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Stability of emulsions formulated with high concentrations of sodium caseinate and trehalose

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ABSTRACT

Stability of emulsions formulated with 10 wt.% oil (concentrated fish oil, CFO, sunflower oil, SFO, or olive oil, OO), sodium caseinate concentrations varying from 0.5 to 5 wt.%, giving oil-to-protein ratios of 20–2, and 0, 20, 30 or 40 wt.% aqueous trehalose solution was studied by Turbiscan. Particle size distribution, microstructure, and small angle X-ray scattering (SAXS) patterns were also obtained. The main mechanism of destabilization in a given formulation strongly depended on oil-to-protein ratio. As evidenced by the *BS*-profile changes with time, emulsions formulated with 0.5 and 1 wt.% NaCas destabilized mainly by creaming while for the 2 wt.% NaCas concentration, both creaming and flocculation mechanisms, were involved. The main destabilization mechanism for the 3, 4 or 5 wt.% NaCas emulsions was flocculation. Stability of emulsions was also affected by the content of trehalose in the aqueous phase. Trehalose diminished the volume-weighted mean diameter ($D_{4,3}$) and greatly improved stability.

1. Introduction

Physical instability results in an alteration in the spatial distribution or structural organization of the molecules. Creaming, flocculation, coalescence, partial coalescence, phase inversion, and Ostwald ripening are examples of physical instability. The development of an effective strategy to prevent undesirable changes in the properties of a particular food emulsion depends on the dominant physicochemical mechanism(s) responsible for the changes. In practice, two or more of these mechanisms may operate in concert. It is therefore important for food scientists to identify the relative importance of each mechanism, the relationship between them, and the factors that influence them, so that effective means of controlling the stability and physicochemical properties of emulsions can be established (McClements, 2005).

Most dispersed multiphase systems are thermodynamically unstable per se and thus require stabilization. In the food industry, actually stabilization of emulsions is obtained by the addition of proteins. Proteins can stabilize emulsions by lowering the interfacial tension due to their adsorption at the interface. Besides, proteins can form films at the interface, providing electrostatic and steric repulsion between droplets (van Aken & van Vliet, 2002). Sodium caseinate is widely used as an ingredient in the food industry due to its functional properties, which include emulsification, water and fat-binding, thickening and gelation (Mulvihill, 1989). The addition of this protein has a double stabilizing effect since it provokes together with other added surfactants an increase of both viscosity of the continuous phase and stability of the interface. Dickinson and Golding (1997a) studying the efficiency of sodium caseinate in stabilizing and destabilizing oil-in-water emulsions found that there exists an optimum protein concentration producing good saturation surface coverage and maximum emulsion stability; smaller amounts of protein are associated with lower surface coverage leading to bridging flocculation and coalescence, while a considerable excess of unadsorbed protein causes depletion flocculation. Dickinson (1999) reported that at even higher protein contents, there is partial restabilization of the flocculated emulsion in the form of a strong particle gel network. Recent studies in sodium caseinate-stabilized emulsions described the behavior for high oil-to-protein ratios (Day, Xu, Hoobin, Burgar, & Augustin, 2007). However, it is also interesting to further characterize emulsions with higher protein concentrations since new protein enriched foods are manufactured now a day.

N-3 Polyunsaturated fatty acids have been shown to have potential beneficial effects for chronic diseases including cancer,

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insulin resistance and cardiovascular disease. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in particular have been studied extensively; however the specific biological effects of alpha-linolenic acid (ALA) are largely unknown. ALA is by far the predominant form of N-3 polyunsaturated fatty acids (PUFA) consumed in some diets such as the typical North American diet, while intakes of EPA and DHA are typically very low. Besides, ALA conversion to EPA and DHA is minimal (Anderson & Ma, 2009). The fish oil used in this study is a commercial ingredient for the food industry. It is a concentrate from fish oil, mainly salmon, which is recommended for formulation of functional foods such as chocolate, yogurt, jam, pastry filling, salad oil, mayonnaise and dressings. The use of this oil permits food manufacturers to ensure a controlled content of omega-3, EPA, DPA and DHA fatty acids, in the final products and supplies vitamin E. Thus, regarding some of these applications it is very interesting to study its behavior in emulsion systems.

Among saccharides, trehalose is particularly effective in terms of its ability to preserve and maintain activity of biomolecules. This sugar has no toxicity and is currently being used in several industries, such as food, medical, cosmetic, and other bio-industries, to preserve biomaterials under dry conditions against evaporation, oxidation, and chemical reaction. Encapsulation properties are in some extent related to the stability of emulsions before dehydration (Cerdeira, Palazolo, Candal, & Herrera, 2007). N-3 polyunsaturated fatty acids are susceptible to oxidation, thus a sugar that has performed well for retaining lipid materials in freeze-drying was selected.

Emulsions have been studied by numerous techniques, such as particle sizing, microscopy, rheology, among others, to characterize their physical properties. Most of these techniques involve some form of dilution. This dilution disrupts some structures that contribute to destabilization. The ability to study the stability of food emulsions in their undiluted forms may reveal subtle nuances about their stability. A relatively recently developed technique, the Turbiscan method, allows scan the turbidity profile of an emulsion along the height of a glass tube filled with the emulsion, following the fate of the turbidity profile over time. The analysis of the turbidity profiles leads to quantitative data on the stability of the studied emulsions and allows making objective comparisons between different emulsions (Chauvierre, Labarre, Couvreur, & Vauthier, 2004).

The aim of the present work was to investigate the effect of sodium caseinate or sugar concentrations and fat phase composition on the stability of emulsions formulated with concentrated fish oil, sunflower or olive oils as fat phase. Creaming and flocculation kinetics were quantified analyzing the samples with a Turbiscan MA 2000.

2. Materials and methods

2.1. 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) were used without any further purification. HPLC water was used for all experimental work. Sodium caseinate (NaCas) was obtained from ICN (ICN Biomedical, Inc., Aurora, Ohio, USA) and used without any further purification. Fat phase was commercial sunflower oil (SFO), olive oil (OO) and a commercial blend of fish oils (CFO) used as ingredient in the food industry in Chile. SFO main fatty acids were identified as C16:0, C18:0, C18:1, and C18:2 with percentages of 6.7%, 3.6%, 21.9%, and 66.3%, respectively. OO main fatty acids were C16:0, C18:0, C18:1, and C18:2 with percentages of 12.1%, 2.6%, 78.3%, and 5.4%, respectively. CFO fatty acids in greater proportions were C14:0, C15:0, C16:1, C17:0 branched, C17:0 linear, C18:0, C20:5, C20:4, C20:2, C20:1, C22:6, C22:5, C22:1, C24:1 in percentages of 11.81%, 2.64%, 2.08%, 2.16%, 2.39%, 6.94%, 10.90%, 4.58%, 3.66%, 7.65%, 7.96%, 5.21%, 5.66%, 2.06%.

2.2. Emulsion preparation

Aqueous phase was a 0, 20, 30 or 40 wt/wt.% solution of trehalose, a known cryoprotectant. Three oil phases were used: commercial SFO, OO and CFO. All emulsions were formulated with a 10 wt/wt.% fat phase. NaCas was used as emulsifier at 0.5, 1, 2, 3, 4, and 5 wt/wt.%, giving oil-to-protein ratios of 20–2. Emulsions were kept at 60 °C during preparation. Then, fat and aqueous phases were mixed using an Ultra-Turrax T8 high speed blender (S 8N-5G dispersing tool, IKA Labortechnik, Janke & Kunkel, GmbH & Co., Staufen, Germany), operated at 20,000 rpm for 1 min. The resultant pre-emulsions were further homogenized for 20 min using an ultrasonic liquid processing VIBRA CELL, VCX model (Sonics & Materials, Inc., Newtown, CT, USA). The temperature of sample-cell was controlled by means of a water bath set at 15 °C. By doing this, sample temperature was around 40 ± 1 °C during ultrasound treatment. Then, they were cooled quiescently to ambient temperature (22.5 °C). Subsequently they were analyzed for particle size distribution, stability in quiescent conditions and microstructure. The pHs of the CFO, SFO, and OO emulsions were 6.63 ± 0.05, 6.66 ± 0.05, and 6.71 ± 0.05, respectively. No buffer was added to emulsions. Experiments were done in duplicate and results were averaged.

2.3. Particle size analysis

The particle size distribution of emulsions was determined immediately after emulsion preparation and after a week of storage at 22.5 °C from the middle part of Turbiscan tube (only when emulsions remained fully turbid) by light scattering using a Mastersizer 2000 with a Hydro 2000MU as dispersion unit (Malvern Instruments Ltd., UK). The pump speed was settled at 1800 RPM. Refraction index for the oil phase was 1.4694. Determinations were conducted in duplicate and values of standard deviations were less than 0.2 μm. Calculation from 0.1 to 10 μm was expressed as differential volume. Distribution width (W) was expressed as:

$$W = [d(v, 0.9) - d(v, 0.1)] \quad (1)$$

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. $D_{4,3}$ parameter, the volume-weighted mean diameter of initial emulsions, obtained from droplet size distribution expressed as differential volume, is more sensitive to fat droplet aggregation (coalescence and/or flocculation) than Sauter mean diameter ($D_{3,2}$) (Relkin & Sourdet, 2005). Moreover, the particle size data were also reported as the volume percentage of particles exceeding 1 μm in diameter ($\%V_{d>1}$) (Thanasukarn, Pongsawatmanit, & McClements, 2006).

2.4. Emulsion stability

The emulsion stability was analyzed using a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France) which was described elsewhere (Pan, Tomás, & Añón, 2002). This equipment allows the optical characterization of any type of dispersion (Mengual, Meunier, Cayré, Puech, & Snabre, 1999). 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 backscattering detector receives the light back-scattered by the sample

(135°). The samples were put in a flat-bottomed cylindrical glass measurement cell and scanned from the bottom to the top in order to monitor the optical properties of the dispersion along the height of the sample placed in the cell. The backscattering (*BS*) and transmission (*T*) profiles as a function of the sample height (total height = 60 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 (Mengual et al., 1999). Thus, by repeating the scan of a sample at different time intervals, the stability or the instability of dispersions can be study in detail. The profiles allow calculation of either creaming, sedimentation, or phase separation rates, as well as flocculation, and the mechanism making the dispersion unstable can be deduced from the transmission or the backscattering data (Chauvierre, Labarre, Couvreur, & Vauthier, 2004). Measurements were performed immediately after preparation of emulsions and at different times for a week.

The curves obtained by subtracting the *BS* profile at $t = 0$ from the profile at $t(\Delta BS = BS_t - BS_0)$, display a typical shape that allows a better quantification of creaming, flocculation and other destabilization processes.

Creaming was detected using the Turbiscan as it induced a variation of the concentration between the top and the bottom of the cell. The droplets moved upward because they had a lower density than the surrounding liquid. When creaming take place in an emulsion, the ΔBS curves show a peak at heights between 0–20 mm. The variation of the peak wide, at a fixed height, during the studied time, can be related to the kinetics of migration of small particles (Mengual et al., 1999). The creaming destabilization kinetics was evaluated by measuring the peak thickness at 50% of the height at different times (bottom zone). The slope of the linear part of a plot of peak thickness vs. *t* gives an indication of the migration rate.

BS mean values (BS_{av}) change with the increase in particle size. Flocculation was followed by measuring the BS_{av} as a function of storage time in the middle zone of the tube. As was theoretically demonstrated by Mengual et al. (1999), the *BS* intensity decreased as the particle size increased (when particle size is higher than the wavelength (λ) of the incident light). It should be mentioned that if the particle size is lower than λ of the incident light, *BS* increase with particle size. This phenomenon was used by several authors to determine flocculation kinetics (Chauvierre et al., 2004; Palazolo, Sorgentini, & Wagner, 2005). The optimum zone was the one no affected by creaming (bottom and top of the tube), that is, the 20–50 mm zone.

2.5. Microstructure

The Olympus FV300 (Olympus Ltd, London, UK) confocal laser scanning microscope (CLSM) with a Ar gas laser ($\lambda = 488$ nm) was used to collect the images. A 10X ocular was used, together with a 60X objective for a visual magnification of 600X. The laser intensity used was 20%. Images were recorded by using confocal assistant Olympus Fluoview versión 3.3 software provided with the FV300 CLSM.

2.6. X-ray studies

The SAXS measurements were made at the DO2A-SAXS2 beamline of the Synchrotron National Laboratory (LNLS, Campinas, Brazil) with a 1.488 Å wavelength. The scattering intensity distributions as a function of scattering angle (2θ) were obtained in the 2θ range between 0.094° and 0.732°. SAXS patterns were recorded with an exposure time of 30 s. A MARCCD 2D detector was used with 2014.73 mm sample detector distance. One-dimensional curves were obtained by integration of the 2D data using the program FIT-2D. Emulsions systems were kept at 60 °C for 5 min, and then mounted on the X-ray cell, placed in a horizontal position, and cooled by using a temperature-controlled sample holder. SAXS patterns were recorded at a constant temperature of 8 °C. The area under the SAXS peak was integrated using commercial software. The diffraction profiles were fitted to a Gaussian equation and the normalized integrated intensity was plotted as a function of reciprocal lattice spacing *q*, where $q = 2\pi/d = 4\pi \sin(\theta)/\lambda$, where *d* is the interplanar spacing and 2θ is the Bragg angle.

2.7. Statistical analysis

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

3. Results

3.1. Emulsions without trehalose

Fig. 1 shows the particle size distribution for the CFO emulsions formulated without trehalose and stabilized by different concentrations of NaCas (0.5–5 wt.%, a–f). $D_{4,3}$, $\%V_{d>1}$, and *W* for emulsions in Fig. 1 are reported in Table 1. When the same processing conditions were used, CFO emulsions showed a monomodal distribution regardless of NaCas concentration. However, 0.5 wt.% NaCas had Gaussian shape while increasing emulsifier led to distributions with a shoulder indicative of a second population. For 5 wt.% NaCas the distribution was almost monomodal with a very small shoulder on the right (f). Distribution in f was quite similar in shape to the one in a. However, $D_{4,3}$, $\%V_{d>1}$, and *W* significantly decreased as NaCas concentration increased ($p < 0.05$) indicating that protein concentration limited the fat globule size in this concentration range.

Fig. 2 shows the *BS* profiles as a function of the sample height (total height = 65 mm) for emulsions in Fig. 1. The initial mean value of back scattering along the entire tube (BS_{av0}, from *BS* profile at $t = 0$) for concentrations of NaCas of 0.5, 1, 2, 3, 4, and 5 wt.% were 82.06%, 80.32%, 77.99%, 77.20%, 76.28%, and 75.92%, respectively. *BS* is a parameter directly dependent on the particle's mean diameter and on the particle volume fraction (ϕ), i.e. $BS = f(D, \phi)$. It was expected that at $t = 0$, the distribution of particles was homogeneous, all emulsions had the same volume fraction and BS_{av0} depended predominantly of mean particle diameter. Clearly *BS* values corresponded to the initial particle size (Table 1): the smaller $D_{4,3}$ the lower *BS*. As was mentioned before, in general but not always, the *BS* flux increases with the particle mean diameter when the particles are smaller than the incident wavelength and it decreases with the mean diameter for particles larger than the incident wavelength (Mengual et al., 1999). The relevant point is that the *BS* value always change (moves up or down) with particle size.

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 (Mengual et al., 1999). The main mechanism of destabilization in a given formulation depended on NaCas concentration (Fig. 2). Emulsions formulated with 0.5 and 1 wt.% NaCas destabilized mainly by creaming (a, b). It is noticed from the decrease in BS_t at the bottom of the tube and a concomitant increase of BS_t in the upper zone attributed to the formation of a cream layer. The top of the profile had a shape indicative of the migration of individual particles. However, the clarification degree was still low after 190 h since the serum phase was still optically opaque and no light reached the transmission detector (transmission $(h_t)\% \approx 0$). All emulsions remained fully turbid along the tube during a week of

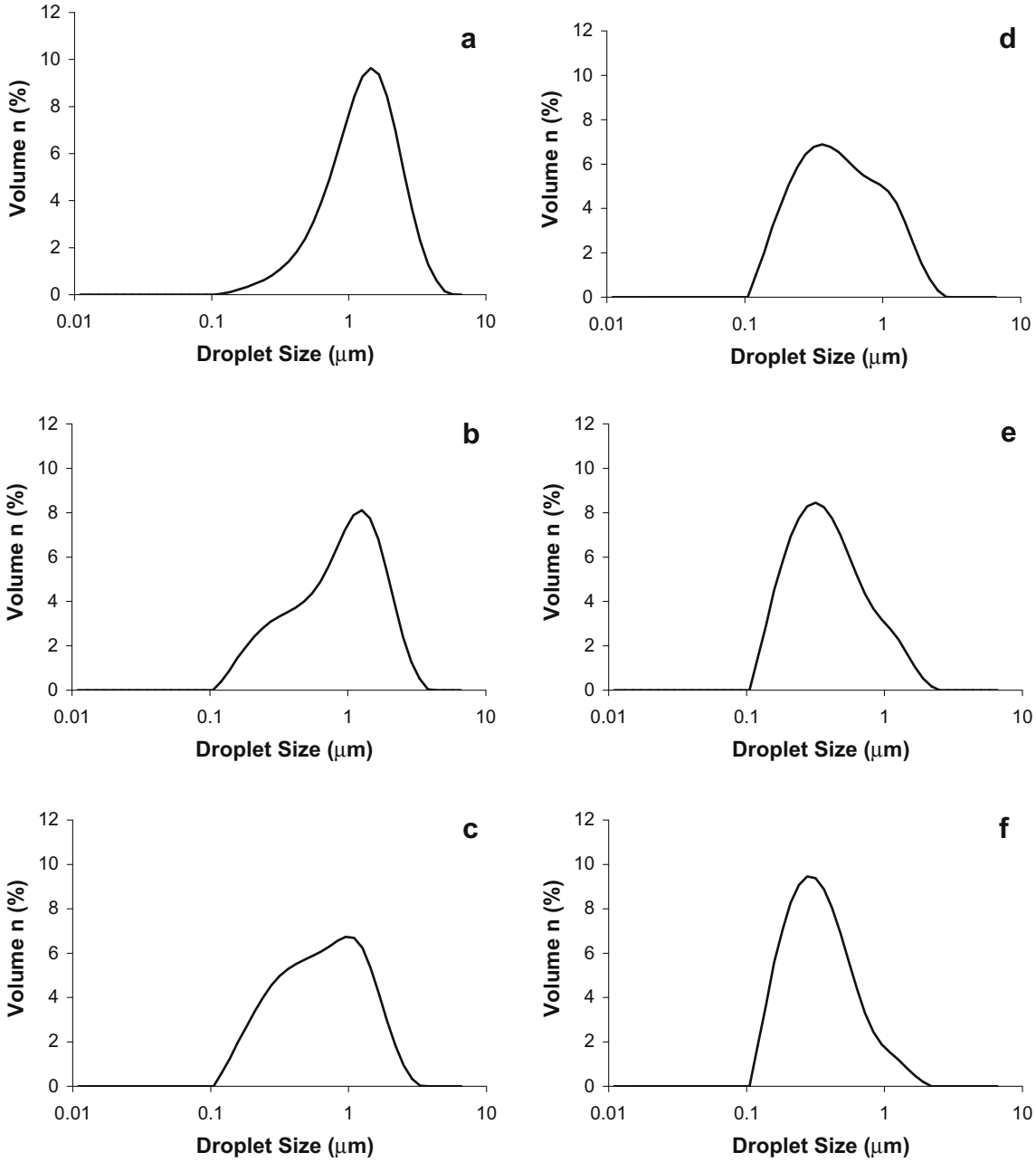

Fig. 1. Particle size distribution of emulsions with CFO as fat phase, no trehalose added to the aqueous phase, and different concentrations of sodium caseinate: (a) 0.5 wt.%, (b) 1.0 wt.%, (c) 2.0 wt.%, (d) 3.0 wt.%, (e) 4.0 wt.%, and (f) 5.0 wt.%. CFO concentrated fish oil.

storage at 22.5 °C. Therefore, the transmission profiles were not reported in this study.

For the 2 wt.% NaCas emulsion, both creaming and flocculation mechanisms, were involved. The *BS* profile showed that creaming process occurred during the first 28 h. The profiles displayed in the reference mode (not showed) had a peak at the bottom of the tube typical of migration of small particles. Then, there was a great increase in *BS* at the top of the tube which suggested migration of aggregates/flocculates. In addition, during that time, there was also a noticeable decrease in BS_{av} indicative of an increment in the mean diameter particle. This behavior may be associated to coalescence or flocculation. To distinguish both alternatives $D_{4,3}$ was measured after a week at 22.5 °C in the middle zone of the tube (20–50 mm). The obtained value was smaller than emulsion initial value (Table 1). Most likely aggregates were separated in the light scattering equipment as consequence of shear forces, exerted during the measurement of particle size in the Mastersizer, which will be sufficient to break-up the weak reversible flocculation. This behavior was previously observed in caseinate-stabilized oil-in-water emulsions (Dickinson & Golding, 1997b). In addition greater particles migrated to the top of the tube as evidenced by the shape of the profile and therefore the $D_{4,3}$ measured after a week in the 20–50 mm zone was smaller. $D_{4,3}$ value supported that destabilization occurred by flocculation and not by coalescence.

As evidenced by the *BS*-profile changes with time, the main destabilization mechanism for emulsions stabilized by 3, 4 or 5 wt.% NaCas was flocculation. The *BS* profiles for the three emulsions did not show a peak in *BS* at the bottom of the tube when they were displayed in the reference mode which indicated that there was no migration of small particles before emulsion floccula-

Table 1
Volume-weighted mean diameter ($D_{4,3}$, μm), volume percentage of particles exceeding 1 μm in diameter ($\%V_{d>1}$), and width of the distribution (W) of emulsions formulated with concentrated fish oil (CFO) as fat phase and different concentrations of trehalose or NaCas immediately after preparation and after a week at 22.5 °C (middle zone of the tube 20–50 mm).

Sample	Emulsions			After 1 week of storage		
	$D_{4,3}$	$\%V_{d>1}$	W	$D_{4,3}$	$\%V_{d>1}$	W
Without trehalose						
NaCas 0.5 wt.%	1.35	64.47	1.87	4.94	47.44	15.90
1.0 wt.%	0.95	36.19	1.57	4.34	33.14	15.34
2.0 wt.%	0.73	21.79	1.25	0.54	4.37	0.43
3.0 wt.%	0.58	12.73	1.02	0.36	5.00	0.50
4.0 wt.%	0.44	5.73	0.73	0.26	1.21	0.24
5.0 wt.%	0.37	2.71	0.52	0.26	0.00	0.29
20 wt.% trehalose						
NaCas 0.5 wt.%	0.88	29.50	1.52	0.76	22.41	1.16
1.0 wt.%	0.85	27.37	1.31	0.76	20.09	1.03
2.0 wt.%	0.63	13.96	1.01	0.47	6.66	0.73
3.0 wt.%	0.52	8.02	0.82	0.37	3.60	0.47
4.0 wt.%	0.42	3.68	0.61	0.30	0.00	0.35
5.0 wt.%	0.35	1.17	0.45	0.34	1.4	0.44
30 wt.% trehalose						
NaCas 0.5 wt.%	0.82	23.08	1.58	0.63	17.01	1.19
1.0 wt.%	0.86	23.51	1.35	0.78	21.41	1.08
2.0 wt.%	0.56	9.78	0.88	0.44	4.06	0.56
3.0 wt.%	0.43	3.79	0.60	0.33	1.25	0.39
4.0 wt.%	0.35	1.18	0.44	0.41	2.67	0.56
5.0 wt.%	0.30	0.43	0.36	0.30	0.42	0.36
40 wt.% trehalose						
NaCas 0.5 wt.%	0.80	16.77	1.35	0.60	15.17	1.18
1.0 wt.%	0.67	15.11	1.10	0.64	14.46	1.06
2.0 wt.%	0.49	5.87	0.69	0.48	5.58	0.68
3.0 wt.%	0.39	2.63	0.49	0.38	2.18	0.47
4.0 wt.%	0.31	0.84	0.36	0.32	0.68	0.37
5.0 wt.%	0.29	0.00	0.33	0.29	0.00	0.33

tion. On the contrary, there was a dramatic change in stability with an important decrease in BS_{av} indicative of flocculation. The shape of the profile at the top of the tube was a consequence of the migration of flocs formed during flocculation. Thus, the mechanism of destabilization in this case was flocculation followed by creaming of flocs. $D_{4,3}$ of 3, 4, and 5 wt.% NaCas emulsions measured after a week at 22.5 °C (Table 1) confirmed the mechanism. Surprisingly, as shown in Fig. 2, destabilization occurred in lesser extent as NaCas concentration increased. The 5 wt.% NaCas emulsion proved to be the most stable of the three formulations. It was reported that the presence of excessive caseinate in an emulsion containing a high concentration of protein can induce depletion flocculation of emulsion droplets, resulting in creaming due to the strong tendency of the casein submicelles to form small protein particles (Dickinson & Golding, 1997b). This was the behavior observed for the 2 wt.% emulsion. However, further addition of NaCas increased both NaCas concentration in the aqueous phase and protein at the interface since $D_{4,3}$ decreased with NaCas concentration. Thus, for a NaCas concentration of 5 wt.% the relationship between these values led to higher emulsion stability.

3.2. Emulsions with trehalose

Fig. 3 shows the particle size distribution for the CFO emulsions formulated with 20 wt.% trehalose and stabilized by different concentrations of NaCas (0.5–5 wt.%, a–f). $D_{4,3}$,$\%V_{d>1}$, and W for emulsions in Fig. 3 are reported in Table 1. Emulsions with trehalose also showed a monomodal distribution but the shapes were more Gaussian than the formulations without trehalose, which is indicative of a more homogeneous population of droplets. $D_{4,3}$ was always smaller than for the emulsions without sugar in the aqueous phase showing that trehalose had strong interactions with the protein influencing droplet size. Distributions for 4 and 5 wt.% NaCas emulsions were very narrow with $D_{4,3}$ of 0.42 and 0.35 μm. As shown in Table 1, 30 and 40 wt.% trehalose addition also cause $D_{4,3}$ diminution.

Fig. 4 shows the *BS* profiles as a function of the tube length for emulsions in Fig. 3. The initial mean value of back scattering along the entire tube (BS_{av0}, from *BS* profile at $t = 0$) for concentrations of NaCas of 0.5, 1, 2, 3, 4, and 5 wt.% were 70.80%, 70.16%, 69.22%, 69.17%, 68.80%, and 66.81%, respectively. These values were lower than the ones found for emulsions without trehalose which is in agreement with the lower $D_{4,3}$ always measured with addition of trehalose. As in the case of emulsions without trehalose, the main mechanism of destabilization in a given formulation depended on NaCas concentration (Fig. 4). However, the rate of destabilization was markedly lower with addition of sugar. The 5 wt.% NaCas emulsion did not flocculate during a week at 22.5 °C. Its *BS* remained unchanged during storage showing a great stability. This sample remained fully turbid and in the liquid state.

3.3. Evaluation of migration rate

The migration of oil droplets in 0.5, 1, and 2 wt.% NaCas-stabilized emulsions led to the appearance of a peak in the 0–20 mm zone that became larger as migration extended in samples. By measuring the peak thickness at different times, it was possible to evaluate the rate of migration. Fig. 5a–c report the evolution of the peak thickness, evaluated from the *BS* profiles, monitored over 100 h. The slopes of the linear zones were calculated and reported in Table 2. As shown in Fig. 5a and b, addition of trehalose to the aqueous phase for the 0.5 and 1 wt.% NaCas stabilized-emulsions diminished creaming rate especially at concentrations of 40 wt.%. For 2 wt.% NaCas emulsion creaming rate of emulsion formulated without sugar addition was notoriously higher for the first 20 h until it reached a plateau ($p < 0.05$). Addition of sugar to aqueous phase of emulsions significantly diminished creaming rate for all sugar concentrations indicating that trehalose should play a very important role in emulsion structure.

3.4. Quantification of flocculation

The variations in the *BS* in the 20–50 mm zone of the emulsions which main destabilization mechanism is flocculation are shown in Fig. 6a–c. When the 40 wt.% trehalose emulsion was stabilized with 3, 4 or 5 wt.% NaCas there was almost no variation in the *BS* with time (changes in *BS* from time zero < 0.5%). The 20 wt.% trehalose emulsion stabilized with 5 wt.% NaCas also showed almost no change in *BS* with time. However, for concentrations of NaCas of 3 or 4 wt.% a decrease in *BS* of 12% or 4%, respectively, was measured after 10 h of storage. Then, the values reached a plateau that remained constant up to 200 h. For 30 wt.% trehalose addition, only the emulsion with 3% NaCas showed a significant decrease in *BS* (8%). The other NaCas concentrations were stable. The 4% NaCas emulsion showed a very small decrease in *BS* of 1.5% while for 5 wt.% NaCas changes in *BS* from time zero were less than 0.5%. It is clear from Fig. 6 that trehalose stabilized emulsions delaying flocculation.

3.5. Microstructure

The images obtained when the 20 wt.% trehalose/10 wt.% CFO emulsions stabilized with different amounts of NaCas were kept at 22.5 °C for 24 h are shown in Fig. 7. The images corresponding to emulsions stabilized with 0.5 or 1 wt.% NaCas showed a homogeneous droplet distribution. Differences in both images are in agreement with the decrease in $D_{4,3}$ with NaCas concentration.

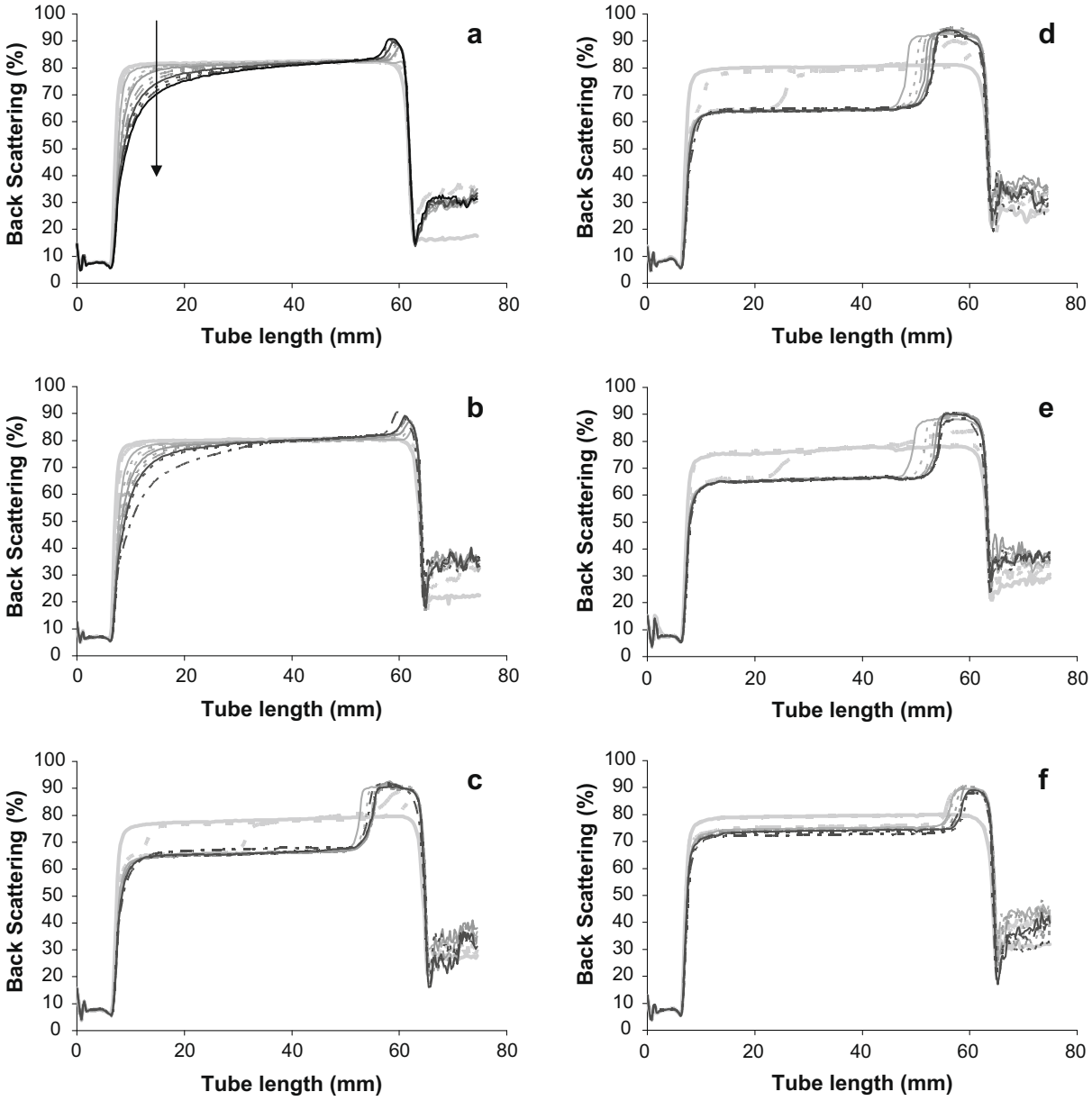

Fig. 2. Changes in back scattering (*BS*) profiles as a function of the tube length with storage time (samples were stored for 1 week, arrow denotes time) in quiescent conditions for emulsions with CFO as fat phase, no trehalose added to the aqueous phase, and different concentrations of sodium caseinate: (a) 0.5 wt.%, (b) 1.0 wt.%, (c) 2.0 wt.%, (d) 3.0 wt.%, (e) 4.0 wt.%, and (f) 5.0 wt.%. Tube length 65 mm. CFO concentrated fish oil.

When protein concentration was 2, 3 or 4 wt.% emulsions presented aggregates/flocculates which were absent in 0.5 and 1 wt.% emulsions. In these emulsions there were higher protein concentrations in the aqueous phase. Surprisingly, a concentration of 5 wt.% NaCas produced an emulsion with a very homogeneous droplet distribution and a lower $D_{4,3}$. In addition to a higher concentration of NaCas in the aqueous phase this emulsion most likely had more protein for the coverage of oil droplet surface. The absence of aggregates/flocculates is in agreement with the great stability of this emulsion as studied by turbiscan.

3.6. Structure of CFO emulsions

Fig. 8 shows the results obtained when the emulsions formulated with 20 wt.% trehalose, 10 wt.% CFO and 2 or 5 wt.% NaCas as stabilizer were analyzed by SAXS. Fig. 8 also shows SAXS patterns for the 20 wt.% trehalose solutions of NaCas at those concentrations (aqueous phase without CFO). The dependence of the position of the peak maxima on NaCas concentration in the aqueous phase was similar to that described by Kalnin et al. (2004). Values of q increased with NaCas concentration showing that the aggregation state of the protein changed as a function of its concentration. For our systems q values were 0.217 and 0.244 nm^{-1} for 2% and 5% NaCas, respectively. According to these authors, peak maxima values for emulsions patterns were very similar to the values for aqueous patterns indicating that micelles organization did not change by the presence of fat phase. Our systems behaved in the same way as their emulsions. Kalnin, Ouattara, and Ollivon (2004) have reported that there was a partition, $K = [\text{NaCas}]_{lip}[\text{NaCas}]^{-1}_{aq}$, between the aqueous phase and the interface depending on the surface area of the interface. According to these authors, both, interface and aqueous NaCas, increased with increase of total NaCas. However, aqueous concentration increased in a quite constant linear way while NaCas at the interface increased at a higher

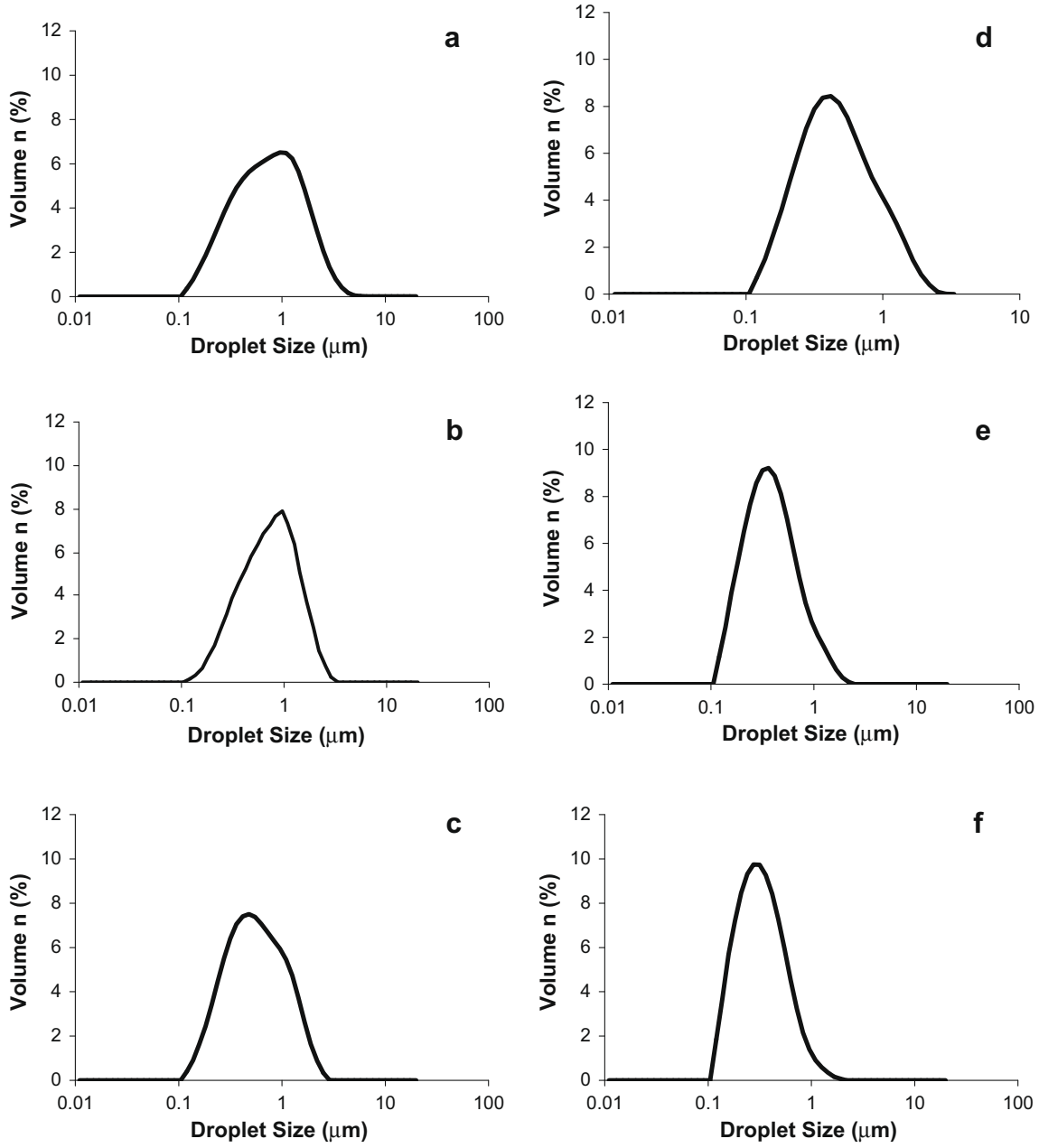

Fig. 3. Particle size distribution of emulsions with CFO as fat phase, 20 wt.% trehalose solution as aqueous phase, and different concentrations of sodium caseinate: (a) 0.5 wt.%, (b) 1.0 wt.%, (c) 2.0 wt.%, (d) 3.0 wt.%, (e) 4.0 wt.%, and (f) 5.0 wt.%. CFO concentrated fish oil.

rate when particle size diminished significantly. Most likely addition of NaCas from 2 wt.% to 5 wt.% had greater effect in aqueous NaCas concentration since changes in $D_{4,3}$, although significant, were very small.

Fig. 9 shows the SAXS patterns obtained when emulsions formulated with 0, 20 or 30 wt.% trehalose, 10 wt.% CFO and 5 wt.% NaCas were analyzed at 8 °C. As was expected addition of sugar diminished the intensity of the signal since electron density of aqueous trehalose solutions increased with concentration and therefore there was less contrast. Values of q were 0.241, 0.248, and 0.252 nm^{-1} for emulsions with 0, 20, and 30 wt.% trehalose, respectively. Some aqueous phase components such as hydrocolloids proved to stabilize emulsions because they increase viscosity. The slightly increased of q values with trehalose addition might suggest that trehalose had an effect further than viscosity changes since the aggregation state of the protein changed with the aqueous phase formulation. These results were in agreement with the small particle size found when trehalose was added to aqueous phase. Changes in fat phase did not affect q values (data not shown). However, patterns had different intensities and shapes. A deeper analysis of the scattering curves would provide more information about the internal organization of casein micelles.

3.7. Formulation with different oils

Table 3 shows $D_{4,3}$, $\%V_{d>1}$, and W for emulsions formulated with OO, SFO, and CFO and 20 wt.% trehalose in the aqueous phase. For the three oils, particle size diminished as NaCas concentration increased. However, there were no significant differences in $D_{4,3}$, $\%V_{d>1}$, and W with oil type. The results of evaluating creaming rate from *BS* profiles are reported in Table 4. For 0.5 and 1 wt.% NaCas concentration, OO and SFO emulsions had a slightly higher cream-

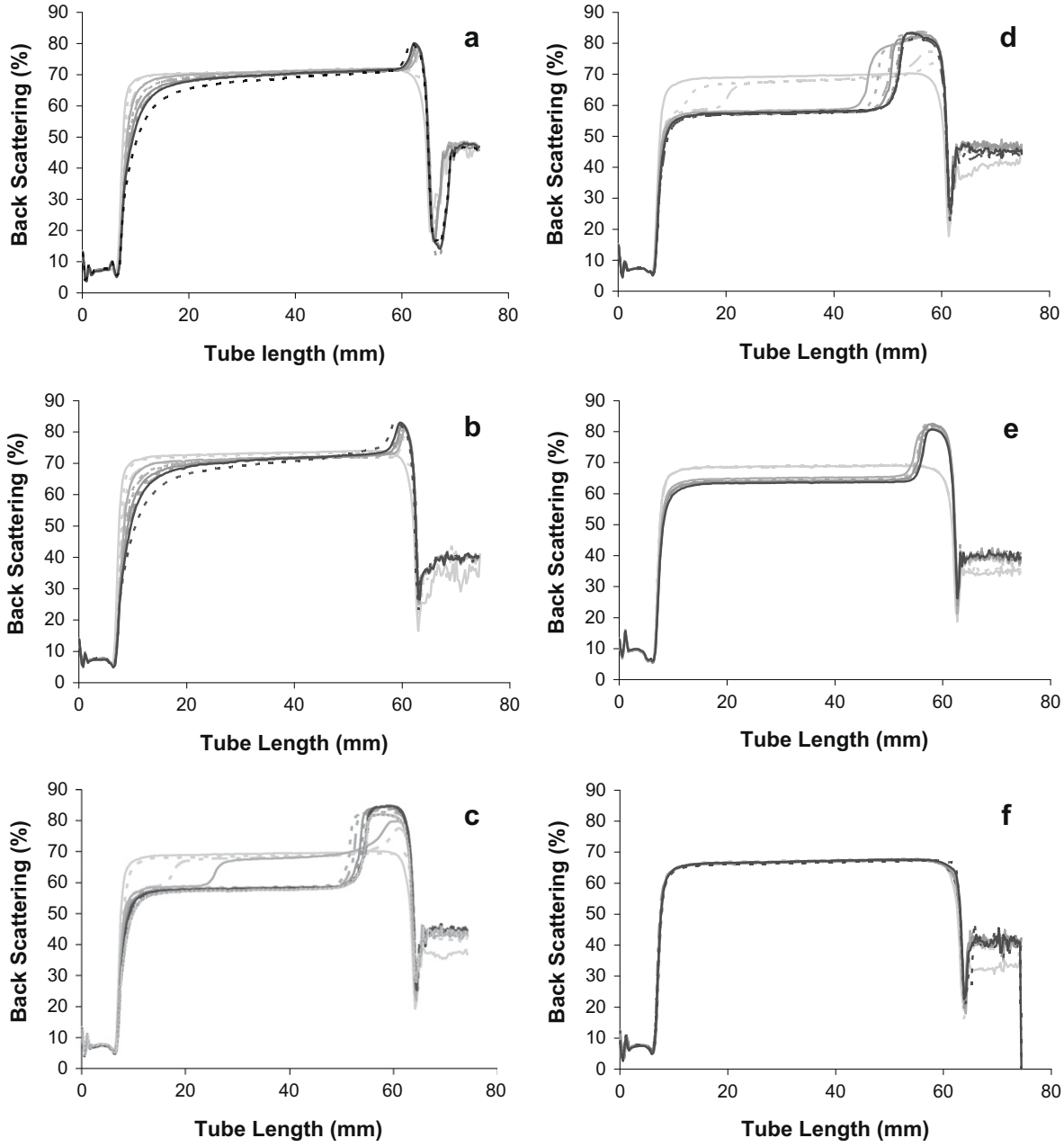

Fig. 4. Changes in back scattering (*BS*) profiles as a function of the sample height with storage time (samples were stored for 1 week, arrow denotes time) in quiescent conditions for emulsions with CFO as fat phase, 20 wt.% trehalose solution as aqueous phase, and different concentrations of sodium caseinate: (a) 0.5 wt.%, (b) 1.0 wt.%, (c) 2.0 wt.%, (d) 3.0 wt.%, (e) 4.0 wt.%, and (f) 5.0 wt.%. Tube length 65 mm. CFO concentrated fish oil.

ing rate than CFO emulsions. For the 2 wt.% emulsion, formulation with OO rapidly destabilized for 7 h and then pick-thickness with time reached an asymptotic value. The slope of the linear zone was markedly higher than for the other two emulsions. CFO showed the lowest creaming rate for this NaCas concentration. Fig. 10 shows the variations in the *BS* in the 20–50 mm zone of the emulsions formulated with different oils which main destabilization mechanism is flocculation. For emulsions stabilized with 3 and 4 wt.% NaCas and formulated with any of the three oils, *BS* with time diminished considerably until reaching an asymptotic value. Values for 3 wt.% NaCas emulsions were the lowest of the three concentrations (3, 4, and 5 wt.%) indicating that flocculation for this level of stabilizer took place in greater extent than for the others. Increasing NaCas concentration improved the stability of emulsions. Formulation with 5 wt.% NaCas concentration was the most stable. *BS* remained unchanged during at least 100 h.

The OO used in this study had a 78.3% content of C18:1 and 5.4% of C18:2, the SFO had values of 21.9% of C18:1 and 66.3% of C18:2 while the CFO had percentages of 10.90% of C20:5, 4.58% of C20:4, 7.96% of C22:6, and 5.21% of C22:5. Unsaturated fatty acids have no linear molecular geometry: the higher the number of instaurations the complex the molecular geometry. We expected different behaviors based on their different chemical composition. CFO the most unsaturated of the three oils was supposed to be less prone to flocculation. Although there were differences among oils, no clear tendencies in *BS* with time were found. The extent of flocculation varied from one combination of oil/stabilizer to the other depending on particular interactions among components of the system.

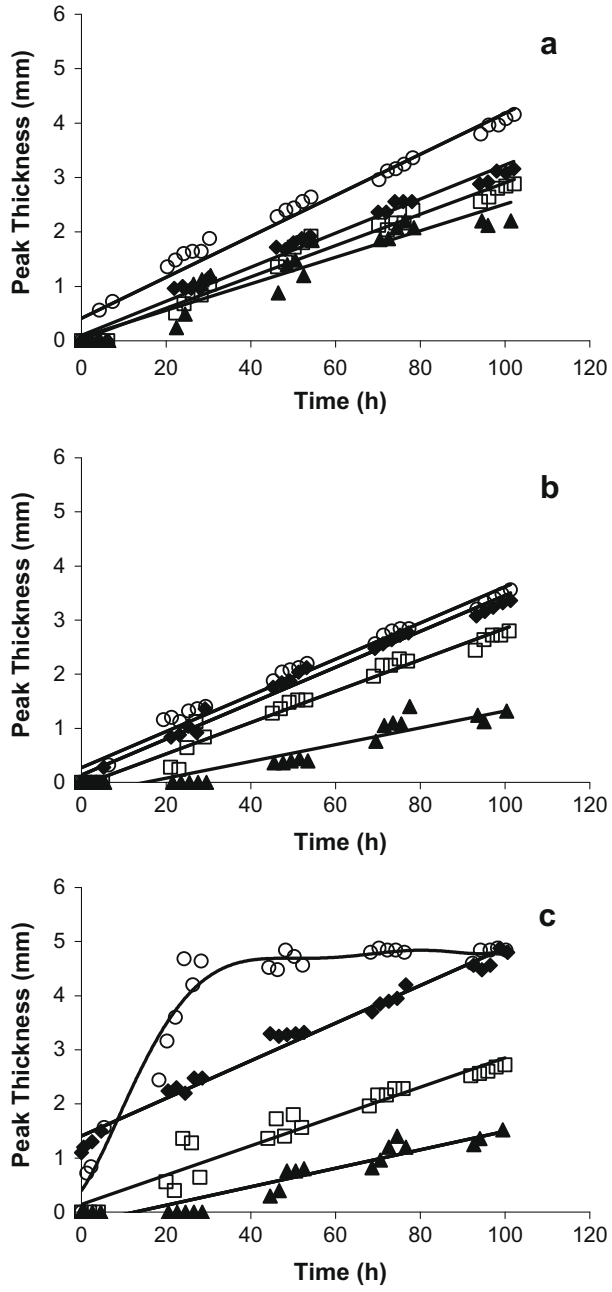

Fig. 5. Peak thickness measured at 50% of the height at different times in the bottom zone of the tube, stored in quiescent conditions at 22.5 °C, for emulsions with CFO as fat phase and different concentrations of sodium caseinate: (a) 0.5 wt.%, (b) 1.0 wt.%, and (c) 2.0 wt.%, monitored over 100 h. Tube length 65 mm. ○ without trehalose, ♦ 20 wt.% trehalose, □ 30 wt.% trehalose, and ▲ 40 wt.% trehalose. CFO concentrated fish oil.

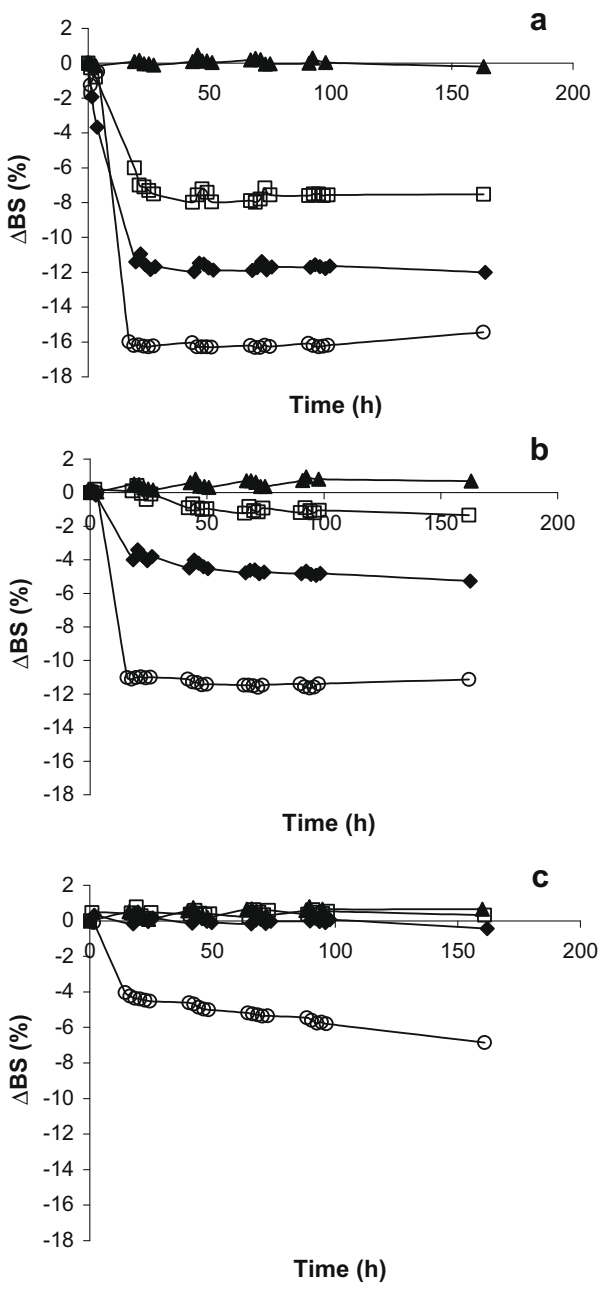

Fig. 6. Variation in *BS* in the 20–50 mm zone monitored over 160 h, for samples stored in quiescent conditions at 22.5 °C. Emulsions were formulated with CFO as fat phase and different concentrations of sodium caseinate: (a) 3.0 wt.%, (b) 4.0 wt.%, and (c) 5.0 wt.%. Tube length: 65 mm. ○ without trehalose, ♦ 20 wt.% trehalose, □ 30 wt.% trehalose, and ▲ 40 wt.% trehalose. CFO concentrated fish oil. *BS* back scattering.

Table 2
Slope of the linear zone (mm/h) and correlation coefficients (R^2) evaluated from the pick-thickness with time curves showing migration in the bottom part of emulsion-samples, as represented in Fig. 5a–c.

Sample	NaCas 0.5 wt.%		NaCas 1 wt.%		NaCas 2 wt.%	
	Slope	R^2	Slope	R^2	Slope	R^2
Without trehalose	0.035	0.972	0.033	0.974	0.149	0.951
20 wt.% trehalose	0.031	0.982	0.033	0.985	0.035	0.981
30 wt.% trehalose	0.029	0.976	0.029	0.976	0.027	0.937
40 wt.% trehalose	0.024	0.901	0.016	0.880	0.017	0.901

4. Discussion

Dickinson (1999) reported that stability of caseinate emulsions at pH 7 was very sensitive to the oil-to-protein ratio. In emulsions containing protein at concentrations well below that required for monolayer saturation coverage, the system exhibited time-dependent bridging flocculation and coalescence. At protein contents around that required for saturation monolayer coverage, the system was very stable towards creaming and coalescence. At higher protein contents, the creaming stability of the system was greatly reduced due to depletion flocculation of protein-coated droplets by

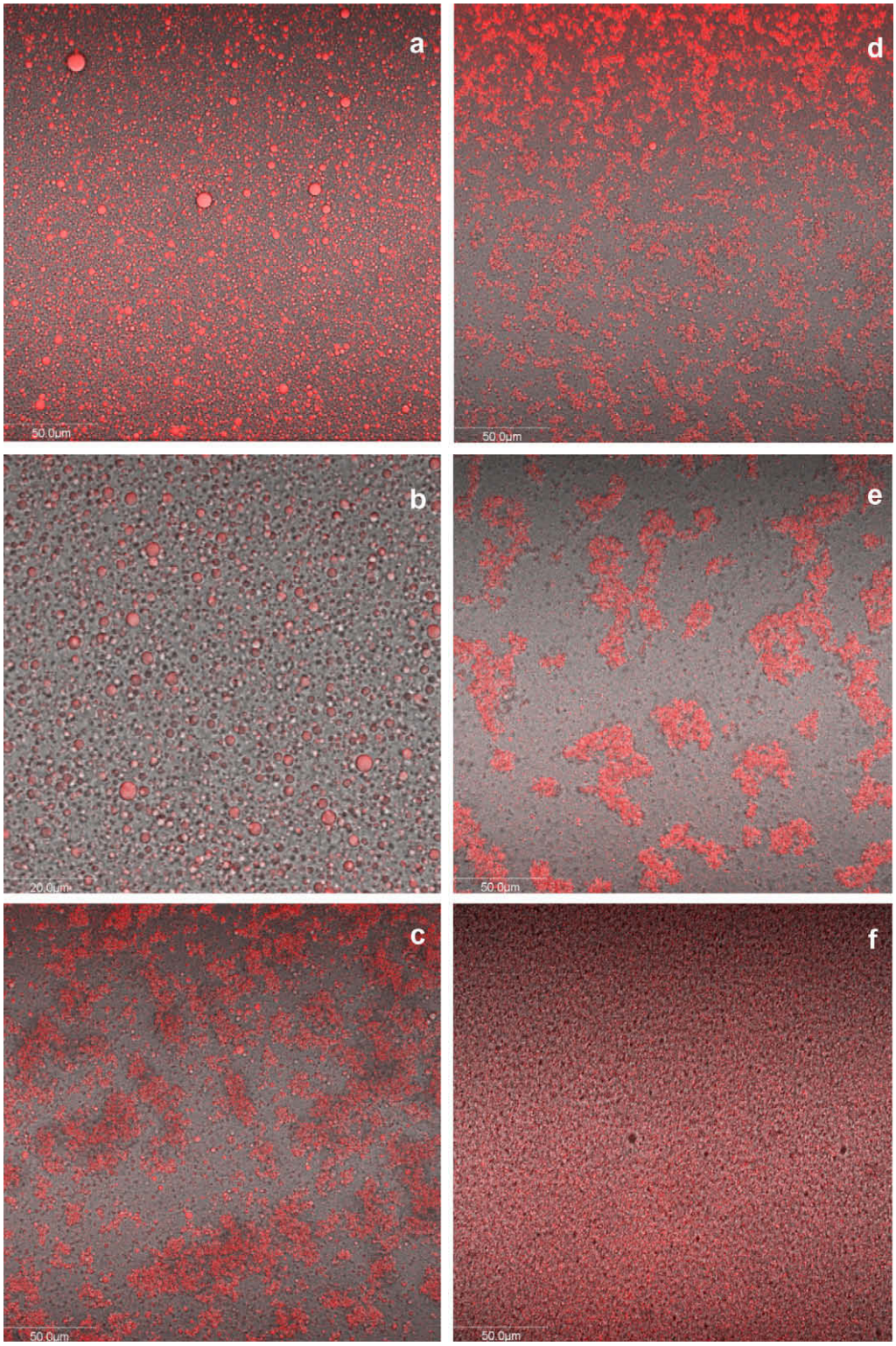

Fig. 7. Confocal laser scattering microscopy (CLSM) images of emulsions with 10 wt.% concentrated fish oil (CFO) as fat phase, 20 wt.% trehalose solution as aqueous phase, and different concentrations of sodium caseinate (NaCas) kept at 22.5 °C for 24 h: (a) 0.5 wt.%, (b) 1.0 wt.%, (c) 2.0 wt.%, (d) 3.0 wt.%, (e) 4.0 wt.%, and (f) 5.0 wt.%. CFO concentrated fish oil.

unadsorbed sub-micellar caseinate. And, at even higher protein contents, there was partial restabilization of the flocculated emulsion in the form of a strong particle gel network. Our systems' behavior proved to be more complicated than that. The oil-to-protein ratio that gave stability also changed with processing conditions and formulation of the aqueous phase.

To determine the oil-to-protein ratio that makes the system very stable, Day et al. (2007) studied fish oil emulsions varying in sodium caseinate concentration (25 wt.% oil and 0.1–1.0 wt.% protein, giving oil-to-protein ratios of 250–25). Although they used a Turbiscan approach, they did not quantify destabilization mechanisms. However, The Turbiscan profiles of these systems clearly

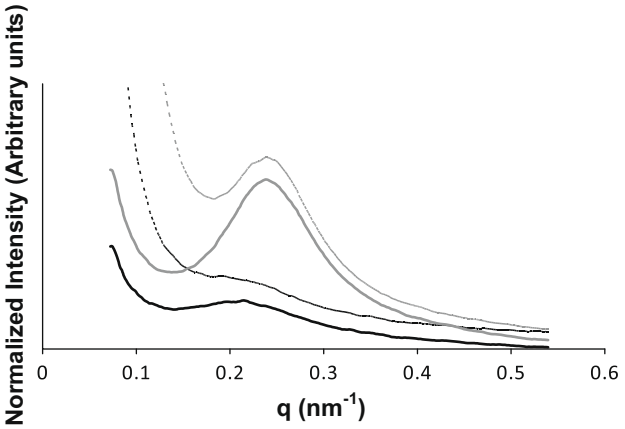

Fig. 8. Normalized intensity vs. reciprocal lattice spacing q, where $q = 2\pi/d = 4\pi \sin(\theta)/\lambda$, where d is the interplanar spacing and 2θ is the Bragg angle. Solid lines: aqueous solutions, black 2 wt.% NaCas, grey 5 wt.% NaCas; dotted lines: emulsions, black 2 wt.% NaCas, grey 5 wt.% NaCas.

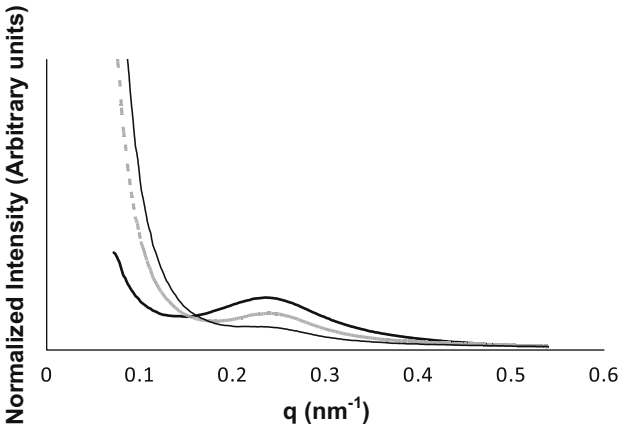

Fig. 9. Normalized intensity vs. reciprocal lattice spacing q for emulsions formulated with 0 (solid black thick line), 20 (dotted gray line) or 30 wt.% (solid black thin line) trehalose, 10 wt.% CFO and 5 wt.% NaCas, analyzed at 8 °C.

showed that for oil-to-protein ratios lower than 100, phase separation occurred in the first 24 h. These authors reported that the presence of excessive protein in an emulsion (i.e., at 1 wt.% NaCas) caused the aggregation of oil droplets through depletion flocculation, resulting in low creaming stability and high low-shear viscosity. At a lower protein concentration (0.1 wt.%), when protein was limited, the emulsion showed greater stability to creaming. However, the 24 h profile of the 250 oil-to-protein-ratio emulsion had a slightly increase in *BS* in the upper zone of the tube indicative of creaming destabilization. As emulsions were no further analyzed

Table 4
Slope of the linear zone (mm/h) and correlation coefficients (R^2) evaluated from the pick-thickness with time curves showing migration in the bottom part of emulsion-samples formulated with olive oil (OO), sunflower oil (SFO), or concentrated fish oil (CFO), and 20 wt.% trehalose.

Sample	NaCas 0.5 wt.%		NaCas 1 wt.%		NaCas 2 wt.%	
	Slope	R^2	Slope	R^2	Slope	R^2
Olive oil	0.039	0.974	0.045	0.971	5.009[a]	0.993
Sunflower oil	0.038	0.984	0.044	0.984	0.072	0.932
Concentrated fish oil	0.031	0.982	0.033	0.985	0.035	0.981

[a] Creaming occurred for the first 7 h then stopped.

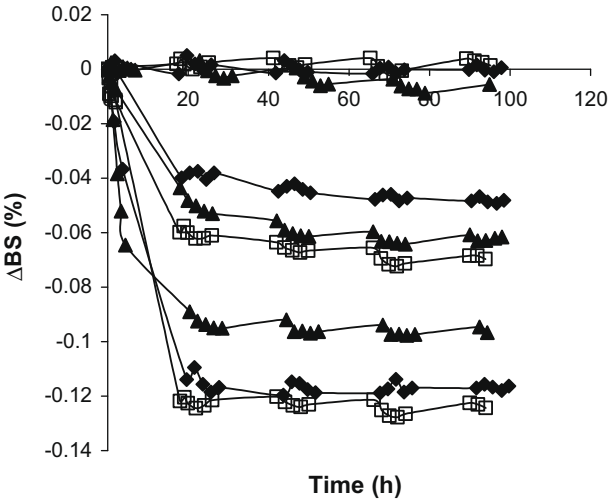

Fig. 10. Variation in *BS* in the 20–50 mm zone monitored over 100 h, for samples stored in quiescent conditions at 22.5 °C. Emulsions were formulated with 20 wt.% trehalose aqueous solution, OO, SFO or CFO as fat phase and different concentrations of sodium caseinate: 3.0 wt.% (lower values), 4.0 wt.% (intermediate values), and 5.0 wt.% (higher values). Tube length: 65 mm. □ olive oil (OO), ▲ sunflower oil (SFO), and ♦ concentrated fish oil (CFO).

it is difficult to predict the behavior for longer times. The Turbiscan profile of the 0.1 wt.% emulsion was very surprising. This condition gave an emulsion with a $D_{4,3}$ of 13.3 ± 3.6 μm, which might be expected to destabilize in minutes. Since emulsions were further homogenized with 14 MPa a lower droplet size should have been obtained, which means that at this oil-to-protein ratio, protein concentration seemed to be below that required for monolayer saturation coverage.

Perrechil and Cunha (2010) studied different ways to stabilize oil-in-water macroemulsions. All the analyzed systems had the NaCas and oil content fixed at 1 wt.% and 30 wt/V%, respectively, giving an oil-to-protein ratio of 30. Emulsions prepared by Ultra Turrax homogenization had mean droplet diameter ($D_{3,2}$) from 6

Table 3
Volume-weighted mean diameter ($D_{4,3}$, μm), volume percentage of particles exceeding 1 μm in diameter ($\%V_{d>1}$), and width of the distribution (W) of emulsions formulated with olive oil (OO), sunflower oil (SFO), and concentrated fish oil (CFO) as fat phase and different concentrations of NaCas immediately after preparation.

Sample	Olive oil			Sunflower oil			Concentrated fish oil		
	$D_{4,3}$	$\%V_{d>1}$	W	$D_{4,3}$	$\%V_{d>1}$	W	$D_{4,3}$	$\%V_{d>1}$	W
NaCas 0.5 wt.%	1.00	29.68	1.72	1.00	36.13	1.50	0.88	29.50	1.52
1.0 wt.%	0.96	33.79	1.42	0.87	28.95	1.34	0.85	27.37	1.31
2.0 wt.%	0.83	18.50	1.15	0.50	8.58	0.86	0.63	13.96	1.01
3.0 wt.%	0.54	10.12	0.91	0.45	5.85	0.73	0.52	8.02	0.82
4.0 wt.%	0.44	4.54	0.65	0.37	2.68	0.53	0.42	3.68	0.61
5.0 wt.%	0.38	2.24	0.51	0.36	2.45	0.50	0.35	1.17	0.45

to 15 μm. To evaluate stability, emulsions were characterized by visual analysis. Most of the systems were not stable, showing phase separation a few minutes after emulsion preparation. However, creaming rate was strongly affected by pH, homogenization pressure or locust bean gum concentration.

In a previous work in our lab (Cerdeira et al., 2007) we study the effect of emulsion stability on initial retention of a low-*trans* fat encapsulated in a trehalose matrix. The emulsion formulated with SFO and NaCas in an oil-to-protein ratio of 8 was very stable after 195 h at 22.5 °C. No creaming and coalescence occurred and, as a result of that, it remained fully turbid and no clarification was detected by the transmission detector. This emulsion also had a 20 wt.% trehalose dissolved in the aqueous phase. In Cerdeira et al. (2007) work the pre-emulsion was further passed through a two-stage valve high pressure homogenizer giving a $D_{4,3}$ of 1.10 ± 0.13. In the present study the pre-emulsions were homogenized for 20 min using an ultrasonic liquid processing. The oil-to-protein ratios selected varied from 20 to 2 giving the $D_{4,3}$ values reported in Table 1. Emulsions were stable only when the oil-to-protein ratio was 2 (Fig. 10). This means that the oil-to-protein ratio that gave long stability depended on processing conditions.

Many studies in literature evaluated emulsions by visual observation. By using this approach, all our emulsions would be considered stable since no phase separation occurred after a week in quiescent conditions at 22.5 °C. This is in agreement with the fact that transmission detector received no light during the time emulsions were analyzed. However, the *BS* detector was able to quantify creaming or flocculation in these systems. Concentrations below 0.5 wt.% NaCas seemed to be below the ones required for saturation monolayer coverage since creaming rate is greater for 0.5 wt.% than for 1 wt.% NaCas. Systems stabilized by these oil-to-protein ratios most likely destabilized by time-dependent bridging flocculation. Further addition of protein led to high instability. The 2–4 wt.% NaCas range was the worst situation. At these protein contents, the creaming stability of the system was greatly reduced probably due to depletion flocculation of protein-coated droplets by unadsorbed sub-micellar caseinate. Although it was reported that at protein contents around that required for saturation monolayer coverage, the system was very stable towards creaming and coalescence (Dickinson, 1999), there was no such oil-to-protein ratio in ours systems between bridging flocculation and depletion flocculation destabilization processes. At even higher protein contents (5 wt.% NaCas concentration), emulsions were very stable especially when the aqueous phase contained trehalose. In these conditions, the systems remained in the liquid state for at least a week, fully turbid, that is no changes were noticed by visual analysis and no creaming or flocculation was detected by the Turbiscan. Although there was no gel formation when emulsions were kept at 22.5 °C for a week, they were very stable. Confocal images obtained after 24 h at 22.5 °C were in agreement with Turbiscan data (Fig. 7). Hiemenz and Rajagopalan (1997) reported that for polymer stabilized emulsions at high polymer concentrations, one may also have what is known as depletion stabilization. The polymer-depleted regions between the particles can only be created by demixing the polymer chains and solvent. In good solvents the demixing process is thermodynamically unfavorable, and under such conditions one can have depletion stabilization. Our systems' behavior might be interpreted with this approach.

In order to slow down the destabilization of emulsions, thickening agents such as polysaccharides and hydrocolloids are frequently used. As may be noticed in Table 1, the effect of trehalose was further than the ability to form viscous solutions since it diminished $D_{4,3}$ values for the same processing conditions. The interactions between protein and sugar, detected by SAXS and particle size analysis, also played an important role in stabilization although was not enough to suppress the depletion effect that led to instability of the emulsions formulated with NaCas concentrations from 2 to 4 wt.%.

From our results and others discussed in literature, it is clear that the stability of caseinate emulsions was strongly affected not only by the oil-to-protein ratio but also by processing conditions and composition of aqueous phase. The structure of the protein and the interactions protein–sugar played a key role in creaming and flocculation processes.

Acknowledgment

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References

Anderson, B. M., & Ma, D. W. L. (2009). Are all n-3 polyunsaturated fatty acids created equal? *Lipids in Health and Disease, 8*, 33–52.
Cerdeira, M., Palazolo, G. G., Candal, R. J., & Herrera, M. L. (2007). Factors affecting initial retention of a microencapsulated sunflower seed oil/milk fat fraction blend. *Journal of the American Oil Chemists' Society, 84*, 523–531.
Chauvierre, C., Labarre, D., Couvreur, P., & Vauthier, C. (2004). A new approach for the characterization of insoluble amphiphilic copolymers based on their emulsifying properties. *Colloid and Polymer Science, 282*, 1097–1104.
Day, L., Xu, M., Hoobin, P., Burgar, I., & Augustin, M. A. (2007). Characterization of fish oil emulsions stabilized by sodium caseinate. *Food Chemistry, 105*, 469–479.
Dickinson, E. (1999). Caseins in emulsions: Interfacial properties and interactions. *International Dairy Journal, 9*, 305–312.
Dickinson, E., & Golding, M. (1997a). Depletion flocculation of emulsions containing unadsorbed sodium caseinate. *Food Hydrocolloids, 11*(1), 13–18.
Dickinson, E., & Golding, M. (1997b). Rheology of sodium caseinate stabilized oil-in-water emulsions. *Journal of Colloidal and Interface Science, 191*, 166–176.
Hiemenz, P. C., & Rajagopalan, R. (1997). *Electrostatic and polymer-induced colloid stability in principles of colloid and surface chemistry* (3rd ed.). New York: Marcel Dekker. pp. 575–625.
Kalnin, D., Ouattara, M., & Ollivon, M. (2004a). A new method for the determination of the concentration of free and associated sodium caseinate in model emulsions. *Progress in Colloid and Polymer Science, 128*, 207–211.
Kalnin, D., Quennesson, P., Artzner, F., Schafer, O., Narayanan, T., & Ollivon, M. (2004b). Monitoring both fat crystallization and self-assembly of sodium caseinate in model emulsions using synchrotron X-ray diffraction. *Progress in Colloid and Polymer Science, 126*, 139–145.
McClements, D. J. (2005). *Emulsion stability in food emulsions, principles, practices, and techniques* (2nd ed.). New York: CRC Press. pp. 269–339.
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.
Mulvihill, D. M. (1989). Casein and caseinates: Manufacture. In P. F. Fox (Ed.), *Developments in dairy chemistry* (pp. 97–130). London: Elsevier Applied Science.
Palazolo, G. G., Sorgentini, D. A., & Wagner, J. R. (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.
Pan, L. G., Tomás, M. C., & Añón, M. C. (2002). Effect of sunflower lecithins on the stability of water-in-oil and oil-in-water emulsions. *Journal of Surfactants and Detergents, 5*, 135–143.
Perrechil, F. A., & Cunha, R. L. (2010). Oil-in-water emulsions stabilized by sodium caseinate: Influence of pH, high-pressure homogenization and locust bean gum addition. *Journal of Food Engineering, 97*(4), 441–448.
Relkin, P., & Sourdet, S. (2005). Factors affecting fat droplet aggregation in whipped frozen protein-stabilized emulsions. *Food Hydrocolloids, 19*, 503–511.
Thanasukarn, P., Pongsawatmanit, R., & McClements, D. J. (2006). Utilization of layer-by-layer interfacial deposition technique to improve freeze-thaw stability of oil-in-water emulsions. *Food Research International, 39*, 721–729.
Van Aken, G. A., & van Vliet, T. (2002). Flow-induced coalescence in protein-stabilized highly concentrated emulsions: Role of shear-resisting connections between the droplets. *Langmuir, 18*(20), 7364–7370.