From Egg Yolk/κ-Carrageenan dispersions to gel systems: Linear Viscoelasticity and Texture Analysis

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Abstract

The effect of pH (3.5-6) and polysaccharide concentration (0-0.5 wt\%) on the linear viscoelastic behaviour of egg yolk/κ-Carrageenan (EY/κC) mixtures in aqueous solution was studied by using small amplitude oscillatory shear (SAOS). Native egg yolk containing 45 wt\% solids was used for all the samples. Thermally set EY/κC gels were also studied by SAOS and texture analysis. A variety of linear viscoelastic behaviours depending on κC concentration and pH were exhibited by EY/κC dispersions that may be explained in terms of the contributions of electrostatic attractive interactions and an exclusion volume effect between protein and polysaccharide macromolecules. This last effect seems to be dominant as pH shifts towards the isoelectric point (IEP) of egg yolk proteins, whereas, at pH far from the IEP a certain enhancement in the degree of compatibility, and even some κC autohydrolysis, seems to take place. The results obtained either from rheological or textural characterization of gels were consistent with that balance. In any case, the results obtained suggest that the microstructure of gels is governed by the protein ability to form gels, where hydrophobically driven interactions and subsequent cross linking among protein segments play a dominant role.
Keywords: Proteins, Polysaccharides, Gels, Viscoelasticity, Texture.

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1. Introduction

Protein and polysaccharides are natural polymers that are widely used as functional ingredients in food materials. The interactions of these biopolymers, which lead to extremely complicated and interesting phenomena in various systems, impart an important effect on the macroscopic properties of food products such as: flow, stability, texture and mouth feel (de Kruif & Tuinier, 2001). Tolstoguzov (2003) stressed out the importance of thermodynamic considerations in protein-polysaccharide interactions to design food formulations with novel or improved properties.

Although for very dilute solutions the system is stable since the mixing entropy dominates and proteins and polysaccharides are co-soluble, from a thermodynamic point of view, proteins and polysaccharides may be compatible or incompatible in an aqueous solution. Thermodynamic incompatibility occurs when the interaction between different biopolymers is energetically less favourable than the average interaction between similar biopolymers. This incompatibility, where biopolymers are mutually segregating one from the other, results in the formation of two separated phases, each being rich in one of the biopolymers. If the interaction between both biopolymers is favoured, a phenomenon known as coacervation or associative phase separation of both biopolymers from a phase mainly containing solvent takes place. The main difference between these two mechanisms is that thermodynamic incompatibility is predominantly entropically driven, whereas complex coacervation, which has been attributed to an electrostatic attraction between
oppositely charged biopolymers is both entropically and enthalpically driven (Turgeon, Beaulieu, Schmitt & Sánchez, 2003; de Kruif, Weinbreck & de Vries, 2004; Tolstoguzov, 2007).

The major non-covalent interactions between proteins and polysaccharides are: electrostatic interactions, steric exclusion, hydrophobic interactions and hydrogen bonding. Thus, associative separation is commonly governed by the electrostatic attraction between molecules with opposite electrical charges, whereas the steric exclusion effect causes segregative separation. The relative importance of these interactions in a particular system depends on the molecules involved, the composition of the aqueous mixture and the environmental conditions. Knowledge of the origin and nature of the interactions involved and the relative importance of these interactions in a particular system can often be used by food scientist to engineer novel structures and physicochemical properties in food systems. Furthermore, by modulating these parameters it is possible to control the interactions between the biopolymers and therefore create microstructures that provide novel textural and sensory properties to food systems, improve emulsion stability to environmental stresses, or enable novel encapsulation-release characteristics (McClements, 2006).

In food technology there has been recent increasing interest in protein-polysaccharide complexes and coacervates derived from novel industrial applications such as micro an nano-encapsulation processes, the design of multi-layers structures, the formation and stabilization of food emulsions, the formation of new food gels and the recovery of protein from industrial by-products (Turgeon, Schmitt & Sánchez, 2007). Thus, the rheological and interfacial properties of protein-polysaccharide complexes might be advantageously used for designing food products with various textures, stability and delivery properties. In addition, a variety of applications of protein-polysaccharide or protein-polyelectrolyte complexes have been described for non-food applications such as microencapsulation for the pharmaceutical industry or its use in the synthesis of biomaterials and the same conclusion might apply to formulate novel delivery systems (Schmitt, 2009).

The objective of this contribution was to analyse the effect of pH and protein-polysaccharide ratio on the linear viscoelastic behaviour of egg yolk/κ-carrageenan mixtures in aqueous solution and gels obtained after application of thermal treatment to these blends in order to obtain information
about the type of microstructures present in the system. Texture properties of gels were also
evaluated.

2 Materials and methods

2.1 Materials

Egg Yolk (EY) was obtained from fresh chicken eggs purchased in a local market according to
grade A and type L (63-73 g) commercial specifications, and all damaged or cracked eggs were
discarded. κ-Carrageenan (κC) (Satiagel™ AMP45) was kindly provided by Degussa (France).
The composition for the counter ion of this κC was 1, 14 and 0.4 wt% of sodium, potassium and
calcium, respectively. Hydrochloric acid (analytical grade) was obtained from Merck (Germany).

2.2 Methods

Chicken eggs were hand broken and the white carefully separated from the yolk using the Harrison
and Cunningham (1986) preparation method. EY/κC mixtures were made by dispersing different
amounts of κC (0-0.5 wt%) in native EY (50.43 ± 0.8 average solid content wt %), under
mechanical stirring (300 rpm, 1h) at room temperature. The natural pH of the system was modified
using 1M and 2M hydrochloric acid as acidulate and demineralised water was adding until 45 wt% in solids EY content.

Small Amplitude Oscillatory Shear (SAOS) measurements of egg yolk dispersions containing 45
wt% in solids at different pH values an κC content, including either stress sweep or frequency
sweep, were performed by means of a controlled-stress rheometer Haake RS-300 (Thermo
Scientific, Germany), using 60 mm and 4º cone-plate geometry with 1 mm gap. A shear stress
sweep test was always done beforehand at 6.3 rad/s to establish the linear viscoelasticity range.
All the dynamic viscoelasticity frequency sweep measurements were carried out at 20ºC and at a
stress clearly lower than the critical value for linear viscoelasticity.

Measurements of the zeta potential of systems containing 45 wt % in EY and 0.3 wt % in κC at
different pH values were performed using a Zetasizer 2000 (Malvern Instruments, U.K.). Aliquots
of 0.5 g were diluted in deionised water up to 100 ml of total volume, keeping adjusted the pH
value of the dispersion with 0.1 N HCl. Prior to analysis, the samples were tempered at 20ºC and
stirred in order to keep the suspension homogeneous, avoiding segregation. The samples were
measured in triplicate and each measurement was an average of five determinations. During the experiments, no flocculation was observed and each measurement was conducted at 20 °C. The zeta potential was calculated from the electrophoretic mobility using the Henry equation and the Smoluchowski approximation.

The gels were obtained by transferring the above samples without entrapping air into a 20 ml plastic bottles (Ø= 20mm, 65mm height) and sealed prior to heat at 90ºC in a thermally controlled water bath during 30 min. These gels were stored overnight at 5ºC in order to allow the maturation of mixed gels. Dynamic linear viscoelastic properties were conducted by frequency sweep tests on thin slices (1.5±0.3mm) of gel samples at 20ºC, using serrated plate-plate geometry with 1 mm gap within the linear response regime. The pH values were also checked after processing and no modification was detected.

Texture properties of the final gels were evaluated using a TA-XT2i (Stable Micro Systems, UK) texturometer. In order to avoid any correction for non-ideal diameter/height ratios, the gel samples were cut into 20mm diameter and 26 mm height specimens (Kuhn, 2000).and lubricated with low viscosity silicon oil. Uniaxial compression tests were performed using a 75mm diameter flat platen (15% strain, 600 s and 0.1 mm/s of crosshead speed). Before performing any measurements, gels were allowed to equilibrate at 20ºC for approximately 3 h in a temperature-controlled room. Textural results for each gel were performed at least three times.

3. Results and discussion

3.1. Linear viscoelastic properties of EY/κC dispersions

Figure 1 displays the influence of both linear viscoelastic moduli (G’ and G’’) on frequency for EY dispersions as a function of κC concentration at pH 6 (Fig. 1A) and pH 3.5 (Fig. 1B). As may be observed in both graphs, in absence of polysaccharide, the EY dispersion showed a predominantly viscous fluid-like behaviour, with both moduli showing higher values for the lower pH, due to the electrostatic interactions among charged protein surfaces. The addition of κC to EY led to a remarkable evolution in both viscoelastic funtions that depends on pH.

Near the mean isoelectric point (IEP) of yolk proteins (pH 6), when 0.15 wt% κC was added, a crossover between G” and G’ took place at high frequency. Although a further increase in κC up to
0.3 wt% yielded similar values of both moduli at high frequency, the behaviour is rather different. Thus, at low frequency \( G'' \) was always higher than \( G' \) for the 0.15 wt\% \( \kappa C/EY \), whereas, the blends containing 0.30 and 0.50 wt\% \( \kappa C \) were predominantly elastic, giving rise to a crossover point and a shift to a predominantly viscous response at higher frequencies. This crossover took place at lower frequency for the highest \( \kappa C \) content and may be related to the increase in repulsive interactions due to increasing negative charges. Thus, the zeta potential value at pH 6 increase from almost 0, corresponding to the IEP in absence of \( \kappa C \), to -26.9 mV for 0.3 wt\% \( \kappa C \) (see Table 1). In any case, no evolution to gel-like behaviour was found near the IEP. This behaviour is similar to that one shown by amorphous uncrosslinked polymers of high molecular weight as described by Ferry (1980). In accordance, the first above mentioned crossover point corresponds to the so-called terminal transition of the generalized mechanical spectrum, whereas the second one corresponds to the beginning of the transition region, with \( G'' \) dominating over \( G' \). For uncrosslinked polymers of high molecular weight, this region is preceded by a predominantly elastic entangled plateau region that in this case was observed at the low frequency regime.

At pH 3.5 protein surfaces exhibit a positive net charge, as may be observed in Table 1. The addition of \( \kappa C \) leaded to an increase in \( G' \) and \( G'' \), as well as to a decrease in their respective frequency slopes and loss tangent and a reduction in the zeta potential value. However since the amount of protein is much higher than the polysaccharide content the electrostatic repulsions are still noticeable. The EY dispersion containing 0.30\% \( \kappa C \) experienced a marked decrease in loss tangent and frequency dependence, showing a weak gel-like behaviour, with \( G' \) higher than \( G'' \) for the whole frequency range studied, which indicates that the presence of charges on protein surfaces promoted the enhancement of network-like structures. A further increase in \( \kappa C \) up to 0.50\% resulted in a weakening of the dispersion leading to linear viscoelastic functions similar to that one shown by the system containing 0.15\% \( \kappa C \). In fact, occurrence of maximum consistency for mixed biopolymers gels has been described in the literature of food hydrocolloids (Turquois, Rochas & Taravel, 1992). Imeson (2009) has recently reviewed the interactions between \( \kappa C \) and other biopolymers, reporting a positive synergism with LBG or konjac glucomannan which yields
maximum gel strength at intermediate κC content for 1% total polysaccharide. A synergism between κC and κ-casein protein was also reported, being attributed to interaction of an electrostatic nature with positively charged amino acids at the surface of κ-casein micelles (Hood and Allen, 1977; Langendorff, Cuvelier, Michon, Launay, Parker & de Kruijff, 2000; Spagnuolo, Dagleish, Goff & Morris, 2005, Tijssen, Canabady-Rochelle & Mellema, 2007).

Figure 2 shows the evolution of tan δ at 1 rad/s as a function of κC content for pH 3.5. Previous results on EY dispersions at acidic pH (3 and 3.5) showed that the behaviour in absence of polysaccharide is quite different from that obtained for 0.3 κC/EY blends, which corresponds to a viscoelastic fluid showing the terminal transition crossover point at high frequency (Guerrero, Carmona, Martinez & Partal, 2004; Aguilar, Cordobés & Guerrero 2007). The results obtained at pH 3.5 suggest that protein-polysaccharide interactions of electrostatic nature may lead to the formation of gel-like network structures, displaying maximum strength at 0.3 wt% κC (minimum loss tangent), at which protein to polysaccharide optimum ratio seems to be found (Schmitt et al. 2009). A possible explanation for this increase in the loss tangent above 0.3% is a phenomenon known as carrageenan autohydrolysis, which occurs at low pH values as carrageenan in the acid form cleaves at the 3,6-anhydrogalactose linkage in the molecule (Hoffmann, Russell & Gidley, 1996). Although this phenomenon is favoured at high temperature, autohydrolysis would also take place to some extent at temperatures above the cold-gelling point as long as κC content is high enough (i.e. 0.5 % κC). The contribution of the decrease in electrostatic repulsions, although moderate, may also be taken into account, being a consequence of the addition of negatively charged κC.

3.2. Linear viscoelastic properties of EY/κC gels

The mechanical spectra of thermally processed EY/κC systems at pH 6 are shown in Figure 3. As may be observed, these systems exhibit a gel behaviour with G’ being much higher than G”. The addition of κC leaded to a clear increase in both moduli, but did not produce any modification on the shape of the mechanical spectra, in spite of the variety of viscoelastic behaviours exhibited by the unheated EY/κC mixtures shown in Fig 1. These results indicate that the viscoelastic behaviour
of EY/κC gels were dominated by the ability of protein macromolecules to form a three-dimensional network, whereas the polysaccharide may contribute to reinforce the consistency of the gel without any further modification on microstructure, which might be related to an exclusion volume effect of incompatible biopolymers in a mixed solution according to the description of the so-called molecular symbiosis reported by Tolstoguzov (2007).

As a consequence of the similar mechanical spectra shown in Fig. 3, a time-concentration superposition method may be applied by normalizing both viscoelastic functions with the storage modulus at 1 rad/s to obtain a master curve. As may be seen in Figure 4A all the mechanical spectra obtained at different κC concentration and pH collapse into the master curve. The values for the normalization parameter (G’ at 1 rad/s), which are shown in Figure 4B, underwent an increase with the addition of κC that became more apparent and steeply as pH was closer to the IEP. In fact, at acidic pH, far from the IEP, this increase is dampened and tends to vanish above 0.15 wt% κC. As a consequence, the higher electrostatic interactions obtained as κC was raised seem to inhibit the enhancement of the gel network. This behaviour may be explained in terms of mutual exclusion of each biopolymer, which seems to be more evident at a low level of electrostatic interactions (pH 6 and 5), at which the evolution of G’, would correspond to an increase in effective concentration of protein. This effect seems to be hindered by a certain degree of compatibility between positively charged proteins (as pH departs from the IEP) and polysaccharide anions or, alternatively, it may be a consequence of the above mentioned autohydrolysis effect found for carrageenan macromolecules.

3.3 Textural properties of EY/κC gels

Figure 5 shows the results of some textural parameters obtained for EY/κC gels under uniaxial compression as a function of κC concentration and pH. Springiness only exhibits a moderate decrease with κC concentration for all the pH values tested. In contrast, the evolution of firmness is more evident, being similar to that one found for viscoelastic properties (i.e. G’ in Fig. 3). Thus, a marked increase in firmness takes place with increasing κC content near the IEP, but this effect attenuated at pH 4.5 and even reversed at the lowest pH. These results of firmness are consistent
with the exclusion volume effect taking place near the IEP and with the reduction produced as a consequence of the increase in electrostatic interactions.

4. Concluding remarks

EY/κC dispersions exhibited a variety of linear viscoelastic behaviours depending on κC concentration and pH, where three regions of the mechanical spectrum may be generally observed: The terminal region obtained at low κC concentration for all the pH values studied; The plateau region, observed at low frequency at 0.3 % κC and above; The beginning of the terminal region at high κC content and frequency, which was particularly pronounced near the IEP.

The second or plateau region was enhanced as pH departs from the IEP even leading to a gel-like behaviour. This change in the microstructure of the system at a controlled κC content and low pH, when protein surfaces exhibited a marked positive net charge, might be due to a certain degree of compatibility between both polymers.

The results obtained either from rheological or textural characterization of gels may be explained in terms of an exclusion volume effect between protein and polysaccharide macromolecules that seems to be more important in absence of net charge at protein surfaces. At pH far from the IEP a certain enhancement in the degree of compatibility also seems to occur, although an autohydrolysis effect typical of carrageenan macromolecules at low pH may also takes place. In any case, as may be inferred from the unique master mechanical spectrum obtained by time-concentration superposition, the microstructure of thermally treated EY/κC systems seems to be governed by the protein ability to form gels, where hydrophobically driven interactions and subsequent cross linking among protein segments play a dominant role.

Acknowledgments

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**FIGURE CAPTIONS**

Fig.1. Evolution of linear viscoelastic moduli of EY/κC dispersions as a function of frequency and κC concentration: A) at pH 6 and B) at pH 3.5.

Fig.2. Evolution of loss tangent values at 1 rad/s for EY/κC systems at pH 3.5, as a function of κC content.

Fig.3. Evolution of the mechanical spectra of EY/κC heat set gels at pH 6, as function of κC content.

Fig.4. Normalized linear viscoelastic functions for EY/κC gels: A) Master mechanical spectrum; B) Normalization parameter as a function of κC concentration and pH.
Fig. 5. Evolution of textural parameters (firmness and springiness) of EY/κC gels as a function of κC concentration at different pH values
Table 1. Z-potential values for EY/kC dispersions as a function of pH and kC concentration

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<thead>
<tr>
<th>pH</th>
<th>kC concentration</th>
<th>Z-Potential</th>
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<tbody>
<tr>
<td></td>
<td>(-) (wt %)</td>
<td>(mV)</td>
</tr>
<tr>
<td>3.5</td>
<td>0</td>
<td>35.8 ± 0.5</td>
</tr>
<tr>
<td>3.5</td>
<td>0.3</td>
<td>35.0 ± 0.8</td>
</tr>
<tr>
<td>3.5</td>
<td>0.5</td>
<td>34.2 ± 0.8</td>
</tr>
<tr>
<td>4.5</td>
<td>0.3</td>
<td>-0.2 ± 0.7</td>
</tr>
<tr>
<td>6.0</td>
<td>0.5</td>
<td>-26.9 ± 1.4</td>
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