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Effect of de-methylesterification on network development and nature of Ca^{2+}-pectin gels: Towards understanding structure–function relations of pectin

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ABSTRACT

Pectins of varying degree and pattern of methylesterification were produced through controlled de-esterification of highly esterified citrus pectin, using carrot pectin methylesterase (PME) (P-pectins), *Aspergillus aculeatus* PME (F-pectins) or sodium hydroxide saponification (C-pectins). Estimation of the degree of methylesterification (DM) and quantification of the pattern of methylester distribution in terms of absolute degree of blockiness (DB_{abs}) enabled the characterisation of pectins. Characterised pectins were used for the preparation of Ca^{2+}-pectin gels with varying calcium ion (Ca^{2+}) concentration. The rheological characteristics of produced gels were evaluated by means of small-amplitude oscillatory tests. During gel formation, gel strength was monitored so as to allow assessment of network development. Based on the evaluation of mechanical spectra, the nature of the cured gels was established. Depending on Ca^{2+} concentration as well as DM and DB_{abs}, gels prepared from specific C-pectins ($48 \geq DM \geq 26\%$; $9 \leq DB_{abs} \leq 37\%$) and F-pectins ($64 \geq DM \geq 29\%$; $9 \leq DB_{abs} \leq 50\%$) showed a striking decrease of the gel strength with time, while gel networks produced from other pectins either displayed a continuous increase of the gel strength or verged towards pseudo-equilibrium within the observation time of 5 h. Furthermore, the DM, DB_{abs} and Ca^{2+} concentration influenced the evolution of Ca^{2+}-pectin networks from "structured liquids" to "strong gels". Based on the experimental results, specific mechanisms of Ca^{2+} interactions with pectins were (also) considered.

1. Introduction

Pectin is a complex polysaccharide abundantly found in the middle lamella of fruit and vegetable tissues. Generally, this macromolecule consists of homogalacturonan (HG) and rhamnogalacturonan I & II domains. Homogalacturonan consists of a linear homopolymer of α(1-4) linked-D-galacturonic acid which, naturally, can be methylesterified at C-6 carboxyl and/or acetylated (Vincken et al., 2003). Owing to the structural features of HG, pectin is used as "textural" polymer in numerous applications. The extent of methylesterification (DM) has been considered as a key parameter defining the gelling properties of the polymer. This functional property of pectin enables the use of isolated commercial pectin as gelling, thickening or stabilising agent in food systems. Generally, high methylesterified pectins (HMPs) with $DM \geq 50\%$ can form gels in the presence of soluble solids such as sugars (Oakenful, 1991; Thibault & Ralet, 2003), whereas low methylesterified pectins (LMPs) of $DM < 50\%$, are associated with gelation by divalent cations (Endress, Mattes, & Norz, 2006; Thibault & Ralet, 2003). Soluble solids induced gelation (for HMPs) requires low pH (COOH groups) while gelation in the presence of divalent cations (specifically Ca^{2+} in food applications) takes place under high pH (COO^- groups) conditions. The latter gelation technique has been largely used in the production of low-calorie healthy foods (Endress et al., 2006). In addition, texture preservation in some processed fruits and vegetables has been related to the formation of *in planta* Ca^{2+}-pectin gel networks,

Abbreviations: DB_{abs}, absolute degree of blockiness; DM, degree of methylesterification; GalA, galacturonic acid; HMP, high methylesterified pectin; LMP, low methylesterified pectin; LVE, linear viscoelastic; NM-GalA, non-methylesterified galacturonic acid; NM-MDT-GalA, non-methylesterified mono-, di- and tri-galacturonic acid; PDP, partially de-esterified pectin; PM, pattern of methylesterification; PME, pectin methylesterase; PG, polygalacturonase.

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which hinder pectin depolymerisation and solubilisation (Sila, Doungla, Smout, Van Loey, & Hendrickx, 2006).

Ca^{2+}-pectin gel networks are generated via "junction zones", whose mechanism of formation is mainly based on the "egg-box" model of Grant, Morris, Rees, Smith, and Thom (1973). This model was used to describe the interaction between some polysaccharides and Ca^{2+}, namely alginate (Morris, Rees, Thom, & Boyd, 1978) and subsequently pectin (Morris, Powell, Gidley, & Rees, 1982; Powell, Morris, Gidley, & Rees, 1982). Junction zone formation through the "egg-box" model entails a cooperative binding of Ca^{2+} to contiguous non-methylesterified galacturonic acid (NM-GalA) residues (referred to as "block") of adjacent pectin molecules. The extent of cooperativeness depends on the number of contiguous NM-GalA residues involved in the formation of individual junction zones. So far, the minimum number of residues required for the formation of highly cooperative or stable junction zones has been rather controversial. Depending on the models used by various research groups, it has been estimated to be 14 (Powell et al., 1982), 9 (Liners, Thibault, & Van Cutsem, 1992), 20 (Braccini & Pérez, 2001) or 6 (Luzio & Cameron, 2008). "Large" NM-GalA blocks cooperatively form "strong associations" with Ca^{2+} (Powell et al., 1982) while short blocks are involved in less cooperative or "less specific interactions" with Ca^{2+} (Durand et al., 1990). The size and thus the distribution of NM-GalA blocks on pectin molecules might play a major role in defining the nature (strength/stability) and number of junction zones occurring in pectin-Ca^{2+} networks. Some mechanical characteristics of polymer gels can be related to the nature and number of junction zones. This suggests that the pattern of methylester distribution greatly influences the characteristics of pectin gels (Speiser, Copley, & Nutting, 1947). A number of researchers provided practical evidence based on works mostly carried out on LMP (Cárdenas, Goycoolea, & Rinaudo, 2008; Fraeye et al., 2009; Powell et al., 1982; Ström et al., 2007). Depending on the methylester distribution pattern, some HMPs might also be predisposed to form Ca^{2+} gels. Studies on the rheological characteristics of Ca^{2+}-HMP gels have been limited to the influence of PMEs (plant and fungal) de-esterified polymers on networks characteristics (Kim & Wicker, 2009; Vincent & Williams, 2009). The effects of the extent and mechanism of de-esterification on most rheological characteristics of pectin gels remains largely unexplored.

Substantial insight in the rheological characteristics of Ca^{2+}-pectin gels requires an in depth assessment of both Ca^{2+}-LMP and Ca^{2+}-HMP gels. Besides, while using pectin as texture "improver" (due to its Ca^{2+} gelling ability) be it *in planta* or as additive, the stability and nature of induced Ca^{2+}-pectin networks might be of great importance to food scientists, technologists and industries. Extensive studies on these rheological characteristics of pectin gels might enable to clearly identify those critical features of pectin responsible for a specific network character. Therefore, the present research aimed at investigating the effect of the degree and pattern of methylesterification on network development and nature of pectin-Ca^{2+} gels. Consequently, starting from parent pectin of very high DM, partially de-esterified pectins of various degrees and patterns of methylesterification (PM) were produced and characterised using state-of-the-art methods. Afterwards, pectin gels were prepared at varied Ca^{2+} concentration. The responses of these gels to dynamic rheological tests were related to the structural characteristics of the polymer.

2. Materials and methods

2.1. Materials

A commercial liquid preparation of recombinant *Aspergillus aculeatus* PME purchased from Novozymes was purified by gel filtration chromatography (Duvetter et al., 2005).

Carrot (*Daucus carota var. Nantes* of Belgian origin) PME was extracted and purified using affinity chromatography (Jolie et al., 2009).

Pure endo-PG from *Kluyveromyces fragilis* was kindly provided by the Laboratory of Food Chemistry of the Wageningen University.

Esterified citrus pectin (DM ~ 94%, GalA content ~85% on dry basis) was purchased from Sigma–Aldrich (P956, lot 077K1432) and encoded M94. Pectins with varying degree and pattern of methylesterification were produced by controlled partial de-esterification of M94 using carrot PME (P-pectins), *A. aculeatus* PME (F-pectins) or NaOH saponification (C-pectins). Detailed production procedures of various PDPs have been described elsewhere (Fraeye et al., 2009; Ngouémazong et al., 2010). Subsequently, all pectins were extensively characterised as described in Ngouémazong et al. (2010).

All chemicals used were of analytical grade.

2.2. Methods

2.2.1. Preparation of Ca^{2+}-pectin gels

For gel preparation, each PDP sample was dissolved in Milli-Q water (18 ΩM) at 4 °C and the pH of the solution was adjusted from ~4.5 to 6.0 (±0.05) using NaOH solutions of concentrations varying from 1.0 to 0.1 M, and under continuous vigorous stirring. The resulting solution was immediately frozen and stored at −40 °C till gel preparation. As the different PDP samples had slightly different GalA content (mol/g pectin; dry matter basis) as a result of de-esterification, the concentration of the pectin solutions was adjusted to constant GalA content (1.68% w/v i.e. 1.68% GalA of unit weight as GalA, 194.14). This correction resulted in an average pectin concentration of 2% w/v (between 1.96 and 2.04% w/v).

A 3 M $CaCl_2$ solution prepared using Milli-Q water was used as $CaCl_2$ stock solution. Prior to gel preparation, this stock solution was diluted so as to prepare gels with defined Ca^{2+} concentration expressed as the stoichiometric ratio ($R = 2[Ca^{2+}]/[COO^-]$), while adding a constant volume of $CaCl_2$ solution. Therefore, the concentration of the $CaCl_2$ solution varied with the DM of PDP samples and the gel *R*-value. Since the calcium content of a 40% (w/v) M94 solution was checked and found to be negligible (<0.00005%), the *R*-value of each prepared gel was varied between 0.0 (no $CaCl_2$ added) and ~5.7. The GalA content of all gels was ~1.53% w/w.

A technique which involved the combination of mild heating and diffusion of Ca^{2+} through microlitres of pectin was used for Ca^{2+}-pectin gel preparation. In this method, Ca^{2+}-pectin gels were prepared on the lower plate of a stress-controlled Physica MCR 501 rheometer (Anton Paar, Austria) (Doungla et al., 2009). More specifically, few microlitres of pectin and $CaCl_2$ solutions were preheated to 50 °C and 30 °C respectively. The Peltier controlled lower plate of the rheometer was preheated to 50 °C. Exactly 262 μl of preheated pectin solution was placed at the centre of the lower plate. Subsequently, 28 μl of preheated $CaCl_2$ was added drop-wise (14 droplets of 2 μl each) over the entire pectin surface. The upper geometry (25 mm Ø parallel plate) was then lowered to the set measuring gap (0.5 mm). The sample surface was covered with light paraffin oil to prevent evaporation during gel development and measurements. In order to limit temperature fluctuations within the gel sample, a Peltier controlled hood was covering the loaded sample.

2.2.2. Small-amplitude oscillatory shear tests

Once loaded (time zero of the experiment), the Ca^{2+}-pectin mixture was allowed to equilibrate for 10 min (to ensure among others complete calcium diffusion through the 262 μl pectin sample) after which it was cooled to 20 °C (0.5 °C/min) for 1 h. The