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Physicochemical characterization of pectin grafted with exogenous phenols

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ABSTRACT

Pectin is a natural polysaccharide, having valuable properties that enable its use in many industrial fields. The aim of this work was to study the impact of pectin modification with phenols, on the properties of this biopolymer. Results suggested that the enzymatic grafting of ferulic acid (FA) oxidation products onto the pectin altered its morphological surface and its thermal properties. Moreover, modified pectin showed a less hygroscopic behavior when water activity is less than 0.50 and a higher ability to bound water above 0.5. Additionally, modified pectin became less viscous than the native pectin and presented different calcium-dependent gelation behavior. Finally, a significant improvement of the antioxidant properties of pectin after functionalization was observed. As a conclusion, the modification of pectin with phenolic compounds appeared as a promising way to produce a polysaccharide with new properties that could enlarge the field of its potential applications.

1. Introduction

Pectin is an anionic high-molecular weight polysaccharide commercially extracted from the cell wall of citrus peel, apple pomace and sugar beet (Qiu, Tian, Qiao, & Deng, 2009). It is composed of a linearly α-1,4-linked D-galacturonic acid residues backbone interrupted occasionally by (1,2)-linked rhamnose residues (Ridley, O'Neill, & Mohnen, 2001). Pectin is well known for its gelling and thickening properties that justify its extensive use as a food ingredient. Many studies reported the existence of a correlation between the chemical structure of pectin and its techno-functional properties (Chen et al., 2015; Sila et al., 2009). The esterification and acetylation degrees of pectin appear as crucial structural characteristics that are mostly exploited to produce biopolymers with modulated properties (Buchholt, Christensen, Fallesen, Ralet, & Thibault, 2004; Cheng & Gu, 2012; Van den Broek & Boeriu, 2013). Such modifications were performed aiming to overcome the major drawbacks of pectin (formation of lumps, swelling behavior, hydrophilic nature) when used in some specific areas (Kurita, Miyake, & Yamazaki, 2012). Another approach was described using a laccase-catalyzed oxidation reaction of feruloylated sugar beet pectin. In this reaction, endogenous ferulic acid units were oxidized leading to reactive semiquinones that can subsequently form ferulic acid dimers allowing pectin crosslinking. The crosslinked structures have showed improved rheological properties (Kuuva, Lantto, Reinikainen, Buchert, & Autio, 2003; Zaidel, Chronakis, & Meyer, 2012), useful for the preparation of *in situ* hydrogels (Takei, Sugihara, Ijima, & Kawakami, 2011) or for the formation of stable emulsions (Zaidel & Meyer, 2013). This approach was also applied to conjugate sugar beet pectin with proteins. In this case, the protein solubility in water was enhanced at its isoelectric point, avoiding its precipitation (Jung & Wicker, 2012). As reported by several studies, laccases could also be used to enrich polysaccharides with phenols. In this instance, semiquinones generated from laccase-catalyzed oxidation of exogenous phenols reacted with nucleophilic functions present in the reaction medium leading to their grafting onto the polysaccharide chains (Elegir, Kindl, Sadocco, & Orlandi, 2008; Y. Liu et al., 2014; Torres et al., 2012).

The main scientific issue related to this work was to compare the physicochemical properties of native pectin with those of pectin modified with exogenous phenolic species issued from laccase-

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catalyzed oxidation of ferulic acid. The color, the thermal and hygroscopic behavior, the morphological state, the rheological properties and the antioxidant capacity were more specifically studied for a qualitative and/or quantitative examination of the polysaccharide.

2. Materials and methods

2.1. Chemicals and enzyme

Citrus pectin with galacturonic acid ≥74.0% (dried basis) and with a methoxylation degree ≥ 6.7%, ferulic acid (FA), and 2,2′-azino-bis (3-éthylbenzothiazoline-6-acide sulfonique) (ABTS) were purchased from Sigma–Aldrich (France). The following chemicals 1,1-Diphenyl-2-picrylhydrazyl (DPPH), methanol, ethanol and acetone were obtained from Carlo Erba (Milwaukee, WI, USA).

An industrial laccase named Suberase® (Novo Nordisk A/S, Bagsvaerdt, Denmark) was bought from the Society Novozymes under liquid form. The Suberase® is a fungal laccase from *Myceliophthora thermophila* sp., which is considered as a member of family of polyphenol oxidases, produced by submerged fermentation of a genetically modified *Aspergillus oryzae*. The enzymatic preparation was supplied as a brown liquid with a density of approximately 1.15 g mL^{-1}. It was completely miscible with water. Laccase activity was 13.5 UI/mL.

The pectin was functionalized with laccase-mediated oxidation products of ferulic acid according to the method described by Aljawish et al. (2012). Briefly, the modification reaction was performed at 30 °C by the addition of 5 ml of methanol solution of ferulic acid 50 mM, 1 g of low methoxyl pectin and 45 ml of phosphate buffer (50 mM, pH 7.5) in the reactor. The addition of 13.5 U/mL of Suberase® (a fungal laccase from *M. thermophila)* trigger the reaction, which was carried out for 4 h. The reaction medium was then kept in a freezer for 24 h, then freeze-dried for 72 h. Next, the powder was washed with organic solvents (methanol, ethanol and acetone) to remove unbounded phenols that could be adsorbed onto pectin through electrostatic interactions. Finally, the powder was kept in a desiccator until use.

2.2. Physicochemical properties

2.2.1. Thermal analyses

Before analysis, all samples were dried at room temperature in a desiccator, containing P_2O_5 as drying agent, for at least one week. The differential scanning calorimetry measurement was carried out using a DSC (DSC 204 F1 Phoenix, Netzsch, Germany) under dynamic inert nitrogen atmosphere, with a flow rate of 4 ml/min. Approximately 10 mg of pectin powders were weighted in an aluminum capsule and placed in the DSC system in parallel to an empty capsule used as reference. The program was fixed to perform a first round of heating from 0 to 200 °C in a 5 K/min rate, followed by a cooling from 200 to 0 °C at the same rate 5 K/min. Then a second heating from 0 to 300 °C at 5 K/min was performed. All runs were performed at least in triplicate.

2.2.2. Surface analyses

The morphologies of native pectin and pectin grafted with phenols were observed using a Hitachi scanning electron microscopy (SEM) S4800. Before testing, the samples were evaporated with carbon and then coated with gold, to make the samples conductive. SEM was performed under high vacuum at an accelerating voltage of 10 kV. The microphotographs were taken using automatic image capture software.

2.2.3. Water sorption isotherms

The Dynamic Vapor Sorption was used to monitor the moisture sorption capacity of pectin powders as a function of water activity (aw). Measuring the water sorption, provided information about the physical and chemical stability of the sample under given storage conditions. Water sorption isotherms were determined gravimetrically using a DVS apparatus (Surface Measurement Systems, London, UK) equipped with a Cahn microbalance. The changes in sample weight over time at 25 °C and at any desired aw (between 0 and 0.9) were recorded. About 70–80 mg of sample were loaded onto the quartz sample pan. The program was initially set to control the water activity at 0 for 12 h (drying phase). This step allowed the sample water activity to decrease to zero and internally equilibrate. The sample was then subjected to successive steps of 0.1 aw increase, up to 0.9. For each step, the mass changes (m) and the rate of mass changes (dm/dt) were plotted against time. The equilibrium was considered to be reached when changes in mass with time were lower than 0.001% total weight/min (i.e. 1 g water/100 g dry basis/day). All experiments were performed at 25 °C and 3 tests were carried out for each sample. The accuracy of the system was ±0.01 aw and ±0.2 °C, respectively.

The rate at which the material equilibrated at each humidity level, as well as the overall shape of the resulting adsorption profile, provided useful information about the structure material and long-term stability.

2.2.4. Rheological measurements

2.2.4.1. Viscosity measurements. The viscosity of solutions prepared in deionized water (pH 6.5) with several concentrations (from 1% to 4% w/v) of native and modified pectin were determined using a Kinexus rotative rheometer (Malvern Instruments, KNX 2100, UK) with a cone-plate geometry (50 mm of diameter, angle of 2°), at constant temperature (25 °C), just after the pectin solutions were prepared. The shear rate was increased from 0.001 to 100 s^{-1}. The Newtonian and power law models were used to analyze the rheological behavior of the samples. Each viscosity measurement was performed in triplicate. The temperature was controlled by a Peltier system and the sample was covered with paraffin oil to avoid evaporation.

2.2.4.2. Oscillation measurements. Oscillatory measurements were used to determine the storage modulus (G′) and loss modulus (G″) of 2% pectin solutions using a 50 mm parallel plate at 25 °C. Strain sweep (0.01–100% at 1 Hz) was applied to test the linear viscoelastic region of the samples. The frequency dependence of G′ and G″ was determined by a frequency sweep (0.1–30 Hz at 1% strain) (Zhang et al., 2013).

2.2.4.3. Gelation rate determination. 2 ml of solutions with 12.5 g of native and modified pectin were prepared in 0.1 M NaCl, in order to screen electrostatic interactions. The pH was adjusted to 6.5 to obtain fully charged chains (Capel, Nicolai, Durand, Boulenguer, & Langendorff, 2006). Then, a certain amount of CaCl2 which corresponded to $R = (2\,[Ca^{2+}])/([COO^-]) = 0.58$ was added to the pectin solutions (Fu & Rao, 2001). The solutions were immediately loaded onto the rheometer cone-plane geometry and *G′* and G″ shear modulus at 1 Hz and 0.001% strain were monitored. The strain was determined by a strain sweep test from 0.0001 to 10%. The frequency was determined by a frequency sweep test from 0.01 to 100 Hz performed on the gels after its formation to determine and compare the storage and loss moduli (G′ and G″, respectively). Rheological measurements were performed in three replicates.