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Influence of pH, pectin and Ca concentration on gelation properties of low-methoxyl pectin extracted from *Cyclea barbata* Miers

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ABSTRACTS

Pectin isolated from *Cyclea barbata* Miers leaf (green jelly leaf) forms a gel at a low pH (~≤3) in the absence of co-solutes and divalent ions at room temperature. These gelling properties are important and particularly useful in the development of low-sugar food systems. In this study, rheological measurements were employed to determine the effect of varying different levels of pH, pectin, urea and divalent ions (Ca^{2+}) concentration towards the gelation properties of the isolated pectin. The gelation time and gel physical properties were investigated by determining the gel-sol transition time and the mechanical spectra (*G'* and *G"*) using oscillatory measurements. This study showed that acid-induced gelation of the extracted pectin was mediated by hydrogen bonding and gelation only occurred at pH ≤3. The results obtained from oscillatory measurements showed that gel strength of green jelly leaf (GJL) pectin was affected by varying pectin concentration where gel strength increased with increasing pectin concentration. However, when compared with a pectin with a lesser charge density (Genu pectin, DE 31%), the gel strength of GJL pectin at different concentrations were lower. Presumably the abrupt formation (due to highly negative charge) of cross-linking junction of GJL pectin molecules had led to unhomogenous gel network and resulting in weaker gels.

1. Introduction

Pectin is a complex polysaccharide of the cell walls of growing plants, and mainly consists of partly methylesterified α-1,4-linked d-galacturonic acid (Mohnen, 2008). Pectins are categorised into three main polymer groups (Ridley, O'Neill, & Mohnen, 2001): homogalacturonan, rhamnogalacturonan I and substituted galacturonan. Homogalacturonan has a backbone with a linear chain of α-(1,4)-linked D-galacturonosyl acid, where Rhamnogalacturonan I has a backbone of α-1,2-linked L-rhamnosyl and α-1, 4-linked D-galacturonosyl acid residues, with neutral sugars such as arabinose and galactose as the side chains.

Pectin is a water soluble polysaccharide (WSP) that is deliberately added to food systems to enhance their functional properties. The most significant functional properties of WSPs are their water binding capacity and their viscosity enhancement. WSPs are widely used as stabilisers and viscosifiers to control the texture of semi-solid foods (Wang & Cui, 2005). Thus, pectin, in general, has three functional properties: viscosity build-up, gel formation and protein stabilisation. Currently apple pomace and citrus peels are the two main sources of commercial pectins (May, 1990). Based on their degree of esterification (DE), pectins are grouped into high methoxyl pectin (HMP, DE > 50%) and low-methoxyl pectin (LMP, DE < 50%) (Rolin & De Vries, 1990). In general, HMP forms a gel in an acidic environment (typically pH ~ 3) and in the presence of low molecular weight co-solutes, such as sucrose, at concentrations of ~65% (Evageliou, Richardson, & Morris, 2000). In contrast, LMP forms a gel in the presence of ionic calcium. However, LMP can also gel in the absence of Ca^{2+} by lowering the pH to below 3.3 (Gilsenan, Richardson, & Morri, 2003). The ability of LMP to gel at acidic condition is related to the progressive reduction of the charge density of pectin with decreasing pH (Capel , Nicolai, Durand, Boulenguer, & Langendorff, 2006) which in turn induces aggregation and eventually gelation.

Vincent, Mansel, Kramer, Kroy, and Williams (2013) describes that the acid-induced gelation of LMP is attributed by hydrogen bonding between protonated carboxylic acid groups on the galacturonic acid units of pectin backbone and also between the hydroxyl groups of neighbouring molecules. This gelation mechanism of LMP depends on intrinsic and extrinsic factors of pectin such as pectin concentration, divalent ions concentration, pH,

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temperature and soluble-solid type and concentration (Axelos & Thibault, 1991).

Cyclea barbata Miers leaf is a climbing shrub grown in many parts of Asia. Traditionally, the water extract from the leaves is consumed in the form of a gel or infusion and is used to treat digestive disorders and fever in some parts of Asia such as Thailand and Indonesia. It is found that the leaves of *Cyclea barbata* Miers form a gel instantly, without addition of other ingredients, when blended with water at room temperature (Kooiman, 1969; Arkarapanthu, Chavasit, Sungpuag, & Phuphathanaphong, 2005). These authors indicated that pectin is the polymer responsible for the gelation to occur.

As the spontaneous gelling of the pectic polysaccharide can be useful in food gel systems that require no heat, additional ingredients or pH reduction, study on gelation rheological properties of pectin extracted from *Cyclea barbata* Miers need to be carried out. The aim of this study is to investigate the effect of varying pH values, pectin concentration, and divalent ion (Ca^{2+}) concentration on rheological properties of gelation of pectin isolated from *Cyclea barbata* Miers leaf.

2. Materials and methods

2.1. Materials

The *Cyclea barbata* Miers leaf was obtained from a plantation area in West Lombok, Indonesia. The leaves were sorted, washed, packaged in a container and freeze (−20 ± 0.4 °C). The pH of the fresh leaf water extract was 6.9 ± 0.2. The purified pectin has a galacturonic acid (GalA) content of 36% w/w, DE 10% and average-weight molecular weight (M_w) of ∼4.4×10^5 g/mol. With regards to the minerals content, it consists of calcium (∼0.57% w/w) and zinc (∼0.54% w/w). Commercial pectin used in this study was Genu® Pectin (DE 31%) obtained from Cp Kelco (Singapore). All chemicals were purchased from Sigma Aldrich (Singapore), unless otherwise specified.

2.2. Extraction of cyclea barbata miers leaf pectin

Sodium citrate, 1% w/v (Danisco, Singapore) in deionised water (DI) was used to extract the pectin from *Cyclea barbata* Miers leaves in order to prevent cation-mediated gelation during the extraction process. The extraction was carried out by blending (Waring Blender 7011 HS, U.S.A) the leaves with the chelator-solution at a ratio of 1:25 (w/v) at low speed for 30 s. The mixture was filtered through three layers of cheesecloths to obtain a clear water extract. The residue was re-dispersed in the chelator-solution and blended again to obtain a second extract. The two filtrate samples were combined. The filtrate was stirred for 1 h at room temperature for the chelating process to take place. The filtrate was then centrifuged at 4000*g* for 15 mins at 20 °C (Biofuge Stratos Haraeus, Buckinghamshire, England) in order to remove any insoluble matter. Ethanol (80% v/v, VWR, Singapore) was used to precipitate the pectin at the ratio of 1:4 (v/v). The recovered precipitated pectin was rinsed twice with 80% (v/v) ethanol, followed by drying in an oven (EU53JOUAN SA, France) at 50 °C ± 0.5 °C for 12 h. The crude pectin extract was further purified by hydrating the pectin in DI water (0.5% w/v) for 2 h. The solubilised crude pectin was then centrifuged at 14,000*g* for 40 mins at 20 °C to remove any insoluble components. The collected supernatant was precipitated and dried as described above to recover the green jelly leaf (GJL) purified pectin.

2.3. Characterisation of isolated purified pectin

The mineral compositions of the GJL and the extracted polysaccharide fractions were determined using an inductively couple plasma spectrophotometry (ICP, Shimadzu ICPE-9000, Singapore) based on the AOAC method (AOAC, 1990). Neutral sugar composition of the GJL polysaccharide fraction was determined by gas liquid chromatography (GLC, BPX-70 column), in the form of alditol acetate derivatives as described by Englyst, Quigley, and Hudson (1994) using an Englyst Kit for NSP determination (Englyst Carbohydrate Ltd, UK). The GLC column temperature was 220 °C, and the injector and detector were maintained at 180 °C. The carrier gas (hydrogen) flow rate was 8 mL/min. The GalA content was determined through colorimetric method as method described by Scott (1979) using an UV-160A spectrophotometer (Shimadzu, Douglas Scientific, Singapore).

The recovered GJL purified pectin was analysed for DE by using infrared spectroscopy (Spectrum Two IR, Perkin Elmer U.S.A) according to the method described by Videcoq, Garnier, Robert, and Bonnin (2011). Briefly, four commercial pectin films with different DE (Sigma P9135, Sigma P8471, Danisco AMD 783 and Genu Pectin CP Kelco) were prepared in duplicate by drying the pectin solutions (0.5% w/w) at 50 °C on petri dishes for ∼18 h. From the spectra of Fourier Transform Infra-Red (FT-IR), the absorptions bands at 1740 and 1624 cm^{-1} are assigned to C=O stretching vibrations of esterified carboxyl groups and carboxylic acid respectively. An equation of prediction was obtained from the standard curve with a coefficient of determination $R^2 = 0.9942$. This analysis was operated using the Spectrum AssureID software (AssureID ES version, Perkin Elmer U.S.A). The spectrum obtained was then compared against the library of spectra from the database (Sadtler Basic Monomers & Polymers, V1, 2012, Perkin Elmer U.S.A).

The size exclusion chromatography coupled to a multi-angle laser light scattering (SEC-MALLS) was used to determine the weight-average molecular weight (M_w) according to the method described by Yuliarti et al. (2015). The eluent used was 0.1 M sodium chloride and 0.02% w/v sodium azide in Milli-Q water. The SEC-MALLS system used in this study comprised of a HPLC quaternary pump (Agilent Technologies 1200 series, U.S.A) connected in series to a Shodex SB-6 guard column, Shodex SB-805 size exclusion column (Shodex, Tokyo, Japan), DAWN™ 8+ MALLS detectors (Wyatt Technology, U.S.A), ViscoStar™II viscometer (Wyatt Technology, U.S.A) and a DRI detector (Agilent Technologies, U.S.A). The software used to operate the HPLC was Agilent Chem Station LC1200 (Agilent Technologies 1200 series, U.S.A) and the light scattering and viscometric data were analysed using Astra 6.1 (Wyatt Technology, U.S.A).

2.4. Rheological measurements

Pectin stock solution (0.5% w/v) of GJL and commercial LMP (Genu® Pectin) was prepared by hydrating the samples in Milli-Q water for 4 h (∼25 °C) to achieve complete dissolution. The pH of the GJL and Genu® Pectin solutions was found to be 6.08 and 4.05 respectively. The pH of Genu® Pectin was adjusted similar to GJL pectin by addition of 0.1 M NaOH. Preliminary experiment was conducted to determine the relation between pH and amount Glucono-delta-lactone (GDL) required using 0.2% w/w pectin concentration at room temperature. GDL was used as an acidulant in this study as GDL slowly hydrolyses in aqueous solution to form gluconic acid, which gives a gradual decrease in pectin solution pH. Small and large deformation oscillatory was performed using a Paar Physica MCR 301 rheometer (Anton-Paar, GmbH, Germany) equipped with a cup and bob measuring system (C-CC27/T200 Cup, B-CC27/Q1 Bob).

The effect of pH on the gelation properties of GJL pectin was studied using 0.2% (w/v) pectin solution and 15%, 7%, 0.7% and 0.075% (w/w) of GDL crystals to achieve the pectin solution final pH of 2, 3, 4 and 5, respectively. To prevent the formation of gel due to