Effect of medium molecular weight xanthan gum in rheology and stability of oil-in-water emulsion stabilized with legume proteins

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Abstract: Xanthan gum is a water-soluble extracellular polysaccharide that has gained widespread commercial use because of its strong pseudoplasticity and tolerance to high ionic strength, which bring unique rheological properties to solutions. This study compares and evaluates the emulsifying properties of oil-in-water (30:70 v/v) emulsions stabilized with lupin and soya protein isolates and medium molecular weight xanthan gum. The protein was obtained by an isoelectric precipitation method and the polysaccharide was produced by Xanthomonas campestris ATCC 1395 in batch culture in a laboratory fermenter (LBG medium) without pH control. The addition of xanthan gum in the emulsion formulation enhances emulsion stability through the phenomenon of thermodynamic incompatibility with the legume protein, resulting in an increase of the adsorbed protein at the interface. The emulsion stability is also enhanced by a network structure built by the polysaccharide in the bulk phase.

Keywords: xanthan gum; stabilizers; lupin; soya; protein isolates; emulsifiers

INTRODUCTION
An important functional property of food biopolymers is the stabilization of food colloids. The two main types of food biopolymers found in oil-in-water emulsions are proteins and polysaccharides and some food emulsions are products containing both types of these macromolecules.1

Polysaccharides are good stabilizing agents because of their hydrophilicity, high molecular weight and gelation behaviour which leads to the formulation of a macromolecular barrier by increasing the viscosity of the aqueous phase and slowing coalescence between dispersed droplets.2,3 Xanthan gum (XG) is a very well known biopolymer that is produced commercially by fermentation on commercial grade glucose or starch, following degradation by a combined acid and enzyme treatment. Recent reports suggested that the gum could be produced in fermentations on whey or chestnut flour.4–6 The polysaccharide finds important uses, especially in the food industry, mainly as an emulsion stabilizer and thickener and, secondarily, as a gelling agent in combination with other polysaccharides. The ability of XG to thicken and stabilize emulsion systems is attributed to a weak gel-like structure in solution, formed by the gum molecules in the emulsion continuous phase, which prevents the oil droplets creaming since the gravitational lift on the droplets is less than the yield stress of the xanthan weak gel.7 The gum is able to form only weak gel structures and therefore it cannot find use as a stabilizing agent on its own, in emulsion systems, unless it is combined with proteins. Because XG is not considered to be surface active, a depletion mechanism is often used to explain the flocculation observed.8,9 However, for food-like systems, strong evidence for depletion flocculation has not presented yet been. Some evidence for adsorption of XG at the interfaces can be found in the literature. Young and Torres10 found that the surface tension (water–air) of 0.1–1.0 wt% XG solutions decreased very slowly with time. Bergenstahl11 showed that XG, along with other polymers, was able to stabilize soya bean oil emulsions (D[3,2] = 0.1–0.8 µm) against flocculation at concentrations much lower than would be necessary to stabilize the emulsion by an increase in viscosity. The amount of XG needed, depending on the emulsifier (type of proteins), was between 10−4 and 10−2 wt%.12 A possible explanation is adsorption of the polymer on the emulsifier layer resulting in the formulation of a so-called bilayer, because XG itself is not a sufficient surface-active agent to co-adsorb on the interface.

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Proteins used are lupin seed protein isolate (LSPI) and soya bean protein isolate (SBPI), produced on a laboratory scale by an isoelectric precipitation method.\textsuperscript{13,14} The use of legume (lupin, soya) products as a source of protein for humans will depend not only upon their nutritional quality, but also on their ability to be used as, or incorporated into, foods which will readily be consumed. Besides the nutritional value of as proteins, their functional properties will also largely determine their acceptability as ingredients in prepared foods.\textsuperscript{13–16} LSPI and SBPI are good emulsifiers (emulsifying agents) because of their substantial hydrophobicity and molecular flexibility which allow rapid adsorption and rearrangement at the interface to give a coherent macromolecular protective layer and an increase in its stability by interfacial action.\textsuperscript{12} Hence, LSPI and SBPI could substitute some animal protein (meat, milk, egg), contributing to the production of new food products and also to the increase of their nutritional quality.

Since the polysaccharides are widely used in the food industry as emulsifiers and thickening agents in protein food systems, our present work aims at evaluating the medium molecular weight XG for its potential commercial applications in stabilizing oil-in-water emulsion, in admixture with legume proteins, such as lupin and soya protein isolates.

**MATERIALS AND METHODS**

**Materials**

Xanthan gum with a molecular weight of around 500 kDa and a pyruvate content of 3.5% was kindly provided by the Department of Chemical Engineering, Aristotle University of Thessaloniki, Greece and was prepared by fermentation on Luria-Bertani (LB) broth, which contained an additional 0.2% glucose (LBG medium) by *Xanthomonas campestris* (LB) broth, which contained an additional 0.2% glucose (LBG medium) by *Xanthomonas campestris* ATCC 1395 according to a method described elsewhere.\textsuperscript{5} Commercial XG, with a molecular weight of around 1000 kDa, was provided by Sigma (St Louis Missouri, USA).

LSPI and SBPI used as emulsifiers for the preparation of oil-in-water emulsions were obtained from lupin seeds (*Lupinus albus* ssp *Gracilus*) and soya beans (*Glycine max*) prepared by an isoelectric precipitation method described elsewhere.\textsuperscript{13,14} The compositions of LSPI and SBPI are presented at Table 1.

**Table 1. Composition (% w/w) of legume protein isolates prepared by isoelectric precipitation**

<table>
<thead>
<tr>
<th>Protein (N × 6.25)</th>
<th>82.1</th>
<th>83.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>6.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Ash</td>
<td>3.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Moisture</td>
<td>5.0</td>
<td>4.9</td>
</tr>
</tbody>
</table>

**Emulsion preparation**

The oil-in-water emulsions (o/w 30/70 v/v) were prepared by adding corn oil into LSPI and SBPI solution which contained legume protein (1% w/v), with or without polysaccharide, and had a pH value 5.5 or 7.0 while mixing with the aid of a mechanical stirrer. In all emulsions series 0.0, 0.1, 0.25 and 0.5 M NaCl was added. The crude emulsion, after mixing for 3 min, was then homogenized with an Ultra-Turrax T-25 homogenizer (IKA Instruments, Germany) equipped with a S25 KG-25P dispersing tool, at a speed of 9500 rev min\(^{-1}\) for 1.5 min. Emulsification conditions were chosen to result in oil droplets with a diameter >1 \(\mu\text{m}\). A small amount of sodium azide (0.1% w/v) was added to the water phase as a preservative. Emulsions were stored at 4°C. The stability against coalescence of the oil-in-water emulsions was studied after 1 and 40 days.

**Average droplet diameter**

Particle size distribution was determined by integrated light scattering using a Malvern Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK). The emulsions were analyzed after 1, 20 and 40 days of preparation, in duplicate. Measurements were performed at room temperature on a dilution approximately 1:1000. The droplet size distribution was then determined using two indexes, \(D[4,3]\) and \(D[3,2]\).\textsuperscript{21}

\[ D[4,3] = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \text{ (μm)} \]  
(1)

where \(n_i\) is the number of droplets of diameter \(d_i\).

\[ D[3,2] = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \text{ (μm)} \]  
(2)

In addition, the specific surface area was calculated according to Walstra.\textsuperscript{22}

\[ S_v = \frac{6 \Phi}{D[3,2]} \text{ (m}^2\text{ ml}^{-1} \text{ emulsion)} \]  
(3)

where \(\Phi\) was the oil volume fraction of the emulsion (0.3).

**Adsorbed protein in emulsions**

In order to determine the amount of protein adsorbed per unit oil droplet surface area, o/w emulsions were prepared by dispersing 30 ml of corn oil into 70 ml of 1% (w/v) LSPI or SBPI protein isolate suspension in deionized water at pH 7.0. In some cases XG was added to the protein suspensions with the aid
of a mechanical stirrer. The crude emulsions were then homogenized for 1.5 min at 9500 rev min⁻¹. Following storage at 4 °C for 24 h, the emulsions were centrifuged for 30 min at 3500 rev min⁻¹ in a S11 Firlabo centrifuge (Firlabo, Meyzieu, France). The cream was collected, washed three times with 100 ml of deionized water and collected by centrifugation, Tris-SDS buffer at pH 6.8 (0.75 M Tris) was added to the cream (in order for the protein to remain dissolved) and finally frozen at -15 °C for 24 h. The frozen cream was then thawed at 50 °C and centrifuged for 30 min at 3500 rev min⁻¹ to break the emulsion. The separated oil was discarded and the water phase collected. The protein, present in the aqueous phase, was analyzed by the Lowry method.¹²

The average surface area (m² ml⁻¹ oil), was calculated using the equation:

\[ S = \frac{6}{D[3,2]} \]  

(4)

The amount of protein adsorbed per unit surface, \( \Gamma_s \), was calculated using the equation:

\[ \Gamma_s \ (\text{mg m}^{-2}) = \frac{\Gamma_T}{S_T} \]  

(5)

where \( \Gamma_T \) is the amount of protein adsorbed and \( S_T \) is the total emulsion surface derived by the equation:

\[ S_T = 30 \ S \]  

(6)

**Surface pressure determination**

Surface pressure development with time at the air–water interfaces was derived from surface tension measurements conducted by applying the ring method with the aid of a Kruss tensiometer (Kruss GmbH-Hamburg, Germany, Model K8). The tensiometer was operated manually. All the experiments were carried out at 25 °C. The surface tension (\( \gamma \)) was calculated by \( \gamma = \gamma_0 - \gamma_t \), where \( \gamma_0 \) and \( \gamma_t \) are the surface tensions of the buffer (72 mN m⁻¹) and the lupin protein solution at time \( t \) at the air/water interface, respectively.

**Rheology of oil-in-water emulsions and xanthan solutions**

A steady-stress rheometer (Brookfield DV-II, LV Viscometer, Brookfield Engineering Laboratories, Middleboro, MA, USA) equipped with the SC4-25/13R small sample adapter was used to determine the viscosity–rate of shear data of the emulsions and XG solutions after 1 or 40 days of storage. All the measurements were carried out at 25 °C.

**Statistical analysis**

All experiments were repeated at least three times and data were analyzed using a one-way ANOVA program. The level of confidence was 95%. Significant differences between means were identified by a LSD procedure.

**RESULTS AND DISCUSSION**

Emulsion stability was evaluated with respect to coalescence (particle size distribution changes through the time) and network creation, which promotes steric stabilization among the oil droplets and enhances stability of the system.

The influence of the type of protein molecules is shown in Fig 1. The pH of the aqueous solution alters, through the charges, the extensibility of the molecules, which has a crucial effect on the absorption and, eventually, on the oil size droplet distribution. It appears that the SBPI proteins at pH 7.0 had for fresh (1 day) and aged (20 days) emulsions the lowest droplet size distribution \( D[3,2] = 7.6 \mu m \) and 8.7 μm, respectively. This may indicate that soya proteins, in the present conditions of extraction and evaluation, are more effective as emulsifying agents.

When XG is added (Fig 2) in 0.1 and 0.25% w/v the creation of a network structure enhances the stability of the system.²⁰ It is important, however, to stress that relatively stable emulsions were prepared in the presence of laboratory xanthan at concentration levels well below the legal limit (1% w/v), considering that in the gum’s absence the systems were relatively unstable.

The effect of the presence of NaCl during emulsion formulation on the average volume surface diameter \( D[3,2] \) of the emulsions made with 0.1, 0.25 and 0.5 M NaCl at pH 5.5 and 7.0 are shown in Fig 3. The LSPI emulsions at pH 5.5 with 0.5 M NaCl seem to be more stable after 1 and 20 days of storage \( D[3,2] = 11.3 \) and 11.6 μm, respectively) than emulsions at pH 5.5 with 0.1 M and 0.25 M

![Figure 1](attachment:figure1.png)

**Figure 1.** Oil droplet size distribution and \( D[3,2] \)um of o/w emulsions prepared with 1% w/v SBPI or LSPI at two pH values, 5.5 and 7.0, after 1 (a) and 20 days ageing, SBPI —— pH 5.5, ——LSPI pH 5.5, —— SBPI pH 7.0, —— LSPI pH 7.0.
emulsions stabilized with or without NaCl and XG. In the present system various factors, such as the origin of protein molecules, its concentration, the pH of the aqueous phase, the type of xanthan gum as well as the addition or not of NaCl in different concentrations, are crucial. However, the build-up of a rigid and viscoelastic film around the oil droplets is important, the mechanical properties of which are decisive for the stability of the system. Different kinds of interparticle interactions lead to different aggregation mechanisms and different aggregation process leads to different kind of colloid structures.24–27

Xanthan and protein are thermodynamically incompatible. Because of unfavourable interactions demixing occurs when xanthan is added in the bulk phase. The addition of XG enhances the adsorption of protein on the oil droplet surface (Γ̃, mg m⁻²), maximizing the protein/surface contact¹¹,¹²,¹⁰,²⁶,²⁷ (Figs 4a, 5a). The NaCl presence increases the amount of protein adsorbed. NaCl, by screening the electric charges, which leads to opening of the protein molecular structure, decreases the barrier to adsorption associated with the electrical potential set up by the protein at the interface. The increase of the amount of protein adsorbed (Fig 4a, treatments C, D and Fig 5a, treatments C, D) in the case of both XG and NaCl being present, is probably due to their simultaneous effect.

The parameter SV (m² ml⁻¹ emulsion) deals with the input of energy during the emulsification process (Figs 4b, 5b). Its values do not correlate with the amount of protein adsorbed but deal with the potential stability of the system. For example in Fig 4a, treatment B, the amount of protein adsorbed, is quite low but the stability of the system is expected to be high (Fig 4b, treatment B). The above observations

NaCl and also than the emulsions stabilized only with 1% LSPI (Fig 1). At pH 7.0, 0.1 M NaCl seems the most promising concentration in comparison with

Figure 2. Oil droplet size distribution and D[3.2]μm of o/w emulsions prepared with 1% w/v LSPI at two pH values, 5.5 and 7.0, containing commercial (Xcom) or laboratory (Xlab) xanthan gum 0.1%w/v (a) and 0.25 %w/v (b). Xlab pH 5.5, Xlab pH 7.0, Xcom pH 5.5, Xcom pH 7.0.

Figure 3. Oil droplet size distribution and D[3.2]μm of o/w emulsions prepared with 1% w/v LSPI at two pH values, 5.5 and 7.0, containing laboratory 0.1%w/v xanthan gum and 0.1, 0.25, 0.5 M NaCl after 1 (a) and 20 (b) days ageing, 0.1 M NaCl pH 5.5, 0.25 M NaCl pH 5.5, 0.5 M NaCl pH 5.5, 0.1 M NaCl pH 7.0, 0.25 M NaCl pH 7.0, 0.5 M NaCl pH 7.0.

Figure 4. Effect of addition of XG and NaCl on the amount of protein adsorbed per unit surface area Γ̃ (mg m⁻²) and their specific surface area SV (m² ml⁻¹ emulsion) in oil-in-water emulsions stabilized with SBPI: A, control, 1% w/v protein; B, 1% w/v protein, 0.25% w/v XG; C, 1% w/v protein, 0.25% w/v XG, 0.1 M NaCl; D, 1% w/v protein, 0.25% w/v XG, 0.5 M NaCl.
are supported by the droplet size distribution data (Fig 2a, b).

Additional experiments, with surface pressure measurements, were carried out in order to investigate the effect of XG and NaCl addition on the protein structure at the air-water interface. The changes in surface pressure during the adsorption of 0.5 × 10⁻²% w/v LSPI with or without XG 0.125 × 10⁻² %w/v and 0.5 M NaCl solutions were studied and the results are shown in Fig 6. All the points represent mean values of three experiments with a standard deviation not exceeding ±0.1. The samples reached near steady values after 20 or 65 min of adsorption. The sample with XG and NaCl reached the higher steady-state values quite rapidly. When XG was added, the steady-state value was higher than with the protein solution without XG. This could be attributed to the maximization of the protein/surface contacts, due to its incompatibility with XG. The results of the surface tension measurements could not be attributed to a potential adsorption of XG to the interface because the Γ values show that the amount of protein adsorbed at the interface, in the presence of XG, is increased.

Figure 7 shows the influence of the type of xanthan and time of storage on the viscosity–rate of shear behaviour of LSPI solutions. It is obvious that, in the absence of gum (Fig 8a), the rheological properties of the emulsions were very low. Following polysaccharide addition, the rheological characteristics of emulsions increased considerably, this being more pronounced in the case of the commercial gum, although the effect of laboratory gum addition at a concentration level of 0.25% w/v was quite spectacular (Fig 8b, c). In general, the presence of XG, up to a certain limit, enhances the stability of emulsion by developing a network structure which holds the oil droplets away.

Figure 9 illustrates the NaCl effect in the rheological properties of emulsions. NaCl, by screening the electrical charges, changes the opening of the protein molecular structure, lowers the barrier to adsorption and so alters interfacial protein coverage as well as the strength of the network structure. In Fig 9a and b the network structure seems to exist in both type of xanthan even after 40 days of storage. As already mentioned (Fig 4a, treatment D and Fig 5a, treatment D), 0.5 M NaCl in the aqueous phase does enhance the protein absorption and the emulsion stability.

The use of a medium molecular weight xanthan gum, as in our case, resulted in acceptable emulsions compared with those prepared with the commercial xanthan in a recent study, Kiosseoglou et al. They concluded that gels prepared with laboratory xanthan will be as firm as those prepared with high molecular weight xanthan, but of lower elasticity, cohesiveness...
and chewiness and therefore more acceptable by the consumer. Preliminary studies conducted at the Department of Chemical Engineering by Liakopoulou have indicated that fermentation by *Xanthomonas campestris* on industrial by-products, such as milk whey or molasses, may result in medium molecular weight xanthan gums which could be useful materials in certain food applications.

**CONCLUSIONS**

The SBPI emulsions, at pH 7.0, exhibit lower droplet size distribution than LSPI. XG enhances the stability of the system when added in 0.1 and 0.25% w/v by creating a network structure. The addition of XG also enhances the adsorption of protein on the oil droplet surface, maximizing the protein/surface contact.

When NaCl (0.5 M) is added to emulsions at pH 5.5 they appear more stable with smaller *D*[3,2] values, even after 20 days of storage, than with 0.1 M or 0.25 M NaCl emulsions. This is more obvious for the LSPI emulsions. The presence of NaCl also increases the amount of protein adsorbed.

As far as the rheological properties of the emulsions are concerned, they are very low in the absence of gum. Following polysaccharide addition, the rheological characteristics of emulsions increased considerably, the result being more pronounced in the case of the commercial gum. Laboratory gum exhibited quite satisfactory properties.

We conclude that laboratory xanthan gum appears a promising food additive for the food industry. The incorporation of xanthan gum and legume proteins to food systems could lead to the creation of new food products with acceptable stability and texture for the consumer.

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Effect of xanthan gum in rheology and stability of oil-in-water emulsions