The complex mechanism of HM pectin gelation: a rheological investigation

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ABSTRACT

Several biopolymers are widely employed in food, pharmaceutical and biomedical sectors by virtue of their ability to generate supramolecular structures, typically physical hydrogels. In the case of high methoxyl pectins (HMP) the gel formation is promoted by the presence of cosolutes (sugars or polyols) and low pH (≤ 3). The present investigation regards the structuring kinetics of aqueous HMP solutions having different polymer concentration and equal glucose content at 20°C. A sequence of consecutive frequency sweep was applied to each sample immediately after its preparation. The time evolution of the linear viscoelastic behavior is described by the sigmoidal profiles of both moduli at each applied frequency and more thoroughly defined through the change of the mechanical spectrum, i.e. the variation of the parameters of the generalized Maxwell model or the Friedrich-Braun model which are both suitable to provide a satisfactory data fitting.

INTRODUCTION

Pectin is a natural branched and heterogeneous polysaccharide obtained from citrus peel and apple pomace under mildly acidic conditions. It is mainly constituted by a linear sequence of 1,4-linked α-D-galactopyranosyluronic acid. In some regions of the backbone, rhamnose residues are present that confer flexibility to the polysaccharide chain introducing a kink to the straight chain and interrupting the galacturonic acid sequence.

Some of the carboxyl group of the uronic acids are esterified with methanol and, depending on their esterification degree (DE), pectins can be classified as high methoxy (HM), having 50% or higher DE, or low methoxy (LM), having 50% or lower DE. The DE, also, determines the gelification behavior of the polysaccharide.

In the case of LM pectin, the gel formation is ionic-mediated through divalent cations, the most relevant being the Ca^{++} and it occurs in a relatively broad pH value (from 2.6 to 7). The proposed mechanism for LM pectin gelation is the so-called “egg-box model” (Grant, Morris, Rees, Smith & Thom, 1973) in which the positive ion lies in the cavity formed by two adjacent pectin ribbons.

HM pectin forms a 3D network at acid pH (lower than 3.5) only in the presence of large amounts of sugars or other co-solutes and its complex gelation mechanism, not yet fully understood, involves the formation of junction zones, formed by two or more polymer chains (Saha & Bhattacharya, 2010) and stabilized by hydrophobic interactions and H-bonds (Oakenful, 1991). A thorough revision of the factors involved in the mechanism of HM pectin gelation is reported in (Agoub, Giannouli, & Morris, 2009).

Sucrose is one the most common additives used for pectin gelification and together they have found large application in food preparation and industry (Bulone, Giacomazza, Manno, Martorana & San Biagio, 2010).

The effect of sucrose has been explained in terms of “preferential hydration” (Lee & Timasheff, 1985) consisting in a heterogeneous distribution of the solvent components around the macromolecules, thus promoting and favoring the crosslinking formation among polymer chains and leading to macroscopic gelation. The preferential hydration theory can explain much experimental data on sucrose effects on protein functionality, but it fails in some cases where there is a tendency to favor the exposure of hydrophobic surfaces (Semenova, Antipova & Belyakova, 2002; Baier & McClements, 2005) that could be attributed to a direct interaction between sucrose and protein.

In the more recent years an attempt to unify the gelation phenomena observed in short-range attractive colloidal mixtures and physical-gel forming systems has been done invoking the glass transition theory. On the basis of this theory the kinetic arrest of the system is due to the achievement of an extremely high viscosity of the surrounding medium, in which the macromolecules are immersed, because of the increase of the co-solute concentration or water content decrease (Del Gado, Fierro, de Arcangelis & Coniglio, 2003).

The glass transition mechanism has been implicated in the anhydrobiosis phenomenon found in several plants, microorganisms and insects. They accumulate large amount of sucrose, to be able to survive, for an extended period, uncomfortable environmental conditions such as too low or too high temperatures and water deficiency in desert or polar regions (Patist & Zoerb, 2005; Watanabe, 2006). These organisms enter into a biological state where their activity is almost completely

suspended, thus preserving the proteins and other biological structures, as long as environmental conditions remain deleterious, and then resume their normal activity (Lerbret et al., 2007).

The present work regards the gelation kinetics of aqueous HMP solutions having different polymer concentration and equal sucrose content. The development of a network structure in gelling systems is usually monitored by oscillatory measurements at constant frequency during temperature ramps at constant heating or cooling rate. An example regarding HM pectins is reported in (Evageliou, Richardson, & Morris, 2000). In this study, we used time resolved mechanical spectroscopy which can represent a more suitable and effective technique on condition that the kinetics is sufficiently slow. So doing, the self-assembly pathway of the polymer can be appropriately described through the time evolution of its mechanical spectra. Both the generalized Maxwell (gM) and the Friedrich-Braun (FB) models provide satisfactory fitting quality along the whole assembly process and far ali the systems examined.

MATERIALS AND METHODS

2.1 Sample preparation

Slow-set high methoxyl pectin (esterification degree 64.5%) was a kind gift from Hercules Inc. (Wilmington, DE). Water was Millipore Super Q filtered with 0.22 ím filters. All other chemical compounds used were analytical grade from Merk Co. A stock solution of pectin at the concentration of 3% w/w was preliminarily prepared by dissolving the powder in pure water at 100 °C for 10 minutes in a high-speed mixer. The solution was then cooled at room temperature overnight, stored at 4 °C, and used up to 2 or 3 days. Samples at different pectin concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.76% w/w) and constant sucrose amount (58% w/w) were prepared by heating at 100 °C, while stirring, the potassium citrate buffer (pH 3.4) with sucrose and the right water quantity. The effects of acid pH and temperature on the rate of sucrose mutarotation have been described by Leininger and Kilpatrick (1938) and Guggenheim and Wiseman (1950). The stock pectin solution was then added at the appropriate amount and boiled for few minutes. quantity. The final buffer concentration was 12 mM. Finally, a few microliters of 50% acid citric solution was added to adjust the pH value to 3.1. All samples were filtered at 80 °C through 0.45 μm Corning filters directly into the rheometry.

2.2 Rheological measurements

The rheological monitoring of the gelation process was performed at 20°C with a rotational

controlled stress rheometer (AR-1000, TA Instruments, UK), equipped with a standard-size double concentric aluminium cylinder (rotor outer radius 21.96 mm, rotor inner radius 20.38 mm, stator outer radius 20.00 mm, cylinder immersed height 59.50 mm, gap 500 μm). A circulating bath thermostat was used for temperature control.

A series of consecutive frequency sweeps (logarithmic sequence of increasing values from 0.02 up to 30 Hz, time needed per sweep: 12 min) was carried out at constant strain amplitude ($4x10^{-3}$) for very prolonged times, ranging from 1400 min (for system 0.76%) to 10400 min (for system 0.1%). The air/sample interface was coated with silicon oil to avoid sample evaporation.

Time-resolved mechanical spectroscopy allows a correct probing of the viscoelastic behavior of transient materials as long as the structural changes occur sufficiently slow in comparison to the experimental time Δt, i.e. the period of the oscillatory shear. In such conditions the shear stress response is still nearly sinusoidal during Δt and correct values of the viscoelastic moduli can still be evaluated. According to the criterium suggested by Mours and Winter (Mours & Winter, 1994), linearity condition is ensured for small values of the dimensionless mutation numbers N_{mu} evaluated for both G' and G" (Winter, Morganelli & Chambon, 1988):

$$N'_{mu} = \frac{2\pi}{\omega G'} \frac{\partial G'}{\partial t}, N''_{mu} = \frac{2\pi}{\omega G''} \frac{\partial G''}{\partial t} \leq 0.15 \qquad (1)$$

In all our tests such a condition was largely satisfied even at the lowest frequencies and the highest concentration which correspond to the smallest experimental times ($t_{exp}=2\pi/\omega$) and the fastest rate of moduli increase, respectively.

RESULTS AND DISCUSSION

The kinetics of the gelation process can be described with acceptable accuracy by the time evolution of the linear viscoelastic moduli determined at the same frequency throughout the whole series of frequency sweeps. If they are plotted in a log-log plot, similar sigmoidal profiles are derived for both moduli at any frequency and for all the systems examined.

Figure 1 A reports the results obtained at five different frequencies for the system 0.5%. It can be easily noticed that at every frequency the elastic component (G') is initially lower than the viscous one (G"), whereas it increases more rapidly than G" in the following times, then passing through a crossover point and eventually tending to an asymptotic value which is almost independent of frequency. At lower frequencies the crossover condition is attained after shorter times, in spite of

the larger distance between the two moduli in the initial state. This is because the structural buildup of the system gives origin to a progressive conversion from liquid-like to gel-like behavior and then a larger and faster increase must be exhibited by the elastic component G'. As it is shown in Fig. 1B, such a conversion becomes more pronounced and faster with increasing polymer concentration and, consequently, the crossover point is recorded at shorter times.

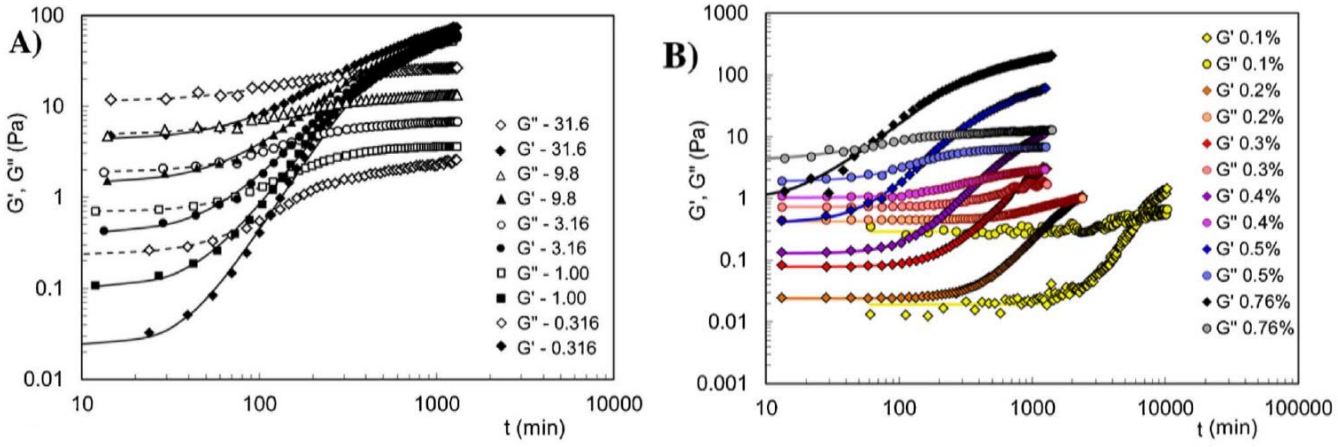

Fig. 1 – Rheological profile of pectin solutions vs time. (A) Time evolution of the viscoelastic moduli at five different frequencies (in rad7s) for system 0.5% w/w. Lines in the figure represent the best fits of the experimental data accordingly with Eq. 2). (B) Time evolution of the viscoelastic moduli at 3.16 rad/s for all the explored concentrations. Lines in the figure represent the best fits of the experimental data accordingly with Eq. 2)

The time evolution of both moduli at constant frequency can be described with good approximation with the following expression:

$$G(t) = G_0 + (G_\infty - G_0) \cdot (t / t_c)^n / (1 + (t / t_c)^n) \qquad (2)$$

This relationship provides better data fitting than those obtained with the Avrami equation and other models suitable to describe similar process kinetics as in the case of crystallization processes in semicrystalline polymers (Supaphol, 2001).

Eq. 2 can be profitably used to calculate the *G'* and *G''* values at any time and all the experimental frequencies considered and, then, to individuate exactly the crossover times t_{cr} (*G'* = *G"*). Even more interesting is to generate 'isochronous' mechanical spectra in order to describe correctly their time evolution along the process.

G_0 and G_∞ are the initial (t = 0) and asymptotic ($t \rightarrow \infty$) values, respectively, and their difference G_∞ - G_0 represents the maximum increase attainable for each modulus along the structural process. The characteristic time t_c corresponds to the mean value between the two extremes, whereas the

exponent n measures the rate of modulus increase.

Obviously, initial and asymptotic moduli increase with increasing frequency and concentration *c*. As it is shown in Fig. 2, within a limited interval (two decades) their frequency dependence is satisfactorily described by a power law equation, whose exponent is higher for initial moduli than for asymptotic ones as expected for a system approaching to the gelling point.

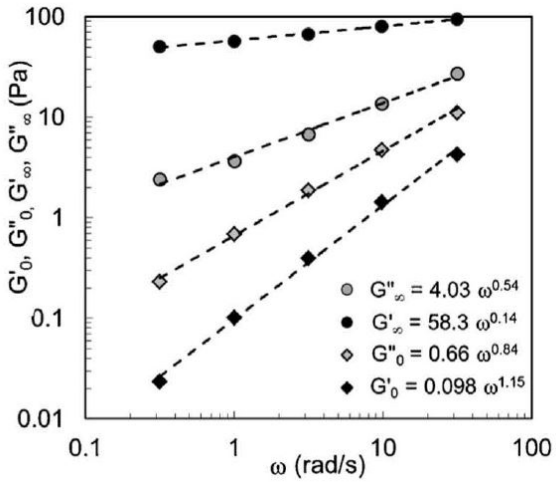

Fig. 2 – Determination of the limiting rheological values. Frequency dependence of G'_0, G'_∞, G''_0 and G''_∞ values for system 0.5% w/w. Lines in the figure are the best fits of the data

At constant frequency, G'_0 and G'_∞ increase with polymer concentration according to a power law equation whose exponent is higher for G'_∞. The concentration dependence of G''_0 and G''_∞ is given by an exponential relationship A exp (B *c*), where for both moduli the parameters A and B increase and decrease with ω, respectively.

The crossover time can be easily individuated at every frequency and concentration by using the sigmoidal model for describing the time evolution of both moduli. Its value slightly increases with increasing frequency while it decreases significantly with polymer concentration *c* according to power laws whose exponents are 0.17±0.06 and -2.55±0.10, respectively.

The frequency dependence of the crossover time clearly confirm that the criterion proposed by Tung and Dynes cannot serve to assess the sol-gel transition of a system and must be replaced by other rheological criteria (Lapasin, 2015).

According to Winter and Chambon (Winter & Chambon, 1986) (Chambon & Winter, 1987), at the gel point the dynamic moduli are parallel with the same power-law frequency or, equivalently, the same value of tan δ is attained over an extended frequency interval (two or more decades).

Alternatively, the sol-gel transition is associated to the onset of an asymptotic non-zero G' for $\omega \rightarrow 0$, which can be assumed as a measure of the gel rigidity and will hereafter indicated as the equilibrium modulus G_e (Djabourov, 1991).

Following the Winter-Chambon criterion, for each system the gel time has been determined with sufficient approximation from the common intersection of the tan δ vs time curves obtained at different frequencies. Its value is inversely proportional to the square of pectin concentration.

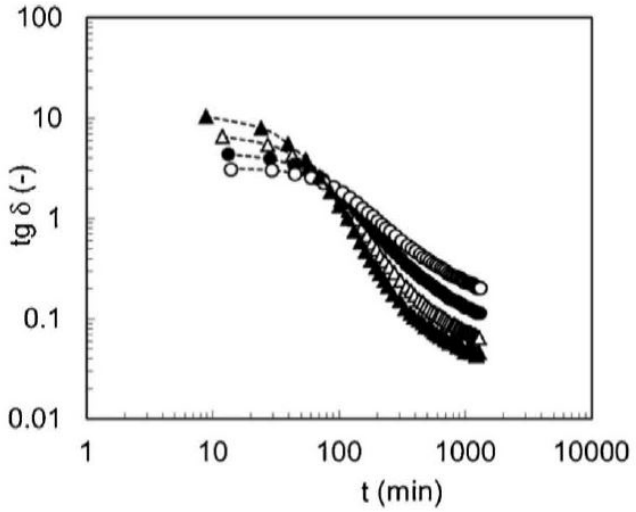

Fig. 3 – Loss tangent evolution. Time behavior of the loss tangent at different frequencies: 0.316 (black triangles), 0.98 (white triangles), 3.16 (black circles) and 9.8 rad/s (white circles) for system 0.5% w/w

Three prerequisites must be satisfied in order to obtain a reliable estimation of the equilibrium modulus through the extrapolation of the storage modulus values to zero frequency and hence to apply correctly the other criterion suggested for individuating the sol-gel transition. The experimental data must cover a sufficiently wide frequency range and this condition is reasonably satisfied. Secondly, a suitable viscoelastic model must be selected to correlate the frequency dependence of both moduli with good approximation. Furthermore, each experimental data set to be used for fitting should refer to the same process time. Such isochronous mechanical spectra can be synthetically built by using eq. (2) to correlate the data obtained for each frequency and for both moduli during the whole process and then to calculate the values of both moduli at the same experimental time. In our case, the data obtained from each frequency sweep of the experimental protocol were collected within narrow time intervals if their duration is compared with the characteristic times of the process kinetics. Thus, they can be reasonably considered almost isochronous and used to describe the time evolution of the mechanical spectrum during the process with sufficient approximation. Such a proximity between experimental and calculated spectra has been proven also in the case of faster structuring kinetics, i.e. at higher pectin concentrations.

Figure 4 illustrates the change of the linear viscoelastic behaviour of the system 0.4% during the process. The mechanical spectra refer to the average times of three different frequency sweeps. It appears quite evident that the loss modulus undergoes a moderate increase without significant changes in its profile while the corresponding increase of the storage modulus is progressively higher with decreasing frequency. Thus, for $\omega \rightarrow 0$, a plateau region tends to appear in the G' profile.

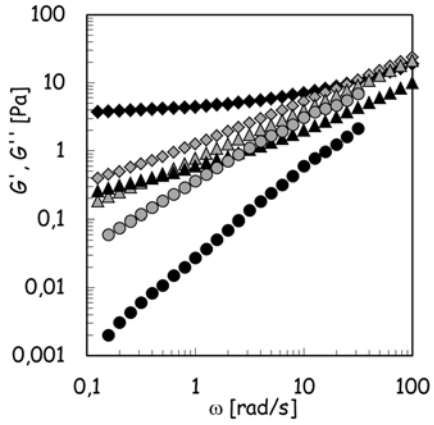

Figure 4. Mechanical spectra evolution. (A) Time development of rheological parameters, G' (black symbols) and G" (grey symbols) for system 0.4% at three different times from the beginning of the experiments: 12 min (circles), 300 min (triangles) and 715 min (diamonds).

The frequency dependence of both moduli at different process times can be satisfactorily described by the generalized Maxwell model (gM) or the Friedrich-Braun model (FB) (Friedrich & Braun, 1992) with similar fitting quality along the whole gelation process and for all the systems examined. Thus, the progressive change of the linear viscoelastic behavior of each system can be properly examined through the time evolution of the parameters of both models.

The gM model results from the parallel combination of N Maxwell elements and a purely elastic contribution. Accordingly, the frequency dependence of both moduli is described through the following relationships:

$$G' = G_e + \sum_{i=1}^{N} \frac{G_i \omega^2 \lambda_i^{\,2}}{1 + \omega^2 \lambda_i^{\,2}} \qquad (3')$$

$$G'' = \sum_{i=1}^{N} \frac{G_i \omega \lambda_i}{1 + \omega^2 \lambda_i^{\,2}} \qquad (3'')$$

where G_i and λ_i are the relaxation modulus and the corresponding relaxation time of the i^{th} Maxwell element, respectively, and G_e is the equilibrium modulus. In order to reduce the correlation degree

between the adjustable parameters, the minimization procedure was performed by adopting the following recurrent constraint for the relaxation times: $\lambda_{i+1} = 10\lambda_i$. Actually, the mechanical spectra were described qualitatively well with five Maxwell elements, i.e. with seven adjustable parameters (G_e, G_i and λ_1).

The traces of both moduli can be described by the expressions derived from the FB model, which is based on fractional derivatives of stress and strain:

$$G'(\omega) = G_e + \Delta G \frac{(\lambda\omega)^d \left[\cos(d\frac{\pi}{2}) + (\lambda\omega)^c \cos((d-c)\frac{\pi}{2}) \right]}{1 + 2(\lambda\omega)^c \cos(c\frac{\pi}{2}) + (\lambda\omega)^{2c}} \qquad (4')$$

$$G''(\omega) = \Delta G \frac{(\lambda\omega)^d \left[\sin(d\frac{\pi}{2}) + (\lambda\omega)^c \sin((d-c)\frac{\pi}{2}) \right]}{1 + 2(\lambda\omega)^c \cos(c\frac{\pi}{2}) + (\lambda\omega)^{2c}} \qquad (4'')$$

where λ is a characteristic relaxation time, G_e is the equilibrium modulus, ΔG is a parameter which rules the magnitude of the viscoelastic response and c and d are the fractional orders of the differential operators. The FB model provides a parsimonious solution for data fitting since it allows to achieve a satisfactory data correlation with only five adjustable parameters.

In the case of the generalized Maxwell model the structural buildup is mainly associated with the onset and the subsequent increase of an equilibrium modulus G_e, which represents the purely elastic contribution, and, secondarily, with the changes of the time relaxation spectrum which characterizes the viscoelastic contribution of the liquid fraction. Figure 5A and B reports the example of the time evolution of the gM parameters for system 0.4%: G_e appears after 200 minutes and then increases with process time, while also the relative weight of the slower relaxation modes parallely increases with the consequent change in the profile of time relaxation spectrum, from wedge to wedge-box distribution.

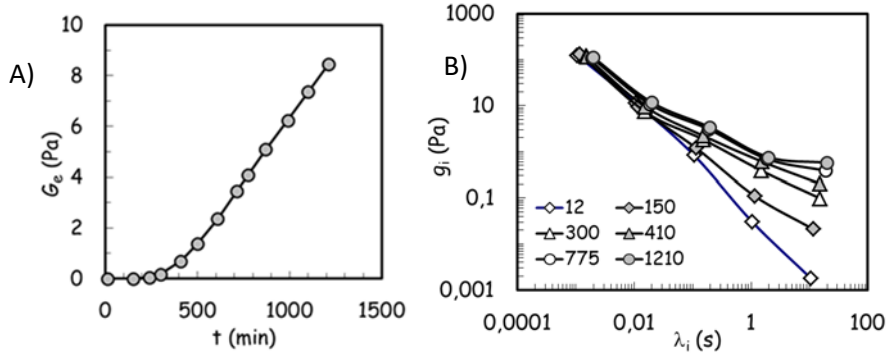

Figure 5: Time evolution of the gM parameters. A) equilibrium modulus G_e vs process time; B) time relaxation spectra at different process times: 12 (white diamonds), 150 (grey diamonds), 300 (white triangles), 410 (grey triangles), 775 (white circles) and 1210 min (grey circles) for system 0.4% w/w.

If the FB model is used for data fitting, the structural buildup is mainly underlined by G_e increase with time and, correspondingly, by the decrease of parameters c and d which are both related to the fractional derivation orders and then measure the proximity of the viscoelastic response to liquid-like or solid-like behaviours. Parallely, in the initial phase of the structural process the parameter ΔG increases while the relaxation time λ_0 decreases and then both remain almost constant after the G_e onset. Figure 7 illustrates all these time variations of the FB parameters for system 0.4%.

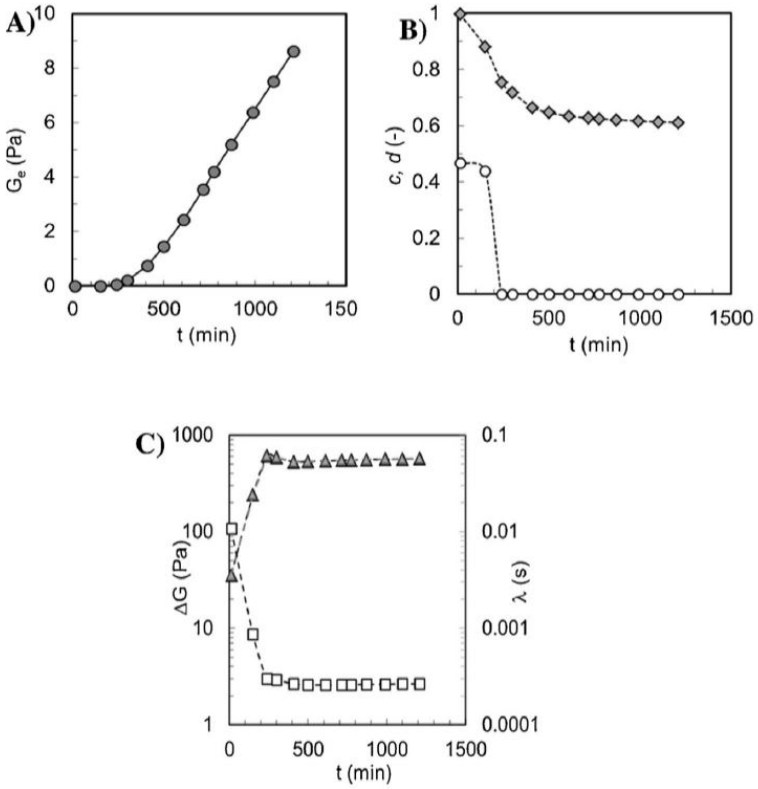

Figure 6: Time evolution of the FB parameters. (A) Equilibrium modulus (G_e); (B) c (white circles) and d (grey diamonds) parameters; (C) relaxation time, λ (white squares) and extent of the viscoelastic response, ΔG (grey triangles) obtained for system 0.4% w/w.

At increasing pectin concentration, the G_e onset and the corresponding c drop are shifted to shorter process times and the extension of the d decrease is smaller.

The G_e values estimated with both models at different process times are close to each other. Moreover, similar G_e vs time profiles are derived at different pectin concentrations and hence a generalized curve can be obtained from them through a double shifting procedure in a log-log plot.

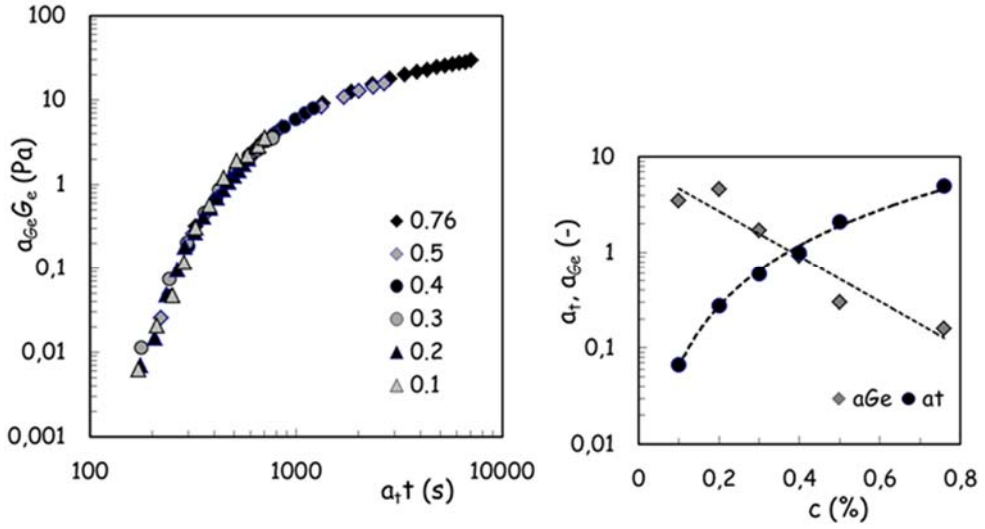

Figure 7. Master curve and shift factors. (A) Generalized plot of G_e (obtained from FB model) vs. time. (B) shift factors a_{Ge} (grey diamonds) and a_t (black circles) vs pectin concentration

Figure 7A reports the generalized plot of G_e vs time obtained using the FB model and the system 0.4% as reference for the shifting procedure. The excellent superposition is quite evident. The variations of the shift factors a_{Ge} and a_t with pectin concentration are given in Figure 7B. Both shift factor are inversely proportional to the individual values of the equilibrium modulus G_e and time t. Thus, the a_{Ge} trend indirectly suggests the exponential increase of G_e with increasing concentration while a_t measures the progressive time shortening of the structural buildup process wich follows a power law profile. The gel time values obtained from the double shift procedure are not far from those reported above.

Quite similar results are obtained using the G_e values derived from data fitting with the generalized Maxwell model.

CONCLUSIONS

The present paper is focused on the kinetics of the structural evolution occurring in aqueous HMP solutions having different polymer concentration and equal sucrose content after their preparation at low pH conditions. As highlighted in a previous work (Bulone, Giacomazza, Manno, Martorana & San Biagio, 2010), sucrose plays a crucial role in determining the structural process which reflects into a progressive change of the mechanical properties in both qualitative and quantitative terms. The sol-gel transition can be more appropriately detected through the time evolution of the mechanical spectrum: a plateau region appears in the G′- ω profile after a critical time and then it

becomes more and more extended, shifting toward higher modulus values with increasing times. For increasing plateau extension, the plateau modulus becomes more and more equivalent to the equilibrium modulus G_e which represents the limiting value of the storage modulus for $\omega \rightarrow 0$ and can be obtained by fitting data to reliable viscoelastic models, like the generalized Maxwell model or the Friedrich–Braun model.

The results hereinbefore discussed to demonstrate that pectin concentration is equally important as sucrose content. Its increase shortens the time scale of the structural buildup process and shifts the plateau modulus towards higher values which are asymptotically attainable at sufficiently long times. Similar profiles are derived for the time evolution of G_e at different pectin concentrations so that a generalized plot of G_e vs time can be obtained through a double shifting procedure in a log–log plot. Thus, both pectin and sucrose concentrations modulate the kinetics and the total amplitude of the structural buildup process in similar manners and comparable extensions, at least above critical threshold concentrations. The transition from sol to gel behavior is due to the combined action of pectin and sucrose and occurs if and when excluded volume effects and attractive interactions are capable to give rise to an incipient three-dimensional network and a structural arrested state is attained, as in attractive glasses or weakly attractive colloidal dispersions (Zaccarelli, Lu, Ciulla, Weitz, & Sciortino, 2008).

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