amount of crystalline fat in the powders was 7.9, 7.3, and 8.7%, respectively. Although the values were similar, particles did not grow after freeze drying in the NaCas emulsion as happened for SE and SE/NaCas-stabilized emulsions. This was in agreement with the reported effect of stabilization provided by some sugars such as sucrose in protein-stabilized emulsions [20].

Effects of All Factors on Retention

When SFO emulsions were encapsulated by freeze drying, initial retention values of the core material were 96.7 ± 0.4, 97.0 ± 0.1, and 97.4 ± 0.8%, for SE, NaCas, and SE/NaCas powders, respectively, while for the 40 wt% SFO-in-HMF blend they were 64.9 ± 0.8, 89.7 ± 0.1, and 55.5 ± 1.0%, respectively. The presence of crystalline material markedly increased the particle size of SE and SE/NaCas powders, which was in agreement with a reduced initial retention of core material found after freeze drying. NaCas/40 wt% SFO-in-HMF blend formulation had a particle size distribution that did not change significantly from emulsion to powder ($p < 0.05$). Particle size remained small as when the core material was SFO. Although the encapsulation efficiency was 7.3% lower than the corresponding SFO emulsion, it was significantly higher than the initial retention measured for 40 wt% SFO-in-HMF blend/SE or SE/NaCas powders.

All emulsions were stable for the first 30 h after preparation (Fig. 4). Then, NaCas with 40 wt% SFO-in-HMF blend, and SE/NaCas emulsions both with SFO or the blend with crystalline material as fat phase, rapidly destabilized. The initial efficiency of encapsulation was the lowest for SE/NaCas/40 wt% SFO-in-HMF blend emulsion. SE/SFO, SE/40 wt% SFO-in-HMF blend and NaCas/SFO emulsions were very stable as evidenced by the low value of h_t% after 195 h (Fig. 4). Although SE/40 wt% SFO-in-HMF blend emulsion was stable, the initial efficiency of encapsulation was low in agreement with the growth of particles during freeze-drying and the presence of crystalline material in the core.

Encapsulation properties were determined by the counteracting effects of particle size and distribution, the presence of crystalline material in the core and interactions among interface components, sugar in the aqueous phase and core material. In addition, retention was less related to emulsion stability. These emulsions were stable for at least 30 h which proved to be enough to obtain a high degree of encapsulation.

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References

1. Rosenberg M, Kopelman IJ, Talmon Y (1990) Factors affecting retention in spray-drying microencapsulation of volatile materials. J Agric Food Chem 38:1288–1294
2. Onwulata C, Smith PW, Criag JC Jr, Holsinger VH (1994) Physical properties of encapsulated spray-dried milkfat. J Food Sci 59:316–320
3. Labrousse S, Roos Y, Karel M (1992) Collapse and crystallization in amorphous matrices with encapsulated compounds. Sci Aliments 12:757–769

4. Wagner LA, Warthesen JJ (1995) Stability of spray-dried encapsulated carrot carotenes. J Food Sci 60:1048–1053
5. Fäldt P, Bergenst?hl B (1995) Fat encapsulation in spray-dried food powders. J Am Oil Chem Soc 72:171–176
6. Keogh MK, O'Kennedy BT (1999) Milk fat microencapsulation using whey proteins. Int Dairy J 9:657–663
7. Hogan SA, McNamee BF, O'Riordan ED, O'Sullivan M (2001) Microencapsulating properties of sodium caseinate. J Agric Food Chem 49:1934–1938
8. Palanuwech J, Coupland JN (2003) Effect of surfactant type on the stability of oil-in-water emulsions to dispersed phase crystallization. Colloids Surf A Physicochem Eng Asp 223:251–262
9. Orthoefer FT (1997) Applications of emulsifiers in baked foods. In: Hasenhuettl GL, Hartel RW (eds) Food emulsifiers and their applications. Chapman & Hall, New York, pp 211–254
10. Pan LG, Tomás MC, Añón MC (2002) Effect of sunflower lecithins on the stability of water-in-oil and oil-in-water emulsions. J Surfactants Deterg 5:135–143
11. Mengual O, Meunier G, Cayré I, Puech K, Snabre P (1999) Turbiscan MA 2000: multiple light scattering measurement for concentrated emulsion and suspension instability analysis. Talanta 50:445–456
12. Anton M, Beaumal V, Brossard C, Llamas G, Le Denmat M (2002) Droplet flocculation and physical stability of oil-in-water emulsions prepared with hen egg yolk. Recent Res Dev Agric Food Chem 37:15–28
13. Palazolo GG, Sorgentini DA, Wagner JR (2005) Coalescence and flocculation in o/w emulsions of native and denatured whey soy proteins in comparison with soy protein isolates. Food Hydrocolloids 19:595–604
14. Relkin P, Sourdet S (2005) Factors affecting fat droplet aggregation in whipped frozen protein-stabilized emulsions. Food Hydrocolloids 19:503–511
15. Thanasukarn P, Pongsawatmanit R, McClements DJ (2006) Utilization of layer-by-layer interfacial deposition technique to improve freeze-thaw stability of oil-in-water emulsions. Food Res Int 39:721–729
16. AOCS (1999) Official methods of the American oil chemists' society, 5th edn. AOCS, Champaign. Method Cd 16b-93 (revised 1997)
17. Palazolo GG, Sorgentini DA, Wagner JR (2004) Emulsifying properties and surface behavior of native and denatured whey soy proteins in comparison with other proteins. Creaming stability of oil-in-water emulsions. J Am Oil Chem Soc 81:625–632
18. Pinfield VJ, Dickinson E, Povey MJW (1997) Modeling of combined creaming and flocculation in emulsions. J Colloid Interface Sci 186:80–89
19. Arima S, Ueji T, Ueno S, Ogawa A, Sato K (2007) Retardation of crystallization-induced destabilization of PMF-in-water emulsion with emulsifier additives. Colloids Surf B Biointerfaces 55:98–106
20. Thanasukarn P, Pongsawatmanit R, McClements DJ (2004) Impact of fat and water crystallization on the stability of hydrogenated palm oil-in-water emulsions stabilized by whey protein isolate. Colloids Surf A Physicochem Eng Asp 246:49–59