HIGH PRESSURE-INDUCED RHEOLOGICAL TRANSITIONS IN EGG PROTEIN DISPERSIONS

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1 INTRODUCTION

Eggs have been one of the first multifunctional ingredients, well known for their whipping, gelling and emulsification properties, where the proteins are the dominant component. Egg yolk is an ingredient of high nutritional value with unique sensory properties and excellent functionality used in the manufacture and stabilisation of egg-containing foodstuffs and provides an inexpensive source of high quality protein. Processing conditions such as temperature and ultra high pressure (UHP) involve complete or partial denaturation of most food systems, including egg yolk. However, pressure processing does not affect the sensory and nutritional characteristics of fresh products (i.e. natural colour, flavour, taste, texture and vitamins) and allows to be obtained gels with a unique texture since pressure is instantaneously uniformly felt through the food. Moreover an increasing demand of high quality, minimally processed, additive-free and microbiologically safe foodstuff have lately made UHP technology fairly attractive. On the other hand, variables related to composition (pH, ionic strength, solids content) can modify the functional properties of the system.

The main objective is to study the influence of UHP processing on the rheology and microstructure of egg yolk dispersions. First of all, this work focuses on pressure-induced transitions. The effect of protein concentration and pH is also evaluated.

2 EXPERIMENTAL

Egg yolk was obtained from fresh, grade A, large hen's eggs using the method of Harrison and Cunningham.[1] Several egg yolk dispersions were prepared by adding water with solids concentrations from 25 to 51%. Modified egg yolk dispersions were sampled in a 20 ml plastic syringe without entrapping air and placed in a pressure reactor (15 cm length and 10 cm in diameter). Finally, the samples were subjected to UHP processing at pressure values between 180-450 ± 10 MPa for 30 min, using a hydrostatic UHP generator (Hidraulica Aguilar, Spain). Different pH values were studied by adding HCl acid (1M). Linear viscoelastic tests were carried out by means of an ARES controlled strain rheometer (TA Instruments, U.K.). Scanning Electron Microscopy (SEM) techniques were used to evaluate the microstructure of the gels formed by means of a Phillips XL-30 microscope.

3 RESULTS AND CONCLUSIONS

3.1 Influence of pressure

Previous results from DSC measurements described a progressive decrease in thermal denaturation entalphy, without any significant change in denaturation temperature, as the pressure level was raised, leading to a complete flattening of the flow signal.[2]

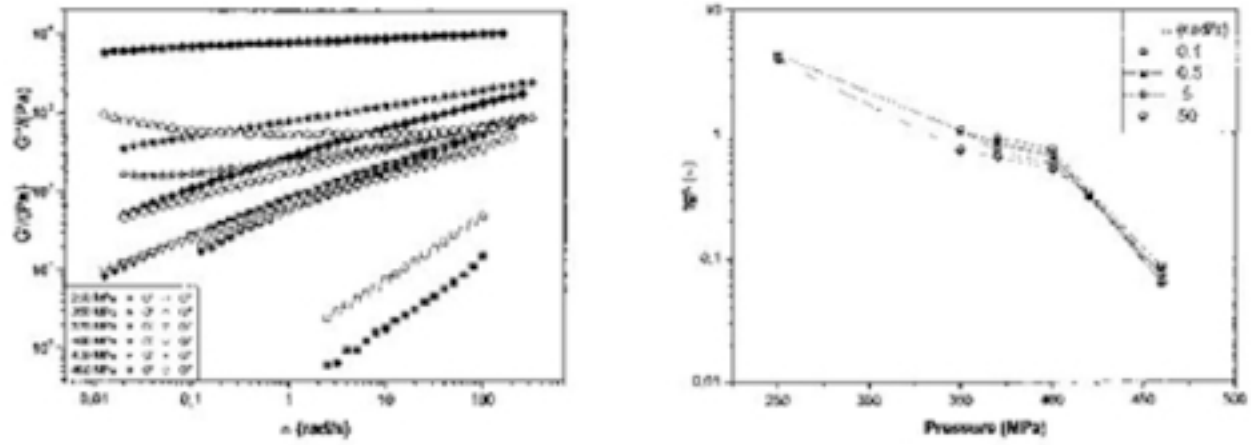

Figure 1 *Evolution of linear viscoelastic properties of egg yolk (45 wt% solids) as a function of frequency and pressure level: a) Storage and loss moduli; b) loss tangent*

Figure 1a shows the results obtained from frecuency sweep tests carried out at a deformation range in the linear visoelastic behaviour. As can be observed, an increase in the pressure of processing produces a remarkable growth in the storage and loss moduli and a dramatic change in the linear viscoelastic behaviour of egg yolk dispersions. Different linear viscoelastic behaviours can be defined as a function of pressure, as can be observed in Figure 1. At pressure levels lower than 320 MPa, a fluid-like behaviour takes place with loss tangent values higher than unity. At pressure levels ranging from 320 to 420 MPa, a fluid-like to gel-like transition takes place, where the loss tangent shows values of unity or slightly lower. As may be seen in Figure 1b, the loss tangent becomes independent on frequency at a pressure slightly lower than 420 MPa, which should correspond to the critical gel point, according to the gel equation given by Winter and Chambon.[3] A further increase in pressure, above 420 MPa, leads to a gel-like behaviour with a dramatic decrease in the loss tangent values.

A Generalized Maxwell model has been used to describe these linear viscoelastic behaviours by means of a simultaneous fitting to the experimental values of G' and G'':

$$G'(\omega) = \sum_{k=1}^{n} G_k \frac{\omega^2 \lambda_k^2}{1+\omega^2 \lambda_k^2} \qquad (1)$$

$$G''(\omega) = \sum_{k=1}^{n} G_k \frac{\omega \lambda_k}{1+\omega^2 \lambda_k^2} \qquad (2)$$

where G_k and λ_k are the relaxation strength and relaxation time for the k-Maxwell element.[4] Other linear viscoelasticity functions such as the relaxation modulus, G(t), may be also calculated from this model as follows:

$$G(t-t') = \sum_{k=1}^{n} G_k \exp(-\frac{t-t'}{\lambda_k}) \quad (3)$$

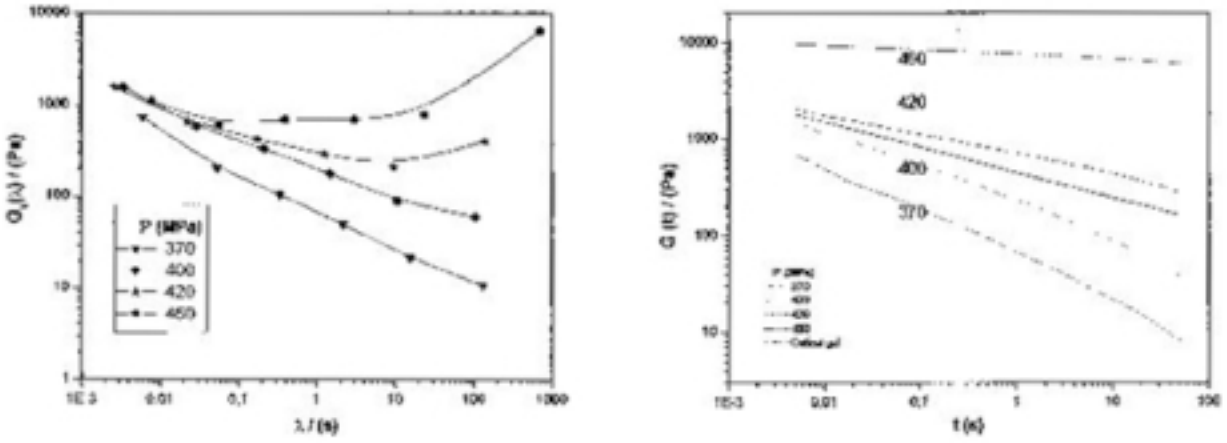

Figure 2 *Influence of pressure on linear viscoelasticity functions: a) Relaxation Spectrum and b) Relaxation Modulus*

Figure 2a displays the discrete relaxation spectra (G_k vs. λ_k) as a function of pressure. These results illustrate the pressure-induced transition from fluid-like to gel-like behaviour, characterized by a reduction in the slope of the spectrum. This evolution is also related to a remarkable increase in the density of entanglements among protein segments. Figure 2b compares the values for the relaxation modulus calculated from equation (3) at different pressure levels and the relaxation modulus for the critical gel that, according to the mechanical definition given by Winter and Chambon[3] follows a power-law equation:

$$G(t) = S \cdot t^{n} \quad (4)$$

where S is the gel stiffness and n is the relaxation exponent. Figure 2b shows the values obtained for parameter S and n obtained by fitting the values for the relaxation modulus to equation (4) at different pressures. The evolution of both parameters leads to an apparent change of behaviour at a pressure between 400 and 420 MPa. Thus, below 400 MPa the gel stiffness is extremely low while the relaxation exponent undergoes a power-law decay above the critical point.

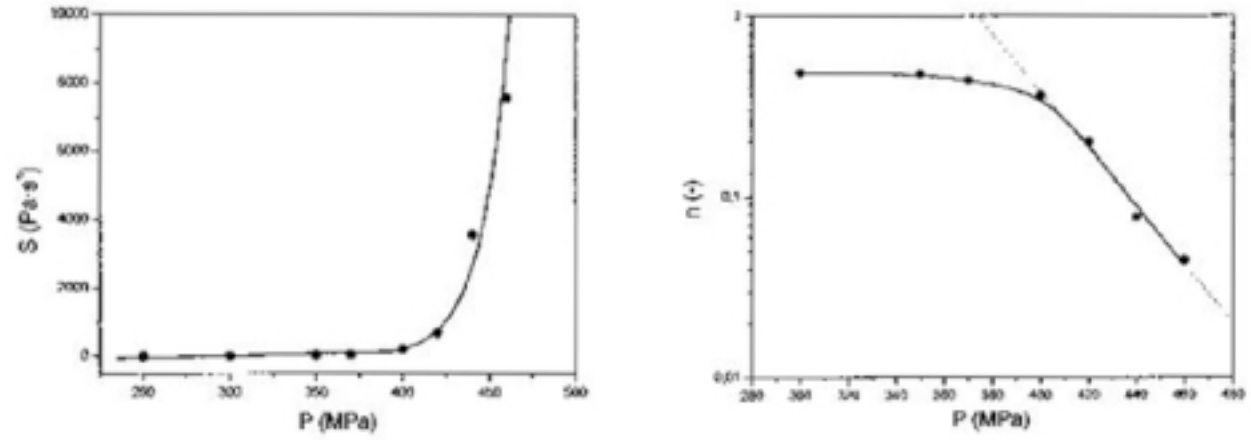

Figure 3 *Influence of pressure on linear viscoelastic parameters of equation (4): a) Gel Stiffness and b) Relaxation Exponent*

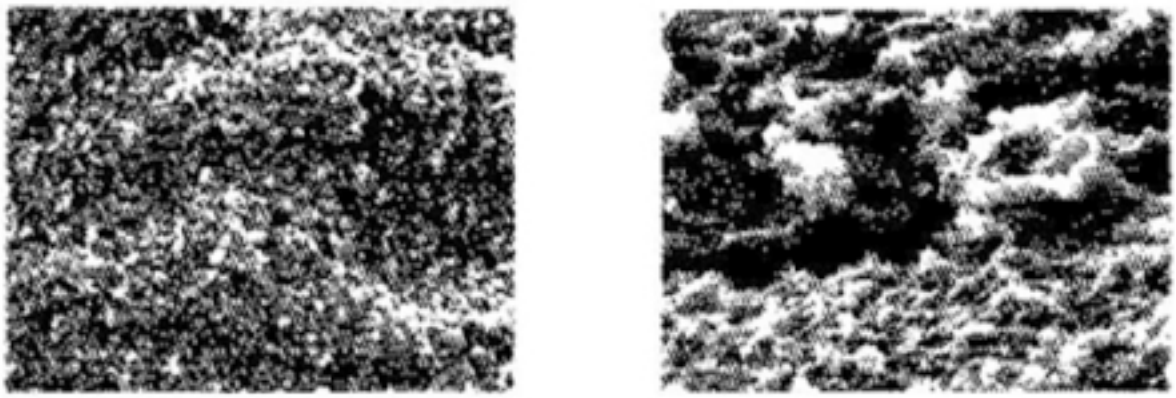

Figure 4 *SEM images for egg yolk containing 45 wt% solids and pH 6, subjected to UHP treatments: a) at 350 MPa and b) at 420 MPa*

Figure 4 compares SEM images obtained after subjecting egg yolk containing 45 wt% solids to UHP treatment at 350 MPa and 440 MPa. The former pressure level corresponds to the fluid-like region whereas the latter is slightly above the gel point. As may be clearly seen, an increase in pressure level leads to an increase in the size of aggregates as well as in the degree of association among protein aggregates that brings about formation of a three-dimensional gel network. These images correspond to pH 6, close to the isoelectric point, at which electrostatic interactions do not participate and protein aggregation is favoured by way of hydrophobic interactions under the effect of a high pressure field.

3.2 Influence of concentration

Figure 5 shows linear viscoelastic properties of egg yolk processed at 440 MPa at different solids concentration. A critical gel behaviour is found at 25 wt% solids, with the loss tangent remaining almost independent on frequency. Thus, the critical pressure undergoes an increase from 410 to 440 MPa as the concentration is reduced from 45 to 25 wt% solids.

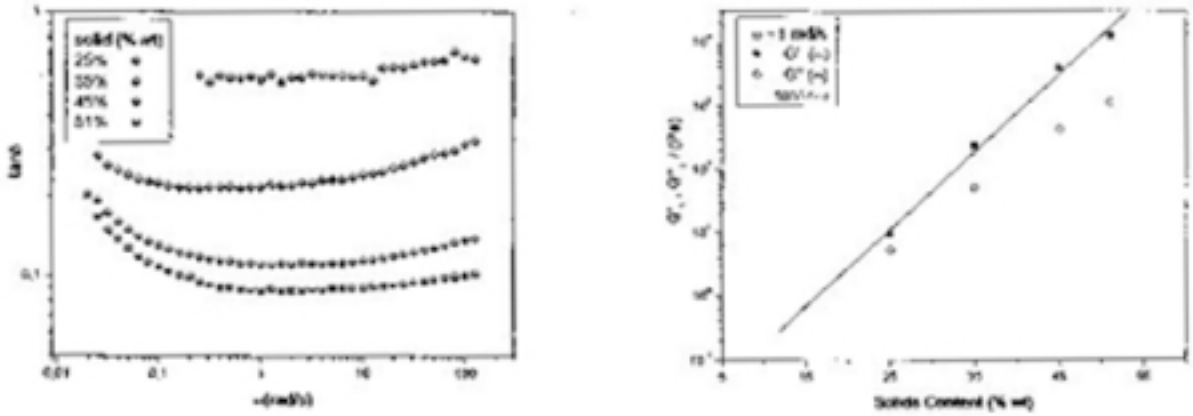

Figure 5 *Influence of concentration of solids on linear viscoelastic properties of UHP-induced egg yolk gels processed at 440 MPa: a) loss tangent vs. frequency and b) storage and loss moduli at 1 rad/s.*

An increase in concentration above the critical value produces a remarkable reduction in tan δ (figure 5a) due to an enhancement of the elastic network. This effect also produces an apparent increase in G' and G'', as may be observed in figure 5b which shows the

evolution of both moduli at 1 rad/s with solids concentration. A tendency to a crossover may be also noticed at low concentration where a fluid-like behaviour should be expected.

3.3 Influence of pH

Figure 6 shows the LV results obtained after application of UHP processing on egg yolk dispersions at different pH values. Egg yolk at pH 6-5 is in the isoelectric point region where the absence of electrostatic interactions allows favourable conditions for hydrophobically driven pressure-induced aggregation, leading to maximum gel strength (i.e. maximum value for G' and minimum value for the loss tangent and the dependency of G' or G'' on frequency).[2] As the pH of egg yolk dispersion is reduced, the net surface charge of proteins increases and hydrophobic interactions are counterbalanced by electrostatic forces leading to a loss of efficiency in aggregation during UHP treatment.[6] A remarkable increase in G' and G'' has been reported when the pH of the egg yolk dispersion is reduced either using citric[7] or HCl acid,[2] eventually leading to a gel-like behaviour at very low pH. This behaviour has been attributed to an increase in the size of aggregates as a consequence of higher denaturation level that may induce gel netwok formation. The behaviour of the protein yolk system after UHP processing at low pH, is governed by the balance between hydrophobic and electrostatic interactions. At pH 3, although hydrophobic interactions are improved, electrostatic repulsions still dominate leading to fluid-like behaviour (with a crossover between G' and G'' at ca. 1 rad/s). At lower pH, a displacement of the balance due to enhancement of hydrophobic interactions takes place, giving rise to weak gel behaviour. Thus, the combination of the above mentioned effects, pH-induced denaturation and UHP-induced aggregation, leads to the minimum in G' and G'' shown in Figure 6 at pH 3.

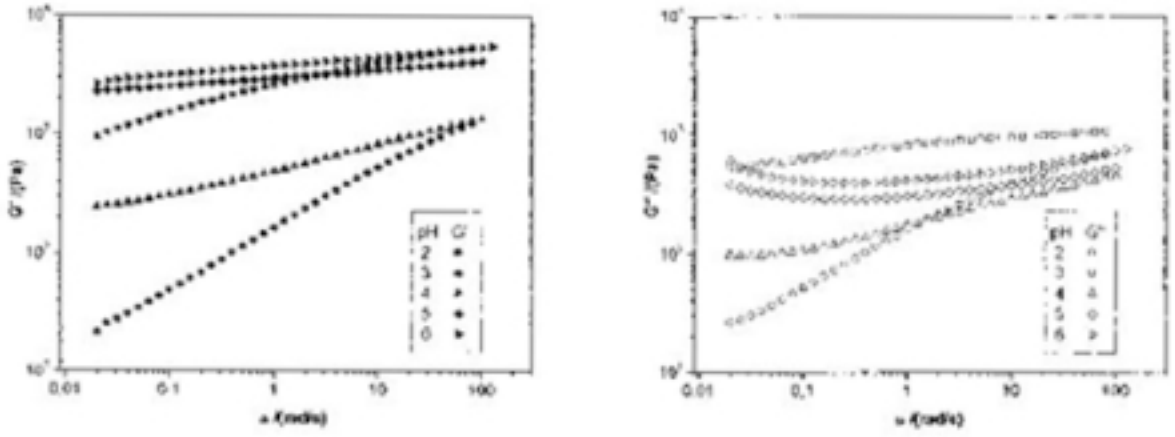

Figure 6 *Influence of pH on linear viscoelasticity functions of egg yolk containing 45 wt% solids, processed at 440 MPa: a) Storage modulus and b) Loss modulus*

4 CONCLUSIONS

High-presure processing produces some relevant modifications on the linear viscoelastic properties of egg yolk dispersions, particularly at pH close to the isoelectric point. These modifications may lead to formation of a gel system above a critical gel point that depends on protein concentration. Thus, the gel point is located at ca. 410 MPa at 45 wt% solids, but higher pressure is required to find the critical gel behaviour at lower protein

concentration (i.e. the gel point is located at 440 MPa at 25 wt% solids). SEM images reveal that an increase in the pressure level promotes an increase in the size of aggregates and a higher degree of association among protein aggregates at pH close to the isoelectric point.

An increase in protein concentration above the critical gel value produces an enhancement of the elastic network leading to higher viscoelastic properties. A change in the pH of the protein system modifies the balance between electrostatic and hydrophobic interactions, which exerts an important role in the transitions taking place during UHP processing. As a consequence, the impact of high pressure on aggregation and network formation can be modulated by pH to a high extent.

Acknowledgements

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