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Short Communication

Effect of dynamic high-pressure treatment on the interfacial and foaming properties of soy protein isolate–hydroxypropylmethylcelluloses systems

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ABSTRACT

The objective of the work was to study the effect of dynamic high-pressure homogenization (HPH) on the interfacial and foaming properties of soy protein isolate (SP) and surface-active polysaccharides (E4M and E15) with different molecular weight.

SP was dispersed with water (2% w/v) together with the polysaccharides (0.3% w/v) and subjected to high-pressure from 0 to 300 MPa, in 100 MPa intervals. After treatment, foam overrun by whipping method, viscosity, particle size distribution and surface pressure at 48 s of drop formation time, of systems were measured.

The effect of HPH of these systems on foam overrun was not directly relation with the effect on the surface pressure at short adsorption time. The viscosity decrease may be explained some of the foaming results together with interfacial performance at longer adsorption time than 48 s which depend on the system and level of pressure applied.

According to the polysaccharide used in this work, interactions between SP and polysaccharides apparently favour the foam overrun on untreated mixed systems; this effect was promoted using HPH particularly in the case of E15 at 300 MPa. The effect of SP–E4M was less pronounced from the one observed for E15. Thus, the molecular weight of polysaccharides is a very important factor of interaction with soy protein isolate under these conditions of high-pressure homogenization.

1. Introduction

The use of soy proteins as functional ingredients in food manufacturing is increasing because of their role in human nutrition and health (Liu, 1997). The major globulins in soy beans are conglycinin (7S) and glycinin (11S).

However, those proteins are easily denatured under some extreme conditions, e.g. acid precipitation and high temperature, during the industrial production of commercial soy protein isolate products. The denatured proteins would further be associated into aggregates, or even precipitates, in the available isolates.

These protein aggregates have limited foaming and emulsifying properties (Kinsella, 1979; Kinsella, 1979; Liu, Lee, & Damodaran, 1999; Utsumi, Matsumura, & Mori, 1997; Yu & Damodaran 1991). However, structural modifications by chemical methods (e.g. deamidation, succinilation, reduction, denaturation, hydrolysis among others) that allow lower protein aggregates size and greater protein conformational flexibility, that may improve the protein surface behaviour and functionality (Carp, Wagner, Bartholomai, & Pilosof, 1997; Martinez, Carrera Sanchez, Rodriguez Patino, & Pilosof, 2009; Wagner & Guéguen, 1999).

Polysaccharides are used in admixture to proteins mainly to enhance stability of dispersed systems. Most high molecular weight polysaccharides, are hydrophilic in nature and do not adsorb to the air–water interface. However, they can strongly enhance the stability of protein foams by acting as thickening or gelling agents at the interface (Dickinson & McClements, 1995).

Hydroxypropyl methyl cellulose (HPMC) is a surface-active cellulose derivate, that is used in the food industry to improve the quality of baked products (Rosell, Rojas, & Benedicto de Barber, 2001) and in the pharmaceutical industries in controlled drug-release matrixes (Ford, 1999; McCrystal, Ford, & Rajabi-Siahboomi, 1997).

HPMC applications are based on the methyl substitutions that constitute hydrophobic zones along the cellulose backbone,

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whereas hydroxypropyl groups are more hydrophilic. The introduction of these hydrophobic groups allows HPMC to behave as a surfactant. Thus, HPMCs are adsorbed at fluid interfaces lowering the surface tension (Daniels & Barta, 1993, 1994; Ochoa-Machiste & Buckton, 1996; Wollenweber, Makievski, Miller, & Daniels, 2000).

Foam formation is influenced by the adsorption of the foaming agent at the air–water interface and its ability to rapidly reduce surface tension. However, foam stabilization requires different surface properties such as the formation of a cohesive viscoelastic film via intermolecular interactions (Dickinson & McClements, 1995).

Protein denaturation by high pressure was first described by Brigman (1914) through the non-thermal coagulation of egg whites. Until recently, the use of high pressure was limited to hydrostatic batch methods or restricted to dynamic systems with relatively low homogenization pressure (e.g. homogenization of milk at <20 MPa to reduce the size of the fat globule). However, recent advances in instrumentation technology made possible the availability of high-pressure homogenizers (HPH) able reach processing pressures greater than 350 MPa. In high-pressure homogenization, a fluid material is subjected to high pressures only for a very short time (milli-seconds), while in high-hydrostatic pressure treatment the exposure time is in the order of minutes or more. Also, fluids passing through a HPH are exposed to cavitation, impingement against static surfaces, high turbulence and shear stress, and increased temperature.

Potential and current applications of HPH include the inactivation of enzymes in milk (Hayes, Fox, & Kelly, 2005), non-thermal treatment of fluid foods, enhancement of rheological and emulsifying properties of whole milk (Kietczewska, Kruk, Czerniewicz, Warmińska, & Haponiuk, 2003), bacterial inactivation (Diels, Callewaert, Wuytack, Masschalck, & Michiels, 2004; Diels, Wuytack, & Michiels, 2003; Wuytack, Diels, & Michiels, 2002), molecular weight reduction of hydrocolloids (Floury, Desrumaux, & Lardières, 2000), production of fine lipid dispersions, and improved efficacy of cosmetic products and pharmaceuticals by the particle size reduction of inactive ingredients (Işcan, Wissing, Hekimoglu, & Müller, 2005; Jia, 2005; Möschwitzer & Müller, 2006).

The modification of protein structure or aggregates size by HPH is also a growing area of interest since protein function is determined by its three-dimensional conformation defined by its tertiary and quaternary structures as well as the aggregated state of proteins (Messens, Van Camp, & Huyghebaert, 1997).

There is a lack of studies on the effect of HPH on systems containing mixed commercial protein isolates and polysaccharides.

The objective of this work was to study the effect of dynamic high-pressure treatment on the interfacial and foaming properties of soy protein isolate–hydroxypropylmethylcelluloses systems at 100, 200 and 300 MPa at pH 7. Accordingly, foam overrun by whipping method, viscosity, aggregates size distribution and surface pressure at short absorption time of mixed systems were studied.

2. Materials and methods

2.1. Materials

Commercial grade soy proteins isolate (SP), Supro 545 (The Solae Company, St. Louis, MO) was used. The isolate composition was: protein, dry basis: 90%; moisture: 6%; fat free: 1%; ash: 5%; pH (5% slurry): 6.9–7.4. The commercial protein isolate was denatured as determined by differential scanning calorimetry (Metler Toledo, DSC 822).

HPMC polysaccharide (PS) (The Dow Chemical Company, Midland, MI) with 2 molecular weights (Methocel E15, 15 mPa s; Methocel E4M, 4000 mPa s) were used in this study to prepare the mixed systems.

2.2. High-pressure homogenization (HPH)

SP dispersions at 4% (w/v) and HPMCs at 0.6% (w/v) were prepared in distilled water and allow to hydrate overnight. The mixed systems (SP–PSs) were prepared from the former solutions with a final concentration of 2/0.3% (w/v) respectively and subjected to high-pressure valve homogenization as well as their controls (protein and polysaccharides alone at 2 and 0.3% (w/v) respectively) (model FPG 12500, Stansted Fluid Power, Essex, UK) at 0, 100, 200, and 300 MPa. Biopolymers concentrations selected were made on the basis of model solutions concentrations representative in food application and they are generally used in the foams produced by whipping method (Britten & Lavoie, 1992; Carp, Baeza, Bartholomai, & Pilosof, 2004; Carp, Bartholomai, Relkin, & Pilosof, 2001; Makri & Doxastakis, 2007; Martínez, Baeza, Millán, & Pilosof, 2005; Martinez et al., 2009; Prins, 1999; Tsaliki, Kechagia, & Doxastakis, 2002; Wagner & Guéguen, 1999).

The operating structure consists of a bench top unit providing synchronized homogenization using two hydraulic intensifiers. Valve temperature was regulated by water bath (Isotemp 3016 D, Fisher Scientific, Pittsburgh, PA) set at 4 °C. Samples treated were collected in a volume of 50 mL per pressure level. Temperature increase with increased pressure was recorded during sample collection using a thermocouple placed immediately after the homogenization valve. After homogenization, samples were cooled using an ice bath and stored at ~4 °C. All the following measures were made on same composition samples obtained by comparing with the corresponding untreated ones.

2.3. Foaming properties: foam overrun

A 40 mL aliquot of each treated and untreated sample was foamed at 25 °C in a graduated cylinder (6 cm diameter) for 3 min using a household mixer (model 727-3, Hamilton Beach, Inc., Washington, NC) equipped with a 25 mm vane rotating at 9000 rpm. Foam overrun (FO) was calculated using equation (1) described by Carp et al. (2001):

$$\mathrm{FO}\ (\%) = [(\text{foam volume} - 40)/40] \times 100 \quad (1)$$

The data reported are means of at least two replicates. The error was less than 10%.

2.4. Viscosity

Rheological measurements were done using a controlled-stress rheometer (AR-2000, TA Instruments, UK) equipped with cone and plate geometry (40 mm diameter, 1° angle, 30 μm truncation). The temperature was controlled at 25 °C by a lower plate Peltier system. Flow curves (shear stress *vs.* shear strain rate) were measured with rate control from 0 to 150 s^{-1} shear strain rate. Newtonian flow behaviour was observed in all treated and untreated samples. The data reported are means of at least two replicates. The error was less than 10%.

2.5. Interfacial properties: surface pressure

Surface tension (σ) protein (2% w/v) and polysaccharides (0.3% w/v) suspensions and in the mixed systems (2% w/v protein + 0.3% w/v polysaccharide) was measured at 20 °C. An automatic pendant drop tensiometer (Easy Drop, DSA10-MK2, Germany) with a 20 μL drop volume, 25 μL/min drop building velocity, and 48 s drop

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formation time where surface pressure was registered for all treated and untreated samples. The surface tension was measured by Laplace equation conversion as follows:

$$\frac{1}{x}\frac{d}{dx}(x \cdot sin\,\theta) = \frac{2}{b} - C \cdot z \quad (2)$$

where x and z (m) are the Cartesian co-ordinates at any point on the drop profile, b (m) is the curvature radius at the drop apex (m), θ (radian) is the angle of the tangent to the drop profile and C is a capillarity constant, $C = g\,\Delta\rho/\sigma$, where $\Delta\rho$ (kg m^{-3}) is the difference between the densities of the solution and the air, σ (N m^{-2}) the interfacial tension, and g (m s^{-1}) is the acceleration of the gravity.

Surface tension was transformed to surface pressure as follow:

$$\pi = \sigma_0 - \sigma \quad (3)$$

where π is the surface pressure, and σ and σ_0 are the surface tension in the presence and in the absence ($\sigma_0 = 73.5$ mN m^{-1}) of biopolymers, respectively. The error was less than 7%.

2.6. Particle size distribution

Particle number size distribution of untreated and pressured samples was determined by static light scattering using a LA-910 particle size analyzer (Horiba Instruments Inc., Irvine, CA). 1 mL of samples were diluted in 100 mL of distilled water. Apparent particle size measurements were made at a 90° scattering angle and 1.08 relative refractive index. All measurements were conducted at room temperature (~25 °C). The data reported are means of at least two replicates. The error was less than 10%.

All measurements were made at least on two samples pressurized on independent homogenizer treatments.

3. Results

3.1. Foaming properties: foam overrun

Fig. 1a, b shows the foam overrun (FO) and viscosity respectively of mixed systems and components alone subjected to HPH in the 0–300 MPa range. The increase in FO (Fig. 1a) in general as a consequence of pressure can be attributed meanly to the viscosity decrease of the solutions which allows air incorporation, as was less limited by the lower viscosity (Fig. 1b). A similar behaviour has been reported for soy protein foams in the presence of polysaccharides (Carp et al., 2001) or κC (Carp et al., 2004). It can be seen that the FO for SP alone was slightly increased from 85 to 114% at 100 MPa, decreased at 200 MPa to 104% and kept constant at that value at higher pressures, whereas the viscosity of soy protein (Fig. 1b) followed a similar behaviour. SP viscosity was reduced significantly from 9.4 to 2 mPa s at 100 MPa and was similar at higher pressures (200 and 300 MPa).

It can be also seen a higher FO of mixed systems (SP–E15 and SP–E4M) compared to SP alone whatever the pressure applied and lower FO of these systems than the PSs alone (Fig. 1a). The FO of mixed systems increase respect to SP is probably due to competitive behaviour between SP and PSs for the interface promoting a foaming ability increase of mixed system. However, it can be seen a different pressure effect for each system.

The SP–E15 system showed a constant increase of FO as determined by HPH. This may be attributed to possible interactions between SP–E15 which favoured a foaming improvement at high pressures and were not present in the protein alone. In the case of SP–E4M, it can be observed an increase of FO at 100 MPa; a decrease at 200 MPa and an increase at 300 MPa. It shows that

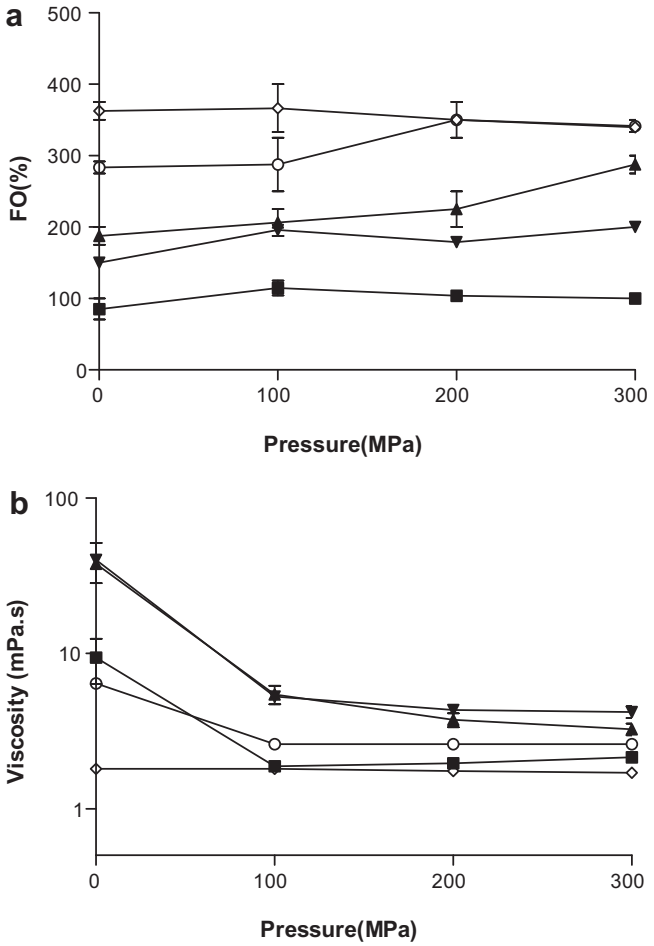

Fig. 1. (a) Foam overrun and (b) viscosity as a function of pressure level homogenization applied for SP (■), E15 (◇), E4M (○), SP–E15 (▲), SP–E4M (▼).

depending to the pressure applied different FO of mixed systems can be obtained determined probably by a particular balance between interfacial behaviour and viscosity effect imparted by the polysaccharide. From untreated SP–E4M system to 200 MPa pressure treatment, it was observed that FO followed the same tendency as viscosity showed (Fig. 1b), it means, it can be possible that this parameter would control the general foam behaviour at these pressures levels. However, at 300 MPa of pressure, an increase of FO was observed as well as their viscosity displayed. Therefore, the interfacial behaviour would be relevant at this point, playing an important role as foaming agent by competence. Martínez, Carrera Sánchez, Pizones Ruiz-Henestrosa, Rodríguez Patino, and Pilosof (2007) found a similar results studying the interfacial of mixed soy protein isolate and polysaccharide systems to gain knowledge on the interactions between these biopolymers at the air–water interface under dynamic conditions at neutral pH. The E4M was used as surface-active polysaccharide. The dynamic surface pressure and rheological properties of films were evaluated with a drop tensiometer at 20 °C, pH 7. It was observed that the presence of E4M greatly increased the surface pressure (at about one hour of adsorption time), which competes for the interface with soy protein, but due to its unusual strong surface activity it could dominate the surface pressure. Although dynamic conditions were study in that work, high-pressure homogenization applied in the present one would alter aggregates structure of systems promoting a faster adsorption to the interface.

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The FO decrease of mixed systems respect to PSs alone at every pressure applied could be determined by the higher viscosity, that precludes air incorporation during the whipping process as was exhibited in the mixed systems of Fig. 1b. Moreover, the viscosity behaviour of PSs conforming pressure treatment increase was different depending on their molecular weight. HPH did not affect the viscosity of the low molecular weight HPMC (E15; average viscosity = 1.71 mPa s). However, the viscosity of the high molecular weight HPMC (E4M) decreased from 6.4 to 2.6 mPa s with increasing pressure treatment, revealing that shear induced reduction in the molecular weight of polysaccharides. Similar results were reported by Camino, Pérez, and Pilosof (2009) where molecular modification of hydroxypropyl methylcellulose by high intensity ultrasound were studied. Changes in viscosity was observed for high molecular weight HPMCs revealing structural modifications that were not apparent for low molecular weight HPMCs as resulted in the present work.

According to the PSs used in this work, interactions between SP–PSs were supposed to favour the FO on untreated mixed systems; this effect was promoted using HPH particularly in the case of E15 at 300 MPa. The effect of SP–E4M was less pronounced from the one observed for E15. Thus, the molecular weight of PS is a very important factor of interaction with soy protein isolate under these conditions of high pressure.

3.2. Interfacial properties: surface pressure

Due to the adsorption at fluid interfaces, protein molecules prevent the re-coalescence of previously created bubbles or droplets. In addition, during the protein adsorption the surface or interfacial pressure of the air–water or/and oil–water interface increases which is an important attribute to optimize the input of energy involved in the foaming or emulsification process (Walstra, 1993) and for the production of smaller bubbles or droplets, which is an important factor for the stability of the dispersions.

The effect of HPH on surface pressure of mixed SP–PSs systems and the surface pressure of the components alone is shown in Fig. 2.

Surface pressure increased with the homogenization treatment for all samples in similar way. It can be also observed that the results became closer as pressure treatment increase denoting a similar tendency.

The pressure increase for all samples may be due to interactions of protein–protein and protein-interfacial film increase as a consequence of molecular flexibility rise with the pressure treatment.

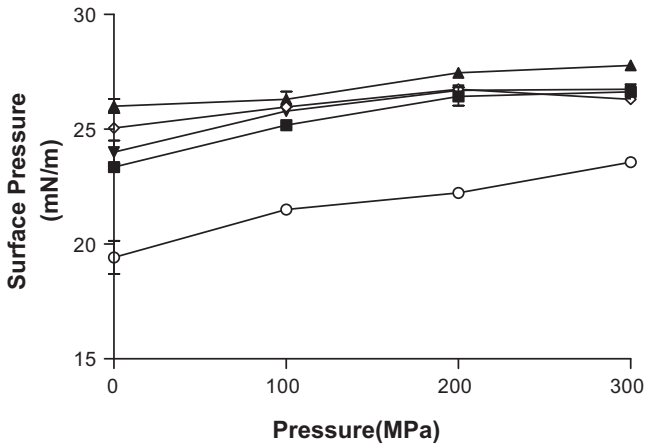

Fig. 2. Surface pressure at 48 s of drop formation as a function of pressure level homogenization applied for SP (■), E15 (◇), E4M (○), SP–E15 (▲), SP–E4M (▼).

Bouaouina, Desrumaux, Loisel, and Legrand (2006) used an ultra high-pressure homogenizer to treat whey protein isolate solutions (3% w/w). The treated solutions (up to 300 MPa) enhanced the kinetic adsorption (surface tension as a function of time) at the fluid interface. A rapid decline in tension (or increase in surface pressure) was observed for treated solutions that may be attributed to increased surface activity (i.e. surface hydrophobicity) as a result of increased surface contact of protein. This could be explained by the decrease of the size of whey protein aggregates leading both to an increase in mobility in the bulk phase and an unmasking of hydrophobic groups.

The size of protein aggregates were also measured in the present work to relation with the surface pressure increase observed. The particle size distributions obtained here are presented as a number that represents the particle size of more frequency for each sample, due to mono-modal distributions for all cases. They are showed in Table 1.

Untreated SP showed a maximum particle number frequency at 0.131 μm. A significant increase in the particle size of 8.816 μm was observed when the pressure increased to 100 MPa. However, above 100 MPa the particle size decreased (0.172–0.150 μm) with increase the pressure (200–300 MPa respectively). This indicates firstly an aggregation of previous existing aggregates follow by a disruption as pressure treatment increased. The temperature increase conforming treatment is an important factor that is frequently reported as responsible for the phenomena described. Moreover, the macromolecules in the valve gap outlet undergo cavitation phenomena, turbulence and impacts which could favour both particle nucleation/aggregation and aggregate disruption. As a consequence, high-pressure homogenization technique may induce changes in protein (techno-) functionality (Grácia-Juliá et al., 2008). Similar further aggregation at 100 MPa was reported by Grácia-Juliá et al. (2008), with dispersions of whey protein isolate (6% or 10% w/w) processed with high-pressure homogenizer. They reached at 250, 275 and 300 MPa and observed an induce marked shifts towards larger sizes, indicating clear aggregation phenomena above 225 MPa at both protein concentrations.

Concerning particle size decrease with pressure level, similar results were observed by Bouaouina et al. (2006) where an ultra high-pressure homogenizing treatment up to 300 MPa induced disruption of large powder protein particles present in a whey protein isolate dispersion. The distribution was bimodal for the non-treated solution with a large amount of “big aggregates”, i.e. over 1 μm. As the pressure increased, the distribution became narrower with particle size less than 1 μm for the most part.

When HPMC was added to SP in the present work, the behaviour of the solutions was different from SP alone.

Table 1 shows also the particle size as a consequence of HPH applied for SP–E15 system. Untreated SP–E15 sample had a particle size predominant of 20.087 μm, which decreased to 0.199 μm at 100 MPa, 0.172 μm at 200 and 300 MPa.

When E4M was added to untreated SP, the system showed a size of 22.797 μm. SP–E4M had a similar behaviour as SP–E15 displayed with HPH. The particle size decreased with increase in pressure

Table 1
Size[a] (μm) corresponding to maximum particle number frequency for SP and their mixed systems at 0, 100, 200 and 300 MPa.

Sample/pressure level	0 MPa	100 MPa	200 MPa	300 MPa
SP	0.131	8.816	0.172	0.150
SP–E15	20.087	0.199	0.172	0.172
SP–E4M	22.797	0.171	0.171	0.226

[a] Mean ± SD % less of 10% for maximum particle number frequency.

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(0.171 μm at 100 MPa; 0.171 μm at 200 MPa and 0.226 μm at 300 MPa). This denotes a clearly disruption effect of aggregates or complex of mixture previously formed by these two biopolymers at untreated conditions.

Galazka, Dickinson, and Ledward (2000) studied the influence of ι-carrageenan (ι-CAR) on the solution with 11S globulin *Vicia faba* before and after high-hydrostatic pressure at pH 8 on interfacial properties. They reported that high-pressure processing induces ι-CAR electrostatic complex(es) formation. In the present work, a probable aggregation representing by a high particle size of untreated mixed systems, could be possible as a consequence of the mechanical forces suffered by the molecules in the narrow gap of the homogenizer. These molecules can develop hydrophobic and/or hydrogen interactions even at 0 MPa with uncharged polysaccharides, promoting temporary linkages. Conforming increase the pressure, the increase in mechanical forces and temperature could favour the disruption of these aggregates.

Galazka et al. (2000), observed that the pressurized (200 MPa for 20 min) 11S exhibited higher surface pressure values at 60 min of adsorption time (dynamic measurements) than native 11S under the same experimental conditions. This correlates with our results, probably due to molecular interactions changes as a consequence of HPH treatment leading to protein flexibility enhancement by the treatment that improves their arrangements at liquid interfaces with time (Ipsen et al., 2001; Miñones Conde & Rodríguez Patino, 2006).

When ι-CAR was mixed with 11S, the biopolymer mixture exhibited a lower surface pressure value than 11S alone. The surface pressure data are indicative of protein–polysaccharide complex formation in bulk solution, which perturbs the dynamic equilibrium of protein between bulk and interface in favour of the bulk, and reduces the number and availability of hydrophobic groups on the protein for adsorbing at the air–water interface. Contrary, in our results the PSs addition promoted a higher surface pressure at short time of adsorption for the two mixed systems. Thus, it is possible a no complex formation at bulk solution between these macromolecules used in the homogenization conditions.

Therefore, the aggregates protein flexibility of samples increase would enhance the adsorption at liquid interfaces and it is probably the mean raison for the surface pressure increment observed, whatever the size of aggregates presented after each pressure applied in the homogenization process.

4. Conclusions

According to the PSs used in this work, interactions between SP and PSs apparently favour the FO on untreated mixed systems; this effect was promoted using HPH particularly in the case of E15 at 300 MPa. The effect of SP–E4M was less pronounced from the one observed for E15. Thus, the molecular weight of PS is a very important factor of interaction with soy protein isolate under these conditions of high pressure.

Therefore, the aggregated protein flexibility of samples increase would enhance the adsorption at liquid interfaces and it is probably the mean raison for the surface pressure increment resulted. This interfacial property increase was observed whatever the size of aggregates resulted after each pressure applied without any change of the flow behaviour as was observed by viscosity measurements.

In conclusion, the effect of high-pressure homogenization of these systems on foam overrun is not directly relation with the effect on the surface pressure at short adsorption time. The viscosity decrease may be explained some of the results together with interfacial performance at longer adsorption times than 48 s such as competence of biopolymers at liquid interfaces which depend on the system and level of pressure applied.

These results suggest that high-pressure homogenization could be used to improve the foam overrun of soy protein–polysaccharides dispersed systems and potentially create new functional aggregates by particle aggregation and aggregate disruption with an appropriate selection of polysaccharide and pressure level.

Acknowledgments

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