Article

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Influence of pH value on microstructure of oil-in-water emulsions stabilized by chickpea protein flour

Manuel Felix[1], Nadia Isurralde[2], Alberto Romero[3] and Antonio Guerrero[1]

Abstract

Food industry is highly interested in the development of healthier formulations of oil-in-water emulsions, stabilized by plant proteins instead of egg or milk proteins. These emulsions would avoid allergic issues or animal fat. Among other plant proteins, legumes are a cost-competitive product. This work evaluates the influence of pH value (2.5, 5.0 and 7.5) on emulsions stabilized by chickpea-based emulsions at two different protein concentration (2.0 and 4.0 wt%). Microstructure of chickpea-based emulsions is assessed by means of backscattering, droplet size distributions and small amplitude oscillatory shear measurements. Visual appearances as well as confocal laser scanning microscopy images are obtained to provide useful information on the emulsions structure. Interestingly, results indicate that the pH value and protein concentration have a strong influence on emulsion microstructure and stability. Thus, the system which contains protein surfaces positively charged shows the highest viscoelastic properties, a good droplet size distribution profile and non-apparent destabilization phenomena. Interestingly, results also reveal the importance of rheological measurements in the prediction of protein interactions and emulsion stability since this technique is able to predict destabilization mechanisms sooner than other techniques such as backscattering or droplet size distribution measurements.

Keywords

Chickpea protein, food emulsions, legumes, microstructure, proteins, rheology

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INTRODUCTION

Legumes include around 17,600 species in about 690 genera. They are dicotyledonous seeds and belong to the Leguminosae family. Among others, soybean, faba bean, pea or chickpea (CP) legumes are the eatable ones. The global production of legumes has an important place on the global production of crops. In fact, they are the fourth food crop most grown in the world (after wheat, rice and corn) (Du et al., 2014). As legume, CP ranks third in most important legume production, based on total grain legume production (Hayta and İşçimen, 2017). It is a rich food source, which contains carbohydrates, proteins, dietary fibres, vitamins and minerals (Du et al., 2014; Huang et al., 2017; Ladjal Ettoumi et al., 2017). They are richer than cereals in proteins amount. In this sense, while cereals typically exhibit 3–7 wt% proteins, legumes have ca. 20 wt% proteins showing similar protein content to meat (De Almeida Costa et al., 2006). In fact, the number of food applications for plant proteins is

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[1]Departamento de Ingeniería Química, Escuela Politécnica superior, Universidad de Sevilla, Sevilla, Spain
[2]Universidad Nacional del Litoral (UNL) – Facultad de Ingeniería Química (FIQ) – Instituto de Investigaciones en Catálisis y Petroquímica (INCAPE-CONICET), Santa Fe, Argentina
[3]Departamento de Ingeniería Química, Facultad de Física, Universidad de Sevilla, Sevilla, Spain

Corresponding author:
Manuel Felix, Escuela Politécnica Superior, Universidad de Sevilla, C/ Virgen de êfrica, 7, Sevilla.
Email: mfelix@us.es

increasing continuously, and they are seen like a real alternative to animal proteins, since they show more drawbacks in human consumption than plant proteins (Ladjal-Ettoumi et al., 2016a; Zhang et al., 2009). In addition, legumes are a cost-competitive product, for instance, Tharanathan and Mahadevamma (2003) indicated that legumes were the cheapest source of supplementary proteins in India. For this reason, they are considered as poor man's meat.

However, nowadays, applications of plant proteins in the food industry are limited to soybean and wheat proteins, despite the fact that other plant proteins such as legume proteins are as available as soybean or wheat proteins. In this sense, CP seeds have been considered as adequate source of proteins in the diet since amino acids are well balanced and their proteins are highly bioavailable (Friedman, 1996). Unfortunately, around 20% CP seeds are sold like a by-product for animal feeding (Ulloa et al., 1988). In fact, the value of these seeds is underestimated since they are a valuable source of proteins that could be used for the development of food products. Apart from nutraceutical properties of these proteins, they are also pH-dependent amphoteric molecules. For this reason, they are able to facilitate the formation of small droplets and prevent destabilization phenomena such as coalescence or creaming (McClements, 2004a; Oboh et al., 2009). Emulsions could be stabilized by plant proteins (i.e. CP) instead of egg or milk proteins, avoiding allergic issues of them and increasing the health of their derivatives.

The aim of this work is to study the effect of the pH value in the formation and stabilization of oil-in-water (O/W) CP emulsions. To achieve this objective, a physicochemical characterization of the protein powder was carried out. Then, emulsions were performed at two protein concentrations (2 and 4 wt%) and at three pH values (2.5, 5.0 and 7.5). Subsequently, the stability of emulsions after 28 days was evaluated by means of backscattering (BS) measurements, droplet size distributions (DSDs) analysis and small amplitude oscillatory shear (SAOS) measurements. Confocal laser scanning microscopy (CLSM) images were obtained to provide useful information on the emulsions microstructure.

MATERIAL AND METHODS

Materials

Preparation of the CP protein concentrate: A protein powder from CP, obtained by direct milling, was supplied by DosBios (San José de la Rinconada, Seville, Spain). To increase the protein concentration, isoelectric precipitation was followed after alkaline solubilization, obtaining similar protein concentration than Karaca (2011). Finally, the supernatant was discarded and the pellet was freeze-dried in a Telstar LyoQuest (Barcelona, Spain), obtaining the CP protein concentrate used in this research.

Emulsions were prepared with high oleic sunflower (Capicua®), which was supplied by Coreysa Company (Sevilla, Spain). All other chemical reagents (i.e. NaOH, HCl) were supplied by Sigma-Aldrich (St Louis, Missouri, USA). Distilled grade water was used for all experiments.

Protein characterization

Protein composition. LECO CHNS-932 (MI, USA) micro analyser was used to carry out the determination of nitrogen content by means of Dumas combustion method. Samples (5 mg) were placed into a porcelain sample holder and they were heated up to 1300 °C. The combustion converted all the nitrogen present in the sample into nitrogen gas (N_2), which was quantitated by conductivity (Etheridge et al., 1998). Moreover, the A.O.A.C. (2000) approved methods were used for the determination of lipids, ashes and moisture of samples.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis. CP soluble protein fraction composition was established by Laemmli SDS-PAGE with some modifications (Cannon-Carlson and Tang, 1997), where continuous and stacking gels were prepared of 10 and 3.5% of acrylamide, respectively. A buffer containing 2.0 M Tris–base, containing 0.15% SDS pH 8.8 was used for the separating gel. The running buffer consisted of 0.027 M Tris–base, 0.38 M glycine, pH 8.3 with the addition of 0.15% SDS was utilized. The equipment was operated at room temperature, whereas the voltage was set at 200 V and the intensity at 30 mA. Coomassie Brilliant Blue was used as staining agent and β-mercaptoethanol was used in the sample buffer. The CP soluble protein fraction was obtained at pH 2.5, 5.0 and 7.5. Precision Plus Protein standards (Bio-Rad-Calibration kit, Richmond, CA, USA) containing 10 protein bands were used as a reference: 10, 15, 20, 25, 37, 50, 75, 100, 150 and 250 kDa.

Protein solubility. CP protein solubility was determined as a function of pH value (from 2 to 12). Aqueous dispersions at 1 mg/ml were prepared and pH of different aliquots was adjusted by using different buffers. Samples were homogenized and centrifuged at 15,000 × g for 15 min at 10 °C. The protein content was determined in triplicate by a modified Lowry

method (Markwell et al., 1978). Results were plotted as percentages of soluble CP protein.

Emulsions preparation

Emulsions were prepared following the two-stage method used by Ladjal-Ettoumi et al. (2016b) for legume-based emulsions. High oleic sunflower oil was gradually blended with aqueous CP protein dispersion (50/50) at the selected pH values: below the isoelectric point (2.5), intermediate pH value (5.0) close to the IEP and above the IEP (7.5). The protein concentration was selected according to the concentration of protein required for the saturation of the O/W interface (results unpublished): (2.0 or 4.0 wt%). Subsequently, blends were subjected to high-shear mixing Ultraturrax T-50 (Staufen, Germany) over 2 min at 5000 r/min (pre-emulsion). Finally, the pre-emulsions were passed through the high-pressure valve homogenizer EmulsiFlex-C5 once at 200 KPa (Avestin, Germany).

Emulsions characterization

BS measurements. A light source on a glass tube, which contains the sample, was applied. The BS and the transmittance were obtained as a function of the tube length. These measurements were carried out with a Turbiscan Lab Expert (L'Union, France). These measurements were carried out for 28 days and the results are useful because it can provide information related to the destabilization phenomena of emulsions. Relative backscattering (ΔBS) as a function of time was defined as follows (equation (1))

$$\Delta BS_{rel}(\%) = \frac{BS_0 - BS_t}{BS_0} \Delta 100 \qquad (1)$$

where BS_0 and BS_t are the mean values for the BS profile obtained at 50 mm tube length the day 0 and after time t (correspond to 1, 7, 14, 21 and 28 days), respectively.

DSD measurements. DSD was determined with the Mastersizer X (Malvern, UK), which is a particle size analyser by means of laser diffraction. To disrupt flocules, 1 wt% of SDS was added to the water/emulsion dispersion, followed by a soft stirring (Puppo et al., 2005). The mean droplet diameter was calculated as follows (equation (2))

$$D[4,3] = \frac{\sum_i^n d_i^4}{\sum_i^n d_i^3} \qquad (2)$$

where n_i is the number of droplets which have d_i as diameter.

The coalescence index ($CI(\%)$) of the emulsions was calculated after 28 days of emulsions preparation as follows (equation (3))

$$CI_{28}(\%) = \left[\frac{D[4,3]_{28}}{D[4,3]_0} - 1\right] \cdot 100 \qquad (3)$$

where $D[4,3]_0$ and $D[4,3]_{28}$ correspond to mean volumetric diameter of emulsions freshly prepared and after storage of 28 days, respectively. In any case, all the emulsions were assessed with SDS (1%) as the diluent.

Linear viscoelasticity. SAOS measurements were carried out using the AR-2000 rheometer (TA instruments, USA). First of all, stress sweep tests were performed at three different frequencies (0.62, 6.20 and 12.52 rad/s) to define the linear viscoelastic region (LVR). Subsequently, frequency sweep tests were carried out from 0.062 to 125 rad/s, at constant stress within the LVR. Serrated plates of 35 mm were used in these measurements to avoid slipping effect.

Additionally, in order to clarify this decrease in the elastic modulus, the relative decrease of G'_1 values was calculated according to equation (4)

$$\Delta G'_1(\%) = \frac{(G'_1)_1 - (G'_1)_{28}}{(G'_1)_1} \cdot 100 \qquad (4)$$

where $(G'_1)_1$ and $(G'_1)_{28}$ are the elastic moduli the day after emulsion preparation and after 28 days, respectively.

CLSM. The ZEISS LSM 7 DUO (Heidelberg, Germany) microscope was used in order to obtain CLSM images. A 100X objective and the Argon laser were used. The exciting wavelength was 488 nm and the emission wavelength was within 520 and 687 nm. It was not necessary to stain the aqueous phase due to the autofluorescent properties shown by the protein used. The green colour of the pictures corresponds to the autofluorescence of proteins when the Argon laser excites tryptophan groups.

Statistical analysis

At least three replicates of each measurement were carried out. Statistical analyses were performed using t-test and one-way analysis of variance (ANOVA, $p < 0.05$) by means of the statistical package Microsoft Excel. Uncertainty was expressed as standard deviation.

RESULTS AND DISCUSSION

CP protein system characterization

The protein content of the CP protein system obtained after alkaline solubilization and isoelectric precipitation method reaches up to 64.5 ± 0.2 wt%, being considered as a protein concentrate according to the Pearson classification (Pearson and Hudson, 1983). On the other hand, the analysis of the lipids, ashes and moisture delivers the following results: 16.4 ± 0.3, 3.8 ± 0.1 and 0.10 ± 0.01 wt%, respectively. Starch can be obtained by difference, showing a total amount of 15.3 wt%. Note that although starch can be hardly adsorbed at O/W interface, polysaccharides may influence the emulsion stability by increasing the emulsion viscosity or by leading to some destabilization phenomena such as depletion flocculation (McClements, 2015). This high protein content together to low ashes suggests that the protein concentrate used (CP) may be suitable for the development of food products.

Figure 1 shows results from the SDS-PAGE electrophoresis at three different pH values (2.5, 5.0 and 7.5 (a)), as well as the protein solubility and the Z-potential of the CP within pH values from 2 to 12 (b).

As may be observed in Figure 1(a), a wide variety of polypeptide subunits is observed, ranging from 9 to 120 kDa. Thus, the bands between 11 and 70 kDa have been previously associated to globular vicilin, whereas the 92 kDa band has been related to lipoxygenase (Taherian et al., 2011). Moreover, the band intensity, which is related to the protein concentration, depends on pH value. In this sense, the bands around 50 and 120 kDa, whose intensity is higher at pH 7.5, have been previously associated to lipoxygenase, covicilin and vicilin (Shand et al., 2007). This result is probably related to the fact that the protein solubility at this pH value is higher (Figure 1(b)). The presence of high molecular weight subunits has been previously related to the stability of emulsions (Dickinson, 2001). The results obtained may suggest that emulsions at pH 5.0 may have some destabilization phenomena, due to the absence of these subunits. On the other hand, lower molecular weight bands (i.e. 30–35 kDa) have been previously associated to globular proteins such as albumin (Sánchez-Vioque et al., 1999).

Figure 1(b) shows that the minimum of solubility is obtained at pH ca. 4.0 and it agrees with the null value found for the Z-potential at this pH value, which indicates this pH value (4.0) corresponds to the IEP for the CP (Petursson et al., 2004). These results agree with previous determination of Z-potential for CP proteins (Boye et al., 2010). Additionally, another minimum of solubility can be observed at ca. pH 7.0, which matches with a plateau zone in the Z-potential value. On the other hand, far from the main IEP (pH 4.0) the solubility of the protein system increases significantly. It is worth noting that the protein solubility and surface charges are important for the stability of emulsions, since proteins tend to stabilize emulsions through the combination of electrostatic and steric repulsions, which depends on pH value and ionic strength (Petursson et al., 2004). Further emulsion stability will be discussed on the basis of electrostatic interactions.

Emulsions characterization

BS measurements. Figure 2 shows BS profiles over 28 days ageing time for the emulsion at pH 2.5

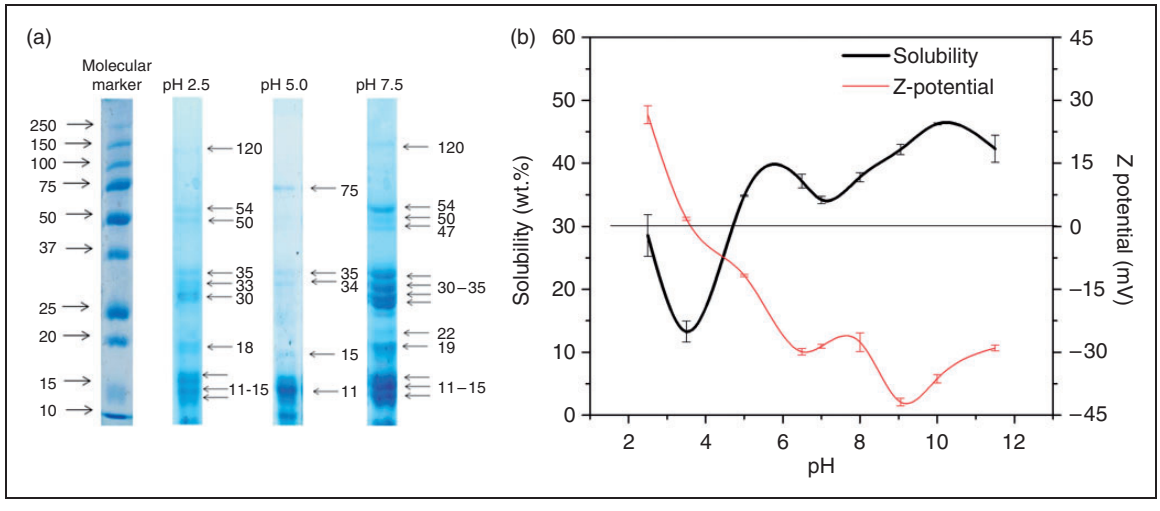

Figure 1. SDS-PAGE gels at three different pH values (2.5, 5.0 and 7.5) (a) and solubility (wt%) as well as Z-potential (mV) from pH 2.0 to 12 (b) for CP water-soluble fractions.

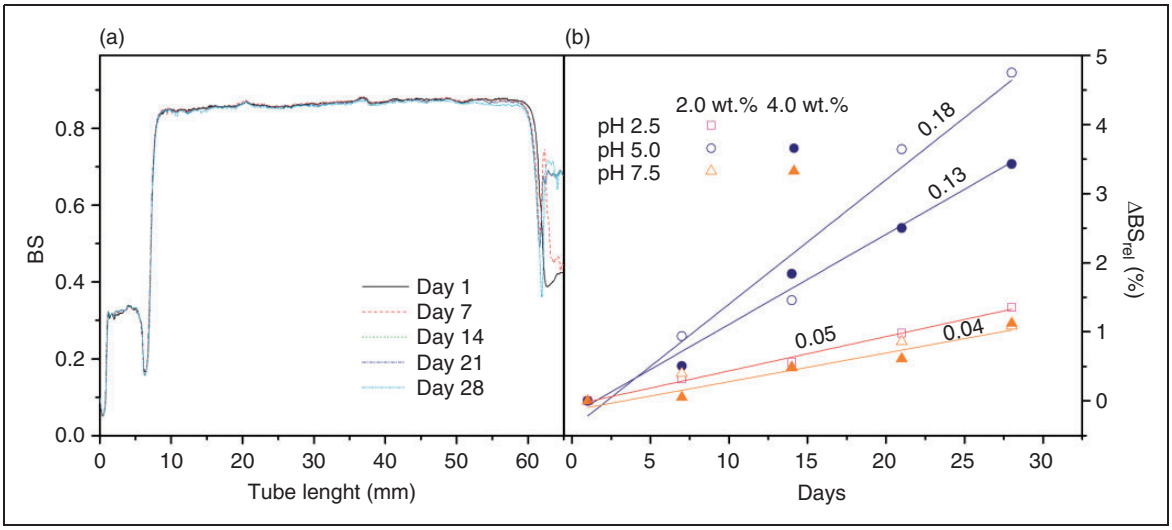

Figure 2. (a) BS profile of CP-based emulsion at pH 2.5 and (b) relative backscattering (ΔBS_{rel} (%)).

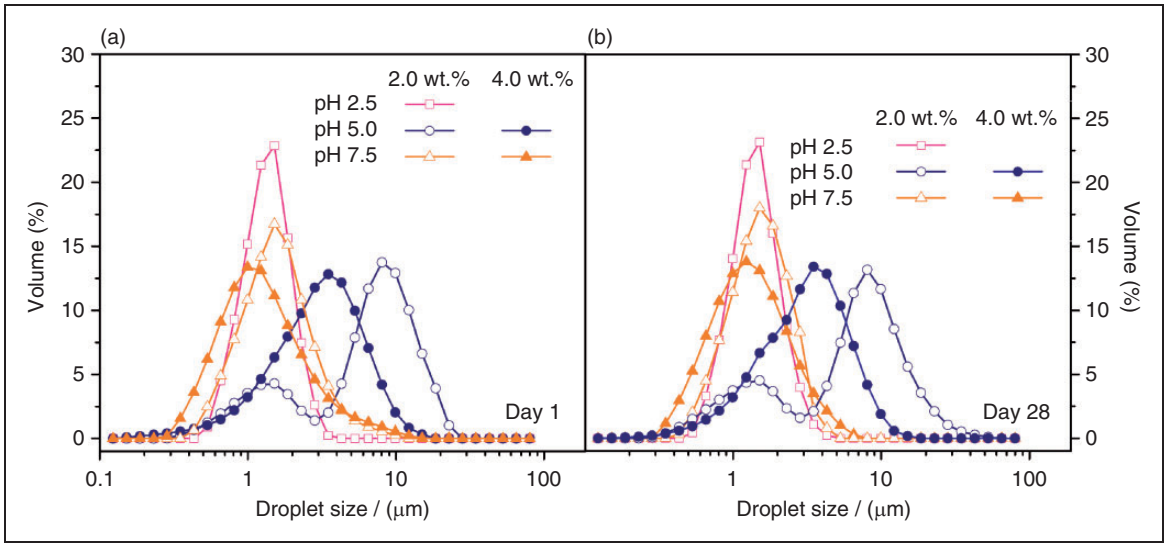

Figure 3. DSD profiles for emulsions the day after emulsion preparation (a) and after 28 days (b) at three pH values (2.5, 5.0 and 7.5) and two protein concentrations (2.0 and/or 4.0 wt%).

(a) and ΔBS_{rel}(%) calculated according to equation (1) for all the emulsions over 28 days storage time (b). Since this technique has provided relevant information related to small changes in emulsion, BS measurements are carried out in order to evaluate the stability of the emulsions over storage time (Mengual et al., 1999).

First of all, it is worth mentioning that all emulsions seem to show a fairly stable BS profile as Figure 2(a) evidences. However, some evolution is possible to be evaluated by ΔBS_{rel} (%) (Figure 2(b)). In fact, according to Figure 2(b), ΔBS_{rel}(%) values depend on the pH value and the protein content. In order to evaluate the dependence of ΔBS_{rel}(%) on storage time, results were fitted to a linear expression whose slope is indicated in the graph. According to these slope values, the greatest changes in emulsions over storage time take place for emulsions at pH 5.0, being more remarkable for the emulsion at lower protein concentration (2.0 wt%). These changes in ΔBS_{rel}(%) are related to a soft decrease of the BS baseline which has been previously related to the occurrence of flocculation and/or coalescence phenomena (Lemarchand et al., 2003; Palazolo et al., 2005). Further measurements (DSD and rheological measurements) will confirm the occurrence of these destabilization processes.

DSD measurements. Figure 3 shows the DSD distributions for emulsions the day after emulsion

preparation (a) and after 28 days (b). Once again, both the pH value and the protein concentration have a strong influence on emulsion microstructure. Thus, the larger sizes are obtained for the emulsion at pH 5.0 and 2.0 wt% CP. Interestingly, this emulsion is also the only one which shows a bimodal DSD distribution, exhibiting the shorter peak at ca. 1.5 μm and the highest one at ca. 9 μm. Thus, according to the electrophoresis measurements (Figure 1(a)), at pH 5.0 only small molecular weight proteins are present. As a result, there is not enough protein available in the continuous phase to stabilize small droplets. The surface area decreases when the droplet size is higher and, consequently droplet sizes shift towards higher sizes (ca. 10 μm) (McClements, 2004b). However, DSD distributions for emulsions at pH 2.5 and 7.5 show a peak around 1 μm, although emulsions at pH 7.5 show a more polydisperse DSD with a wider peak. Once again, emulsion with lower CP concentration leads to emulsions with higher droplet sizes.

Figure 3(b) shows DSD for CP-based emulsions after 28 days. As may be observed, the shape of the DSD is practically the same as the one obtained the day after emulsion preparation for all the systems studied. This apparent absence of changes is important since it may indicate that emulsions do not suffer any significant change in their droplet distribution, which may indicate that the macroscopic properties have not changed (Bengoechea et al., 2009), and, consequently, CP-based emulsions are stable in the experimental time range (28 days).

Table 1 shows parameters ($D[4,3]_0$, $D[4,3]_{28}$ and $CI_{28}(\%)$) obtained from DSD profiles for emulsions as a function of pH values (2.5, 5.0 and 7.5) and protein concentration (2 and 4 wt%). As may be observed, the smallest initial $D[4,3]$ ($D[4,3]_0$) corresponds to emulsions carried out at pH 2.5 and 7.5. Moreover, in order to evaluate the emulsion stability, $D[4,3]$ after 28 days ($D[4,3]_{28}$) and $CI_{28}(\%)$ parameters were calculated. Results put forward that emulsions with higher protein concentration (4.0 wt% CP) exhibit the highest stability since no significant differences in $D[4,3]$ and lowest $CI_{28}(\%)$ (always lower than 4.0%) can be found. In any case, $CI_{28}(\%)$ range between 3.4 and 7.8, which evidence that DSD analysis is not highly affected by emulsion destabilization.

Linear viscoelasticity. Figure 4 shows the frequency sweep tests carried out within the linear viscoelastic range (LVR) for selected emulsions at different CP concentrations and pH values: 2.0 wt% CP for pH 2.5 and 4.0 wt% for pH 5.0 and 7.5.

This figure indicates that the viscoelastic responses of CP-based emulsions generally can be related to a gel-like structure since G′ is always above G″ within the overall frequency range. In addition, there is not a frequency dependence of viscoelastic moduli (G′ and G″), regardless of the pH, CP concentration or storage time. This behaviour has been previously related to the plateau region of the mechanical spectrum (Ferry, 1980), and it has been previously found for a wide variety of polydisperse systems, being related to the development of an entanglement region (Bengoechea et al., 2006; Quintana et al., 2002). These mechanical spectra are typical for extensively flocculated emulsions that are able to develop a fairly strong elastic gel network of small droplets. This behaviour typically confers high emulsion stability, depending on the values of the elastic modulus. Figure 4(a) also indicates that the emulsion below the IEP (pH 2.5) seems to have higher viscoelastic properties, exhibiting the viscoelastic moduli around one order of magnitude higher than the ones at pH 5.0 and 7.5. As for the influence of storage time on emulsions, Figure 4(b) shows that all systems suffer a decrease in both moduli. However, the decrease suffered by the system at pH 5.0 is the most remarkable, which evidences that the emulsion at pH 5.0 presents the weakest structure and, consequently, it undergoes the highest destabilization.

In order to compare all systems studied over the ageing time, Table 1 shows the elastic moduli (G′) at

Table 1. DSD and rheological parameters ($D[4,3]_0$,$D[4,3]_{28}$, $CI_{28}(\%)$, $(G'_1)_1$, $(G'_1)_{28}$ and $G'_1(\%)$) for emulsions at three pH values (2.5, 5.0 and 7.5) and two protein concentrations (2.0 and/or 4.0 wt%)

pH	[C] wt%	$D[4,3]_0$ (μm)	$D[4,3]_{28}$(μm)	CI_{28} (%)	$(G'_1)_1$ (Pa)	$(G'_1)_{28}$ (Pa)	$\Delta G'_1$ (%) (Pa)
2.5	2.5	1.29a ± 0.01	1.35b ± 0.01	4.4 ± 0.1	2195A ± 7	1570B ± 42	28.5 ± 0.5
5.0	2.5	6.40a ± 0.02	6.94b ± 0.01	7.8 ± 0.1	82A ± 3	30B ± 5	63.4 ± 0.1
	4.0	3.29a ± 0.12	3.42a ± 0.08	3.9 ± 0.2	251A ± 12	118B ± 2	53.0 ± 0.1
7.5	2.5	1.66a ± 0.01	1.75b ± 0.01	5.4 ± 0.1	34A ± 2	20B ± 3	41.2 ± 0.1
	4.0	1.94a ± 0.03	2.01a ± 0.05	3.4 ± 0.2	575A ± 35	360B ± 28	37.4 ± 0.7

DSD: droplet size distribution.
Different letters indicate statistically significant differences between systems over storage time ($p < 0.05$).

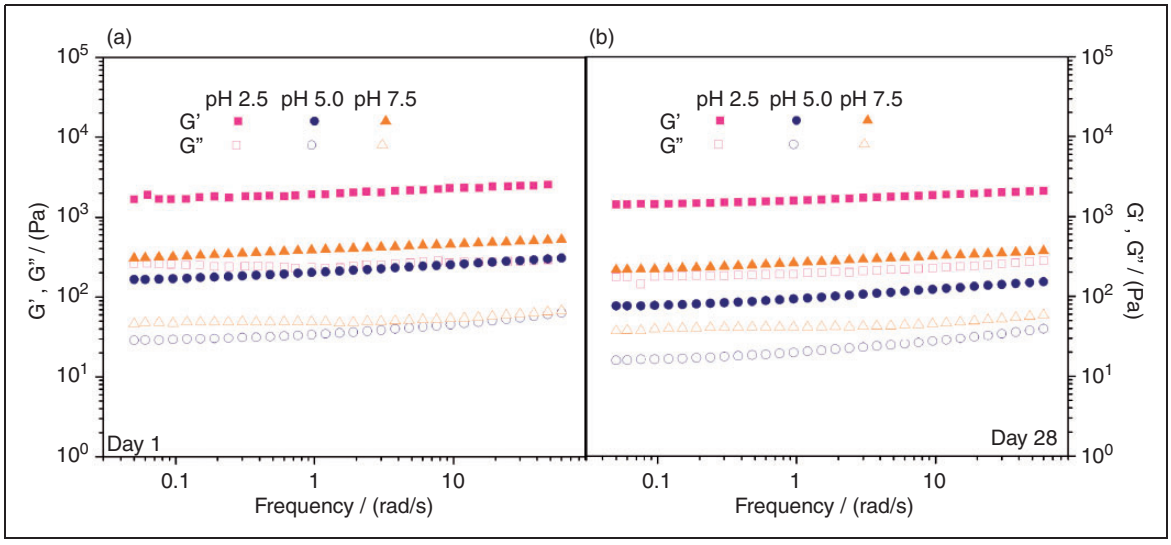

Figure 4. Frequency sweep tests for emulsions the day after emulsion preparation (a) and after 28 days (b) at three pH values (2.5, 5.0 and 7.5) and two protein concentrations (2.0 and/or 4.0 wt%).

1 Hz (G′1) the day after emulsion preparation and after 28 days ((G′1)1 and (G′1)28, respectively) as well as Δ(G′1)28(%) (equation (4)). As can be observed, G′1 values are significantly higher for the emulsion at pH 2.5 in comparison with other emulsions studied (pH 5.0 and 7.5). Additionally, as it can be observed in Table 1, G′1 significantly decreases after 28 days regardless of the system, being more evident at pH 5.0, probably as a consequence of microstructural changes (McClements, 2015). The higher stability of emulsions at pH 2.5 and 7.5 can be related to higher surface charges (Figure 1(b)), which avoid the coalescence phenomena and may contribute to hold the structure of the system (Chang et al., 2015). In any case, these changes after 28 days are related to the emulsion stability assessment, which is much more evident by rheological characterization than by the other techniques used (BS and DSD).

CLSM. Figure 5 shows the visual appearance of emulsions as well as confocal images the day after emulsion preparation. Both pictures are shown as a function of pH value (2.5, 5.0 and 7.5), whereas the CP concentration was 2.0 wt% CP for pH 2.5 and 4.0 wt% for pH 5.0 and 7.5.

The analysis of the macroscopic images of emulsions indicates that the pH value involves noticeable changes in the visual appearance, which are in agreement with rheological properties (Figure 4). Thus, the emulsion at pH 2.5 seems to have the highest viscosity (Figure 5(a)), which corresponds to the highest viscoelastic moduli found in the rheological characterization. On the other hand, confocal images evidence the formation of an O/W emulsion, where the continuous and dispersed phases correspond to water protein dispersion and oil, respectively. Moreover, confocal images indicate that the pH value involves huge changes in droplet sizes. Thus, the biggest sizes are obtained for

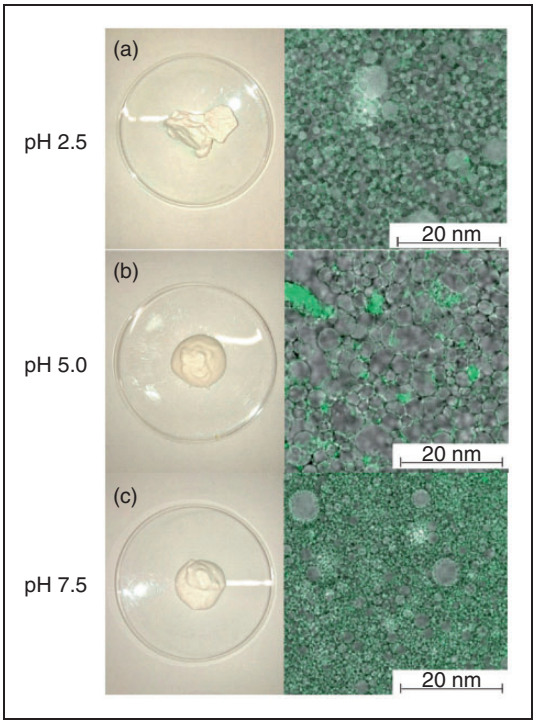

Figure 5. Visual appearance and confocal images of emulsions the day after emulsion preparation as a function of pH value (2.5, 5.0 and 7.5). The CP concentration was 2.0 wt% for pH 2.5 (a) or 4.0 wt% for pH 5.0 and 7.5 (b) and (c), respectively.

emulsions at pH 5.0, while the smallest sizes and the highest uniformity for the systems at pH 2.5 and 7.5. These results are also in accordance with those obtained from DSD measurements. Moreover, the lower solubility of the CP protein system may be also observed at pH 5.0, where inhomogeneous structures or aggregates can be observed with higher green intensity. Consequently, a lower emulsion stability can be found at this pH value.

CONCLUDING REMARKS

A CP protein concentrate is obtained by alkaline solubilization followed by isoelectric precipitation. The results obtained in this work demonstrate that this CP is suitable for the development of highly stable O/W emulsions. Electrophoresis reflects the presence of protein fractions typically found in legumes, with higher molecular weight units at pH 2.5 and 7.5. Moreover, Z-potential also indicates that surface charges are higher at pH 2.5 and 7.5, which is related to the distance to the IEP.

In any case, the stability and microstructure of these emulsions strongly depend on the pH value and the protein concentration. Overall, it can be concluded that CP-based emulsion obtained at pH 2.5 and 2.0 wt% CP corresponds to be the most stable. This system exhibits a proper visual appearance with highest viscoelastic properties and, practically, no apparent destabilization by BS or DSD measurements are observed. By contrast, the emulsions at pH 5.0 show the poorest microstructural characteristics, especially at 2.0 wt% CP, probably due to their closeness to the IEP. At this pH value, oil droplets after emulsification process are the biggest ones, as well as, comparing with pH 2.5 and 7.5, the droplet coalescence found at pH 5.0 is greater. Thus, results from DSD and BS measurements by different parameters and calculations are required to elucidate the stability of these emulsions, and they are not able to determine clearly changes in emulsion microstructure. However, a simple observation of viscoelastic moduli along time concludes that all emulsions suffer significant changes on their structure after 28 days storage time. According to these results, the usefulness of rheological measurements in order to assess the emulsion stability has been proved, anticipating much better structural changes in emulsions.

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DECLARATION OF CONFLICTING INTERESTS

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ORCID ID

Manuel Felix http://orcid.org/0000-0002-3608-7035

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