



Effect of salt content and type on emulsifying properties of hull soy soluble polysaccharides at acidic pH



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ABSTRACT

Hull soluble polysaccharide (HSPS) is a novel product consisting in a mixture of polysaccharides and proteins extracted from soy hulls by using a methodology based on the extraction of citric pectins. In this work we studied the effect of the addition of two different salts (NaCl and CaCl₂) on the emulsifying properties of HSPS at acidic conditions. Low and high homogenization energies were used, obtaining coarse and fine emulsions, respectively. Mean droplet size, the stability against destabilizing processes (creaming, flocculation and coalescence) and the rheological properties of the emulsions were analyzed. Also, the rheology of the O/W interface was studied by using du Noüy ring geometry. Coarse HSPS emulsions were unstable to creaming, being more stable in the presence of salts. In contrast, fine HSPS emulsions showed long-term creaming stability similar to those performed with commercial citric pectin (CCP), although they differ in particle size distribution and flocculation degree. The presence of CaCl₂ reduced the mean size of droplets in fine HSPS emulsions and improved their stability to flocculation and coalescence. Significant differences were observed in the rheological behavior of O/W emulsions and interfaces of HSPS and CCP with respect to the salt addition.

Our results indicate that HSPS can be used in the formulation and stabilization of acidic O/W emulsions. Besides, HSPS generates emulsions with different characteristics than those obtained with citric pectins. The use of HSPS provides a suitable alternative in food engineering contributing to the exploitation and valorization of soy hulls, which represents an important waste material in soybean processing.

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1. Introduction

Proteins and polysaccharides are widely used in the formulation and stabilization of food emulsions. Proteins are surface-active molecules that reduce the interfacial tension at the oil-water interface and protect droplets against coalescence immediately after emulsification. Then, long-term stability is due to the ability of adsorbed molecules to generate a viscoelastic structural barrier at the droplet surface or in the spaces between them (McClements, 2005). Other mechanisms for emulsion stabilization include the use of insoluble particles that generate a steric barrier by pickering stabilization (Dickinson, 2012), or polymeric materials that form a cross-linked network of polymers, called microgel, with characteristics of a colloidal dispersion of gel-like particles (Dickinson, 2015).

Abbreviations: HSPS, hull soluble polysaccharides; CCP, commercial citric pectins; %DE, degree of esterification; %FD, degree of flocculation; %CI, coalescence index; O/W emulsions, oil-in-water emulsions.

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Polysaccharides generally exhibit little surface activity at the oil-water interface due to its predominantly hydrophilic character. In some cases, these macromolecules are chemically modified by the addition of non-polar hydrocarbon chains covalently attached to the polysaccharide chain, as in the case of modified starch and modified cellulose (McClements & Gumus, 2016). In other cases, polysaccharides are naturally associated to a proteinaceous moiety responsible for anchoring to the droplet surface, as in the case of soybean soluble polysaccharide (Nakamura, Maeda, & Corredig, 2006) or pectins (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). The hydrophobic character of the acetyl groups in pectins also contributes to its emulsifying capacity (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002). Despite these cases, polysaccharides are commonly used as stabilizers, and emulsifying properties are normally associated with proteins. Nevertheless, proteins have limitations at pH values around its isoelectric point (pI), where they have a net charge near zero. At pI, if the adsorbed layer thickness is not large enough to cover the interface, droplet aggregation can take place.

In a recent work, we obtained soy hull soluble polysaccharides (HSPS) by using a method of acid solubilization of citric pectins. Its protein content, sugar composition and the degree of esterification were

reported (Porfiri, Cabezas, & Wagner, 2016). In that work we demonstrated that HSPS consists in a mixture of high methoxyl pectin-like polysaccharides and proteins (~41 wt%), and the ability of HSPS to form and stabilize acidic oil-in-water (O/W) emulsions was analyzed.

Nowadays, there is an increasing interest in the isolation, characterization and use of new types of natural emulsifier in food engineering (McClements & Gumus, 2016). In this sense, HSPS is a natural protein-polysaccharide complex, that provides good functional properties at extreme pHs. Indeed, it represents a natural alternative to the common emulsifiers and contributes to the environment care and to the revalorization of the residues from the food industry, since it is obtained from soy hulls, a waste material of the soybean processing.

The first objective of the present work was to further study the ability of HSPS to form stable acidic O/W emulsions in the presence of different salts. The second objective was to compare emulsions formulated using HSPS with a formulation using high-methoxyl commercial citric pectin (CCP). A complementary study of the oil/water interfacial rheology was also made.

2. Materials and methods

2.1. Materials

Soy hulls were provided by Terminal 6 S. A. (Santa Fe, Argentina). High-methoxyl citric pectin (Genu®, type 105, CP Kelco, Brazil, %DE 67–73%, protein ≈ 3%) was gently donated by Gelfix S. A. (Buenos Aires, Argentina). The chemical reagents used in this work were all of analytical grade.

2.2. Methods

2.2.1. Extraction of the HSPS

Soy hulls were ground to a particle size of <500 µm in all-purpose high speed smashing machine (Chincan, FW, China) for 5 min. The soluble fraction (HSPS) was obtained by adapting the methods of acid extraction and alcohol precipitation of pectins optimized by Kalapathy and Proctor (2001) and modified by our research group (Porfiri et al., 2016). Briefly, ground soy hulls were dispersed in 0.1 M HCl (1:15), homogenized in a high-speed blender (25,000 rpm for 5 min, Ultraturrax 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, cooled to room temperature and centrifuged at 7000 × g for 15 min at 4 °C. 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 absolute 2-propanol. The precipitate was dried in drying oven with forced air circulation at 40 °C.

2.2.2. Preparation of coarse and fine O/W emulsions

HSPS and CCP aqueous dispersions (3% w/w) were prepared by dissolving the sample in 8 mM sodium citrate buffer at pH 3.0, in the absence and presence of NaCl or CaCl₂ at different concentrations (0.05, 0.1 and 0.2 M). Sodium azide 0.02% w/v was added in order to retard the microbial growth. The samples were stirred with a magnetic stirrer until complete dispersion and were immediately used as aqueous phase in the preparation of O/W emulsions.

Coarse O/W emulsions were prepared at 25 °C by mixing aqueous dispersions of HSPS or CCP and refined sunflower oil (oil mass fraction, Φ_m = 0.3) in a high-speed blender (Ultraturrax T-25, S 25 N-10 G dispersing tool) at 24,000 rpm for 2 min. The behaviour of coarse emulsions was analyzed at 25 °C during 22 h immediately after emulsion formation.

In order to obtain fine emulsions, coarse emulsions at selected conditions 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 in a glass beaker of 28 mm diameter for 2 min (Cabezas, Madoery, Diehl, & Tomás, 2012). The behaviour of fine emulsions was analyzed at 25 °C during 30 days immediately after emulsion formation.

2.2.3. Gravitational separation

Just after preparation (time zero), emulsions were placed into a 10 ml graduated tube and the progress of the creaming process was followed by calculating the percentage of aqueous phase (%) as a function of time (Dagorn-scaviner, Gueguen, & Lefebvre, 1987), according to Eq. (2):

$$\text{Aqueous phase(\%)} = \frac{V}{10} \times 100 \quad (2)$$

where V is the volume of the separated aqueous phase.

2.2.4. Particle size distribution

Particle size distribution of emulsions was determined by laser diffraction with a particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, U.K.) in the diameter range between 0.1 and 1000 µm. Emulsions were drooped in the water bath of the dispersion unit (Hydro 2000MU) and optical parameters applied were: refractive indexes of sunflower oil and water 1.47 and 1.33, respectively; adsorption 0.001. De Brouckere mean diameters of the droplets (D_{4,3}) were determined given its high sensibility to an increment of droplet size by flocculation and/or coalescence processes (Cortés-Muñoz, Chevalier-Lucia, & Dumay, 2009). Also, particle size distribution and D_{4,3} were measured after 2 min of sonication at 40 W with the ultrasonic probe of the dispersion unit. The purpose of this low energy sonication was to break down the flocs that would be present in the emulsions (Higashitani, Yoshida, Tanise, & Muratac, 1993). Flocculation degree (% FD) was calculated according to Eq. (3) (Porfiri et al., 2016):

$$\% \text{ FD} = \frac{D_{4,3} - D_{4,3-S}}{D_{4,3-S}} \times 100 \quad (3)$$

where D_{4,3} and D_{4,3-S} are De Brouckere mean diameters of the droplets before and after the sonication treatment, respectively.

The coalescence index (% CI) was calculated according to Eq. (4):

$$\% \text{ CI} = \frac{D_{4,3-S} - D_{4,3-S,\text{initial}}}{D_{4,3-S,\text{initial}}} \times 100 \quad (4)$$

where D_{4,3-S,initial} and D_{4,3-S} are the volume weighted diameters measured in initial and stored emulsions after the sonication process, respectively.

2.2.5. Oscillatory rheology

Oscillatory rheology of the emulsions was studied in an AR G2 rheometer (TA Instruments; New Castle, DE, USA) with parallel-plate geometry (gap 1000 µm; diameter 40 mm). The temperature (25 °C) was controlled by a water bath (Julabo ACW100, Julabo Labortechnik; Seelbach, Germany) associated to the rheometer. Experimental data were obtained by recording G' and G'' as a function of the oscillation strain in the range between 0.1 and 100% at constant frequency of 1 Hz, which was measured to be within the linear viscoelasticity range. Tangent of delta (tan δ) was calculated according to Eq. (5):

$$\tan \delta = \frac{G''}{G'} \quad (5)$$

2.2.6. Interfacial oil-water rheology

Rheological properties of the oil-water interface generated by HSPS and CCP in the absence and presence of the different salts were studied

by using an AR-G2 rheometer (TA Instruments, New Castle, DE) equipped with a du Noüy ring (platinum ring) geometry. For this, 30 ml of aqueous dispersion of HSPS or CCP 3% w/w at pH 3.0, in the absence and presence of NaCl or CaCl₂ 0.2 M were placed in a beaker (6.7 cm in diameter) and the ring was lowered to make contact with the surface. In order to increase the repeatability the gap was zeroed and kept constant at the position of 10,900 μm . The same volume of sunflower oil was carefully placed above the aqueous phase, generating an oil-water interface. Oscillatory shear measurements were conducted at constant frequency of 0.1 Hz and strain of 5%, which was measured to be within the linear viscoelastic regime. The temperature was set to 25 °C. In order to follow the overall evolution of the adsorption of HSPS or CCP at the interface, experimental data were obtained by recording G' and G'' as a function of time immediately after interface generation.

2.2.7. Optical microscopy

Optical microscopy was used to analyze the microstructure of initial emulsions, which were diluted with distilled water (1:10 w/w) immediately before their observation in the microscope. Micrographs of the diluted emulsions were obtained with an optical microscope operating at 400 \times magnification and fitted with a digital camera (Canon A570 289 IS; Malaysia) at 4 \times optical zoom.

2.2.8. Statistical analysis

Experiments were done at least in duplicate and reported results represent the mean from two or more calculated values and their standard deviations. Data were evaluated using analysis of variance (ANOVA) using the statistical program Statgraphics Plus 5.1 (Statgraphics Corporation; USA). For this purpose, differences were considered significant at $p < 0.05$.

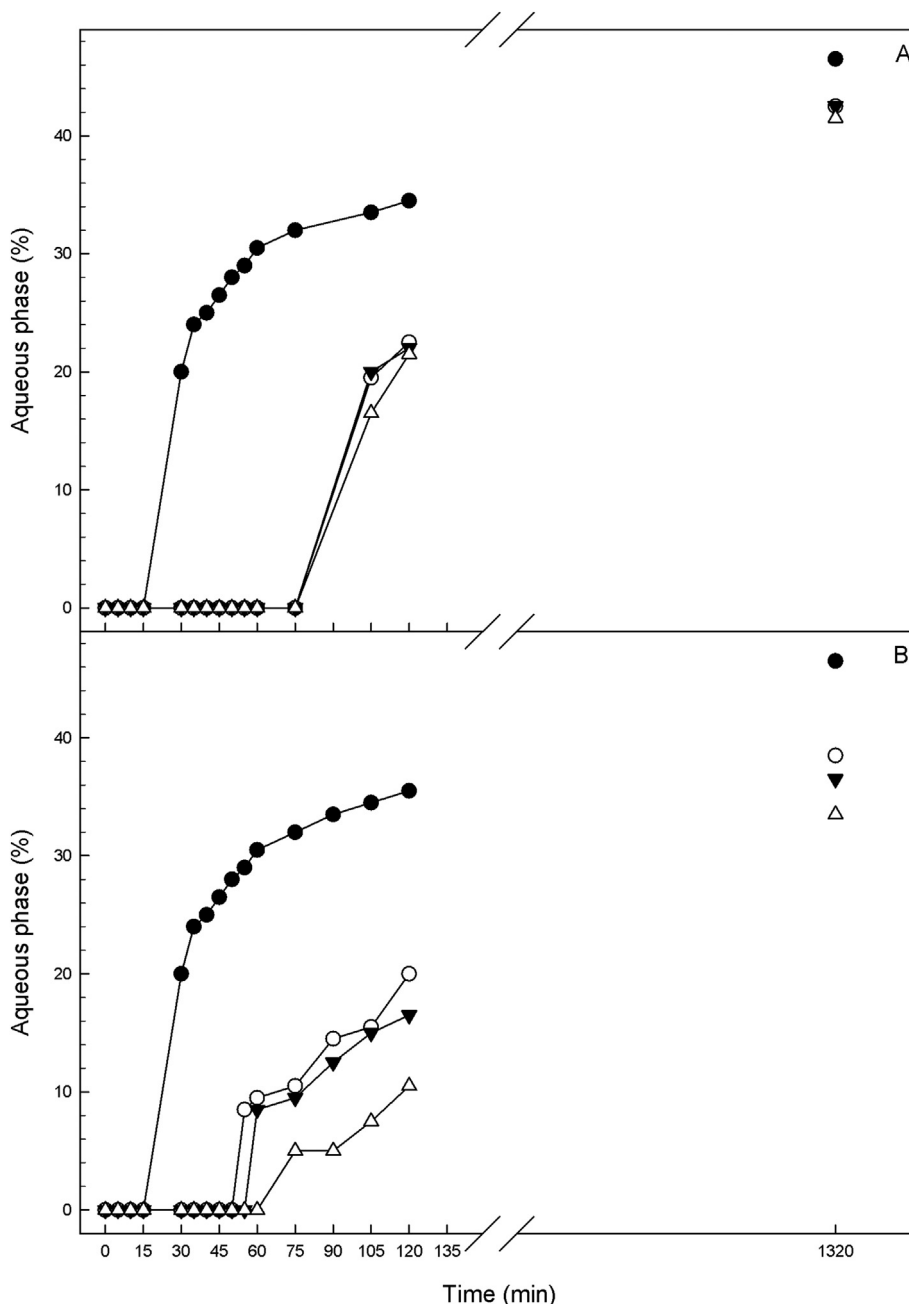


Fig. 1. Aqueous phase (%) as a function of the storage time for HSPS emulsions at pH 3.0 and different NaCl (A) or CaCl₂ (B) concentrations: 0 M (●), 0.05 M (○), 0.1 M (▼), 0.2 M (△).

3. Results and discussion

3.1. Coarse emulsions

Fig. 1 shows the kinetics of the creaming process of coarse HSPS emulsions expressed as separated aqueous phase (%) versus time, without salts and in the presence of NaCl (Fig. 1A) and CaCl₂ (Fig. 1B). All the systems were unstable to creaming. However, the presence of NaCl or CaCl₂ retarded the onset of the creaming process and reduced the aqueous phase percents after 22 hs. These effects would be the result of polysaccharide and/or protein structuring in the presence of salts, generating a more hydrated cream phase. In addition, at increasing concentrations of CaCl₂ both effects were accentuated.

Table 1 shows D_{4,3} values of initial emulsions (t₀) and after 22 hs of storage (t₂₂) at pH 3.0 and at different NaCl or CaCl₂ concentrations. For initial emulsions, NaCl generated a decrease in particle size in all assayed concentrations, which would explain the decrease in the creaming rate in these emulsions (Fig. 1). However, only in the presence of CaCl₂ 0.05 M a significant decrease in the D_{4,3} value was observed, in comparison to emulsion without salt. It is already known that the formation of reversible electrostatic complexes between pectins and proteins is pH dependent (Thakur, Singh, & Handa, 1997). The formation of these complexes takes place at pH values where protein and pectins have opposite net charge, mainly at pHs below the isoelectric point of proteins. According to Clark and Glanz (1990), ionic strength reduces the electrostatic attraction between protein and an oppositely charged polymer due to the shielding effect. In this sense, at pH 3.0 the presence of NaCl or CaCl₂ would reduce the magnitude of the interaction between proteins and pectic polysaccharides in HSPS. Then, protein is more available in the aqueous phase to interact at the interface generating emulsions with lower droplet size. On the other hand, higher CaCl₂ concentrations produced emulsions with the particle size similar to the emulsion without salt addition. This behaviour would suggest that the effect of ionic interactions induced by Ca⁺² ions overcomes the effect of charge screening above mentioned.

Regarding the stored emulsions, only those with 0.1–0.2 CaCl₂ added exhibited an increase in particle size after 22 days of storage, indicating a coalescing and/or flocculation process. In these emulsions the effect of salt interactions would promote the generation of hydrated flocs, and this flocculation would retard the creaming process due to the formation of a three-dimensional network of aggregated droplets (Degner, Chung, Schlegel, Hutkins, & McClements, 2014). Low energy sonication was applied on initial and stored coarse HSPS emulsions in order to verify the presence of flocs. However, due to the low stability of coarse emulsions, the application of low energy sonication during the particle size measurement caused destabilization and coalescence of the droplets, so that D_{4,3-s} values could not be determined under these conditions.

Aqueous dispersions of CCP presented high viscosity, which generated coarse emulsions with large D_{4,3} values, and therefore low stability to creaming. As a consequence, coarse CCP emulsions were not analyzed.

3.2. Fine emulsions

It has been observed in coarse HSPS emulsions that different NaCl concentrations did not affect emulsion stability but a lower rate of

creaming was reached in the case of CaCl₂ 0.2 M. So, fine O/W emulsions were formulated at pH 3.0 with HSPS or CCP in the absence and presence of NaCl or CaCl₂ 0.2 M.

Fig. 2 shows the photographs of initial emulsions formulated with HSPS (Fig. 2A) and CCP (Fig. 2B) in the absence and presence of NaCl and CaCl₂. The colors of the emulsions were somewhat different between those formulated with HSPS and CCP, but it was not altered by the addition of the salts in any case. Also, visual appearance of emulsions remained with negligible changes along 30 days of storage. In this sense, %FA values were close to zero during that period, so both fine HSPS and CCP emulsions were stable to creaming.

Fig. 3 shows the particle size distributions of fine emulsions, expressed as volume frequency, before and after the breakdown of flocs by sonication. Emulsions with HSPS in the absence of salts (Fig. 3A), in the presence of NaCl (Fig. 3B) and CaCl₂ (Fig. 3C) exhibited bimodal particle size distributions before deflocculation and monomodal distributions after 120 s of sonication. Peaks shifted toward smaller particle sizes as the sonication proceeds and remained constant after 120 s of sonication, which suggests complete disruption of the flocs. At this time, emulsions exhibited a narrower distribution with a particle population in the range between 1 and 10 μm (without salt) or below this range (with NaCl or CaCl₂). On the other hand, particle size distributions of emulsions with CCP in the absence (Fig. 3D) and presence of NaCl

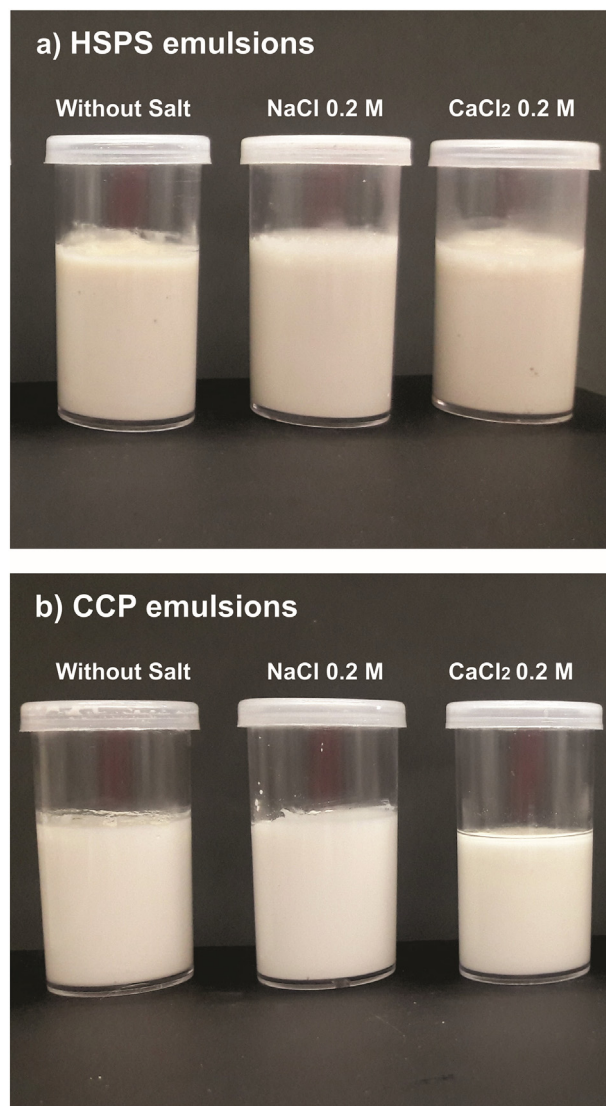


Fig. 2. Photographs of initial fine emulsions formulated with HSPS (A) and CCP (B).

Table 1
De Brouckere mean diameters (D_{4,3}) of coarse emulsions with HSPS: initial (t₀) and at 22 hs (t₂₂) of storage.

Salt	D _{4,3} -t ₀ (μm)	D _{4,3} -t ₂₂ (μm)
–	40.4 ± 0.7	39.4 ± 0.1
NaCl 0.05 M	33.2 ± 0.2	33.2 ± 0.1
NaCl 0.1 M	32.9 ± 0.1	32.3 ± 0.1
NaCl 0.2 M	32.6 ± 0.4	32.7 ± 0.5
CaCl ₂ 0.05 M	32.5 ± 0.1	34.0 ± 0.8
CaCl ₂ 0.1 M	35.5 ± 0.6	45.7 ± 0.6
CaCl ₂ 0.2 M	41.3 ± 2.4	44.0 ± 1.0

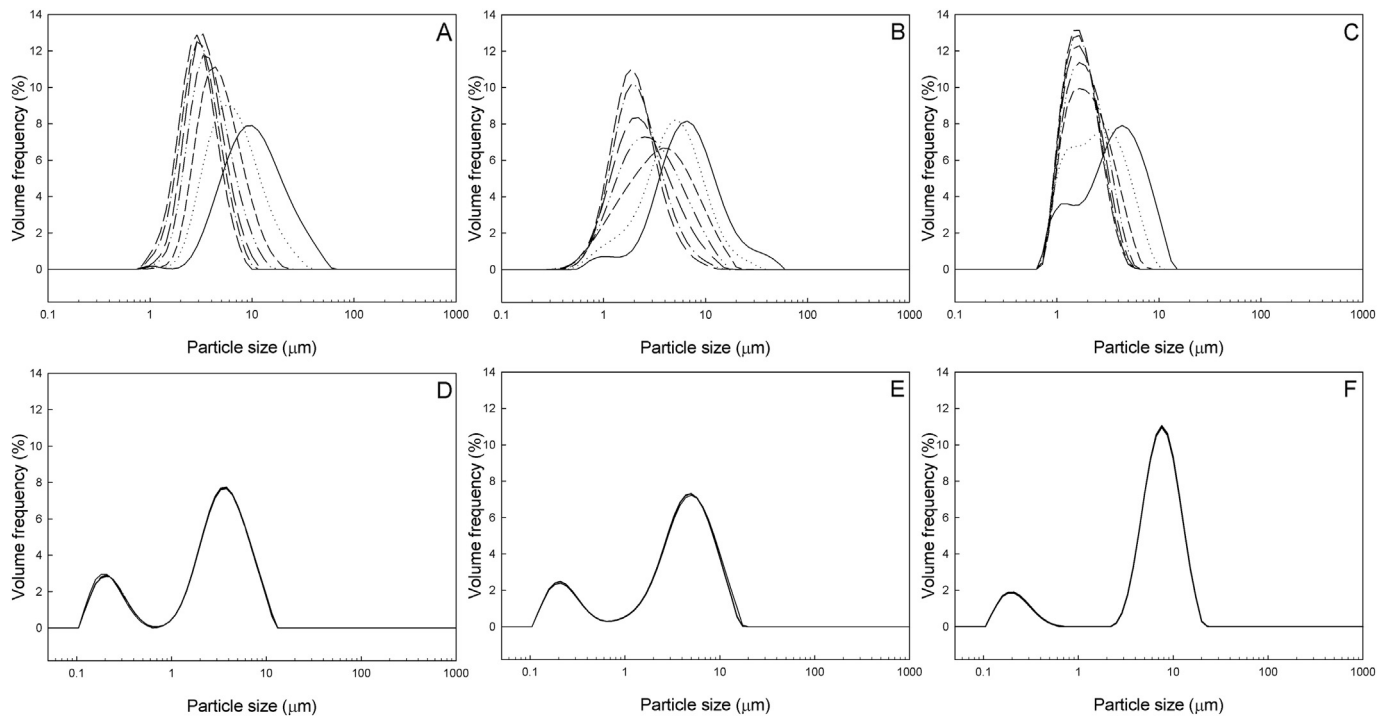


Fig. 3. Particle size distribution (expressed as volume frequency) of fine emulsions; pH 3.0: HSPS (A), CCP (D); pH 3.0, NaCl 0.2 M: HSPS (B), CCP (E); pH 3.0, CaCl₂ 0.2 M: HSPS (C), CCP (F). Deflocculation by sonication at 40 W, time (s): 0 (—), 30 (···), 60 (---), 90 (— · —), 120 (— — —), 150 (— · —), 180 (— — —).

(Fig. 3E) and CaCl₂ (Fig. 3F) were not affected by sonication, suggesting that these emulsions did not show a significant flocculation. In this case, bimodal particle size distributions were obtained with a main particle population between 1 and 11 μm and a secondary smaller population in the range between 0.1 and 0.6 μm with or without salts.

Fig. 4 shows $D_{4,3}$ and $D_{4,3-5}$ of fine emulsions versus storage time. Initially, HSPS emulsions exhibited droplets with $D_{4,3-5}$ values approximately lower than 5 μm in the absence of salts (Fig. 4A), decreasing slightly in the presence of NaCl (Fig. 4B) and even more in the presence

of CaCl₂ (Fig. 4C). As in coarse emulsions (Section 3.1.), the effect of salts on the droplet size would be attributed to a charge screening effect, which improves the interaction of the protein moiety of HSPS at the droplet surface, also at a concentration of NaCl or CaCl₂ 0.2 M. In all cases, a significant decrease in mean diameters of droplets was observed after sonication as a result of the breakdown of flocs. The presence of flocs in emulsions with HSPS was expected given the large shifts in particle size distributions by sonication, previously observed in Fig. 3. This observation was confirmed by optical micrographs of the

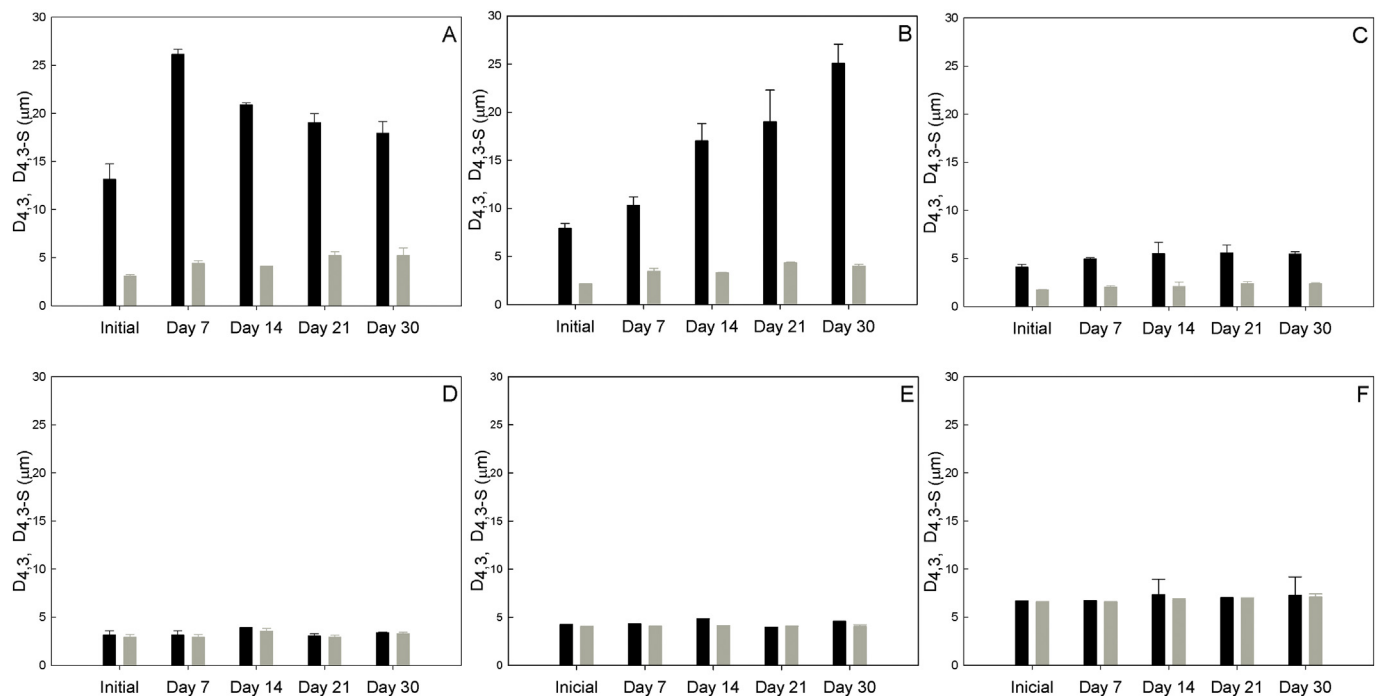


Fig. 4. De Brouckere mean diameters of fine emulsions without ($D_{4,3}$ ■) and with ($D_{4,3-5}$ ▒) a subsequent deflocculating process by low energy sonication; pH 3.0: HSPS (A), CCP (D); pH 3.0, NaCl 0.2 M: HSPS (B), CCP (E); pH 3.0, CaCl₂ 0.2 M: HSPS (C), CCP (F).

emulsions (Fig. 5), were the presence of flocs was effectively observed. This generates high values of %FD in HSPS emulsions (Table 2). These micrographs further confirm the reported particle sizes (Fig. 3) and the decrease in the %FD in the presence of salts (Table 2). The proximity between flocculated droplets would induce or facilitate the onset of a coalescence process. In this sense, a slight tendency of the droplet to coalescence was observed after 30 days of incubation (Fig. 4), presenting %CI values of 70 ± 4 , 86 ± 5 and 38 ± 3 , without salt, with NaCl and CaCl₂, respectively. However, the increase in droplet sizes was at most 2 μm at 30 days of storage, which means that these emulsions showed only an incipient coalescence. Emulsions with NaCl presented more gradual flocculation and coalescence processes than emulsion without salt, and with CaCl₂ both processes were slowed. In this sense, CaCl₂ promotes the generation of droplets with smaller mean diameters and higher stability to flocculation and coalescence. Thus, in HSPS emulsions Ca⁺² ions provides improved stability against environmental stresses, such as extreme pH.

Fig. 4 also shows $D_{4,3}$ and $D_{4,3-5}$ versus time in CCP emulsions in the absence of salts (Fig. 4D), in the presence of NaCl (Fig. 4E) and CaCl₂ (Fig. 4F). Mean sizes of droplets increase slightly with NaCl and significantly with CaCl₂, in comparison to emulsions without salts. As salt is added, the higher viscosity of the aqueous phase would make more difficult the generation of interfacial area during homogenization (McClements, 2005), giving rise to higher droplet sizes. In all cases, mean droplet sizes were not significantly affected by sonication, which suggests the absence of populated flocs. This was confirmed by optical micrographs of the emulsions (Fig. 5), and the low values of %FD calculated in CCP emulsions (Table 2). This figure also confirms the increase in droplet size in CCP emulsions in the presence of salts. Besides, $D_{4,3-5}$ showed low oscillations along time (Fig. 4), which demonstrates that CCP emulsions were quite stable to coalescence. This is confirmed by the low values of %CI at 30 days of storage: 11 ± 1 , 2 ± 0.4 and 7 ± 1 , without salt, with NaCl and CaCl₂, respectively.

Pectins would generate a “gel like” structure in the aqueous phase of emulsions (Dickinson, 2015). So, in CCP emulsions this would contribute to keep oil droplets more dispersed making flocs less populated (Zimmerer & Jones, 2014). Also, structured pectins could affect the viscosity of CCP aqueous phase generating higher sizes of droplets, mainly using Ca⁺² ions. Finally, structured pectins would provide long-term stability to CCP emulsions. Because of its stability was not affected by the presence of salts, the steric factor would be predominant in the stabilization of CCP emulsions (Nakamura, Takahashi, Yoshida, Maeda, & Corredig, 2004). On the other hand, HSPS has a large content of proteins,

Table 2

Flocculation degree (%FD) of fine emulsions formulated with HSPS and CCP at pH 3.0 as a function of the storage time.

%FD (HSPS)					
Salt	Initial	Day 7	Day 14	Day 21	Day 30
–	320 \pm 30	460 \pm 40	404 \pm 4	260 \pm 40	240 \pm 20
NaCl 0.2 M	270 \pm 20	200 \pm 10	420 \pm 50	340 \pm 10	526 \pm 5
CaCl ₂ 0.2 M	130 \pm 10	144 \pm 7	92 \pm 5	133 \pm 7	130 \pm 10
%FD (CCP)					
Salt	Initial	Day 7	Day 14	Day 21	Day 30
–	8 \pm 1	7.5 \pm 0.6	11 \pm 1	3.9 \pm 0.8	3.9 \pm 0.2
NaCl 0.2 M	4.41 \pm 0.08	6.17 \pm 0.08	16.45 \pm 0.02	14 \pm 2	12 \pm 1
CaCl ₂ 0.2 M	1.0 \pm 0.4	1.5 \pm 0.2	7.3 \pm 0.4	1.5 \pm 0.2	2.2 \pm 0.4

which may be involved in the generation of flocs by bridging flocculation. Emulsion stability was sensitive to the salt addition, which means that the steric barrier provided by structured polysaccharides was not the only factor involved in emulsion stabilization. Even, the effect of salts on the viscosity of the aqueous phase was less evident than with CCP, due to both cations, Na⁺ and Ca⁺², generated emulsions with lower droplet sizes in comparison to emulsions without salts. However, as in the case of CCP, in the presence of Ca⁺² structured polysaccharides would contribute to keep oil droplets more dispersed, making flocs less populated, and to stabilize emulsions against coalescence.

Fig. 6 shows the dependence of G' and G'' as a function of the strain (%) in fine emulsions. In emulsions with HSPS without salts (Fig. 6A), in the presence of NaCl (Fig. 6B) and CaCl₂ (Fig. 6C) viscoelastic parameters remained constant up to a certain deformation and then a significant fall was detected (mainly in G'). Taking into account that HSPS emulsions were flocculated in a large extent, flocs would be resistant to low deformation forces. However, these forces became significant at higher deformations and some breakdown of the emulsion structure might occur above a critical value, possibly causing the rupture of flocs and consequently the decrease in rheological parameters (McClements, 2005; Tadros, 1994). In these emulsions, G' were higher than G'' before structure breakdown, which indicates that the systems have higher elastic than viscous characteristics. $\tan \delta$ values were lower than 1 (1% of strain): 0.48 ± 0.08 without salts, 0.19 ± 0.00 with NaCl and 0.18 ± 0.01 with CaCl₂, indicating that the presence of salts provided higher elastic characteristics. In addition, in the presence of salts higher G' and G'' were obtained, mainly with CaCl₂, contributing

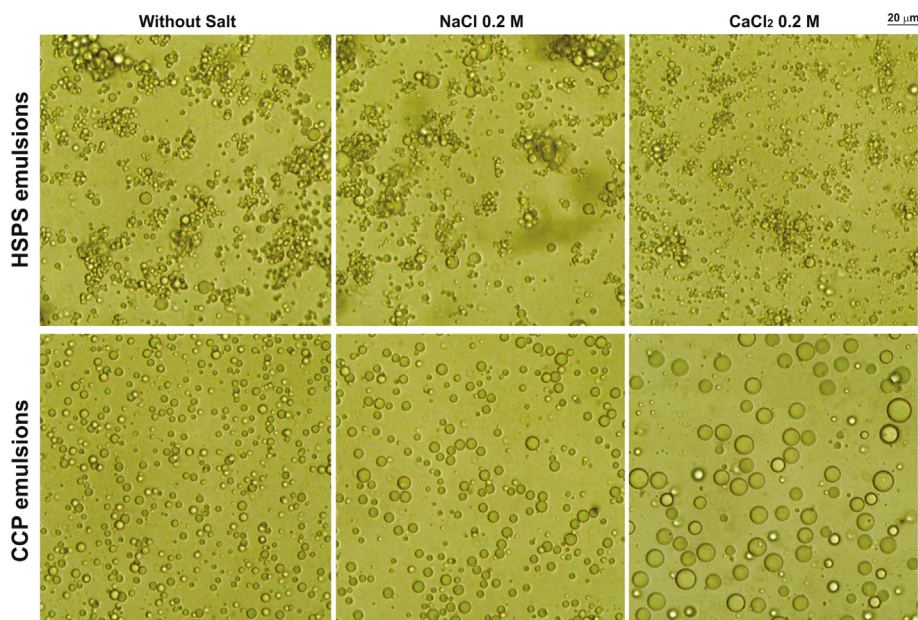


Fig. 5. Optical microscopy images of initial emulsions formulated with HSPS and CCP at pH 3.0 in the absence and presence of NaCl and CaCl₂ 0.2 M. Dilution 1:10 w/w. Bar = 20 μm .

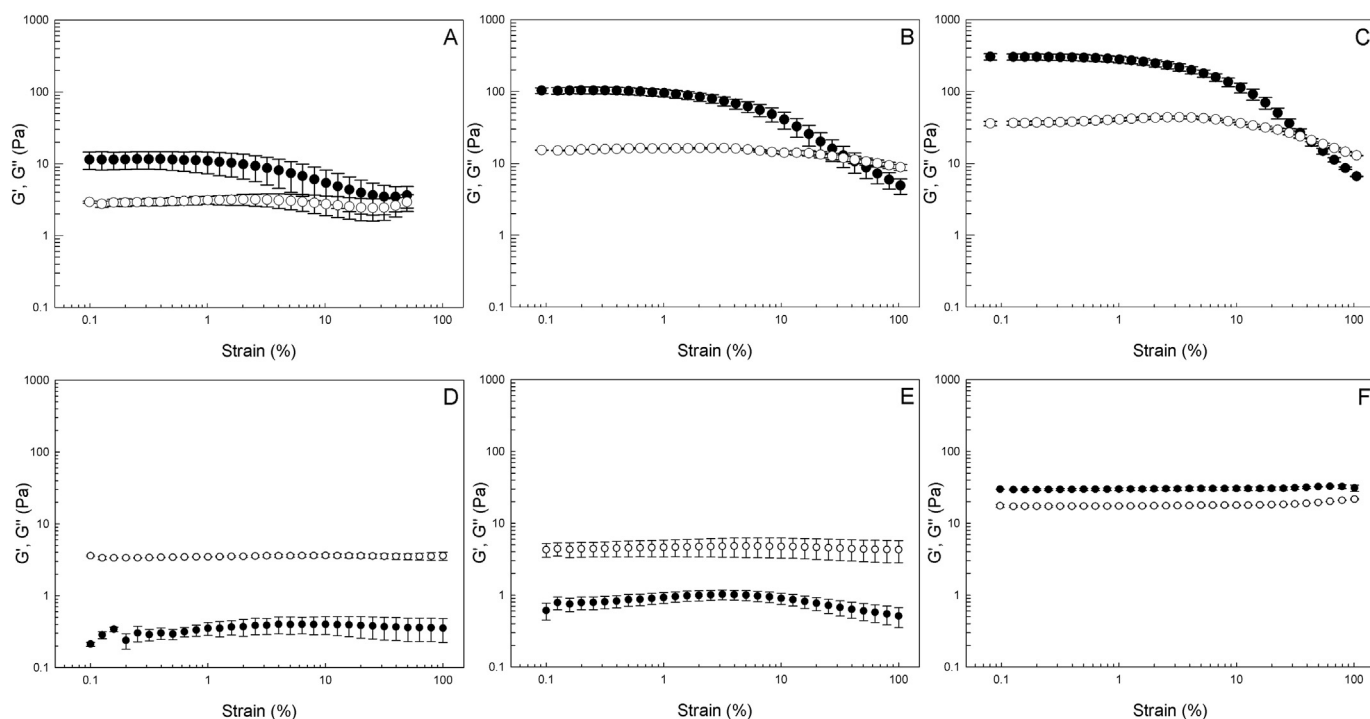


Fig. 6. Elastic modulus (G' ●) and viscous modulus (G'' ○) of fine emulsions as a function of the strain (%); pH 3.0: HSPS (A), CCP (D); pH 3.0, NaCl 0.2 M: HSPS (B), CCP (E); pH 3.0, CaCl₂ 0.2 M: HSPS (C), CCP (F). Frequency 1 Hz.

to a hydrated gel behaviour. Generally, emulsions containing three-dimensional networks of aggregated droplets present an elastic threshold at high shear stresses, above this value emulsions behave as a liquid (more fluid). Furthermore, this threshold depends strongly on the structural organization of the network (McClements, 2005). This would explain the transition from predominantly elastic to predominantly viscous response in the presence of salts (Fig. 6B and C). Furthermore, in the presence of CaCl₂ this transition is shifted toward higher strains (%), which denote a more structured network.

For the three emulsions formulated with CCP in the absence of salts (Fig. 6D), in the presence of NaCl (Fig. 6E) and CaCl₂ (Fig. 6F) G' and G'' were remained constant along all the range of strain (%). This behaviour could be explained by the low tendency of CCP emulsions to form stable flocs, in accordance with the hypothesis that the decrease in rheological parameters is due to the breakdown of flocs, previously stated for HSPS emulsions. $\tan \delta$ values (1% of strain) were: 5.70 ± 0.28 , 6.60 ± 0.53 and 0.66 ± 0.03 without salts, in the presence of NaCl and CaCl₂, respectively. In emulsion prepared without salts and with NaCl, G'' values were higher than G' , meaning that systems had more viscous than elastic characteristics resulting in values of $\tan \delta$ higher than 1, in contrast to emulsions with CaCl₂.

When comparing emulsions formulated with HSPS and CCP, those samples with HSPS presented higher viscoelastic parameters than those with CCP, which was also related to the different flocculation behaviour. It was reported elsewhere that 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 continuous phase trapped within it, increasing the emulsion viscosity (Marquez & Wagner, 2010).

3.3. Oil-water Interface

Interfacial G' , G'' and $\tan \delta$ versus time are shown in Fig. 7. In all cases, the adsorption of the sample to the interface started immediately after the interface generation. However, in some cases they did not reach a constant rheological behaviour during the time of the experiment (60 min), which could be indicative of an incomplete adsorption

and/or interface restructuring due to interaction between adsorbed components (Dickinson, 1992).

In emulsions with HSPS (Fig. 7A and B), G' was significantly higher than G'' generating $\tan \delta$ values lower than 1. This indicates that gel-like interfacial films were formed at the oil-water interface. However, in any case a significant difference was observed when the salts were added. The emulsifying activity of HSPS would be ascribed to its protein fraction, which adsorbs at the droplet surface generating a predominantly proteic interfacial film. Then, the polysaccharide portion would be involved in a stabilizing role, as with other cases reported in literature (Nakamura et al., 2006). Given that at pH 3.0 proteins are positively charged, the addition of cations did not affect the rheological behaviour of the interfacial film. On the other hand, cations interact with the negatively charged polysaccharides of the aqueous phase, reducing its interaction with the interfacial film (Nguémazong, Christiaens, Shpigelman, VanLoey, & Hendrickx, 2015). This also would lead to a reduction of bridging flocculation (Table 2).

In CCP emulsions (Fig. 7C and D), G' was also predominantly higher than G'' , giving rise to $\tan \delta$ values lower than 1. Due to the hydrophobic character of the acetyl groups in pectins and the lower protein content in CCP in comparison with HSPS, pectins would also be adsorbed at the droplet surface, contributing to the emulsifying capacity of CCP samples (Akhtar et al., 2002; Nguémazong et al., 2015). In this sense, the presence of cations affected the interfacial film rheology. Comparison of $\tan \delta$ suggests that the presence of NaCl or CaCl₂ promoted a stronger gel-like structure of the interfacial film (lower $\tan \delta$), predominantly with Ca²⁺, as expected given the effect of this ion on the gelation behaviour of pectins (Picout, Richardson, Rolin, Abeysekera, & Morris, 2000; Willats, Knox, & Mikkelsen, 2006). This provides stability to coalescence even with higher droplet sizes (Fig. 4F), where collision efficiency is enhanced (McClements, 2005).

4. Conclusions

HSPS is a soy hull polysaccharide/protein mixture. It was obtained by a method based on the extraction of citric pectins, but it confers different characteristics to O/W emulsions in comparison with CCP. When extracting HSPS, a large proportion of protein was solubilized together

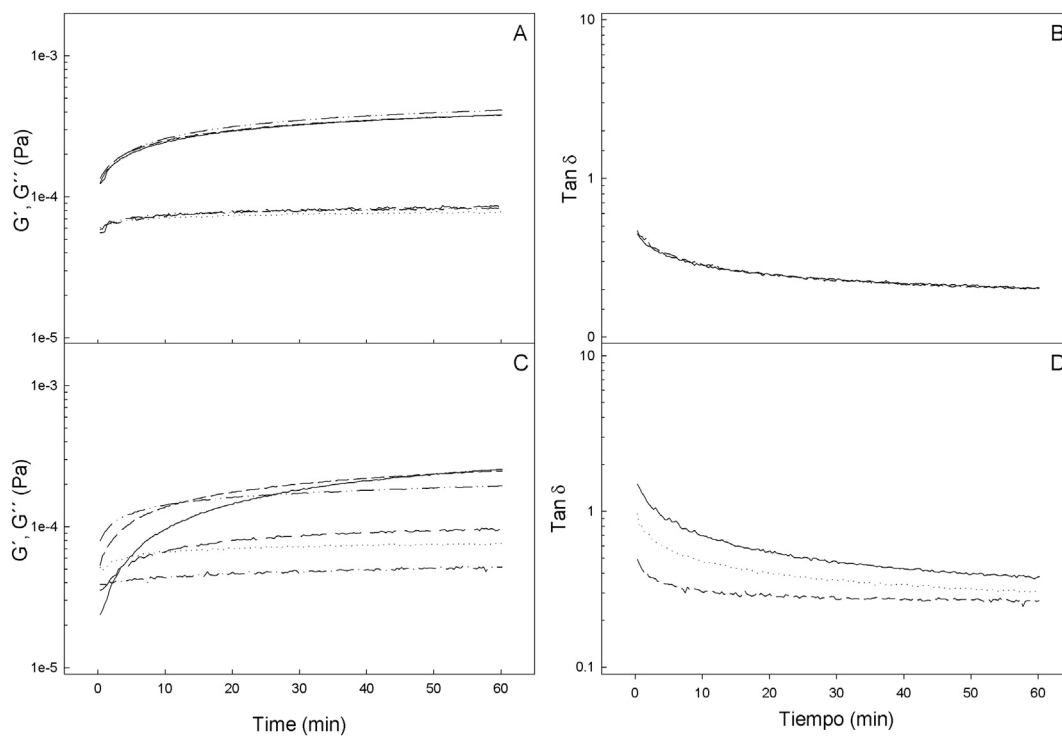


Fig. 7. Elastic (G') and viscous (G'') interfacial shear module at the oil-water interface for HSPS (A) and CCP (C): pH 3.0 G' (—) G'' (---); pH 3.0, NaCl 0.2 M G' (---) G'' (- · - ·); pH 3.0, CaCl₂ 0.2 M G' (- · · -) G'' (- · - ·). Tan δ for HSPS (B) and CCP (D): pH 3.0 (—); pH 3.0, NaCl 0.2 M (- · - ·); pH 3.0, CaCl₂ 0.2 M (- - -).

with the polysaccharides, which affected significantly its emulsifying and stabilizing properties. HSPS forms viscoelastic films at the oil-water interfaces in acid medium; hence it could be used as an effective emulsifier in acidic O/W emulsions. Interfacial rheology was not significantly affected by the presence of salts. Evidently, interactions between droplets and the structuring of polysaccharides in the aqueous phase were involved in the overall behaviour of HSPS emulsions. In CCP emulsions, instead, pectin structuring at the aqueous phase would provide a steric factor that predominate in the stabilization of these emulsions.

HSPS is a natural polysaccharide-protein mixture providing a novel alternative to formulate and stabilize acidic O/W emulsions. Moreover, the addition of Ca²⁺ reduced the mean size of droplets and increased the stability against flocculation and coalescence processes under stress conditions, such as extreme pH and prolonged storage. This makes HSPS a suitable alternative in food emulsion engineering. Besides, it is economically convenient, since it is obtained from a byproduct of soybean processing, soy hulls.

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