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# Influence of pectin structure on texture of pectin–calcium gels

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ABSTRACT

A library of pectins with varying degree and pattern of methoxylation was produced by demethoxylating a parent pectin by use of NaOH or pectinmethylesterase from plant or fungal origin. Additionally, pectin was chemically depolymerised by a heat treatment. The resulting pectins were characterised in terms of degree and pattern of methoxylation ("(absolute) degree of blockiness") and the extent of depolymerisation. Pectin–calcium gels were prepared and their texture was studied by performing compression tests. From the resulting force vs. distance curves, the modulus of elasticity under low strain and the fracture stress and strain were determined. The modulus of elasticity under low strain increased with decreasing degree of methoxylation. At very low degree of methoxylation, gels were brittle, resulting in low fracture stress. Both modulus of elasticity and fracture stress correlated more with degree of blockiness and absolute degree of blockiness as compared to degree of methoxylation. Gel strength increased with increasing $Ca^{2+}$ or pectin concentration. Depolymerisation of pectin resulted in formation of brittle gels.

*Industrial relevance:* Pectin with low degree of methoxylation can form a gel in presence of calcium. Therefore, it is widely used in the food industry. In addition, pectin-calcium interactions are of importance for the texture of fruits and vegetables, since crosslinked pectin in the cell wall provides cell-cell adhesion and mechanical strength of tissues. This research is focused on textural characteristics of pectin-calcium gels. It is shown that pectin structural properties influence texture of gels. As a result, control of pectin structure allows fine-tuning of functional properties.



## 1. Introduction

Pectin is a cell wall polysaccharide with complex chemical and macromolecular organisation, the exact architecture is still being explored (Vincken et al., 2003; Coenen, Bakx, Verhoef, Schols, & Voragen, 2007). One of the main domains in pectin is homogalacturonan, a linear chain consisting of α-(1,4)-linked galacturonic acid (GalA) residues, part of which can be esterified with methanol. The percentage of GalA that is methoxylated is defined as the degree of methoxylation (DM). The DM of pectin is generally considered to be one of the main parameters determining its functionality (Thibault & Ralet, 2003). In the presence of sugars, pectin with high DM can form a gel in acidic media. On the other hand, pectin with low DM contains a higher amount of free carboxylic acid groups, which can interact with divalent ions such as $Ca^{2+}$, resulting in the formation of a continuous network. The interactions between low DM pectin and $Ca^{2+}$ ions are often explained in terms of the "egg-box" model (Morris, Powell, Gidley, & Rees, 1982; Powell, Morris, Gidley, & Rees, 1982). According to this model, two pectin chains in which a number of adjacent residues are non-methoxylated, chelate $Ca^{2+}$ ions. The minimum number of successive non-methoxylated GalA residues (NMG) necessary to form a cooperative egg-box is subject of debate and has been estimated to be 6–13 (Luzio & Cameron, 2008), 9 (Liners, Thibault, & Vancutsem, 1992), 14 (Powell et al., 1982) or up to 20 (Braccini & Pérez, 2001). The gel properties are mainly determined by the ability to form stable egg-box-type junction zones, hence the availability of blocks of NMG, long enough for cooperative binding of $Ca^{2+}$ ions. As a result, gel properties depend not only on the degree, but also on the pattern of methoxylation (Powell et al., 1982; Thibault & Rinaudo, 1985; Thibault & Rinaudo, 1986; Willats et al., 2001; Strom et al., 2007; Luzio and Cameron, 2008; Fraeye et al., 2009a, 2009b).

Because of its gel-forming ability, low DM pectin is often used in the food industry. Extracted pectin usually has a high DM. DM can be lowered, allowing formation of a pectin–calcium gel, without addition of sugar (Thibault and Ralet, 2003; Sila et al., 2009). Typically, DM is lowered chemically. Unfortunately, this process is accompanied

*Abbreviations:* DB, degree of blockiness; $DB_{abs}$, absolute degree of blockiness; DM, degree of methoxylation; DP, degree of polymerisation; GalA, galacturonic acid; HPAEC-PAD, high performance anion exchange chromatography pulsed amperometric detection; HPSEC, high performance size exclusion chromatography; NMG, non-methoxylated galacturonic acid residues; PME, pectinmethylesterase; PG, polygalacturonase.

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by depolymerisation of pectin, which could induce a change in gel properties. As an alternative, demethoxylation could be performed by use of pectinmethylesterase (PME), which offers the advantage that depolymerisation is avoided (Savary, Hotchkiss, Fishman, Cameron, & Shatters, 2003). Furthermore, enzymatic demethoxylation gives rise to a different pattern of methoxylation, i.e. a different distribution of NMG on the resulting pectin, which could further influence the functional properties. Chemical demethoxylation generates a completely random pattern of methoxylation (Limberg, et al., 2000a,b). PME with acidic pI (predominantly fungal PME) converts short sequences of GalA residues before attacking a next chain, while PME with neutral or alkaline pI (mostly plant PME) demethoxylates in a more blockwise mode, demethoxylating large parts of a single chain before attacking the next chain (Denes, Baron, Renard, Pean, & Drilleau, 2000; Limberg et al., 2000a,b; Duvetter et al., 2006; Fraeye, et al., 2007), although the exact mechanism is still under debate (Luzio and Cameron, 2008).

In addition to the importance of extracted pectin for many food products, this polymer also determines the texture of fruits, vegetables and derived products *in situ*. As a cell wall polysaccharide, pectin contributes to cell–cell adhesion and mechanical strength. Depolymerisation of pectin, for example as a result of thermal processing, can result in uncontrolled texture softening (Van Buren, 1979). Such texture loss can be partly compensated by the action of PME which is naturally present in plant systems, or can be added exogenously. PME hydrolyses methoxyl esters with formation of carboxylic acid groups which can interact with $Ca^{2+}$ ions, forming a calcium–pectate network (Fraeye et al., 2009a, 2009b; Van Buggenhout et al., 2009).

It can be summarised that pectin–calcium interactions are of importance both in the area of fruit and vegetable processing, and for application of extracted pectin in various food products. The objective of this research was to investigate the influence of intrinsic (degree and pattern of methoxylation, degree of polymerisation) and extrinsic ($Ca^{2+}$ and pectin concentration) parameters on the texture of pectin–calcium gels.

## 2. Materials and methods

### 2.1. Materials

Apple pectin with a DM of 79% was purchased from Fluka (Buchs, Switzerland). Several modified pectins were produced starting from this parent pectin.

For isolation of tomato PME, fresh tomatoes (*Lycopersicon esculentum*) were cut into pieces, frozen using liquid nitrogen and stored at −40 °C until use. PME was extracted from these tomatoes and purified using affinity chromatography as described by Fachin et al. (2002). Recombinant *Aspergillus aculeatus* PME was purified from a commercial liquid preparation (Novoshape, Novozymes, Bagsvaerd, Denmark) by gel filtration chromatography as described by Duvetter et al. (2005). After purification, both pectin preparations were free of any contaminating enzymes (Fachin et al., 2002; Duvetter et al., 2005).

Endo-polygalacturonase of *Kluyveromyces fragilis* was kindly donated by the Laboratory of Food Chemistry of the Wageningen University.

All chemicals were of analytical grade.

### 2.2. Pectinmethylesterase activity assay

Activity of the purified *A. aculeatus* PME and tomato PME solutions was determined by following the release of acid as a function of time (during 10 min) using an automatic pH stat titrator (Metrohm, Herisan, Switzerland) at constant temperature (22 °C) and pH (pH = 8.0 for tomato PME, pH = 4.5 for *A. aculeatus* PME, optimal pH for activity of the respective enzymes). PME-solution (250 µL) was added to a reaction mixture consisting of 30 mL of a 3.5 g/L apple pectin (DM = 79%) solution and 0.117 M NaCl. The $pK_a$ of GalA has been estimated as 3.5. In case of *A. aculeatus* PME, the activity measurement was carried out at pH 4.5. At this pH, a fraction of $10^{(pK_a-pH)} = 0.1$ of the carboxyl groups released by the enzyme was not dissociated, hence this fraction was not titrated. A correction for this incomplete dissociation of demethoxylated carboxyl groups was made by multiplying the apparent activity with a factor $[1 + 10^{(pK_a-pH)}]$ (Christgau et al., 1996). One unit (U) of PME activity is defined as the amount of enzyme capable of catalysing the release of 1 µmol methyl-ester bonds per minute under above mentioned standard assay conditions.

### 2.3. Pectin preparation

Pectins with varying degree and pattern of methoxylation were prepared by demethoxylation of the parent apple pectin with a DM of 79% (denoted as P79). Pectin was treated with purified tomato PME (T-pectins), with purified *A. aculeatus* PME (A-pectins) or chemically (C-pectins).

For production of A-pectins, P79 was dissolved (0.8% w/w) in Na-acetate buffer (0.1 M; pH 4.5). Pyrex tubes were filled with 25 mL of the pectin solution. After addition of 15 U of PME, the tubes were capped, the content was mixed and the tubes were incubated in a thermostated water bath at 30 °C. After a preset incubation time, tubes were placed in a water bath at 85 °C during 4 min. This heat shock was sufficient to inactivate the enzyme. Then, tubes were cooled in ice water.

T-pectins were prepared by dissolving P79 in water (0.8% w/w), adjusting the pH to 7.0 with NaOH and adding tomato PME (15 U per 25 mL) at room temperature. Production of T-pectins was not performed at optimal pH for tomato PME activity (pH 8.0), but at pH 7.0 in order to reduce unwanted depolymerisation. Enzymatic demethoxylation of pectin resulted in a pH decrease, which was titrated with NaOH. The amount of NaOH added during titration was directly related to the amount of esters hydrolysed. When the desired DM was reached, the reaction was stopped by lowering the pH to 4.5 with a 0.1 M HCl solution. The solution was divided over pyrex tubes with cap (25 mL per tube) which were put in a water bath at 85 °C during 4 min to inactivate the enzyme. Tubes were cooled in ice water.

C-pectins were prepared by dissolving P79 in water (0.8% w/w) and increasing the pH to 11.0 with NaOH. At these alkaline conditions, methyl esters were saponified by $OH^-$-ions, resulting in a pH decrease. This decrease was titrated with NaOH. The procedure was performed in an ice bath to eliminate undesired depolymerisation reactions. The reaction was stopped by decreasing the pH to 4.5 with a 0.1 M HCl solution.

A-, T-, and C-pectins were extensively dialysed (MWCO = 12–14 kDa) against demineralised water during 48 h with 10 changes of water, lyophilised and stored in a vacuum desiccator over phosphorous pentoxide until use.

Two of the C-pectins (DM = 30% and DM = 24%) were depolymerised to create pectins with lower DP (D-pectins). The C-pectins were dissolved (0.3% w/w) in phosphate buffer (0.1 M; pH 6.0). The solution was divided over pyrex tubes with cap (25 mL per tube) and heated in an oil bath at 130 °C for preset treatment times up to 20 min. Under these conditions, β-elimination takes place. Tubes were subsequently cooled in ice water. These D-pectins were dialysed (MWCO = 3.5 kDa) against demineralised water during 48 h with 10 changes of water, lyophilised and stored in a vacuum desiccator over phosphorous pentoxide until use.

### 2.4. Determination of degree of methoxylation

DM of the pectins was expressed as the molar ratio of the amount of methanol esters to the amount of galacturonic acid residues. To estimate the amount of methyl esters, a pectin solution was