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Creaming stability of oil in water (O/W) emulsions: Influence of pH on soybean protein–lecithin interaction

D.I. Comas[a], J.R. Wagner[b], M.C. Tomás[a,*]

[a]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) (UNLP-CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), 47 and 116 (1900) La Plata, Provincia de Buenos Aires, Argentina
[b]Departamento de Ciencia y Tecnología Universidad Nacional de Quilmes UNQ, Bernal, Provincia de Buenos Aires, Argentina

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Abstract

In the present work, the influence of pH on stability of oil in water O/W (25:75 w/w) emulsions prepared with soy isolates and lecithin (Lec) was studied. Emulsions were prepared using native (NSI) and denatured (DSI) soybean isolates, Lec and sunflower oil, with a protein–Lec ratio of 10:1. Dispersions of soybean proteins were adjusted to pH 2.0–6.2. Emulsions were optically characterized by droplet size distribution and using a vertical scan analyzer (Quick Scan) to determine the creaming destabilization and the corresponding kinetics involved.

At pH 2, a negative effect was observed on droplet size distribution and stability of Lec emulsion. Changes in droplet size and creaming rate as a function of pH value were observed for NSI–Lec and DSI–Lec emulsions in comparison with their corresponding control systems (Lec, NSI, DSI). NSI–Lec emulsions at pH 2.0 presented an important initial emulsifying activity, but the creaming rate recorded was faster than that corresponding to pH 5.5–6.2. For DSI–Lec systems, the stability increased at pH 2.0 and 6.2, values away from the isoelectric point. The presence of Lec enhanced both the initial characteristics reflected in BS_0 and droplet size, and the stability against the creaming process.

Keywords: Protein–lecithin interaction; Soybean isolates; Phospholipids; O/W emulsion stability; Vertical scan analyzer

1. Introduction

Functional foods are made up of natural components that impart nutritional benefits to human health. Proteins and phospholipids are examples of these components which, in addition to their inherent nutritional value, also contribute to the taste and texture of foods.

Among proteins of vegetable origin, soy protein isolates have good functional properties for food processing (Kinsella, 1979). These proteins are used as emulsifiers in food emulsions due to the surface active properties of their constitutive proteins, the storage globulins 7S (β-conglycinin) and 11S (glycinin) (Aoki, Taneyama, Orimo, & Kitagawa, 1981). Recent research demonstrated that thermally denatured soy isolates (DSI) gave more stable emulsions against creaming and coalescence processes (Palazolo, Sorgentini, & Wagner, 2004, 2005).

Natural or modified soybean lecithins (Lec) have been included in many technical processes due to their versatile role as emulsifiers, viscosity regulators and dispersing agents in bakery goods, chocolate, instant products (milk powder), margarines and mayonnaise (Hassenhuettl & Hartel, 1997; McClements, 1999; van Nieuwenhuyzen, 1999).

Interactions between proteins and phospholipids may lead to changes in surface activity, modifications on protein structure and net charge, and incorporation of protein into surfactant miscelles and vesicles (van Nieuwenhuyzen & Szuhaj, 1998). Research on the interaction between soy proteins and phosphatidylcholine (PC) has shown the existence of a protein–lipid complex with a different extent of association for both 7S and 11S globulin preparations (Beckwith, 1984). Soybean protein–Lec complex enhanced

*Corresponding author. Tel./fax: +54 221 4254853/4249287/4890741.
E-mail address: mabtom@hotmail.com (M.C. Tomás).

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the emulsification activity after heat treatment, mainly attributed to 11S denaturation (Hirotsuka, Taniguchi, Narita, & Kito, 1984). Nevertheless, further research is necessary to understand soy protein–Lec interactions in terms of emulsion stability.

Emulsions are thermodynamically unstable systems from a physicochemical point of view, rapidly or slowly separating into two immiscible phases according to the kinetic stability. Physical destabilization mechanisms of emulsions include oil droplets size variation processes such as flocculation and coalescence and particle migration phenomena like sedimentation and creaming.

Soy proteins isolates and Lecs have interesting surface active properties which enable them to achieve emulsion stability of different products (Ohtsuru & Kito, 1983). Recent studies demonstrated that thermally DSIs gave more stable emulsions against creaming and coalescence processes (Palazolo et al., 2004, 2005). Also, native (NSI) or denatured (DSI) soy protein isolates presented a different behavior as emulsifier agents, either alone or with its interaction with PC. The presence of this phospholipid diminishes the creaming rate in both systems (NSI-PC and DSI-PC), producing creamed phases with different characteristics (Comas, Wagner, & Tomás, 2004; Scuriatti, Tomás, & Wagner, 2003).

In food emulsions, some components such as proteins and phospholipids possess charges or have the capability to be ionized. Then, pH level can modify their surface active behavior and hydrodynamic interactions between oil droplets in emulsions (McClements, 1999).

The main focus of the present work was to study the effect of pH on soybean proteins–Lec interaction considering the protein structure and the emulsifier agents ratio in order to evaluate the stability of oil in water (O/W) emulsions.

2. Materials and methods

2.1. Materials

2.1.1. Soybean proteins

NSI was prepared according to Sorgentini and Wagner (1999). The defatted soybean flour was extracted for 2 h at room temperature with water adjusted to pH 8.0 with 2 N NaOH (water:flour ratio, 10:1). The mixture was centrifuged at 10,400*g* for 15 min at 20 °C (GSA rotor, Sorvall RC 5B refrigerated Superspeed Centrifuge). The supernatant was adjusted to pH 4.5 with 1 N HCl, then kept for 2 h at 4 °C and subsequently centrifuged at 10,400g for 20 min at the same temperature (Sorvall, GSA-rotor). The precipitate was washed with water, resolubilized in water by neutralization to pH 8.0 with 2 N NaOH at room temperature and freeze-dried (Thermovac Industries Corp. freeze-dryer). NSI composition (% p/p) was: 89.7 ± 0.3 proteins, 5.0 ± 0.1 carbohydrates, 3.45 ± 0.05 ions (from ash values). Electrophoretic patterns of NSI proteins were similar to those reported by Sorgentini and Wagner (1999). DSI was obtained by heating NSI dispersion at 90 °C for 5 min at pH 7.0.

2.1.2. Lecithins

Soybean Lec in a powder form with purity greater than 98% was provided by Bunge Alimentos (Sao Paulo, Brazil). The phospholipid composition, determined by HPLC-ELSD according to Pan, Tomás, and Añón (2002), was phosphatidylethanolamine 31.9%, phosphatidylinositol 13.4%, PC 44.1%, phosphatidic acid and phosphatidylserine 10.4%.

2.2. Preparation of O/W emulsions

O/W emulsions (25:75 w/w-100 g) were prepared at room temperature by homogenization in an ULTRA-TURRAX T25 homogenizer at a rate of 20,000 rpm for 30 s. NSI and DSI dispersions (1 mg/ml) in 0.01 M sodium phosphate, adjusted to pH 2.0, 5.5 and 6.2 with HCl, were used as aqueous phase. Refined sunflower oil without (protein control) or with the addition of Lec in a protein–Lec ratio 10:1 was used as oil phase. Emulsion systems prepared without protein in aqueous phase were also carried out (Lec control).

2.2.1. Emulsion stability

All emulsions immediately after homogenization were optically characterized using a Vertical Scan Analyzer (QuickScan, Beckman Coulter) as described previously (Pan et al., 2002). Curves of back scattering (BS %) with an interval of 1 min as a function of the sample height (in mm) were obtained. Initial back scattering (BS_0) and creaming kinetics were recorded plotting the mean values of backscattering as a function of time at the bottom (zone 8–10 mm) by duplicate.

The creaming constant ($K_{0.1}$) was evaluated as proposed by Palazolo et al. (2005). The value of $K_{0.1}$ was defined as

$$K_{0.1} = 1/\mathrm{BS}_0 t_{0.1},$$

where BS_0 is the initial mean value of BS ($t = 0$ min) and $t_{0.1}$ is the time for which $BS = 0.1BS_0$.

2.2.2. Particle size distribution

Immediately after homogenization, aliquots of emulsions were analyzed using a Mastersizer Micro Particle Analyzer (Malvern Instruments Ltd.). Oil droplet size distribution at $t = 0$ was recorded; Sauter Mean Diameter $D[3,2]$ and $D[4,3]$ were determined as droplet mean values for surface and volume distribution, respectively.

2.2.3. Statistical analysis

Data were analyzed by analysis of variance and significant difference between the Fisher's test (Systat, version 5.0). An alpha level of 0.05 ($\alpha = 0.05$) was used to determine significance.

3. Results and discussion

Fig. 1A shows the comparative particle size distribution at pH 2.0 for the different systems studied. At this pH, Lec presents a poor behavior as emulsifier, which it is possible to observe in the highest droplet size population, corresponding to a $D[4,3]$ value of $97.7 \pm 4.4\,\mu m$. There are not significant differences in size distribution for NSI and DSI with respect to NSI–Lec and DSI–Lec emulsions, with a similar $D[4,3]$ values ranging from 40 to 42 μm. Taking into account that NSI is a non-DSI, at pH 2.0, it exhibits a similar behavior to the heat-denatured DSI sample due to 11S-glycinin fraction is completely denatured at this acidic pH (Wagner, Sorgentini, & Añón, 1996).

At pH 5.5, Lec exhibited a better emulsifying behavior ($D[4,3] = 37.4 \pm 1.9$) in comparison at pH 2.0, and similar droplet size distribution that those of DSI and DSI–Lec (Fig. 1B). However, NSI and NSI–Lec systems exhibited a bimodal distribution of droplets: a particle population with droplet size $>100\,\mu m$ and another one $<10\,\mu m$, which produce a $D[4,3] > 100\,\mu m$. This fact suggests a different behavior between NSI and DSI and their interaction with Lec. At pH 6.2 (Fig. 1C), both size distribution and $D[4,3]$ values for all systems were similar to those observed at pH 5.5.

In Fig. 2, the influence of pH on $D[3,2]$ value (mean droplet size in surface distribution) for the different systems can be observed. When pH was changed from 6.2 to 2.0, an increase in $D[3,2]$ was observed for Lec system as a consequence of a diminution in their surface activity or due to the fact that the interfacial film formed was less resistant to avoid the coalescence during homogenization. This behavior could be related to the swelling of phospholipids which become more hydratable compounds after the addition of acids (Diez, 1995), diminishing their capacity as an emulsifier agent.

In the case of NSI, no significant differences ($\alpha < 0.05$) in $D[3,2]$ were observed between 5.5 and 6.2, with an increase in this parameter at pH 2.0. For NSI–Lec emulsions, $D[3,2]$ at pH 5.5 was the lowest, likely because the pH is close to the isoelectric point of the soy proteins, suggesting a high interaction between less charged 7S–11S proteins and Lec. DSI exhibited the highest $D[3,2]$ values at pH 5.5 with a

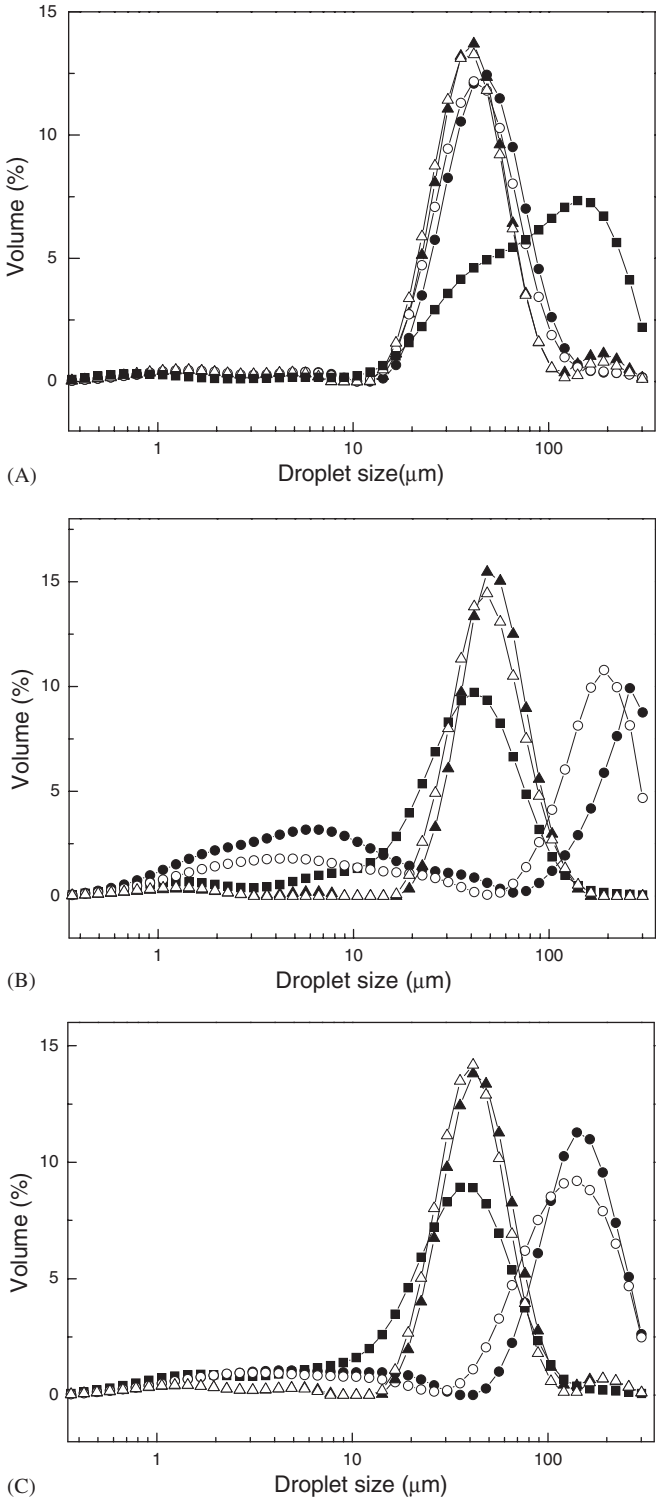

Fig. 1. Particle size distribution at different pH values for O/W emulsions. (A) pH 2.0, (B) pH 5.5, (C) pH 6.2. (-●-●-) NSI; (-○-○-) NSI–Lec; (-▲-▲-) DSI; (-△-△-); DSI–Lec; (-■-■-) Lec.

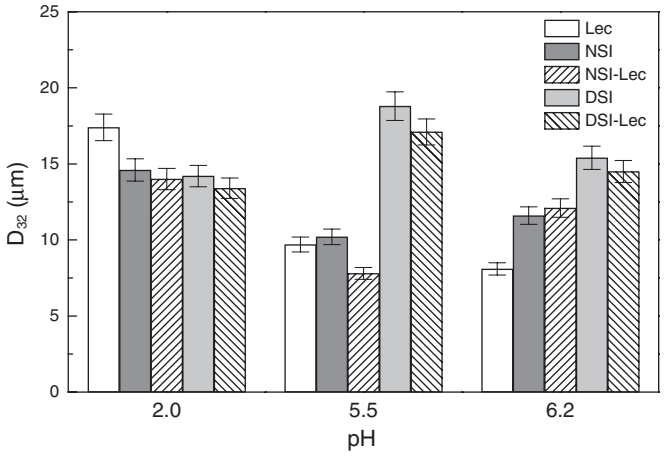

Fig. 2. Mean diameter $D[3,2]$ as a function of pH for O/W emulsions. The values are the mean of at least two determinations. Vertical bars: standard deviation.

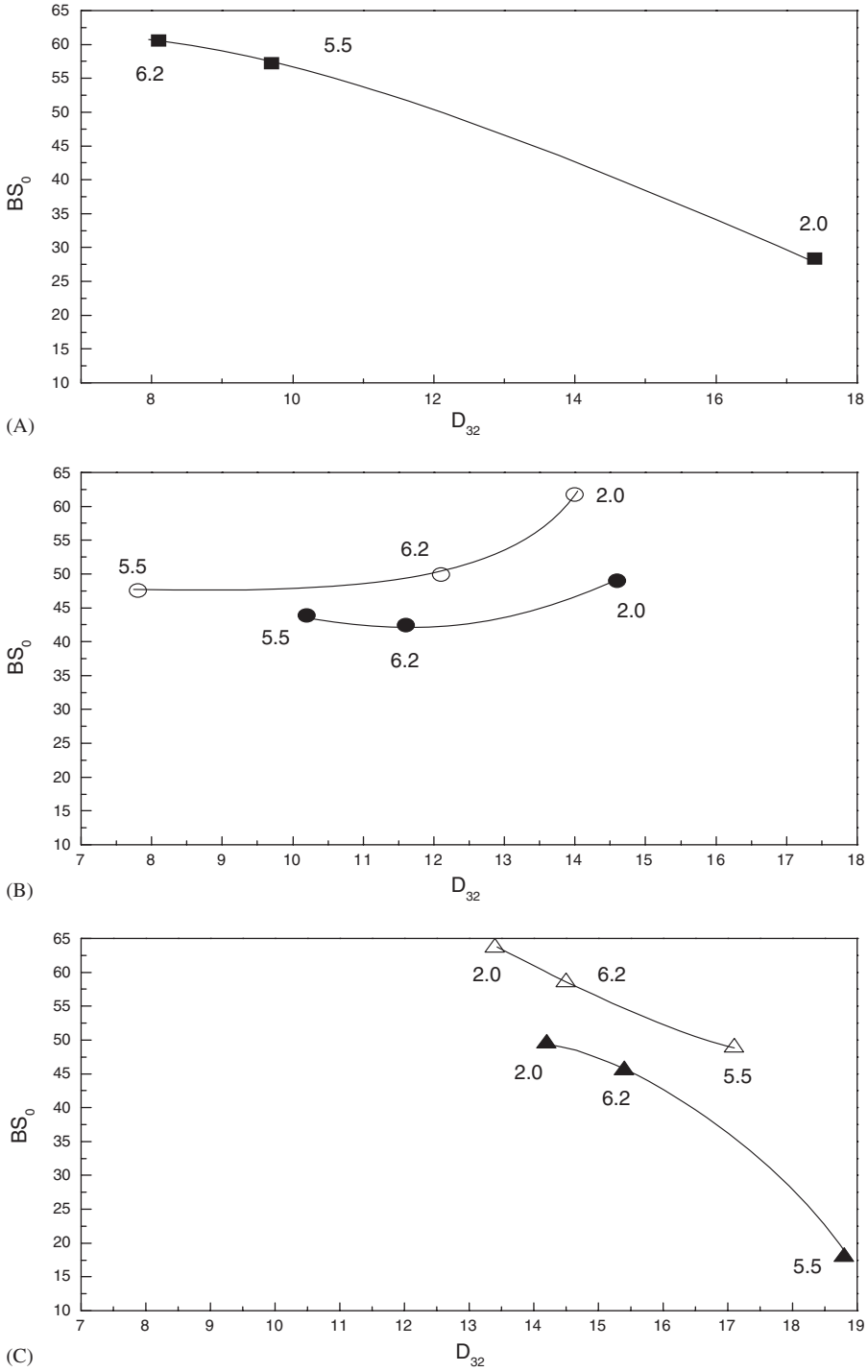

Fig. 3. Relationship between BS_0 and $D[3.2]$ as a function of pH value: (A) (-■-■-) Lec emulsions; (B) (-●-●-) NSI, (-○-○-) NSI–Lec;(C) (-▲-▲-) DSI, (-△-△-) DSI–Lec.

slight decrease in presence of Lec. These results were well correlated with low solubility of denatured soy proteins at pH close to the isoelectric point (Molina Ortiz & Wagner, 2002).

On the other hand, the optical characterization of O/W emulsions using a QuickScan was carried out. The relationship between BS_0 and $D[3,2]$ for the different emulsions can be observed in Fig. 3A–C. BS_0 is a value related to the size and density of particles in emulsions; BS_0 enhances as the increase of the number of particles, and decreases as a function of the size of individual droplets and/or presence of flocs (Palazolo et al., 2004, 2005). For Lec systems, there is a significant diminution ($\alpha < 0.05$) of BS_0 with an increase of $D[3,2]$ as a function of pH value from 6.2 to 2.0, indicating that it was difficult for this emulsifier agent to create a surface area (Fig. 3A). In case

of NSI, the addition of Lec produced an increase of BS_0 at all pH values, mainly at pH 2.0 and an important decrease in D[3,2] at pH 5.5 (Fig. 3B). These results correlate with those observed in Fig. 1A–C.

In contrast, for DSI and DSI–Lec, lower values of D[3,2] were observed relative to the NSI and NSI–Lec systems which correlates to the higher BS_0 levels at pH values not close to the isoelectric point. DSI–Lec systems also exhibited higher BS_0 values than the DSI control systems. For DSI and NSI, the increase in BS_0 upon addition of Lec could be attributed to the disruption of flocs, mainly when D[3,2] was not significantly modified (pH 2.0) (Fig. 3C).

Figs. 4A–E show the creaming destabilization kinetics of O/W emulsions measured by %BS decrease as a function of time. Fig. 4A shows the behavior of emulsions prepared only with Lec. It is important to note the different behavior recorded at pH 5.5 and 6.2 instead of 2.0, in which the BS% decreases faster than the other ones. These results are in agreement with the droplet size distribution previously analyzed (Figs. 1A and 2). In all conditions studied, these emulsions presented a destabilization mechanism consisting in simultaneous creaming and coalescence processes, the latter one very fast in comparison with the same process in protein stabilized emulsions. This behavior was equivalent with those recorded by Scuriatti et al. (2003).

Figs. 4B and C show the creaming kinetics corresponding to emulsions formulated with NSI and NSI–Lec at the different pH values assayed. Firstly, a marked diminution of BS% as a function of time for NSI emulsion, mainly at pH 2.0, was observed. In the case of NSI–Lec, at this condition it is possible to observe that the creaming process is faster than the others. Nevertheless, the presence of Lec produced a decrease in creaming kinetics. This behavior suggests the occurrence of a marked interaction between

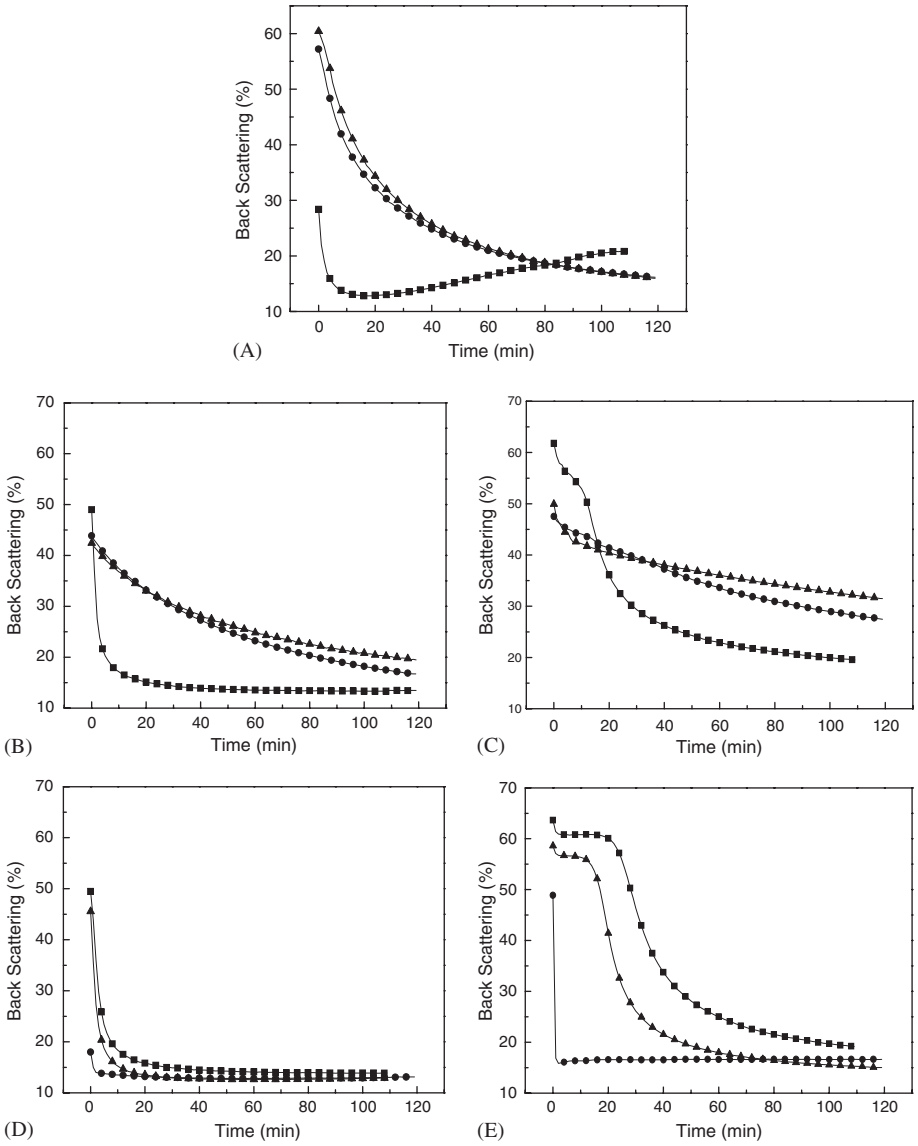

Fig. 4. Creaming destabilization kinetics of O/W emulsions. (A) Lec, (B) NSI, (C) NSI–Lec, (D) DSI, (E) DSI–Lec. (-■-■-) pH 2.0; (-●-●-) pH 5.5; (-▲-▲-) pH 6.2.

these emulsifier agents. This fact also could be attributed to the negative influence of the acidic medium on Lec. This negative effect is more important than the enhancement of hydrophobicity evidenced by the 11S fraction (glycinin) which could improve the protein–Lec interaction (Beckwith, 1984; Chen & Soucie, 1985; Mitidieri & Wagner, 2002). For all pH values studied, it was possible to observe an increase in the initial BS% and the emulsions stability against creaming process. However, this interaction was less important for the same systems at the other pH level used.

The comparative evolution of creaming kinetics corresponding to DSI which all protein fractions are denatured, can be visualized in Fig. 4D. It is interesting to note the rapid diminution of the initial BS% in all cases. Higher BS initial and the constant value during a longer period for DSI–Lec at pH 2 than the others, suggests that the interaction of denaturated soy proteins with Lec is promoted (Fig. 4E). In this case, the negative effect of pH on Lec behavior was not evidenced. On the other hand, at pH 5.5, the pH value close to the isoelectric point of storage soybean proteins, the major destabilization of the DSI emulsions studied was observed.

Creaming constant ($K_{0.1}$) values as a function of pH for all emulsions studied are presented in Fig. 5. It is possible to observe that the presence of Lec enhances the emulsion stability of DSI system at all pH assayed, while for NSI only at pH 2.0. If $K_{0.1}$ values are analyzed taking into account D[3,2] values (see Fig. 2), it is possible to observe the following: (a) Very low stability ($K_{0.1} > 0.05$) correlates with a D[3,2] > 17 μm, in the case of Lec at pH 2, DSI and DSI–Lec at pH 5.5; (b) For each system, $K_{0.1}$ increases as a consequence of a significant increase of D[3,2] values; (c) Emulsions with mean droplet size D[3,2] in the range 7–12 μm, exhibited a similar stability, $K_{0.1} < 0.01$; (d) Emulsions with intermediate D[3,2] values (12.1–15 μm) were not significantly different with respect to mean droplet size ($\alpha < 0.05$), however, exhibited significant differences in their stability. As an example of this interesting condition, NSI and DSI emulsions at pH 2.0 showed a significant increase in BS_0 (see Fig. 3) and a significant diminution in $K_{0.1}$ (Fig. 5) with similar D[3,2] values ($\alpha < 0.05$) with respect to NSI–Lec and DSI–Lec, respectively. Similar results were observed between DSI and DSI–Lec at pH 6.2. These results suggesting that the charge contribution of Lec prevents the aggregation phenomena, evolve to a more stable emulsion.

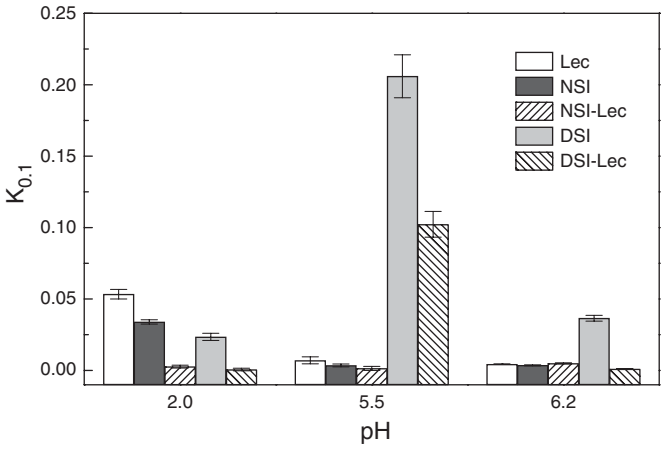

Fig. 5. Creaming constant $K_{0.1}$ as a function of pH for O/W emulsions. The values are the mean of at least two determinations. Vertical bars: standard deviation.

4. Conclusions

In conclusion, pH is an important factor in emulsifying activity of soy protein and Lec. Soy protein isolates, NSI or DSI, displayed a different behavior as emulsifier agents, either alone or with its interaction with Lec. Differences between NSI–Lec and DSI–Lec systems were observed due to the protein structure and pH value. DSI–Lec systems showed more stability against the creaming process at pH not close to the isoelectric point of proteins. The presence of Lec enhances the initial characteristics of emulsions (high density of small and low flocculated particles) and diminishes the creaming rate in both systems, NSI–Lec and DSI–Lec. The role of Lec could be related to its charge contribution in the interface instead of an increase in surface activity.

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