

# Relationship of rheological and microstructural properties with physical stability of potato protein-based emulsions stabilized by guar gum



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## ABSTRACT

Despite potato protein is a great potential ingredient in food products due to its nutritional quality, it is not useful for the formation of sufficiently stable emulsions. For this reason, a widely used polysaccharide in the food industry, Guar gum, was added. The addition of guar gum to potato protein-based emulsions results in enhanced stability as demonstrated by the cooperative information provided by the combination of different techniques (rheology, optical microscopy and multiple light scattering). We established a relationship between a critical time for the onset of creaming and two rheological functions, namely the zero shear viscosity and the storage modulus. In addition, we propose a fast rheological method consisting of non-linear creep tests to detect shear-induced microstructural transitions.

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## 1. Introduction

Emulsions are complex systems comprised of an oil-dispersed phase and an aqueous continuous phase that may contain a variety of ingredients (sugars, salts, acids, surfactants, polysaccharides, etc.) (McClements, 2005). Emulsions tend to destabilize due to some physicochemical mechanisms such as creaming, flocculation, coalescence, phase inversion or Ostwald ripening. The shelf-life of these dispersed systems is principally determined by their physical stability (McClements, 2005; Walstra, 1996). The interfacial region between the two phases may contain a mixture of various surface-active components including proteins, surfactants and phospholipids (Dickinson, 2003). Proteins reduce interfacial tension and form viscoelastic films; therefore it can be effective at producing and stabilizing dispersed systems such as emulsions and foams (Prins, Bos, Boerboom, & van Kalsbeek, 1998). Polysaccharides increase the long-term stability against creaming.

Potato protein is a potential ingredient in food products because it possesses a nutritional quality higher than most major plant proteins and close to that of egg proteins (Van Gelder & Vonk, 1980). About 1–2% of the human population has food-related allergies, with egg, gluten, soy, fish and nuts being among the most

common. These are all important food protein sources, and many are being used as emulsifiers, gelling and foaming agents in food systems. Allergy towards potato protein is much less common (Castells, Pascual, Esteban, & Ojeda, 1986), and potato protein concentrates can therefore be an interesting replacement for the aforementioned proteins as food hydrocolloids. Furthermore, there is an increasing interest in the application of vegetable proteins in food products because they help to prevent some gastrointestinal diseases, some types of cancer, and cardiovascular diseases (Serra & Aranceta, 2005). In this sense, potato may be regarded as a potential source to produce plant protein-based food products with high valorisation standard (Calero, Muñoz, Cox, Heuer, & Guerrero, 2013), whose ability in the stabilization of O/W interfacial layers has been already put forward (Romero et al., 2011).

Polysaccharides are used to increase the viscosity of the continuous aqueous phase of the emulsion and to control their rheological properties. For this reason, polysaccharides are widely used in the food industry in many sauces. However, the majority of these polysaccharides show little surface activity. (Erçelebi & Ibanoglu, 2009; Neiryneck, Dewettinck, & Van Der Meeren, 2007; Tuinier, Ten Grotenhuis, & DeKruif, 2000).

Today, xanthan gum, guar gum, modified starch as well as carboxymethyl cellulose are the most common polysaccharides being used in concentrated emulsion products such as mayonnaises and salad dressings. Such polysaccharides impart important product characteristics including creaming mouthfeel, thickness and, to

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certain extent, long-term stability (Erçelebi & Ibanoglu, 2009; Nor Hayati, Che Man, Tan, & Nor Aini, 2009).

Proteins tend to be better than polysaccharides at producing small emulsion droplets, whereas polysaccharides tend to be better than proteins at producing emulsions that are stable to a wider range of conditions (McClements, 2005). The use of polysaccharides to enhance the stability of emulsions that contain proteins has been previously reported in many studies (Dickinson, 2011; Murray, 2011; Schmitt & Turgeon, 2011).

In addition, there is an increase of social awareness about health problems due to obesity. For this reason, an increase in consumption of low-fat food is occurring nowadays (Vázquez, De Cos, & López, 2005). High-oleic sunflower oil is very important for the diet because it contains a high percentage of mono-unsaturated fatty acids (MUFA) like oleic acid (at least 82%). These acids, in contrast with the saturated acid, have health benefits and positively influence many digestive functions (Serra & Aranceta, 2005).

This study focuses on emulsions formulated with a) high-oleic sunflower oil, with dietary benefits supported above, b) vegetable protein (potato protein isolate which may provide many health benefits) and c) Guar gum which is a well-known stabilizer and thickener in the food industry. To be more precise, the specific goal of this project was to study the effect of guar gum concentration on the stability, microstructural and rheological properties of O/W potato protein-stabilised emulsions.

## 2. Materials and methods

### 2.1. Materials

Potato Protein Isolate (PPI) was supplied by Protastar (Reus, Barcelona, Spain). The chemical composition of potato protein isolate was determined in a previous study (Romero et al., 2011):  $80 \pm 2\%$  wt% protein;  $3.1 \pm 0.4\%$  lipids;  $5.9 \pm 0.6\%$  wt% carbohydrates;  $0.8 \pm 0.1\%$  wt% ashes;  $10 \pm 2\%$  wt% moisture. Guar Gum (GG) was purchased from Sigma Chemical Company.

### 2.2. Preparation of oil-in-water (O/W) emulsions

Oil-in-water emulsions were prepared following the procedure described by Calero et al. (2013). Potato protein stock dispersion was prepared by dispersing 5 wt% potato protein isolate powder in water. Then it was adjusted to pH 11.5 with 1 M NaOH in order to improve protein solubility.

Guar Gum stock solution was prepared by dissolving 2.5 wt% Guar powder in water containing 0.2 wt%  $\text{NaN}_3$ . GG solution was stirred for at least 3 h at 700 rpm to ensure complete dissolution. The stock solution was diluted to prepare the rest of guar gum solutions (0.1 wt%, 0.2 wt%, 0.3 wt% and 0.5 wt%). The system was left to stand for 48 h at 7 °C for complete hydration of the polymer and the removal of bubbles.

Concentrated high-oleic sunflower oil-in-water emulsions (50 wt% oil, 5 wt% PPI as emulsifier) was prepared by gradually blending 50 wt% high-oleic sunflower oil with 50 wt% aqueous potato protein dispersion (pH 11.5) using a high-shear mixer (Silverson L 5 M). The mixture was stirred for 3 min at 8600 rpm (pre-emulsion 1). The mixture was passed through a high-pressure valve homogenizer once at 105 KPa.

Subsequently diluted emulsions were prepared by diluting concentrated emulsions with guar gum solutions. The concentrated emulsion and guar gum solutions were mixed manually using a spatula (final emulsion).

Final emulsion contained 40 wt% high-oleic sunflower oil, 40 wt% aqueous potato protein dispersion (2 wt% potato protein) and

20 wt% GG solution (0 wt%, 0.1 wt%, 0.2 wt%, 0.3 wt%, 0.5 wt%). The pH of final emulsions is 8.

### 2.3. Rheological characterization

The Thermo Haake MARS rheometer was used for all rheological measurements. Oscillatory, step-wise flow curves and stress jumps were carried out for final emulsions with different concentrations of guar gum at 20 °C, using a plate-and-plate geometry with a rough surface (60 mm diameter).

Stress sweeps at a frequency of 0.62, 6.20 and 12.52 rad/s were performed for all systems studied to estimate the dynamic linear viscoelastic range. Frequency sweep tests (from 0.1 to 100 rad/s) were performed selecting a stress well within the linear range. Shear flow tests were carried out from 0.05 to 100 Pa. Creep experiments were performed for constant shear stresses for all emulsions in the range of 10–60 Pa.

### 2.4. Microstructural observation

The microstructure of emulsions was observed at room temperature using an optical microscope Axio Scope A1 (Carl Zeiss) equipped with an AxioCam camera. Microphotographs were taken of all emulsions before and after stress jump tests with a 63× objective. To improve the view of the flocs, all samples were diluted to 1:20 in distilled water.

### 2.5. Physical stability

Backscattered light measurements with Turbiscan Expert Lab-Measuring were used in order to study the destabilization of the emulsions. Measurements were carried out for 15 days to study the influence of the concentration of GG on the stability of the emulsions and to determine the predominant mechanism of destabilization in each case as well as the kinetics of the destabilization process.

## 3. Results and discussion

### 3.1. Oscillatory measurements

Dynamic frequency sweep tests were performed in the linear viscoelastic range (LVR) to determine the frequency dependence of

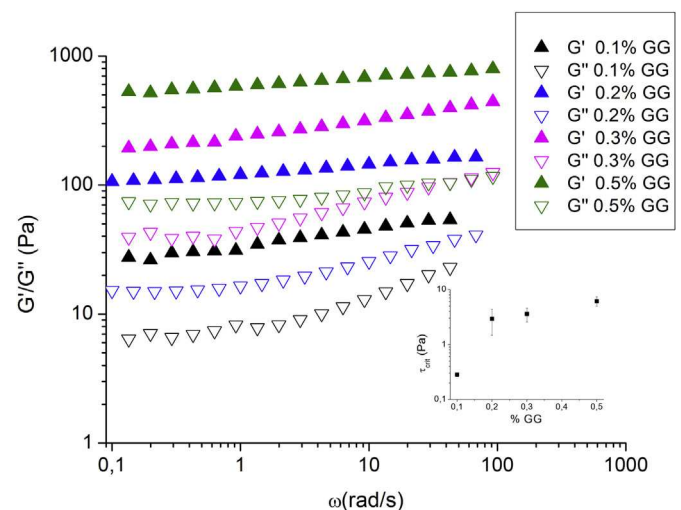


Fig. 1. Influence of Guar gum concentration on the mechanical spectra for the studied emulsions. Inset: evolution of the critical shear over gum concentration.

the storage modulus ( $G'$ ) and loss modulus ( $G''$ ). Fig. 1 shows the mechanical spectra of emulsions as a function of Guar Gum concentration, as well as the evolution of the critical shear over gum content. The response of the emulsion without guar gum is not shown because it was probably outside of the LVR at the lowest stress allowed by the rheometer. In other words, its structure was broken down by applying a shear stress as low as 0.05 Pa. In fact, a dramatic reduction in the critical stress may be noticed when the GG concentration decreased below 0.2%, which also corresponded to a decrease in the critical strain (i.e. the critical strain took a value of 0.01, being constant between 0.2 and 0.5% GG, and 0.007 at 0.1% GG).

Emulsions containing 0.1 wt%, 0.2 wt%, and 0.3 wt% GG showed a weak gel behaviour where  $G' > G''$  in the studied frequency range.  $G'$  is only slightly dependent on  $\omega$  while  $G''$  exhibited a bit more marked dependency on  $\omega$ . A region characterized by a relatively low slope of  $G'$  versus frequency and by a trend to reach constant values of  $G''$  is observed in all aforementioned emulsions. This corresponded to the plateau zone of the mechanical spectrum. This window of the mechanical spectrum is related in polymer rheology to the occurrence of macromolecular entanglements (Ferry, 1980) and has also been found in concentrated emulsions (Calero et al., 2013), concentrated solutions of polymers (Nandan, Kandpal, & Mathur, 2004), micellar solutions (Calero, Alfaro, Lluch, Berjano, & Muñoz, 2010), and in a protein-polysaccharide system in aqueous dispersions (Aguilar et al., 2011). It is worth noting that the emulsion containing 0.5% GG exhibited  $G'$  and  $G''$  almost independent of frequency; this is attributed to a formation of a biopolymer gel network (Clark & Ross-Murphy, 1987).

Furthermore, a gradual change of the relative values of  $G'$  and  $G''$  was observed as the guar gum concentration was increased. The addition of guar gum caused the formation of a much stronger (more elastic) structure. This structure formed in the presence of polysaccharides can be attributed either to the formation of a highly flocculated droplet network or to the formation of a gel-like structure in the continuous phase (Kontogiorgos, Biliaderis, Kiosseoglou, & Doxastakis, 2004). The viscoelastic properties at 1 rad/s,  $G'_1$  and  $G''_1$  were fitted to a potential function, as follows:

$$V = A \cdot C_{GG}^b \tag{1}$$

Parameters  $A$  and  $b$  are included in Table 1. As may be seen in this table all the data fit the power-law equation fairly well since  $R^2$  is always higher than 0.995.

A stronger dependence of  $G'$  and  $G''$  with guar gum concentration was observed, which leads to a decrease in  $\tan \delta_1$ . This is a consequence of the formation of a structure possessing a stronger elastic component.

### 3.2. Flow properties

Fig. 2 shows the flow curves of emulsions at different concentrations of guar gum at 1 day after emulsion preparation. Flow curves exhibited a shear thinning behaviour and a trend to reach a Newtonian region at low shear rate enabling the estimation of a zero-shear viscosity ( $\eta_0$ ).

**Table 1**  
Viscoelastic properties fitting parameters for the potential model.

Fitting parameters	$G'$	$G''$
$A$	2014 ± 47	200 ± 11
$b$	1.76 ± 0.02	1.4 ± 0.01
$R^2$	0.999	0.997

The shear rate dependence of viscosity fitted fairly well the Carreau-Yasuda model ( $R^2 > 0.99$ ).

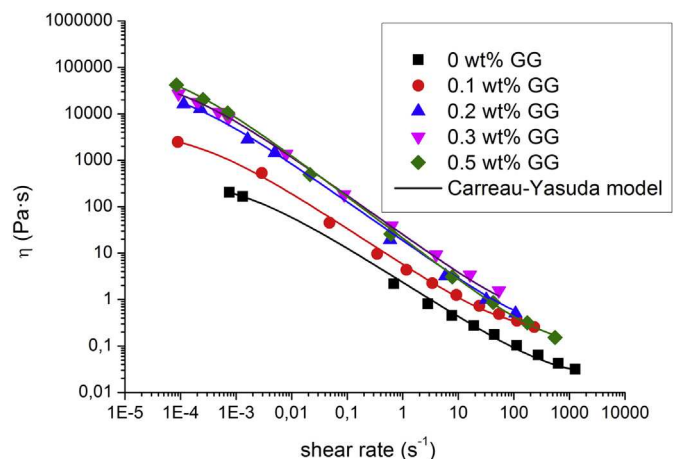
$$\eta = \eta_\infty + \frac{(\eta_0 - \eta_\infty)}{\left[1 + \left(\frac{\dot{\gamma}}{\dot{\gamma}_c}\right)^p\right]^{1-n}} \tag{2}$$

where  $\eta_0$  are the viscosities at infinite and zero shear rates, respectively.  $\dot{\gamma}_c$  is the critical shear rate for the onset of the non-Newtonian region, and  $n$  is the flow index.

The parameters obtained from this model are shown in Table 2. The zero-shear viscosity increases with the concentration of GG. This increase may be explained by the fact that polysaccharides have a thickening effect in the bulk phase. In addition, a characteristic time ( $\lambda$ ) was calculated as the inverse of the critical shear rate obtained from equation (2). In this case, an increase of the characteristic time with guar gum concentration was observed. As previously reported by Santos et al., the characteristic time may also be correlated with the instability of emulsions against creaming. Thus, emulsions exhibiting higher characteristic times resulted in better stabilities against creaming, which is a consequence of stronger droplet–droplet interactions (Santos, Trujillo, Calero, Alfaro, & Muñoz, 2013). The flow index decreases with increasing GG concentrations indicating a reinforcement of the shear-thinning character of the emulsion as a result of the increase in consistency of the continuous phase. The shear thinning character may be associated with the flocculation of droplets. This is usually the case with weakly flocculated emulsions or those to which a thickener is added (Tadros, 2004). Flocculation increases the apparent dispersed phase volume and leads to the formation of nonspherical aggregates. Both factors contribute to increase the zero shear viscosity. This behaviour has been previously reported for emulsions containing potato protein and chitosan (Calero et al., 2013).

### 3.3. Non-linear creep tests

Fig. 3 shows some selected data obtained from non-linear creep tests performed for the emulsion 0.3 wt% GG. Fig. 3a plots the creep tests in terms of shear creep compliance versus time as a function of shear stress, whereas Fig 3b shows the steady state viscosity obtained from the same tests as a function of shear stress. These tests were carried out in order to detect some possible shear-induced transitions. As may be observed in Fig. 3a, the creep flow response is always linear since the applied shear stress is much



**Fig. 2.** Flow curves as a function of Guar gum concentration in the (0–0.5) wt% range. Continuous lines illustrate data fitting to the Carreau-Yasuda model.

**Table 2**  
Flow curves fitting parameters for the Carreau-Yasuda model.

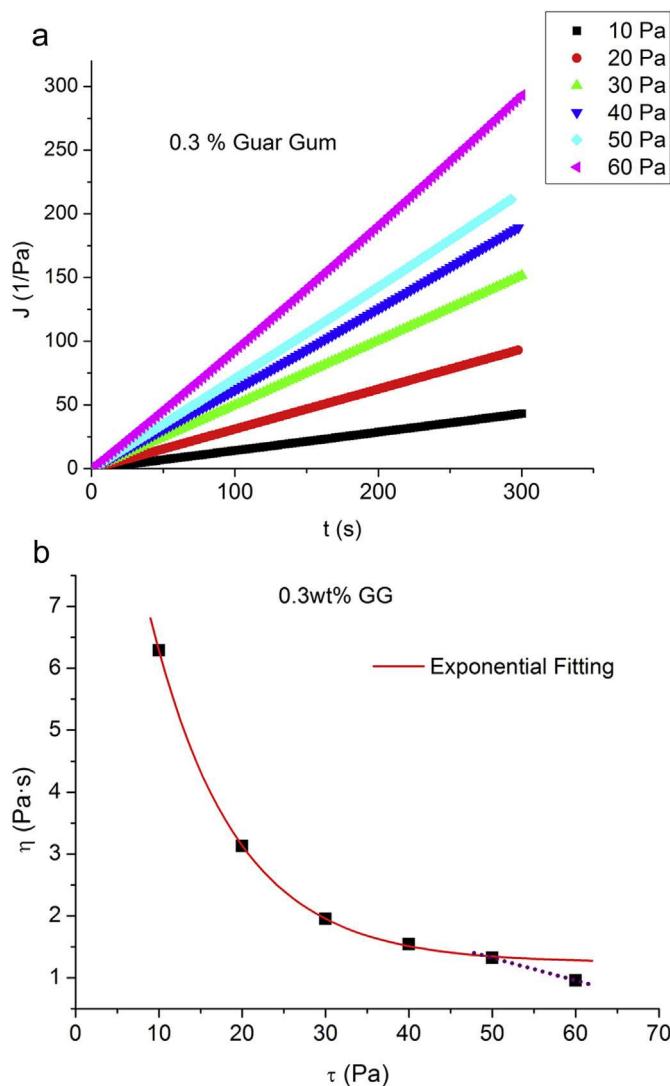
% Guar Gum	$\eta_{\infty}$ (Pa s)	$\eta_0$ (Pa s)	$\dot{\gamma}_c$ ( $s^{-1}$ )	$n$	$p$
0%	0.02	434	1.05 E-3	0.24	0.60
0.1%	0.20	4684	2.00 E-4	0.21	0.60
0.2%	0.20	49,685	8.33 E-5	0.16	0.60
0.3%	0.15	75,404	8.06 E-5	0.15	0.60
0.5%	0.10	142,725	5.49 E-5	0.10	0.60

Standard deviation of the mean (3 replicates) for  $\eta_0 < 8\%$ .

Standard deviation of the mean (3 replicates) for  $\dot{\gamma}_c < 7\%$ .

Standard deviation of the mean (3 replicates) for  $n < 8\%$ .

higher than the critical value for the onset of the non-linear viscoelastic region, as may be deduced from the inset of Fig. 1. It is interesting to note that  $dJ/dt$  increased with the shear stress and therefore the associated viscosity decreased, which supports the results obtained by flow curves. In addition, Fig. 3b shows by way of example that viscosity follows an exponential decay with shear stress from 10 Pa to 50 Pa. This could not be observed by analysing the step-wise flow curves.



**Fig. 3.** a) Creep tests in terms of shear creep compliance versus time as a function of shear stress for the emulsion containing 0.3 wt% GG. b) Steady-state viscosity obtained from creep tests versus shear stress for the 0.3 wt% GG emulsion.

Further information was provided by microphotographs taken before and after creep tests. This microscopic observation suggested that a critical stress was needed to induce coalescence in all emulsions studied. The critical stress for coalescence, obtained by comparing the different microphotographs, was plotted as a function of the concentration of GG (Fig. 4).

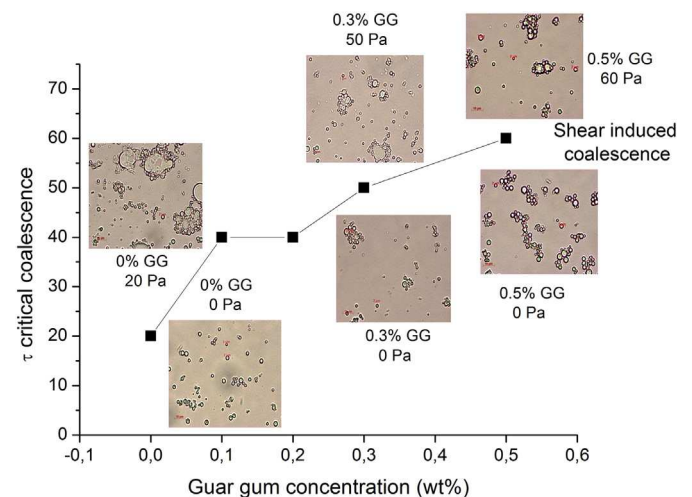
It must be pointed out that both critical values for the change in the steady state viscosity dependence on shear stress and for the onset of coalescence are consistent.

An analysis of the results obtained revealed that the critical stress depended on GG concentration, as is also the case for the change in behaviour found from the viscosity-stress plot. Thus, the critical stress for coalescence increased with GG concentration. The microphotographs inset in Fig. 4 showed the microstructure of the samples before application of any shear stress and after the creep test for a stress equal to or higher than the critical stress for coalescence. In the absence of shearing, the microphotographs confirm the occurrence of flocs for all concentrations of guar gum studied (inset micrographs). This was particularly obvious for the emulsion with 0.5% of guar gum where more numerous and larger elongated flocs may be observed, congruently with a depletion flocculation mechanism. This fact along with the increasing thickening effect of GG in the continuous phase may be responsible for both its more elastic behaviour and higher viscosity values. This may minimize the mobility of droplets and flocs under shear, which hinders the collision frequency and hence reduces shear-induced droplet coalescence.

### 3.4. Physical stability

Fig. 5 shows a plot of backscattering (BS) versus measuring cell height in the reference mode (BS–BS at the beginning of the study) for the emulsion with 0.2% GG. A backscattering decrease was observed for the lower and intermediate zones of the vial. All the emulsions exhibited the same qualitative behaviour but displayed different intensities.

The drop of backscattering detected at the bottom of the measuring cell clearly indicated the occurrence of a destabilization mechanism by creaming. This is a consequence of the dispersed phase possessing a lower density than the continuous phase (McClements, 2005). The fact that the backscattering decreased for the intermediate zone suggests that destabilization mechanisms



**Fig. 4.** Critical shear stress for coalescence as a function of concentration of GG. Inset: Photomicrographs for 0, 0.3 and 0.5 wt% GG emulsions before and after critical shear stress for coalescence.

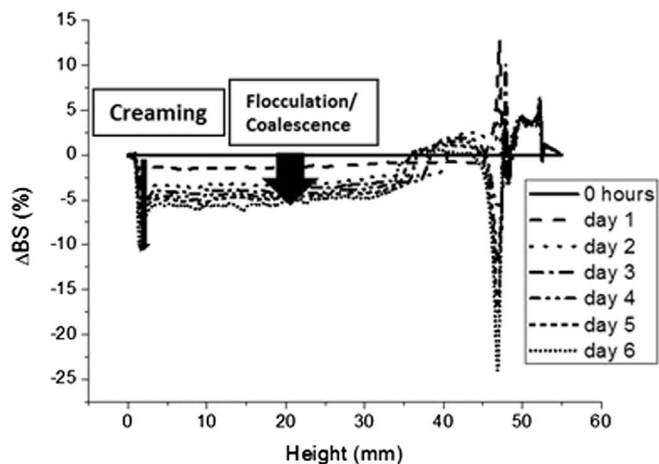


Fig. 5. Increase of the backscattering (BS) versus measuring cell height as a function of ageing time for the 0.3 wt% GG emulsion at 25 °C.

such as flocculation and coalescence were significant. Nevertheless, oiling-off was not visually observed for the higher zone of the vial whereas creaming was clearly observed by detecting a layer of aqueous phase at the bottom of the measuring cell.

Fig. 6 shows the increment of height of creaming as a function of ageing time. The emulsion without guar gum showed a creaming phenomenon immediately after its preparation where the increment of height of creaming followed a linear relationship with ageing time, while the other emulsions did not show this linear trend, exhibiting a delay time. In any case, their dependences of height of creaming on ageing time were weaker than for that in absence of guar gum. It should be noted that emulsion containing 0.3 and 0.5% GG exhibited the same trend, reaching an asymptotic value of height of creaming.

The delay time increased with the concentration of guar gum. Emulsion 0.1%GG has a short delay time. Also, although emulsions containing 0.3 and 0.5 %GG showed the same trend, they displayed different values of delay time.

For further information of creaming phenomenon, a reference height of creaming was fixed as a reference (0.4 mm). The ageing time associated with that critical height of creaming was called reference time for creaming,  $t_{ref}$ , whose values are shown in Table 3.

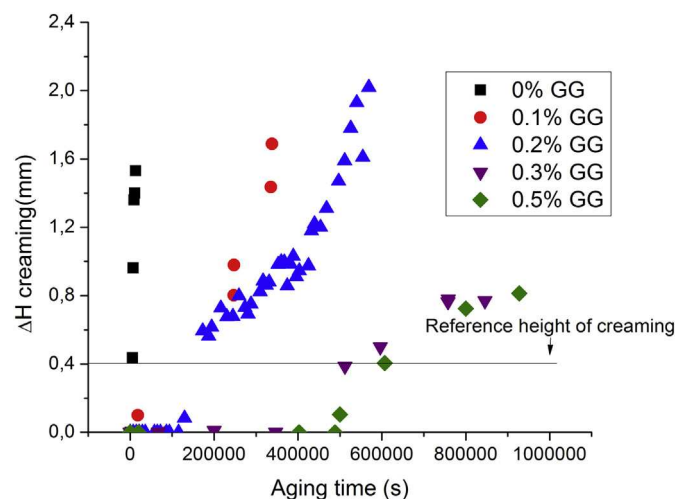


Fig. 6. Increase of the height of serum layer (bottom zone) versus ageing time for 0, 0.1, 0.2, 0.3 and 0.5 wt% GG emulsions at 25 °C.

Table 3  
Reference time for the onset of creaming as a function of Guar gum concentration.

%wt GG	$t_{ref}$ (s)
0	300
0.1	60,000
0.2	160,000
0.3	520,000
0.5	620,000

An increase of wt% GG provoked an increase of the  $t_{ref}$ . This rise was more pronounced for 0–0.3 % wt of GG range while from 0.3 to 0.5, only a slightly increase was found. The increase of the delay time for creaming in the emulsions containing guar gum was probably due to the immobilization of dispersed oil droplets in a weak gel-like network of aggregated droplets (Dickinson, 2003; Meller & Stavans, 1996). A three-dimensional droplet network was also observed by other researchers for emulsions stabilized with sodium caseinate and xanthan gum (Hemar, Tamehana, Munro, & Singh, 2001) or with whey protein isolate and xanthan gum (Aguilar et al., 2011; Sun, Gunasekaran, & Richards, 2007).

Fig. 7 shows the relationship between rheological parameters such as  $\eta_0$  and  $G'$  (1 rad/s) and the stability parameter,  $t_{ref}$ . An increase of the rheological parameters caused an increase of the critical time for creaming. The increase of zero shear viscosity with guar gum concentration was due to the increase of viscosity of the dispersed phase, which is directly related to the creaming rate by Stokes law. The droplets move hardly upward due to the occurrence of the more viscous continuous phase. Therefore as expected, the emulsions exhibiting higher zero shear viscosity had better stability against creaming. Storage moduli show a similar tendency to zero shear viscosity as shown in Fig. 7. Emulsions with higher storage moduli possess more compact structures, which are able to reduce creaming. Thus, emulsions with higher storage moduli and zero shear viscosities (0.3 wt% and 0.5 wt% GG) are those with the best critical times for creaming.

#### 4. Conclusions

O/W emulsions containing PPI exhibited poor stability (no longer than 12 h after preparation). The addition of guar gum to PPI-based emulsions has been proven to be a good strategy to

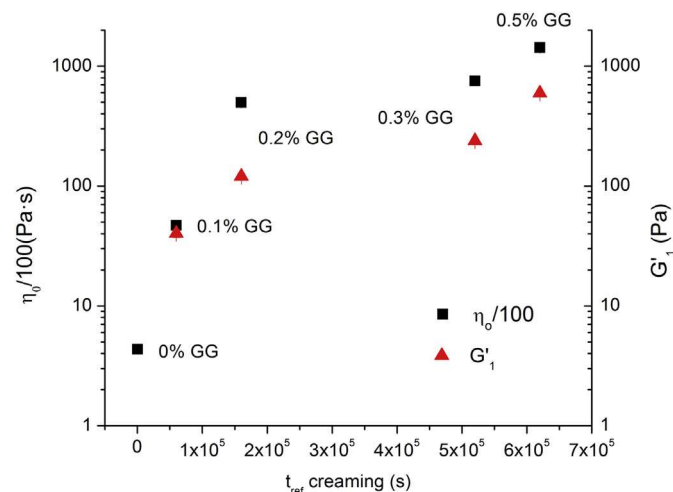


Fig. 7. Relation of zero shear viscosity, and storage moduli (1 rad/s) with reference time for creaming for 0, 0.1, 0.2, 0.3 and 0.5 wt% GG emulsions. Standard deviation of the mean (3 replicates) for  $\eta_0 < 8\%$  Standard deviation of the mean (3 replicates) for  $G'_1 < 10\%$ .

improve stability. Thus, emulsion without GG did not show viscoelastic properties at the lowest stress allowed by the rheometer used, whereas emulsions containing 0.1 wt%, 0.2 wt%, and 0.3 wt% GG showed a weak gel behaviour, which turned out to be markedly stronger for the most concentrated emulsion.

Studied emulsions exhibited shear thinning behaviour and a trend to reach a Newtonian region at lower shear rate at 1 day after preparation. The Carreau-Yasuda model fitted fairly well the flow behaviour in the whole shear rate range. The critical shear rate derived from aforementioned model allowed a characteristic time ( $\lambda$ ) to be calculated, which correlated with the stability against creaming.

Non-linear creep tests were carried out in order to detect some possible shear-induced transitions. Thus, a change of tendency in viscosity with shear stress allowed a critical stress for the onset of shear-induced coalescence to be calculated as a function of GG concentration. In addition, the critical stress was also checked by optical microscopy. Interestingly both critical values were consistent to each other. As a consequence, we propose a fast non-linear creep test as a novel powerful tool to get relevant information on shear-induced transitions in emulsions.

The results derived from multiple light scattering revealed that backscattering decreased for the lower, which indicates the occurrence of a clear creaming destabilisation process. In addition, the drop of backscattering at the intermediate zone of the measuring cell and the lack of oiling-off supports the existence of a parallel flocculation mechanism. The addition of guar gum to these emulsions results in significant enhanced physical stability as indicated by the increase in the delay time for creaming with GG concentration. The stabilising role of GG has been illustrated by the correlation between a stability parameter,  $t_{ref}$ , and  $\eta_0$  and  $G'$  values could be demonstrated. Thus, the reference time for creaming increases with the values of the zero shear viscosity and storage modulus regardless the frequency considered.

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