Chickpea protein-stabilized emulsions: from interfacial to bulk rheology

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ABSTRACT

The objective of this work is to assess the relationship between interfacial and bulk rheology of O/W chickpea-based emulsions, both being related to emulsion stability, as a function pH. Whereas dilatational rheology is related to short-term stability, interfacial shear rheology provides useful information related to the long-term stability of emulsions.

INTRODUCTION

Plant proteins have the ability not only to produce emulsions, but also to provide the stability required for commercial purposes. These properties are related to their ability to be absorbed at the oil/water (O/W) interfaces, reducing the interfacial tension. Thus, the adsorption of proteins molecules at O/W interface is well-known for model proteins (i.e. β-Lactoblobulin, ovalbumin or bovine serum albumin) and the nature of protein interactions at the interface is highly complex[1]. This complexity may involve protein denaturation, interactions among denatured protein molecules, formation of protein aggregates, as well as interactions with other biopolymers and/or colloidal particles at the fluid-fluid interface. All these events, which in turns determine the microstructure and rheological properties of the interface, will affect emulsion stability. Therefore, interfacial rheology may be regarded as a powerful tool dominating the dynamics of complex fluid-fluid interfaces. More specifically, rheological properties from shear measurements have been postulated as the most useful technique for the assessment of the microstructure of complex fluid-fluid interfaces, and its relationship to long-term emulsion stability[2]. In contrast, rheological properties from dilatational measurements are reported to be related to short-term stability of emulsions, as well as to the emulsification ability, which in turn depend on the capacity of proteins to reduce the interfacial tension[3]. In addition, it is widely recognised the close relationship between emulsion rheology, microstructure and stability. Interestingly, however, the links between interfacial and bulk emulsion rheology have not yet been extensively studied, although they must be highly promising in view of their respective links to emulsion stability.

Food industry is highly interested in the development of low-fat (O/W) emulsions in order to obtain healthier food emulsions. Proteins have been widely used for (O/W) emulsions since they facilitate breakup of oil drops and prevent droplets coalescence over emulsification and storage by increasing repulsion forces between droplets. The strength of the interface stabilised is related to the cohesive interactions between protein molecules[4,5]. Most of commercial food emulsions are stabilised by milk or eggs proteins. However, their prices are high and they show some allergic problems. Hence,

food industry is interested in plant proteins, such as legume proteins as low-cost and healthier substitutes for animal and soybean proteins[6,7]. In this regard, understanding of factors affecting the functional properties of legume proteins will enable better control of these properties, which will eventually facilitate the development of novel food products based on these proteins. In fact, the current international market of legume proteins is growing, which is driven by the increase of applications in food products[8]. Among these proteins from legume sources, chickpea is fairly attractive in view of the nutritional quality of the proteins present in the raw product.

The objective of this work was the evaluation of the relationship between interfacial and bulk rheology of O/W chickpea protein-based emulsions as a function of pH.

MATERIALS AND METHODS

Materials

A protein powder from chickpea milled was supplied by DOSBIO (San José de la Rinconada, Seville, Spain). This chickpea powder was dispersed into water (10 wt.%) and the pH value was adjusted at 8.0 using 4 M NaOH (alkaline solubilization). Then, the powder dispersion was stirred for 30 min and the dispersion was centrifuged for 15 min at 15000 × g and 10ºC. The supernatant was separated from the pellet and the pH value was adjusted at 3.5 using 4M HCl (isoelectric precipitation). Dispersion was again centrifuged for 15 min at 15000 × g and 10ºC. The pellet was freeze-dried using a Telstar LyoQuest (Barcelona, Spain).

Methods

Interfacial O/W tension was measured by means of a droplet pendant tensiometer (Tracker, IT Concept, France) that can be also used to measure interfacial dilatational viscoelastic properties. The droplet profile was digitized and analysed through a CCD camera. Droplet profiles were processed according to the Laplace equation as was described by Castellani et al.[9]. The complex viscoelastic modulus of protein-absorbed O/W layers (E_i^*) were determined at 10% strain amplitude after reaching a pseudo equilibrium state (i.e. after 10,800 s), as a function of frequency, ranging from 0.0075 to 0.1 Hz. All the experiments were carried in an optical glass cuvette which contains the oil phase, which was thermoset at 20.0 ± 0.1 °C.

Interfacial shear rheology was carried out using a double-wall-ring geometry (DWR) attached to a sensitive magnetic air bearing stress-controlled rheometer (DHR-3, TA Instruments, USA). The procedure was carried out as described by Vandebril et al[10]. Results of the complex shear modulus (G_i^*) for the O/W interface were obtained from frequency sweep tests (from 0.0075-1 Hz) after reaching the pseudo equilibrium state (i.e. after 10,800 s).

After the interfacial characterization, different emulsions were prepared using a high-pressure homogenizer EmulsiFlex-C5 (Avestin). First of all, a pre-emulsion was obtained with a 50/50 (O/W) mixing ratio using a protein dispersion containing 2 wt% protein (at a selected pH value) as the continuous phase and sunflower oil. The mixture was homogenized in a Ultraturrax® mixer for 2 min.

Droplet size distributions (DSD) of the emulsions were determined with a particle size analyser based on laser diffraction technique (Mastersizer X, Malvern). To avoid the presence of floccules, emulsions were diluted 1:10 into 1 wt.% SDS solution (pH 8.0), and then softly stirred. The volumetric mean droplet diameter ($D_{4,3}$) was calculated as follows:

$$D_{M,N} = \left[\frac{\int D^M n(D) dD}{\int D^N n(D) dD}\right]^{\frac{1}{M-N}} \qquad (1)$$

where *M* and *N* are 4 and 3, respectively, for the volumetric mean diameter ($D_{4,3}$).

The uniformity parameter was also obtained from DSD results. The uniformity index is related to the polidispersity of the different droplet sizes and is defined by the following expression:

$$U = \frac{\sum V_i |d(v,0,5) - d_i|}{d(v,0,5) \sum V_i} \qquad (2)$$

where *d(v,0,5)* is the median for the distribution, and *Vi* is the volume of droplets with a diameter *di*.

Small Amplitude Oscillatory Shear tests (SAOS) of the emulsions were carried out in a stress-controlled rheometer (AR-2000, TA Instruments) using serrated plate-plate geometry to avoid slipping effect. The complex modulus from the bulk was obtained after performing frequency sweep tests (from 0.05 to 50 rad/s), the day after the emulsification and 30 days later.

Finally, photographs were taken to show the visual appearance of emulsions.

Statistical analysis

At least three replicates of each measurement were carried out. Uncertainty was expressed as standard deviation.

RESULTS

Interfacial characterisation

Figure 1A shows the interfacial tension (σ) over the protein adsorption time as a function of pH value (2.5, 5.0 and 7.5). In all cases, the adsorption of the protein at O/W interface is characterized by a rapid decrease in interfacial tension, which is followed by a slower evolution and a tendency to reach a constant value (σ_{eq}). Achieving a pseudo-equilibrium value as low as possible is quite important, since it would involve an enhancement in the ability of the protein to stabilize the interface, obtaining smaller oil droplets. Thus, while the surface tension of the systems at pH 2.5 and 7.5 is around 7 mN/m, the final surface tension for the system at pH 5.0 is ca. 4 mN/m.

Figure 1B shows the complex modulus (E_i*), which was obtained after reaching the above-mentioned pseudoequilibrium state from interfacial dilatational measurements as a function of frequency. The response of the interface against the stress applied indicates the occurrence of strong protein interactions, which can be related to the bending rigidity and/or deviatoric stresses[2]. Despite the fact that the interface is strong in all cases studied, the pH value has a marked effect on the strength of the interface.

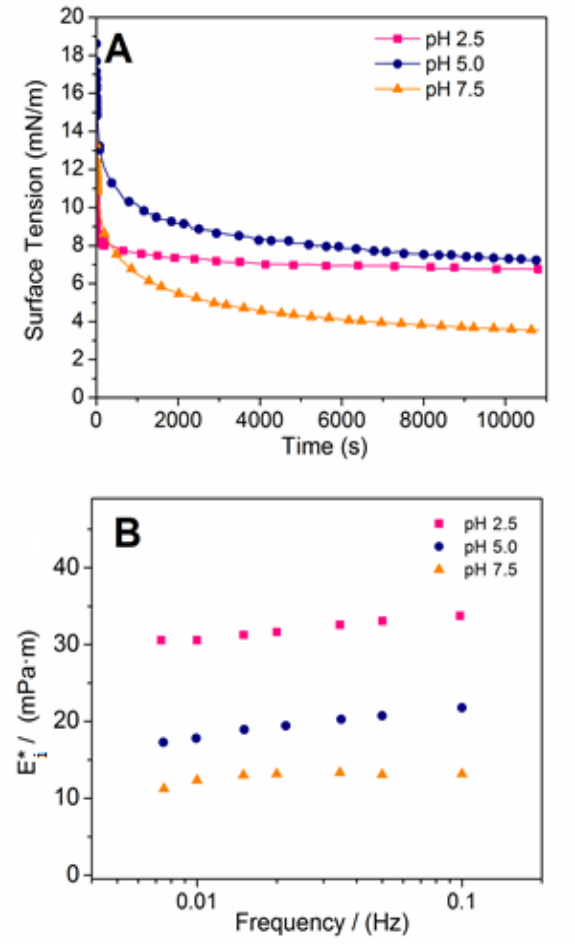

Figure 1. Interfacial properties as a function of frequency, at 2.5, 5.0 and 7.5): (A) Interfacial tension along protein adsorption. (B) Complex modulus (E_i*) after protein adsorption

Thus the highest value was obtained for the system at pH 2.5, whereas the lowest was obtained for the system at pH 7.5,

which also showed a deviation at high frequencies values, denoting certain gel weakness. A similar behaviour was found in previous papers for other protein systems, where an enhancement of E_i* was related to protein unfolding preferentially taking place at low pH[11,12].

Subsequently, interfacial shear rheology was used to characterize the interfacial O/W layer. Figure 2 shows the complex modulus (G_i*), obtained from interfacial SAOS tests, as a function of pH (2.5, 5.0 and 7.5). This figure corroborates not only the presence of protein interactions taking place at the O/W interface after protein adsorption, but also its strong dependence on pH.

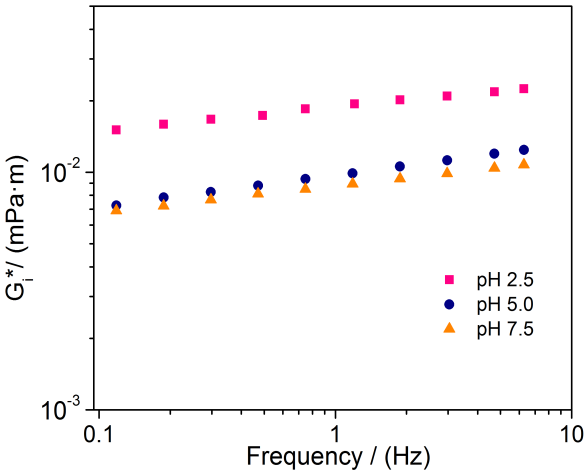

Figure 2. Complex modulus (G_i*) obtained from SAOS measurements after protein adsorption as a function of frequency at three different pH values (2.5, 5.0 and 7.5).

As may be observed, results from shear and dilatational measurements are consistent since the highest and lowest values of G_i* correspond again to pH 2.5 and 7.5, respectively. These results seem to confirm that the gel strength of the interface obtained at pH 2.5 is related to the presence of strong protein interactions. However, the values of E_i* from pendant drop were higher at pH 5.0 than 7.5.

This different response may be related to the nature of the protein interactions. The interfacial response to SAOS measurements is related to proteins interactions, while dilatational measurements give an overall response of the interface strength. Among others, values obtained from the dilatational measurements have been related to bending rigidity of the interfacial layer, which does not provide a proper long-term emulsion stability[12]. In any case, the values of G_i* are higher than other obtained previously for rice protein adsorbed at O/W interface, where the pH exhibited a similar effect[13].

Emulsion characterisation

Table 1 shows parameters from DSD profiles ($D_{4,3}$ and U) as a function of pH value. First of all, it is worth pointing out the dependence of droplet sizes on pH value.

Table 1. DSD parameters obtained from Eq. 1 ($D_{4,3}$) and Eq. 2 (U) for emulsions the day after preparation and 30 days later as a function of pH (2.5, 5.0 and 7.5).

	$D_{4,3}$ (μm)		U (-)	
	Time (days)		Time (days)	
pH	1	30	1	30
2.5	1.3±0.1	1.4±0.2	0.24±0.1	0.25±0.2
5.0	6.4±0.2	6.9±0.2	0.36±0.2	0.45±0.2
7.0	1.7±0.1	2.0±0.1	0.49±0.2	0.63±0.3

Thus, whereas the lowest values were obtained far from the isoelectric point (IEP) at pH 2.5 and 7.5, the most stable system was found for that one corresponding to the highest E_i*. This higher stability can be seen clearly with the uniformity index (U), which increases over ageing time for systems at pH 5.0 and 7.5 (reflecting broader peaks, as a consequence of coalescence phenomena). Note that the uniformity index is a useful tool that reflects the polydispersity of the samples, for which low values are desirable. In any case, it is noticeable that the mean

volume particle diameters were in the same range than those values obtained for other protein-based emulsions[11,14–16].

Dynamic frequency sweep tests were carried out for all the emulsions studied to determine the frequency dependence of complex modulus (G*). Figure 3 shows G* corresponding to the day after emulsion preparation as well as 30 days later at three different pH values (2.5, 5.0 and 7.0). On the one hand, the pH value has a strong influence on the G* obtained. The highest values were obtained for the emulsion at pH 2.5, whereas emulsions at pH 5.0 and 7.5 exhibit a G* modulus around one order of magnitude lower.

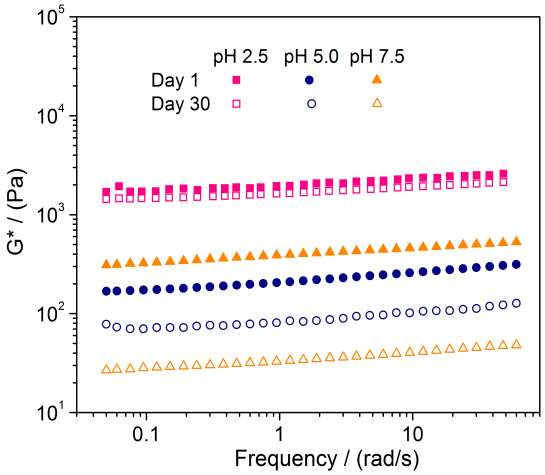

Figure 3. Complex modulus (G*) from SAOS measurements the day after emulsion preparation and 30 days later at three different pH values (2.5, 5.0 and 7.5)

In addition, these emulsions show a moderate dependence on frequency. This behaviour corresponds to the plateau region of the mechanical spectrum and has been previously found for a wide variety of polydisperse systems. The occurrence of a plateau zone has been related to the development of entanglements among macromolecules[17]. The effect of pH found in these systems was previously found for other protein systems, and it was attributed to modifications in the interactions among protein side chains. Thus, at low pH value (below the IEP), the net surface charge of the protein is positive, while at higher pH values (above the IEP), the negative charges are predominant on protein surfaces[18].

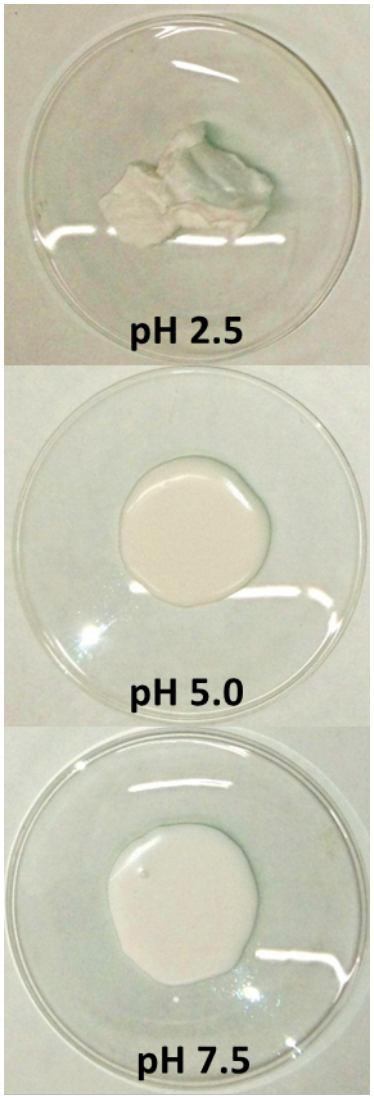

Figure 4. Visual appearance of emulsions one day after emulsion preparation, as a function of pH

On the other hand, Figure 3 also reflects the higher stability for the system at pH 2.5 over ageing time, which suffers a slight decrease of G* after one month. In addition, it is also noticeable that despite the fact that the initial values for G* at pH 7.5 is higher than that one found for the system at pH 5.0,

after 30 days emulsion storage, G* goes down faster at pH 7.5 than at pH 5.0.

Finally, visual appearance of emulsions was evaluated in order to determine the suitability of these emulsions for being the basis of food products (Figure 4). According to the microstructure characterization carried out by means of bulk rheology, the emulsion at pH 2.5 seems to be the most consistent, for this reason its use as mayonnaise could be advisable. However, the consistency of emulsions at pH 5.0 and 7.5 seems to be lower. In this case, these emulsions could be more appropriate to be used in salad dressings.

CONCLUSIONS

Chickpea proteins provide suitable interfacial properties for stabilizing O/W interface, decreasing the interfacial tension as the same time as increasing the complex interfacial moduli (G_i* and E_i*). Thus, despite the fact that the interfacial response is highly dependent on pH, the best response was obtained at pH 2.5 in all cases, showing better interfacial and bulk rheological properties and lower DSD distributions. Initially, however at pH values 5.0 and 7.5 the interfacial properties were poorer, which also provide worse macroscopic properties to the final emulsion (lower viscosity and higher droplet sizes).

The higher strength found by dilatational measurements should be related to bending rigidity, instead of to protein interactions (which is the actual responsible for emulsion stability). The visual appearance of emulsions indicates that the pH value not only may modulate both the interfacial and microstructure properties, but also may lead the final use of these systems as food products.

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