Microstructure and Kinetic Rheological Behavior of Amidated and Nonamidated LM Pectin Gels

Caroline Löfgren,[†] Stéphanie Guillotin,[‡] and Anne-Marie Hermansson*,[†]

SIK, The Swedish Institute for Food and Biotechnology P.O. Box 5401, SE- 402 29 Göteborg, Sweden, and Wageningen University, Agrotechnology and Food Sciences, Laboratory of Food Chemistry, P.O. Box 8129 6700 EV Wageningen, The Netherlands

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The microstructure, kinetics of gelation, and rheological properties have been investigated for gels of nonamidated pectin (C30), amidated pectin (G), and saponified pectin (sG) at different pH values, both with and without sucrose. The low-methoxyl (LM) pectin gels were characterized in the presence of Ca^{2+} by oscillatory measurements and transmission electron microscopy (TEM). The appearance of the gel microstructure varied with the pH, the gel structure being sparse and aggregated at pH 3 but dense and somewhat entangled at pH 7. During gel formation of pectins G and C30 at pH 3 there was a rapid increase in G' initially followed by a small increase with time. At pH 7 G' increased very rapidly at first but then remained constant. The presence of sucrose influenced neither the kinetic behavior nor the microstructure of the gels but strongly increased the storage modulus. Pectins G and C30 showed large variations in G' at pH values 3, 4, 5, and 7 in the presence of sucrose, and the maximum in G' in the samples occurred at different pH values. Due to its high Ca^{2+} sensitivity, pectin sG had a storage modulus that was about 50 times higher than that of its mother pectin G at pH 7.

I. Introduction

Pectins are frequently studied natural polysaccharides with useful gelling and thickening properties for many application areas. Pectin is composed of α-(1-4) D-galacturonic acid units, interrupted by insertion of rhamnose units and with side chains of neutral sugars attached to the backbone. The carboxyl groups can be substituted with methyl esters or with amide groups, which play a decisive role in the functional properties of pectin. High-methoxyl (HM) pectins, with more than 50% of the carboxyl groups esterified, form gels mainly by hydrophobic interactions and hydrogen bonds at acidic pH and in the presence of more than 55% sugar or similar cosolute.[1] Low-methoxyl (LM) pectins usually form gels in the presence of Ca^{2+} ions and over a wider range of pH values, with or without sugar.[2]

The gel formation ability of LM pectin is a combination of several mechanisms. The efficient Ca^{2+} binding is an important factor both at high and low pH values.[3] Moreover, under acidic conditions cross-links supported by hydrophobic interactions and hydrogen bonds may also strengthen the gel formation of LM pectin.[4] Amidated LM pectins need less calcium for gel formation, and they are also less prone to precipitation at high Ca^{2+} concentrations.[5]

The pH value affects the functional properties of LM pectins, and it has been suggested from potentiometry, CD spectroscopy, and calorimetry of pectins in solution that the polygalacturonate chains undergo a conformational transition when the pH is changed.[6,7] At neutral pH values the chains are highly extended and stiffened due to intramolecular electrostatic repulsions, with geometry close to a 2-fold helix structure. Reducing the pH can lead to a conformational transition into a more compact 3-fold helix structure.[4] Gilsenan et al. suggest that gel formation of LM pectin under acidic conditions occurs by association of 3-fold helices enabled by the suppression of electrostatic repulsion and by allowing the carboxyl groups to act as hydrogen-bond donors.[4] The influence of pH and Ca^{2+} concentration on the gel properties of amidated and nonamidated LM pectins has been studied by Lootens et al.[8] They found that the gel temperature increased when the Ca^{2+} concentration was increased. Furthermore, they report that the gel formation of amidated LM pectins was promoted by a reduction of pH (≤ 3.5) presumably by hydrogen bonds to the amide groups. The pectin and sugar concentrations are also known to affect the gel formation of LM pectins.[9,10] Increasing pectin concentration results in higher storage modulus, whereas the effect of sugar varies, largely depending on the type of sugar and its specific structural characteristics.[11−13] By increasing the sucrose concentration higher storage modulus is obtained for LM pectin gels.[11]

The kinetic behavior during gelation of pectins is an important factor in the processing of many food products. For example, too slow gel formation can result in phase separation in a fruit jam product. On the other hand, very rapid kinetic behavior can result in undesirable air bubbles entrapped in the gel product. Moreover, the kinetic behavior can affect the functionality and the microstructure of the resulting gel. A recent study focused on the kinetic effects and the microstructure of HM pectin gels having the same characteristics but differing in the internal distribution of the methyl esters.[14] It was shown that, in the absence of Ca^{2+}, the shortest gelation time was obtained for the most blockwise-distributed sample. The kinetic behavior was also strongly affected by the Ca^{2+} concentration and by rather small changes in pH. The presence of Ca^{2+} resulted in faster gel formation for both random and blockwise-distributed HM pectins at pH 3. When pH was increased from 3.0 to 3.5, slower gel formation and also lower G' values were obtained for the HM pectin gels in the presence of Ca^{2+}. Furthermore, transmission electron microscopy revealed that the presence of Ca^{2+}

* Corresponding author. Phone: +46 31 335 56 58. Fax: +46 31 83 37 82. E-mail: amh@sik.se.
[†] SIK, The Swedish Institute for Food and Biotechnology.
[‡] Wageningen University.

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Table 1. Chemical Characteristics of the Pectins

pectin	DM %[a]	DAm %[b]	DS % (DM + DAm)[c]	DB %[d]	GalA content (%)	MW (kDa)
C30	30	0	30	16.5	78.5	
G	31	18	49	10	70	87
sG	0	18	18	16	69	69

[a] Degree of methyl esterification. [b] Degree of amidation. [c] Degree of substitution. [d] Degree of blockiness.

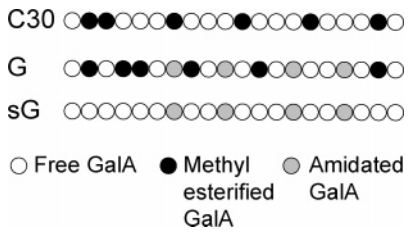

Figure 1. Schematic representation of the substitution groups in pectins C30, G, and sG.

caused an inhomogeneous gel microstructure for the blockwise-distributed HM pectin.

In many applications it can be useful to have mixtures of different pectins to obtain products with certain functional properties. It was shown previously that large variations in microstructure and rheological properties were obtained in mixtures of HM/LM pectin gels by altering the gel formation conditions.[15] The mixed gels were also compared with pure HM and LM pectin gels. Results from transmission electron microscopy revealed that the microstructure of the pure and mixed pectin gels differed greatly, which implied that the kinetic properties had a strong influence on the organization of the gel network. Large variations in kinetic behavior were also found for the different preparations at pH 3.[16] The gel formation of HM pectin in the presence of 60% sucrose was rather slow, whereas rapid gel formation was obtained for LM pectin. Consequently, the mixed HM/LM pectin gel in the absence of Ca^{2+} showed characteristics of both HM and LM pectin gelation with a rapid initial increase in G' followed by a slight progressive increase during the latter part of the measurement.

The microstructure of LM pectin gels has previously been studied in the presence of sucrose at acidic pH.[15,17] Both a nonamidated LM pectin gel at pH 3[15] and an amidated LM pectin gel at pH 4[17] showed rather homogeneous gel structures with pores in the range of 200−500 nm.

In the present investigation, the microstructure and the kinetic behavior were examined for three LM pectins differing in methyl esters and amide groups. The objective was to outline the effect of pH, sucrose, and substitution groups on the microstructure and functional behavior of LM pectin gels.

II. Materials and Methods

II.A. Materials. The chemical characteristics of the three pectin samples are summarized in Table 1, and a schematic representation of the substitution groups are shown in Figure 1. Pectin C30 was produced by Copenhagen Pectin A/S (Lille Skensved, Denmark), pectin G was produced by Degussa Texturant Systems (Redon, France), and pectin sG was obtained by alkaline saponification of pectin G. For this purpose, pectins (4 g) were dissolved in 500 mL of water at 40 °C. An equal volume of 0.1 M sodium hydroxide was added under cold conditions (4 °C) to avoid β-elimination. After 24 h, the samples were neutralized by adding 500 mL of acetic acid. Pectins were then ultrafiltrated (Millipore Pellicon membrane; 10 kDa) and freeze-dried. The MW of the samples was determined with high-performance size exclusion chromatography (HPSEC), and the uronic acid content was determined by the automated colorimetric *m*-hydroxydiphenyl method,[18,19] where pectins (60 μg/mL) were boiled (1 h), cooled, and then saponified with sodium hydroxide (40 mM). The degree of blockiness (DB) was determined by digestion of the pectins with polygalacturonase of *Kluiveromyces fragilis* (PG*kf*) which needs four or more free galacturonic acid units (GalA) to act. Degradation products were analyzed by HPAEC at pH 5. The degree of blockiness is defined as the amount of nonesterified mono-, di-, and trigalacturonic acid residues released by the enzyme relative to the total amount of nonesterified galacturonic acid units in the pectin.[20] A high DB value thus indicates a blockwise distribution of nonesterified GalA residues in a pectin.

II.B. Sample Preparation. Pectin solutions with concentrations of 0.4% and 0.8% (w/w) were prepared by dispersing the required amount of pectin in 0.1 M NaCl. The samples were stirred at room temperature for at least 90 min. The pH was set to 3.0, 4.0, 5.0, or 7.0 with NaOH and HCl before the samples were heated to 80 °C. Sucrose was added to some samples to a concentration of 10% or 30% (w/w), and then a preheated solution of $CaCl_2$ was slowly added to give a final concentration of 0.05% (w/w) $CaCl_2 \cdot 2H_2O$ (3.4 mM) in the 0.4% pectin sample and 0.1% (w/w) $CaCl_2 \cdot 2H_2O$ (6.8 mM) in the 0.8% pectin sample. The weight of each sample was adjusted with distilled water, and the samples were reheated to 80 °C before they were poured into the rheometer cup. To prepare them for microscopy, the samples were poured into cylindrical moulds and stored at room temperature overnight.

II.C. Rheological Measurements. Dynamic rheological measurements were carried out in a Stresstech rheometer (Reologica Instruments, Lund, Sweden) with strain-controlled oscillation in a cup and bob geometry at 25 °C. The surface of the sample was covered with paraffin oil to avoid evaporation. A temperature of 25 °C was reached after ~9 min. Gel formation measurements were performed at a frequency of 1 Hz and a strain of 2×10^{-3} for 3 and 10 h.

II.D. Microscopy. Small gel cubes ~$1 \times 1 \times 1$ mm were carefully cut from the gels the day after the sample preparation. The gels were fixed in an aldehyde solution, based on 0.1 M NaCl, 2% glutaraldehyde, and 0.1% ruthenium red. Four different NaCl solutions were used for fixation depending on the composition of the samples: NaCl solution with or without 30% sucrose at pH 3.0 and pH 7.0, respectively. The gel cubes were placed in the fixation solution for 2 h. The samples were rinsed twice in NaCl solution for 15 min. The samples were dehydrated with ethanol, and then the alcohol was replaced with the acrylic resin LR white (for details, see previous work).[15] The polymerization of LR white was obtained at 60 °C for about 20 h. Thin sections (~97 nm) were cut with a diamond knife. The sections were transferred to gold grids and stained with periodic acid, thiosemicarbazide, and silver proteinate.[21] The samples were examined with a transmission electron microscope (LEO 906E, LEO Electron Microscopy Ltd., Cambridge, England) at 80 kV. Each microscopy preparation was conducted in at least two replicates.

III. Results and Discussion

The gel microstructure and the kinetic rheological behavior were characterized for the nonamidated pectin C30 and the amidated pectin G at pH 3 and 7 with and without 30% sucrose. The effect of sucrose on the storage modulus of these two pectins was also studied at pH 3, 4, 5, and 7. Finally, the microstructure and gel rheology were investigated for the saponified pectin G at pH 7. Pectin is a weak acid with reported pK_a values ranging from 3.4 to 4.5.[5,22,23] Thus, at pH 3 the majority of the carboxyl groups are undissociated, while at pH 7 the majority of the carboxyl groups are dissociated.

III.A. Gel Microstructure and Kinetic Behavior at Acidic and Neutral pH. *III.A.1. Microstructure at pH 3.* The gel network of 0.8% pectin G was characterized at different length

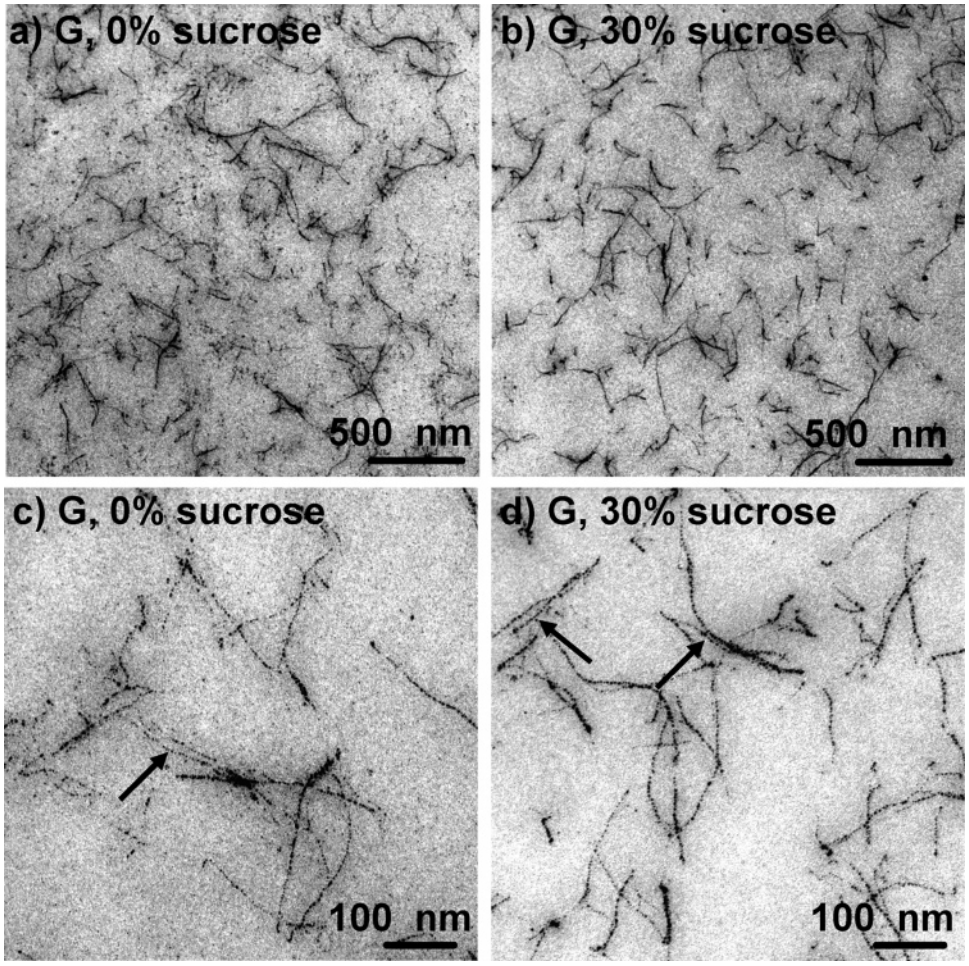

Figure 2. Gel microstructure of 0.8% pectin G at pH 3 with and without sucrose: (a and c) 0% sucrose; (b and d) 30% sucrose.

scales by TEM both in the presence and in the absence of 30% sucrose. The general nature of the gels at pH 3 is shown at the lowest magnification in Figure 2, parts a and b. Both with and without sucrose a sparse, aggregated, and homogeneous network structure is revealed. At the higher magnification in Figure 2, parts c and d, details of the network can be seen. The strands appear stiff and straight, and some of the strands are arranged in parallel, as indicated by arrows in Figure 2, parts c and d. The same gel network characteristics were also found for an amidated pectin in the presence of 0.1% $CaCl_2$ and 20% sucrose at pH 4 by Walkenström et al.[17] That gel was also homogeneous and composed of rather stiff strands. A slightly less homogeneous network has been found previously for a nonamidated LM pectin in the presence of 0.15% $CaCl_2$ and 30% sucrose at pH 3.[15] The nonamidated pectin gel structure showed similar dimensions to those reported here, but the strands appeared more flexible and branched.

III.A.2. Microstructure at pH 7. Figures 3 and 4 reveal that the microstructure of 0.8% pectin G and 0.8% pectin C30 at pH 7 is much denser than the structure of pectin G at pH 3. A rough estimation of the pore size indicates that gels at pH 7 contain many pores in the range of 100 nm, whereas the gels at pH 3 contain pores in the range of 300−400 nm. Furthermore, at the highest magnification both pectins G and C30 show fine, branched structures with flexible strands connected in loose aggregates, in contrast to the stiff and straight appearance of the strands occurring at pH 3 (compare Figures 3c,d, and 4c,d with Figure 2c,d). In similarity to the gels at pH 3, the presence of sucrose does not affect the microstructure of either the amidated or the nonamidated gels at pH 7. Both with and without 30% sucrose homogeneous, dense structures can be seen for the pectins.

The degree of substitution is 30% for pectin C30 and 49% for pectin G. Thus, the amount of charged carboxyl groups at pH 7 is quite different for the pectins (70% for C30 and 51% for G). However, the difference in charge density of the pectins does not give rise to any larger structural variations among the gels.

III.A.3. Kinetic Rheological Behavior. The kinetic behavior during gel formation of pectin G at pH 3 can be seen in Figure 5a. The amidated pectin shows rapid gel formation with $G' > G''$ already from the beginning of the measurements both with and without 30% sucrose. A sharp initial increase in G' during the first ~0.5 h is followed by a small increase during the latter part of the measurements. The same behavior is found both with and without sucrose, although in the presence of sucrose the G'_{10h} is about 4 times higher than in the absence of sucrose. Figure 5c shows normalized values of G' for both pectins G and the nonamidated C30 with and without 30% sucrose. Each measurement point on the G' curve is divided by the maximum value during the first 3 h of the measurement (G'_{max}). The results in Figure 5c show that the normalized (G'/G'_{max}) curves for pectin G both with and without sucrose and for pectin C30 with sucrose coincide, whereas the normalized curve for pectin C30 without sucrose is somewhat lower.

Another kinetic behavior is found at pH 7 for pectins G and C30 both with and without sucrose (Figure 5, parts b and d). Figure 5b shows the absolute values of G' and G'' for pectin G. The initial G' increase is very rapid, and the storage modulus reaches a plateau within the first 20 min and thereafter remains

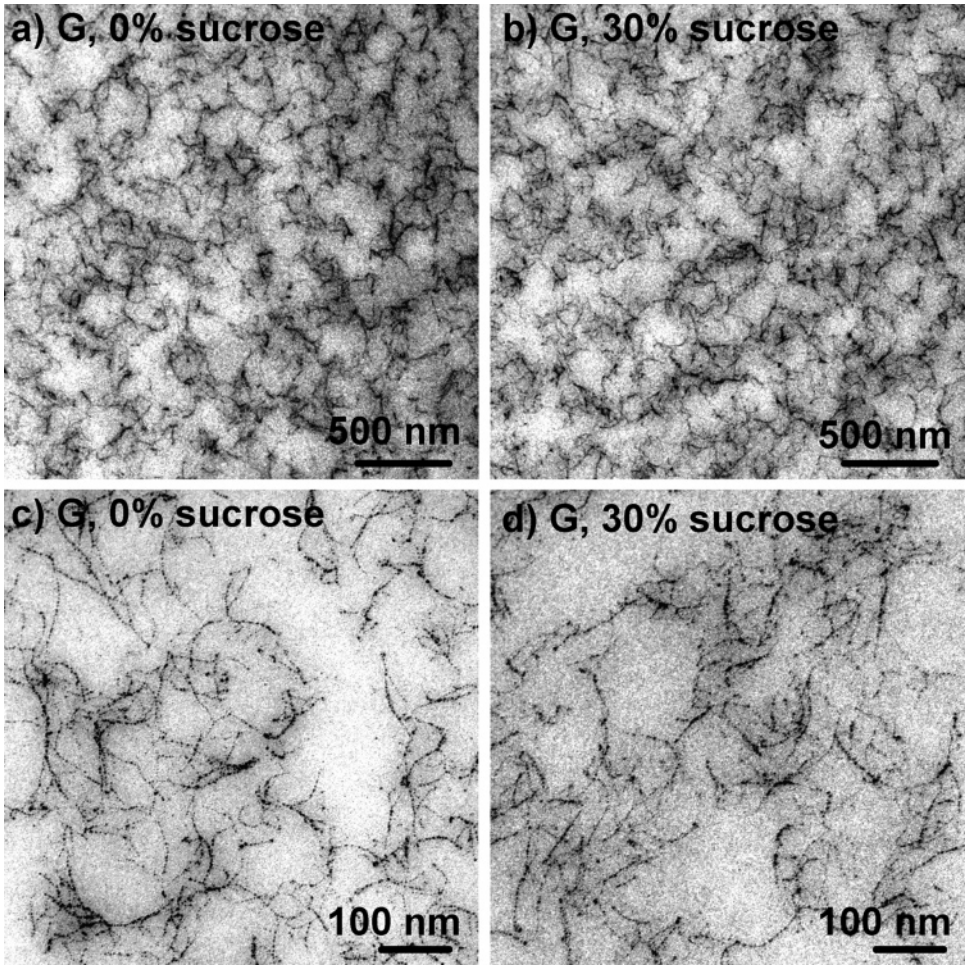

Figure 3. Gel microstructure of 0.8% pectin G at pH 7 with and without sucrose: (a and c) 0% sucrose; (b and d) 30% sucrose.

constant during the latter part of the measurement. The same rapid kinetic behavior occurs both with and without sucrose, but for pectin G the presence of 30% sucrose results in a 4 times higher G'_{10h} value than in the absence of sucrose. The normalized (G'/G'_{max}) curves for pectins G and C30 in Figure 5d coincide, which indicates similar kinetic behavior for both pectins at pH 7.

The loss modulus (G'') of pectin G is higher with sucrose than without both at pH 3 and pH 7, as can be seen in Figure 5, parts a and b. Furthermore, the phase angle (δ) ($\tan \delta = G''/G'$) was slightly affected by the presence of sucrose and the pH change. Although the differences were small ($\delta \sim 2-5$), the same behavior was observed for both pectins G and C30, values being higher with sucrose than without and also at pH 3 than at pH 7. The behavior of the phase angle implies that gels of a slightly higher viscous character are obtained at pH 3 than at pH 7, which agrees well with recent results on citrus LM pectin.[3] Cardoso et al. report that a change in pH from 7 to 3 clearly decreases the differences between the storage and loss modulus for an LM pectin gel in the absence of sucrose. Moreover, they found similar kinetic behavior both at pH 3 and pH 7 during LM pectin gel formation but at a lower Ca^{2+} concentration at pH 7 than at pH 3.

It is interesting that pectins G and C30, although they are of different origin and cannot be completely comparable, appear very similar both in gel microstructure and kinetic behavior at pH 3 and pH 7.

III.A.4. Sucrose Structure Relations. The presence of 30% sucrose does not affect the gel structure or the kinetic behavior of pectin G or C30, but the storage modulus is, as expected, strongly affected by the presence of sucrose. The similarity of the gel network and the kinetic behavior with and without sucrose indicates that the network structure is formed independently of the sucrose content.

One function of sucrose in the gel formation of pectins is to reduce the water content, thereby promoting polymer−polymer interactions rather than polymer−solvent interactions.[12,13] It has also been suggested that sucrose, with its specific spacing of the hydroxyl groups, stabilizes the cross-linking junctions in the gel.[1,10] The present results imply that sucrose strengthens the pectin gel on a molecular level without any detectable effect on the overall gel network structure.

III.A.5. pH Structure Relations. The structural variations at pH 3 and 7 (Figures 2−4) might be a result of (i) the differing kinetic behavior or (ii) the conformational transition of the pectin backbone that is associated with a pH change.

(i) At pH 3 the gel structure of pectin G is coarse, open, and with large aggregates of stiff strands. Since the storage modulus increases sharply during the first 30 min of the measurement and thereafter continues to increase throughout the measurement, it can be assumed that the gel network develops continuously during a long period of time. However, previous microscopy results showed that an HM pectin gel at pH 3 of similar dimensions was unaffected between 4 and 20 h after the beginning of the measurement, despite a large increase in G' (40%) during the same period of time.[14] From that result it was concluded that the basic network structure was developed during the initial 4 h of the gel formation process and that the later

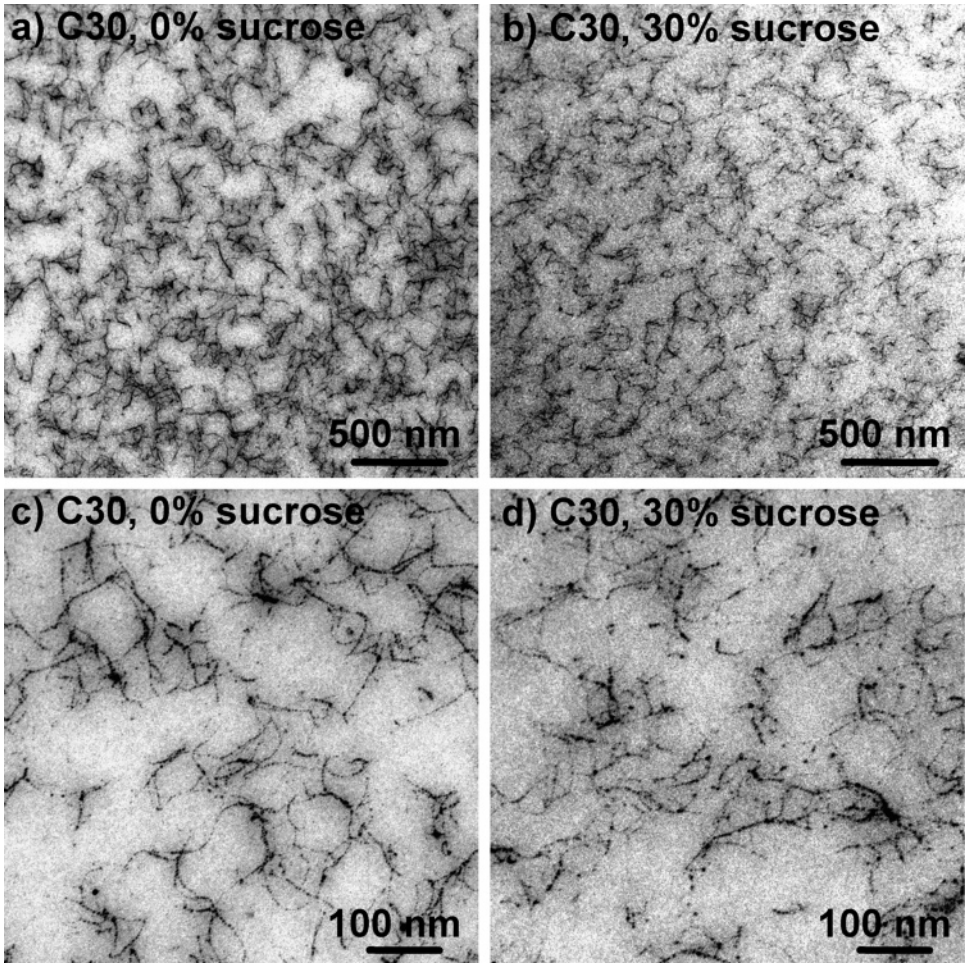

Figure 4. Gel microstructure of 0.8% pectin C30 at pH 7 with and without sucrose: (a and c) 0% sucrose; (b and d) 30% sucrose.

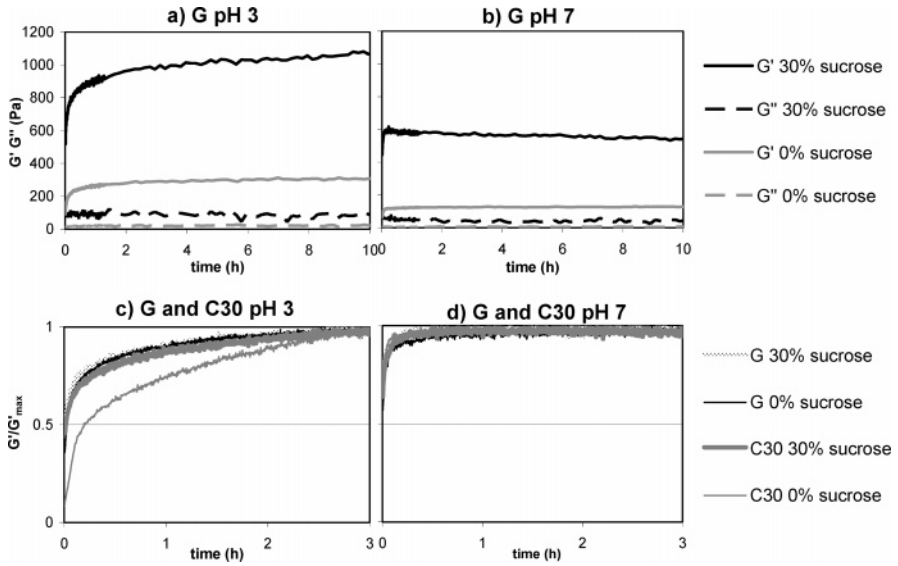

Figure 5. *G′* and *G″* during gel formation of 0.8% pectin G at (a) pH 3; (b) pH 7. Normalized *G′* values during gel formation of 0.8% pectin G and 0.8% pectin C30 at (c) pH 3; (d) pH 7. The initial 9 min involves a temperature decrease from 80 to 25 °C.

increase in *G′* was related to internal strengthening of the gel network by intermolecular forces. One may speculate that the coarse aggregated network structure of pectin G at pH 3 is developed rather early, when the largest *G′* increase occurs, followed by a strengthening of the network by intermolecular forces within the formed aggregated strands. At pH 7 the gel formation is far more rapid than at pH 3. *G′* reaches its final value almost instantaneously without any further increase during the latter part of the measurement. The micrographs at pH 7 reveal a dense entanglement-like structure composed of fine, flexible strands. It is possible that the initial gel structure is fixed within the first 15−20 min and that no further aggregation of the strands occurs after that.

(ii) It has been reported that pectin in solution undergoes a conformational transition from a highly extended 2-fold helix structure at neutral pH to a compact 3-fold structure at acidic

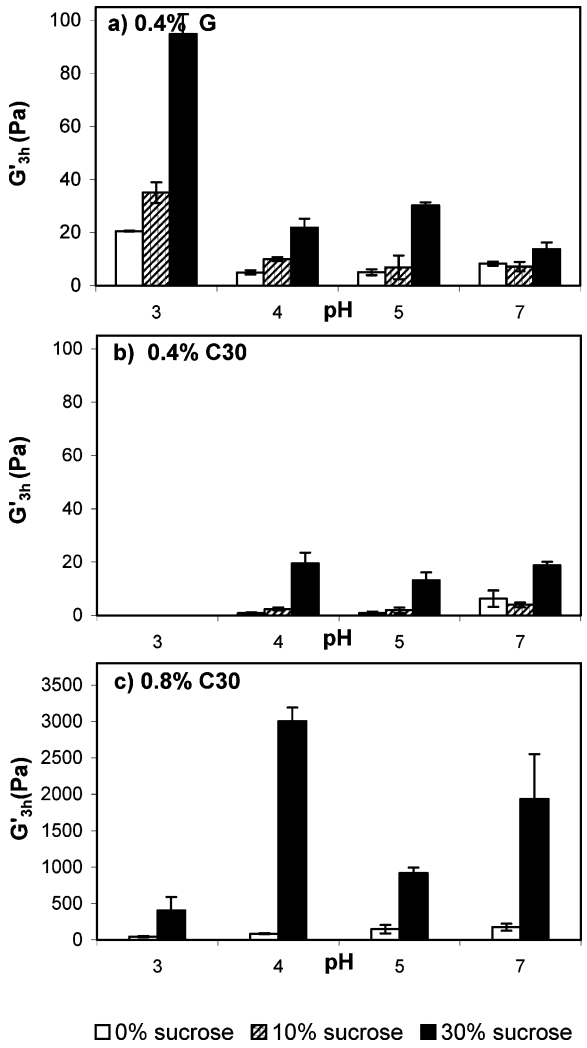

Figure 6. Effects of pH and sucrose on G'_{3h} for (a) 0.4% pectin G; (b) 0.4% pectin C30; (c) 0.8% pectin C30.

pH.[4,6,7] At neutral pH values, where most of the carboxyl groups are dissociated, inter- and intramolecular electrostatic repulsions occur, and cross-links between the pectin chains are possible through Ca^{2+} bindings to the carboxyl groups. It is possible that the extended conformation and the electrostatic repulsions have a bearing on the dense, entanglement-like network structure at pH 7 seen in Figures 3 and 4.

Reducing the pH can lead to a conformational transition into a more compact 3-fold helix structure. It is suggested that the gel formation of LM pectin under acidic conditions can occur by association of 3-fold helices enabled by the suppression of electrostatic repulsion and by allowing the carboxyl groups to act as hydrogen-bond donors.[4] The highly aggregated network structure seen in Figure 2 might be a consequence of low charge density and the compact conformation of the pectin molecules.

III.B. Gel Rheology for Amidated and Nonamidated Pectins — Effect of pH and Sucrose. The variation in the storage modulus 3 h after gel formation (G'_{3h}) was investigated at pH 3, 4, 5, and 7 for pectins G and C30. At 0.4% pectin the influence of pH was studied in the presence of 0%, 10%, and 30% sucrose (Figure 6, parts a and b). Large differences in G' can be observed for both G and C30 when pH is changed.

For the amidated pectin G, the highest storage modulus is obtained at pH 3. At this pH it is likely that the amide groups strengthen the gel network by hydrogen bonds. At pH 4, 5, and 7 the storage moduli are considerably lower, which might be a consequence of increased electrostatic repulsions between the pectin chains, preventing hydrogen bonds from being formed. At pH 4, 5, and 7 the gel formation is facilitated by the increased dissociation of carboxyl groups, which favors the Ca^{2+}-induced gel formation.

For the nonamidated pectin C30, no gel formation occurs at pH 3 at 0.4% pectin due to the weakened pectin−Ca^{2+} interaction when the undissociated form of the carboxyl groups dominates. At pH 4, 5, and 7 the dissociation of the carboxyl groups is higher, which results in gel formation.

A large variation in G' is observed for 0.8% C30 in the presence of 30% sucrose (Figure 6c) when the pH is changed. The same variation is also seen at the lower pectin concentration but not in the absence of sucrose. At pH 3 the storage modulus is rather low at 0.8% pectin, according to the weak pectin−Ca^{2+} interaction. A large effect on G' is observed when the pH is changed from 3 to 4. The highest storage modulus is found at pH 4, followed by a lower value at pH 5. G' is higher at pH 7 than at pH 5, probably as a result of strong Ca^{2+} interaction with the dissociated carboxyl groups. In contrast to the gels with 30% sucrose, a small progressive increase in G' can be observed for pectin C30 in the absence of sucrose when the pH increases from 3 to 7, which may be ascribed to the increased dissociation of the carboxyl groups.

III.C. Gel Rheology and Microstructure in the Absence of Methyl Esters. The rheological properties were also investigated for the saponified pectin (sG) at a concentration of 0.4% at pH 3 and 7. At pH 3 the samples phase-separated into microgel droplets in a viscous phase both in the presence and the absence of 30% sucrose. The elimination of the methyl esters of pectin sG changed the gel-forming ability at pH 3 compared to its mother pectin G. It seems possible that a strong aggregation of the polymer chains may occur in the presence of Ca^{2+} due to the high Ca^{2+} sensitivity of pectin sG as the degree of substitution is low (18%). In addition, the suppressed electrostatic repulsions that occur at pH 3 may also contribute to aggregation and microgel formation. Pectin G forms coherent gels with high storage moduli at pH 3, both with and without sucrose. The presence of methyl esters in pectin G will give rise to hydrophobic interactions and also constitute a sterical hindrance preventing complete Ca^{2+}-mediated aggregation and microgel formation.

At pH 7 the gel-forming ability of pectin sG is high. In the absence of sucrose G'_{3h} is 590 (±100) Pa, and in the presence of 30% sucrose G'_{3h} is 1130 (±260) Pa for gels with a pectin concentration of 0.4%. In comparison, pectin G at pH 7 forms rather weak gels with $G'_{3h} \sim 10$ Pa (Figure 6a).

An interesting difference can be observed in the microstructure of pectins G and sG at pH 7. Figure 7 shows micrographs of pectins G and sG at a concentration of 0.8% pectin in the presence of 30% sucrose. Both pectins form a gel network of similar dimensions, but the structure of G is homogeneous with small pores surrounded by loose aggregates of strands. In contrast, the structure of sG is more inhomogeneous with both large and small pores (Figure 7, parts a and b). Furthermore, the aggregates of pectin sG are mainly composed of rodlike strands with a clear tendency toward parallel alignment (marked by arrows in Figure 7d). The variations in network density observed for pectin sG is probably an effect of the high Ca^{2+} sensitivity that exists for the saponified pectin. When the Ca^{2+} solution is added dropwise to the sample, rapid gel formation occurs in the vicinity of the droplets. As a comparison, an inhomogeneous gel structure was also observed for a highly Ca^{2+}-sensitive HM pectin with a blockwise distribution of the

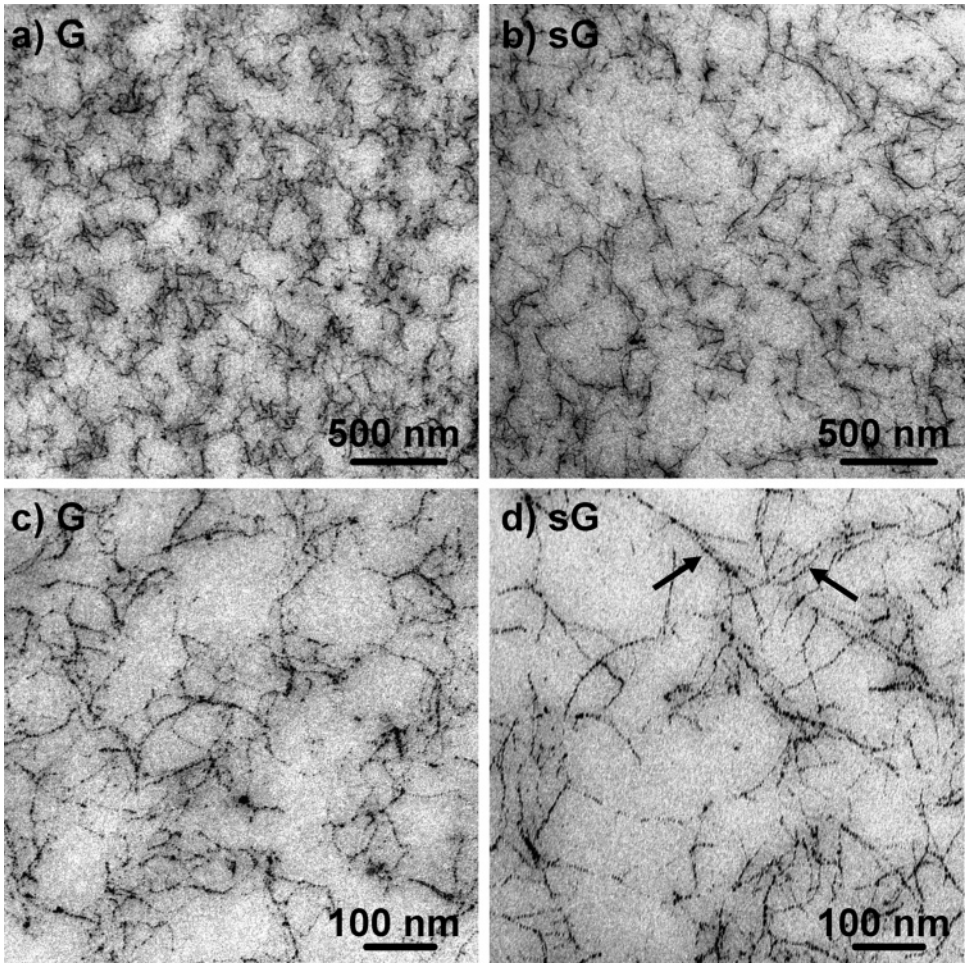

Figure 7. Gel microstructure of 0.8% pectin G and 0.8% pectin sG at pH 7 in the presence of 30% sucrose: (a and c) pectin G; (b and d) pectin sG.

methyl esters at pH 3 and 60% sucrose.[14] In the presence of 0.25% $CaCl_2 \cdot 2H_2O$, large differences were revealed in the network density, whereas the corresponding gel in the absence of Ca^{2+} was homogeneous.

The big difference in G'_{3h} for pectin G and sG at pH 7 may be a combination of several factors. At pH 7 the carboxyl groups are dissociated, and the Ca^{2+}-induced binding dominates the gel formation. Pectin sG has a larger number of carboxyl groups accessible to Ca^{2+} binding than pectin G, which results in a higher G'_{3h} for pectin sG. In addition, the high degree of substitution in pectin G may also cause sterical hindrance of the pectin strands, resulting in a more flexible appearance of the gel structure, which limits the possibility of long Ca^{2+}-mediated assemblies. Consequently, the lower degree of substitution of pectin sG may enable the strands to adopt a somewhat more stretched and straight form, which can affect both the storage modulus and the gel structure.

A comparison between pectin sG and its mother pectin G reveals differences both in structural appearance and the storage modulus. The results indicate that the elimination of methyl esters strongly influences the Ca^{2+} interaction, thereby affecting the functionality of the gels.

IV. Conclusions

We have shown that the microstructure and the kinetic behavior of LM pectin gels differed at pH 3 and at pH 7. Gels at pH 3 were open and highly aggregated, whereas gels at pH 7 showed dense, entanglement-like structures.

The kinetic behavior during gel formation was similar for both the amidated pectin G and the nonamidated pectin C30. Rapid gel formation occurred both at pH 3 and pH 7, but the shape of the G' curves differed. Furthermore, our results showed that the presence of 30% sucrose influences neither the kinetic behavior nor the microstructure of the gels but that it did, as was expected, enhance the storage modulus. The storage moduli of the amidated and the nonamidated pectins showed large variations in the presence of sucrose when pH was changed, and the maximum in G' of the samples occurred at different pH values. A comparison of the three pectins, which differed in their substitution groups, showed a clear difference between pectin sG and the other pectins in the storage modulus. The storage modulus for pectins G and C30 at pH 7 in the presence of sucrose was ~10−15 Pa, whereas G' for pectin sG was ~1100 Pa. In contrast, at pH 3 gel formation was only possible for pectin G, facilitated by the amide groups. Pectins G and sG revealed some small differences in microstructure at pH 7. Pectin sG was slightly inhomogeneous with a more rigid appearance of the pectin strands compared to those of pectin G. The results display the large influence of substitution groups and pH on the functional properties of LM pectin gels.

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References and Notes

(1) Oakenfull, D.; Scott, A. Hydrophobic interaction in the gelation of HM pectins. *J. Food Sci.* **1984** *49*, 1093−1098.

(2) Axelos, M. A. V.; Thibault, J.-F. The chemistry of low-methoxyl pectin gelation. In *The Chemistry and Technology of Pectin*; Walter, R. H., Ed.; Academic Press: New York, 1991; pp 109−118.

(3) Cardoso, S.; Coimbra, M.; Lopes da Silva, J. A. Temperature dependence of the formation and melting of pectin−Ca^{2+} networks: a rheological study. *Food Hydrocolloids* **2003**, *17*, 801−807.

(4) Gilsenan, P. M.; Richardson, R. K.; Morris, E. R. Thermally reversible acid-induced gelation of low-methoxy pectin. *Carbohydr. Polym.* **2000**, *41*, 339−349.

(5) Racape, E.; Thibault, J.-F. Properties of amidated pectins. II. Polyelectrolyte behavior and calcium binding of amidated pectins and amidated pectic acids. *Biopolymers* **1989**, *28*, 1435−1438.

(6) Paoletti, S.; Cesaro, A.; Delben, F.; Ciana, A. Ionic effects on the conformation, equilibrium, properties and rheology of pectate in aqueous solutions and gels. In *Chemistry and Function of Pectins*; Fishman, M. L., Jen, J. J., Eds.; American Chemical Society: Washington DC, 1986; pp 73−87.

(7) Thibault, J.-F.; Rinaudo, M. Interactions of counterions with pectins studied by potentiometry and circular dichroism. In *Chemistry and Function of Pectins*; Fishman, M. L., Jen, J. J., Eds.; American Chemical Society: Washington DC, 1986; pp 61−72.

(8) Lootens, D.; Capel, F.; Durand, D.; Nicolai, T.; Boulenguer, P.; Langendorff, V. Influence of pH, Ca concentration, temperature and amidation on the gelation of low methoxyl pectin. *Food Hydrocolloids* **2003**, *17*, 237−244.

(9) Clark, A. H.; Farrer, D. B. Shear modulus−concentration relationships for low DE pectin−calcium gels in the temperature range 20−85 °C. *Food Hydrocolloids* **1996**, *10* (1), 31−39.

(10) Fu, J.-T.; Rao, M. A. Rheology and structure development during gelation of low-methoxyl pectin gels: the effect of sucrose. *Food Hydrocolloids* **2001**, *15*, 93−100.

(11) Grosso, C. R. F.; Rao, M. A. Dynamic rheology of structure development in low-methoxyl pectin + Ca^{2+} + sugar gels. *Food Hydrocolloids* **1998**, *12*, 357−363.

(12) Tsoga, A.; Richardson, R. K.; Morris, E. R. Role of cosolutes in gelation of high-methoxy pectin. Part 1. Comparison of sugars and polyols. *Food Hydrocolloids* **2004**, *18*, 907−919.

(13) Evageliou, V.; Richardson, R. K.; Morris, E. R. Effect of pH, sugar type and thermal annealing on high-methoxy pectin gels. *Carbohydr. Polym.* **2000**, *42*, 245−259.

(14) Löfgren, C.; Guillotin, S.; Evenbratt, H.; Schols, H.; Hermansson, A.-M. Effects of calcium, pH and blockiness on kinetic rheological behavior and microstructure of HM pectin gels. *Biomacromolecules* **2005**, *6* (2), 646−652.

(15) Löfgren, C.; Walkenström, P.; Hermansson, A.-M. Microstructure and rheological behavior of pure and mixed pectin gels. *Biomacromolecules* **2002**, *3* (6), 1144−1153.

(16) Löfgren, C.; Hermansson, A.-M. Microstructure and kinetic behavior of pure and mixed pectin gels. In *Gums and Stabilizers for the Food Industry 12*; Williams, P. A., Phillips, G. O., Eds.; The Royal Society of Chemistry: London, 2004; pp 153−159.

(17) Walkenström, P.; Kidman, S.; Hermansson, A.-M.; Rasmussen, P. B.; Hoeg, L. Microstructure and rheological behaviour of alginate/pectin mixed gels. *Food Hydrocolloids* **2003**, *17* (5), 593−603.

(18) Ahmed, A. E. R.; Labavitch, J. M. A simplified method for accurate determination of cell wall uronide content. *J. Food Biochem.* **1977**, *1*, 361−365.

(19) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *12*, 484−489.

(20) Daas, P. J. H.; Meyer-Hansen, K.; Schols, H. A.; De Ruiter, G. A.; Voragen, A. G. J. Investigation of the nonesterified galacturonic acid distribution in pectin with endopolygalacturonase. *Carbohydr. Res.* **1999**, *318*, 135−145.

(21) Thiery, J. P. Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microsc. (Paris)* **1967**, *6*, 987−1018.

(22) Michel, F.; Thibault, J.-F.; Doublier, J.-L. Viscometric and potentiometric study of high methoxyl pectins in the presence of sucrose. *Carbohydr. Polym.* **1984**, *4*, 283−297.

(23) Plaschina, I. G.; Braudo, E. E. Tolstoguzov, V. B. Circular dichroism studies of pectin solutions. *Carbohydr. Res.* **1978**, *60*, 1−8.

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