

CHAPTER 11

Influence of pH and type of acid anion on the linear viscoelastic properties of Egg Yolk

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Introduction

The native conformation of a protein can be explained in terms of a net balance between attractive and repulsive forces. Proteins present a maximum stability at the isoelectric point. Thus, any change in the environmental conditions (as pH or chaotropic salts) can modify and modulate the functional properties of proteins to a large extent.

Extreme pH values cause a high net charge leading to electrostatic repulsion between neighbour molecules and modifying the charge distribution among the amino acid side chains. As a consequence, changing pH to extreme values is a well known method for protein denaturation at low temperature [1].

Ion effects in biological systems were firstly investigated by Hofmeister and co-workers. They published a series of papers in which they showed that salts could be classified according to their salting-in and salting-out behaviour against proteins precipitation [2]. Nowadays, this specific ion effects make up the Hofmeister phenomena and the series of cations and anions are usually given in terms of the ability of the ions to stabilize the structure of proteins [3]. However, there is not a single and unique Hofmeister series, and, in general, cation effects are less pronounced than specific anion effects [4].

The objective of this study is to assess the influence of pH, as well as the contribution of each anion to the linear viscoelastic properties of egg yolk.

Experimental

Materials

Egg Yolk (EY) was obtained from grade A, type L (63-73 g) fresh chicken eggs according to EU specifications. Any cracked or damaged egg was discarded and

the yolk was carefully extracted from the eggs by the Harrison & Cunningham preparation method [5]. Analytical grade Hydrochloric acid (37%) was obtained from Fisher Scientific (France). Chemically pure grade (purissimus) Citric acid anhydrous and Orthophosphoric acid (85%) were obtained from Panreac (Spain).

Methods

EY dispersions were obtained by dilution of native EY (50-52 wt% in solid content). The native pH of the system (ca. pH 6) was modified using solutions (1-5 M) of citric, or hydrochloric, or orthophosphoric acid. Samples with pH values 2-6 were prepared by adding the suitable acidulant and demineralised water in order to reach the desired pH (± 0.2 pH units) in a EY dispersion with a 45 wt% in solids content. Samples were refrigerated overnight at 4°C.

Frequency sweep tests of egg yolk dispersions containing 45 wt% in solids at different pH values were performed by means of an AR-2000 (TA Instruments, UK) using a low-inertia 60 mm aluminium plate-plate geometry with 1 mm gap. As the viscoelastic response is different in the experimental frequency range ($10^{-2} - 10^2$ rad/s), it was subdivided in several intervals overlapping at the ends of the range. Any measurement was carried out at 20 °C and at a stress clearly lower than the critical value for linear viscoelasticity. All samples were placed at room temperature for 30 min before their loading on the sensor. Afterwards, they were sealed with vaseline oil to prevent their drying during the test of samples. At least two replicates were performed for each rheological test.

Results and Discussion

Figure 1 shows stress sweep tests of EY (45 wt%) at 1 rad/s. It can be clearly seen that the three dispersions exhibit a predominantly viscous behaviour at pH 4.

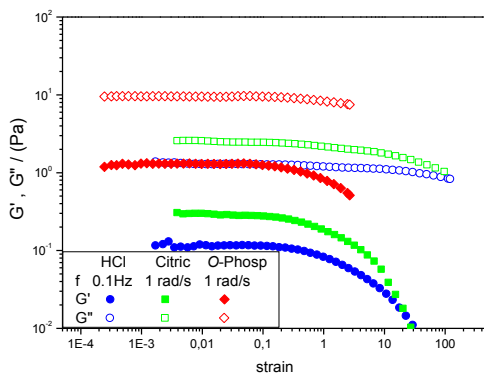


Figure 1. Stress Sweep Tests at 1 rad/s and 2 °C for EY (45 wt%) acidified until pH 4 using different acids.

A wide LVR is always found, although the critical strain depends on frequency, pH and the type of acid (i.e. a slightly smaller value may be observed in Fig. 1 when ortho-phosphoric acid is used).

Figure 2 and 3 compare mechanical spectra of EY dispersions for the three acids studied, at pH 4 and 2, respectively. The native EY (45 wt% at pH 6) is also plotted in both figures as no acid. As may be appreciated the ortho-phosphoric acid leads to moderate changes in the viscoelasticity behaviour of native EY, that become more apparent at low pH.

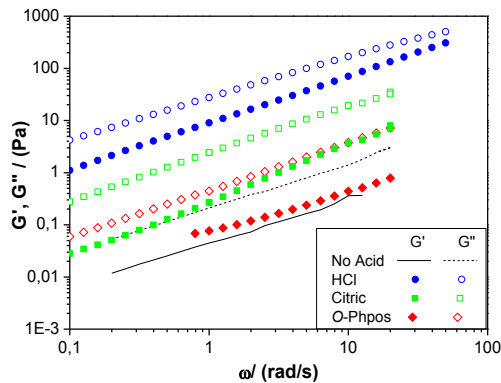


Figure 2. Mechanical Spectra at 20 °C for EY dispersions (45 wt% solids content) acidified until pH4 using different acids.

All the mechanical spectra plotted in Fig. 2 correspond to a predominantly viscous behaviour in which G'' is always higher than G' within the experimental frequency range. This behaviour is typical of relatively dilute dispersions such as that corresponding to the native EY. Only a certain tendency to find a crossover between both moduli at high frequency may be noticed for the HCl-acidified dispersion.

On the other hand, the behaviour shown by EY at pH 2 (acidified by HCl or citric acids) is completely different to that one exhibited at pH 4 or 6. Both acids lead to a gel behaviour characterized by values of G' being much higher than G'' where both functions show almost no dependence on frequency. This behaviour contrasts with the fluid-like behaviour displayed by the dispersion acidified with ortho-phosphoric acid.

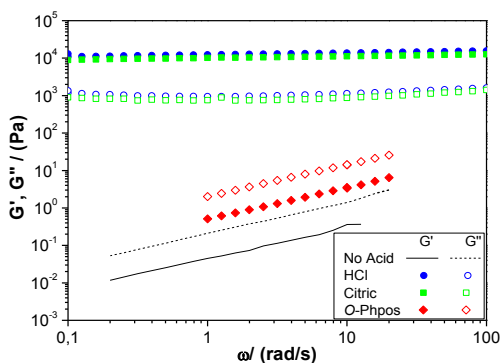


Figure 3. Mechanical Spectra at 20 °C for EY dispersions (45 wt% solids content) acidified until pH 2 using different acids.

Figure 4 shows the evolution of the complex modulus at 1 rad/s (G_1^*) as a function of pH for EY dispersions (45 wt% solids content) using the three acids under study. The higher values of this parameter correspond to the use of hydrochloric acid, whereas the use of *o*-phosphoric acid produced the lower values. In any case, all studied acids exhibit an increase of this parameter as the pH decrease. However, a higher slope can be observed when the pH decrease from moderate pH values (3 or 4) to pH 2. This effect is still more pronounced for citric and hydrochloric acids, for which the complex modulus, G_1^* , increases in several orders of magnitude. However, it should be noted that this evolution doesn't correspond to the original sequence established by Hofmeister, according to which, citric and *o*-phosphoric acid should exhibit a higher structure-making effect, thus limiting the amount of available water to hydrate protein molecules. This difference may be related to the fact that under the experimental conditions, proteins present a positive surface charge, whereas in the original sequence the protein surface was negatively charged. This difference may be regarded as responsible for the inversion of the sequence, which would explain the higher values (except for pH 2) corresponding to hydrochloric acid.

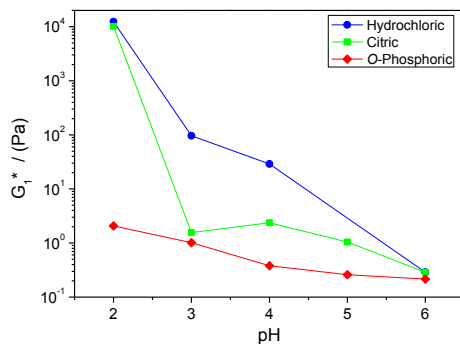


Fig. 4 Evolution of complex modulus values at 1 rad/s for EY (45 wt%) dispersions as function of pH and type of acidulant.

Furthermore, citric acid is hardly dissociated at pH 2 and present low ability to neutralize the charged protein residues, whereas hydrochloric, being a strong acid, is fully dissociated at all pH values. As for the *ortho*-phosphoric acid, it exhibits a unique anionic species in the experimental pH range. The sharply G_1^* profile found for citric acidified EY has to be related to a higher amount of anionic species with increasing pH.

The evolution of loss tangent at 1 rad/s as a function of pH is plotted in Figure 5. As shown, most of the systems under study exhibit a clearly predominant viscous component over the elastic one, with loss tangent values between 3 and 9. However, when the pH value decreases to 2, the values of this parameter fall to values less than one, leading to a predominantly elastic behaviour.

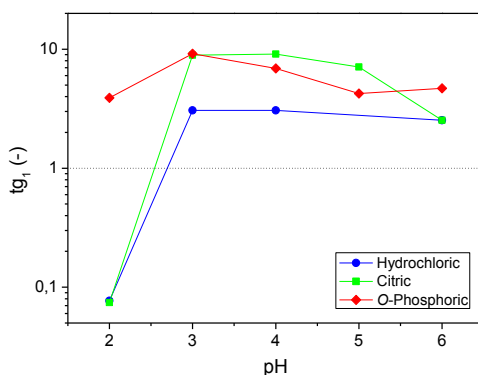


Fig. 5 Evolution of loss tangent values at 1 rad/s for EY dispersions (45 wt%) as a function of pH and type of acid.

Conclusions

The reduction of pH media leads to an increase on the viscoelastic properties of EY dispersions for the three acids under study. This increase depends on the pH value and the type of acid used.

The addition of Hydrochloric or Citric acid showed a similar behaviour leading to a gel formation when the pH 2 is reached. But, this change is more sharply when the citric acid is used, which may be related to the different dissociation pattern of its ionic species.

On the other hand, the addition of *ortho*-Phosphoric acid showed a more moderate dependency of the viscoelastic properties on the pH values, without any sol-gel transition taking place within the experimental pH range. These results suggest that dihydrogen-phosphate anion exerts a certain inhibition effect on the hydrophobic interactions and hydrogen bonds between protein segments.

Acknowledgements

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