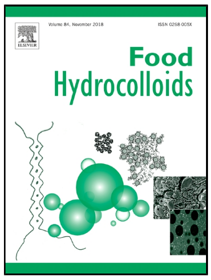
Accepted Manuscript



Heat-induced gelation of egg yolk as a function of pH. Does the type of acid make any difference?

J.M. Aguilar, F. Cordobés, C. Bengoechea, A. Guerrero

PII: S0268-005X(18)30547-2

DOI: 10.1016/j.foodhyd.2018.07.045

Reference: FOOHYD 4574

To appear in: *Food Hydrocolloids*

Received Date: 29 March 2018

Accepted Date: 25 July 2018

Heat-induced gelation of egg yolk as a function of pH. Does the type of acid make any difference?

Aguilar J.M., Cordobés F., Bengoechea C., Guerrero A.

Department of Chemical Engineering, Escuela Politécnica Superior, Universidad de Sevilla. Sevilla (Spain)

---

*A. GUERRERO

Departamento de Ingeniería Química,
Escuela Politécnica Superior, Universidad de Sevilla
Virgen de África, 7
41011 Sevilla (Spain)
E-mail: mfelix@us.es
Phone: +34 954557179; Fax: +34 954556441.

Abstract

The objective of this study was to assess the influence of reducing pH from its native value on the heat-induced gelation behaviour of egg yolk by monitoring its linear viscoelastic properties. Three acids differing in their location in the Hofmeister series were used (hydrochloric, citric, and phosphoric acids) at pH between 2 and 6. The viscoelastic measurements were carried out under small amplitude oscillatory shear, using parallel plate geometry by means of: (i) stress sweep tests to delimit the linear viscoelastic range at different temperatures; (ii) temperature ramp tests to monitor egg yolk protein gelation; and (iii) frequency sweep tests at 20ºC, after the thermal cycle. The microstructure of gels was also evaluated by Cryo-scanning Electronic Microscopy (CryoSEM). Egg yolk undergo dramatic changes in rheological and microstructural properties, when processed at high temperature, depending on pH and the type of acid used. Generally, four different regions take place over heat treatment: (i) a fluid-like region showing a moderate decrease in viscoelastic properties with temperature (ii) a sol-gel transition region involving denaturation, aggregation of protein molecules and association of aggregates to form a gel network; (iii) a plateau region for G' and G'' and (iv) a reinforcement of the gel network through the regeneration of physical interactions during cooling. This pattern may show a strong dependence on pH and the acid involved. Both effects tend to decrease as the thermal treatment proceeds. Heat treatment also reveals large differences in gel microstructure, depending on pH and on the type of acid used.

Keywords: Egg yolk; Linear viscoelasticity; Thermal gelation; Acid gelation; Gel microstructure

1. Introduction

Egg yolk is a well-known multifunctional ingredient in many food products (Kiosseoglou, 2003), as well as in some medical, pharmaceutical, and cosmetic applications (Laca, Paredes, Rendueles & Diaz, 2015). The yolk itself may be regarded as an oil in water emulsion, in which different fat particles are suspended in a protein solution known as plasma (Kiosseoglou, 2003; Pisuchpen Chaim-ngoen, Intasanta, Supaphol & Hoven, 2011). Thus, egg yolk is a complex system integrated by lipids (33 wt%), proteins (17 wt%) and water (50 wt%).

In rheological terms, egg yolk (containing 45 wt% solids) behaves as a non-Newtonian fluid being a slightly shear-thinning material with an apparent viscosity at 1 s^{-1} around 0.4 Pa·s, a flow index around 0.94 and a surface tension around 0.044 $N \cdot m^{-1}$, at room temperature and native pH (Miranda, Partal, Cordobés, & Guerrero, 2002). Being a protein system, it may turn into a gel with higher viscoelastic properties when treated under certain processing conditions, for example by reducing pH. Several protein systems as soy or whey protein have been gelled through a process that involves acidification (Perrechil, Braga & Cunha, 2013; Cavallieri & Cunha, 2008; Ju & Kilara, 1998). The isoelectric point of egg yolk has been reported to be between 5-6 (Navidghasemizad, Temelli & Wu, 2015). Even if most of the research focused on the gelation properties of egg yolk is centred on heat-set gels (Raikos, Campbell & Euston, 2007), the lowering of the pH into the isoelectric point through the addition of an acid might possess a potential cold-set gelation effect on egg yolk.

In this study, three different acids, which are generally used in the food industry, have been used: citric acid, hydrochloric acid and phosphoric acid. Citric acid is an organic weak acid with the following values for the dissociation constants (K_a): $K_{a1} = 7.1 \cdot 10^{-4}$; $K_{a2} = 1.7 \cdot 10^{-5}$; $K_{a3} = 4.0 \cdot 10^{-7}$. Citric acid may be found naturally in fruits and vegetables and could be useful as a mild, nutritionally harmless, and water-soluble gelation agent. Its potential as coagulant in protein systems has already been studied by Cao et al., (2017). Phosphoric acid is a weak inorganic acid (K_{a1} = 7.25·10^{-3}; K_{a2} = 6.31·10^{-8}; K_{a3} = 3.98·10^{-13}) which is mainly used in the production of fertilizers. However, phosphoric is after citric acid the second most widely used acidulant in the food industry, particularly in cola, root beer and other carbonated soft drinks (Dziezak, 2016). Hydrochloric acid on the other hand is a strong common inorganic acidifier used in many industrial applications, including the production of water, beverages, pharmaceuticals and foods. It should be kept in mind that, according to Kunz (2010), protein gelation will be influenced by the different nature of the negatively charged ions present in those acids (e.g. net charge, molecular size).

Previous work has been focused on the gelation properties of several egg yolk dispersions through different methods like thermal processing, addition of polysaccharides (e.g. k-carrageenan) at different pH values, or high-pressure treatment (Cordobés, Partal & Guerrero, 2004; Guerrero, Carmona, Martínez, Cordobés & Partal, 2004; Aguilar, Cordobés, Raymundo, & Guerrero, 2017; Aguilar et al., 2011; Aguilar, Cordobés, Jerez & Guerrero, 2007; Cordobés et al., 2004). The main objective of the present work has been to evaluate the effect of different acids (hydrochloric acid, citric acid, phosphoric acid) on the rheological properties and microstructure of egg yolk over heat-induced protein gelation.

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 (63–73g) commercial specifications, being discarded all damaged or cracked eggs. In order to minimize variations in the composition of eggs, a particular brand was always purchased checking that they were collected from the same laying hen farm through the identification code marked on their shells. Anhydrous citric acid, Hydrochloric acid and Phosphoric acid (analytical grade) were purchased from Panreac (Spain), Merck (Germany) and Panreac (Spain), respectively, and were used as acidifiers.

2.2. Preparation of Egg Yolk dispersions

Chicken eggs were broken by hand and then yolk was carefully collected following a method proposed by Harrison & Cunningham (1986), forming parts of the same batch.

EY solid content wt% was estimated through drying some aliquots at 105±2°C in a temperature-controlled oven at atmospheric pressure for 24 h (Miranda et al., 2002). Native EY was found to contain 50.43±0.8wt% solids.

Native EY pH (close to 6.0) was determined for each sample using a digit 501 pH-meter (Crison, Spain). The initial pH of each aliquote from the sample batch was modified by using the needed amount of the corresponding acid (1 M, 2M, 3M, and 5 M solutions) up to the desired pH value (i.e. 2, 3, 4, 5 or 6), and adding later the amount of demineralised water required to raise a 45 wt% solids content.

2.3. Linear viscoelasticity measurements

Small-amplitude oscillatory shear (SAOS) measurements, including either stress sweep, frequency sweep, were performed by means of controlled-stress rheometer AR2000 (TA Instruments, USA). A 60 mm hard anodized aluminium plate–plate geometry with 1 mm gap was selected for all measurements. In order to reach temperature and structure equilibrium, all the samples were kept for 20 min in the sensor system before running any rheological test. To ensure correct stress control, temperature ramp experiments, performed from 20 to 90 ºC, were carried out into three zones using dynamic viscoelasticity monitoring (Cordobés et al., 2004) at 1.5ºC/min and constant frequency (1 Hz). A shear stress sweep test was previously performed at a frequency of 1 Hz for each region and sample to keep measurements within the linear response regime. Similarly, all the dynamic viscoelasticity frequency sweep measurements (10^{-2}-10^{2} rad/s) were carried out within the Linear Viscoelastic Region (LVR).

2.4. Microstructure

Cryo-Scanning Electron Microscopy (CSEM) was used in collaboration with the Microscopy Service (CITIUS, Universidad de Sevilla), to evaluate the microstructure of egg yolk dispersions and gels samples as a function of pH and the type of acid used. Small pieces of the samples (2–3mm) were frozen in nitrogen slush (−210°C), transferred quickly to the cryo specimen chamber, etched at −90°C for 7 min in order to remove surface ice. Then, samples were gold coated and examined using a ZEISS EVO at −120°C. The microscopy was operated at an acceleration voltage of 8kV with a beam current of 70 pA, a working distance of 6mm, and analyses were carried with ca. 4500× magnification.

2.5. Statistical analysis

The statistical analysis of the results obtained was performed by using t-test and one-way analysis of variance (ANOVA, $p < 0.05$). At least three replicates of each measurement were carried out. Uncertainty for each result was expressed as the standard deviation.

3. Result and discussion

3.1. Thermal-induced gelation of acidified egg yolk

In spite of the strong influence that pH exerts on the complex modulus of egg yolk dispersions before applying heat treatment, a general evolution over the thermal cycle applied can be observed in Figure 1. Four different regions can be distinguished in this figure: (i) The first region is characterised by a decrease in viscoelastic properties (represented by the complex modulus) as the temperature is raised at constant heat rate, reflecting a typical increase in mobility induced by thermal agitation (Rocha, Teixeira, Hilliou, Sampaio & Gonçalves, 2009; Cordobés et al. 2004). This region comes to an end when a minimum in G* appears. (ii) The second region shows a fast sigmoidal evolution (S-shaped) where a dramatic increase in viscoelastic properties takes place, which can rise up to four orders of magnitudes regardless of the acid used. A rather fast sol-gel transition, takes typically place in this region (Clark, Kavanagh, & Ross-Murphy, 2001; Cordobés et al., 2004). In any case, a crossover point between G' and G'', which may be observed in the inserts of Figure 1 at the point where tan δ=1, is often found within this region. In addition, a remarkably fast reinforcement of the gel network can be observed. This effect is more apparent near the IEP and can be related to some crosslinking between protein residues. (iii) In the third region, which is roughly coincident with the isothermal stage, a tendency to a plateau value in G* takes place. (iv) A fourth region comes eventually about, coinciding with the cooling stage. The response of gels in this region has been found to be either reinforcement or weakening of the protein network, depending on pH. When the pH is not far from the IEP the cooling stage brings about an increase in viscoelastic properties related to the recovery of physical interactions (e.g. hydrogen bonds) that lead to a further reinforcement of the gel network (Romero et al. 2009). It is worth mentioning that near the IEP repulsive electrostatic interactions are so weak that they can hardly oppose the effect of attractive interactions. As a result, heat treatment is particularly efficient for gel development near the IEP. On the other hand, the reduction in linear viscoelastic properties observed upon cooling at low pH may be related to an enhancement of electrostatic repulsions that tend to overcome the effect of hydrophobic interactions, eventually leading to the weakening of the gel network.

It is worth mentioning that the above-described general behaviour has been previously explained for globular proteins in terms of a multistage mechanism consisting of thermal induced protein denaturation, followed by aggregation of partially denatured protein molecules and random association of the aggregates formed (Clark et al., 2001). More specifically, this mechanism has been successfully applied to describe heat-induced egg yolk gelation (Aguilar et al. 2017; Cordobés et al. 2004). However, as may be observed in Fig. 1A, 1B and 1C, regardless of the type of acid used, a reduction in pH gives always rise to a reduction in the heat-induce overall reinforcement of the gel. This effect may be explained by the fact that protein surfaces exhibit a larger amount of positive charges giving rise to two effects: an increase in initial consistency caused by the increase in electrostatic interactions and the disturbance that those interactions produce on the development of the gel network. The combination of both effects is particularly dramatic when pH 2 is achieved using hydrochloric acid (Fig. 1A), such that the heat treatment leads to an overall weakening, rather than causing any enhancement, which is in fact the most typical behaviour.

Figure 2 shows the evolution of different parameters extracted from temperature ramp tests as a function of pH, for the three acids studied. More specifically, this figure shows the temperature at which the complex modulus reaches a minimum value (T^*_m) as well as the temperature at which the crossover between G' and G'' ($\tan \delta=1$) takes place (T_c). First of all, it has to be taken into account that the effect of the type of acid on T^*_m and particularly on T_c is completely different at pH 2 as compared to the rest of pH values. Thus, no crossover point has been observed at pH 2 when using hydrochloric or citric acid (i.e. no values for T_c has been obtained). Once this difference has been established, a statistical analysis has been carried out between pH 3 and 6.

The results obtained from the ANOVA test performed between pH 3 and 6 reveal that T^*_m significantly depend on pH ($p<10^{-5}$). On the other hand, the type of acid does not show any significant effect ($p>0.4$) on this parameter. In other words, the reduction of the pH from the native value of egg yolk yields a significant decrease in the value of T^*_m, regardless of the acid used. This effect may be also observed in Figure 1 since an anticipation of the beginning of the second region takes always place upon decreasing pH.

The reduction in pH also leads to a decrease in the crossover temperature, showing similar values for the three acids used, which is confirmed by the results from the ANOVA tests, where the probabilities for the effect of pH and the type of acid are respectively $p<10^{-4}$ and $p>0.3$, respectively.

This effect indicates that egg yolk gelation is anticipated by reducing pH. In fact, as mentioned above, the values of T_c at pH 2 are not shown when hydrochloric or citric acids are used since their elastic properties are already predominant prior to heat processing.

These results reflect the relevance of the electrostatic interactions on the rheology of unprocessed dispersions, which also applies to the early heating stage. Therefore, at low pH, at which electrostatic interactions become stronger, both the beginning of region (ii) and the crossover point are anticipated (i.e. taking place at lower T^*_m and T_c, respectively).

Figure 2 also includes the results obtained for a linear least squares regression for each of these two parameters as a function of pH, in the range from pH 3 to 6. Since no significant differences between the type of acid used was observed in this range, both regressions were carried out using the values obtained for the three acids. The linear equations obtained for both parameters were:

$$T^*_m = 9.49 + 9.43 \cdot pH$$

$$T_c = 47.3 + 5.00 \cdot pH$$

The coefficient of determination (R^2) were 0.98 and 0.96, respectively. These results suggest that the effect of pH is so strong that it masks the influence or the type of acid between 6 and 3.

As for the results obtained at pH 2, the type of acid shows a significant effect, where T^*_m follows the reverse sequence of the Hofmeister series, as has been reported for protein dispersions when the pH is lower than the IEP (Finet, Skouri-Panet, Casselyn, Bonneté & Tardieu, 2004). Thus, the strongest promotion for aggregation corresponds to hydrochloric acid, followed by citric acid with phosphoric being the acid showing the weakest tendency to promote aggregation.

3.2. Linear viscoelasticity behaviour of egg yolk gels

The mechanical spectra of the gels formed upon application of the above-described thermal cycle to egg yolk dispersions are shown in Figure 3A, 3B and 3C, as a function of pH for the three acids. All the mechanical spectra correspond to a well-developed gel network, with G' much higher than G'' within the experimental frequency window and with both functions showing a moderate frequency dependence. However, it may be also noticed that a decrease in pH from the native value leads to a remarkable decrease in rheological consistency of the egg yolk gel network, irrespectively of the acid used. This effect seems to eventually lead to a weakening of the gel network in the case of the lowest pH using hydrochloric acid (Fig. 3A), at which both viscoelastic moduli show a certain trend to reach a crossover at high frequencies. These results confirm the disturbing effect of electrostatic interactions, driven by a reduction in pH, on the formation of the gel network as discussed in the previous section.

3.3. Comparative effect of the type of acid on egg yolk systems

Figure 4 compares the values of some parameters obtained at 1 Hz from the initial value of the temperature ramps (before thermal treatment) and from the thermal-induced egg yolk gels (Fig. 1), as a function of pH for the three acids used. It may be noted that these later values are similar to those obtained from the mechanical spectra of egg yolk gels (Fig. 3)

The parameters plotted are the complex modulus, G_1^* (Fig. 4A), the loss tangent, tan δ_1 (Fig. 4B) and a strengthening index, I_1^* (Fig. 4C), defined from the complex modulus as follows:

$$I_1^* = \frac{G_{1f}^*}{G_{1i}^*} \qquad (1)$$

where G_{1i}^* and G_{1f}^* are the values for the complex modulus at 1 Hz and 20ºC obtained before and after application of the thermal cycle, respectively. A similar definition for this parameter has been previously used (Bengoechea et al. 2010; Aguilar, Jaramillo, Cordobés & Guerrero, 2010). Figure 4A also shows the values of G_{1c}^* obtained from the temperature ramps carried out at 1 Hz (Fig. 1). It may be noticed that these values are obtained always at tan δ_1=1 but correspond to different temperatures as may be inferred from the values of T_c shown in Fig. 2. The values of G_{1c}^* for the hydrochloric or citric acids at pH 2 are not shown since egg yolk already show a gel behaviour under such conditions.

Parameter G_{1i}^* is generally dominated by the viscous component (excepting at pH 2 for hydrochloric and citric acids) and always exhibit a strong dependence on pH, being quite different for unprocessed and thermally processed acidified egg yolk systems (Fig. 4A). In the former case, G_{1i}^* yields an apparent increase after a reduction in the pH value. However, the values of G_{1f}^*, for heat-induced egg yolk gels, which are always dominated by the elastic response, undergo a decrease with a reduction in pH, which may even lead to lower values than those corresponding to unprocessed acidified egg yolk (when hydrochloric or citric acids are used). This divergence in behaviour put forward the different effect of the increase in electrostatic interactions, being positive for the reinforcement of fluid-like structures of unprocessed egg yolk, but negative for the formation of the heat-induced gel network. In addition, it is worth mentioning that the effect of the type of acid on G_1^* is quite prominent for unprocessed egg yolk, particularly for phosphoric acid at low pH, but rather moderate for egg yolk gels. Moreover, this buffering effect is already noticed in region (ii) since the influence of the type of acid on parameter G_{1c}^* is also moderate. In other words, the profile for the crossover point, which roughly marks the thermal induced sol-gel transition, clearly depends on pH but not on the type of acid used, except when HCl or citric acids are used at pH 2 at which EY already exhibits a gel-like behaviour.

Furthermore, as may be observed in Fig. 4B, the effect of the type of acid on tan δ_1 values is again noticeable before heat treatment (particularly at low pH) but is clearly reduced for the heat-induced gels. In addition, the effect of pH on $\tan\delta_{1i}$ or $\tan\delta_{1f}$ is moderate excepting at pH 2. At this pH, a dramatic reduction for unprocessed egg yolk can be found when HCl or citric acids are used as the acidulant. As for the heat processed gels, only a reduction in $\tan\delta_{1f}$ can be observed when hydrochloric acid is used.

The strengthening index also shows a strong dependence on pH, as may be observed in Fig. 4C, where an increase in pH, which involves weaker electrostatic interactions, generally leads to higher values of this parameter. However, in this case the type of acid used also yields remarkable differences, particularly at the lowest pH. Thus, a weakening rather than reinforcement of the protein gel network occurs after the thermal process carried out at pH 2 by using

HCl or citric acids as acidulant (i.e. I_1^* is lower than unity). In contrast, the same thermal cycle, at the same pH, produces a three hundred-fold reinforcement in the gel network by using phosphoric acid.

3.4. Microstructure of acidified egg yolk dispersions and gels

Figure 5 shows the images of egg yolk samples, before and after heat-induced gelation, obtained by Cryo-scanning electron microscopy (Cryo-SEM) at different pH values, achieved with hydrochloric acid. All the images were taken using cryogenically fixed samples with high moisture content (ca. 55%). As may be observed, unprocessed samples (UP-A, UP-B and UP-C) are similar regardless of the pH, showing a random distribution of egg yolk aggregates. Image UP-C, which corresponds to frozen native egg yolk, shows some protein aggregates of approximately 300 nm in size (probably corresponding to yolk granules) that seem to be suspended into the plasma. A decrease in pH from 6.0 to 4.0 seems to give rise to a higher density of clusters. In fact, the increase in positive charges achieved at acidic pH induces electrostatic repulsions that results in the disruption of granules (Anton 2007). In addition, protein denaturation may also occur as pH is being reduced, leading to an increase in aggregate size, as well as an enhancement of the protein-aggregate network, particularly when a decrease in pH from 4.0 to 2.0 (UP-B and UP-A) takes place. This enhancement is far more apparent when comparing linear viscoelastic properties as a function of pH at the start-up of thermal processing at 15°C (Fig. 1A). It is worth bearing in mind that egg yolk samples are liquid dispersions at pH 4.0 and 6.0, whereas they show gel behaviour when using hydrochloric acid at pH 2.0 (Guerrero et al. 2004).

In any case, heat treatment always produces a dramatic change in the microstructure of native egg yolk or acidified yolk using hydrochloric acid, which is also reflected on the viscoelastic properties of the gel obtained (Fig. 1A), particularly at pH 4 and 6. It is worth mentioning that the image observed at pH 2 (Fig. 5TP-A) is associated to a weaker gel microstructure than those observed at pH 4 (Fig. 5TP-B) and pH 6 (Fig. 5TP-C). The former image (TP-A) seems to correspond to a particulate gel network (i.e. formed by association between 1-3 μm protein particles). At the other end, image TP-C shows how small protein aggregates self-assemble into a three-dimensional network, also incorporating some protein particles. Image TP-B reflects an intermediate microstructure between the two systems, alternating zones with protein particles and self-assembling aggregates.

Figure 6 shows Cryo-SEM images of unprocessed egg yolk dispersions and heat-processed gels at pH 4 using different acids. Images for unprocessed samples suggest that a change in the acid type form hydrochloric to citric leads to an increase in the size of aggregates, but also to a wider distribution of sizes. Similarly, a change from citric to phosphoric acid gives rise to a further increase in aggregate size and to a wider aggregate size distribution. This evolution may be related to the decrease in the complex modulus at the start-up (15°C) of thermal processing observed in Fig. 1a, 1b and 1c. In any case, the microstructure of the three unprocessed samples does not differ to a large extent from each other, thus explaining why they show similar fluid-like behaviour (data not shown).

However, it is worth mentioning that thermal processing may induce large differences on microstructure depending on the type of acid used. Thus, thermal

ACCEPTED MANUSCRIPT

processing induces a dramatic change in microstructure when hydrochloric acid was used. However, cryo-SEM images only reflect moderate microstructural changes when citric or phosphoric acid was used. Hence, it seems that citric or phosphoric anions (being larger than hydrochloric anion) interfere on the growth of aggregates, preventing formation of large protein particles. In this way, the protein network formed is quite different from that obtained in hydrochloric medium. In the former case, the protein network has been described above in terms of a combination of protein particles and self-assembling aggregates. The later protein gel networks rather consist of a three-dimensional association of clusters (average size about 300 nm). A disruption of Low Density Lipoproteins and a subsequent rearrangement into clusters of this size has been reported to be a consequence of heat treatment (Anton, Martinet, Dalgalarrondo, Beaumal, David-Brian, Rabesona, 2003). It may be noted that a few particles (about 2 μm in size) may be also formed during heat processing.

It is also worth pointing out that in spite of the difference found in microstructure, the viscoelastic properties of the final gels are rather independent on the type of acid used. This similarity may be expected when using citric or phosphoric acids, but is quite striking for hydrochloric acid, taking into account the dramatic different found in the morphology of the gel.

4. Concluding remarks

From the results obtained in this study it is apparent that pH exerts a strong influence on the linear viscoelastic properties of egg yolk. However, the evolution of these LVE properties upon heating can be generally described by a similar pattern. Thus, heat processing generally yields an initial moderate decrease in the VLE moduli, driven by thermal agitation, which is followed by a dramatic increase that reflects a remarkable strengthening of the protein gel network. The subsequent isothermal region gives rise to a plateau value for each pH, whereas the cooling stage may lead to either a reinforcement or a weakening of the gel network, depending on pH.

Heat processing is particularly efficient for gel development when electrostatic interactions are weak (i.e. near the IEP) such that hydrophobic interactions are predominant. As pH is reduced from the IEP, electrostatic interactions among positively charged protein surfaces become progressively more relevant, leading to an apparent increase in the linear viscoelastic properties of egg yolk which may be even dramatic, depending on pH and the acid anion used. At the isothermal stage the sequence of the complex modulus is reverted, leading to a decrease as pH of egg yolk is reduced from its native value (i.e. near the IEP), which may be associated to the disturbing effect exerted by electrostatic repulsions on protein gelation. The cooling stage typically brings about an enhancement of linear VLE properties, related to the recovery of physical interactions (e.g. hydrogen bonds), which is clear near the IEP. However, this effect is counterbalanced and even reverted at low pH, when electrostatic repulsions are dominant. This being the case at pH 2 achieved with hydrochloric or citric acids, heat processing seems to impair the acidic-induced egg yolk gel network.

Heat processing may also induce large differences on microstructure, particularly when hydrochloric acid is used to reduce pH. However, such differences are not apparently reflected on the linear VLE properties of the final egg yolk gels.

In summary, the type of acid used exerts a remarkable influence on the viscoelastic properties of egg yolk at room temperature. This influence is still apparent at the first stages of thermal gelation of egg yolk but progressively tends to decrease as the thermal treatment proceeds.

Acknowledgements

This work has been part of a research project No. TEP-6134 supported by the Spanish by the CICE (Andalousian Government, Spain). The authors gratefully acknowledge their financial support. The authors also acknowledge to the Microscopy Service from CITIUS (Universidad de Sevilla) for providing full access and assistance to the CryoSEM equipment.

References

Aguilar, J.M., Batista, A.P., Nunes, M.C., Cordobés, F., Raymundo, A., Guerrero, A. (2011). From egg yolk/κ-Carrageenan dispersions to gel systems: Linear viscoelasticity and texture analysis. Food Hydrocolloids, 25, 654-658.

Aguilar, J.M., Cordobés, F., Jerez, A., Guerrero, A. (2007). Influence of high pressure processing on the linear viscoelastic properties of egg yolk dispersions. Rheologica Acta, 46, 731-740.

Aguilar, J.M., Jaramillo, A., Cordobés, F., Guerrero, A. (2010). Influencia del pocesado térmico sobre la reología de geles de albumen de huevo. Afinidad, 47 (545), 28-32.

Aguilar, J.M., Cordobés, F., Raymundo, A., Guerrero, A. (2017). Thermal gelation of mixed egg yolk/kappa-carrageenan dispersions. Carbohydrate Polymers, 161, 172-180.

Anton, M. (2007). Low-density Lipoproteins (LDL) or Lipovitellenin Fraction. In Huopalahti, R. & López-Fandiño, R. & Anton, M. & Schade, R. (Eds.), Bioactive Egg Compounds (pp. 1-24), Springer Verlag.

Anton, M., Martinet, V., Dalgalarrondo, M., Beaumal, V., David-Briand, E., Rabesona, H. (2003). Chemical and structural characterisation of low-density lipoproteins purified from hen egg yolk. Food Chemistry, 83 (2), 175-183.

Bengoechea, C., Romero, A., Aguilar, J.M., Cordobés, F., Guerrero, A. (2009). Influence of pH and droplet size distribution of highly concetrated O/W crayfish flour-based emulsions. Food Hydrocolloids, 23 (2), 244-252.

Cao, F.H., Li, X.J., Luo, S.Z., Mu, D.D., Zhong, X.Y., Jiang, S.T., Zheng, Z., Zhao, Y.Y. (2007). Effects of organic acid coagulants on the physical properties of and chemical interactions in tofu. LWT Food Science and Technology, 85, 58-65.

Cavallieri, A.L.F., Cunha, R.L. (2008). The effects of acidification rate, pH and ageing time on the acidic cold set gelation of whey proteins. Food Hydrocolloids, 439-448.

Clark, A. H., Kavanagh, G. M., Ross-Murphy, S. B. (2001). Globular protein gelation - theory and experiment. Food Hydrocolloids, 15, 383-400.

Cordobes, F., Partal, P., Guerrero, A. (2004). Rheology and microstructure of heat-induced egg yolk gels. Rheologica Acta, 43(2), 184-195.

Dziezak J.D., Acids: Natural Acids and Acidulants. In Encyclopedia of Food and Health. Caballero B., Finglas P.M. and Toldrá F. (eds.), Academic Press (2016), pp. 15–18.

Finet, S., Skouri-Panet, F., Casselyn, M., Bonneté, F., Tardieu, A. (2004). The Hofmeister effect as seen by SAXS in protein solutions. Current Opinion in Colloid & Interface Science, 9(1-2), 112-116.

Guerrero, A. Carmona, J.A., Martínez, I., Cordobés, F., Partal, P. (2004). Effect of pH and added electrolyte on the thermal-induced transitions of egg yolk. Rheologica Acta 43, 539-549.

Harrison, L.J., Cunningham, F.E. (1986). Influence of salt on properties of liquid yolk and functionality in mayonnaise. Poultry Science, 65 (5), 915-921.

Ju, Z.Y., Kilara, A. (1998). Gelation of pH-Aggregated Whey Protein Isolate Solution Induced by Heat, Protease, Calcium Salt, and Acidulant. Journal of Agriculture and Food Chemistry, 46, 1830-1835.

Kiosseoglou V. (2003). Egg yolk protein gels and emulsions. Current Opinion in Colloid and Interface Science, 8, 365–370.

Kunz, W. (2010). Specific ion effects in colloidal and biological systems. Current Opinion in Colloid & Interface Science, 15, 34-39.

Laca, A., Paredes, B., Rendueles, M., Diaz, M. (2015). Egg yolk plasma: Separation, characteristics and future prospects. LWT - Food Science and Technology, 62, 7–10.

Miranda, J., Partal, P., Cordobés, F., Guerrero, A. (2002). Rheological characterization of egg yolk processed by spray-drying and lipid-cholesterol extraction with carbon dioxide. Journal of the American Oil Chemists Society, 79 (2), 183-190.

Navidghasemizad, S., Temelli, F., Wu, J. (2015). Phase separation behavior of egg yolk suspensions after anionic polysaccharides addition. Carbohydrate Polymers, 117, 297-303.

Perrechil, F.A., Braga, A.L.M., Cunha, R.L. (2013). Acid gelation of native and heat-denatured soy proteins and locust bean gum. International Journal of Food Science and Technology, 48, 620-628.

Pisuchpen, T., Chaim-ngoen, N., Intasanta, N., Supaphol, P., Hoven, V.P. (2011). Tuning hydrophobicity and water adhesion by electrospinning and silanization. Langmuir, 27 (7), 3654–3661.

Raikos, V., Campbell, L., Euston, S. (2007). Rheology and texture of hen's egg protein heat-set gels as affected by pH and the addition of sugar and/or salt. Food Hydrocolloids, 21 (2), 237-244.

Rocha, C., Teixeira, J.A., Hilliou, L., Sampaio, P., Gonçalves, M.P. (2009). Rheological and structural characterization of gels from whey protein hydrolysates/locust bean gum mixed systems. Food Hydrocolloids, 23 (7), 1734-1745.

Romero, A., Cordobés, F., Puppo, M.C., Villanueva, A., Pedroche, J., Guerrero, A. (2009). Linear viscoelasticity and microstructure of heat-induced crayfish protein isolate gels. Food Hydrocolloids, 23 (3), 964-972.

Figures captions

Figure 1. Evolution of the linear viscoelastic complex modulus over heat-induced gelation of egg yolk at different pH values using different acids: **(A)** Hydrochloric acid; **(B)** Citric acid; **(C)** Phosphoric acid. The line represents the thermal cycle applied. The inserts plot the values of $\tan\delta$ as a function of temperature obtained in the heating part of the cycle (between 50 and 90ºC). The point at which $\tan\delta$ equals unity corresponds to the crossover point (T_c).

Figure 2. Influence of pH and the type of acid (hydrochloric, citric and phosphoric) on the temperature at the minimum complex modulus (T^*_m) and at the crossover point (T_c) obtained from temperature ramp tests. The lines correspond to a linear regression obtained by fitting the values of T^*_m and T_c for the three acids, between pH 3 and 6.

Figure 3. Linear viscoelastic moduli of thermally processed egg yolk gels at 20ºC, as a function of frequency and pH using different acids: **(A)** Hydrochloric acid; **(B)** Citric acid; **(C)** Phosphoric acid.

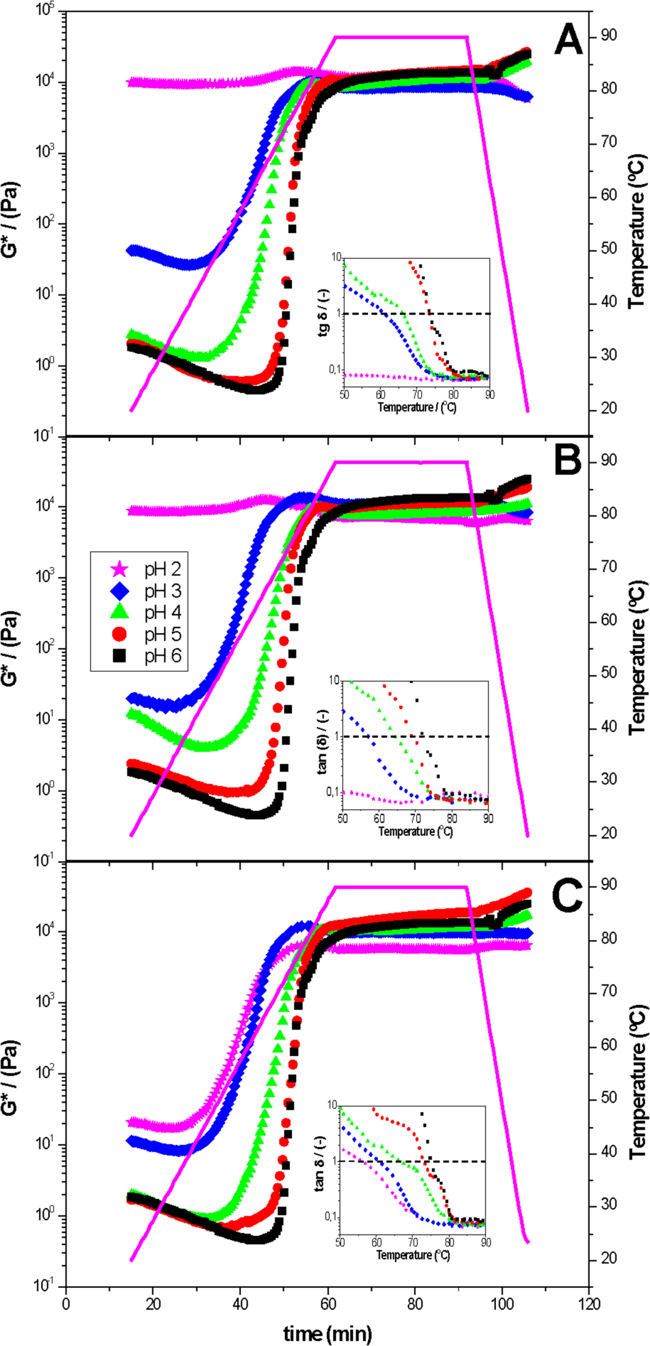
Figure 4. Linear viscoelastic parameters obtained over thermal processing of egg yolk systems at 1 Hz, as a function of pH using different acids: **(A)** G^*_1 before, G^*_{1i}, and after heat treatment, G^*_{1f}, at 20ºC, as well as the thermal-induced crossover point, G^*_{1c}; **(B)** $\tan\delta_1$ before, $\tan\delta_{1i}$, and after heat treatment, $\tan\delta_{1f}$, at 20ºC; **(C)** thermal-induced strengthening index, I^*_1.

Figure 5. CryoSEM images of unprocessed (UP) and thermally processed (TP) egg yolk systems at different pH values, using hydrochloric acid: **(A)** pH 2; **(B)** pH 4; **(C)** pH 6.

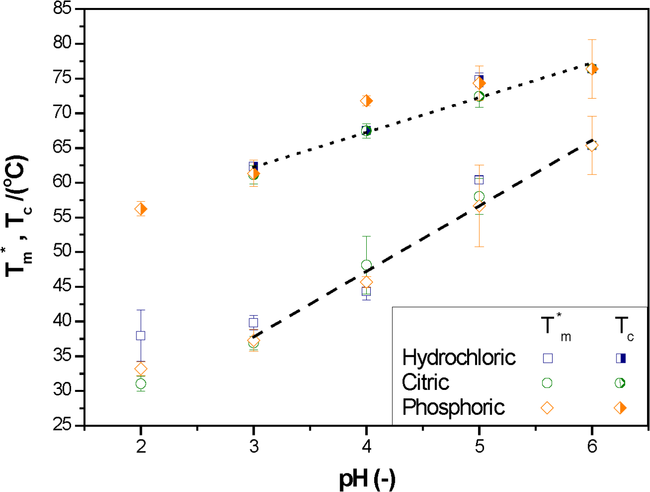
Figure 6. Cryo-SEM images of unprocessed EY dispersions (UP) and thermally-processed (TP) EY gels at pH 4, using different acids: **(A)** hydrochloric acid; **(B)** Citric acid; **(C)** Phosphoric acid.

[Highlights]

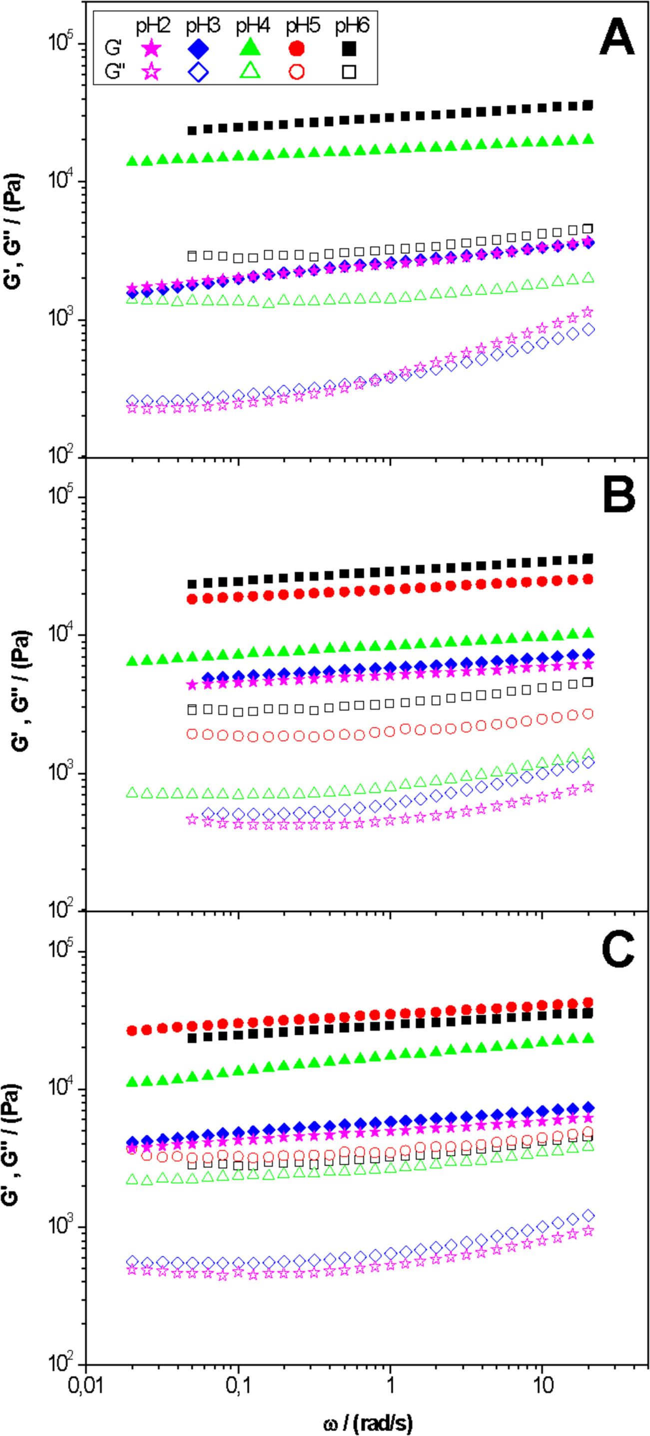
- Egg yolk dispersions may form a strong gel at pH 2 depending on the type of acid
- The thermal gelation process is highly dependent on pH and the type of acid used
- Viscoelastic moduli are strongly affected by pH and acid type before heat processing
- The effect of pH or acid type tends to decrease as the thermal treatment proceeds
- The heat cycle at pH 2 with hydrochloric or citric acid slightly impairs gel strength



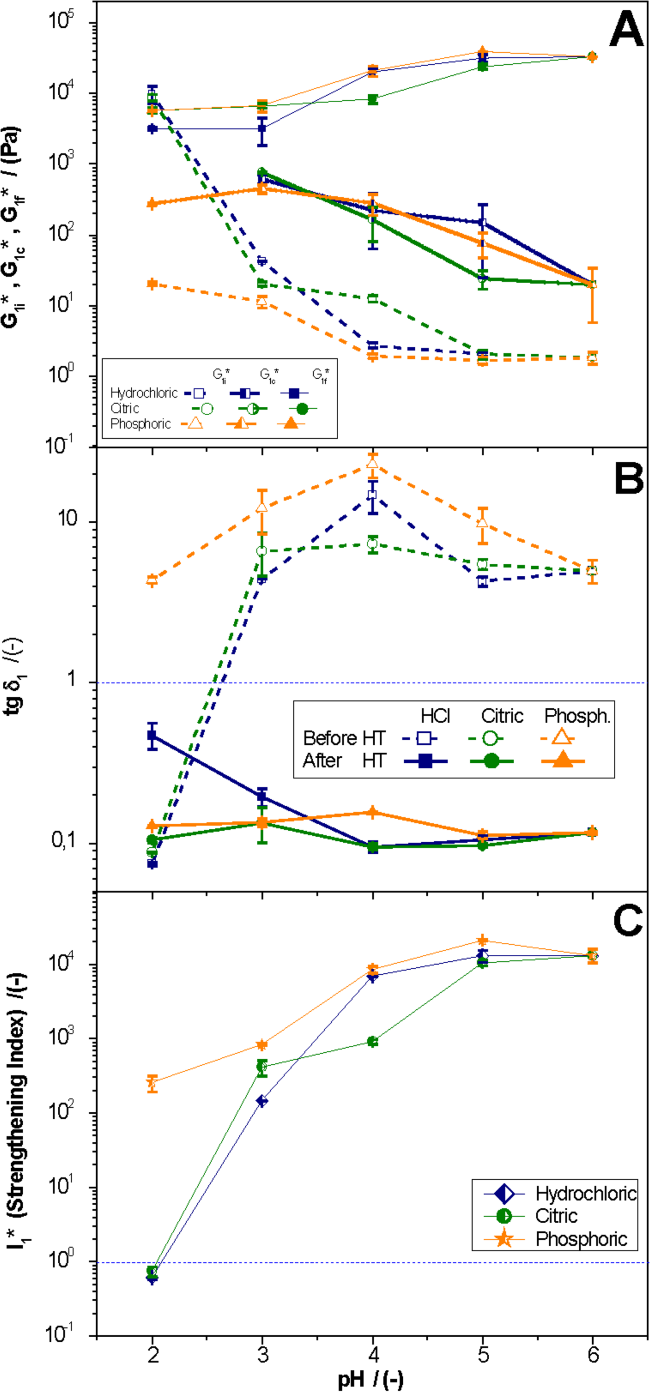
A
B
C
G* / (Pa)
Temperature (ºC)
time (min)
tg δ / (-)
tan (δ) / (-)
tan δ / (-)
Temperature / (°C)
Temperature (°C)
pH 2
pH 3
pH 4
pH 5
pH 6



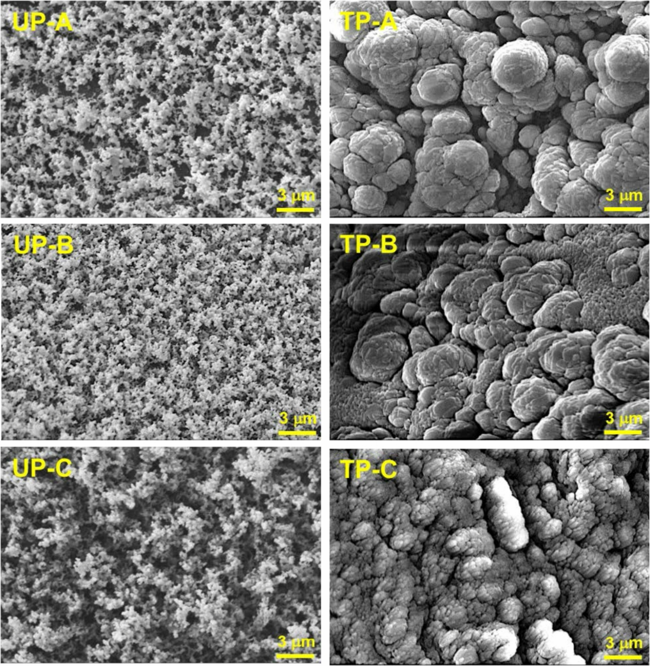
85
80
75
70
65
60
55
50
45
40
35
30
25
2
3
4
5
6
pH (-)
T*m , Tc /(ºC)
T*m
Tc
Hydrochloric
Citric
Phosphoric

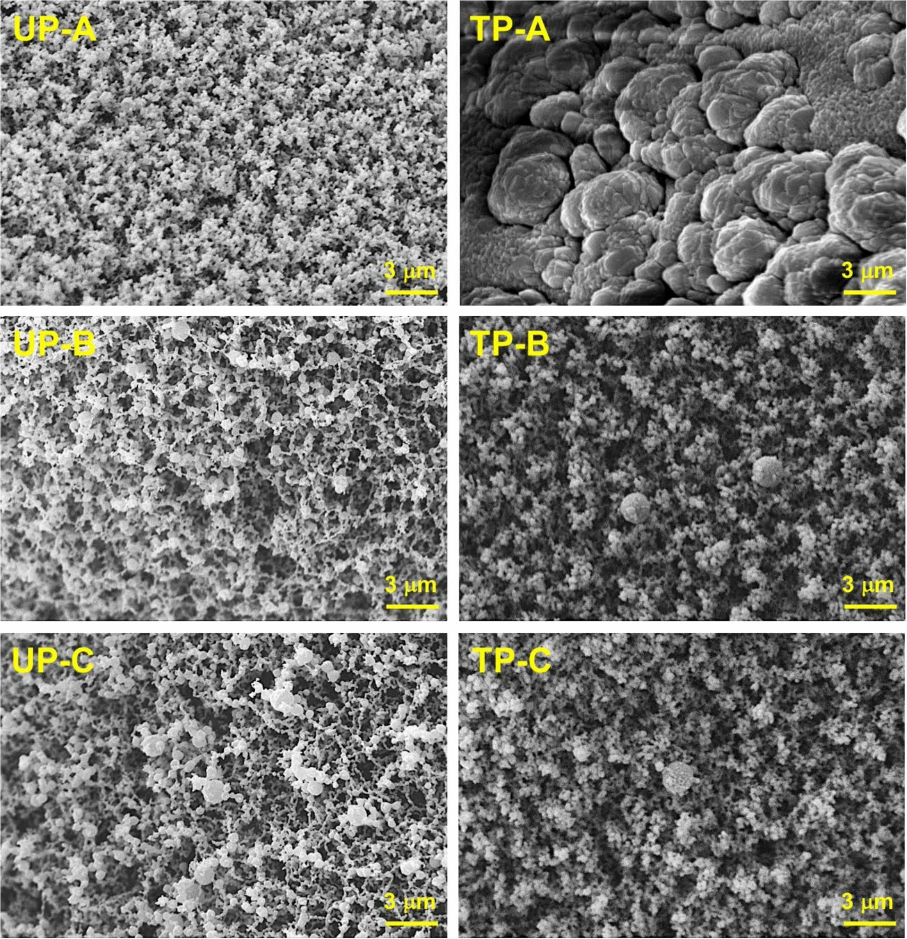


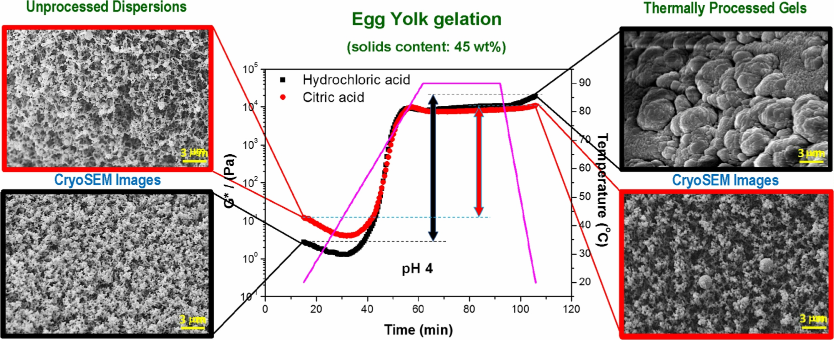
pH2
pH3
pH4
pH5
pH6
G'
G''
A
B
C
G' , G'' / (Pa)
10^5
10^4
10^3
10^2
0,01
0,1
1
10
ω / (rad/s)



A
B
C
G_{1i}^*, G_{1c}^*, G_{1f}^* / (Pa)
G_{1i}^* G_{1c}^* G_{1f}^*
Hydrochloric
Citric
Phosphoric
tg δ_1 /(-)
HCl Citric Phosph.
Before HT
After HT
I_1^* (Strengthening Index) /(-)
Hydrochloric
Citric
Phosphoric
pH / (-)


UP-A
3 μm
TP-A
3 μm
UP-B
3 μm
TP-B
3 μm
UP-C
3 μm
TP-C
3 μm


UP-A
3 μm
TP-A
3 μm
UP-B
3 μm
TP-B
3 μm
UP-C
3 μm
TP-C
3 μm


Unprocessed Dispersions
Egg Yolk gelation
(solids content: 45 wt%)
Thermally Processed Gels
Hydrochloric acid
Citric acid
CryoSEM Images
CryoSEM Images
pH 4
G* / (Pa)
Temperature (°C)
Time (min)
3 μm
3 μm
3 μm
3 μm