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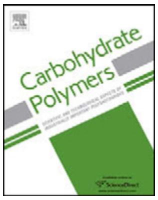

Rheological behaviour and microstructure of microfibrillated cellulose suspensions/low-methoxyl pectin mixed systems. Effect of calcium ions

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In this study, the influence of the presence of low-methoxyl pectin (LM pectin) on the rheological and microstructural properties of microfibrillated cellulose suspensions was elucidated in order to create new structures with new and interesting textures. For that purpose, the rheological properties of the cellulose/LM pectin mixtures in variable proportions were compared with those of the individual biopolymers. The influence of the presence of calcium and/or sodium ions on the properties of the mixed systems was studied. The microstructure of the resulting system was studied by transmission electron microscopy and confocal laser scanning microscopy. It was found that, in the presence of LM pectin, a synergistic effect was observed when calcium ions were also present, leading to increased rheological properties of the composites. Indeed, addition of calcium to the mixtures induced LM pectin gelation, which was favoured in the presence of sodium, the pectin network contributing to the formation of a stronger cellulose/LM pectin composite gel. The presence of LM pectin alone in the microfibrillated cellulose suspensions does not significantly modify the viscoelastic and microstructural properties of microfibrillated cellulose suspensions. Whether calcium was added to the mixtures or not in water, the viscoelastic properties of the mixtures are mainly controlled by cellulose. The same behaviour was observed for the mixtures in NaCl without added calcium. Contrary to this observation, it was noticed that in presence of both sodium and calcium ions, the viscoelastic properties of the mixtures are largely governed by LM pectin. On the other hand, it was showed that the flow behaviour of microfibrillated cellulose suspensions is modified in the presence of LM pectin with an increase in thixotropic character shear-thinning behaviour, which was more pronounced in the presence of NaCl. It was also shown, from TEM observations, that an interpenetrating network formed in cellulose/LM pectin composites gel in the presence of calcium ions. In the same way, the CLSM observations allowed the separate localization of cellulose and LM pectin within the composite systems to be highlighted. The results obtained suggests that it is possible to thus create new structures with new interesting textures, by mixing microfibrillated cellulose suspensions and LM pectin in suitable proportions in the simultaneous presence of both sodium and calcium ions.

1. Introduction

Polysaccharides and mixed polysaccharide systems are used as raw materials in a variety of food or non-food applications, either to modify flow behaviour, enhance mechanical strength, or improve adhesion performance (Pérez & Mazeau, 2005). Due to this extensive use, many academic and industrial scientists wish to better understand the behaviour of mixed polysaccharide systems in order to optimize their use and find new structures or potentially novel applications.

Cellulose and pectins, the major components of most plant primary cell walls, are polysaccharides having different structures and very diverse properties (Carpita & Gibeaut, 1993; Talbott & Ray, 1992). Cellulose is biosynthesized not only by higher plants but also by a wide variety of bacteriae, algae and fungi and by some animals (tunicates for instance) (Attala, 1999; Horii, 2000; Salmon & Hudson, 1997). It is a linear homobiopolymer with high molecular weight composed of long chains of D-glucose units joined together by β-1,4-glucosidic bonds, which are tightly linked by hydrogen bonds to form cellulose microfibrils (Kirk & Othmer, 1967). By using high-pressure homogenization, a process commonly used in the food industry, it is possible to disintegrate the cellulose structure into suspension of myofibrils to obtain what is known as microfibrillated cellulose (MFC) (Herrick, Casebier, Hamilton, & Sandberg, 1983; Turbak, Snyder, & Sandberg, 1983). Cellulose nanofibers

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materials have excellent mechanical and thermal properties. They have a high Young's modulus of 138 GPa for the crystalline regions, in the longitudinal direction (Sakurada, Nukushina, & Ito, 1962) and a very low thermal expansion coefficient (10^{-7} K^{-1} in the longitudinal direction) (Nishino, Matsuda, & Hirao, 2004). Therefore cellulosic nanofibers were used in many applications as a reinforcing phase in nanocomposite films (Azizi Samir, Alloin, & Dufresne, 2005). In addition to their exceptional mechanical and thermal properties, when properly dispersed in water, cellulosic nanofibers 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; Pääkkö et al., 2007). These interesting mechanical properties of MFC suspensions make them a promising nanofiber for use in future materials. Besides cellulose, pectins, the other major component of primary cell wall, are anionic polysaccharides, which can be commercially extracted from fruit industry waste such as citrus peels and apple pomace. These polysaccharides are composed of a backbone of $(1 \rightarrow 4)$-linked α-D-galacturonic acid units that can be methyl esterified and acetylated. This linear chain is interspersed with α-$(1 \rightarrow 2)$-linked L-rhamnopyranosyl residues, to which are linked neutral sugar side-chains, composed mostly of arabinose and galactose (Ralet & Thibault, 2002; Thakur, Singh, & Handa, 1997; Voragen et al., 2005). The degree of methylation (DM), i.e., the proportion of galacturonic acids esterified by methyl groups (on the carboxylic acid) 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%). 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.

Recently, Zykwinska, Ralet, Garnier, & Thibault (2005) showed that pectins can bind *in vitro* to cellulose via their neutral sugar side chains. On the other hand, Chanliaud and Gidley (1999) reported that commercial citrus pectins which are well-known to contain very limited amounts of neutral sugar side chains due to their extraction under harsh acidic conditions, do not bind to cellulose. Even though pectins low in neutral sugar side chains do not bind to cellulose, their viscosifier effect or gelling properties could be used in order to find new structures.

Great attention has been given to the possibility of using mixtures of polysaccharides to improve gel strength. Up to now, research into the assembly of cellulose and pectins has mainly focused on the way in which they interact in the plant primary cell walls. However, although LM pectins and cellulose suspensions display interesting gel-like behaviour, the rheological behaviour of cellulose/LM pectins mixed systems in presence or not of NaCl and/or $CaCl_2$ has not been a topic of previous studies and little information on their microstructure in relation with rheological properties exists.

This study focuses on a better understanding of the properties of model systems composed of microfibrillated cellulose, and an anionic polysaccharide, a low methoxyl pectin (LM pectin) in order to propose controlled "cellulose/pectins" model systems, having interesting texturing properties. The rheological and microstructural properties of the mixtures 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 were compared to the properties of each biopolymer.

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). The obtained cellulosic residue contained 80.3% of glucose and a low amount of galacturonic acid (1.2%) (Agoda-Tandjawa et al., 2010).

The commercial citrus LM pectin sample (LMNA DE 28, no. 13827), kindly given by Cargill Texturant systems France SAS (Baupte, France), had a degree of etherification of 28.5% and 80.6% of galacturonic acid content. Pectin powder was purified by washing with acidic ethanol (EtOH/HCl: 5%/70%) in order to eliminate ions in excess and to obtain the polysaccharide under an acidic form.

Calcium content of both biopolymers was determined by atomic absorption spectroscopy. Results were 3.75×10^{-2} mM in purified cellulosic residue and 3.31×10^{-2} mM in purified LM pectin powder.

2.2. 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 bars and 5 other passes from 380 to 400 bar) as described elsewhere (Agoda-Tandjawa et al., 2010).

The purified LM pectin powder was dispersed in deionized water (2.5%, w/w) under gentle stirring at room temperature for 4 h. 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 respectively through a 8, 3, 0.8 and 0.45 μm filter to remove the non-dissolved particles and aggregates. Sodium azide (0.02%) was added to the cellulose suspension and the LM pectin solution to prevent from bacterial contamination. Concentrations were calculated from the determination of the dry matter.

The suspensions of cellulose microfibrils and LM pectin solutions were mixed 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 to obtain 1% (w/w) of total biopolymer concentration while $CaCl_2$ would be added or not. It was verified that the pH after mixing was still 6.8. All the samples were then homogenized with the high intensity mixer (Polytron PT 45/80, Kinematica, Switzerland) at 20 000 rpm for 5 min. The mixtures were then stirred 25 min at 80 °C before addition of a hot $CaCl_2$ solution and then let to equilibrate at this temperature for 5 min. $CaCl_2$ solution was added to obtain 3 mM $CaCl_2$ in the medium at a total biopolymer concentration of 1% (w/w). All the prepared systems are shown in Table 1.

2.3. Rheological measurements

The rheological measurements were carried out using an ARES (TA Instruments) 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/LM pectin system or the individual component, prepared as described above, was poured onto the ARES plate heated at 80 °C. Samples were covered with paraffin oil to prevent 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