Journal Article

The influence of pH and monovalent ions on the gelation of pectin from the fruit seeds of the creeping fig plant

Chen, R., Ratcliffe, I., Williams, P., Luo, S., Chen, J., Liu, C

The influence of pH and monovalent ions on the gelation of pectin from the fruit seeds of the creeping fig plant

Ruiyun Chen[a], Ian Ratcliffe[b], Peter A Williams[b,*], Shunjing Luo[a], Jun Chen[a], Chengmei Liu[a,*]

a State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, 330047, China

b Centre for Water Soluble Polymers, Glyndwr University, Plas Coch, Mold Road, Wrexham, LL11 2AW, United Kingdom

* Corresponding author: Peter A Williams

Highlights

- LM Pectin was obtained from creeping fig seeds by water extraction.
- The pectin formed 'acid gels' on reducing the pH to below 3.5
- Gelation of pectin solutions was observed on addition of monovalent salts at pH 4.5
- Thermal hysteresis is observed for salt induced gels

ABSTRACT:

Pectin from the fruit seeds of the creeping fig plant was extracted and its chemical composition and rheological properties determined. It was found to consist of ~87% galacturonic acid with a degree of methoxylation of ~20%. The polysaccharide produced a viscous solution at pH 4.5 and was shown to form strong gels when the pH was reduced by the addition of glucono-delta lactone. It was concluded that as the pH was lowered, the reduction in electrostatic repulsions between the pectin chains facilitated chain association mainly through hydrogen bonding. The rate of gelation increased considerably as the pH was reduced. Although the pectin was in the form of a solution at pH 4.5, the addition of Na^+ and K^+ salts resulted in gel formation. The strength of the gels was found to be dependent on both the concentration and nature of the added electrolyte in accordance with the Lyotropic series. It has been suggested that the role of the electrolyte was to reduce the electrostatic repulsions between the carboxylate groups along the pectin chains thus facilitating chain association. Association is promoted by the presence of a low concentration of Ca^{2+} ions (1.88% w/w) naturally present in the extracted material which facilitated the crosslinking of the pectin chains in addition to the association through hydrogen bonding.

Keywords: Creeping fig seeds; Pectin; Gelation; Rheology

1. Introduction

Creeping fig (*Ficus pumila* Linn.), also known as climbing fig and creeping rubber plant, is a species of perennial plant of the mulberry family. It grows on trees or rocks in the warm and humid regions of Asia, such as southwestern China, Taiwan, Japan and India. The plant produces a fruit and the fruit seeds have a long history of use for making summer drinks (locally referred to as "Liangfen", a transparent gel-like food) in China. The seeds produce a viscous fluid after repeated scrubbing in water and then Ca-containing substances such as plant ash are added and a gel is formed after standing for a while.

To date, there have been very few reported studies on the physicochemical properties and characteristics of this viscous fluid (Chen, et al., 2014; Liang, et al., 2012). Liang, et al., (2012) extracted the material from the seeds using water at 25 ^{o}C, 80 mM ammonium oxalate and 30 mM HCl. Characterisation of the sample extracted using water showed that it contained ~87% galacturonic acid with a degree of methoxylation (DM) of 14.6%, together with small amounts of rhamnose, arabinose and mannose (0.74%, 1.77% and 0.81% respectively) indicating that it was a type of pectin. The samples extracted using ammonium oxalate and HCl were found to contain 85.8% and 77.9% galacturonic acid respectively with DM values of 14.2% and 42.6%.

In the Food Industry pectin is classified into low methoxyl (LM) and high methoxyl (HM) pectins. LM pectins have a DM, below 50% and typically 20-50%, while HM pectins have a DM above 50% and typically 50-80% (Rolin, 1993; Endress, 2011). It is well known that LM pectins form gels in the presence of divalent ions, typically at pH values in the range 3-5. The affinity of pectin for Mg ions is significantly less than for Ca, Sr, and Ba ions (Kohn, 1975; Thom, Grant, Morris & Rees, 1982). The mechanism of gel formation for LM pectin and also for alginate, a structurally similar polysaccharide, has been described by the "egg-box model" (Grant, Morris, Rees, Smith, & Thom, 1973; Thom, Grant, Morris & Rees, 1982). It was proposed that the linear chains of (1,4) linked α-D-galacturonic acid residues, in the case of pectin and the (1,4) linked α-L-guluronic acid chains in the case of alginate adopt 2-fold screw symmetry. This gives rise to a buckled chain which facilitates coordination of the Ca^{2+} ions through the oxygen atoms of the pyranose ring in addition to electrostatic interaction with the uronic acid groups which facilitates crosslinking of the polysaccharide chains. Differences in the behaviour observed between alginate and pectin by circular dichroism were reported to indicate greater selectivity of ion binding for alginate. Braccini & Pérez, (2001) investigated the binding of Ca^{2+} ions to both linear (1,4) linked α-D-galacturonic acid and (1,4) linked α-L-guluronic acid chains through molecular modelling studies and also found that Ca^{2+} ions did not bind selectively to polygalacturonate but did to polyguluronate. They concluded that whereas the "egg-box model" adequately describes the gelation of alginate it was not entirely appropriate for the gelation of pectin. Siew, Williams & Young studied the binding of Mn^{2+} ions with pectin and alginate by EPR spectroscopy and found that the amount of Mn^{2+} ions bound to the chains corresponded to $[Mn^{2+}]$: $[COO^{-}]$ ratios of ~0.25 and 0.2 respectively. In addition it was noted that maximum binding was achieved when the effective linear charge parameter, ξ_{eff}, reduced to a value of ~1.

Liang, et al., (2012) reported that the water extracted pectin from creeping fig seeds was found to contain mainly K^{+} and Ca^{2+} ions as counterions. Some preliminary experiments were carried out to evaluate the gel forming properties of the pectin using a Brookfield CT3-100 texture analyser. Solutions were produced at concentrations of 0.2%-1% in the presence of 1 mM $CaCl_2$ and 0.1 M NaCl and were found to form gels. The gel strength increased with pectin concentration and time. The fact that gels were formed at all is surprising since the overall concentration of Ca^{2+} ions present was low. For example, for the 1% solution the [Ca^{2+}]:[COO^-] ratio has been calculated to be ~0.1:1 which is far lower than expected for gel formation (Siew, Williams, & Young, 2005; Han et al., 2017). It is likely, therefore, that the presence of 0.1 M NaCl has a significant role to play in the gelation process.

Interestingly, other workers have reported on the gelation of pectin by addition of sodium and potassium salts. Ström, Schuster, & Goh, (2014) investigated the rheological behaviour of two LM pectin samples (DM 37%) and with degrees of blockiness (DB) of 40% and 57%. The high DB pectin produced solutions with a higher viscosity at pH 3 compared to pH 5. Small deformation oscillation measurements showed that the mechanical spectrum of a 3% solution at pH 3 was characteristic of a weak gel structure with a G' value of ~150 Pa at a frequency of 1 Hz. The value of G' for a 1% solution was found to increase in the presence of monovalent ions (0.05 M) with the greater effect occurring in the order $Li^+ < Na^+ < K^+$. More recently, Wang, et al., (2019) studied the gelation of citrus pectin in the presence of monovalent cations under alkaline conditions. Experiments were performed on a sample of citrus pectin DM ~80% and Mw 714 kg/mol. Pectin solutions were prepared at concentrations of 1-3% at varying concentrations of NaCl and KCl, (0.2-1.4 M) and NaOH or KOH (0.3-0.5 M). It was found that the addition of NaOH + KCl, KOH + NaCl and NaOH +

NaCl to 1% pectin resulted in gelation within 1 min. However, addition of KOH + KCl did not lead to gelation at 1% pectin but gels were formed at 2% pectin and above. Gelation did not occur when KOH, NaOH, KCl and NaCl were added alone. The strength of the gels formed depended on the nature and concentration of the electrolytes added. It was found that Na^+ ions produced stronger gels than K^+ under alkaline conditions. The effect of the alkali on the DM and molar mass was not reported.

It is known that pectin and alginate will form gels in the absence of Ca^{2+} ions at pH values below the pKa (~3.5) but there have been relatively few studies reported on this (Atkins, Mackie, Parker, & Smolko, 1971; Draget, Bræk, & Smidsrød, 1994; King, 1983; Gilsenan, Richardson & Morris, 2000). It has been postulated that gelation is brought about by association of the polysaccharide chains at low pH by hydrogen bonding. The pectin from creeping fig seeds has a very low DM and it is likely that it will be able to form gels under acid conditions. The aim of the present work, therefore, is to gain a fundamental understanding of the gelation mechanism of the pectin from creeping fig seeds and in particular to evaluate the role of pH and monovalent ions on the gelation process. The results will be relevant to the behaviour of LM pectins generally.

2. Materials and Methods

2.1. Materials

The fruit from creeping fig plants was picked in Nantian Village, Jishui County, Ji'an City, Jiangxi Province, China in July 2019 and was cut open to collect seeds. The fresh seeds were dried at 60 °C for 5 h, packed in a vacuum bag and stored at room temperature until use.

2.2. Extraction and purification of pectin

Extraction and purification was carried out according to the method of Liang, et al., (2012) with some modifications. The dried creeping fig seeds were heated at 95 ^{o}C for 90 min to inactivate the pectinase activity. The seeds were placed in distilled water at a ratio of 1:20 (w/v) and slow stirring for 30 min at room temperature. The mixture was filtered through a fine-pore nylon cloth to obtain a clear water extract. The residue was re-dispersed in distilled water and stirred again to obtain a second extract. The two filtrates were combined and precipitated by the addition of ethanol to obtain a final concentration of 50% (v/v). The precipitate was washed successively with 70%, 80%, 90% and absolute ethanol and air-dried in a fume hood at room temperature.

The sample was further purified by placing 20 g of the above pectin sample in a Buchner funnel and passing through 4 × 50 mL aliquots of the solution containing a 50:50 vol% solution of [5 mM EDTA and 0.25 M NaCl]: isopropanol. This was followed by 4 × 50 mL aliquots of 50% aqueous ethanol and then 2 × 50 mL absolute ethanol. The pectin sample was collected and placed in an oven at 40 ^{o}C overnight to dry.

The DM was determined by titration (Mizote, Odagiri, Tôei, & Tanaka, 1975) and found to be 20.63 ± 0.12%. The galacturonic acid content was determined using the meta-hydroxy diphenyl method (Blumenkrantz & Asboe-Hansen, 1973) and found to be 87.03 ± 0.72%. The protein content was 1.61 ± 0.37% (N × 6.25) as determined by the Kjeldahl procedure. The ferulic acid content of 0.22 ± 0.04% was determined by measuring the absorbance at a wavelength of 310 nm (ultraviolet-visible spectroscopy, Pgeneral T6, China) (Siew & Williams, 2008). The metal ion content of the sample was determined by Nu-Instruments, Wrexham UK using an Attom ES high resolution ICP-MS using a 0.2% solution and it was found to contain 2.99% Na^+, 0.09% K^+, 0.78% Mg^{2+}, 1.88% Ca^{2+}, 0.03% Ba^{2+} and 0.01% Sr^{2+} (w/w%).

2.3. Preparation of pectin solution

1% pectin solution was prepared by dispersing 1.00 g of pectin in 100 ml of distilled water and stirred slowly overnight at room temperature. The dispersion was centrifuged at 2500 rpm for 30 min to obtain a clear pectin solution. The actual concentration of pectin was determined by gravimetric analysis by drying clear pectin solution in an oven at 40 ^{o}C overnight. The concentration of the pectin solution was found to be 8.53 ± 0.02 mg/mL.

2.4. Preparation of pectin gel

2.4.1. Effect of pH on the gelation properties of pectin

The initial pH of the pectin solution was 4.50 and was adjusted by the addition of various concentrations of glucono-delta-lactone (GDL) which slowly hydrolysed to form gluconic acid, thus reducing the pH. The GDL (0.5 - 7% w/v) was added at room temperature and stirred rapidly for 1 min giving final pH values of 3.70 -2.57.

2.4.2. Effect of monovalent ions on the gelation properties of pectin

The effect of monovalent ions on the gelation properties of pectin was studied by the addition of various amounts of 2 M salt (NaCl, NaI, $NaNO_3$, KCl, KI, EDTA) stock solutions. The final concentration for salt used was 35 mM, 70 mM and 105 mM. The appropriate amount of salt stock solution was added to the pectin solution and stirred rapidly for 1 min at room temperature.

2.4.3. Effect of NaCl at various pH on the gelation properties of pectin

The effect of the NaCl at various pH on the gelation properties of pectin was studied by adjusting the pH of the pectin solution to 5.0, 5.5 and 6.0 using 1 M NaOH. The appropriate amount of NaCl stock solution was then added with rapid stirring for 1 min to make a final NaCl concentration of 70 mM.

2.4. Rheological measurements

The rheological properties were measured using an advanced rheometer AR 2000ex (TA instruments, New Castle, DE, USA) equipped with two different geometry systems (vane rotor and cross-hatched parallel plate). The vane rotor was used for time sweep and frequency sweep measurements and the cross-hatched parallel plate was used for temperature sweep measurements. The vane rotor geometry consists of four thin blades arranged at equal angles around a small cylindrical shaft: the radius of the blades was 14 mm and the height of the blades 42 mm. It was immersed in the sample contained in a cylindrical cup with a 15 mm radius. The cross-hatched parallel plate geometry was composed of a lower stationary steel plate and an upper cross-hatched plate with a 40 mm diameter and 0.5 mm separation.

The time sweep test was performed at 20 ^{o}C to investigate the time dependence of the storage and loss moduli (G' and G") of the pectin gels. Approximately 30 ml of the sample prepared as described in section 2.4 was loaded into a pre-equilibrated (20 ^{o}C) cylindrical cup. The blade was lowered and the gap set to 2000 μm. Samples were subjected to a time sweep over 180 min after equilibration for 5 min. An oscillation frequency of 1 Hz and a strain amplitude of 1% was used in order to be within the limit of the linear viscoelastic regime.

The frequency dependence of the moduli of the pectin gels were determined using a frequency sweep from 0.01 to 10 Hz at a constant 1% strain.

The temperature sweep was used to determine the effect of temperature on the storage and loss moduli, G’ and G”. Approximately 2 ml of 1% pectin solution containing varying amounts of NaCl prepared as described in section 2.4 was heated to 80 ^{o}C then placed onto the plate (preheat to 80 ^{o}C) and the gap was set at 1000 μm. After equilibrium for 5 min, temperature sweeps were carried out from 80 to 20 ^{o}C and 20 to 80 ^{o}C, with cooling/heating rates of 0.5 ^{o}C/min at a constant frequency of 1 Hz and a strain of 1%.

2.5. Statistical analysis

All of the experiments were carried out in triplicate. Statistical analysis was carried out using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY). The results were expressed as mean ± standard deviations.

3. Results and Discussion

3.1. Influence of pH on the gelation properties of pectin

Various amounts of GDL solid were added to the pectin solutions in order to adjust the pH and the values of G’ and G” at a frequency of 1 Hz were determined as a function of time. The results are presented in *Figure 1*.

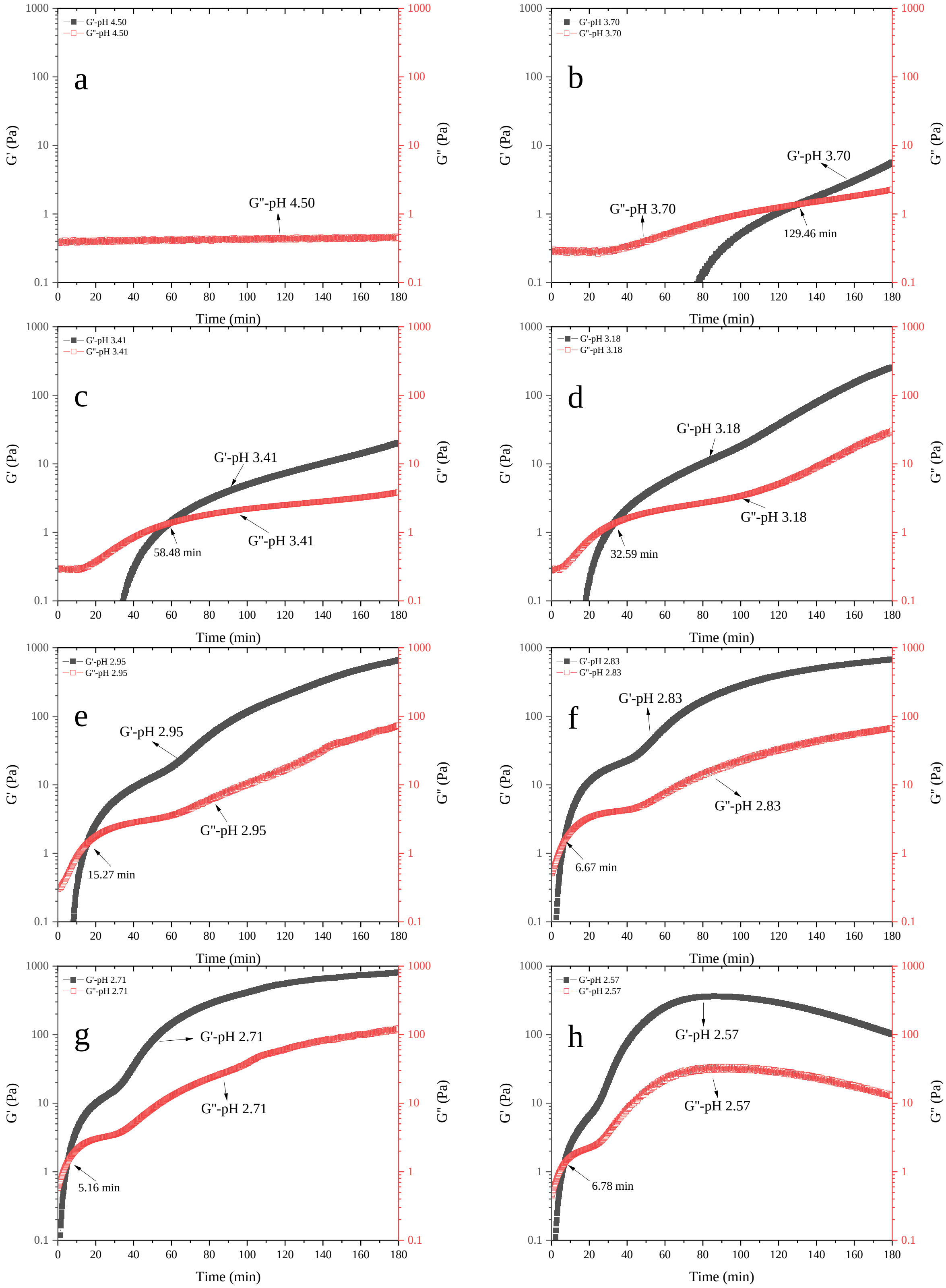


Figure 1. G' and G" for 1% pectin solutions at varying pH as a function of time, the letters a-h represent pH 4.50, 3.70, 3.41, 3.18, 2.95, 2.83, 2.71 and 2.57 respectively. (pH adjustment was made by addition of various concentrations of solid GDL).

The initial solution without GDL addition had a pH of 4.50 and was a liquid with G” greater than G’ over the frequency range studied. G” had a value of ~0.4 Pa and G’ a value of ~0.1 Pa (not shown). As noted in the Materials section the sample does contain a small amount of Ca^{2+} and Mg^{2+}ions. The amonut of Ca^{2+} ions present in our sample is equivalent to a stoichiometric ratio of ~0.09:1 [Ca^{2+}]:[COO^{-}] and is insufficient to induce gelation at pH 4.5. Ratios greater than 0.3:1 are required for network formation to occur (Siew, Williams & Young, 2005; Han et al., 2017). Also as noted above, it has previously been reported that Mg^{2+} ions are not effective at inducing pectin gelation (Kohn, 1975; Thom, Grant, Morris & Rees, 1982).

On the addition of GDL, it is seen that the values of G’ and G” increased over time and that G’ became greater than G” indicating that gelation had occurred. The G’ and G” values at a frequency of 1 Hz for the pectin samples after 180 min are shown as a function of pH in *Figure 2a*. It is clearly seen that the values increased significantly below the pKa value of 3.50 (Han et al., 2017) as the carboxylate groups became less ionised. The values of G’ and G” are shown as a function of frequency in *Figure 2b* at the various pH values. G’ is greater than G” for all samples (apart from the sample at pH 4.50) and both are only slightly dependent on frequency. This is clear evidence for the formation of strong gels (Williams & Phillips 2009).

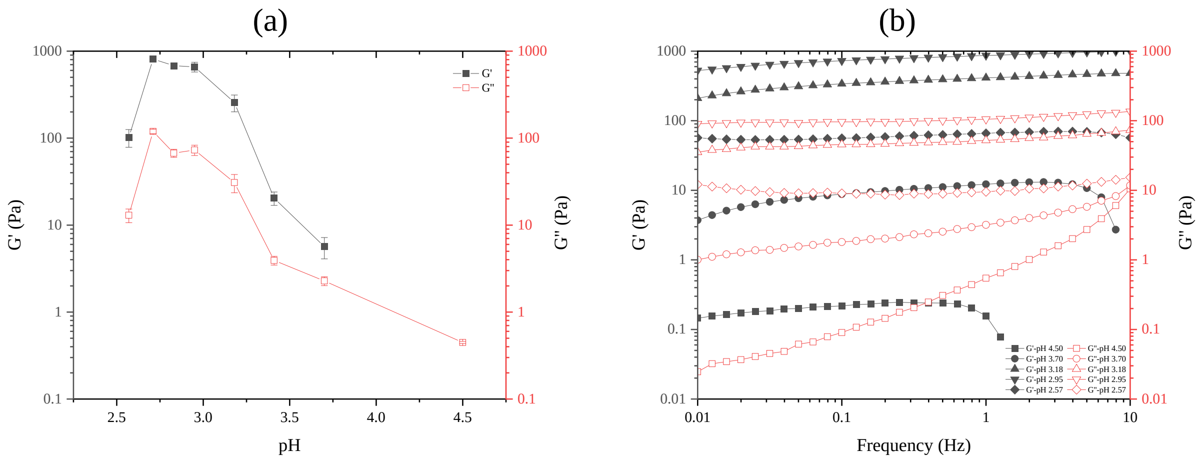


Figure 2. G' (solid symbols) and G" (open symbols) for 1% pectin solutions (a) at a frequency of 1Hz after 180 min as a function of pH (b) as a function of frequency, pH 4.50 (square), pH 3.70 (circle), pH 3.18 (up-triangle), pH 2.95 (down-triangle), pH 2.57 (diamond).

The time to reach the G' and G" crossover point (*Figure 1*) is seen to decrease gradually with increasing GDL concentration and consequently to a decrease in pH. For the system at pH 3.76, for example, the crossover point occurs after 129 min and the G' and G" values were 5.6 Pa and 2.2 Pa respectively while for the sample at pH 2.71 the crossover occurs after 5.16 min and G' and G" have values of 812.8 Pa and 119.5 Pa respectively. A similar behaviour was initially observed for the system at pH 2.57 with G' and G" increasing but over time both G' and G" values were found to decrease. This may be a consequence of the high concentration of GDL is this system which is equivalent to ~0.4 M gluconic acid. It is interesting to note that Draget, et al., (1994) carried out similar experiments on the gelation of alginate on the addition of GDL and observed similar findings. They found that G' increased significantly with time up to ~180 min and then less slowly. They also reported that the value for Young's modulus decreased at higher additions of GDL (0.8 M). The kinetic plot showing the time for the G' and G" crossover as a function of pH is given in *Figure 3a*. A theoretical plot showing the percentage dissociation of the carboxyl groups calculated from the pKa is given as a function of pH is given in *Figure 3b*.

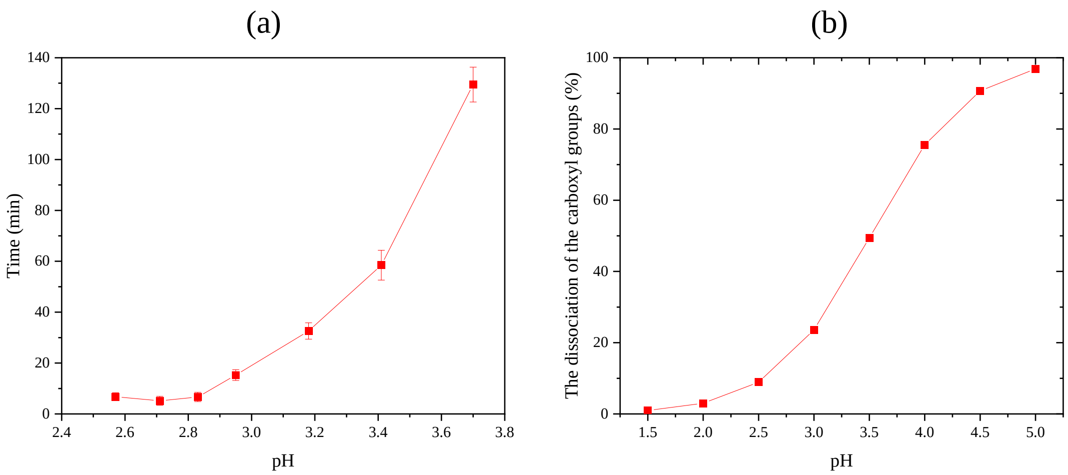


Figure 3. (a) Time for the crossover of G' and G" and (b) the dissociation of the galacturonic acid groups as a function of pH.

The strongest gels were formed at pH values below 2.5 corresponding to degrees of dissociation of less than ~10%. It is evident that gelation occurs as a consequence of the association of the pectin chains brought about through hydrogen bonding. The results are in accordance with those of Gilsenan, Richardson & Morris, (2000) who determined the mechanical spectra of LM pectin with DM 31% as a function of pH. They reported that G' and G" values increased as the pH was lowered from 4 to 1.6 by addition of HCl. At pH values of 4 and 3 the mechanical spectra were typical of a dilute solution with low G' and G" values and both frequency dependent. When the pH was lowered further to 2 and 1.6, the mechanical spectra were typical of strong gels with G' values greater than G" over the frequency range studied. Since X-ray fibre diffraction analysis had indicated that pectic acid had three-fold symmetry it was suggested that gelation resulted from the association of three-fold helical chains through hydrogen bonding. We argue that the association is facilitated by the reduction in the electrostatic charge repulsions between the pectin chains as the degree of dissociation of the carboxylate groups is reduced enabling them to approach each and for interaction to occur. The reduction in electrostatic repulsions also influences the rate of gelation. The lower the proportion of anionic charges the faster is the rate of gelation. It is also worth noting that a small percentage of the galacturonic acid residues are methoxylated and it is possible, therefore, that some hydrophobic interaction could also occur through the methoxyl groups following the association of the pectin chains particularly if the methoxyl groups occur in blocks. The pectin also contains a small percentage of ferulic acid which could also give rise to hydrophobic interaction. Since the carboxyl groups along the pectin chain are virtually all in the non-ionised form any Ca^{2+} ions present cannot be involved in the gelation process.

3.2. Influence of monovalent cations on the gelation properties of pectin

The effect of the addition of various concentrations of NaCl on the values of G' and G" at 1 Hz for pectin solutions at pH 4.50 are given as a function of time in *Figure 4a*. It is noted that both G' and G" increase rapidly initially with time and tend towards a plateau value over the course of the experiment. The values increase with increasing NaCl concentration. G' and G" are plotted as a function of frequency in *Figure 4b* and it is noted that G' is significantly greater than G" for all of the samples and that they are both independent of frequency which is typical of a strong gel. The G' values obtained at a NaCl concentration of 105 mM are similar to the values obtained for the sample at pH 2.71 in the absence of NaCl.

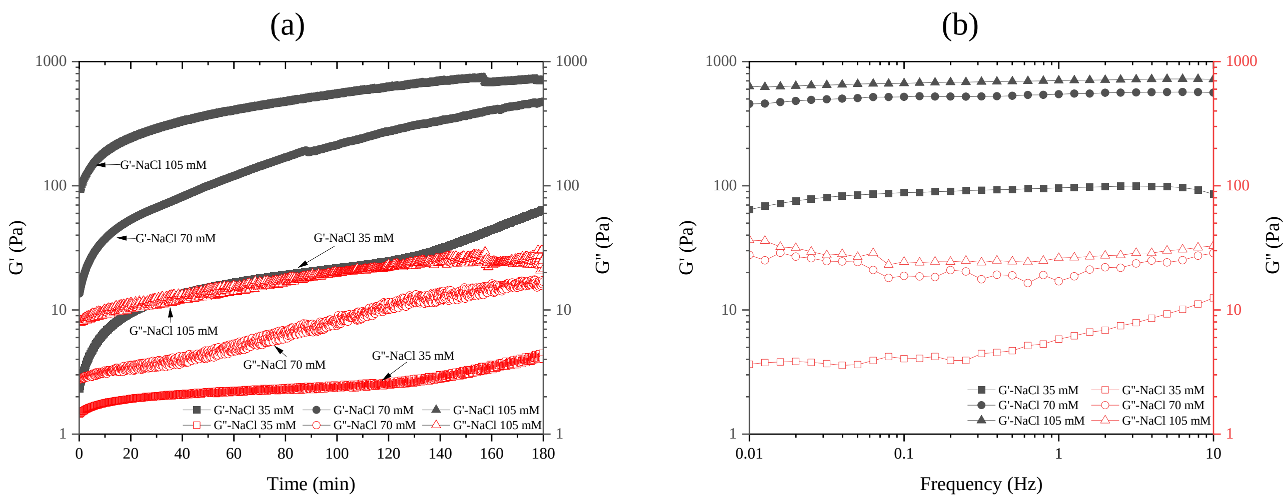
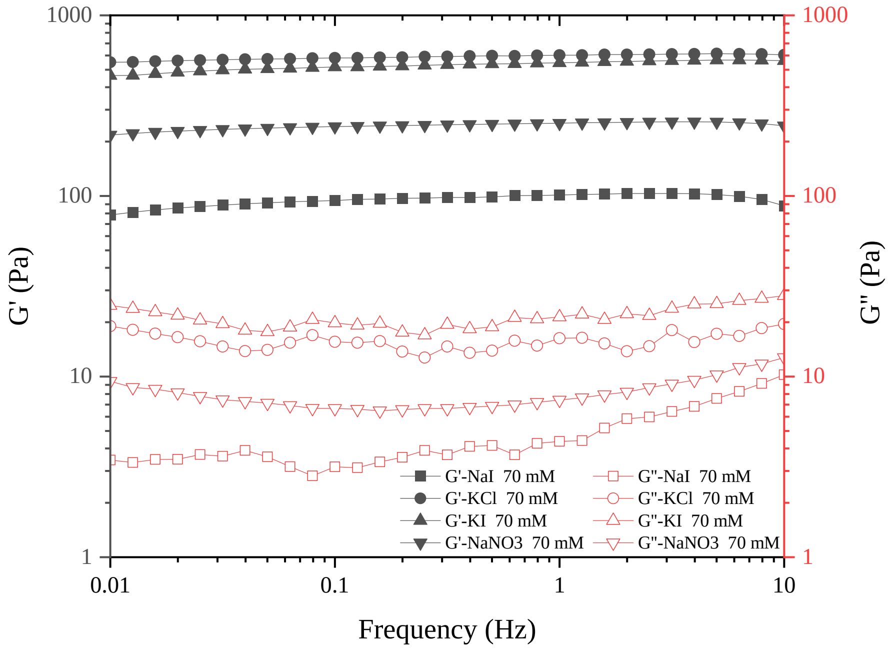


Figure 4. G' (solid symbols) and G" (open symbols) for 1% pectin solutions at pH 4.5 in the presence of varying concentrations of NaCl (a) as a function of time and (b) as a function of frequency. The concentrations of NaCl 35 mM (square), 70 mM (circle), 105 mM (up-triangle).

At pH 4.50 the galacturonic acid residues will be largely dissociated *(Figure 3b)* and there will be strong repulsions between the pectin chains. On the addition of NaCl the repulsions between the chains will be screened which will enable the chains to associate and form a three-dimensional gel network. Further experiments were carried out on 1% pectin solutions at pH 4.50 in the presence of a number of different salts and the plots of G' and G" as a function of frequency are presented in *Figure 5* and the values of G' at a frequency of 1Hz are given as a function of ionic strength are shown in *Figure 6*.



Figures 5. G' (solid symbols) and G" (open symbols) for 1% pectin solutions at pH 4.50 in the presence of different electrolytes concentrations of 70 mM as a function of frequency. NaI (square), KCl (circle), KI (up-triangle), $NaNO_3$ (down-triangle).

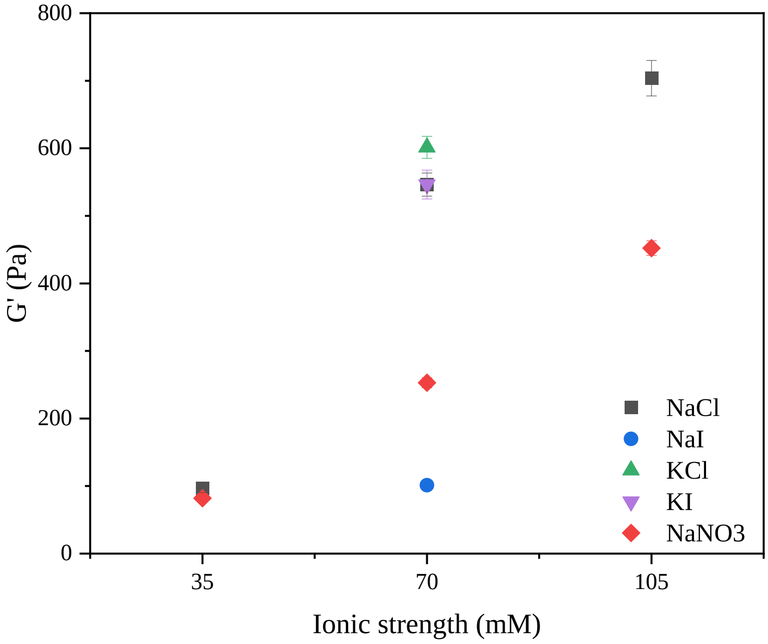


Figure 6. G' at a frequency of 1 Hz for 1% pectin solutions at pH 4.50 in the presence of various electrolytes as a function of ionic strength. NaCl (square), NaI (circle), KCl (up-triangle), KI (down-triangle), $NaNO_3$ (diamond).

It is seen that the values of G' are influenced by the nature of the ions present. G' increases in the order $I^- < NO_3^- < Cl^-$ and the values are higher for K^+ salts than Na^+ salts at the different ionic strengths in accordance with the Lyotropic series. The effect of K^+ and Na^+ ions on the gelation process follows the trend reported by Ström, Schuster, & Goh, (2014) but is contrary to the findings of Wang, et al., (2019) as reported in the Introduction. The Lyotropic series ranks anions and cations according to their ability to influence water structure at the polymer-water interface and is a consequence of their adsorption or exclusion and their influence on the interfacial energy (Hyde, et al., 2017; Piculell & Nilsson, 1990).

It is well known that the gelation of LM pectins is highly sensitive to the presence of Ca^{2+} ions (Rolin, 1993). As mentioned above the sample contains a small amount of Ca^{2+} ions and it is, therefore, likely that Ca^{2+} ions also have a role to play in gel formation for systems at pH 4.50. As discussed previously (Siew, Williams & Young, 2005) the specific binding of Ca^{2+} ions to the pectin chain can give rise to mono-complex formation where one Ca^{2+} ion interacts with one carboxylate ion on the pectin chain resulting in localised charge reversal and the formation of positive electrostatic patches. This occurs because the spacing between the carboxylate groups along the pectin chain is too large for one Ca^{2+} ion to interact with two carboxylate ions on the same chain. For our system, these patches are not sufficient to induce gelation at pH 4.50 since the pectin chains are highly charged with ~90% of the carboxylate groups dissociated and electrostatic repulsions prevent them from associating. However, it is evident that when electrolyte is added the electrostatic repulsions will be screened sufficiently to enable the pectin chains to approach each other for association to occur through Ca^{2+} ion crosslinking. The Ca^{2+} ions bound to one chain are able to interact with carboxylate groups on another pectin chain as previously described (Siew, Williams & Young, 2005). There is also the possibility of additional association through hydrogen bonding. In order to further investigate the role of the Ca^{2+} ions we studied the effect of EDTA on the gelation process. The plots of G' and G" on the gelation of 1% pectin solutions in the presence of NaCl and EDTA are presented in *Figure 7*.

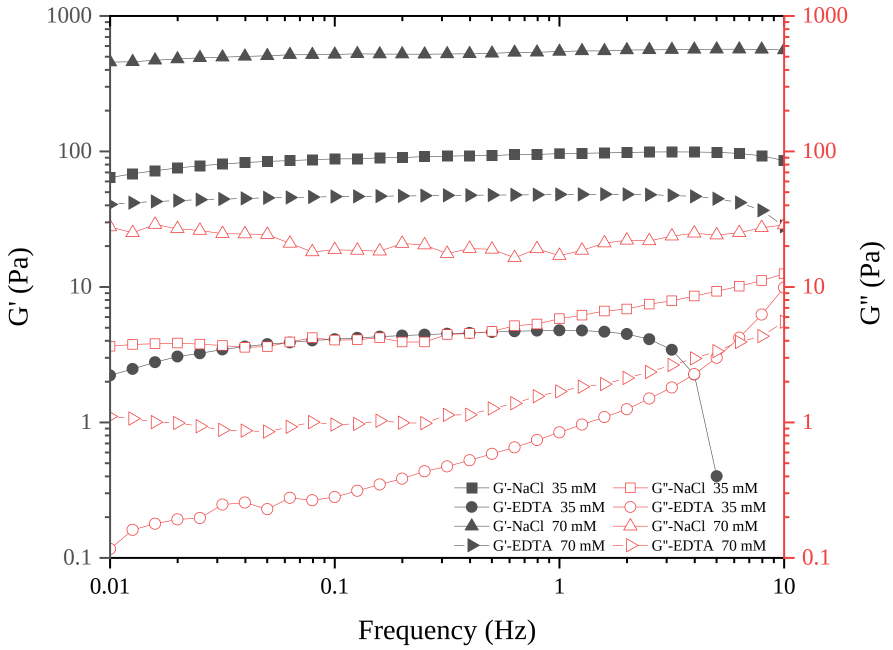


Figure 7. G' (solid symbols) and G" (open symbols) for 1% pectin solutions at pH 4.50 in the presence of NaCl and EDTA as a function of frequency. NaCl 35 mM (square), EDTA 35 mM (circle), NaCl 70 mM (up-triangle), EDTA 70 mM (right-triangle).

EDTA is expected to chelate the Ca^{2+} ions present and thus eliminate crosslinking of the pectin chains. It is clearly seen that the values of G' and G" are significantly reduced in the presence of the EDTA compared to systems containing NaCl but that gelation still occurs. It is evident, therefore, that the Ca^{2+} ions are involved in the gelation process. The fact that G' and G" increase in value as the EDTA concentration increased from 35 mM to 70 mM indicates that the resultant additional charge screening facilitates increased pectin chain association through hydrogen bonding.

3.3. Influence of NaCl at various pH on the gelation properties of pectin

The pH of pectin samples containing 70 mM NaCl was adjusted by adding 1 M NaOH and the G' and G" values were determined. The plots are shown in *Figure 8* and it is noted that as the pH of the system increased the values of G' and G" decreased and above pH 6.0

gelation did not occur. It is evident from Figure 3b that the degree of dissociation of the carboxylate groups is >90% above pH 4.5. This being the case there will only be a marginal increase in the electrostatic repulsions between the pectin chains as the pH increases which will have a limited effect on molecular association. It is possible that the decrease in G' and G" at pH 5 and above is due to de-esterication of some of the carboxylate groups present and also to depolymerisation of the pectin chains.

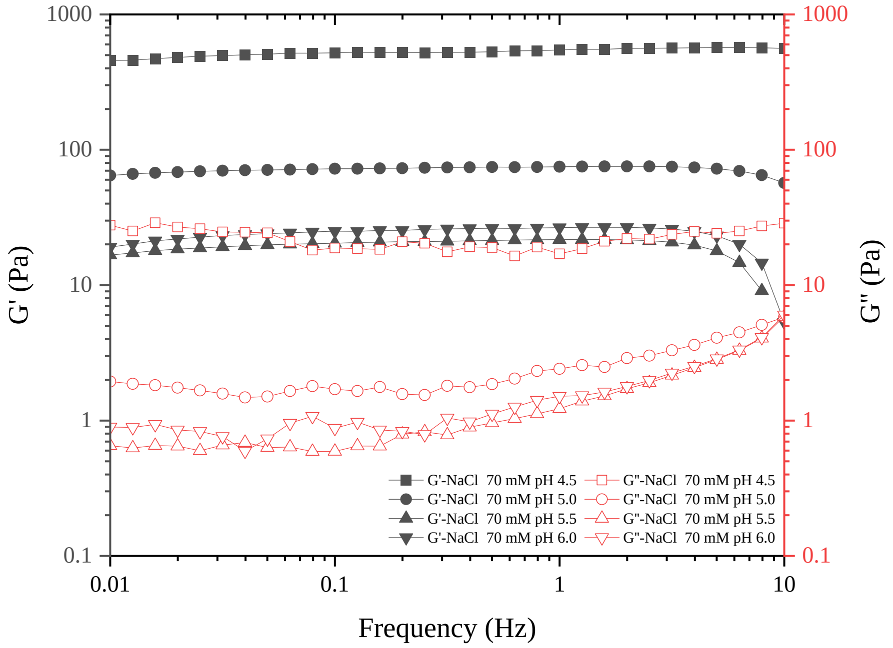


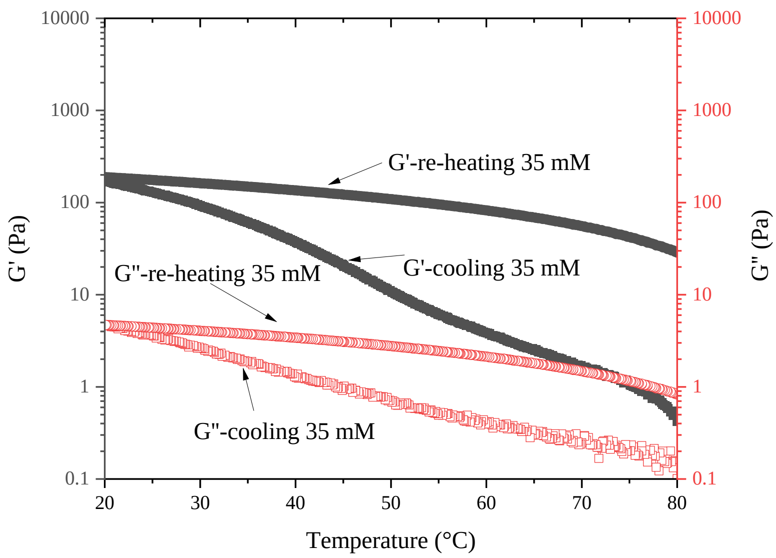
Figure 8. G' (solid symbols) and G" (open symbols) for 1% pectin solutions in the presence of 70 mM NaCl at various pH as a function of frequency. pH 4.5 (square), pH 5.0 (circle), pH 5.5 (up-triangle), pH 6.0 (down-triangle).

3.4. Influence of temperature on the gelation properties of pectin

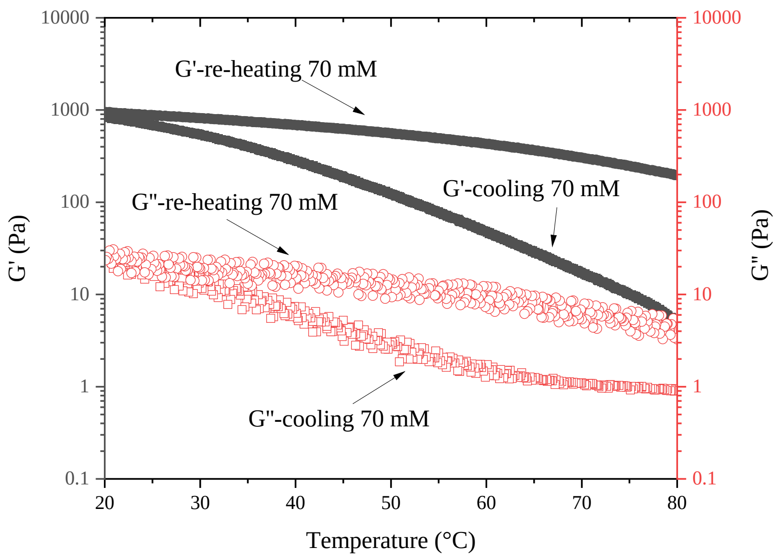
The effect of temperature on the viscoelastic properties of the pectin solutions was studied. 1% pectin solutions were prepared at pH 4.50 in the presence of various concentrations of NaCl and heated to 80 °C. The values of G' and G" were determined at a frequency of 1 Hz on cooling to 20 °C and then reheating to 80 °C. The results are reported in

Figures 9. For all samples both G' and G" are seen to increase as the temperature is reduced and G' is significantly greater than G" at all temperatures indicating gel-like characteristics. As might be expected, the reduction in the molecular mobility of the pectin chains as the temperature is reduced facilitates an increase in the number and/or length of the junction zones forming the three-dimensional gel network. On reheating the G' and G" values are seen to decrease but it is interesting to note that the values are higher than on initially cooling. It is evident that the higher values are due to the increased molecular chain association which is not fully disrupted on heating.

(a)



(b)



(c)

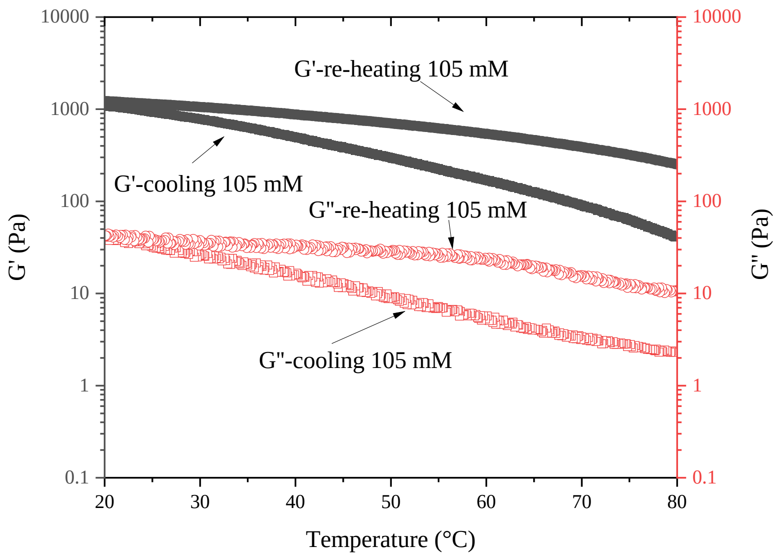


Figure 9. G' and G" for 1% pectin solutions at pH 4.50 containing varying concentrations of NaCl 35 mM (a), 70 mM (b) and 105 mM (c) as a function of temperature on cooling and reheating.

Gilsenan, Richardson & Morris, (2000) also observed thermal hysteresis for LM pectin gels formed at low pH and concluded that this was due to the extensive aggregation of the pectin chains. In a further experiment we investigated the influence of temperature and shearing on gel formation and this is illustrated in the schematic shown in *Figure 10*. In the first experiment NaCl solid was added to 1% pectin solution at pH 4.50 at room temperature to give a 0.5 M solution and a gel was formed. The system was heated to 80 ^{o}C and continuously sheared slowly with a magnetic stirrer and it became liquid-like. On cooling the system remained as a liquid and the pectin was seen to form a precipitate after 24 h. It is argued that on shearing at 80 ^{o}C the gel is disrupted but the molecules are still highly aggregated and on cooling the aggregated molecules are unable to form a network. In the second experiment the sample was heated to 80 ^{o}C with shearing and then the NaCl solid to give a 0.5 M solution was added. The sample was liquid-like at this stage and on cooling to

20 °C the sample formed a gel. In this case the molecules were not in an aggregated state prior to cooling and hence were able to associate and form a gel.

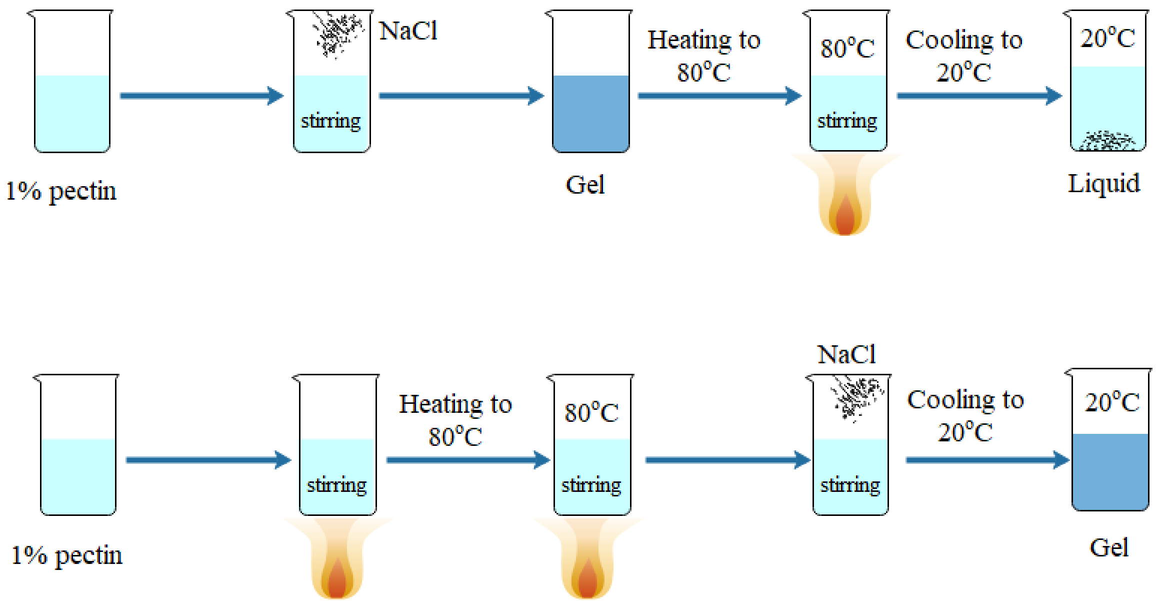


Figure 10. The schematic diagram of the effects of temperature and shearing on pectin gel formation.

4. Conclusions

The viscous fluid that exudes from the seeds of the fruit of the creeping fig plant contains pectin. It has a low degree of methoxylation and is able to form gels at low pH values. Gelation arises due to the association of the pectin chains brought about through hydrogen bonding. The rate of the gelation process increases as the pH is reduced. At pH 4.5, where the degree of dissociation of the carboxylate ions is greater than ~90%, the pectin forms a viscous solution. Unusually, the addition of Na^{+} and K^{+} salts to the solution results in gel formation. Gelation is due to the fact that the added electrolyte screens the electrostatic repulsions between the pectin chains facilitating chain association. The small number of Ca^{2+} ions naturally present in the pectin gives rise to positively charged patches which promote crosslinking of the pectin chains. The strength of the gels varies with the nature of the salt added and is in accordance with the Lyotropic series. The pectin could be of commercial interest since most pectins tend to have a high methoxyl content and are often de-esterified for particular applications.

CRediT authorship contribution statement

Ruiyun Chen: Conceptualization, Methodology, Investigation, Data Curation, Writing - Original Draft. **Ian Ratcliffe:** Conceptualization, Methodology. **Peter A Williams:** Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing. **Shunjing Luo:** Methodology, Investigation. **Jun Chen:** Resources, Methodology. **Chengmei Liu:** Conceptualization, Funding acquisition.

Acknowledgements

This study was supported financially by the National Natural Science Foundation of China (No. 31660488), and the PhD Students Abroad Visiting Program of Nanchang University (2019).

References

Atkins, E., Mackie, W., Parker, K., & Smolko, E. (1971). Crystalline structures of poly-D-mannuronic and poly-L-guluronic acids. *Journal of Polymer Science Part B: Polymer Letters, 9*(4), 311-316.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical biochemistry, 54*(2), 484-489.

Braccini, I., & Pérez, S. (2001). Molecular basis of Ca2+-induced gelation in alginates and pectins: the egg-box model revisited. *Biomacromolecules*, *2*(4), 1089-1096.

Chen, J., Wu, S.-S., Liang, R.-H., Liu, W., Liu, C.-M., Shuai, X.-X., & Wang, Z.-J. (2014). The effect of high speed shearing on disaggregation and degradation of pectin from creeping fig seeds. *Food chemistry, 165*, 1-8.

Draget, K., Bræk, G. S., & Smidsrød, O. (1994). Alginic acid gels: the effect of alginate chemical composition and molecular weight. *Carbohydrate polymers, 25*(1), 31-38.

Endress, H. (2011). Pectins: Production, properties and applications. In *Renewable resources for functional polymers and biomaterials* (pp. 210-260).

Gilsenan, P.M., Richardson, R.K. and Morris, E.R. (2000) Thermally reversible acid-induced gelation of low-methoxy pectin *Carbohydrate Polymers* 41 339-349

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J., & Thom, D. (1973). Biological interactions between polysaccharides and divalent cations: the egg-box model. *FEBS letters*, *32*(1), 195-198.

Han, W., Meng, Y., Hu, C., Dong, G., Qu, Y., Deng, H. & Guo,Y. (2017) Mathematical model of Ca2+ concentration, pH, pectin concentration and soluble solids (sucrose) on the gelation of low methoxyl pectin *Food Hydrocolloids* 66 37-48.

Hyde, A. M., Zultanski, S. L., Waldman, J. H., Zhong, Y.-L., Shevlin, M., & Peng, F. (2017). General principles and strategies for salting-out informed by the Hofmeister series. *Organic Process Research & Development*, *21*(9), 1355-1370.

King, A. H. (1983). Brown seaweed extracts (alginates). *Food hydrocolloids, 2*, 115-188.

Kohn, R. (1975). Ion binding on polyuronates-alginate and pectin. *Pure and Applied Chemistry, 42*(3), 371-397.

Liang, R.-h., Chen, J., Liu, W., Liu, C.-m., Yu, W., Yuan, M., & Zhou, X.-q. (2012). Extraction, characterization and spontaneous gel-forming property of pectin from creeping fig (Ficus pumila Linn.) seeds. *Carbohydrate polymers, 87*(1), 76-83.

Mizote, A., Odagiri, H., Tôei, K., & Tanaka, K. (1975). Determination of residues of carboxylic acids (mainly galacturonic acid) and their degree of esterification in industrial pectins by colloid titration with Cat-Floc. *Analyst, 100*(1196), 822-826.

Piculell, L., & Nilsson, S. (1990). Effects of salts on association and conformational equilibria of macromolecules in solution. In *Surfactants and Macromolecules: Self-Assembly at Interfaces and in Bulk* (pp. 198-210): Springer.

Rolin, C. (1993) Pectin, Industrial Gums; Polysaccharides and their derivatives (third edition). In: Academic Press, London (pp. 257-288).

Siew, C. K., & Williams, P. A. (2008). Role of protein and ferulic acid in the emulsification properties of sugar beet pectin. *Journal of agricultural and food chemistry, 56*(11), 4164-4171.

Siew, C. K., Williams, P. A., & Young, N. W. (2005). New insights into the mechanism of gelation of alginate and pectin: charge annihilation and reversal mechanism. *Biomacromolecules, 6*(2), 963-969.

Ström, A., Schuster, E., & Goh, S. M. (2014). Rheological characterization of acid pectin samples in the absence and presence of monovalent ions. *Carbohydrate polymers, 113*, 336-343.

Thom, D, Grant, G.T., Morris, E.R. & Rees, D.A. (1982) Characterisation of cation binding and gelation of polyuronates by circular dichroism. *Carbohydrate Research, 100*(1), 29-42.

Wang, H., Wan, L., Chen, D., Guo, X., Liu, F., & Pan, S. (2019). Unexpected gelation behavior of citrus pectin induced by monovalent cations under alkaline conditions. *Carbohydrate Polymers, 212*, 51-58.

Williams, P.A. and Phillips, G.O. (2009) 'Introduction to food hydrocolloids' In Handbook of hydrocolloids eds Phillips, G.O. and Williams, P.A. Woodhead Publishing Ltd Cambridge, UK, pp 1-22