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Gelation properties of extruded lemon cell walls and their water-soluble pectins [†]

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Abstract

The amount of water-soluble pectins was largely increased after extrusion-cooking of lemon fibres. These pectins showed the ability to form a gel in the presence of sucrose and at acidic pH. The gels obtained with the water-extracted pectins after extrusion-cooking and with pectins acid-extracted on a laboratory scale were softer than those prepared with commercial citrus pectins. The water-extracted pectins after extrusion-cooking and the pectins acid-extracted on a laboratory scale contained long neutral side-chains and required a higher sucrose concentration to gel than the commercial citrus pectins. The extruded lemon fibres showed the ability to form gels in the presence of sucrose and at acidic pH. The gels obtained with the extruded fibres containing some water-soluble pectins of high molecular weight were stronger than those obtained with the extruded fibres containing higher amounts of more depolymerised water-soluble pectins. The extruded fibres containing 12.5–14.9% of water-soluble pectins of high molecular weight (intrinsic viscosity: 413–504 mL/g) were those showing the better gelation properties.

1. Introduction

Pectins have such properties as gelation and emulsion stabilisation which make them useful in the food industry. These polysaccharides contain a linear backbone of D-galacturonic acid residues partially esterified by methanol and occasionally interrupted by L-rhamnose residues. Other neutral sugars, mainly L-arabinose and

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[†] Pectins of Lemon Cell Walls, Part II. For Part I, see ref 9.

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D-galactose, constitute the neutral side-chains of the pectic substances [1,2]. It has been established that the neutral sugars are grouped in "hairy" regions containing few galacturonic acid residues, while the bulk of the galacturonic acid residues were recovered in "smooth" homogalacturonic regions [3,4].

Pectins are commercially extracted from such wastes of the fruit juice industry as citrus peels and apple pomace. The raw material is treated with hot dilute mineral acid at pH ~ 2; pectins are then released and can be recovered by alcoholic precipitation [2,5]. This acid extraction has several practical disadvantages, such as water pollution and corrosion. The resulting pectins have a high degree of methylation (dm, mol of MeOH per 100 mol of galacturonic acid); they can form gels [2,5,6] at a pH of 2.2-3.5 if a cosolute such as sucrose is present at a concentration of $> 55\%$ by weight. It has been shown [7,8] that the network in gels of high methoxyl pectins is stabilised both by hydrogen bonds between undissociated carboxyl and secondary alcohol groups and by hydrophobic interactions between methoxyl groups. The requirement of a high concentration of sucrose can be explained by the stabilisation of hydrophobic interactions or by a marked decrease in the water-activity of the system.

In the accompanying paper [9], we have shown that appreciable amounts of pectins could be extracted by water from lemon fibres after extrusion-cooking. These pectins had particularly long neutral side-chains, a very high dm, and molecular weights close to those usually observed for acid-extracted pectins. We have therefore investigated the rheological properties of gels made with pectins water-extracted from lemon fibres after extrusion-cooking. These gels were compared with those made with pectins acid-extracted on a laboratory scale and with those made with commercial lemon pectins.

Moreover, since extruded lemon fibres contained large amounts of water-soluble pectins of high molecular weight, we combined the two steps of pectin extraction and gelation in one, and studied the gelation abilities of various extruded lemon fibres under the standard conditions of gelation of high methoxyl pectins.

2. Materials and methods

Pectins and lemon fibres.—Commercial citrus HM pectins (C73, GENU-X-0907) were from the Copenhagen Pectin Factory (Denmark). They were previously characterised [10]. Lemon fibres were extruded as previously described [9], giving LF1-LF9 samples. Water-soluble pectins (EP) and acid-extracted pectins (HP) were obtained from LF3 and the initial lemon fibre. Purification of EP and HP was performed by ion-exchange chromatography [9]. Some physico-chemical parameters of the different pectins are given in Table 1.

Preparation of the gels and phase diagrams.—Aqueous solutions containing different concentrations of pectins were prepared. Various quantities of sucrose were then added and each mixture was heated at 100°C for 30 min with magnetic stirring. Citric acid (0.5 M) was then added slowly to reach 0.05 M in the final

Table 1
Physico-chemical characteristics of the pectin samples

Pectin [a]	GalA (%)	Neutral sugars (%)	Dm	Intrinsic viscosity (mL/g)
C73 (a)	76.3	10.2	73	562
HP (b)	51.6	22.6	81	588
Purified HP (b)	76.3	14.3		
EP (b)	49.1	29.4	91	450
Purified EP (b)	73.3	14.6		

[a] (a) Ref 10; (b) ref 9.

slurry. The concentration of sucrose is expressed in % (w/w). The slurry was poured into a cylindrical glass mould (2 × 1.1 cm), previously oiled. The surface of the slurry was covered with paraffin oil in order to avoid evaporation and the mixture was left for 16 h at ambient temperature. The sol and gel phases were estimated visually after removal from the mould. When the system was seen to flow, it was said that the system was still a sol; the system was considered as gelled when it kept the shape of the mould.

The same method was applied to the gelation trials on extruded fibres. Fibres were suspended in distilled water at 20°C for 30 min with magnetic stirring; sucrose was then added and the mixture heated, acidified, and poured as described above.

Dynamic rheological mesurements.—These were determined with a Carri-med CS-50 controlled stress rheometer, using a temperature-regulated cone-plate device (radius, 2.5 cm; cone-plate angle, 4°). An oscillatory movement of small selected maximum amplitude of a given frequency was imposed on the upper cone about a vertical axis, which determines a sinusoidal shear stress within the sample. The cone is linked to a stress-controlling device that adjusts the torque necessary to maintain the required deformation. The pectin/sucrose solution was prepared as described above and acidified, and the resulting slurry was poured onto the Carri-med plate heated at 90°C. Paraffin oil was layered onto the plungered cone and sample to prevent evaporation of the sample, and the system was cooled to 20°C. The kinetics of gel formation at 20°C were monitored by measuring the storage modulus (G') and the loss modulus (G'') at 0.125 Hz with a low deformation of 4%. When the kinetics reached a plateau, the system was considered to be at equilibrium and mechanical spectra were recorded over the frequency range $5 \cdot 10^{-3}$–5 Hz. For the extruded fibre gels, a plate–plate device (radius, 2.5 cm; gap, 1 mm) was chosen in order to minimise the effects of the heterogeneity of the slurry. Kinetics of gel formation and mechanical spectra were recorded at 20°C on the extruded fibres gels. It was checked that the viscoelastic behaviour was linear at the deformation imposed (up to 20%) for all the systems studied.

Compression measurements.—Pectins or fibre slurries (50 g) were prepared as previously described and poured rapidly into cylindrical moulds (3 × 3 cm) of which the top edge was raised with a sticky tape. The surface of the gel was layered with paraffin oil in order to avoid dehydration, and the gels were kept for 16 h at ambient temperature. Before performing measurements, the sticky tape was removed and the gel in excess above the top edge of the mould was carefully cut off with a thin steel wire. The gel was removed from the mould and covered with paraffin oil. An Istron apparatus was used. A compression was applied for 30 s at a rate of 2 mm/min, leading to a low (~ 3.3%) deformation in order to record the Young elastic modulus (E). The compression was then followed until the breaking point of the gel was reached.

3. Results

Phase diagrams.—The sol/gel transition lines are plotted in Fig. 1 against sucrose concentration for the pectins (EP, HP, and C73) and for one of the extruded lemon fibres (LF3).

Whatever the sucrose concentration, the transition lines for HP, C73, and EP have similar shapes. For a sucrose concentration above 54%, the pectin concentration required to obtain a gel was independent of the sucrose concentration. This minimal concentration was, however, slightly higher for EP and HP (0.15%) than for C73 (0.05%). Below 55% of sucrose, the minimal pectin concentration able to promote gelation increased when the sucrose content diminished. No gels could be obtained under 2% of pectins for a sucrose concentration below 40 and 45% for C73, and for EP and HP, respectively.

Gelation tests at pH 3 with 55% of sucrose have been done on the initial and extruded lemon fibres (1-5%, w/w). No gels could be obtained under these conditions with initial lemon fibres; in contrast, all the extruded fibres tested showed the ability to form gels. The phase diagram of LF3 was composed of two perpendicular straight lines. For a concentration in sucrose ≥ 50%, the minimal

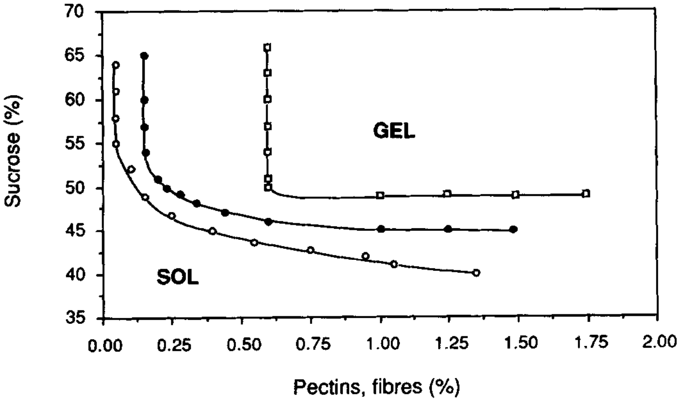

Fig. 1. Phase diagrams of pectins and extruded fibres. Determination at pH 3 of the critical gel concentration as a function of sucrose concentration: ●, EP and HP pectins; ○, C73; □, LF3 extruded fibres.

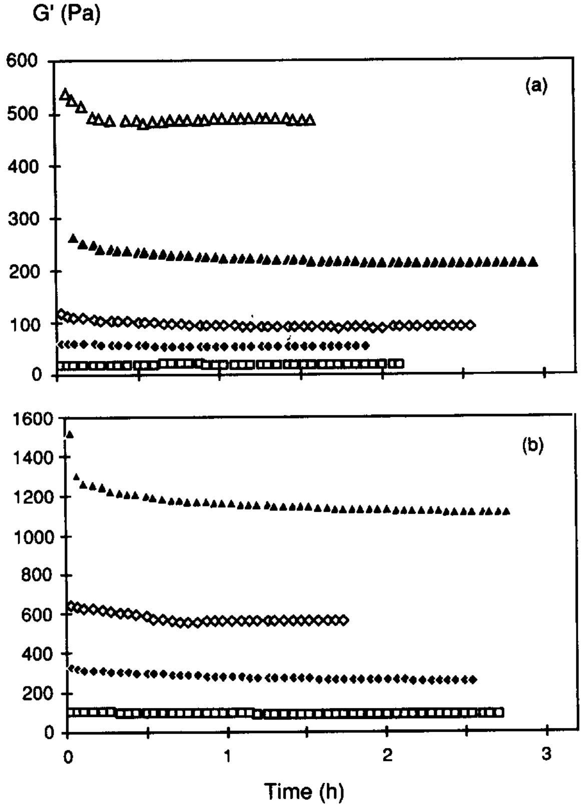

Fig. 2. Gelation kinetics at pH 3, 20°C, and 60% of sucrose for (a) EP: △, 0.8%; ▲, 0.6%; ◇, 0.4%; ◆, 0.3%; and □, 0.2% of pectin; and (b) C73: ▲, 0.6%; ◇, 0.4%; ◆, 0.3%; and □, 0.2% of pectin.

concentration able to promote gelation (0.6%, w/w) was independent of the sucrose content. Below 49% of sucrose, no gels could be obtained in the concentration range tested.

Effect of pectin concentration.—Gels formed at pH 3 for 60% of sucrose and 0.1–0.8% of pectins were studied. The gelation kinetics of EP and C73 are shown in Fig. 2. It appeared that the gels were already totally formed and stable when the mixture reached 20°C, and generally no time dependence of the storage moduli was observed after the first hour whatever the pectin concentration. A slight decrease in the storage modulus at the beginning of the measurement was only noticed for concentrations of pectins > 0.3%; this was probably the consequence of a limited syneresis. HP displayed similar kinetics (data not shown). Whatever the pectin concentration, C73 formed the strongest gels, followed by HP and finally EP (Fig. 3). The last two pectins contained [9] ca. 20% of extraneous neutral polysaccharides and the values of concentrations were corrected for these impurities. For the purified pectins, measurements of the storage moduli at equilibrium gave the

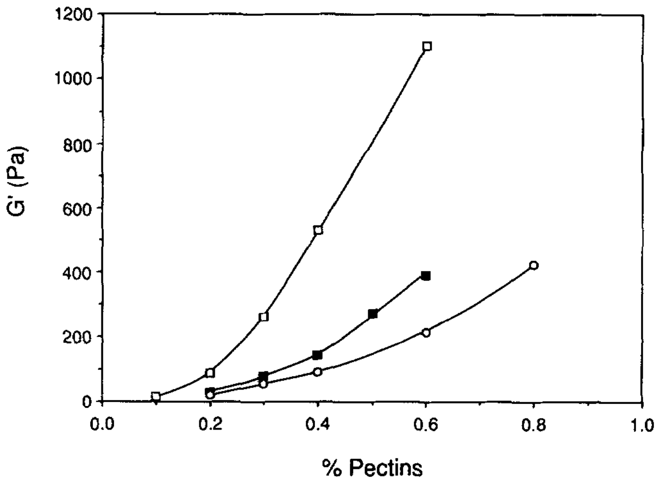

Fig. 3. Evolution of the storage moduli with the pectin concentration: ○, EP; ■, HP; □, C73.

same values, showing that the storage modulus values are not influenced by this contamination.

Mechanical spectra obtained for 0.2 and 0.6% of EP are shown in Fig. 4. The constant value of *G′* and the high *G′/G″* ratio, observed for the mechanical spectra in the frequency range explored, showed that these systems were viscoelastic solids referred to as true gels. Similar mechanical spectra were observed with the other pectin samples (data not shown).

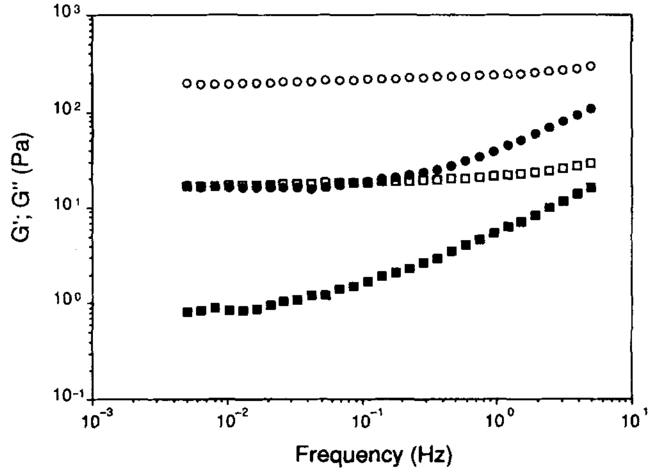

Fig. 4. Mechanical spectra of EP gels at pH 3, 20°C, and for 60% of sucrose: ○, *G′* for 0.6% of pectins; ●, *G″* for 0.6% of pectins; □, *G′* for 0.2% of pectins; ■, *G″* for 0.2% of pectins.

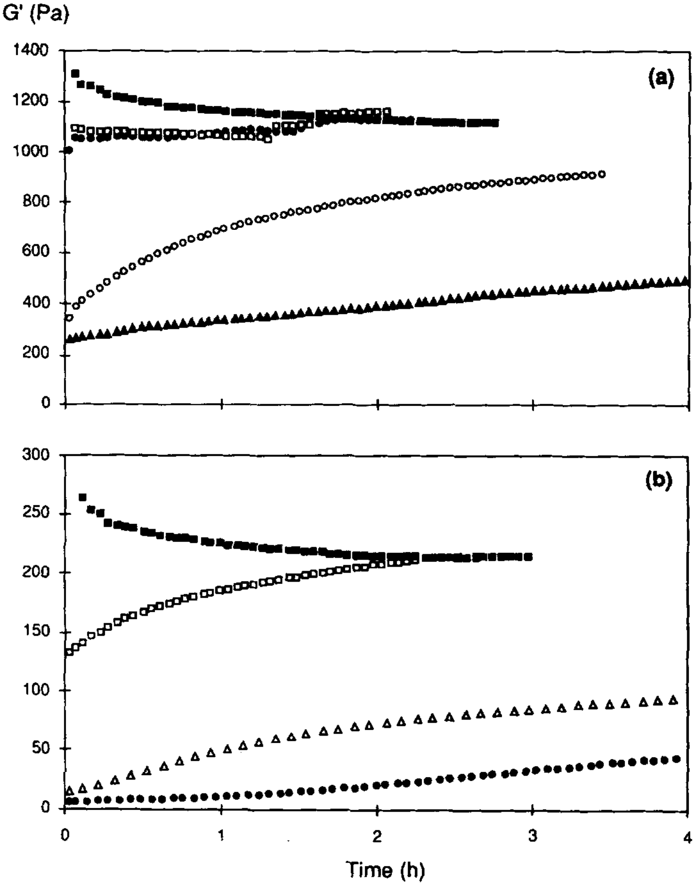

Fig. 5. Gelation kinetics of pectins (0.6%) at pH 3, 20°C, and for various sucrose concentrations: (a) C73: ■, 60% of sucrose; □, 55% of sucrose; ●, 50% of sucrose; ○, 45% of sucrose; ▲, 42.5% of sucrose; (b) EP: ■, 60% of sucrose; □, 55% of sucrose; △, 52.5% of sucrose; ●, 50% of sucrose.

Effect of the sucrose concentration.—Gelation kinetics of C73 and EP at a concentration of 0.6%, with various sucrose concentrations, are shown in Fig. 5. For C73, it appeared that, for 50 to 60% of sucrose, the values of the storage moduli merged after 2 h and remained constant. Nevertheless, for 60% sucrose, the beginning of the kinetics showed a decrease of G'. In contrast, the value of G' continuously increased for sucrose concentration lower than 50% and it required more and more time to reach the plateau as the sucrose concentration decreased. No storage modulus was measurable for 40% sucrose. EP and HP (data not shown for HP) behaved similarly to C73; however, the values of the plateau were much lower and no storage modulus was measurable if the sucrose concentration was lower than 50%.

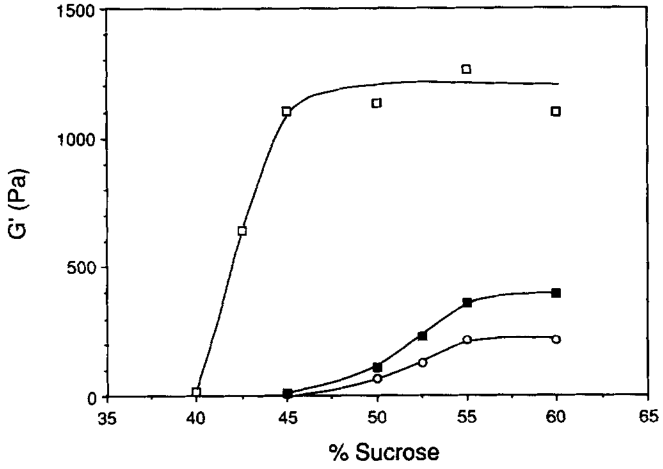

Fig. 6. Evolution of the storage moduli with the sucrose concentration: ○, EP; ■, HP; □, C73.

The dependence of the storage moduli at the plateau value on sucrose concentration is indicated in Fig. 6. For C73, *G′* increased markedly from 40–45% of sucrose and thereafter remained constant at a value of ~ 1100 Pa. For EP or HP, *G′* increased more slowly from 45–55% of sucrose and the value of the plateau was only 200 and 350 Pa, respectively.

The mechanical spectra (data not shown) were similar to those reported in Fig. 4 for a sucrose concentration between 45 and 60% for HP and EP, and between 40 and 60% for C73. It was concluded that these systems behave as viscoelastic solids.

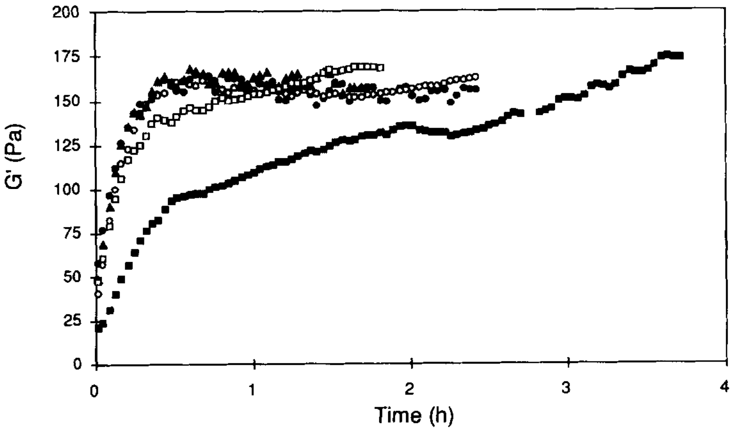

Fig. 7. Gelation kinetics of extruded fibres (1.5%) at pH 3, 20°C, and for 60% of sucrose: ●, LF1 fibres; ○, LF2 fibres; ▲, LF3 fibres; □, LF5 fibres; ■, LF9 fibres.

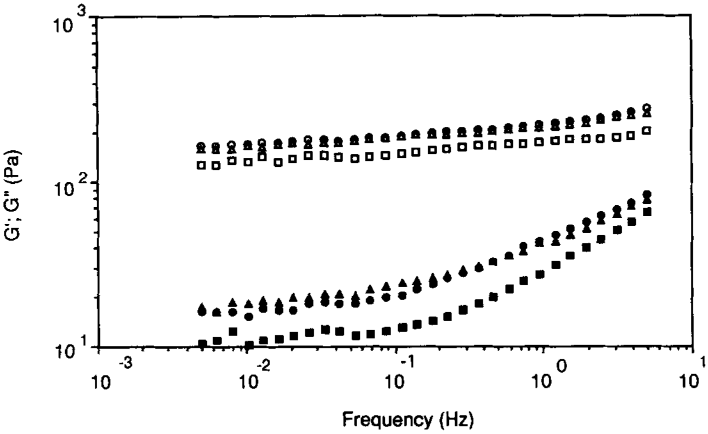

Fig. 8. Mechanical spectra of extruded fibre gels at 1.5%, pH 3, 20°C, and for 60% of sucrose: □, G′ for LF1; ■, G″ for LF1; ○, G′ for LF3; ●, G″ for LF3; △, G′ for LF9; ▲, G″ for LF9.

Gelation kinetics of the fibre gels.—Gelation kinetics at pH 3, for 60% of sucrose and 1.5% of different extruded fibres, are shown in Fig. 7. In contrast to the pectin gels at the same sucrose concentration, the fibre gels were still being formed at the beginning of the measurement at 20°C. The storage moduli reached a plateau after ~ 30 min for the fibres LF1, LF2, and LF3, which were extruded under mild conditions; ~ 1 h for the fibres LF5 extruded under medium conditions; and ~ 4 h for the fibres LF9 extruded under drastic conditions. LF1, LF2, and LF3 fibres contained [9] fair amounts of water-soluble pectic substances (12.5–14.2%) of high molecular weight (intrinsic viscosities, 413–460 mL/g). LF5 fibres contained [9] slightly higher amounts (15.1%) of more depolymerised water-soluble pectins (intrinsic viscosity, 305 mL/g), and LF9 fibres [9] very high amounts (29.4%) of severely depolymerised water-soluble pectins (intrinsic viscosity, 191 mL/g). Although there seemed to be a clear correlation between the stabilisation time of the gels and the degree of polymerisation of the water-soluble pectins present in the extruded fibres, the storage modulus values at equilibrium were similar for all the fibres studied. The mechanical spectra (Fig. 8) showed that the G' values were similar in the frequency range explored for all the fibres studied; a very slight decrease in G' can be observed with frequency, which may be attributed to the presence of fibres embedded in the network giving rise to a dispersion-like behaviour. The experiments followed with the controlled-stress rheometer did not provide a clear differentiation between the extruded fibres. Compression experiments were therefore carried out in order to obtain more information.

Instron 1122 experiments.—The compression tests have been performed on gels obtained with various concentrations of different extruded fibres (LF1, LF3, LF5, LF6, LF7, and LF9) at pH 3 and for 60% (w/w) of sucrose. The elastic Young moduli (E) and the breaking strength of these gels were determined and compared to values obtained with C73 gels (0.4 and 0.6%) at pH 3 and 60% of sucrose (Table 2).

Table 2
Young moduli and breaking strength of pectin gels and of extruded fibre gels for 60% sucrose at pH 3 for different concentrations

Gel	*c* (%)	Content in water-soluble pectin (%)	Intrinsic viscosity (mL/g)	*E* (Pa)	Breaking strength (N)
C73			562		
	0.6			2439	6.9
	0.4			1382	6.2
LF1		12.5	460		
	1.5			2160	0.4
	2.0			2165	1.0
	2.5			2594	1.4
	3.0			2444	2.2
LF3		14.2	450		
	1.0			1212	0.3
	1.5			2320	0.6
	2.0			2103	1.0
	2.5			2224	1.7
	3.0			2388	2.6
LF5		15.1	305		
	2.0			2121	0.9
	3.0			2496	2.2
LF6		21.1	314		
	2.0			1873	1.0
	3.0			2705	2.4
LF7		23.3	243		
	1.5			1631	0.4
	2.0			1886	0.7
	2.5			2256	1.1
	3.0			2239	1.5
LF9		29.4	191		
	2.0			1617	0.3
	3.0			2483	1.2

The gels obtained with 3% of extruded fibres had similar Young modulus values (2239–2505 Pa), close to the value observed for the gels obtained with 0.6% of C73. When the fibre concentration was diminished to 2%, the Young modulus was slightly decreased for LF1, LF3, and LF5 fibres, and markedly decreased for the fibres extruded in more severe conditions (LF6, LF7, and LF9). As reported above, the various extruded fibres cannot be clearly differentiated by their elastic moduli (E or G'). It must also be noted that $E = \sim 2.3\ G'$ for the C73 pectin gels, while $E = \sim 12\ G'$ for the extruded fibre gels. This large difference between E and G' for the LF samples may be attributed to the composite nature of these gels which are far from an ideal network of long chains in which entropic effects govern the elasticity.

The breaking strengths of the fibre gels were low compared to those of the pectins and varied with the extruded fibres studied. For LF1, LF3, LF5, and LF6 fibres, extruded under mild or medium conditions, breaking strengths of 2.2–2.6 N were observed, while for LF7 and LF9 fibres, extruded under more severe conditions, the breaking strengths obtained were much lower (1.5 and 1.2 N, respectively).

4. Discussion

All the pectins studied gelled under the classical conditions of gelation of the HM pectins (pH 3, 60% of sucrose). However, it appeared that EP and HP formed weaker gels than C73 and that they needed more sucrose to gel. The sucrose concentration is known to be of critical importance in the gelation of high methoxyl pectins [8,11,12]. There are more differences between HP and C73 than between EP and HP, although HP and C73 were obtained by acid extraction. Discrepancies between HP and C73 may be ascribed to different raw materials and/or different extraction conditions.

The pectins tested were only slightly different in terms of average molecular weight [9,10] and therefore differences in gelation ability or in the gel strength can not be ascribed to differences in the molecular weight [13–16]. In contrast, the pectins tested differed significantly [9,10] in their dm and the length of the neutral sugar side-chains. It is well known that an increase in the dm increases the maximum pH at which gelation occurs and the setting temperature [17]. An increase in the dm seems also to lead to weaker gels [7,8,17]. The differences in the dm of our pectins could therefore partly explain their different rheological behaviours. On the other hand, EP and HP contain much longer (or more numerous) arabinan side-chains than C73. The effects that the neutral sugar side-chains and their distribution may have on gel formation is presently unknown [2,18]. They might limit the extent of interchain association, and thus formation of junction zones for gelling may be inhibited [15]. Other studies [18] have shown, in contrast, that more branched pectins gave higher storage moduli than the less-branched pectins, suggesting that the side-chains of pectins contribute to the elastic properties of the gels. The separate effects of the dm, and the length and the number of the neutral side-chains, on the gelation properties of pectins are difficult to distinguish. The exact role of side-chains in gelation can only be elucidated through a better knowledge of the topology of the branching.

Extrusion-cooking applied to lemon fibres led to a large solubilisation of pectic substances. Unlike the untreated lemon fibres, extruded lemon fibres were able to gel at an acidic pH (3.0) for a sucrose concentration ≥ 50%. The gels obtained had high and close elastic moduli, the values being dependent on the amount of soluble pectins present in the system rather than on their molecular weight. The values are similar to those obtained for the gel from C73 at 0.6%. In contrast, the breaking strengths were much lower than those obtained with pectin gels. Furthermore, the values of the different fibre gels depend largely not only on the fibre concentration but also on the intensity of the extrusion-cooking treatment applied. It was therefore clear that the amount of water-soluble pectins in the extruded fibres, as well as the degree of polymerisation of these pectins, determined the physical characteristics of the heterogeneous fibre gels.

The possibility of obtaining gelled systems directly with extruded fibres through their water-soluble pectins has the advantage of avoiding the acid extraction of the pectins. The extruded fibres could be used in various preparations, such as jams or jellies, traditionally requiring high methoxyl pectins as gelling agent. The final products could therefore be enriched in dietary fibres and devoid of additives *sensu stricto*.

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