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Highly acetylated pectin from cacao pod husks (*Theobroma cacao* L.) forms gel

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ABSTRACT

A pectin (OP) obtained from cacao pod husk with a high acetyl content, which is a structural feature that could disturb the pectins' gel formation, was able to form gels at low pH and a high sucrose content. Pectin gels (1.32% GalA equivalent, w/w) were prepared at pH 2.5–3.3 in the presence of 60% sucrose (w/w). Rheological analyses were performed to determine the optimal pH for further studies. Next, the OP samples were prepared at pH 2.7 in concentrations ranging from 0.33 to 1.98% GalA (w/w) with 60% sucrose (w/w) and subjected to rheological analysis. Dynamic oscillatory experiments at 25 °C indicated the presence of gels for all of the analysed concentrations. Measurements of the elastic (G') and viscous (G'') moduli at 25 °C also indicated that increasing the pectin concentration resulted in stronger gels. Rotational experiments revealed a shear-thinning behaviour in which the apparent viscosities of the samples increased as the concentration increased. Although the OP had a high degree of acetylation, this pectin was able to form gels, which suggests its potential for use as a gelling and thickening additive.

1. Introduction

Cacao pod husks (*Theobroma cacao* L.), which are considered an undesirable waste product of the cocoa industry, have been investigated as a new source of pectins. Pectins have been isolated from cacao pod husks by aqueous (Vriesmann, Amboni, & Petkowicz, 2011) and acid extractions (Vriesmann, Teófilo, & Petkowicz, 2011). Experimental design and statistical approaches using response surface methodology were employed to optimise the nitric-acid-mediated pectins' extraction from the cacao pod husks to improve the pectin yield and uronic acid content (Vriesmann, Teófilo et al., 2011). The optimised pectin (OP) was composed of a homogalacturonan (degree of esterification, DE 56.6 ± 1.4%) with rhamnogalacturonan insertions possessing side chains primarily containing galactose. The polymers were highly acetylated (degree of acetylation, DA 17.1 ± 0.5%) indicating a chemical pattern that differs from that of commercial pectins.

In addition to the temperature, pH, pectin concentration and presence of co-solutes and ions, pectin gelation is affected by several parameters related to the pectin structure, including the molar mass, charge distribution along the backbone, presence of side chains, DA and DE. DE is a particularly important parameter; low-methoxyl (LM pectins: DE < 50%) and high-methoxyl (HM pectins: DE > 50%) pectins have distinct gel formation mechanisms (Axelos & Thibault, 1991; O'Neill, Darvill, & Albersheim, 2001; Rolin, 1993).

After heating, the HM pectins gel at a low pH (frequently pH < 3.5) in the presence of high co-solute concentrations (typically 60–65% sucrose), whereas the gelation of LM pectins occurs in the presence of calcium ions (O'Neill et al., 2001; Oakenfull, 1991; Rolin, 1993; Thakur, Singh, & Handa, 1997). In the first case, the gelling mechanism primarily involves hydrogen bonding combined with hydrophobic interactions (Oakenfull, 1991), while ionic interactions play an important role in the gelation of LM pectins (Axelos & Thibault, 1991).

HM pectins are very important in the food industry because many processed fruit products are acidic and contain a high sucrose content (e.g., jams and jellies) (O'Neill et al., 2001). Therefore, several studies have investigated the gelling properties of low-pH, high-sugar HM pectin gels by varying the sucrose concentration (El-Nawawi & Heikel, 1997; Evageliou, Richardson, & Morris, 2000; Sharma, Liptay, & Le Maguer, 1998; Vriesmann, Silveira, & Petkowicz, 2010), pH (El-Nawawi & Heikel, 1997; Evageliou et al., 2000; Sharma et al., 1998), pectin concentration (Ptitchkina, Danilova, Doxastakis, Kasapis, & Morris, 1994; Sharma et al., 1998; Vriesmann et al., 2010) and the type of co-solute employed (Evageliou et al., 2000; Tsoga, Richardson, & Morris, 2004).

However, few studies have investigated the gelling properties of HM pectins with high acetyl content (high DA), which is known to

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modify pectin solubility and gelation properties (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995; Willats, McCartney, Mackie, & Knox, 2001). A content value of 4% acetyl was considered the critical limit for gel formation for previously studied systems (Iglesias & Lozano, 2004; Pilnik & Voragen, 1992).

Therefore, a high degree of acetylation appears to be important for the gelling capacity of pectin. This factor has been a limitation in the use of sugar beet pectin as a gelling additive (Mc Cready, 1966; Pippen, McCready, & Owens, 1950; Rolin, 1993). Many studies have investigated the chemical or enzymatic removal of acetyl groups from sugar beet pectins and various extraction procedures to improve their gelling properties (Faulds & Williamson, 1990; Matthew, Howson, Keenan, & Belton, 1990; Oosterveld, Beldman, Searle-van Leeuwen, & Voragen, 2000; Pippen et al., 1950; Turquois, Rinaudo, Taravel, & Heyraud, 1999, 2000; Williamson et al., 1990).

Because the characteristics of pectin can determine its applications, e.g., as a gelling agent, the analysis of its physicochemical properties is essential for its optimal use. Therefore, we report the rheological properties of the highly acetylated pectin (OP) previously obtained from cacao pod husk under optimised conditions.

2. Materials and methods

2.1. Pectin sample

OP is a highly acetylated HM pectin (DE 56.6 ± 1.4%; DA 17.1 ± 0.5%). The OP used in this study was obtained by the optimised nitric-acid-mediated extraction from cacao pod husk and previously characterised (Vriesmann, Teófilo et al., 2011).

2.2. Preparation of pectin for rheological analysis

First, OP was solubilised in deionised water at 5% (w/w) with agitation over 16 h at 25 °C. The solution was allowed to rest for 4 h prior to the rheological experiments.

The OP samples were then processed under the appropriate conditions to obtain HM pectin gels. The OP solutions were prepared at 1.32% (as galacturonic acid equivalents in the fraction, w/w) with 60% sucrose (w/w) at various acidic pH values (2.5–3.3) to determine the appropriate pH for future experiments. Weighed amounts of the sample were hydrated in aqueous citric acid with agitation over 16 h at 25 °C. After solubilisation, the mixtures were heated and sucrose was added in the appropriate proportion. The mixtures were heated in a boiling bath with continuous stirring for 15 min and cooled to room temperature. Next, their pH was measured, and the samples were stored under refrigeration for 16 h and analysed at 25 °C. The OP solutions were also prepared with different GalA quantities (0.33–1.98%) at pH 2.7 and processed as described above.

2.3. Rheological analyses

The rheological measurements were performed using a RheoStress1 rheometer (Haake GmbH, Germany) equipped with a Haake DC30 circulating bath at 25 °C using a C60/2° spindle (cone and plate geometry) with a 1 mm gap between the cone and the plate. The mechanical responses of the materials were determined by subjecting them to a frequency sweep from 0.01 to 10 Hz at 25 °C under strain in the viscoelastic-linear region ($\gamma < 5\%$; obtained by strain sweep tests at 1 Hz) where the network structure was preserved. The flow behaviour of the samples was evaluated using continuous shear rate curves in the CR mode (controlled shear rate). The sensor was programmed to increase the shear rate from 0 to 300 s^{-1} over 360 s at 25 °C. The data from the rheological measurements were evaluated using the HAAKE RheoWin 4 software. All of the experiments were repeated at least twice. The reported results are the averages of these multiple trials and exhibited excellent reproducibility.

3. Results and discussion

Rheological analysis was employed to characterise the OP in solution and as a gel and will provide important information for future studies on their properties and applications.

3.1. OP solution

The viscosity curve of an aqueous solution containing 5% OP (w/w) is shown in Fig. 1. OP exhibited a low initial viscosity (<1 Pa s), which is similar to the 5% pectin solutions chemically extracted from apple pomace (Min et al., 2011). A non-Newtonian shear-thinning behaviour was observed for OP, with decreases in the viscosity with increases in shear rate, as described for other pectin solutions (Evageliou, Ptitchkina, & Morris, 2005; Hwang & Kokini, 1992; Min et al., 2011; Sengkhamparn et al., 2010; Singthong, Ningsanond, Cui, & Goff, 2005).

The viscoelastic properties of the 5% OP were evaluated using frequency sweeps (Fig. 2). G' refers to the elastic (solid) behaviour, and G'' refers to the viscous (liquid) behaviour. The 5% OP solution exhibited $G'' > G'$ in the analysed frequency range, which indicated the predominance of the liquid behaviour. The value of both moduli increased proportionally with the frequency.

The predominance of the liquid behaviour was also observed for the 5% solutions of the pectins from apple pomace (58 and 69% DE; Min et al., 2011). However, the apple pomace samples exhibited higher elastic properties at higher frequency oscillations.

3.2. OP gels

The OP is composed of highly acetylated HM pectins and is expected to form gels at low pH in the presence of a high content of low-molecular-weight co-solutes (i.e., sucrose). Acidic pH (<3.5) leads to the protonation of the carboxylate groups of the unesterified galacturonate units, reducing the negative charge along the pectin chain and minimising the electrostatic chain repulsion. In addition, a high sucrose concentration decreases the water activity,

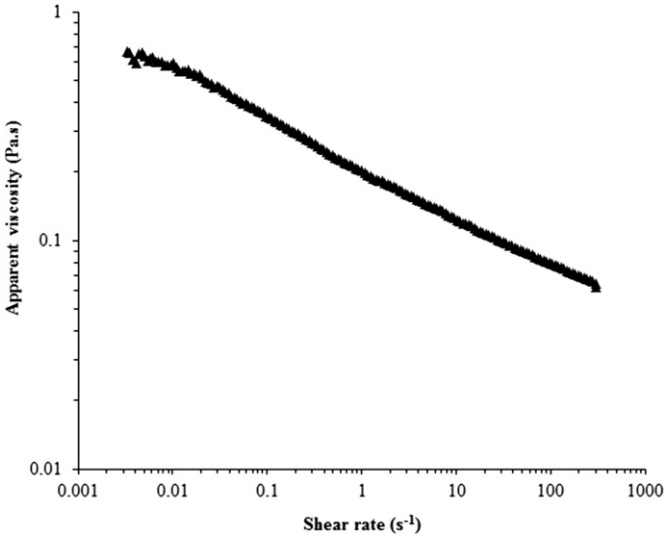

Fig. 1. Influence of shear rate on the apparent viscosity of the 5% OP aqueous solution at 25 °C.