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Properties of cellulose/pectins composites: Implication for structural and mechanical properties of cell wall

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ABSTRACT

The primary cell wall of dicotyledonous plants can be considered as a concentrated polymer assembly, containing in particular polysaccharides among which cellulose and pectins are known to be the major components. In order to understand and control the textural quality of plant-derived foods, it is highly important to elucidate the rheological and microstructural properties of these components, individually and in mixture, in order to define their implication for structural and mechanical properties of primary plant cell wall. In this study, the rheological and microstructural properties of model systems composed of sugar-beet microfibrillated cellulose and HM pectins from various sources, with varied degrees of methylation and containing different amounts of neutral sugar side chains, were investigated. The influence of the presence of calcium and/or sodium ions and the biopolymer concentrations on the properties of the mixed systems were also studied. The characterizations of the mixed system, considered as a simplified model of primary plant cell wall, showed that whatever the structural characteristics of the pectins, the ionic conditions of the medium and the biopolymer concentrations, the gelation of the composite was mainly controlled by cellulose. Thus, the cellulose network would be the principal component governing the mechanical properties of the cell walls. However, the neutral sugar side chains of the pectins seem to play a part in the interactions with cellulose, as shown by the interesting viscoelastic properties of cellulose/apple HM pectins systems. The rigidity of cellulose/pectins composite was strongly influenced by the structural characteristics of pectins. The particular properties of primary plant cell walls would thus result from the solid viscoelastic properties of cellulose, its interactions with pectins according to their structural characteristics (implication of the neutral sugar side chains and the specific potential calcic interactions) and of the distribution of the components in separate phases.

1. Introduction

The primary cell wall of dicotyledonous plants is mainly composed of polysaccharides, among which cellulose, pectins and hemicelluloses. It is the major textural component of many plant-derived foods. Indeed, changes in the structure and organization of primary cell wall polymers influence the mechanical properties of fruits and vegetables (Vanstreels et al., 2005; Waldron, Parker, & Smith, 2003). To understand and control the textural quality of plant-derived foods, it is highly important to elucidate the rheological and microstructural properties of the major polysaccharidic components of primary cell wall, individually and in mixture, in order to define their implication for structural and mechanical properties of primary plant cell wall.

Cellulose is a linear homobiopolymer constituted of long chains of D-glucose units joined together by β-1,4-glucosidic bonds, which are tightly linked by hydrogen bonds to form microfibrils. It is the primary structural element of the plant cell (Carpita & McCann, 2000). Indeed, it was shown that when cellulose fibres are properly dispersed in water by shearing forces, the obtained cellulosic microfibrils interact with each other to build a strong network that provides a gel-like structure to the aqueous medium, even at low concentration (Agoda-Tandjawa et al., 2010; Lowys, Desbrieres, & Rinaudo, 2001; Pääkkö et al., 2007; Tatsumi, Ishioka, & Matsumoto, 2002). Besides cellulose, the other major polysaccharide of primary cell wall is pectin. The pectic polysaccharide, a highly complex and heterogeneous carbohydrate polymer, is mainly composed of a backbone of $(1\rightarrow4)$-linked α-D-galacturonic acid units that can be methyl esterified and acetylated. This linear chain can be interspersed with α-$(1\rightarrow2)$-linked L-rhamnopyranosyl residues, to which are linked neutral sugar side-chains, composed mostly of arabinose and galactose. The degree of methylation (DM), *i.e.*, the proportion of galacturonic acids esterified by methyl groups is of great importance for the use of pectins as gelling agents. Depending on the degree of methyl esterification (DM), they are classified into high methoxyl (HM) pectin ($DM > 50\%$) and low methoxyl (LM) pectin ($DM < 50\%$). HM pectin gelation occurs in an acidic environment ($pH < 3.5$) and in the presence of a cosolute, usually

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sugar, typically more than 60% (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). LM pectins are well-known to form gels in the presence of calcium ions over a wide range of pH values (Axelos & Thibault, 1991). It was established that the affinity of pectins towards calcium ions becomes greater when the degree of methylation of pectins and the ionic strength of the systems decreased (Garnier, Axelos, & Thibault, 1994). On the other hand, the affinity of pectic chains towards calcium ions also increased with polymer concentration. According to Garnier et al. (1994), pectin–calcium interactions are anti-cooperative in water and become cooperative in the presence of 0.1 M NaCl. In the primary plant cell wall, pectins are characterized by a high quantity of neutral sugar side chains, among which arabinan and galactan are the most abundant (Schols & Voragen, 1994). It has been shown that pectins can play a determining role in structuring the plant cell walls through the formation of calcium pectate gel *in vivo* controlled by the action, within the wall, of pectin methylesterase enzymes (PME) and their inhibitors PMEI (Vincken et al., 2003).

Primary cell walls have been studied mainly in terms of their spatial organization; for example, the way in which the biopolymers are assembled within the cell wall has been the subject of numerous studies (Bootten, Harris, Melton, & Newman, 2004; Carpita & Gibeaut, 1993; Cosgrove, 2000; Keegstra, Talmadge, Bauer, & Albersheim, 1973). They allowed to distinguish three macromolecular networks (regarded sometimes as independent) in the cell wall: a cellulose/hemicellulose network, a protein network and a pectic network, the formation of which is generally allotted to ionic linkages through calcium cross-links; for this last network, other kinds of interactions such as hydrogen, hydrophobic and ester-linkages have not however to be excluded. Indeed, Zykwinska, Ralet, Garnier, and Thibault (2005) recently highlighted the possibility for pectins rich in neutral sugar side chains to bind *in vitro* to cellulose microfibrils by adsorption. All these interactions determine the final structure, the texture and the stability of primary plant cell wall. However, the role of each parietal polysaccharide on the structural and mechanical properties of the plant cell wall are still little known. Up to now, research into the structural and mechanical properties of the plant cell wall was based on model cell wall made by cellulose synthesized by bacteria in culture medium containing xyloglucans and/or pectins (Chanliaud, Burrows, Jeronimidis, & Gidley, 2002; Chanliaud & Gidley, 1999; Cybulska et al., 2010; Cybulska, Zdunek, & Konstankiewicz, 2011). Recently, Szymańska-Chargot, Cybulska, & Zdunek (2011) showed that addition of only xyloglucan or only pectin to pure bacterial cellulose both resulted in a slight decrease of crystallinity. On the contrary, culturing bacterial cellulose in the presence of mixtures of xyloglucan and pectins resulted in an increase of crystallinity. Moreover, it was shown that the crystallinity of bacterial cellulose can be reduced to levels found in primary cell wall by interactions with hemicelluloses (Whitney, Brigham, Darke, Reid, & Gidley, 1995, 1998). Although the composites based on bacterial cellulose allowed to study the mechanical properties of artificial mimetic cell walls, only the composite made by bacterial cellulose cultured in presence of xyloglucan and pectin had a structure similar to those observed in natural primary cell walls (Szymańska-Chargot et al., 2011). This context leads to more investigations of the properties of cell wall analogues made by polysaccharides extracted mainly from higher plants where cellulose is much less crystalline than bacterial cellulose. Moreover, in the major cases, only slightly methylesterified pectins were used for the mechanical tests of the cellulose/pectins composites (Chanliaud et al., 2002; Chanliaud & Gidley, 1999; Cybulska et al., 2010) whereas pectins exist in the form of highly methoxylated pectins in primary plant cell walls (Carpita & Gibeaut, 1993; McCann & Roberts, 1996; O'Neill & York, 2003). Because of the complex organization of primary plant cell wall, it is obvious to privilege an approach of study based on simple model systems. Since cellulose microfibrils and pectins are the principal components of primary cell wall where they play a significant role in the structuration, one can suppose that the mechanical properties of the composite cellulose/pectins in presence or not of calcium ions could give informations on the properties of primary plant cell wall.

This study focuses on better understanding the properties of model systems composed of microfibrillated cellulose and HM pectins from various sources, with varied degrees of methylation and containing different amounts of neutral sugar side chains, in order to get a better insight in the mechanical properties of primary cell wall, in particular the mechanisms responsible for their structuration and the role of each polysaccharidic component. Cellulose was extracted from parietal material of higher plants to put itself under the true conditions of primary plant cell wall. Rheological and microstructural characterization of the mixtures in various proportions were studied in water or in 0.1 M NaCl, in the presence or not of 3 mM $CaCl_2$ at pH 6.8 and compared to the properties of pure microfibrillated cellulose suspension. The approach was performed completely *in vitro* in order to control perfectly all the intrinsic parameters (structure of the polysaccharides on various scales) and the extrinsic ones (biopolymer concentrations, presence or not of ions) defining the model system.

2. Materials and methods

2.1. Materials

Sugar-beet cellulose was extracted from dried sugar-beet pulp provided by S.I.D.E.S.U.P (sugar factory in Engenville, France) as described elsewhere (Agoda-Tandjawa et al., 2010).

Three pectins of various characteristics, a commercial citrus pectin, a sugar-beet and an apple pectin, with various degrees of methylation were used. The commercial citrus pectin was from Danisco (Copenhagen, Denmark), and those of sugar-beet and apple from Cargill Texturant Systems France SAS (Baupte, France). Pectins were purified as described elsewhere (Agoda-Tandjawa, Durand, Gaillard, Garnier, & Doublier, 2012) in order to eliminate ions in excess and to obtain polysaccharide under an acidic form.

2.2. Chemical characterization

Individual neutral sugars were analyzed as their alditol acetate derivatives by gas–liquid chromatography (Blakeney, Harris, Henry, & Stone, 1983) after hydrolysis by 2 N H_2SO_4 at 100 °C for 2 h. The cellulose residue was first prehydrolyzed in 72 (w/v)% H_2SO_4 for 3 h at 25 °C. Inositol was used as an internal standard. The uronic acid content was determined colorimetrically by the automated m-hydroxybiphenyl method (Thibault, 1979).

Methanol was determined colorimetrically by using N-methylbenzothiazolinone-2-hydrazone in conjunction with alcohol oxidase as described elsewhere (Anthon & Barrett, 2004). The degree of methylation (DM) of the various pectins was calculated as the molar ratio of methanol to galacturonic acid.

Weight-average molar mass and intrinsic viscosity of the pectins were determined by high-pressure size-exclusion chromatography (HPSEC) carried out on two columns (Shodex-OH-Pak SB-804 HQ and OH-Pack SB-805 HQ) equipped with one Shodex OH SB-G precolumn as described previously (Hellin, Ralet, Bonnin, & Thibault, 2005). Pectin solutions at ~5 mg/mL prepared in 50 mM sodium nitrate containing 0.02% sodium azide as a preservative were analyzed. The weight-average molar mass (M_w) and the intrinsic viscosity $[\eta]$ were calculated using the Astra software (Wyatt, Santa Barbara, CA) and the TriSEC software (Version 3.0, Viscotek, Houston, TX), respectively. All the analyses were performed in triplicate.

2.3. Samples preparation and biopolymer mixtures

The aqueous suspensions of cellulose microfibrils (microfibrillated cellulose suspensions) have been obtained by treating aqueous cellulose suspension through a Rannie two-stages homogenizer (5 passes at 300 bar and 5 other passes from 380 to 400 bar) as described elsewhere (Agoda-Tandjawa et al., 2010).

Each purified pectin powder was dispersed in deionized water (2.5%, w/w) as described elsewhere (Agoda-Tandjawa et al., 2012). The pH of the solution was adjusted at 6.8 with NaOH, then left overnight at 4 °C under gentle magnetic stirring to carry on the solubilization. After this process, it was verified that the pH was still 6.8 and the solution was filtered successively through 8, 3, 0.8 and 0.45 μm filters to remove the non-dissolved particles and aggregates. Sodium azide (0.02%) was added to the cellulose suspension and to pectin solution to prevent from bacteria contamination. Concentrations were calculated from the determination of the dry matter.

1% (w/w) cellulose and pectin mixtures were prepared in variable proportions (0.25/0.75, 0.5/0.5 and 0.75/0.25) at room temperature, in water or in 0.1 M NaCl, at pH 6.8, and were heated at 80 °C to allow in some cases homogeneous addition of 3 mM $CaCl_2$, as described elsewhere (Agoda-Tandjawa et al., 2012). Microfibrillated cellulose suspensions alone were also prepared at the corresponding concentrations in the same conditions. The heating step did not significantly affect the rheological properties neither of cellulose alone nor of the mixtures in absence of calcium, which was taken as a guarantee of the same obtained ultrastructure (Agoda-Tandjawa et al., 2010, 2012). The shortcuts of the mixed systems are: C-HM.Pc, C-HM.Pa and C-MM.Psb for the mixtures of cellulose-citrus high methoxyl pectin, cellulose-apple high methoxyl pectin and cellulose-sugar-beet medium methylated pectin, respectively.

2.4. Rheological measurements

The rheological measurements were carried out using an ARES (TA Instruments, Guyancourt, France) controlled strain rheometer equipped with a 40 mm teflon plate-and-plate geometry and a Peltier temperature controller. The gap was fixed at 1 mm. The cellulose/pectin mixtures or the microfibrillated cellulose suspensions, prepared as described above, was poured onto the ARES plate heated at 80 °C. Samples were covered with paraffin oil to prevent from evaporation during measurements, and the system was cooled to 20 °C. The kinetics of gel formation at 20 °C was monitored by measuring the storage modulus (G') and the loss modulus (G'') at 1 rad s^{-1}. When the moduli reached a plateau (after ~20 h), the system was considered to be at equilibrium and mechanical spectra were recorded over the frequency range 100–0.01 rad s^{-1}. In all experiments, the measurements were performed at a 0.7% strain, which was in the linear viscoelasticity domain. Each measurement was performed in triplicate ($n = 3$).

2.5. Microscopic observations

2.5.1. Observation by TEM (transmission electron microscopy)

For these observations, samples were prepared as described elsewhere (Agoda-Tandjawa et al., 2012). A drop of each diluted aqueous dispersion was first placed on a carbon-coated TEM copper grid (Quantifoil, Germany) and let to air-dry. The sample was then negatively stained with phosphotungstic acid. For that, the sample-coated TEM grid was successively placed on a drop of an aqueous solution of phosphotungstic acid (2%, w/w) and on a drop of distilled water. The grid was then air-dried before introduction in the electron microscope. The samples were viewed using a JEOL JEM-1230 TEM (JEOL, Tokyo, Japan) operating at 80 kV.

2.5.2. Observation by CLSM (confocal laser scanning microscopy)

CLSM was used in the fluorescence mode. Observations were made with a Nikon A1 (Nikon, Tokyo, Japan) microscope with 20× objective. As microfibrillated cellulose and pectins do not exhibit intrinsic fluorescence, it was necessary to label them.

Cellulose and pectins were covalently labelled, respectively with rhodamine B isothiocyanate (RITC) and with fluoresceinamine (FA), as described elsewhere (Agoda-Tandjawa et al., 2012). The shortcuts of the labelled mixtures are: cellulose/FA-pectin, RITC-cellulose/pectin and RITC-cellulose/FA-pectin, for the mixtures of unlabelled cellulose/labelled pectin, labelled cellulose/unlabelled pectin, and both labelled cellulose and pectin, respectively.

The warm labelled samples (80 °C), prepared as for rheological measurements, were poured between a preheated concave slide and a coverslip, then hermetically sealed to avoid dehydration. It was verified that labelling did not change the rheological behaviour of the systems (results not shown). The systems were cooled from 80 to 20 °C and the CLSM observations were performed at 20 °C after 20 h. A laser beam at the wavelengths of 561 nm and 488 nm was used to excite RITC and FA, respectively, and the emissions of fluorescence were recorded between 570 and 620 nm, and 500 and 550 nm, respectively. In this work, all observations reported were taken at the same depth into the sample (approximately 40 μm under the surface). All the observations (TEM and CLSM) were performed in triplicate, from new sample preparation, as for rheological measurements. At least 15 images per sample were taken. A representative image of the ultrastructure of the sample is presented here.

3. Results and discussion

3.1. Results

3.1.1. Chemical and macromolecular features of the biopolymers studied

Chemical characteristics of commercial citrus high methoxyl pectin (HM.Pc), apple high methoxyl pectin (HM.Pa), medium methylated pectin of sugar-beet (MM.Psb), and those of cellulose residue from sugar-beet used in this study are presented in Table 1. The physicochemical properties of the pectins are also shown in this table. The degree of methylation (DM) of HM.Pc, HM.Pa and MM.Psb were 72%, 69.2% and 51%, respectively. Only sugar-beet MM pectins were acetylated for a total amount of 31%. The galacturonic acid contents were 85.1%, 61% and 57.7%, respectively. In term of total neutral sugars, the citrus pectin contained less neutral sugars than those of sugar-beet and apple (7.4%, 23.5% and 38.86%, respectively). These chemical characteristics were close to those already observed in the literature for sugar-beet pectins (Levigne, Ralet, & Thibault, 2002; Ralet, Thibault, Hallaert, Vandamme, & Van Loo, 1990), apple pectin (Renard, Lemeunier, & Thibault, 1995; Renard & Thibault, 1993) and that of citrus (Ralet & Thibault, 1994). The total carbohydrate content of HM.Pc, HM.Pa and MM.Psb were 93%, 99% and 81%, respectively. The low amount of total carbohydrate content of sugar-beet pectin can probably be related to ash content of the pectin powder, as in sugar-beet pulps, due to the persistence of soil particles after the industrial process, as mentioned previously (Agoda-Tandjawa et al., 2010). The rhamnose, galactose, glucose and xylose contents were higher in apple pectin than in that of sugar-beet, whereas arabinose was more present in sugar-beet pectin. However, glucose content was much higher in apple pectin than that of sugar-beet and citrus. This high glucose content in apple pectin residue can originate either from remaining starch or from co-extracted polysaccharides, as for example hemicelluloses, as observed elsewhere (Garna et al., 2007). Despites the relatively high level of glucose, all these observations suggest an

Table 1
Neutral sugar and galacturonic acid contents of the citrus, apple and sugar-beet pectins, and that of the cellulosic residue of sugar-beet, and some physicochemical features of the various pectins.

	Citrus HM pectin (HM.Pc) (% dry weight)	Apple HM pectin (HM.Pa) (% dry weight)	Sugar-beet FM pectin (FM.Psb) (% dry weight)	Cellulose (% dry weight)
Rhamnose	1.35	4.66	3.10	0
Fucuse	0	0.30	0	0
Arabinose	1.89	3.20	11	0.50
Xylose	0.22	2.70	0.20	3.93
Mannose	0	0	0	1.75
Galactose	3.43	15.90	8.60	0.72
Glucose	0.51	12.10	0.60	80.33
Galacturonic acid	85.10	61	57.70	1.20
DM (mol%)	72	69.20	51	–
DAc (mol%)	–	–	31	–
M_w (kg/mol)	174	376	259	–
η (dL/g)	5.32	7.25	1.91	–
R_g (nm)	26	37	19	–

DM, degree of methylation; DAc, degree of acetylation; M_w, weight-average molar mass; [η], intrinsic viscosity in 50 mM $NaNO_3$; R_g, radius of gyration.

important level of neutral sugar rich side chains in sugar-beet and apple pectins, compared to citrus pectin. That indicates that pectins were totally different one from another by their side chains content and composition. In the same way, the three pectin samples differed clearly by their macromolecular characteristics. Indeed, the weight-average molar mass (M_w) of the citrus, apple and sugar-beet pectins were 174 kg/mol, 376 kg/mol and 259 kg/mol, respectively. Apple pectins had the highest intrinsic viscosity [η] (7.25 dL/g), followed by that of citrus (5.32 dL/g) and sugar-beet (1.91 dL/g). The radius of gyration (R_g) values of the pectins were extremely different one from another. These values are relatively low, ranging from 19 to 37 nm, suggesting rather flexible and/or branched macromolecules. All these differences of structures and physicochemical characteristics will allow to compare their behaviour in the presence of cellulose.

Concerning the cellulose residue, its chemical characteristics shows that glucose was the main neutral sugar in the cellulose residue, in agreement with previous results (Zykwinska et al., 2005). The low amount of galacturonic acid (1.2%) and of the other residual neutral sugars indicates respectively low pectin and hemicellulose contents. The low pectin content is in agreement with previous results (Foster, Ablett, McCann, & Gidley, 1996). Indeed, by performing nuclear magnetic resonance study of the sequential chemical extraction of onion cell wall material, Foster et al. (1996) suggested that galactans could fill many of the network pores and that extractability of pectins was not dependent on segmental mobility. Furthermore, part of pectic backbones (and not side chains) were found strongly associated with cellulose. In addition, it is important to specify that the residual galacturonic acid content gives a negative surface charge to cellulose fibres. It can then be thought that electrostatic repulsions could perturb interactions between the two negatively charged species. However, in a previous study performed with the same cellulose sample in presence of LM pectins, more charged than the ones used here, no influence of the addition of LM pectin on the rheological behaviour of the cellulose sample was evidenced (Agoda-Tandjawa et al., 2012). Even though the cellulose used in both studies is not molecularly pure, it appears that the amount of charges carried by cellulose microfibrils seems too low to disturb the organization and the properties of the mixed system.

3.1.2. *Viscoelastic behaviour of cellulose/pectins mixed systems*

Fig. 1 shows the mechanical spectra at 20 °C obtained for cellulose/citrus HM pectin, cellulose/apple HM pectin and cellulose/sugar-beet MM pectin mixtures at the mixing ratio of 0.5/0.5, at the end of the gelation kinetics (after 20 h at 20 °C), in water or in 0.1 M NaCl with and without 3 mM $CaCl_2$, together with those of pure cellulose suspensions (0.5%, w/w), taken as reference. The variations of G' (the storage modulus) and of G'' (the loss modulus) are plotted as a function of angular frequency in the range 10^2–10^{-2} rad s^{-1}.

Whatever the ionic conditions of the medium, all the systems displayed a typical solid-like behaviour with $G' > 10G''$ and relatively frequency-independent moduli. This behaviour was also observed for mixtures at mixing ratios of 0.25/0.75 and 0.75/0.25, and for the pure cellulose suspension at the corresponding concentrations (results not shown). It is clearly seen that the profiles obtained for the mixtures were qualitatively similar to those obtained for pure cellulose suspensions, under the same salt conditions. The properties of the different systems seem then to be mostly governed by the ones of cellulose microfibrils, which can be considered as dispersed in a continuous aqueous phase containing pectins. In water with and without NaCl, the G''/G' ratios of the mixed systems in the presence of calcium did not differ very much from those obtained without calcium ions (of the order of 0.1) meaning that all the systems are structured on the same way than cellulose suspension network.

However, differences among the ionic strength of the system or the origin of pectins can be evidenced. For cellulose/citrus HM pectin systems (Fig. 1a and b), it can be seen that in water without NaCl (Fig. 1a), the G' and G'' moduli obtained for the mixture are nearly superimposed with those of pure cellulose suspension at the corresponding concentration (0.5%, w/w). This indicates that the presence of citrus HM pectin within the cellulose network had little effect on the viscoelastic properties of the cellulose network. Under these conditions, pectins in solution do not intervene in the viscoelastic properties of the mixed system, only cellulose microfibrils contribute to the rheology of the system. On the other hand, in presence of Ca^{2+} ions, a slight increase of the moduli G' and G'' was observed. In NaCl (Fig. 1b), an opposite tendency was observed in absence of Ca^{2+} ions. The G' and G'' moduli obtained for the mixture were lower than those of pure cellulose suspension, due to the screening of electrostatic repulsions. In addition, the presence of Ca^{2+} ions did not induce any modification of the mixture moduli.

For cellulose/apple HM pectin systems (Fig. 1c and d), it is clearly seen that in water without NaCl (Fig. 1c), the presence of apple HM pectin in cellulose suspension (0.5/0.5) without added Ca^{2+} ions induced an important increase in the G' and G'' moduli throughout the overall mechanical spectrum meaning that the viscoelastic properties of cellulose suspension were reinforced. On the other hand, in presence of calcium, the G' values are superimposed on those obtained for the corresponding pure cellulose suspension, whereas G'' was higher and approaches the values obtained for the mixture without calcium. The elastic character is thus attenuated.

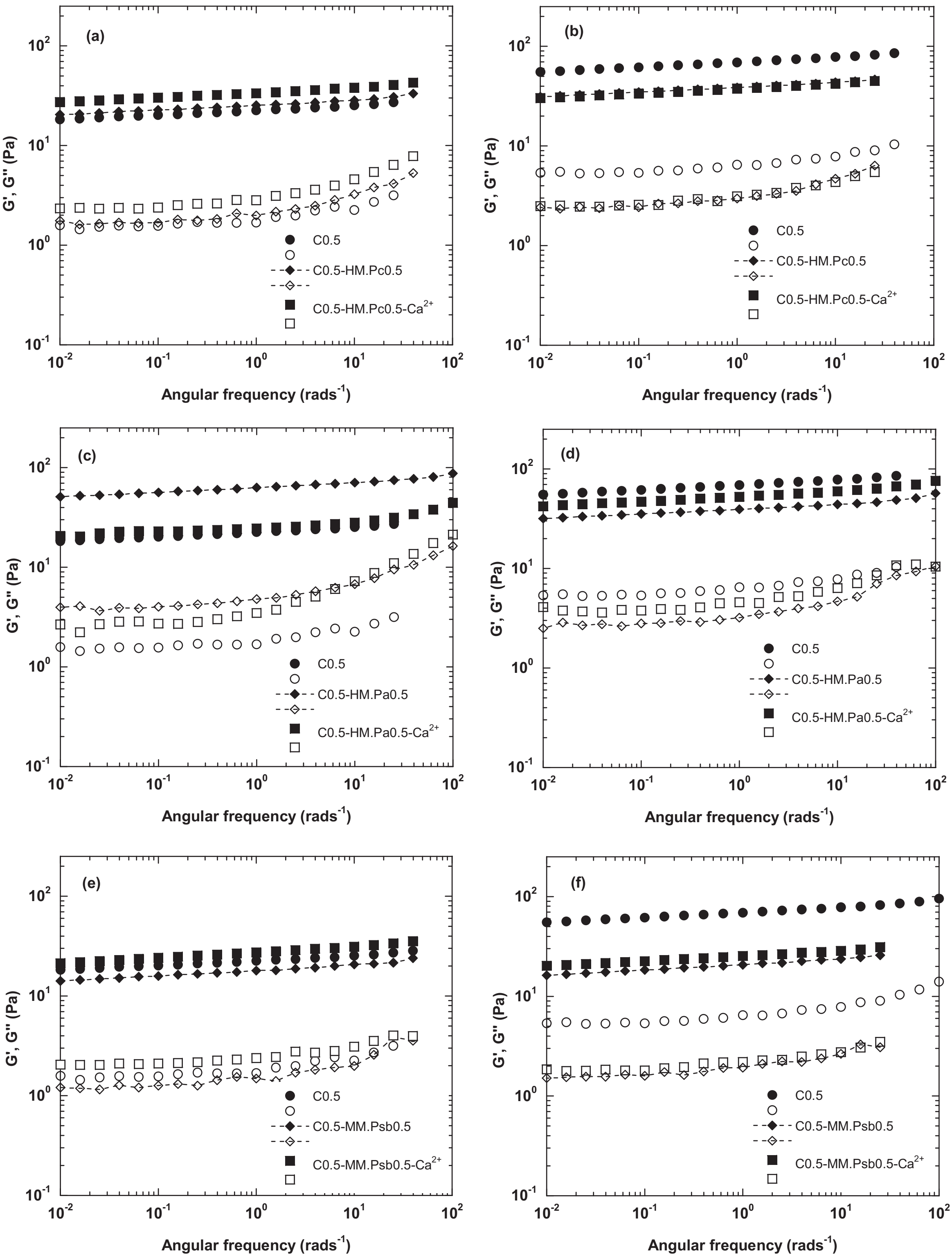

Fig. 1. Mechanical spectra of cellulose/citrus HM pectin (a and b), cellulose/apple HM pectin (c and d) and cellulose/sugar-beet MM pectin (e and f) mixtures in presence or not of 3 mM $CaCl_2$ and in water without NaCl (first column; a, c and e) and in water with 0.1 M NaCl (second column; b, d and f) at a mixing ratio of 0.5/0.5, and those of pure cellulose suspensions at the corresponding concentration, taken as references, at pH 6.8. Measurement temperature, 20 °C; strain, 0.7%. G', filled symbols; G'', empty symbols.

Contrary to the former case, in NaCl with or without added calcium (Fig. 1d), the G' and G'' moduli obtained for the mixture are decreased compared to the pure cellulose suspension. However, the moduli remained slightly higher in the presence of calcium.In addition, it is noticeable for the cellulose/sugar-beet MM pectin systems (Fig. 1e and f) that in water without NaCl (Fig. 1e) the moduli G' and G'', in presence of calcium, decreased slightly compared to pure cellulose suspension, whereas the addition of calcium to the mixture allowed to find moduli rather close to those of pure cellulose suspension. On the other hand, Fig. 1f shows that in 0.1 M NaCl, the moduli G' and G'' obtained in absence of Ca^{2+} ions are decreased compared to the pure cellulose suspension. The presence of Ca^{2+} ions produces a light increase of G' and G'' values, the moduli remaining much lower than those of the pure cellulose suspension.

In all the cases, it was found that the moduli G' and G'' of the systems in water were always lower than those obtained in the case of 0.1 M NaCl, indicating that the systems were strengthened in 0.1 M NaCl compared to pure water without NaCl. This reinforcement of the viscoelastic behaviour of the systems in NaCl can be related to the decrease of electrostatic repulsions between biomacromolecules.

3.1.3. Effects of pectin characteristics, of biopolymer concentrations and of Ca^{2+} ions on the viscoelastic behaviour of cellulose/pectins systems

In order to get a better understanding of the effects of the pectin structural characteristics and of the biopolymer concentrations on the viscoelastic properties of cellulose/pectin mixed systems, the values of the storage modulus, G', obtained at 0.1 rad s^{-1} from mechanical spectra at 20 °C, were plotted *versus* the cellulose concentration and the pectin concentration in the mixtures (total polymer concentration of 1%, w/w) (Figs. 2 and 3).

Whatever the salt conditions of the medium and the structural characteristics of the pectins, G' decreased when pectin concentration in the mixtures increased. These results show that, in these conditions, microfibrillated cellulose in suspension govern the viscoelastic properties of the composites.

In water (Fig. 2a), no major modification of the viscoelastic properties of cellulose suspensions was observed when citrus HM and sugar beet MM pectins were added to cellulose in the range 0.25/0.75–0.75/0.25. Although no interaction between cellulose microfibrils and citrus HM pectins (poor in side chains) should be possible (Chanliaud & Gidley, 1999; Zykwinska et al., 2005), the addition of HM citrus pectins could stabilize the system at the mixing ratios of 0.5/0.5 and 0.25/0.75, because of their viscosifier effect. The presence of a low quantity of citrus pectin disturbs the cellulose network, which can be explained by a dilution effect of the cellulose microfibrils. Moreover, electrostatic repulsions can play a role in this system; this citrus HM pectin being particularly rich in galacturonic acid (85.1%) even if 72% of them are methylated. On the contrary, an important increase of the viscoelastic properties of the mixed system was observed at the mixing ratios of 0.5/0.5 and 0.25/0.75 in the case of apple HM pectin. This could be related to the possibility of apple HM pectins, rich in neutral sugars side chains, to bind to microfibrillated cellulose (Zykwinska et al., 2005). Moreover, the relatively high radius of gyration value (37 nm) of this pectin can play a part in the reinforcing behaviour of the composite, due to excluded volume effect. The fact that no modification of the viscoelastic properties of cellulose suspension when low amount of apple HM pectin (0.25/0.75) was added can be explained by the fact that interactions through pectin side chains compensate the dilution effect described for citrus pectin. On the other hand, although one could expect a reinforcement of the mixed system in presence of sugar-beet MM pectin also rich in side chains, a weakening effect compared to cellulose alone is observed. This could be related to their content in acetyl groups which would disturb the establishment of a significant number of interactions between both polymers.

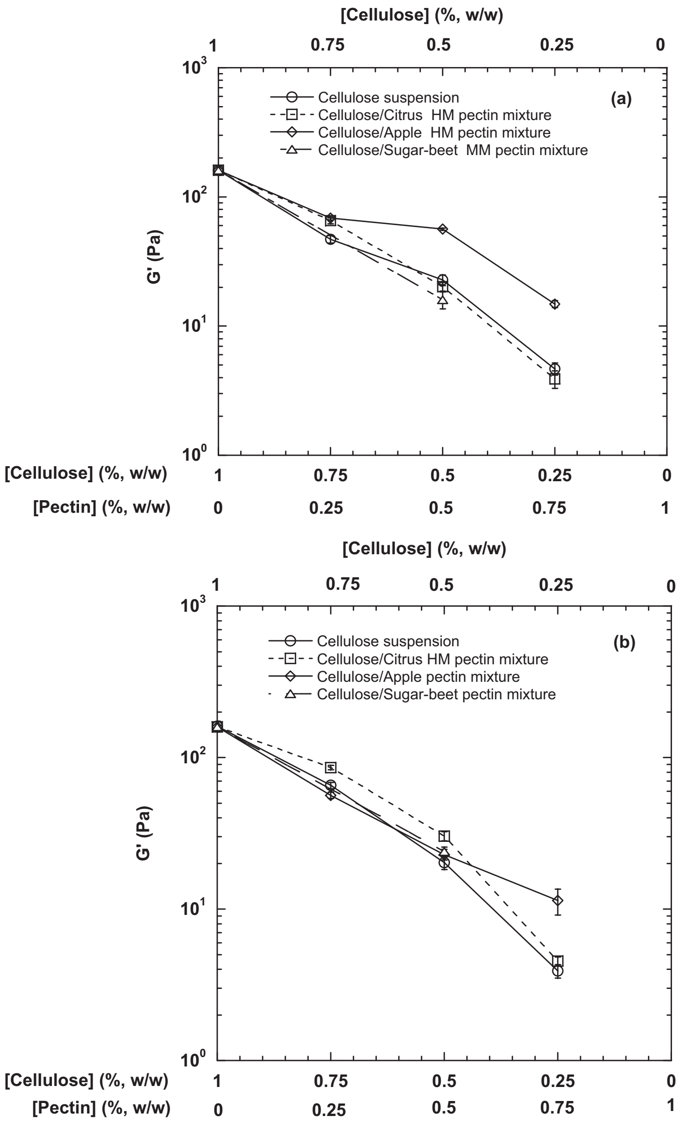

Fig. 2. Variations of G' as a function of cellulose/pectins mixing ratio at a total polymer concentration of 1% (w/w) in water without added salts (a) and in water with added 3 mM $CaCl_2$ (b), and those of G' obtained for cellulose suspensions alone in water (from 0.25 to 1%, w/w), taken as references, at 20 °C and at an angular frequency of 0.1 rad s^{-1}. The data are average obtained from three repetitions. The error bar is standard deviation.

When water contained Ca^{2+} ions (Fig. 2b), one can observe a light improvement of the viscoelastic properties of the mixed system cellulose/citrus HM pectin for all the mixing ratios studied. This observation suggests a possible existence of specific calcic interactions between cellulose and citrus HM pectin, or between pectin chains in these conditions, because of the high percentage of galacturonic acid content of these pectins. No major change of G' moduli of cellulose suspensions was observed in presence of apple HM pectin and of sugar-beet at the mixing ratios of 0.75/0.25 and 0.5/0.5, whereas an increase of the moduli is noticed for apple HM pectin at the mixing ratio of 0.25/0.75. A decrease of the viscoelastic properties of the 0.5/0.5 cellulose/apple HM pectin mixed system in presence of calcium is observed compared to the same system without calcium, whereas no major modification of the properties is noticed at other mixing ratios. At a close mixing ratio, Chanliaud and Gidley (1999) also highlighted a decrease of the mechanical properties, using uniaxial tensile tests, of a composite made by bacterial cellulose and apple HM pectin (DM = 67%) with a relatively low content of neutral sugars (10%) in presence of calcium. Even though this was not observed under small deformation conditions like those used here, this behaviour in presence of Ca^{2+} ions is questionable. The fact that the presence of Ca^{2+} ions involves a weakening effect of the mixed system could be explained by disturbances due to the competitions between formation of specific and privileged interactions between polymers through the side chains of pectins and cellulose/pectin interactions through Ca^{2+} ions. At

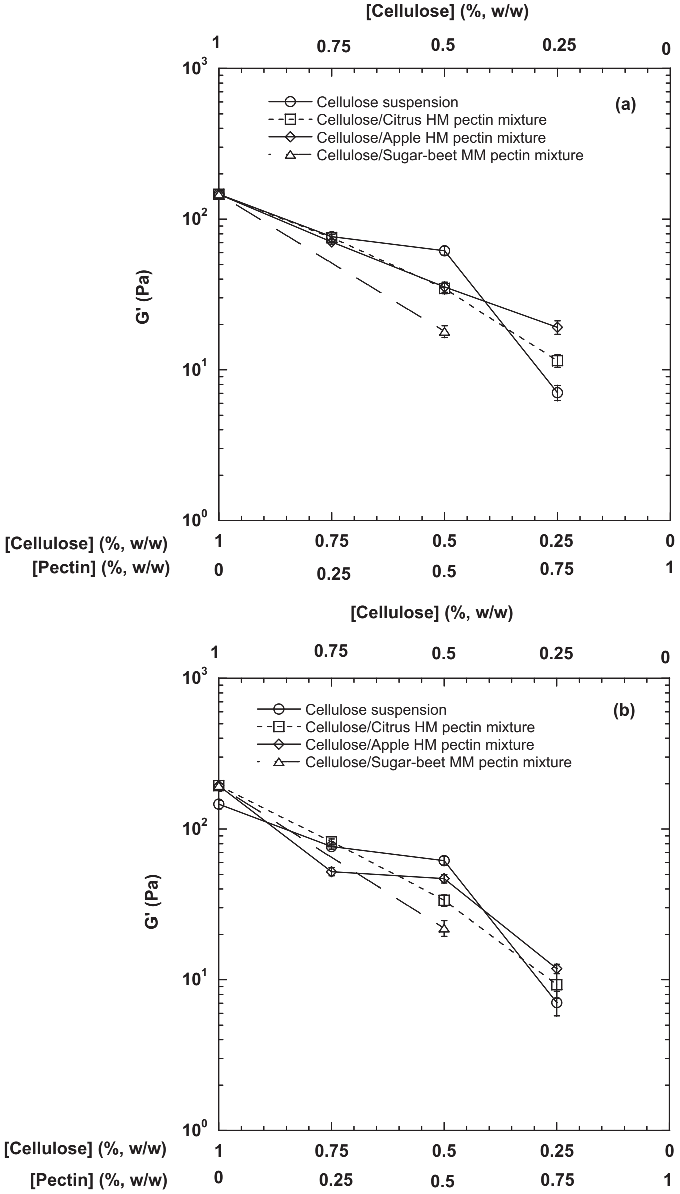

Fig. 3. Variations of G' as a function of cellulose/pectins mixing ratio at a total polymer concentration of 1 (w/w%) in 0.1 M NaCl without added $CaCl_2$ (a) and in 0.1 M NaCl with added 3 mM $CaCl_2$ (b), and those of G' obtained for cellulose suspensions alone 0.1 M NaCl (from 0.25 to 1, w/w%), taken as references, at 20 °C and at an angular frequency of 0.1 rad s^{-1}. The data are average obtained from three repetitions. The error bar is standard deviation.

low and high concentrations of apple HM pectin in the mixture (0.75/0.25 and 0.25/0.75), these effects were less marked.

In 0.1 M NaCl, in absence of Ca^{2+} ions (Fig. 3a), synergistic effects take only place for systems in which cellulose concentration is low (0.25%, w/w), and were higher in presence of apple HM pectin than in presence of citrus HM pectin. At low pectin concentration, the same behaviour was observed for citrus and apple pectin, highlighting charge screening by NaCl. A more pronounced decrease of the composite elasticity was observed in presence of sugar-beet MM pectin at the mixing ratio of 0.5/0.5. In contrast to what was observed in pure water, the viscoelastic properties of the 0.75/0.25 cellulose/citrus HM pectin mixed system were reinforced and do not present anymore a weakening effect. In the same way, the rigidity of cellulose/apple HM pectin composite at the same mixing ratio was reinforced and remained equivalent to that of the corresponding cellulose suspensions. Moreover, at the mixing ratio of 0.5/0.5, the composites containing citrus or apple HM pectins show lower G' values than cellulose alone in NaCl, which was not the case in water. On the other hand, the stabilizing or reinforcing effect respectively observed in the case of apple and citrus HM pectins in water at the mixing ratio of 0.25/0.75 was more pronounced in presence of NaCl. All these behaviours can be explained on the basis of NaCl effect which would strongly affect the electrostatic interactions taking place in the systems. At high concentration in cellulose (0.75/0.25), the presence of NaCl contributed to stabilize the system, with favoured microfibril/microfibril interactions (Agoda-Tandjawa et al., 2010). At equal proportions in biopolymers (0.5/0.5), charge screening could disturb interactions, which would weaken the composite. On the other hand, when citrus and apple HM pectins concentrations are high to (0.25/0.75) in presence of NaCl, the viscosifier effect of pectins becomes significant and then induced a reinforcement of the composite.

In NaCl containing Ca^{2+} ions (Fig. 3b), and whatever the citrus HM pectin concentrations in mixture, the same profile of G' variation as the one obtained in NaCl without Ca^{2+} ions was observed for the composites. However, these composites exhibited a higher elasticity than observed in water containing Ca^{2+} ions, evidencing again the NaCl effect. Moreover, the fact that the viscoelastic properties of cellulose/citrus HM pectin composites in NaCl were not importantly affected in presence of Ca^{2+} ions, suggests that calcic interactions between polymers are not favoured in presence of Na^+ ions. For composites containing apple HM pectins, a decrease of G' values at the mixing ratios of 0.75/0.25 and 0.5/0.5 was observed whereas a slight increase of the modulus was obtained at 0.25/0.75. When comparing the properties of cellulose/apple HM pectin mixed system in NaCl with and without calcium ions, a light improvement of the viscoelasticity was observed at equal proportions of the biopolymers (0.5/0.5), whereas a decrease at the mixing ratios of 0.75/0.25 and 0.25/0.75 appeared. Looking at the results obtained with the corresponding systems in water in presence or not of Ca^{2+} ions, it seems that at equal proportions in biopolymers, the presence of the two cations stabilizes the system. The calcic interactions are thus favoured in presence of NaCl in this particular system.

3.1.4. *Microscopic structures in cellulose/pectin mixtures*

The variations in viscoelastic behaviours of cellulose/pectin mixtures due to the structural characteristics of the pectins (citrus HM pectin poor in side chains, apple and sugar-beet pectins rich in side chains), the Ca^{2+} and/or Na^+ addition together with the mixing ratios of both biopolymers raised the question about microstructural variations among the composite. Thus, in order to understand these differences, TEM and CLSM were performed to visualize the ultrastructural arrangement within cellulose/pectin mixed systems at two observation scales. As the viscoelastic properties of the mixed system made by cellulose and apple HM pectin seemed to be different from the other ones considered, due to the structural characteristics of the apple HM pectin, we chose specifically to examine this system in presence or not of NaCl and/or $CaCl_2$.

TEM micrographs of microfibrillated cellulose suspension, apple HM pectin and cellulose/apple HM pectin mixtures at a mixing ratio of 0.5/0.5 in water with and without 3 mM $CaCl_2$, are shown in Fig. 4. Pictures a and b of Fig. 4 show the microstructure of microfibrillated cellulose in water without and with $CaCl_2$, respectively. In water, the cellulose suspension observation revealed a network structure of well-defined and packed fibrils randomly dispersed with a diameter of 2–15 nm and a length of up to 10 μm, as observed previously (Agoda-Tandjawa et al., 2010). In presence of calcium ions (Fig. 4b), the network structure of microfibrillated cellulose suspension does not change a lot in nature, indicating that 3 mM $CaCl_2$ are not enough to induce strong interactions between

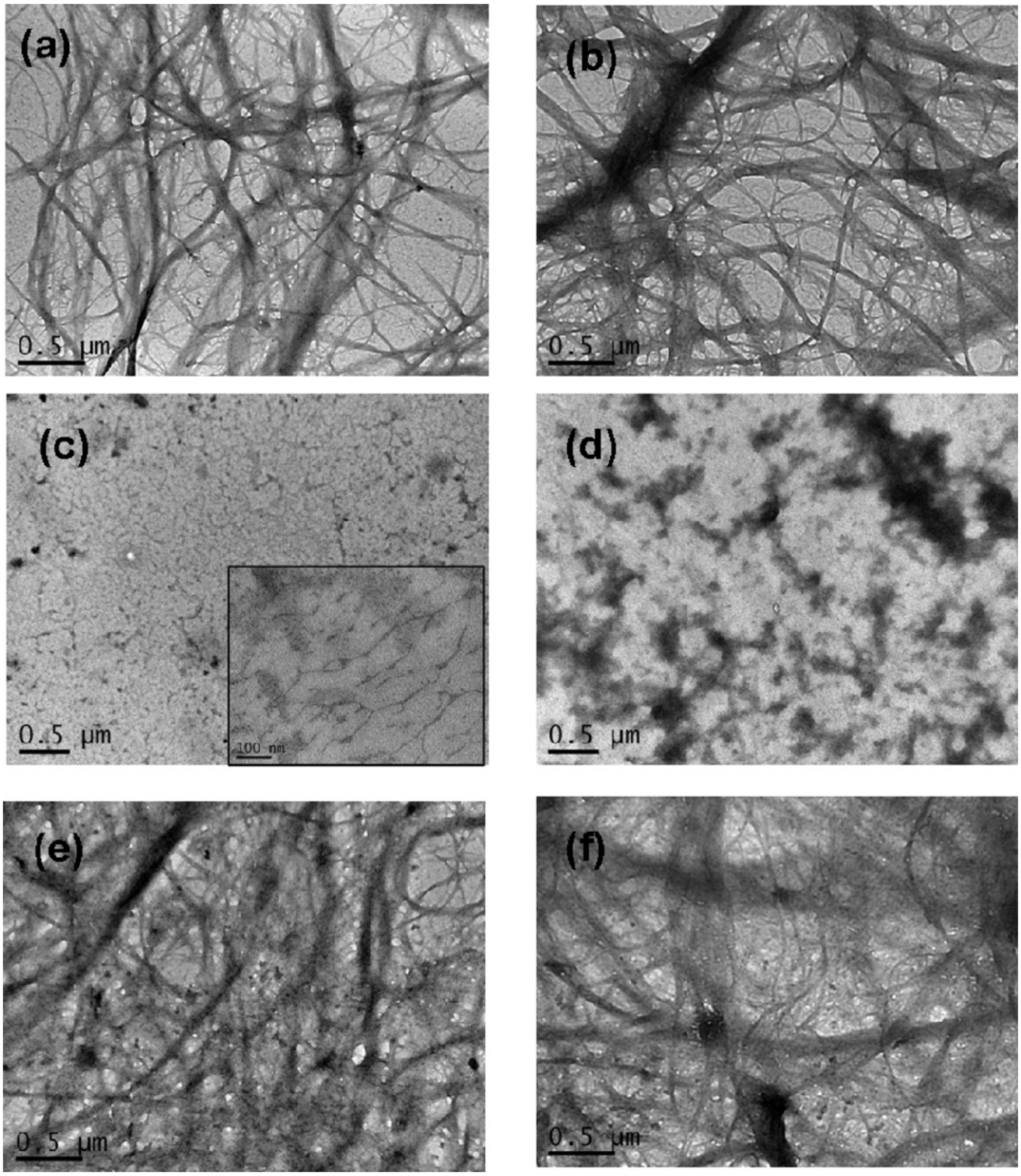

Fig. 4. TEM micrographs of cellulose suspension (a and b), apple HM pectin (c and d), and of cellulose/apple HM pectin mixtures (e and f) at a mixing ratio of 0.5/0.5 without added salts (first column) and with 3 mM $CaCl_2$ (second column) in water at pH 6.8. Scale bar = 0.5 μm.

microfibrils. This observation is supported by the reinforcement of the viscoelastic properties of cellulose suspension in presence of 100 mM $CaCl_2$ as shown elsewhere (Agoda-Tandjawa et al., 2010). The micrographs obtained for apple HM pectin alone in water (Fig. 4c), show clearly small chains distributed rather regularly. In presence of 3 mM $CaCl_2$ (Fig. 4d), dense dark zones distributed in a rather regular way were observed, which suggests that pectin are rather strongly aggregated under these conditions. From these results, it appears that the presence of side chains does not prevent pectins from aggregation under these conditions. When apple HM pectins are added to cellulose suspension, in water only and in presence of calcium ions (Fig. 4e and f, respectively), a drastic change was observed in the microfibrillated cellulose network. Indeed, the structure became denser than observed for the cellulose alone. That was related to the viscoelastic properties, the values of G' modulus obtained for cellulose suspension increasing in presence of apple HM pectin at the corresponding concentration. The open porous network seen in pure microfibrillated cellulose suspension with and without calcium ions disappeared in the presence of apple HM pectins. Whether calcium was added to the mixture or not, a network structure qualitatively similar was observed. Some aggregated pectins, due to calcium presence, were seen in micrograph 4f. Contrary to this result, from TEM observations, Chanliaud and Gidley (1999) showed that the presence of apple HM pectins in bacterial cellulose at the mixing ratio of 71/29 with addition of 12.5 mM $CaCl_2$ did not significantly modify the cellulose network structure. However, it is important to notice that the sample preparation used in the two studies and overall conditions were different, which could explain the discrepancies observed.

CLSM micrographs of cellulose/FA-apple HM pectin mixtures in water or in NaCl with and without 3 mM $CaCl_2$, are shown in Fig. 5. On the four micrographs, the same ultrastructural organization of cellulose microfibrils and apple HM pectin in the mixtures is clearly observed. Indeed, in all conditions, inhomogeneities in the distribution of non-labelled cellulose were evidenced by the presence of dark cotton-like zones, more or less aggregated, embedded in a continuous fluorescent phase corresponding to apple HM pectin. These observations provide evidence that cellulose microfibrils and

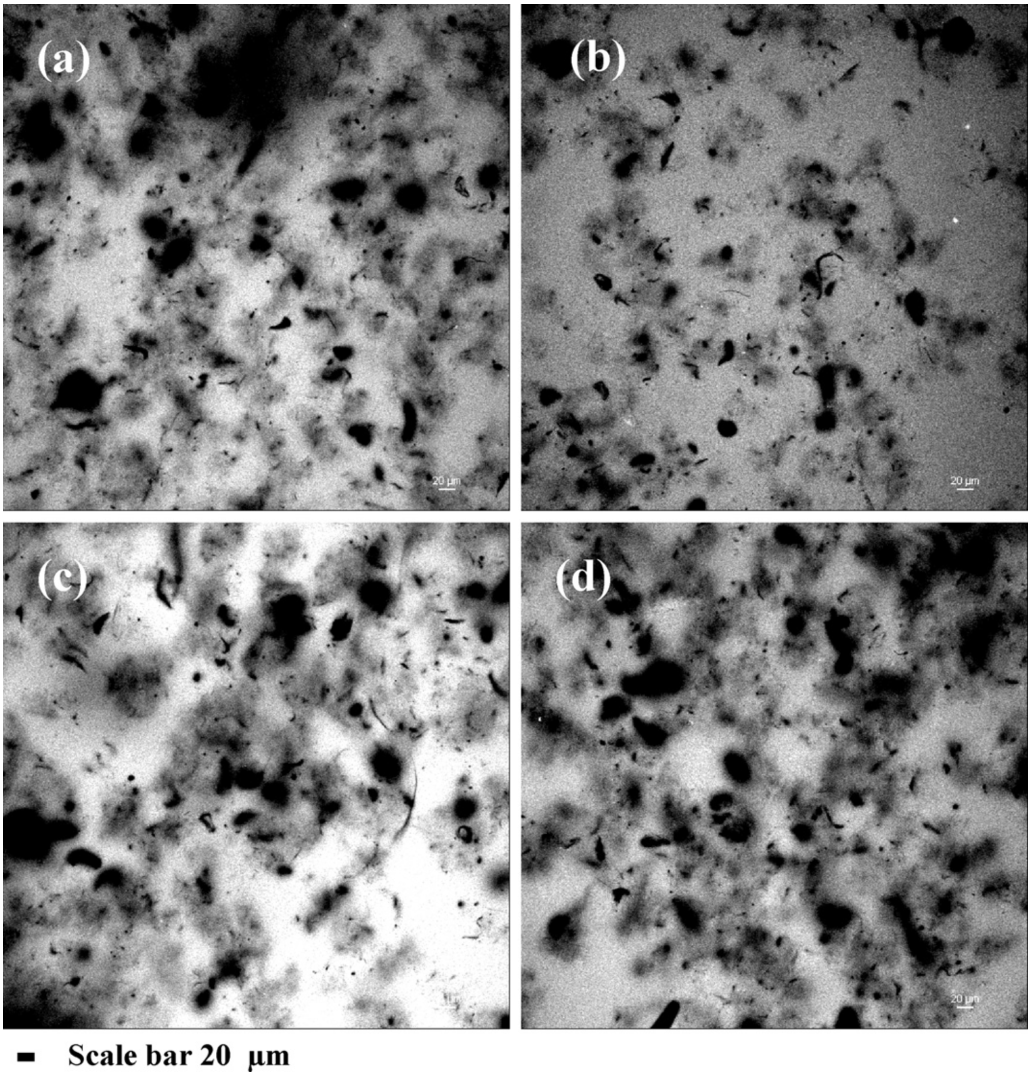

Fig. 5. CLSM micrographs of cellulose/FA-apple pectin mixtures at a mixing ratio of 0.5/0.5: in water without added salts (a), in water with 3 mM $CaCl_2$ (b), in 0.1 M NaCl (c), and in 0.1 M NaCl with 3 mM $CaCl_2$ (d), at pH 6.8. Scale bar = 20 µm.

apple HM pectin are mainly localized in different phases in the system. The same global organization was seen in double localization experiments using both labelled cellulose and apple HM pectin and in composites made with the other pectins (results not shown).

3.2. Discussion

In all the composites and whatever the medium ionic conditions, rheological characterizations showed that microfibrillated cellulose in suspension impose their rheological properties. That can be related to the morphology of the cellulose microfibrils (lengthened and rigid), which would form a rigid entangled fibre network. Similar features have previously been observed for bacterial cellulose and cellulose/xyloglucans composites in small deformation oscillatory rheology tests and uniaxial tensile testing (Whitney, Gothard, Mitchell, & Gidley, 1999). They also showed that tomato cell walls retained rheological properties close to the ones of cellulose after sequential extraction of much of the pectin and hemicellulose components, thus showing that plant cell wall behaviour was dominated by cellulose one under small deformation conditions. This fact is moreover independent of the cellulose origin.

Globally, in NaCl and in presence or not of calcium ions, at a mixing ratio of 0.5/0.5, the addition of pectins to cellulose suspensions tends to weaken the cellulose network. In these salt conditions, whatever their structural characteristics, pectins thus seem to disturb the microfibrils network, which is difficult to highlight by TEM and CLSM observations. In water containing calcium ions, only the viscoelastic properties of cellulose/citrus HM pectin composites were slightly increased, which corresponds to the thickening of the network observed in TEM (results not shown). The mixed systems made by the other pectins do not show significant change of their viscoelastic properties, in correlation with a little modified microstructure.

In water without added salts, interesting modifications of the rheological properties are observed only for the systems made with apple HM pectins. These pectins under these conditions show a very dispersed structure which makes them more likely to interact with the microfibrils through the side chains. The ultrastructure of the mixed systems shows a predominant cellulose microfibrils network in the meshes of which were trapped structures potentially ascribable to pectins. These structures seem to cover the cellulose microfibrils. Nevertheless, at this scale, no information on the mechanism of interactions between cellulose microfibrils and this type of pectic chains can be definitively given. However, both mechanical properties and microstructure are in agreement with the hypothesis made by Zykwinska et al. (2005) on the possibility of interaction between cellulose and pectins *via* their side chains. The observations carried out in CLSM show that cellulose and pectins are globally located in separate phases, suggesting a two-level organization.

By extrapolating these results on the scale of primary plant cell wall, cellulose network appears to be the principal component governing the mechanical properties of plant cell walls. This observation is in agreement with previous studies based on large deformation tensile extension which highlighted a decrease of the mechanical properties of bacterial cellulose in a pre-formed pectin gel, and a more pronounced decrease in presence of both pectins and xyloglucans (Chanliaud et al., 2002; Chanliaud & Gidley, 1999). However, this behaviour was not observed under small deformation conditions. In addition, the viscoelastic properties of cellulose/pectin composites were seen to be influenced by the biopolymers concentrations, the structural characteristics of pectins (low or rich in neutral sugar side chains), and the presence or not of ions Ca^{2+} and/or Na^{+}; this enables us to suggest that it could give information for the understanding of mechanical properties of primary plant cell wall.

Moreover, rheological characterizations combined to TEM and CLSM observations of cellulose/pectin model systems suggest that structuration of primary plant cell walls could imply the solid-like behaviour of cellulose, its interactions with pectins according to their structural characteristics (implication of the neutral sugar side chains and specific calcium cross-links) and distribution of the components in separate phases. In addition, MacDougall, Rigby, and Ring (1997) highlighted the immiscibility of pectic polysaccharides (an high-esterified and relatively unbranched one and a highly branched and low-esterified other one) and xyloglucans, in absence of calcium ions. That allowed the authors to suggest the existence of phase separation between pectic polysaccharides and xyloglucans within cell wall. In their analysis, the authors suggested that phase separation could occur during the period before enzymatic de-esterification of the pectins, leading to their incorporation into the gel network, before some other forms of interaction takes place. These observations allowed to not exclude the contribution of phase separation phenomenon to the particular structuration of primary plant cell walls.

4. Conclusion

In this study, rheological and microstructural properties of model systems composed of sugar-beet microfibrillated cellulose and pectins from various sources, with varied degrees of methylation and containing different amounts of neutral sugar side chains, were investigated in order to define its implications for structural and mechanical properties of primary plant cell wall. Through this work, we showed that whatever the structural characteristics of the pectins, the ionic conditions of the medium and the biopolymer concentrations, the gelation of the composite was mainly controlled by cellulose. Thus, the cellulose network would be the principal component governing the mechanical properties of the cell walls. However, the neutral sugar side chains of pectins seem to play a part in the interactions with cellulose, as shown by the interesting viscoelastic properties of cellulose/apple HM pectins systems. Microscopic observations suggested a two-level organization. The particular properties of primary plant cell walls would thus result from the solid viscoelastic properties of cellulose, its interactions with pectins according to their structural characteristics (implication of the neutral sugar side chains and the specific potential calcic interactions) and of the distribution of the components in separate phases.

To deepen the knowledge of the mechanical properties of primary cell wall, further studies need to be done considering the importance of incorporation the other plant cell wall polysaccharides (hemicelluloses and lignins) in the artificial plant cell wall analogues. On the other hand, it should be useful to take into account how the degree of crystallinity of cellulose from various plant sources influences the mechanical properties of primary cell walls and what would be the composite properties in presence of higher levels of calcium.

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References

Agoda-Tandjawa, G., Durand, S., Berot, S., Blassel, C., Gaillard, C., Garnier, C., et al. (2010). Rheological characterization of microfibrillated cellulose suspensions after freezing. *Carbohydrate Polymers*, *80*(3), 677–686.

Agoda-Tandjawa, G., Durand, S., Gaillard, C., Garnier, C., & Doublier, J. L. (2012). Rheological behaviour and microstructure of microfibrillated cellulose suspensions/low-methoxyl pectin mixed systems. Effect of calcium ions. *Carbohydrate Polymers*, *87*(2), 1045–1057.

Anthon, G. E., & Barrett, D. M. (2004). Comparison of three colorimetric reagents in the determination of methanol with alcohol oxidase. Application to the assay of pectin methylesterase. *Journal of Agricultural and Food Chemistry*, *52*(12), 3749–3753.

Axelos, M. A. V., & Thibault, J.-F. (1991). The chemistry of low-methoxyl pectin gelation. In R. H. Walter (Ed.), *The chemistry and technology of pectin* (pp. 109–118). New York: Academic Press.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research*, *113*(2), 291–299.

Bootten, T. J., Harris, P. J., Melton, L. D., & Newman, R. H. (2004). Solid-state C-13-NMR spectroscopy shows that the xyloglucans in the primary cell walls of mung bean (*Vigna radiata* L.) occur in different domains: A new model for xyloglucan–cellulose interactions in the cell wall. *Journal of Experimental Botany*, *55*(397), 571–583.

Carpita, N., & Gibeaut, D. (1993). Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. *The Plant Journal*, *3*(1), 1–30.

Carpita, N., & McCann, M. (2000). The cell wall. In B. B. Buchanan, W. Gruissem, & R. L. Jones (Eds.), *Biochemistry and molecular biology of plants* (pp. 52–108). Rockville, MD: American Society of Plant Physiologists.

Chanliaud, E., Burrows, K. M., Jeronimidis, G., & Gidley, M. J. (2002). Mechanical properties of primary plant cell wall analogues. *Planta*, *215*(6), 989–996.

Chanliaud, E., & Gidley, M. J. (1999). In vitro synthesis and properties of pectin/acetobacter xylinus cellulose composites. *The Plant Journal*, *20*(1), 25–35.

Cosgrove, D. J. (2000). Expansive growth of plant cell walls. *Plant Physiology and Biochemistry*, *38*(1–2), 109–124.

Cybulska, J., Vanstreels, E., Ho, Q. T., Courtin, C. M., Craeyveld, V. V., Nicolaï, B., et al. (2010). Mechanical characteristics of artificial cell walls. *Journal of Food Engineering*, *96*(2), 287–294.

Cybulska, J., Zdunek, A., & Konstankiewicz, K. (2011). Calcium effect on mechanical properties of model cell walls and apple tissue. *Journal of Food Engineering*, *102*(3), 217–223.

Foster, T. J., Ablett, S., McCann, M. C., & Gidley, M. J. (1996). Mobility-resolved 13C NMR spectroscopy of primary plant cell walls. *Biopolymers*, *39*.

Garna, H., Mabon, N., Robert, C., Cornet, C., Nott, K., Legros, H., et al. (2007). Effect of extraction conditions on the yield and purity of apple pomace pectin precipitated but not washed by alcohol. *Journal of Food Science*, *72*(1), C1–C9.

Garnier, C., Axelos, M. A. V., & Thibault, J. F. (1994). Selectivity and cooperativity in the binding of calcium-ions by pectins. *Carbohydrate Research*, *256*(1), 71–81.

Hellin, P., Ralet, M. C., Bonnin, E., & Thibault, J. F. (2005). Homogalacturonans from lime pectins exhibit homogeneous charge density and molar mass distributions. *Carbohydrate Polymers*, *60*(3), 307–317.

Keegstra, K., Talmadge, K. W., Bauer, W. D., & Albersheim, P. (1973). The structure of plant cell walls: III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. *Plant Journal*, *51*(1), 188–197.

Levigne, S., Ralet, M. C., & Thibault, J. F. (2002). Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. *Carbohydrate Polymers*, *49*(2), 145–153.

Lowys, M. P., Desbrieres, J., & Rinaudo, M. (2001). Rheological characterization of cellulosic microfibril suspensions. Role of polymeric additives. *Food Hydrocolloids*, *15*(1), 25–32.

MacDougall, A. J., Rigby, N. M., & Ring, S. G. (1997). Phase separation of plant cell wall polysaccharides and its implications for cell wall assembly. *Plant Physiology*, *114*(1), 353–362.

McCann, M. C., & Roberts, K. (1996). Plant cell wall architecture: The role of pectins. In J. Visser, & A. G. J. Voragen (Eds.), *Pectins and pectinases* (pp. 91–126). Amsterdam: Elsevier Science B.V.

O'Neill, M. A., & York, W. S. (2003). The composition and structure of primary cell walls. In J. K. C. Rose (Ed.), *The plant cell wall* (pp. 1–54). Boca Raton, FL: CRC Press.

Pääkkö, M., Ankerfors, M., Kosonen, H., Nykänen, A., Ahola, S., Österberg, M., et al. (2007). Enzymatic hydrolysis combined with mechanical shearing and high-pressure homogenization for nanoscale cellulose fibrils and strong gels. *Biomacromolecules*, *8*(6), 1934–1941.

Ralet, M. C., & Thibault, J. F. (1994). Extraction and characterization of very highly methylated pectins from lemon cell-walls. *Carbohydrate Research*, *260*(2), 283–296.

Ralet, M.-C., Thibault, J.-F., Hallaert, J., Vandamme, E., & Van Loo, J. (1990). Characteristics of pectins extracted from sugar-beet pulp by *Geotrichum penicillatum*. *Sciences Des Aliments*, *10*, 865–876.

Renard, C., Lemeunier, C., & Thibault, J. F. (1995). Alkaline extraction of xyloglucan from depectinised apple pomace: Optimisation and characterisation. *Carbohydrate Polymers*, *28*(3), 209–216.

Renard, C., & Thibault, J. F. (1993). Structure and properties of apple and sugar-beet pectins extracted by chelating-agents. *Carbohydrate Research*, *244*(1), 99–114.

Schols, H. A., & Voragen, A. G. J. (1994). Occurrence of pectic hairy regions in various plant cell wall materials and their degradability by rhamnogalacturonase. *Carbohydrate Research*, *256*(1), 83–95.

Szymańska-Chargot, M., Cybulska, J., & Zdunek, A. (2011). Sensing the structural differences in cellulose from apple and bacterial cell wall materials by Raman and FT-IR Spectroscopy. *Sensors*, *11*(6), 5543–5560.

Tatsumi, D., Ishioka, S., & Matsumoto, T. (2002). Effect of fiber concentration and axial ratio on the rheological properties of cellulose fiber suspensions. *Journal of the Society of Rheology Japan*, *30*(1), 27–32.

Thibault, J.-F. (1979). Automatisation du dosage des substances pectiques par la méthode au méta-hydroxydiphényl. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology*, *12*, 247–251.

Vanstreels, E., Alamar, A. C., Verlinden, B. E., Enninghorst, A., Loodts, J. K. A., Tijskens, E., et al. (2005). Micromechanical behaviour of onion epidermal tissue. *Postharvest Biology and Technology*, *37*(2), 163–173.

Vincken, J. P., Schols, H. A., Oomen, R., McCann, M. C., Ulvskov, P., Voragen, A. G. J., et al. (2003). If homogalacturonan were a side chain of rhamnogalacturonan. I: Implications for cell wall architecture. *Plant Physiology*, *132*(4), 1781–1789.

Voragen, A. G. J., Pilnik, W., Thibault, J.-F., Axelos, M. A. V., & Renard, C. M. G. C. (1995). Pectins. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 287–339). New York: Marcel Dekker.

Waldron, K. W., Parker, M. L., & Smith, A. C. (2003). Plant cell wall and food quality. *Comprehensive Reviews in Food Science and Food Safety*, *2*(4), 101–119.

Whitney, S. E. C., Brigham, J. E., Darke, A. H., Reid, J. S. G., & Gidley, M. J. (1995). In vitro assembly of cellulose/xyloglucan networks—Ultrastructural and molecular aspects. *Plant Journal*, *8*(4), 491–504.

Whitney, S. E. C., Brigham, J. E., Darke, A. H., Reid, J. S. G., & Gidley, M. J. (1998). Structural aspects of the interaction of mannan-based polysaccharides with bacterial cellulose. *Carbohydrate Research*, *307*(3–4), 299–309.

Whitney, S. E. C., Gothard, M. G. E., Mitchell, J. T., & Gidley, M. J. (1999). Roles of cellulose and xyloglucan in determining the mechanical properties of primary plant cell walls. *Plant Physiology*, *121*(2), 657–663.

Zykwinska, A. W., Ralet, M.-C. J., Garnier, C. D., & Thibault, J.-F. J. (2005). Evidence for in vitro binding of pectin side chains to cellulose. *Plant Physiology*, *139*(1), 397–407.