Comparative study of emulsifying properties in acidic condition of soluble polysaccharides fractions obtained from soy hull and defatted soy flour

María Cecilia Porfiri, Darío Marcelino Cabezas & Jorge Ricardo Wagner

Journal of Food Science and Technology

Association of
Food Scientists &
Technologists (India)

AFSTI

🦄 Springer

🦄 Springer

Springer

ORIGINAL ARTICLE

Comparative study of emulsifying properties in acidic condition of soluble polysaccharides fractions obtained from soy hull and defatted soy flour

María Cecilia Porfiri[1,2] · **Darío Marcelino Cabezas**[1,2] · **Jorge Ricardo Wagner**[1,2]

Abstract The present study compares the emulsifying properties in acidic conditions of hull soluble polysaccharides (HSPS), soybean soluble polysaccharides (SSPS) and its mixtures. These fractions were obtained from byproducts of soybean processing industry (soy hull and residual fiber after isolation of soy cotyledon protein, respectively). Although SSPS is already characterized, HSPS is a novel fraction which has not been studied in deep and it is still unexplored as emulsifier. Dispersions of both fraction and a mixture 50:50 of them at pH 3.0 were used as aqueous phase (1.0–3.0 % *w/w*) in coarse and fine oil-in-water emulsions (oil mass fraction = 0.3). Its stability was evaluated through the evolution of backscattering profiles (%BS), particle size distribution and mean particle diameters. The rheology of the emulsions was also analyzed. Both fractions provided stability to creaming when increasing the polysaccharide concentration and energy of homogenization. While coarse emulsions were unstable systems, fine emulsions were stable enough and allowed a deeper analysis of the destabilizing processes. A bridging flocculation phenomenon in the presence of HSPS and HSPS/SSPS mixtures is suggested, which influences the creaming and rheological behavior. Also, coalescence index increases according HSPS and HSPS/SSPS concentrations, but particle sizes reached were smaller than in SSPS emulsions. Fine emulsions with 3 % of HSPS/SSPS mixtures yielded the best results on the overall stability at 28 days. So, functional properties of the fractions may improve by the formulation of emulsions consisting in mixtures of them. These results are of interest to the manufacturing of acidic foods, taking advantage of obtaining byproducts from residual materials.

Highlights 1. HSPS and SSPS were extracted from seed coat and cotyledon of soybean respectively

2. Both fractions were effective in the formulation and stabilization of acidic O/W emulsions

3. Higher concentrations stabilize emulsions to creaming and reduces mean droplet sizes

4. The presence of a mixture of the fractions improved the stability of the emulsions

5. This is of interest to the manufacturing of acidic foods making use of residual materials

María Cecilia Porfiri and Darío M. Cabezas contributed equally to this work.

✉ Darío Marcelino Cabezas
  dario.cabezas@unq.edu.ar

1 Laboratorio de Investigación en Funcionalidad y Tecnología de Alimentos (LIFTA), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, B1876BXD, Bernal, Provincia de Buenos Aires, Argentina

2 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

Abbreviations

HSPS	Hull soluble polysaccharides
SSPS	Soybean soluble polysaccharides
HSPS/SSPS	Mixtures 50/50 of hull soluble polysaccharides and soybean soluble polysaccharides
O/W emulsion	Oil-in-water emulsion
%BS profiles	Profiles of backscattering

Introduction

Soybean seed is one of the major food sources providing protein, oil, carbohydrates, isoflavones, and many other

nutrients to humans and animals. This seed contains approximately 38 % protein, 18 % oil, 15 % insoluble carbohydrates (dietary fiber) and 15 % soluble carbohydrates (including oligosaccharides) (Hoogenkamp 2005). Besides proteins, the application of different soy derivates in the food industry has gained importance worldwide, given its high fiber content and the presence of various biologically active phytochemicals (Kumar et al. 2010; Rodríguez et al. 2006; Tripathi and Misra 2005).

The seed coat of soybeans (also known as soy hull) is the major co-product of the soybean processing industry. Soy hulls constitute about 8 % of the whole seed and contain about 86 % complex carbohydrates thus making them a source of dietary fiber. Proteins and galactomannans are also present in soy hull (Magdel-Din Hussein et al. 1998; Santos et al. 2008). Numerous studies have investigated the processing of soy hull as a source of pectins. Usually, the extraction of these polysaccharides is based on its solubility in acid medium at high temperature (Monsoor 2005; Gnanasambandam and Proctor 1999; Kalapathy and Proctor 2001). Although these works analyze the effect of different solubilization and precipitation conditions in the main properties of these polysaccharides (methoxyl and galacturonic acid content, degree of esterification, structure, solubility, etc.), it has not yet been studied in depth issues such as the presence of proteins and sugars composition. Their interfacial and emulsifying properties are also unknown, as if it was already made with most frequently studied soluble polysaccharide from cotyledon of the soybeans (soybean soluble polysaccharides, SSPS). In this way, soy hull is potentially a natural and inexpensive source of pectin like polysaccharides (in this work, hull soluble polysaccharides, HSPS) which represents a novel and unknown sample in the field of emulsion formulations.

SSPS, instead, is extracted from the residual fiber after isolation of soy protein. SSPS is a protein associated polysaccharides and its chemical structure has been extensively characterized (Nakamura et al. 2003). It is known that SSPS stabilizes oil-in-water (O/W) emulsions in a wide range of pH and at lower concentrations than those reported for other polysaccharides such as gum arabic or modified starch (Nakamura et al. 2004; Nakauma et al. 2008).

Proteins and polysaccharides play a key role in the structure formation and stabilization of food colloid systems. Proteins are normally used as emulsifying agents while polysaccharides, given its predominantly hydrophilic character, generally exhibit little surface activity at the oil–water interface. Only polysaccharides directly complexed with proteins, or those with sufficient hydrophobic character to adsorb strongly at the oil–water interface, are capable of making and stabilizing oil-in-water emulsions. Mixtures of both biopolymers can be found in the ingredients of a wide range of food colloids ranging from mayonnaise to ice cream (Akhtar et al. 2002; Evans et al. 2013; Neirynck et al. 2007).

Some food emulsions are acidic because they contain ingredients like citrus juice, vegetable juice or vinegar (e.g. dressings and sauces), or because of the addition of organic acids to enhance the microbiological stability. As a consequence, there is a need to find alternative emulsifiers/stabilizers with good properties in acidic conditions.

The aims of the present work were to analyze the emulsifying properties of HSPS in comparison with a reference soy polysaccharide, SSPS, and mixtures of them, and to evaluate its stabilizing effect on acidic oil-in-water emulsions.

Materials and methods

Materials

Defatted soybean flour was provided by Terminal 6 S. A. (Santa Fe, Argentina) and soy hull from Bunge Argentina S. A. (Santa Fe, Argentina). Refined sunflower oil (Molinos Cañuelas, Argentina) was purchased in a local supermarket. The chemical reagents used in this work were of analytical grade.

Preparation of polysaccharide fractions

Extraction of soy hull soluble polysaccharides

The soy hull was ground to a particle size lesser than 500 μm. HSPS was obtained by adapting the acid extraction and alcohol precipitation method optimized by Kalapathy and Proctor (Kalapathy and Proctor 2001). Ground soy hull was dispersed in 0.1 M HCl (1:15), homogenized in a high-speed blender (25,000 rpm for 5 min, Ultra-turrax T-25, S25-20NK-19G dispersing tool, IKA Labortechnik, GmbH & Co, Staufen, Germany) and adjusted at pH 1.0. The dispersion was incubated at 90 °C for 45 min, allowed to cool to room temperature and centrifuged at $7000 \times g$ for 15 min at 4 °C and a supernatant enriched in polysaccharides was obtained. In order to precipitate the HSPS, the supernatant was collected and dispersed in an equal volume of 2-propanol, the pH was adjusted to 3.5 and allowed to settle for 4 hs. The pellet was collected, centrifuged, dispersed in 70 % 2-propanol, stirred for 30 min and centrifuged. The washing was repeated with 70 % 2-propanol and then with 100 % 2-propanol (Kalapathy and Proctor 2001). The precipitate was dried at 40 °C in drying oven with forced air circulation.

Extraction of soluble soybean polysaccharides

Defatted soybean flour was ground to a particle size lesser than 150 μm. SSPS was prepared by adapting the extraction method developed by (Nakamura et al. 2012) with modifications. Protein and water-soluble substances in the defatted soybean flour were extracted twice at 50 °C for 30 min with 11 times the weight of distilled water, after the pH had been adjusted to 9.0 with 5 M NaOH. The residue obtained as a precipitate after centrifugation (7000 × g, 4 °C, 15 min) was suspended in distilled water at a concentration of 25.0 % w/w, adjusted at pH 3.5 with HCl, and heated in autoclave at 120 °C for 2 h. After that it was centrifuged at 7000 × g at 4 °C for 15 min and a supernatant enriched in polysaccharides was obtained. In order to precipitate the SSPS, the alcohol precipitation method was used as in the HSPS extraction previously described (Kalapathy and Proctor 2001).

Chemical composition

Each fraction (ca. 3 mg) was hydrolyzed with 2 M trifluoroacetic acid (TFA, 1 mL) for 90 min at 120 °C in closed-cap vials. The TFA was eliminated by evaporation, and the resulting monosaccharides were reduced to alditols using $NaBH_4$ and converted to alditol acetates to determine the neutral sugar composition of both fractions (Ponce et al. 2010). Consequently, these compounds were analyzed by using a Hewlett-Packard 5890 gas chromatograph (Agilent Technologies, Inc.) fitted with a capillary column 30 m × 0.25 mm i.d. 0.20 μm, SP-2330 (Supelco Inc.) and equipped with a FID operated at 240 °C. The injector temperature was 240 °C, and the oven temperature was kept isothermally at 220 °C. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. Aliquots were injected with a split ratio of ca. 80:1. *myo*-Inositol was used as the internal standard, and the different alditol acetates were identified by comparison with standards. The percentage of the different monosaccharides was calculated by considering that the FID responses are proportional to the molecular weight of the alditol acetates.

The protein content of samples was determined by Micro-Kjeldahl (N × 6.25) using a colorimetric procedure for determining the nitrogen content calibrated with ammonium sulphate (Nkonge and Ballance 1982).

Uronic acid contents of the sample were determined by a colorimetric method using m-hydroxydiphenyl, and expressed as galacturonic acid (GalA) (Blumenkrantz and Asboe-Hansen 1973).

Degree of esterification was expressed as percent methoxy groups as determined by a titration method (Lira-Ortiz et al. 2014).

Preparation of aqueous dispersions of polysaccharide fractions

Aqueous dispersions of polysaccharide fractions (1.0, 2.0 and 3.0 % w/w) were prepared by dissolving the HSPS and SSPS in 8 mM sodium citrate buffer pH 3.0, with the addition of 0.02 % w/v sodium azide to retard the microbial growth. These aqueous samples were immediately utilized after their preparation.

Preparation of coarse O/W emulsions

Refined sunflower oil was used to prepare O/W emulsions (oil mass fraction, $\Phi_m = 0.3$). Coarse emulsions were prepared at room temperature in an Ultra-turrax T-25 homogenizer using S 25 N-10 G dispersing tool at 25,000 rpm for 2 min, with different aqueous dispersions of polysaccharides and a mixture of them (50:50), in a range of 1.0–3.0 % w/w, as continuous phase. The behavior of these emulsions as a function of the storage time was analyzed for 1 day at 24 °C..

Preparation of fine O/W emulsions

Coarse emulsions, previously obtained in Ultra-turrax high-speed blender, were sonicated in an ultrasound homogenizer (SONICS 135 Vibra Cell VCX750) at a power level of 70 % (525 W), with the standard tip immersed 1/3

Table 1 Sugar composition, degree of esterification and protein content of soluble polysaccharides fractions obtained from soy hull and defatted soy flour

Polysaccharides	Neutral Sugar Composition (Mol %)								%DE	Protein content (wt%)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	GalA		
HSPS	11.1 ± 1.4	1.9 ± 0.3	4.0 ± 0.7	7.6 ± 1.0	22.8 ± 0.2	22.5 ± 0.1	4.6 ± 0.3	25.5 ± 1.3	77.8 ± 1.0	41.0 ± 2.1
SSPS	4.5 ± 0.4	2.5 ± 0.1	13.7 ± 2.7	7.1 ± 0.9	<LOD	42.3 ± 3.4	9.4 ± 0.5	20.5 ± 2.2	72.0 ± 2.3	6.2 ± 0.8

Mean values (n = 2) ± standard deviation

Rha rhamnose; *Fuc* fucose; *Ara* arabinose; *Xyl* xylose; *Man* mannose; *Gal* galactose; *Glc* glucose; *GalA* galacturonic acid; *LOD* limit of detection; *%DE* degree of esterification

in a glass 28 mm diameter for 2 min (Cabezas et al. 2012). The behavior of these emulsions was analyzed for 28 days of storage at 24 °C.

Optical characterization of O/W emulsions

The backscattering (%BS) of light was measured using a vertical scan analyzer (QuickScan, Beckman-Coulter, Fullerton, USA). The backscattering of monochromatic light ($\lambda = 850$ nm) from the emulsions was determined as a function of the height of the sample tube (ca. 65 mm). This methodology allowed us to discriminate between particle migration (creaming) and particle size variation (flocculation, coalescence) processes (Cabezas et al. 2012). The creaming process was analyzed from the %BS profiles of each emulsion obtained at different storage times at 24 °C. In this sense, the kinetics of the creaming process was evaluated through the percentage of emulsion whose %BS is lower than 60 % ($\%E_{60}$) and 85 % ($\%E_{85}$) from the initial %BS, as a function of time.

Particle size determination

Particle size distribution (expressed in % surface) and De Brouckere ($D_{4,3}$) mean diameters of particles of the emulsions were determined by laser diffraction with a particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, U.K.). $D_{4,3}$ are more sensitive to an increment of droplet size by flocculation and/or coalescence processes than Sauter mean diameters ($D_{3,2}$) (Cortés-Muñoz et al. 2009). Optical parameters applied were: refractive indexes of sunflower oil and water 1.47 and 1.33, respectively; adsorption: 0.001. Samples were diluted in the water bath of the dispersion system (Hydro 2000MU).

Particle size distribution and $D_{4,3}$ were measured after 2 min of sonication with the ultrasonic probe of the dispersion system at 40 W. The purpose of this low energy sonication was the break down the flocs that would be present in the emulsions (Higashitani et al. 1993). Subsequently, the flocculation degree (%FD) and coalescence index (%CI) were analyzed in the emulsions
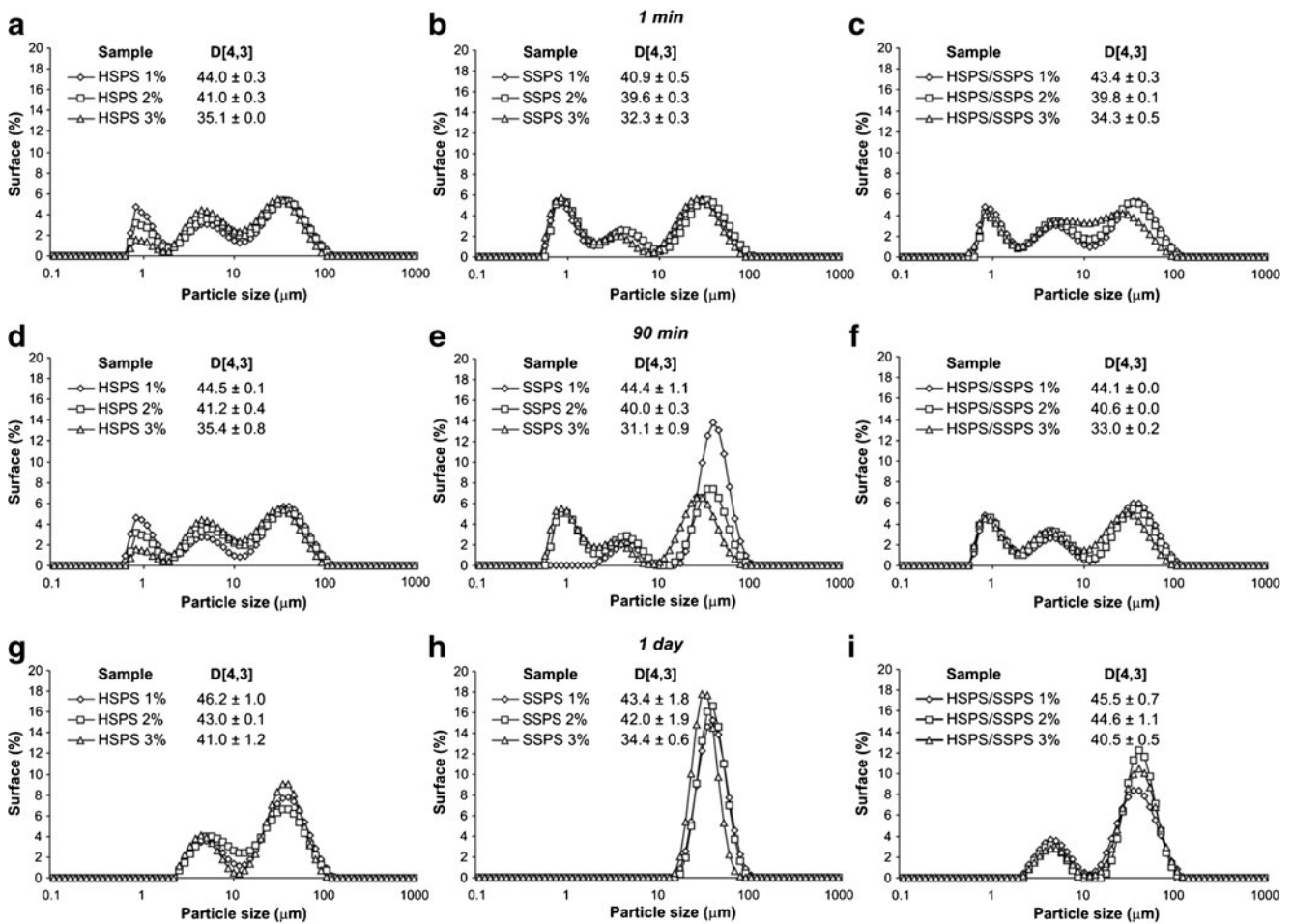
Fig. 1 Surface particle size distribution and De Brouker ($D_{4,3}$) mean diameters for O/W emulsions ($\Phi_m = 0.3$) obtained in Ultra-Turrax homogenizer with the addition of different soybean soluble polysaccharide fractions (SSPS, HSPS, HSPS/SSPS). Mean values ($n = 3$) $\pm$ sd

according to Palazolo et al. (Palazolo et al. 2005). The %FD was calculated by Eq. (1):

$$\%FD = \frac{D_{4,3} - D_{4,3sonic}}{D_{4,3sonic}} \times 100 \qquad (1)$$

Where $D_{4,3}$ and $D_{4,3\,sonic}$ are the volume-weighted diameters, measured before and after the sonication process, respectively.

The %CI was calculated by Eq. (2):

$$\%CI = \frac{D_{4,3sonic} - D_{4,3sonic,\,initial}}{D_{4,3sonic,\,initial}} \times 100 \qquad (2)$$

Where $D_{4,3\,sonic,\,initial}$ and $D_{4,3\,sonic}$ are the volume-weighted diameters measured in initial and stored emulsions after the sonication process, respectively.
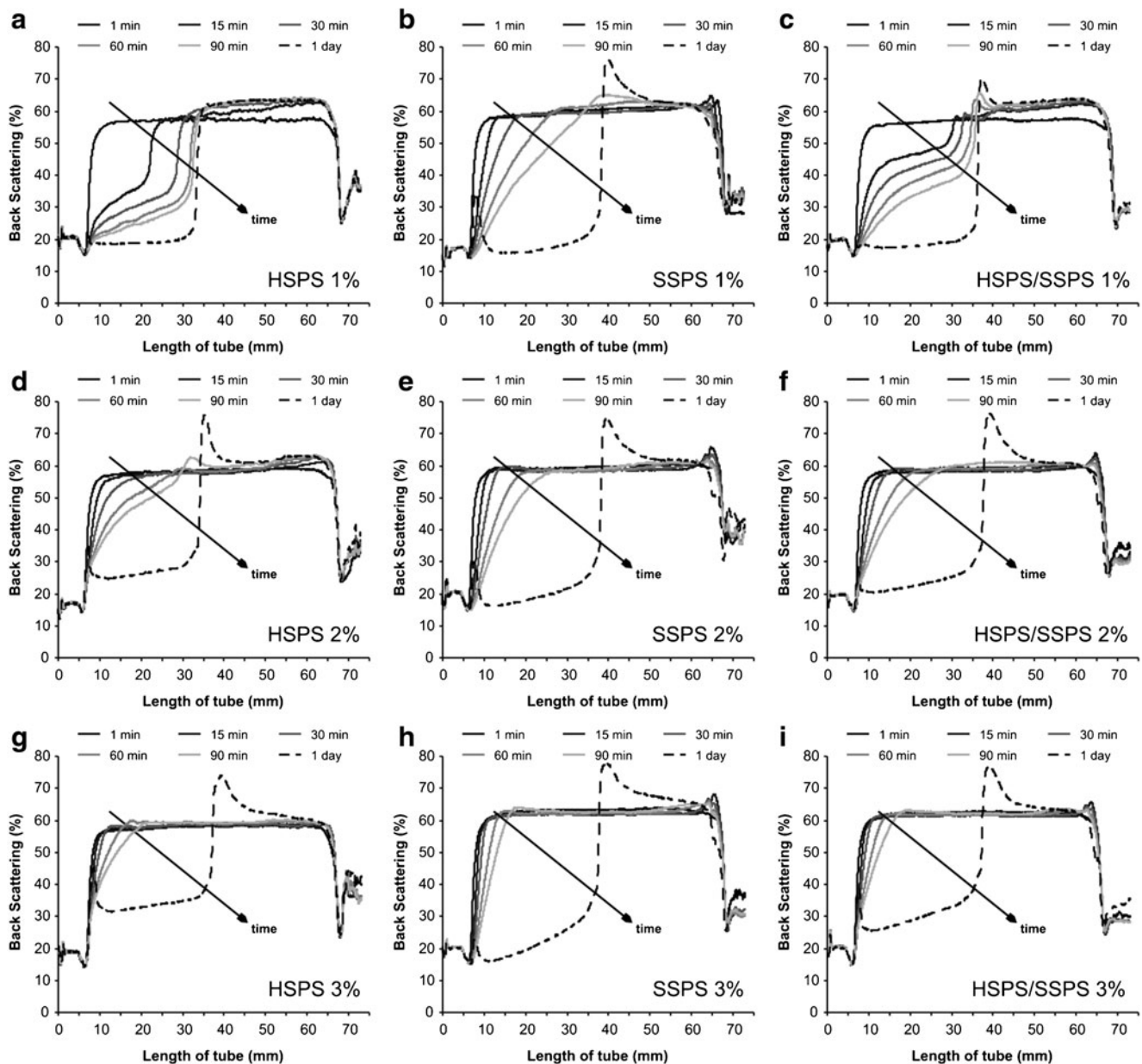
Fig. 2 Changes in back scattering (%BS) profiles as a function of the tube length with storage time (samples were stored for 1 day, arrow denotes time) for O/W emulsions ($\Phi_m = 0.3$) obtained in Ultra-Turrax homogenizer with the addition of different soybean soluble polysaccharide fractions (SSPS, HSPS, HSPS/SSPS). Mean values ($n = 3$) ± sd

Light microscopy

Emulsions were diluted with distilled water (1:10 v/v) immediately before analysis. Micrographs of these samples were obtained with an optical microscope operating at 400× magnification and fitted with a digital camera (Canon A570 289 IS; Malaysia) at 4× optical zoom.

Rheology

Oscillatory rheology of the emulsions was studied using an AR-G2 rheometer (TA Instruments; New Castle, DE, USA) with parallel-plate geometry (gap 1000 μm; diameter 40 mm).

Temperature (24 °C) was controlled with a water bath (Julabo ACW100, Julabo Labortechnik; Seelbach, Germany) associated with the rheometer. Experimental data for emulsions with different polysaccharide fractions at 3 % w/w were obtained by recording the storage or elastic modulus (G′), the loss or viscous modulus (G″) and tan δ (G″/ G′) as a function of oscillation frequency (0.1–100 Hz range) within the linear viscoelasticity range (strain 1 %) previously determined by stress-sweeps.

Statistical analysis

Assays were conducted at least in triplicate. Data were evaluated using analysis of variance (ANOVA) with the
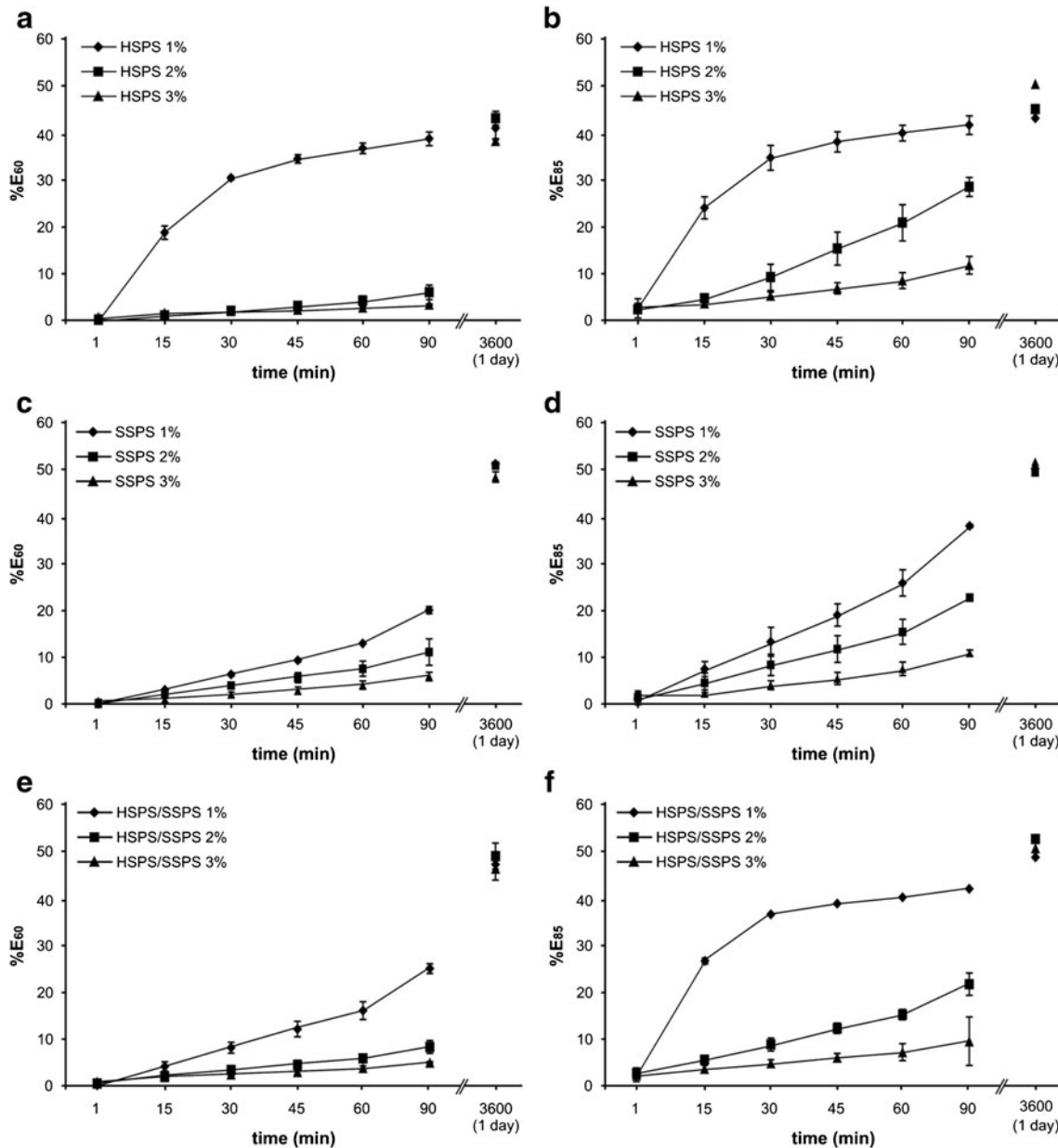
Fig. 3 $\%E_{60}$ and $\%E_{85}$ of O/W emulsions ($\Phi_m = 0.3$) obtained in Ultra-Turrax homogenizer with the addition of different soybean soluble polysaccharide fractions (SSPS, HSPS, HSPS/SSPS). Mean values ($n = 3$) ± sd

Statgraphics Centurion XV software (StatPoint Inc. 2005, USA). For this purpose, differences were considered significant at $p < 0.05$.

Results and discussion

Compositional analysis

Table 1 shows the compositional analysis of HSPS and SSPS. They contain high concentration of galactose and galacturonic acid. The esterification degree of the HSPS was higher than SSPS, although it was above 70 % in both cases. HSPS showed high content of protein and mannose. The content of this sugar is in concordance with the presence of galactomannans previously described in the literature (Magdel-Din Hussein et al. 1998).

Coarse O/W emulsions

Particle size distributions expressed as surface frequency for HSPS, SSPS and HSPS/SSPS coarse emulsions are shown in Fig. 1. Initially, all the systems showed a trimodal distribution of particles and similar $D_{4,3}$ values (Fig. 1a–c). SSPS emulsions were more sensitive to storage than HSPS and HSPS/SSPS. At 90 min, the smallest population of droplets disappeared using 1 % of SSPS (Fig. 1e), generating a change in the particle size distribution towards larger particle sizes. From 1 day of storage (Fig. 1h) smaller populations (<10 μm) disappeared leading to a monomodal distribution of particles. This behavior could be correlated with a flocculation process of the smaller droplets, affecting only slightly the $D_{4,3}$ values along time. In HSPS and mixture HSPS/SSPS emulsions, instead, only the population with lower particle size disappears at 1 day of storage (Fig. 1g, i). In all the emulsions, regardless of the sample concentration in aqueous phase, a slight increase in $D_{4,3}$ values is observed at 1 day of storage. Also, in all cases the distributions displaced toward smaller particle diameters when sample concentration increases, in accordance to a decrease in $D_{4,3}$ values (Fig. 1).

Sonication at low energy was applied in order to verify the presence of flocs. However, this was not possible in these coarse emulsions, given that the energy applied was sufficient to cause destabilization, mainly due to the tendency to coalesce of the higher droplets (>10 μm). Coarse emulsions had similar initial particle size distributions, however, the stability against the creaming process varied significantly depending on the type and concentration of the polysaccharides fractions, mainly at 1 % concentration. These differences can be
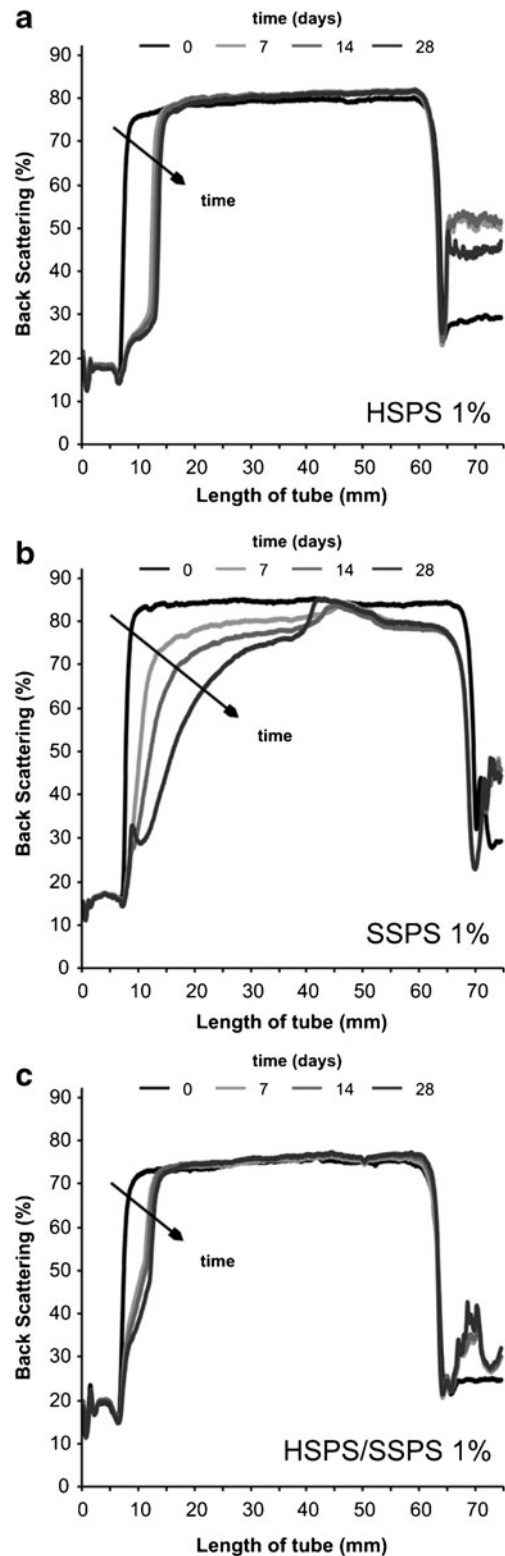
Fig. 4 Changes in back scattering (%BS) profiles as a function of the tube length with storage time (samples were stored for 28 day, arrow denotes time) for O/W emulsions ($\Phi_m = 0.3$) obtained in ultrasound homogenizer with the addition of 1 % of different soybean soluble polysaccharide fractions (SSPS, HSPS, HSPS/SSPS). Mean values ($n = 3$) ± sd

observed in the backscattering profiles (Fig. 2) and the creaming kinetics evaluated through the increase of the $\%E_{60}$ and $\%E_{85}$ values as a function of time (Fig. 3). Emulsions with SSPS showed profiles with a gradual change of the BS along the tube length (Fig. 2b, e, h), resulting in an increase of the $\%E_{60}$ parameter lower than the $\%E_{85}$ (Fig. 3c, d). This could be related to the creaming of the larger droplets (>10 μm), staying the smaller droplets distributed in the lower region of the tube. This non-homogeneous distribution of oil droplets along the sample tube was confirmed by the presence of a peak with a maximum of backscattering, related to the accumulation of smallest droplets in the bottom of the cream phase around 40 mm (higher concentration of dispersed oil phase) (Fig. 2b, e, h). Stability at higher concentrations (2 and 3 % SSPS) increases due to the smaller droplet size at initial time (Fig. 1).

HSPS 1 % emulsion showed an abrupt creaming process (Fig. 2a), reflected in similar $\%E_{60}$ and $\%E_{85}$ kinetics (Fig. 3a, b), in comparison to the gradual creaming mechanism of the SSPS emulsions previously described (Fig. 2b, e, h). This emulsion creates an interface that can be seen with the naked eye with a low concentration of dispersed oil phase in the aqueous phase (low values of %BS at the bottom of the tube). The creaming process of the HSPS 1 % emulsion could be influenced by the generation of flocs, which migrate faster than the individual droplets. These results could be due to a mechanism of bridging flocculation mediated by the high protein content presented on the HSPS fraction. This type of flocculation tends to occur when a biopolymer is used as an emulsifier and there is insufficient to completely cover the oil–water interface formed during homogenization (Mc Clements 1999; Walstra 1996). The formation of a floc structure observed through the microscope could explain the absence of a peak of maximum backscattering observed in the SSPS emulsions confirming a uniform distribution of the oil particles throughout the length of the cream phase (Fig. 2a). Higher concentration (2 and 3 % HSPS) caused more extensive flocculation of droplets, observed through the microscope (data
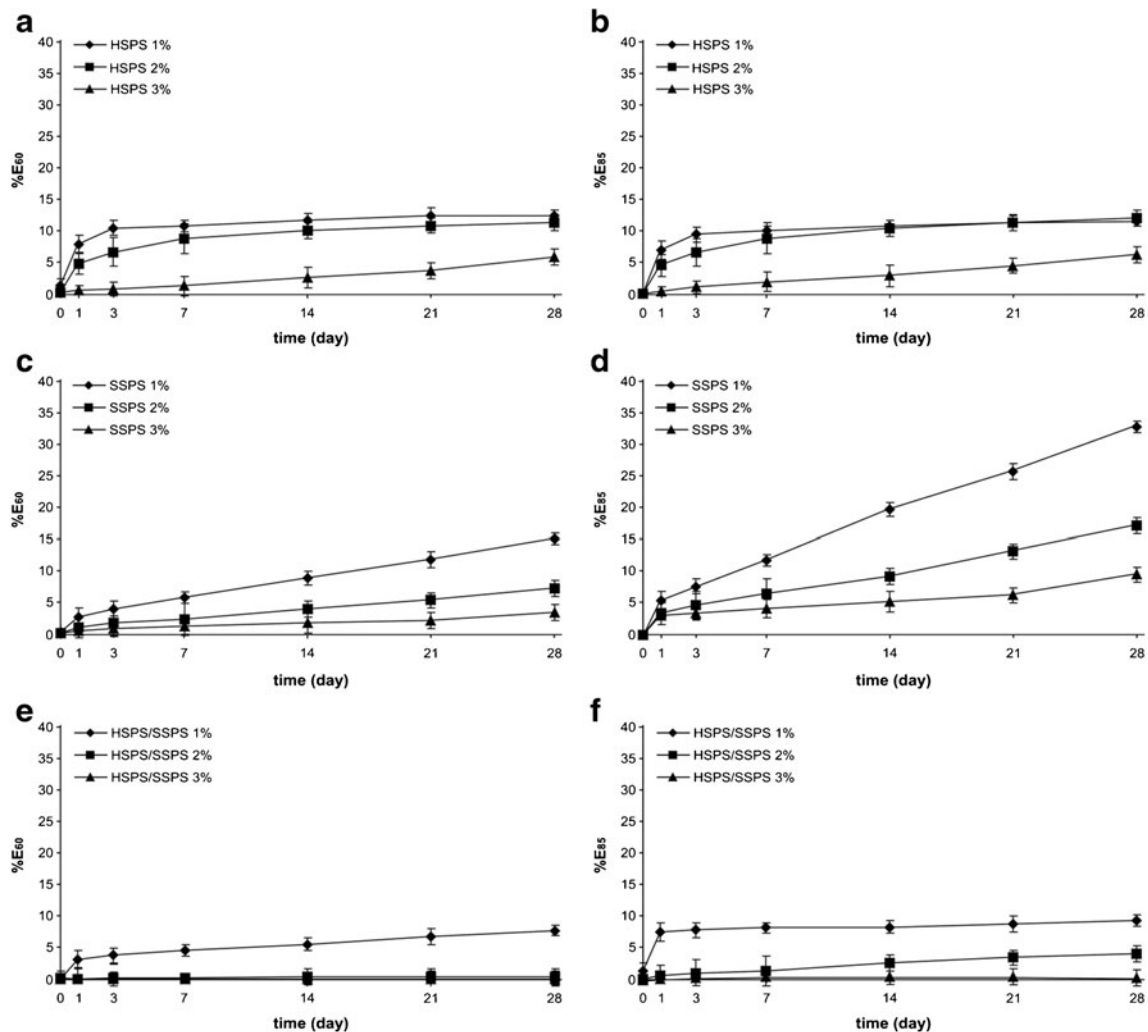
Fig. 5 $\%E_{60}$ and $\%E_{85}$ of O/W emulsions ($\Phi_m = 0.3$) obtained with the addition of different soybean soluble polysaccharide fractions (SSPS, HSPS, HSPS/SSPS). Mean values ($n = 3$) ± sd

not shown), but improved the creaming stability of the emulsions probably due to an increase in the viscosity of the continuous phase. This slows down the diffusion of the droplets and the flocs, and hence the rate of creaming (Figs. 2d, g and 3a, b). These results are consistent with those obtained by other researchers on different polysaccharide-containing emulsions (Cao et al. 1990; Hemar et al. 2001).

It is interesting that HSPS-SSPS mixtures presented an intermediate behavior between the individual polysaccharides fractions in both the %BS profiles (Fig. 2c, f, i) and the creaming kinetics (Fig. 3e, f).

Fine O/W emulsions

Fine O/W emulsions were studied recording the %BS profiles for 28 days (Fig. 4). The kinetics of creaming (%E_{60} y %E_{85}) presented a similar mechanism between coarse and fine emulsions (Figs. 3 and 5). SSPS emulsions showed a continuous creaming process over time (Fig. 4b), without allowing the formation of a defined cream phase (Fig. 5c and d). In contrast, the HSPS and HSPS/SSPS ones lead to the formation of a perceptible interface between the oil and the aqueous phases, mainly at 1 %, corroborated by the back scattering profiles (%BS) as a function of the tube length (Fig. 4a, c) and by the constant values of %E_{60} and %E_{85} from the third day (Fig. 5a, b, e, f). In accord with coarse emulsions, there was an improvement in creaming stability with increasing the polysaccharide concentration. Emulsions with 3 % of HSPS/SSPS were stable to creaming during the time of storage, and presented %E_{60} and %E_{85} values near to zero (Fig. 5e, f). As other authors demonstrated, the presence of an appropriate combination of emulsifiers would offer a promising way to improve emulsion stability (Aoki et al. 2005).
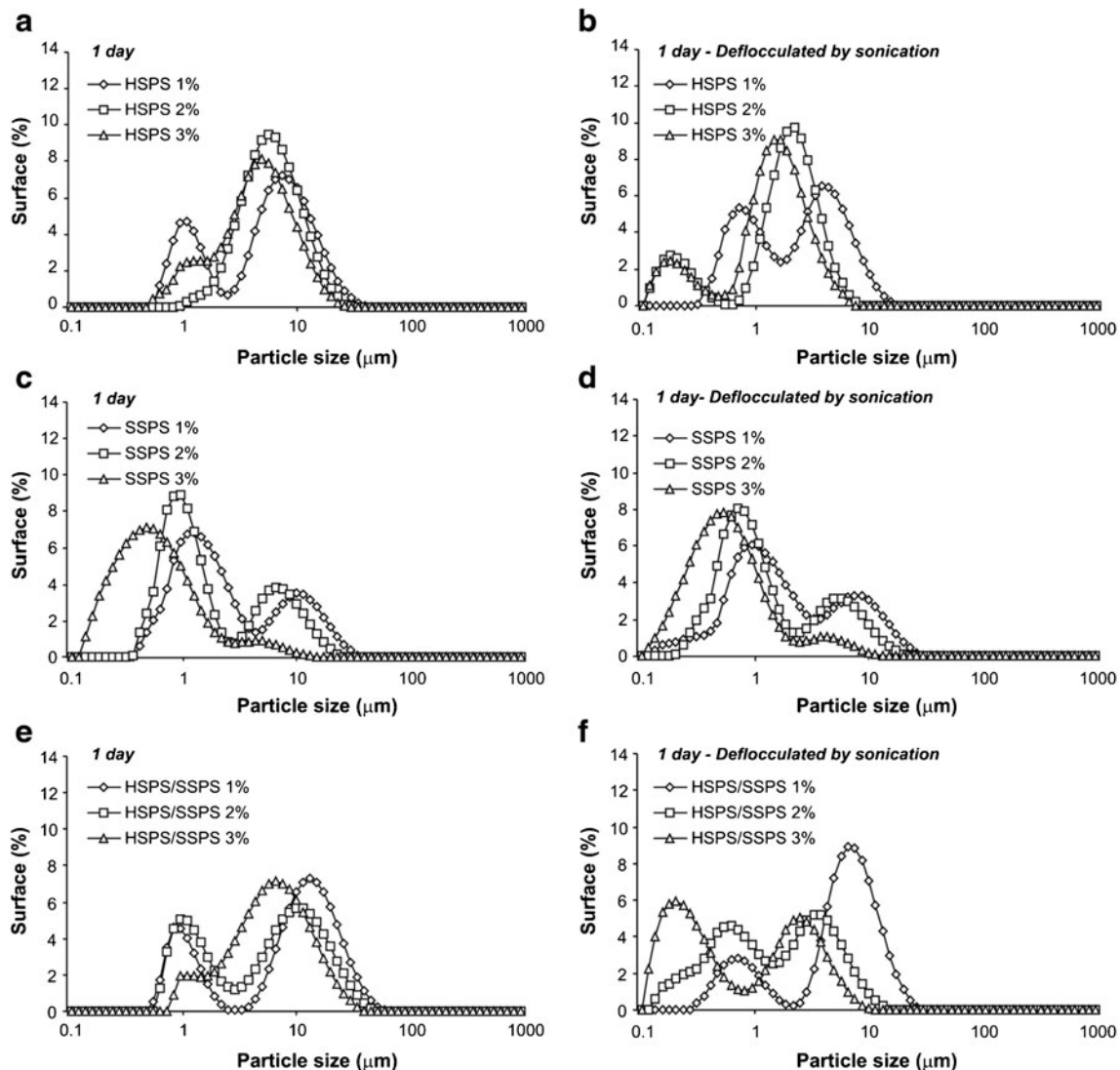
Fig. 6 Surface particle size distribution for O/W emulsions ($\Phi_m = 0.3$) obtained in ultrasound homogenizer with the addition of different soybean soluble pol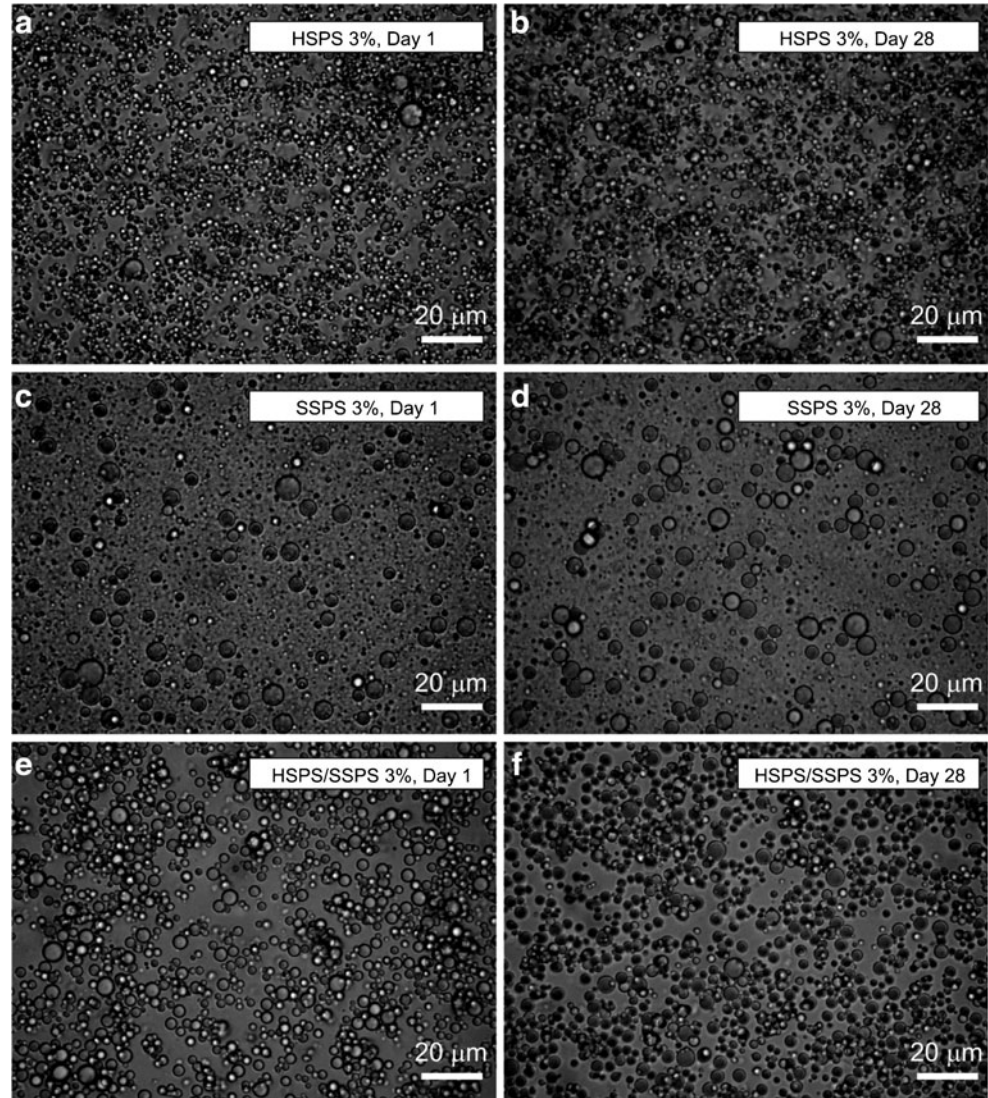ysaccharide fractions (SSPS, HSPS, HSPS/SSPS) without (**a**, **c**, **e**) and with (**b**, **d**, **f**) a subsequent deflocculation process by low energy sonication after 1 day of storage. Mean values ($n = 3$) ± sd

The particle size distribution of fine O/W emulsions at 1 day of storage, before and after low energy sonication, shows a bimodal behavior (Fig. 6). The increase in the concentration of SSPS generated a shift of the distribution towards smaller particle sizes and the application of additional low energy ultrasound did not generate substantial modifications in the particle size distributions (Fig. 6c, d). However, increasing the HSPS and HSPS/SSPS concentration decreases the population of smaller particles (Fig. 6a, e). Interestingly, in contrast to the SSPS emulsions, the application of an additional low energy ultrasound generated substantial changes in their particle size distributions as the appearance of a droplet population smaller than 1 μm mainly at concentration of 2-3 % (Fig. 6b, f). These results suggest the presence of flocs in these emulsions, which are disaggregated by this treatment. This observation was corroborated by optical micrographs of the emulsions (Fig. 7) and by calculating the %FD through the $D_{4,3}$ values before and after low energy sonication (Table 2),

which varied between 0 and 22.1 for SSPS or 78.8 and 225.9 for HSPS and HSPS/SSPS emulsions. As observed in coarse emulsions, the area created by sonication generates a deficiency of HSPS or HSPS/SSPS inducing aggregation by bridging flocculation (Walstra 1996). Should be noted, increasing the concentration of these fractions also decreased the droplet size producing more created area (Table 2). The presence of flocs would explain the creaming behavior of emulsions with HSPS and HSPS/SSPS, protecting them against creaming restricting the mobility of the droplets (Mc Clements 2000).

The majority of emulsions presented values of $D_{4,3}$ practically constant along time (Table 2), which indicates that the initially flocs do not disintegrate during storage. In contrast, the microstructure of such flocs was modified during storage depending on the type of emulsifier. This can be seen by analyzing the deflocculated emulsions and the corresponding coalescence index ($D_{43,\ sonic}$ and %CI, Table 2). HSPS and HSPS/SSPS presented a more advanced coalescence at higher

Fig. 7 Optical microscopy images of emulsion stabilized with 3 % of different soybean soluble polysaccharide fractions (SSPS, HSPS, HSPS/SSPS) after 1 day (a, c, e) and 28 days of storage (b, d, f). Bar = 20 μm

Table 2 De Brouker mean diameters without ($D_{4,3}$) and with ($D_{4,3\ sonic}$) a subsequent deflocculating process by low energy sonication, Flocculation degree (%FD) and coalescence index (%CI) for O/W emulsions ($\Phi_m = 0.3$) obtained in ultrasound homogenizer with the addition of different soybean soluble polysaccharide fractions (SSPS, HSPS, HSPS/SSPS)

HSPS/SSPS	Storage time (Day)											
	1				14				28			
	$D_{4,3}$	$D_{4,3\ sonic}$	%FD	CI %	$D_{4,3}$	$D_{4,3\ sonic}$	%FD	CI %	$D_{4,3}$	$D_{4,3\ sonic}$	%FD	CI %
HSPS 1 %	9.5 ± 0.0	5.1 ± 0.9	86.3	–	9.3 ± 0.0	5.2 ± 1.0	78.8	2.0	9.7 ± 0.1	5.4 ± 0.6	79.6	5.9
HSPS 2 %	7.0 ± 0.4	2.8 ± 0.3	150.0	–	7.4 ± 0.4	2.9 ± 0.2	155.2	3.6	9.6 ± 0.1	3.2 ± 0.1	200.0	14.3
HSPS 3 %	6.0 ± 0.5	2.2 ± 0.1	172.7	–	6.3 ± 1.2	2.5 ± 0.2	152.0	13.6	8.8 ± 0.6	2.7 ± 0.1	225.9	22.7
SSPS 1 %	9.4 ± 0.7	7.7 ± 0.8	22.1	–	9.5 ± 1.3	7.9 ± 0.8	20.3	2.6	11.2 ± 1.6	9.2 ± 1.9	21.7	19.5
SSPS 2 %	6.4 ± 0.9	5.8 ± 0.5	10.3	–	7.1 ± 0.6	6.0 ± 0.7	18.3	3.4	7.6 ± 0.9	6.5 ± 2.2	16.9	12.1
SSPS 3 %	4.3 ± 0.2	4.3 ± 1.7	0.0	–	4.3 ± 0.4	4.3 ± 0.4	0.0	0.0	4.5 ± 0.6	4.3 ± 0.5	4.7	0.0
HSPS/SSPS 1 %	18.85 ± 1.1	9.0 ± 0.4	108.9	–	18.8 ± 0.8	9.0 ± 0.1	108.9	0.0	24.9 ± 1.4	9.4 ± 0.1	164.9	4.4
HSPS/SSPS 2 %	13.0 ± 0.4	4.3 ± 0.3	202.3	–	13.2 ± 0.1	4.6 ± 0.3	187.0	7.0	13.7 ± 0.7	5.1 ± 0.3	168.6	18.6
HSPS/SSPS 3 %	9.6 ± 0.0	3.1 ± 0.0	209.7	–	9.6 ± 2.3	3.7 ± 0.1	159.5	19.4	10.1 ± 1.1	3.9 ± 0.1	159.0	25.8

Mean values ($n = 3$) ± sd

concentration of polysaccharide, and lower values of $D_{43,\ sonic}$ particle sizes in relation to SSPS emulsions.

Additionally, the rheological behaviors of the different samples was evaluates at 0.1 Hz and 1 % strain. SSPS emulsions *(G'(Pa): 0.15, G"(Pa): 0.09 and tan(δ): 0.65)* were almost creamy liquid, which correspond to a non-flocculate system, while HSPS *(G'(Pa): 190.97, G"(Pa): 42.50 and tan(δ): 0.23)* and HSPS/SSPS *(G'(Pa): 28.76, G "(Pa): 6.79 and tan(δ): 0.24)* emulsions were gel-like systems. G′ was higher than G″ for all the systems, giving values of tan δ lower than 1. This indicates that the systems have higher elastic than viscous characteristics. HSPS and HSPS/SSPS have a more important effect on the magnitude of the viscoelastic parameters, probable due to the higher flocculation degree which was previously evidenced by the %FD values. Flocculation increases the viscosity of an emulsion because the effective volume fraction of a floc is greater than the sum of the volume fractions of the individual droplets due to the presence of the continuous phase trapped within it (Marquez and Wagner 2010).

Conclusions

This study provides information on stability of emulsions prepared with different soy polysaccharide/protein fractions and its mixtures. The fractions extracted from hull (HSPS) and cotyledon (SSPS) differs in their sugar composition and protein content, which considerably affects its surface behavior and consequently the stability that they confer to the emulsions.

Although coarse and fine emulsions have similar creaming behavior, the increase in surface area in fine emulsions profoundly affects its characteristics. In coarse emulsions, a rapid creaming phenomenon and high droplet sizes resulted in unstable systems, impeding further analysis such as flocculation and rheology. In fine emulsions, instead, both fractions provided lower droplet sizes and stability to the emulsions, delaying significantly the main destabilization processes (creaming and coalescence) without need to add another stabilizing agent. On the other hand, the different fractions vary significantly the properties of the emulsions. Although in all cases higher concentrations of the fractions retard the cream formation, in HSPS and HSPS/SSPS emulsions a deficient amount of emulsifying agent could induce a bridging flocculation phenomenon which is correlated with the high %FD values obtained. This also influences the rheology of the emulsions, showing with HSPS and HSPS/SSPS mixtures a gel-like behavior with higher viscoelasticity than those prepared with SSPS. Besides, in accord with previous studies by other authors (Nakamura et al. 2004) higher concentrations of SSPS improves coalescence stability. On the contrary, in HSPS and HSPS/SSPS emulsions higher concentrations induces coalescence of the droplets, correlated with higher %FD.

Although HSPS and SSPS contribute in a different manner to the stability and properties of the emulsions, when a mixture of them is used their properties combine and at 3 % the best results on creaming stability are reached. At this concentration, it is possible that polysaccharides form a three-dimensional network that trap the droplets and effectively inhibit their movement, retarding the creaming process. In this sense, HSPS gives to the mixture the capacity to flocculate and consequently the creaming and coalescence behaviors, and also the viscoelastic characteristics.

These results are of interest to the food industry, taking into account the use of functional products obtained from residual materials.

References

Akhtar M, Dickinson E, Mazoyer J, Langendorff V (2002) Emulsion stabilizing properties of depolymerized pectin. Food Hydrocoll 16: 249–256

Aoki T, Decker EA, McClements DJ (2005) Influence of environmental stresses on stability of O/W emulsions containing droplets stabilized by multilayered membranes produced by a layer-by-layer electrostatic deposition technique. Food Hydrocoll 19:209–220

Blumenkrantz N, Asboe-Hansen G (1973) New method for quantitative determination of uranic acids. Anal Biochem 54:484–489

Cabezas DM, Madoery R, Diehl BWK, Tomás MC (2012) Emulsifying properties of different modified sunflower lecithins. J Am Oil Chem Soc 89:355–361

Cao Y, Dickinson E, Wedlock DJ (1990) Creaming and flocculation in emulsions containing polysaccharide. Food Hydrocoll 3:185–195

Cortés-Muñoz M, Chevalier-Lucia D, Dumay E (2009) Characteristics of submicron emulsions prepared by ultra-high-pressure homogenization: effect of chilled or frozen storage. Food Hydrocoll 23:640–654

Evans M, Ratcliffe I, Williams PA (2013) Emulsion stabilization using polysaccharide-protein complexes. Curr Opin Colloid Interface Sci 18:272–282

Gnanasambandam R, Proctor A (1999) Preparation of soy hull pectin. Food Chem 65:461–467

Hemar Y, Tamehana M, Munro PA, Singh H (2001) Influence of xanthan gum on the formation and stability of sodium caseinate oil-in-water emulsions. Food Hydrocoll 15:513–519

Higashitani K, Yoshida K, Tanise N, Muratac H (1993) Dispersion of coagulated colloids by ultrasonication. Colloids Surf A Physicochem Eng Asp 81:167–175

Hoogenkamp HW (2005) Soy protein essentials. In: Soy protein and formulated meat products. Cabi Publishing, Oxfordshire, pp 7–18

Kalapathy U, Proctor A (2001) Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin. Food Chem 73:393–396

Kumar V, Rani A, Dixit AK, Pratap D, Bhatnagar D (2010) A comparative assessment of total phenolic content, ferric reducing-anti-oxidative power, free radical-scavenging activity, vitamin C and isoflavones content in soybean with varying seed coat colour. Food Res Int 43:323–328

Lira-Ortiz AL, Reséndiz-Vega F, Ríos-Leal E, Contreras-Esquivel JC, Chavarría-Hernández N, Vargas-Torres A, Rodríguez-Hernández AI (2014) Pectins from waste of prickly pear fruits (Opuntia albicarpa Scheinvar 'Reyna'): chemical and rheological properties. Food Hydrocoll 37:93–99

Magdel-Din Hussein M, Helmy WA, Salem HM (1998) Biological activities of some galactomannans and their sulfated derivatives. Phytochemistry 48:479–484

Marquez AL, Wagner JR (2010) Rheology of double (W/O/W) emulsions prepared with soybean milk and fortified with calcium. J Texture Stud 41:651–671

Mc Clements DJ (1999) Food emulsions: principles, practice and techniques. CRC Press, Florida

Mc Clements DJ (2000) Comments on viscosity enhancement and depletion flocculation by polysaccharides. Food Hydrocoll 14:173–177

Monsoor MA (2005) Effect of drying methods on the functional properties of soy hull pectin. Carbohydr Polym 61:362–367

Nakamura A, Furuta H, Kato H, Maeda H, Ngamatsu Y (2003) Effect of soybean soluble polysaccharides on the stability of Milk protein under acidic conditions. Food Hydrocoll 17:333–343

Nakamura A, Takahashi T, Yoshida R, Maeda H, Corredig M (2004) Emulsifying properties of soybean soluble polysaccharide. Food Hydrocoll 18:795–803

Nakamura A, Fujii N, Tobe J, Adachi N, Hirotsuka M (2012) Characterization and functional properties of soybean high-molecular-mass polysaccharide complex. Food Hydrocoll 29:75–84

Nakauma M, Funami T, Noda S, Ishihara S, Al-Assaf S, Nishinari K, Phillips GO (2008) Comparison of sugar beet pectin, soybean soluble polysaccharide, and gum arabic as food emulsifiers. 1. Effect of concentration, pH, and salts on the emulsifying properties. Food Hydrocoll 22:1254–1267

Neirynck N, Van der Meeren P, Lukaszewicz-Lausecker M, Cocquyt J, Verbeken D, Dewettinck K (2007) Influence of pH and biopolymer ratio on whey protein-pectin interactions in aqueous solutions and in O/W emulsions. Colloids Surf A Physicochem Eng Asp 298:99–107

Nkonge C, Ballance GM (1982) A sensitive colorimetric procedure for nitrogen determination in Micro-Kjeldahl digests. J Agric Food Chem 30:416–420

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 isolate. Food Hydrocoll 19:595–604

Ponce NMA, Ziegler VH, Stortz CA, Sozzi GO (2010) Compositional changes in cell wall polysaccharides from Japanese Plum (Prunus salicina Lindl.) during growth and on-tree ripening. J Agric Food Chem 58:2562–2570

Rodríguez R, Jiménez A, Fernández-Bolaños J, Guillén R, Heredia A (2006) Dietary fibre from vegetable products as source of functional ingredients. Trends Food Sci Technol 17:3–15

Santos PO, Santos IS, Gomes VM, Machado OLT, Fernandes KVS, Xavier-Filho J, Oliveira AEA (2008) In vitro evaluation of antifungal activity of soybean (Glycine max) seed coat proteins. J Stored Prod Res 44:310–315

Tripathi AK, Misra AK (2005) Soybean- a consummate functional food: a review. J Food Sci Technol 2:42–46

Walstra P (1996) Emulsion stability. In: Becher P (ed) Encyclopedia of emulsion technology, vol 4. Marcel Dekker, New York, pp 33–42