Emulsifying Properties and Surface Behavior of Native and Denatured Whey Soy Proteins in Comparison with Other Proteins. Creaming Stability of Oil-in-Water Emulsions

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ABSTRACT: In this work a comparative study of emulsifying and surface behaviors of native whey soy proteins (NWSP) and denatured whey soy proteins (DWSP) with those of native soy isolates, denatured soy isolates (DSI), and sodium caseinate was done. These samples showed different molecular mass distributions in gel filtration profiles. Dissociation and soluble high-M.W. species in DWSP and DSI were observed. Lower interfacial and surface pressure values were obtained with native samples. Thermal treatment and salt addition enhanced tensioactivity in all fractions. Backscattering measurements of all oil-in-water emulsions, which exhibited a trimodal size distribution of droplets, showed the existence of a negative correlation with the median diameter of droplets. Greater droplet sizes were observed with NaCl addition. The NWSP emulsion had the lowest stability against creaming. Denaturation of this sample increased stability and favored air incorporation in emulsions. Destabilization depends not only on median droplet size but also on floc formation and structure. NaCl addition negatively affected the creaming stability only in emulsions formulated with soy isolates. The use of denaturation to enhance the surface and emulsifying properties of whey soy proteins would allow their use in food emulsions.

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KEY WORDS: Creaming stability, droplet size, flocculate, interfacial behavior, oil-in-water emulsions, whey soy proteins.

Emulsions bear great importance in the food industry, being ubi-quitous in many natural and processed products. From the thermodynamic viewpoint, emulsions are unstable and tend to destabilize by creaming, flocculation, coalescence, and phase inversion. However, they can be kinetically stabilized by emulsifying agents such as proteins (1). Creaming exerts an undesirable effect on food emulsion texture, taste, and mouthfeel, and it is influenced by several factors such as droplet size, emulsion polydispersibility, continuous phase viscosity, and floc formation (2). Previous works have reported that creaming rate is strongly affected by floc size and structure, a phenomenon mainly determined by the nature of colloidal interactions between droplets (3–5). Dagorn-Scaviner *et al.* (6) have evaluated the stability of emulsions formulated with pea globulins during creaming and flocculation and measured the volume of the separated aqueous phase as a function of time. Likewise, destabilization of an emulsion can be followed in an accurate and reproducible form (7) from the beginning, using a vertical optical analyzer (Quick Scan), without disturbing the system.

Storage and whey proteins are the main protein groups in soybean seeds. Whey proteins are the soluble fraction separated by isoelectric precipitation of globulins from the aqueous soluble extract of soy proteins, and include 2S and 7S fractions. The main components of these fractions are the Kunitz trypsin inhibitor (KTI) and lectin (L) (8) with M.W. of 20 and 120 kDa, respectively (9,10). The antinutritional effect of these proteins and their thermolability have been reported previously (11), so they must be thermally treated before they can be used in formulated foods. Globulins and whey proteins were reported to differ in their surface aromatic hydrophobicity, DSC characteristics, surface behavior, and in the way in which these parameters vary with ionic strength (8,12). This work evaluates the stability of emulsions prepared with whey soy proteins undergoing creaming and flocculation. The effects of thermally induced denaturation and NaCl addition were also analyzed. To undertake a comparative study, two protein fractions widely employed in the food industry also were included: native soy isolate, which possesses the storage proteins glycinin (11S) and β-conglycinin (7S), and sodium caseinate (SC).

MATERIALS AND METHODS

Materials. Defatted, solvent-free soy flour, prepared under controlled, nonthermal conditions (not inactivated to avoid protein denaturation), was provided by Santista S.A. (San Pablo, Brazil). SC (Sigma Co., St. Louis, MO) was utilized without further purification; the divalent ion (Ca^{2+}, Mg^{2+}) content was 0.7 g%, as determined following a method by Ntailianas and Whitney (13). All the other reagents were analytical grade.

Native soy isolate (NSI), native whey soy proteins (NWSP), and the corresponding denatured fractions (DSI, DWSP). Preparation of NSI and NWSP was as described in previous work (8): The defatted soybean flour was extracted at room temperature with water adjusted to pH 8.0 and centrifuged

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(10,400 × *g* for 15 min at 20°C). The supernatant was adjusted to pH 4.5 and centrifuged (10,400 × *g* for 20 min at 4°C). The precipitate was washed with water, adjusted to pH 8.0, and freeze-dried, resulting in the NSI sample. To obtain NWSP, the filtered supernatant at pH 4.5 obtained from the NSI preparation was brought to pH 8.0 and centrifuged (12,400 × *g* for 15 min at 20°C). The clarified supernatant was precipitated with ammonium sulfate to 90% saturation and centrifuged (12,400 × *g* for 15 min at 20°C). The precipitate was washed with water, dialyzed for 24 h at 4°C, and freeze-dried. The protein content in NSI and NWSP was 90.0 ± 0.1 and 99.0 ± 0.5% w/w (N × 6.25), respectively. For DSI and DWSP preparations, total protein denaturation was achieved by heating the respective solutions (NSI and NWSP, 1 mg/mL, 0.01 M sodium phosphate buffer, pH 7.0) to 90ºC for 5 min. Denatured fractions (DSI and DWSP) were either used immediately after preparation or else freeze-dried for gel filtration assays.

The following surface hydrophobicity (H_0) values of protein samples, determined by fluorescence probe 1-anilino-8-naphthalene sulfonate, were reported in a previous work (12): 98 ± 7, 350 ± 12, <10, 142 ± 9, and 220 ± 11 for NSI, DSI, NWSP, DWSP, and SC, respectively. In the presence of 500 mM NaCl, H_0 values for these samples were 191 ± 10, 521 ± 15, 63 ± 6, 178 ± 12, and 340 ± 10, respectively.

The solubility of aqueous dispersions (1 mg/mL, 0.01 M sodium phosphate buffer, pH 7.0) for all samples was >99%, as determined by the experimental procedure described by Mitidieri and Wagner (12).

Gel filtration chromatography [fast protein liquid chromatography (FPLC)]. Freeze-dried samples of SC, NSI, NWSP, DSI, and DWSP were dissolved in 0.01 M sodium phosphate buffer, pH 7.0 (13.75 mg/mL), stirred for 15 min in a MS1-Minishaker (IKA), and centrifuged at 14,000 × *g* for 20 min (IEC Centra MP4R microcentrifuge; International Equipment Company, Boston, MA). The supernatant was filtered and applied to a Superose 6B HR 10/30 column (Pharmacia LKB, Uppsala, Sweden), with a flow rate of 0.2 mL/min, operating at a maximum pressure of 1 MPa. Samples were eluted with the same buffer used in their preparation. Fractions were received in a FRAC-100 collector (Pharmacia LKB, Uppsala, Sweden) and monitored by measuring absorbance at 280 nm (UV-1 detector; Pharmacia LKB). The column was calibrated with the following known molecular mass (MM) proteins: blue dextran (void volume), tyroglobulin (669 kDa), alcohol dehydrogenase (150 kDa), serum bovine albumin (66 kDa), and carbonic anhydrase (30 kDa) (Sigma Co).

Adsorption behavior. Interfacial tension measurements of NSI, DSI, NWSP, DWSP, and SC dispersions (1 mg/mL, sodium phosphate buffer 0.01 M, pH 7.0) were performed in a CSC–DuNouy 70.535 tensiometer, according to the ring method. The interfacial tension, modified by a correction factor (14), decreases from the clean interface value γ_o to a value γ. Therefore, the interfacial pressure was calculated as:

$$\pi = \gamma_o - \gamma \quad \text{(mN/m)} \qquad [1]$$

Determinations were conducted at least in duplicate.

Preparation of oil-in-water (o/w) emulsions. NSI, NWSP, DSI, DWSP, and SC were dispersed in 0.01 M sodium phosphate buffer pH 7.0 (with and without NaCl 500 mM) at a final concentration of 0.1% (1 mg/mL). The emulsions were prepared with 10 mL of one dispersion and 5 mL of commercially refined sunflower oil (oil volume fraction, $\Phi = 0.33$) by homogenization at 20,000 rpm for 60 s (UltraTurrax T-25, S25N10G device). Tests were done at least in duplicate.

Determination of droplet size distribution. The droplet size distribution was determined immediately after emulsion preparation by light scattering using a Mastersizer Micro Particle Analyzer (Malvern Instruments Ltd., Malvern, United Kingdom). Calculation from 0.003 to 300 μm was expressed in differential volume. The span (S) was expressed as $S = [d(v, 0.9) - d(v, 0.1)]/d(v, 0.5)$, where $d(v, 0.9)$ and $d(v, 0.1)$ are the 90 and 10% volume percentiles of the size distribution, whereas $d(v, 0.5)$ is the 50% volume percentile, also known as the median of the distribution. The v in the expression refers to the volume distribution. S indicates the width of the distribution regardless of the median size. Determinations were conducted at least in duplicate.

Stability studies of emulsions. The emulsion stability was analyzed using a vertical scan analyzer (Quick Scan; Beckman Coulter, Fullerton, CA). Samples were put in a cylindrical glass measurement tube to study the backscattering (BS) profiles as a function of the sample height (in mm). Measurements were performed at 1-min intervals for 60 min. Creaming kinetics was followed by measuring the mean values of BS (BS_{av}) as a function of time for a given zone in the sample. To estimate the creaming constant (K), the bottom zone of tubes (zone 10–20 mm) was analyzed. The value of K was defined as

$$K = \frac{1}{BS_0 t_{1/2}} \qquad [2]$$

where BS_0 is the initial mean value of back scattering (t = 0 min) and $t_{1/2}$ is the time for which $BS_{av} = BS_0/2$. This equation is similar to that used by Wagner *et al.* (15) to calculate the specific liquid drainage rate in foams. In samples where creaming leads to total clarification in the measuring zone, correction of overestimated BS_{av} values for reflected light were performed. The cross section of the measurement tube is uniform; therefore, the volume of the cream phase relative to the total emulsion volume (V_r) can be determined from the BS profile at 24 h, as the length ratio of cream phase and entire emulsion (cream phase + serum phase). At least duplicate determinations were carried out.

Emulsion microstructure. A 20 μL aliquot of a given emulsion was placed on a glass slide and covered with a 22 × 22 mm cover slip. Samples were observed with a light microscope (DC 100; Leica, Wetzlar, Germany) fitted with an adapted digital camera, and operating at 100× magnification.

Statistical analysis. Data were analyzed by ANOVA and significant difference between the Fisher's test (Systat, 5.0;

Systat, Point Richmand, CA). An alpha level of 0.05 was used to determine significance.

RESULTS AND DISCUSSION

Structural characteristics and surface behavior of protein samples. To analyze the MM distribution and the differences between protein fractions of samples, molecular exclusion chromatography tests were done using the same elution buffer as that used to prepare the o/w emulsions; the results are shown in Figure 1. This determination, together with surface properties, was useful in understanding the behavior of protein samples as emulsifier agents.

In a previous work (16), the electrophoretic profiles of NSI and NWSP samples were compared with those of control proteins [7S and 11S fractions, L, KTI, Bowman-Birk trypsin inhibitor (BBTI) and urease (U)]. NSI is composed mostly of globulins 7S (α, α′, and β subunits) and 11S (A and B polypeptides). In turn, NWSP consist mainly of KTI and L subunits: 20 and 35 kDa bands, respectively. This electrophoretic study allowed us to interpret the results obtained by FPLC. The NWSP chromatographic profile (Fig. 1) shows three elution peaks: The one of lower MM corresponds to L (120 kDa tetramer) and the others to high- and low-M.W. aggregates of L and KTI. Denaturation decreases the proportion of the heaviest component and is accompanied by the appearance of low-M.W. polypeptides, corresponding to free antitryptic factor and lectin monomer (MM = 20–30 kDa). The NSI elution profile shows the characteristic peaks of 15S (11S aggregates), 11S, 7S, and 4S fractions with MM > 700, 360, 270, and 60 kDa, respectively (17). Denaturation leads to the almost complete disappearance of the 15S peak, reduction of that for 11S, and increase of species of MM < 100 kDa (subunits of 11S and 7S globulins). SC is mainly constituted by α_{s1}, α_{s2}, β, and κ-casein, with MM ≈ 20–25 kDa (18). However, the observed FPLC peaks indicated that this sample contains high-M.W. components corresponding to micellar aggregates of various sizes.

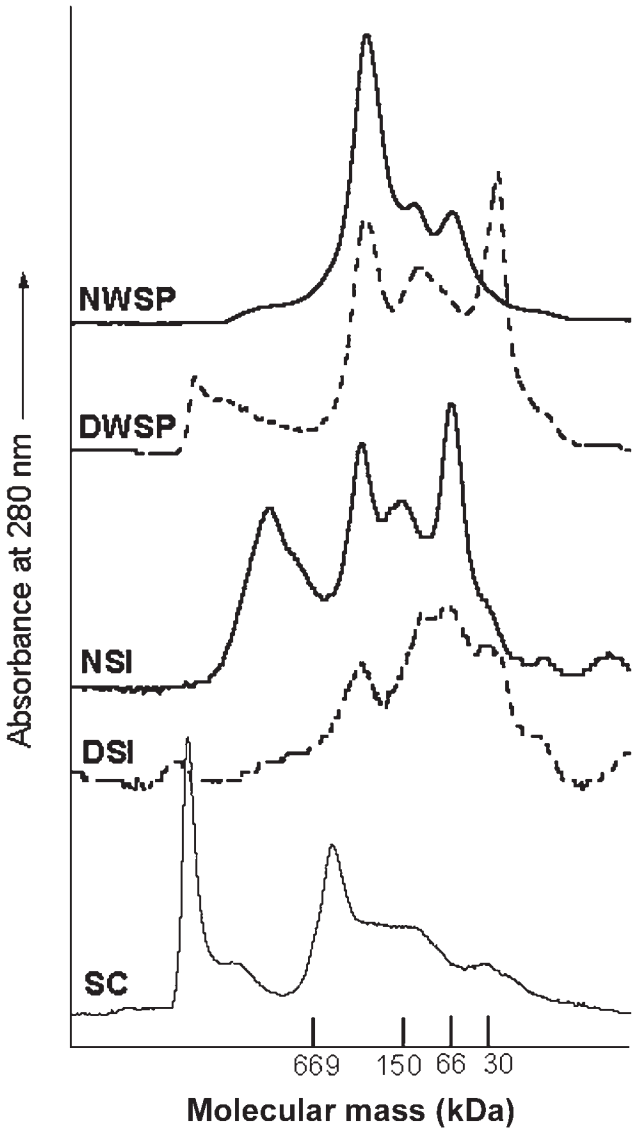

FIG. 1. Gel filtration profiles of samples on a Superose 6B column (Pharmacia LKB, Uppsala, Sweden) using sodium phosphate buffer, 0.01 M, at pH 7.0 as eluent. Flow rate: 0.2 mL/min. NWSP, native whey soy protein; DWSP, denatured whey soy protein; NSI, native soy isolate; DSI, denatured soy isolate; SC, sodium caseinate.

In considering that the decrease in interfacial tension is necessary, although not enough, to form and keep an emulsion stable (2), the interfacial pressure of the aqueous dispersions (π_i) were determined for each sample (Table 1). In the absence of NaCl, NWSP showed the lowest value of π_i ($\alpha < 0.05$). Thermally denatured whey proteins (DWSP) significantly improved the π_i value. Likewise, DSI was more tensioactive than NSI ($\alpha < 0.05$). The tensioactivity improvement obtained as a consequence of thermal treatment would be caused by a higher surface hydrophobicity (H_0) as well as by the dissociation degree of denatured proteins. Values of H_0 for NSI and NWSP were 98 ± 7 and <10, respectively, and they increased four- to sixfold after denaturation (12). Detection of low-MM species in denatured samples (Fig. 1) would confirm the oligomer dissociation in DWSP and DSI. The SC sample, which already exhibited high H_0 in the native state (close to that for DSI), was the most tensioactive despite being formed by micellar aggregates of high MM (Fig. 1). Addition of NaCl increased H_0, as previously observed (12,19), and caused a significant increase ($\alpha < 0.05$) in π_i values for denatured samples and SC as well as a stronger increase for NWSP and NSI (Table 1).

Characteristics of initial o/w emulsions. The emulsifying properties of NWSP and DWSP were compared with those of native (NSI) and denatured (DSI) soy isolates. SC was employed as the control sample owing to its suitable emulsifying behavior.

Emulsions prepared with the samples just mentioned showed a similar rheological behavior: Newtonian fluids with viscosities below 10 cP. Bearing this in mind and considering the fixed emulsification conditions used, one could say that the dissimilar stability of the emulsions prepared with the different proteins is given by other factors: interfacial activity,

TABLE 1
Interfacial (π_i) and Surface Pressures (π_s) of NWSP, DWSP, NSI, DSI, and SC Dispersions (1 mg/mL) With and Without NaCl Addition; Median Diameter ($D_{v,0.5}$); and Span (*S*) of Droplet Size Distribution of o/w Emulsions Prepared with These Protein Samples

Protein sample	NaCl addition (500 mM)[a]	Tensioactivity of dispersions		Droplet size parameters of emulsions	
		π_i (mN/m)[b]	π_s (mN/m)[b]	$D_{v,0.5}$[c] (μm)	Span (*S*)[c] (dimensionless)
NWSP	–	12.2 ± 0.3	19.2 ± 0.5	41.0	1.62
	+	14.0 ± 0.6	21.1 ± 0.4	55.0	1.32
DWSP	–	14.3 ± 0.1	21.9 ± 0.3	42.4	1.20
	+	14.7 ± 0.3	23.1 ± 0.2	47.6	1.19
NSI	–	13.2 ± 0.1	21.0 ± 0.3	43.7	1.10
	+	14.6 ± 0.2	23.0 ± 0.4	50.9	0.23
DSI	–	14.2 ± 0.1	21.7 ± 0.3	47.0	1.14
	+	14.7 ± 0.2	23.2 ± 0.2	50.7	0.99
SC	–	15.2 ± 0.1	24.6 ± 0.5	38.8	1.23
	+	15.8 ± 0.2	25.2 ± 0.2	40.4	1.19

[a]Salt concentration in the aqueous phase.
[b]Each value was the mean of at least three determinations.
[c]Maximum SD was 5%. LSD (α = 0.05): 0.4, 0.3, 1.8, and 0.07 for π_s, π_i, $D_{v,0.5}$, and *S*, respectively. NWSP, native whey soy protein; DWSP, denatured whey soy protein; NSI, native soy isolate; DSI, denatured soy isolate; SC, sodium caseinate; o/w, oil-in-water.

nature of the film, and colloidal interactions between droplets. In previous work (12), the droplet size distribution was trimodal only for whey soy protein emulsions, results attributed to the homogenization method used. In the present study, all emulsions showed trimodal distributions. These results could be attributed to the smaller device used (for small sample volume). In the absence of NaCl, the span of droplet size distribution (*S*) of NWSP was significantly greater ($\alpha < 0.05$) than that observed in all other samples. On the other hand, in salt-added samples, *S* followed the order NWSP > DWSP ≈ SC > DSI ≈ NSI (Table 1). Emulsions prepared with denatured samples of whey proteins and soy isolates (DWSP and DSI) exhibited greater median droplet sizes ($D_{v,0.5}$) than emulsions containing native samples (NWSP and NSI). In the presence of salt, an increase of droplet size was observed. In this condition, only DWSP emulsions possess a $D_{v,0.5}$ value close to those of SC (Table 1). During emulsion homogenization, collisions between droplets are very frequent owing to intense mechanical stirring (3). In comparison with NWSP and NSI, film viscoelasticity in emulsions prepared with denatured proteins DWSP and DSI is low and favors a phenomenon known as droplet recoalescence (12). This would explain the large $D_{v,0.5}$ observed in DWSP and DSI emulsions despite the high π_i of their constitutive proteins (Table 1). The lack of correlation between droplet size and equilibrium interfacial tension could be attributed to the low film resistance, according to results obtained by Walstra (20).

BS profiles (BS%) were analyzed as a function of the length of the measuring tube (mm). Figure 2 shows the initial (t = 1 min) BS_0% profiles for emulsions prepared with native proteins (NWSP, NSI), denatured proteins (DWSP, DSI), and SC without (Fig. 2A) and with NaCl (Fig. 2B). SC emulsions, in both conditions, presented the highest BS_0, possibly because of a greater number of droplets. This result can be explained by the fact that SC is composed of hydrophobic proteins (α_{s1}, α_{s2}, β, and κ-casein) which, on interacting with the o/w interface, are easily adsorbed by micellar dissociation. The other emulsions, without salt added, were different only in the upper part of the tube, even though they were prepared with proteins with different π_i values. In the presence of salt, BS_0 differed not only from one type of sample to the other but also along the entire tube length. The decreasing order for BS_0 was SC > DWSP > DSI > NSI > NWSP and showed the

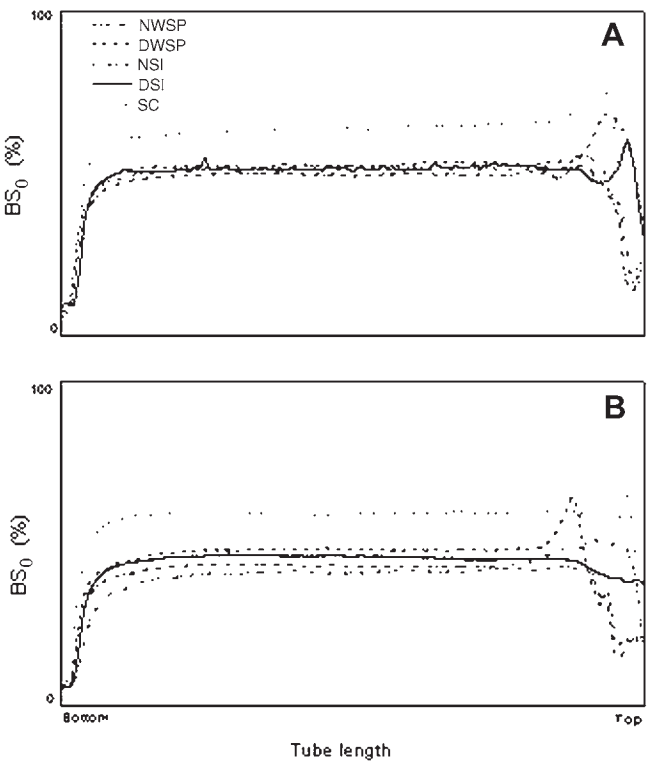

FIG. 2. Initial backscattering (BS_0) profiles of oil-in-water (o/w) emulsions. (A) Without NaCl, and (B) with 500 mM NaCl in the aqueous phase. Tube length: 60 mm. For other abbreviations see Figure 1.

same tendency to π_i values. Thus, high interfacial activity may be achieved by low-M.W. species and high H_0 generated by thermal denaturation (DSI and DWSP) or by dissociation of micellar aggregates while interacting with the interface (SC). Values of $BS_{0,av}$ (zone 10–20 mm) can be utilized to estimate the median diameter droplet size because of the negative correlation ($r = -0.84$) observed in Figure 3. In all emulsions, the presence of NaCl increased droplet size, in agreement with previous results (12), as was evidenced by a decrease in $BS_{0,av}$.

Owing to the homogenization method employed, the amount of air incorporated during emulsification depends on the protein used. In the liquid emulsions studied here, the flotation rate of air bubbles is much higher than required for emulsion creaming (21). Therefore, in emulsions where air was incorporated to form stable bubbles, foam buildup in the upper part of the tube was observed at short times (1–2 min). This is visualized as a peak in the BS_0 profile, its area being a direct measure of the amount of the foam formed (Fig. 2). As in emulsions, foam stability against collapse depends not only on the surface tension–depression capacity of the protein but also on the rheological properties of the interface (22). Figure 2A shows that NWSP did not form foam at the top of the tube, in agreement with their very low H_0 and surface pressure (π_s) (Table 1). NSI emulsions were also observed to show a very low tendency to form and stabilize foam. In turn, denaturation of globular protein positively influences foam-forming capacity (23), as evidenced in DWSP and DSI emulsions by a pronounced peak in BS_0 profiles (Fig. 2). Tendency to foam formation by DWSP and SC emulsions was similar, although weaker in DSI. Graham and Phillips (24) showed that β-casein, a protein of high structural flexibility and a main constitutive protein of SC (18), possesses a high foam formation capacity though with low stability.

No changes in the upper part of BS_0 profiles for NWSP and DWSP were observed as a consequence of salt addition, and this result demonstrated a low effect of ionic strength on foam stability in both emulsions (Fig. 2B). As it was observed at low ionic strength, DWSP exhibited the greatest foam formation. The wide peak observed in the salt-added NSI emulsion indicated a strong foaming and stabilizing capacity possibly caused by greater tensioactivity (Table 1) and a higher degree of interfacial organization (22–24) than those without salt. Although a similar tensioactivity increase was observed for salt-added NWSP, the π_s value reached was similar to that for unsalted NSI (Table 1). In comparison with native isolate proteins, this feature of NWSP together with their low H_0 and lower M.W. (Fig. 1) would prevent formation of a film sufficiently organized to stabilize the foam. Although the tensioactivity of DWSP, DSI, and SC samples was higher in the presence of salt (Table 1), the foam peak on the BS_0 profiles did not change in DWSP, decreased in SC, and was not observed in DSI (Fig. 2B).

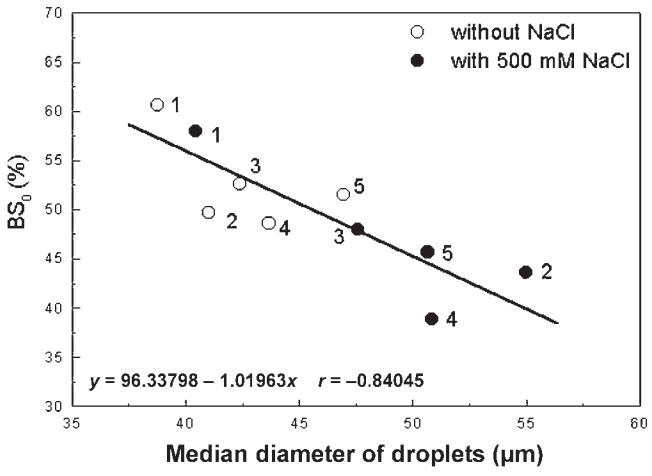

FIG. 3. Correlation between $BS_{0,av}$ and median diameter of droplets. $BS_{0,av}$ is the average of BS_0 values at zone 10 to 20 mm of the measurement tube. (1) SC; (2) NWSP; (3) DWSP; (4) NSI; (5) DSI. For other abbreviations see Figure 1.

Under the emulsification conditions employed, a competition was established for migration to air/water and oil/water interfaces. For DSI, considering the marked increase of H_0 induced by NaCl addition (12), these denatured proteins are likely to have a high affinity for the oil/water interface owing to the energetically favorable interaction of nonpolar amino acid residues with the lipid phase. Thus, as in the case of SC, the decrease in concentration of available protein to adsorb at the air/water interface would explain the weaker tendency of DSI samples to which salt had been added to form foam during the emulsification process.

Creaming kinetics of emulsions. To study the stability of emulsions against the creaming process, the BS% profiles were analyzed at different times. In Figure 4, typical BS profiles of NWSP, NSI, and SC emulsions are plotted for 1 (initial) to 60 min. Data recorded at 15-min intervals were selected for illustrative purposes. The 10–20 mm zone was chosen to estimate the destabilization degree by creaming. Both NWSP and NSI emulsions were very unstable as evidenced by a rapid shift in the BS front (Fig. 4A,B). By contrast, the SC emulsion was the most stable (Fig. 4C).

Creaming kinetics for all emulsions were evaluated from the BS profiles by recording the average BS values (BS_{av} 10–20 mm zone) as a function of time (Fig. 5A). The sharp decrease of BS_{av} reflected the instability observed for NWSP and NSI (Figs. 4A,B), in comparison with the slow decrease observed for SC (Fig. 4C). The stability increase caused by thermal denaturation was considerable in DSI and less noticeable for DWSP. Besides, in the latter emulsion, a notable clarification of the lower aqueous phase occurred, leading to BS_{av} close to zero. The stability of every sample was expressed by its creaming constant K (Table 2), which decreased as stability increased. The lack of correlation between K and the $D_{v,0.5}$ means that droplet size differences among the several emulsions is not the sole factor affecting this destabilization mechanism. According to McClements (2), floc formation and structure are important influences on emulsion stability against creaming. Optical micrographs of initial emulsions (t = 0) showed that most samples had a tendency to flocculate

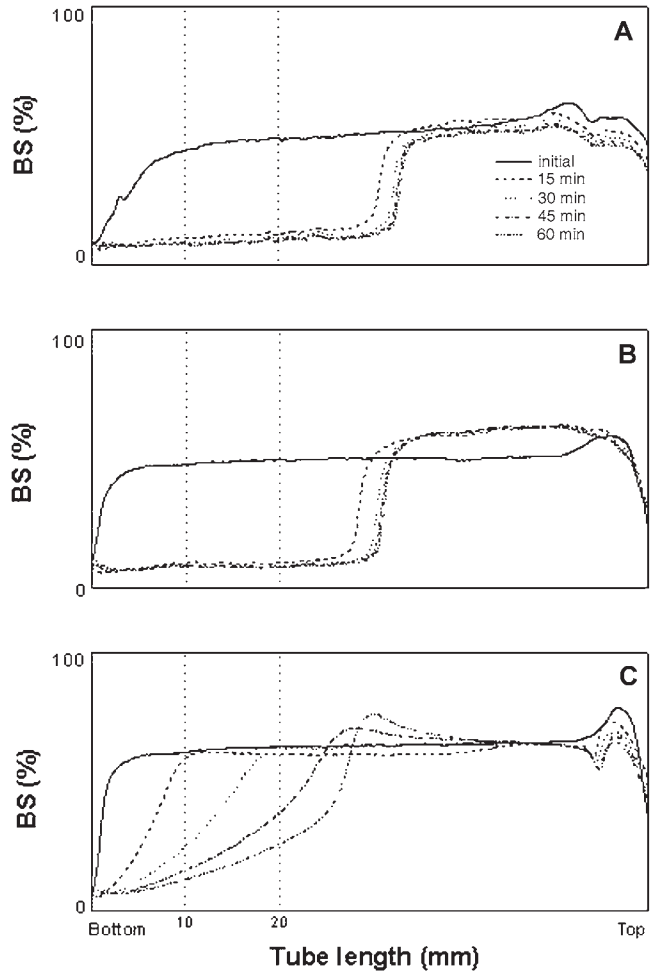

FIG. 4. Backscattering (BS) profiles of (A) NWSP, (B) NSI, and (C) SC o/w emulsions. Measurement tube length: 60 mm. For abbreviations see Figures 1 and 2.

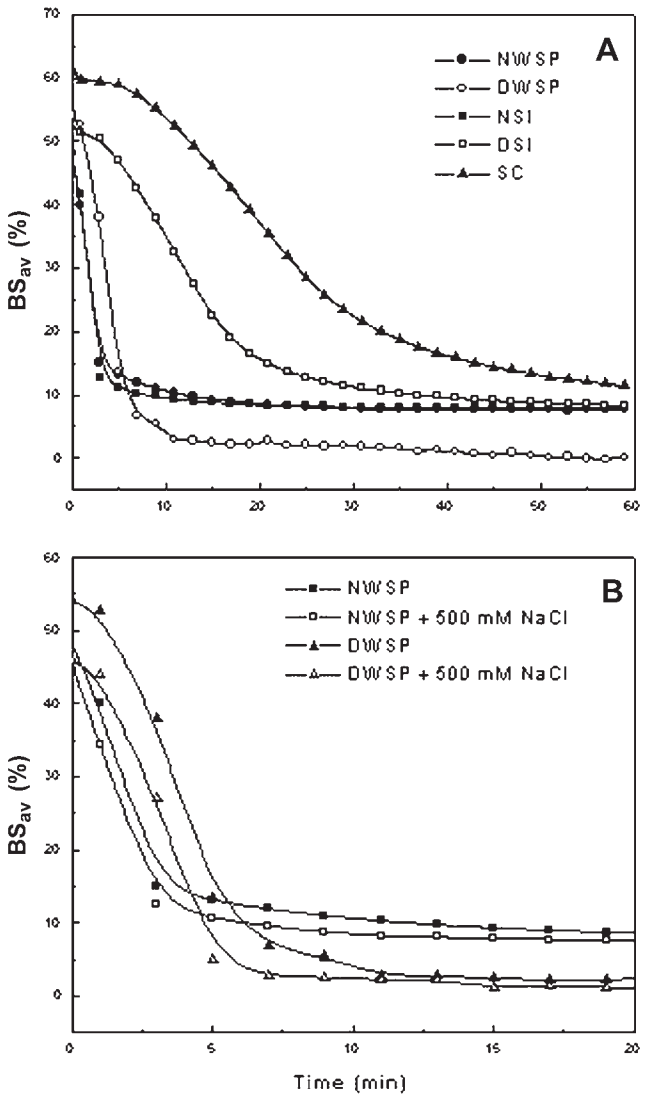

FIG. 5. Creaming destabilization kinetics of o/w emulsions. Zone 10–20 mm. Values are the means of at least three determinations. For abbreviations see Figures 1 and 2.

TABLE 2
Effect of NaCl Addition (500 mM in the aqueous phase) on Creaming Stability and Volume of Cream Phase (V_r, relative to total volume) of o/w Emulsions Prepared with NWSP, DWSP, NSI, DSI, and SC

Sample	NaCl	Creaming constant K (min^{-1})	V_r[a] (dimensionless)
NWSP	0 mM	11.28 ± 0.14	0.37
DWSP		3.32 ± 0.01	0.50
NSI		8.59 ± 1.51	0.38
DSI		1.48 ± 0.11	0.41
SC		0.68 ± 0.01	0.42
NWSP	500 mM	12.38 ± 0.94	0.31
DWSP		3.35 ± 0.01	0.47
NSI		27.62 ± 4.11	0.39
DSI		3.81 ± 0.69	0.42
SC		0.97 ± 0.01	0.41

[a]Maximum SD was 5%. LSD (α = 0.05): 0.02 for V_r. For abbreviations see Table 1.

(Fig. 6). Although the flocs are unstable under droplet size measurement conditions (high dilution and turbulent flux) at low or intermediate oil volumetric fractions (ϕ = 0.33) and low protein concentrations (25), these aggregates exist in quiescent conditions. The flocs of open structure, enclosing a great volume of continuous phase, have a density close to that of the continuous phase (3,5). These open network-structured flocs were observed in SC emulsions at low ionic strength (Fig. 6A). As mentioned earlier, SC exhibits a micellar structure (18) with a high value for H_0. In this case, flocculation is favored not only by colloidal hydrophobic interactions but also by the effect of Ca^{2+} (0.7 g%, Materials and Methods section), which is responsible for the formation of saline bonds at low ionic strength. Therefore, the very low K for the SC emulsion could be explained by the presence of hydrated flocs (V_r = 0.42), which are stabilized by hydrophobic and ionic interactions and composed of droplets with the lowest $D_{v,0.5}$ (Tables 1 and 2).

On the other hand, for highly unstable NWSP and NSI emulsions (K = 11.28 and 8.59 min^{-1}, respectively), although the micrograph shows the presence of flocs (Fig. 6A), these do not constitute a reticular structure. By entrapping a lower

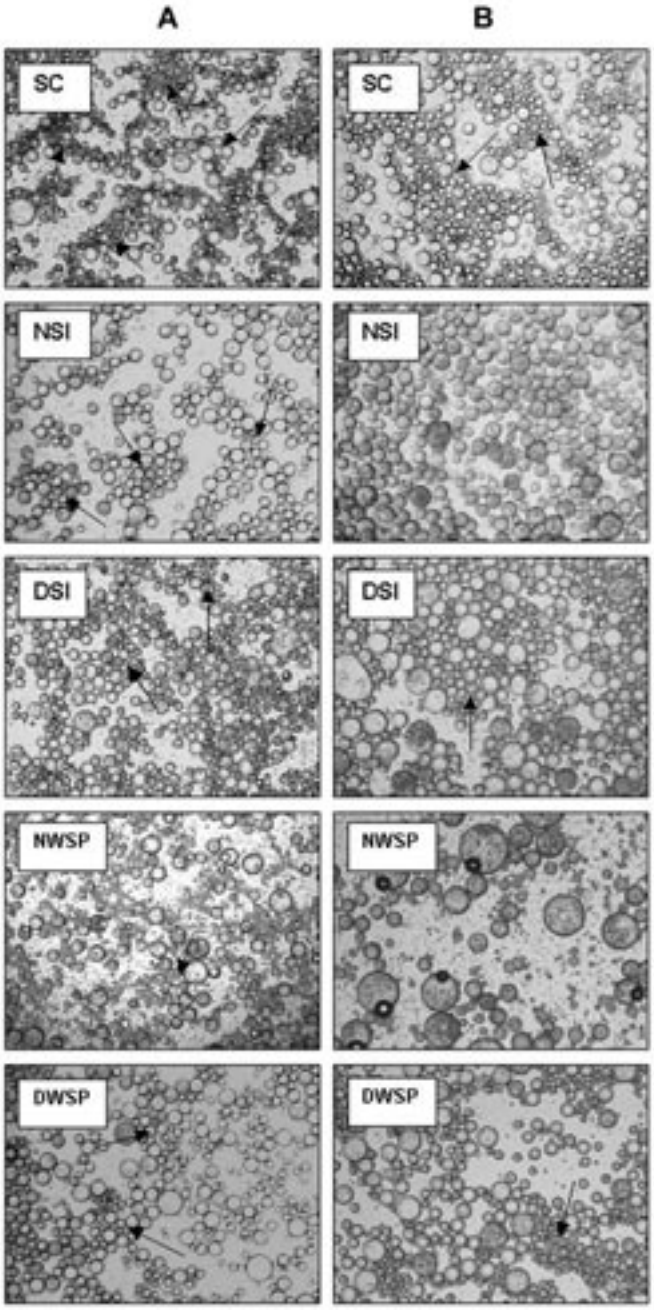

FIG. 6. Photomicrographs of undiluted o/w emulsions at 100× magnification. (A) Without salt, (B) with 500 mM NaCl added to the aqueous phase. For abbreviations see Figures 1 and 2.

volume of continuous phase and behaving as droplets of high effective size, this structure would increase the creaming rate (5). This could explain the lower hydration degree of the cream phase in NWSP and NSI compared with SC, and its low stability against creaming (Table 2). Emulsions prepared with DWSP and DSI, in comparison with the SC emulsion, also showed open-structured flocs and lower *K* values (Fig. 6A, Table 2). H_0 values for DWSP and DSI are high, and this is caused by exposure of nonpolar amino acidic residues, initially hidden in the native structure of constitutive proteins (12). Thus, hydrophobic colloidal interactions between droplets would favor their aggregation. The hydration degree of the cream phase was similar for DSI and SC ($\alpha < 0.05$). The use of DWSP led to even more hydrated creams, occupying 50% of the total volume (Table 2).

The increase of ionic strength exerts an important influence on colloidal interactions between droplets (2). In the presence of NaCl, the NWSP emulsion showed a slight increase of *K* whereas for the DWSP emulsion, this parameter did not vary significantly (Table 2, $\alpha < 0.05$). On the other hand, NaCl addition had a marked effect on creaming stability of emulsion prepared with isolate proteins, showing a twofold rise in NSI and a threefold rise in DSI (Table 2). Salt addition in NSI did not alter the tendency to flocculate or the hydration of flocs (Fig. 6B, Table 2). Besides, as indicated by measurements of $D_{v,0.5}$, NSI and DSI emulsions with NaCl exhibited a higher droplet size than these emulsions without salt added (Table 1, Fig. 3). This fact could explain the decrease in creaming stability due to NaCl addition. An ionic strength increase had little effect on flocculation of SC emulsions (Fig. 6B) and on V_r (Table 2, $\alpha < 0.05$). In view of the observation that Ca^{2+} considerably influences flocculation for SC emulsions, the increase of *K* (Table 2) may be ascribed to the negative effect of Na^+, which shifts the bivalent ions from its binding sites toward the caseins (18). Salt addition would decrease the formation of Ca^{2+}-induced intermolecular bridge bonds, thus contributing to weak floc structure. Therefore, in high ionic strength conditions, only the hydrophobic interactions seem to be relevant during flocculation of SC emulsions.

As previously mentioned, the creaming constants of NWSP and DWSP emulsions did not vary greatly after salt addition (Table 2). Figure 5B shows the creaming kinetics of whey protein emulsions in the presence and absence of NaCl. In the presence of salt, NWSP showed a low tendency to flocculate and a considerable increase in $D_{v,0.5}$ (Fig. 4, Table 1). The lowest degree of hydration of the cream phase ($V_r = 0.31$, $\alpha < 0.05$) may be attributed to the exclusion of aqueous phase favored by the presence of large droplets.

On the other hand, in the presence of NaCl, the DWSP emulsion evidenced a high tendency to flocculate and an increased $D_{v,0.5}$ and a large V_r value (Fig. 3, Tables 1 and 2). Under these conditions, clarification occurred in the lower part of the tube, leading to BS_{av} values around zero (Fig. 5B). Whey protein emulsions contained a great volume of lipids in the population of small droplets (5–10 μm). This distribution explains the high *S* addressed earlier (Table 1). In salt-added DWSP emulsions, small droplets would be part of the flocs migrating together with droplets of larger size, thus explaining the total clarification observed in the 10–20 mm zone. By contrast, weakly flocculated small droplets in NWSP have very low individual creaming rates, so they would remain in that zone for a much longer period. Although the *K* value in NWSP was about four times as high as in DWSP, no clarification was observed, and the BS_{av} decreased only to 10–15% (Fig. 5B).

Thermal treatment is a fundamental process in using whey soy proteins in formulated foods (11). For instance, it

increases tensioactivity in constitutive proteins by thermal denaturation, which can be important for formulating aerated emulsions. Unlike storage proteins, the stability against creaming was improved slightly compared with the thermally untreated whey protein sample, and it was not affected by the NaCl addition. Low protein concentrations were used in the continuous phase, the better to ascertain the differences between samples. On the other hand, the high tendency toward flocculation and formation of a highly hydrated creaming phase in denatured whey protein emulsions may stabilize them against coalescence.

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