Effect of debranching on the rheological properties of Ca²⁺-pectin gels

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1 **Effect of debranching on the rheological properties of Ca²⁺-pectin gels**

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Abstract

Water-soluble pectin (WSP) extract was subjected to controlled carrot pectin methylesterase treatment, thereby producing de-esterified pectin (DEP). Both WSP and DEP were incubated with a mixture of endo-arabinanase and α -L-arabinofuranosidase to yield partially debranched pectins (WSP_{DBr} and DEP_{DBr} respectively). Pectin samples were characterised in terms of degree of methylesterification (DM), neutral sugar content, and degree of branching (DBr). The characterised pectins were used for the preparation of pectin gels with high and low calcium ion (Ca^{2+}) concentrations. The rheological characteristics of the produced gels were evaluated by means of small-amplitude oscillatory tests. These characteristics include network development of the gel, gel strength (G'), gel elastic character and gel type. Partial debranching of pectin resulted in a reduction of the arabinose content (by approximately 50%) and caused a slight decrease in polymer DBr. Gels produced from semi-dilute solutions of partially debranched pectins showed network development profiles similar to those prepared from semi-dilute solutions of the corresponding non-debranched polymers. Yet, the former gels showed lower G' values, poor gel elastic character and a “weaker” nature as compared to the latter. Next to Ca^{2+} cross-links, the presence of long arabinose-containing side chains is suggested to play an important role in the rheological characteristics of Ca^{2+} -pectin gels.

Keywords: Pectin - Rhamnogalacturonan I - Degree of branching - Ca^{2+} -pectin gels - Egg-box model – Rheology

39 **List of abbreviations**

40 AGI = type I arabinogalactan

41 AGII = type II arabinogalactan

42 AFASE = α -L-arabinofuranosidase of type B

43 ARASE = endo-arabinanase

44 DBr = degree of branching

45 DEP = partially de-esterified pectin

46 DEP_{DBr} = partially de-esterified and debranched pectin

47 DM = degree of methylesterification

48 Endo-PG = endo-galacturonase

49 GaIA = galacturonic acid

50 HG = homogalacturonan

51 MM = average molar mass

52 MWCO = molecular weight cut off

53 NMGaIA = non-methylesterified galacturonic acid

54 NS = neutral sugars

55 PL= polymer linearity

56 PME = pectin methylesterase

57 RGI = rhamnogalacturonan I

58 RGII = rhamnogalacturonan II

59 WSP = water soluble pectin

60 WSP_{DBr} = partially debranched water soluble pectin

61

1. Introduction

Pectin is a polysaccharide located in the cell wall of dicotyledonous plants. Generally, this macromolecule consists of linear and branched domains whose detailed structures can be very heterogeneous, even within a single cell wall (Willats, Knox and Mikkelsen, 2006; Voragen, Coenen, Verhoef and Schols, 2009). The structural features of endogenous and extracted pectins play a major role in determining the texture of (processed) fruits and vegetables as well as that of any pectin-containing system (such as food products) (Sila, Doun gla, Smout, Van Loey and Hendrickx, 2006; Fraeye, Duvetter, Doun gla, Van Loey and Hendrickx, 2010). This functionality of pectin is attributed to the gel-forming ability of the polymer. Specifically, in the presence of calcium ions (Ca^{2+}), pectin can form Ca^{2+} -pectin gels (Powell, Morris, Gidley, and Rees, 1982; Capel, Nicolai, Durand, Boulenguer and Langendorff, 2006) which greatly contribute to the rheological character of pectin-containing products. The Ca^{2+} -induced gelation of pectin depends several intrinsic and extrinsic parameters (Fraeye et al., 2010). The intrinsic factors that influence the rheological characteristics of Ca^{2+} -pectin gels include the polymer average molar mass (MM) and the structural features of both the linear and branched domains.

Homogalacturonan (HG), the linear structural element of pectin, consists of a homopolymer of $\alpha(1\rightarrow4)$ -linked-D-galacturonic acid (GalA) which, naturally, can be methylesterified at the C-6 carboxyl (Waldron, Parker and Smith, 2003). The amount of methylesters occurring on this pectic domain defines the degree of methylesterification (DM) of the polymer. In addition to HG, a predominant, heterogeneous and branched domain known as rhamnogalacturonan I (RGI) has been identified as an important

pectin structural element. RGI is generally composed of a backbone of the repeating disaccharide [$\rightarrow$ 2)- α -L-rhamnopyranose-(1 $\rightarrow$ 4)- α -D-GalA-(1 $\rightarrow$], sometimes substituted at the O-4 position of the rhamnose residue with neutral sugar side chains. These substitutions are mainly single galactose units (β -D-galactopyranose-(1 $\rightarrow$ 4)) as well as polymeric side chains of arabinan and arabinogalactan (Ridley, O'Neil and Mohnen, 2001; Voragen et al., 2009). The arabinan side chains are linear or branched polymeric structural elements (Ridley et al., 2001), while the arabinogalactans (AG) are either of type I (AGI) or type II (AGII). AGI are rather simple polysaccharides in which short ramifications of α -(1 $\rightarrow$ 5)-L-arabinofuranose residues generally occur on the galactose backbone (Schols & Voragen, 2003; Voragen et al., 2009). On the contrary, AGII, which are more common in plants are extremely branched polysaccharides composed of highly ramified chains of β -D-galactosepyranose (Schols and Voragen, 2003). The proportions, chain lengths and further ramifications of RGI and its side chains vary from one pectin source to another (Willats et al., 2006; Verhoef et al., 2009). Specifically, carrot pectin contains predominantly AGII and smaller proportions of arabinan and AGI (Schols and Voragen, 1994; Verhoef, Knox, Voragen and Schols, 2009). Generally, the side chain extent of pectin is quantified using an indicative parameter termed degree of branching (DBr) (Boeriu, Stolle-Smits, and Van Dijk, 1998; Kaaber, Kaack, Kriznik, Bràthen and Knutsen, 2007).

In Ca^{2+} -pectin gelation, adjacent pectin molecules bearing contiguous non-methylesterified galacturonic acid (NMGalA) residues (referred to as “block”) are cross-linked via Ca^{2+} bridges as described by the mechanism of the egg-box model, thereby yielding junction zones (Powell et al., 1982; Luzio and Cameron, 2008). This implies

that, of all pectin structural elements, only the non-substituted and non-methylesterified portions of HG are involved in Ca^{2+} dimerisation. Until now, the influence of most HG structural features (methylesterification, acetylation) on the rheological characteristics of Ca^{2+} -pectin gels has been extensively explored (Powell et al., 1982; MacDougall, Needs, Rigby, and Ring, 1996; Luzio and Cameron, 2008; Fraeye et al., 2010). The contribution of the branched region of pectin to the rheological properties of Ca^{2+} gels has merely been based on numerous speculations (Hwang, Pyun and Kokini, 1993; Cardoso Coimbra and Lopes Da Silva 2003) or studies involving combined modifications of HG and RGI side chains (Mathew, Howson, Keenan and Belton, 1990; Budolfson and Heldt-Hansen, 1997; Oosterveld, Beldman, Searle-van Leeuwen and Voragen 2000; Schmelter, Wientjes, Vreeker and Klaffke, 2002). As clear evidence of the effect of RGI neutral sugar side chains on the rheological properties of Ca^{2+} -pectin gels has been scarcely provided, this study aims at investigating the influence of the partial removal of the RGI neutral sugars side chains on the rheological characteristics of Ca^{2+} -pectin gels. Accordingly, water-soluble pectins (relatively rich in neutral sugar side chains) of high and low degree of methylesterification, were subjected to partial enzymatic (endo-arabinanase and α -L-arabinofuranosidase) depolymerisation of RGI side chains to yield partially debranched polymers. After structural characterisation of the polymers, Ca^{2+} -pectin gels were prepared and their responses to dynamic rheological tests were evaluated. Using this state-of-the-art approach, comprehensible structure-function relations of pectin were established.

2. Materials and methods

2.1 Materials

Carrots (*Daucus carota* var. Nantes of Belgian origin) were used for the extraction of water soluble pectin (WSP) (see section 2.3) and carrot pectin methylesterase (PME). Extracted PME was purified using affinity chromatography (Jolie, Duvetter, Houben, Clynen, Sila, Van Loey and Hendrickx, 2009).

Commercial liquid preparations of endo-arabinanase (ARASE) and α -L-arabinofuranosidase of type B (AFASE), purchased from Megazyme (UK), were appropriately characterised. Arabinan, purchased from Sigma (Germany) was used for the activity assays of both enzymes.

All chemicals used were of analytical grade.

2.2. Analysis of commercial debranching enzyme preparations

Prior to the activity assays of the debranching enzymes, commercial enzyme preparations were subjected to a series of tests in order to check their purity level. First, sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a Phast-system (GE Healthcare Bio-Sciences AB, Sweden) with PhastGel homogeneous 20 and PhastGel SDS buffer strips. Commercial preparations were further analysed for other pectinases by means of various enzyme activity measurements. Specifically, endo-polygalacturonase (endo-PG) and PME activities were assayed since the presence of these enzymes in any preparation can, during debranching, result in depolymerisation of HG and hydrolysis of methylester bonds respectively. Endo-PG activity was measured based on the determination of reducing

end groups produced in poly-D-GalA solution, at pH 5.0 (Ngouémazong, Tengweh, Duvetter, Fraeye, Van Loey, Moldenaers, and Hendrickx, 2010). PME activity was analysed at pH 7.0 by means of automatic titration of carboxyl groups produced using 0.01 M NaOH (Ly-Nguyen, Van Loey, Fachin, Verlent, Indrawati, and Hendrickx, 2002).

Endo-arabinanase (ARASE) and α -L-arabinofuranosidase (AFASE) activities were determined using arabinan (2% (w/v) in a 0.2 M sodium acetate buffer, at pH 4.0) as substrate. ARASE digests arabinan while AFASE acts both on arabinan and arabinose-containing oligosaccharides, releasing arabino-oligosaccharides and terminal arabinose residues, respectively (De Vries & Visser, 2003). Following each enzymatic digestion, the amount of reducing end groups released per time unit at 40 °C was quantified based on the 2-cyanoacetamide method (Gross, 1982). One unit (U) of ARASE or AFASE activity is defined as the amount of enzyme catalysing the release of 1 μ mol of reducing end groups per minute under the given assay conditions.

2.3 Preparation of pectin samples

Sample preparation involved the production of water-soluble pectin (WSP) from carrots followed by its partial de-esterification using carrot PME to yield pectin samples of lower DM (DEP). Both WSP and DEP samples were then treated with debranching enzymes to yield partially debranched pectins (WSP_{DBr} and DEP_{DBr}, respectively).

Using alcohol-insoluble residue from frozen carrot tissues (Mc Feeters and Armstrong, 1984), the water-soluble fraction of pectin was extracted based on a modified hot water extraction procedure (Sila et al., 2006). After the vacuum filtration of the extract, the filtrate (WSP) was extensively dialysed (MWCO = 12-14 kDa) against

de-mineralised water for 24 h with 5 changes of dialysing water. Dialysed samples were then lyophilised and stored in a desiccator above P_2O_5 at room temperature.

Partial de-esterification of WSP was carried out using purified carrot PME at pH 7.0 (Ngouémazong et al., 2010). Regarding pectin debranching, samples (WSP and DEP) were treated with a mixture of endo-arabinanase and α -L-arabinofuranosidase, according to Budolfson and Heldt-Hansen (1997). Exactly 2.5 U of each enzyme was rapidly added to 10 mL of 0.25% (w/v) WSP or DEP, dissolved in a 0.2 M phosphate buffer (pH 4.0). The enzymes-substrate mixture was placed in a thermo-stated shaking water bath (Haake W13, Belgium) at 40 °C for 24 h. At the end of the incubation time, the solutions were immediately placed in a thermo-stated water bath (Haake W13, Belgium) at 85 °C for 4 min, so as to inactivate the enzymes. The resulting solutions were then rapidly cooled in an ice bath and dialysed (MWCO = 12-14 kDa) against de-mineralised water to remove sodium salts and oligosaccharides from the partially de-branched pectin samples. Dialysed samples were then lyophilised and stored in desiccators above P_2O_5 at room temperature. Partially de-branched samples from WSP and DEP were labeled WSP_{DBr} and DEP_{DBr}, respectively.

Prior to biochemical analysis and gel preparation, all samples (WSP, WSP_{DBr}, DEP, DEP_{DBr}) were subjected to ethanol/acetone precipitation. The precipitation step further ensured the removal of oligosaccharides which failed to pass through the dialysis membrane. This step entailed first the centrifugation (using a Microfuge 22R centrifuge, Beckman Coulter Inc.) of a mixture of pectin solution and 95% ethanol at 14000 rpm (21920 g) for 40 min, followed by the centrifugation (under similar conditions) of a mixture of ethanol precipitated material and acetone. Thereafter, the acetone precipitated material was dried in an oven at 40°C for 48 h. All dried samples

were then stored in a desiccator above P_2O_5 until analysis and gel preparation were carried out.

2.4 Structural characterisation of pectins

Pectin polymers were characterised in terms of molar mass distribution profile, degree of methylesterification (DM), neutral sugar (NS) content and degree of branching (DBr). The last two characteristics portray the structural features of RGI and its side chains. The average molar mass (MM) of the starting materials used for debranching (WSP and DEP) was also determined. The estimation of the parameters used for pectin characterisation was carried out in duplicate.

2.4.1 Molar mass distribution profile of pectin samples

The molar mass distribution profile of pectin samples was examined by high performance size exclusion chromatography (HPSEC) using an Akta™ Purifier 10 (GE Healthcare, Sweden), coupled with a Shodex R101 refractive index detector (Showa Denka, K.K, Japan). The estimation of the MM of WSP and DEP was achieved by means of a log-linear standard curve between the MM and elution time of eight pullulan standards (MM range between 5900 and 788000 Da) (Sila et al., 2006). Pullulan displays similar structural and hydrodynamic characteristics as poly-D-GalA, a linear polymer which is comparable with only the HG domain of pectin. Nonetheless, as both WSP and DEP polymers consist of HG and branched RGI, any effect of the RGI side chains on the estimated MM was assumed to be similar.

2.4.2 Determination of degree of methylesterification (DM)

The DM of all pectin samples (WSP, DEP, WSP_{DBr}, DEP_{DBr}) was calculated as the ratio of the molar amount of methyl-groups (as methanol) to the molar amount of GalA per gram sample and expressed in percentage. The methylesters of all pectin samples were hydrolysed and the methanol produced during pectin saponification was determined based on the colorimetric method of Klavons and Bennet (1986). In order to determine the GalA content of the pectins, dissolved samples were subjected to sulfuric acid hydrolysis. Subsequently, the GalA concentration was determined by the colorimetric m-hydroxyl-diphenyl method (Blumenkrantz & Asboe-Hansen, 1973). All concentrations were determined on dry basis.

2.4.3 Determination of degree of branching (DBr)

The DBr of pectin is a numerical value that represents a measure of the side chains extent (Boeriu et al., 1998). The neutral sugar (NS) contents of RGI form the basis of DBr quantification. Therefore, in order to determine this parameter, the NS contents of pectin samples were determined. This entailed a thorough hydrolysis of pectin (to release the individual NS) using 4 M tri-fluoro-acetic acid at 110 °C for 1.5 h, followed by an analysis of the released NS of pectin digests via high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). NS separation and detection were carried out at pH 12.0 using a Dionex-DX600 system (Dionex Corporation) equipped with a 4 × 250mm CarboPac PA1 column and guard column. The identification and quantification of individual NS was based on the elution of commercial NS standards (L-rhamnose, L-arabinose, D-galactose, D-glucose,

L-fucose and L-xylose) at varying concentrations (1-10 ppm). Mixed external standards of specific concentrations ensured accurate quantification.

Although the definition of DBr is generally approved, the estimation of this parameter remains controversial. Boeriu et al. (1998) calculated DBr as the ratio of the molar amounts of GalA to the sum of the molar amounts of RGI neutral sugars ([GalA] / ([rhamnose] + [galactose] + [arabinose])), while Kaaber et al. (2007) determined DBr as the ratio of the molar amounts of either galactose or arabinose to the molar amounts of rhamnose ([galactose] / [rhamnose] or [arabinose] / [rhamnose]). However, as rhamnose constitutes the branching point of RGI backbone while both galactose and arabinose are the major side chain NS, DBr was determined, in this study, as the ratio of the sum of the molar amounts of side chain NS over the molar amount of rhamnose as follows:

$$\text{DBr} = \frac{[\text{galactose}] + [\text{arabinose}]}{[\text{rhamnose}]}$$

2.5 Rheological characterization of Ca²⁺-pectin gels

2.5.1 Determination of concentration regime of pectin solutions

The concentration regime of polymer solutions partially determines the behaviour of polymer chains (in solution) with respect to each other, thus the ability of the chains to be entangled (Mascosko, 1994; Teraoka, 2002). Generally, the concentration regime is determined based on polymer concentration (in solution) and intrinsic viscosity ([η]) (Macosko, 1994). The latter parameter is a measure of the hydrodynamic volume of the polymer, which depends on its MM and conformation. Pectin debranching does not have any significant effect on the polymer MM and its conformation (Hwang & Kokini,

1992; Schmelter et al., 2002). Consequently, using the determined MM of WSP and DEP, $[\eta]$ was calculated using the Mark-Houwink equation ($[\eta] = k \cdot (MM)^a$), which is used for the determination of polymers' MM from $[\eta]$ data and vice versa. Berth, Anger and Linow (1977) estimated the constants k (216×10^{-7} l/g) and a (0.78) for high methylesterified pectins with MM in the range of 20-200 kDa. As Mark-Houwink constants can be used irrespective of pectin source and DM (Schmelter et al., 2002), the aforementioned constants were used for estimating the $[\eta]$ of carrot pectins.

Thus estimated, the $[\eta]$ of non-debranched polymer enabled to calculate the coil overlap parameter ($C \cdot [\eta]$, where C represents polymer concentration (g/L) and $[\eta]$ (L/g) corresponds to polymer intrinsic viscosity), which forms the basis of the classification of polymer solutions into dilute ($C[\eta] < 1$), semi-dilute ($1 < C[\eta] < 10$) or concentrated ($C[\eta] \geq 10$) regimes (Macosko, 1994).

2.5.2 Preparation of Ca^{2+} -pectin gels

For gel preparation, each pectin sample (WSP, DEP, WSP_{DBr} or DEP_{DBr}) was dissolved in Milli-Q water (18 M Ω .cm resistance, Millipore, USA) at 4 °C. The pH of the solution was adjusted to 6.0 ($\pm$ 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 until gel preparation. Since NMGalA drives gel formation and the GalA content (mol/g pectin; dry matter basis) of the different pectin samples showed some differences as a result of de-esterification and de-branching, the concentration of the pectin solutions was adjusted to constant GalA content (0.2% (w/v)). This resulted in pectin concentrations of 0.96 and 0.54% (w/v) for WSP and

WSP_{DBr} and concentrations of 0.72 and 0.52% (w/v) for DEP and DEP_{DBr}, respectively.

A 3 M CaCl₂ stock solution was prepared using Milli-Q water. Prior to its use, this stock solution was diluted so as to produce gels with defined Ca²⁺ concentration expressed as the stoichiometric ratio ($R = 2[Ca^{2+}] / [COO^-]$) (Capel et al., 2006). The R-value of each prepared gel was either 0.5 or 2.0 for low and high Ca²⁺ gels, respectively.

A technique involving the combination of mild heating and Ca²⁺ diffusion through microlitres of pectin was used for Ca²⁺-pectin gel preparation (Doungla, Vandebril, Duvetter, Van Loey, Moldenaers and Hendrickx, 2009). In brief, Ca²⁺-pectin gels were prepared on the lower plate of a stress-controlled Physica MCR 501 rheometer (Anton Paar, Austria). Few microliters of pectin and CaCl₂ solutions were preheated to 50°C and 30°C respectively. Meanwhile, the Peltier controlled lower plate of the rheometer was preheated to 50°C. Exactly 262 µl of the preheated pectin solution was placed at the centre of the lower plate. Subsequently, 28 µl of the preheated CaCl₂ 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 formation and measurements. In order to limit temperature fluctuations within the sample, a Peltier controlled hood was lowered over the loaded sample.

2.5.3 Small amplitude oscillatory shear tests

Once loaded (time zero of the experiment), the Ca²⁺-pectin mixture was allowed to equilibrate for 10 min (to ensure complete calcium diffusion through the 262 µl pectin

solution), after which it was cooled to 20 °C (0.5 °C/min) within 1 h. Afterwards, the sample was let to evolve for 5 h at 20 °C to ensure complete gel development. Preliminary stress sweep tests (at 1 rad/s) were performed in order to define the linear viscoelastic region of the gels. All amplitude oscillatory shear tests were then carried out within this region, thereby excluding effects of shear-induced gelation or structure break-down. The network development of various Ca²⁺-pectin systems was assessed by means of a time sweep test (angular frequency of 1 rad/s). Subsequently, a frequency sweep test (0.1-10 rad/s) was performed so as to investigate the frequency dependence of the moduli of the formed gel. Although the aforementioned gel preparation method and rheological measurements have been reported to be reproducible (Dounghla et al., 2009), most tests were performed in duplicate, on freshly prepared gels.

3. Results and discussion

3.1 Assessment of the purity of commercial debranching enzyme preparations

Prior to partial debranching of the RGI side chains, the purity of the commercial enzyme preparations was evaluated. For both ARASE and AFASE preparations, single major bands, whose estimated MM correspond to literature data were observed after SDS-PAGE (results not shown). This is an indication that a single protein or proteins with similar MMs are present in these enzyme preparations.

Upon treating arabinan, poly-D-galacturonic acid and apple pectin solutions with each of the enzyme preparations, it was observed that both preparations could depolymerise arabinan whereas they could cause neither the hydrolysis of poly-D-

galacturonic acid nor the de-esterification of apple pectin (Fig. 1). Consequently, it can be concluded that, the commercial preparation of ARASE or AFASE contain individual corresponding enzyme but both preparations are considered free from endo-PG and PME contamination.

3.2 Structural characterisation of pectins

3.2.1 Molar mass distribution profile of pectins

The extracted carrot WSP is characterised by a single polymeric population, having an average molar mass (MM) of approximately 140.68 ± 14.24 kDa (elution time of 11.15 ± 0.08 min). A comparable MM has been reported in literature for carrot WSP (Sila et al., 2006). After molecular modification, the WSP polymeric population did not show any clear shift in elution time (11.22 ± 0.04 , 11.25 ± 0.01 and 11.24 ± 0.05 min for DEP, WSP_{DBr} and DEP_{DBr} samples respectively). These results indicate that no major change in polymer hydrodynamic volume occurred during de-esterification and/or debranching, thereby suggesting that no significant pectin backbone depolymerisation occurred in the course of enzymatic modifications, as earlier reported by Schmelter et al. (2002).

3.2.2 Neutral sugars analysis of pectins

After pectin hydrolysis, the neutral sugars (NS) were determined via HPAEC techniques. Generally, all pectins display the same NS which include fucose, rhamnose, arabinose, galactose, glucose and xylose. Similar NS have been reported in literature for carrot WSP (De Roeck, Sila, Duvetter, Van Loey and Hendrickx, 2008). After

structural modifications, although the amount of each sugar type show either a decrease or remain constant, the total amount of NS display a decrease.

Fucose, a neutral sugar which is usually located on the RGII side chain of pectin (Schols & Voragen, 2003), display quite constant concentrations after pectin de-esterification or debranching. This suggests that the RGII side chains, which contain negligible amounts of NS, did not undergo relevant depolymerisation during partial debranching. The presence of glucose (~5% of WSP total NS) possibly indicates the extraction of non-pectic polysaccharides along with water soluble pectin. The detection of considerable amounts of xylose (~20% of total NS content) in WSP might either suggest a high level of xylosidation of carrot WSP or the extraction of some xylose-containing non pectic polysaccharides together with WSP. Both the glucose and the xylose content of WSP decreased during pectins' enzymatic modifications.

Apart from fucose, glucose and xylose, the other NS (rhamnose, galactose and arabinose) were considered as emanating from RGI. Fig. 2 depicts the content of individual NS of RGI and the total amount of NS released after the digestion of pectin samples. Rhamnose, the RGI backbone neutral sugar, represents approximately 5% of the total NS content of WSP. This sugar reveals rather constant concentrations after pectin modification processes, suggesting limited (if any) depolymerisation of the RGI backbone.

Galactose is the predominant NS of the RGI side chains (~50 % of total NS of carrot WSP), while arabinose accounts for about 20% of the total NS content of WSP, agreeing well with the results reported for the modified hairy regions (enzyme resistant pectin mostly made up of RGI) of carrot tissues (Verhoef et al., 2009). De-esterification

384 does not cause any major change in the galactose and arabinose contents of pectin
385 samples. Upon debranching, the galactose content of the polymers remains rather
386 constant whereas the amount of arabinose decreases by half. This is an indication that
387 the combined activity of ARASE and α -L-AFASE enzymes results in a considerable
388 degradation of the arabinan side chains and/or arabinose-containing branches of the
389 AGs. The glycosidic bonds that are cleaved by ARASE and AFASE are described by De
390 Vries and Visser, (2003). ARASE only acts on the α -L-arabinofuranose-(1 $\rightarrow$ 5) bond of
391 arabinan. Apart from breaking the α -L-arabinofuranose-(1 $\rightarrow$ 5) bond of arabinan and
392 AGI side chains, AFASE also acts on α -L-arabinofuranose-(1 $\rightarrow$ 2) and α -L-
393 arabinofuranose-(1 $\rightarrow$ 3) bonds of arabinan and AGII. Since arabinan and its side chains
394 are composed of all the listed bonds, it is proposed that a 24 h digestion of pectin (with
395 ARASE and AFASE) results in an extensive degradation of arabinan. As the galactan
396 backbone of AGI is not degraded by any of these enzymes, only certain AGI side
397 branches were depolymerised during partial debranching. The AGII structural element is
398 composed of numerous bonds including α -L-arabinofuranose- (1 $\rightarrow$ 3) linkages, which
399 are found in the end branches of extremely ramified AGII (Schols & Voragen, 2003;
400 Voragen et al., 2009). The location of these bonds indicates that the degradation of AGII
401 likely occurred at the extreme ends of the ramifications. This suggests that, during
402 partial debranching of pectin, the degradation of arabinose-containing branches of AGII
403 did not affect the interior and exterior β -galactose backbones, implying a rather poor
404 depolymerisation of AGII and thus, a limited release of arabinose from this structural
405 element. This is consistent with the comparable galactose content observed in both
406 non-debranched and debranched pectin samples. Therefore, partial debranching likely

yields pectins with extensively degraded arabinan side chains, partially and poorly depolymerised AGI and AGII, respectively.

3.2.3 Overview of structural characteristics of pectins

Although the structural characteristics of pectin samples display some alterations depending on the modification process, the total composition of each polymer remains quite constant (Table 1).

The GalA content of WSP (~20%) is less than half of the major NS content of the polymer (~55%), indicating that RGI constitutes the largest proportion of carrot WSP. Generally, partial enzymatic debranching with a mixture of ARAse and AFase causes some changes in the structural characteristics of the pectins. The GalA content of the debranched pectin samples is increased as a result of the decrease in the total NS content of the polymer, as earlier observed by Hwang and Kokini (1992). In addition, a slight decrease of the degree of branching (DBr, as expressed in 2.4.3) is observed upon debranching. The insubstantial alteration of the DBr is a reflection of the poor degradation of AGII, the predominant side chain polysaccharide of carrot pectin. Furthermore, the DM of the non-debranched pectin and that of its corresponding debranched polymer show comparable values, indicating that no further de-esterification took place during debranching.

3.3 Rheological characterisation of Ca²⁺-pectin gels

3.3.1 Concentration regime of pectin solutions

The rheological characteristics of pectin solutions and Ca²⁺-pectin gels partially

depend on the concentration regime of the pectin solutions (Hwang & Kokini, 1992; Rao, 1999; Capel et al., 2006). In view of maintaining a similar GalA content in all gels, the polymer concentration (C) of the debranched pectin solutions was taken lower than that of the corresponding non-debranched pectin solutions (partial debranching resulted in an increase of the sample GalA content). Therefore, it became imperative to estimate the concentration regime of the pectin solutions, in order to perform a reliable evaluation of the effect of debranching on the rheological characteristics of Ca^{2+} -pectin gels.

The intrinsic viscosity ($[\eta]$) of carrot WSP and DEP is estimated (see section 2.5.1) to be 0.22 and 0.21 L/g, respectively. All pectin solutions show rather low values of coil overlap parameter (2.15 ± 0.17 , 1.19 ± 0.1 , 1.47 ± 0.07 , 1.07 ± 0.05 , for WSP, WSP_{DBr}, DEP, DEP_{DBr} respectively). Polymer solutions with $C \cdot [\eta]$ in the range from 1 to 10 are known to be in the semi-dilute regime (Macosko, 1994; Teraoka, 2002). This implies that, despite the variations in polymer concentration, all pectin solutions used for gel preparation are semi-dilute. Depending on the polymer MM, semi-dilute solutions consist mainly of either overlapping chains or of fully entangled chains (Macosko, 1994). For pectin polymers with MM of approximately 100 kDa, semi-dilute solutions reveal chains which are predominantly entangled from $C \cdot [\eta] \approx 4$ onwards (Hwang & Kokini, 1992). In our study, all pectin (WSP, DEP, WSP_{DBr}, DEP_{DBr}) solutions display $C \cdot [\eta] < 4$. This suggests that in the pectin solutions used for gel preparation, polymer chain overlapping dominates over chain entanglements.

3.3.2 Effect of de-branching on the rheological characteristics of Ca^{2+} -pectin gels

Upon Ca^{2+} addition to the pectin solutions, the development of various networks

was assessed as a function of time. Subsequently, the mechanical spectra and $\tan\delta$ (ratio of G'' to G') of the formed gels enabled to evaluate the gel type (“weak” vs. “true” gels) and gel elastic character, respectively. Furthermore, from the gel mechanical spectra, the gel strength (G' at 1 rad/s) of Ca^{2+} -pectin gels was determined. In view of establishing qualitative structure-function relations of pectin, the structural characteristics of the different pectin samples, as established in section 3.2, were used to elucidate the rheological properties of the Ca^{2+} -pectin gels.

3.3.2.1 Effect of debranching on the rheological characteristics of Ca^{2+} -high-methylesterified pectin gels

Fig. 3 displays the structure development of the gels prepared from non-debranched and debranched high-methylesterified pectins, at low and high Ca^{2+} concentrations. At a given Ca^{2+} concentration and regardless of the polymer DBr, the gels of high-methylesterified pectin show rather similar development profiles with time. This is possibly an indication that these gel networks are governed by the same type of interactions. At low Ca^{2+} concentration ($R=0.5$), gels prepared from WSP and WSP_{DBr} solutions show similar strength. Whereas at a higher Ca^{2+} concentration ($R=2.0$), Ca^{2+} -WSP gel display a higher strength than Ca^{2+} - WSP_{DBr} gel ((Fig. 4). More specifically, the difference in the gel strength between these samples is approximately 25%. Using solutions of the same concentration, Schmelter et al. (2002) reported about 98% decrease in G' for Ca^{2+} gels produced from arabinanase treated high-methylesterified (DM~80%) citrus pectin. The discrepancies between both findings might result, among others, from the different method (penetration test) or ratio of Ca^{2+} to polymer

concentrations (R-value) used by Schmelter and his co-workers.

In the present study, although both WSP and WSP_{DBr} show comparable DM values and likely have similar pattern of methylester distribution (since no further de-esterification occurred during debranching), the latter pectin sample display less arabinose (Fig. 2) and a slightly lower DBr value (Table 1). This suggests that the lower G' value observed for Ca²⁺-WSP_{DBr} gel (at higher Ca²⁺ concentration) can be related to the reduced length of the arabinose-containing sides chains of WSP_{DBr}.

Irrespective of the polymer DBr and Ca²⁺ concentration, the mechanical spectra of all gels display a large dependence of the dynamic moduli on frequency (data not shown), pointing out network relaxation processes and thereby implying “weak” gel behaviour (Rao, 1999; Mezger, 2006). Furthermore, all gels show a limited elastic character ($\tan\delta \geq 0.3$), indicating a loss of a high percentage of energy in viscous dissipation, as a result of molecular mobility. Pectins of high DM are devoid of large NMGalA blocks (Ngouémazong et al., 2010), which are a prerequisite for the formation of stable Ca²⁺-NMGalA junction zones (Powell et al., 1982; Fraeye et al., 2010). Therefore, irrespective of DBr, the aforementioned weak gel behaviour is mostly related to the inability of pectin polymers of very high DM to form stable junction zones. Moreover, the presence of polymer chain entanglements is also an important prerequisite in the formation of Ca²⁺-pectin gels (MacDougall et al., 1996). Since the polymer chains of WSP and WSP_{DBr} solutions are expected to have limited entanglements (mainly overlapping chains), the generation of Ca²⁺ gel networks from these solutions is further limited. Consequently, in Ca²⁺-high-methylesterified pectin gels, any effect of pectin debranching might either be poorly expressed or very limited.

3.3.2.2 Effect of de-branching on the rheological characteristics of Ca^{2+} -low-methylesterified pectin gels

Fig. 5 portrays the structure development of the gels prepared from non-debranched and debranched low-methylesterified pectins, at low and high Ca^{2+} concentrations. Generally, irrespective of pectin DBr and Ca^{2+} concentration, all gels show a similar development profile, characterised by a relatively constant gel strength as a function of time. The rather instantaneous development of these networks is an indication of the rapid gel formation through cooperative binding of the Ca^{2+} to the NMGalA blocks of pectin chains. However, irrespective of the Ca^{2+} concentration, DEP gels display similar strength, indicating an unavailability of unbound NMGalA and thus a saturation of gel with Ca^{2+} . This fast Ca^{2+} saturation of the gels is probably caused by the low polymer concentration of the pectin solutions.

Regardless of the Ca^{2+} concentration, gels produced from debranched pectin samples show more than 85% reduction in G' as compared to those prepared from non-debranched polymers (Fig. 6). This observation indicates that a 50% decrease in the arabinose content (as a result of the depolymerisation of mainly AGI and arabinan) yields polymers whose Ca^{2+} gels display drastically lower gel strength. Remarkably, this decrease is more pronounced than the one observed in Ca^{2+} -WSP_{DBr} gels. Low-methylesterified pectins produced through controlled plant PME de-esterification (as is the case for DEP) are characterised by the occurrence of large NMGalA blocks on many pectin chains (Ngouémazong et al., 2010). In the presence of sufficient Ca^{2+} , rapid cooperative binding of Ca^{2+} to the NMGalA blocks is stimulated throughout the polymer, thereby generating numerous energetically stable and strong junction zones

(Powell et al., 1982). As DEP_{DBr} was produced from the partial debranching of DEP using a non-PME contaminated enzyme preparation, both samples display a comparable DM (Table 1) and likely reveal similar size and distribution of NMGalA blocks throughout the polymer. This suggests that both DEP and DEP_{DBr} could stimulate cooperative binding of Ca²⁺ to a similar extent. Since the same R-values were used for the preparation of Ca²⁺-DEP and Ca²⁺-DEP_{DBr} gels containing an equivalent GalA content, the extremely low G' values observed in Ca²⁺-DEP_{DBr} gels can be ascribed to the extremely reduced Ca²⁺ dimerisation of pectin chains which results from the very low extent of polymer chain entanglements in the semi-dilute DEP_{DBr} solutions. Based on experimental results, it has indeed been postulated that in debranched polymer (such as pectin) solutions, the decrease of the length of the branches to a size shorter than a certain critical dimension hinders side chain entanglements, thereby reducing the overall entanglement of the polymer (Graessley, 1977; Schmelter et al., 2002). In carrot pectin, AGI and arabinan, which represent the side chain population with higher MM (between 80 and 300 kDa) compared to the AGII polymeric population (between 30 and 80 kDa) (Verhoef et al., 2009), are depolymerised during debranching with a mixture of ARASE and AFASE. This can result in a drastic reduction of the length of side chains, possibly below the critical dimension required for side chains entanglements. The reduction or lack of side chain entanglements might lead to a net decrease in entanglements in semi-dilute DEP_{DBr} solutions. This further implies that in Ca²⁺-DEP gels, the high G' values can be associated with the enhanced ability of the DEP polymer and solution (semi-dilute) to form Ca²⁺ gels, through the combined effect of the presence of numerous large NMGalA blocks on the polymer and a solution of

relatively entangled polymer chains. Therefore, a part from NMGalA blocks, the arabinose-containing RGI side chains play an important role in defining the gel strength of Ca^{2+} -pectin gels.

It can be noted that the difference in coil overlap parameter ($C[\eta]$) of DEP and DEP_{DBr} solutions (1.47 and 1.07 respectively), might suggest that the extent of polymer chain entanglements resulting from the amount of polymer in individual solution is higher in DEP solutions. Hereto, DEP gels were prepared from solutions with pectin concentration similar to that of DEP_{DBr} solution (0.52% w/v, ie. the DEP solution has lower GalA content than the DEP_{DBr} solution). Interestingly, despite the lower GalA content of these DEP gels, they still show higher G' values compared to those of DEP_{DBr} gels (Fig. 7). This is an indication that the lower strength of Ca^{2+} - DEP_{DBr} gels can not be attributed to the lower pectin content of DEP_{DBr} solutions.

Furthermore, the reduced aptitude of DEP_{DBr} solutions to form Ca^{2+} gels is confirmed by the very “weak” nature and poor elastic character of Ca^{2+} - DEP_{DBr} gels (judged by a high dependence of dynamic moduli on frequency and $\tan\delta$ of approximately 0.3, respectively), compared to the “improved” gel nature (particularly at high R-value) and good elastic character ($\tan\delta \sim 0.1$) of Ca^{2+} -DEP gels (Fig.8). Interestingly, at a slightly lower polymer concentration (0.52% as the concentration of DEP_{DBr}) and irrespective of Ca^{2+} concentration, the frequency dependence of the moduli and the elastic character of DEP gels remained rather unchanged (Fig. 9). This is an indication that the poor elastic behaviour of Ca^{2+} - DEP_{DBr} gels can not be attributed to the lower pectin concentration of DEP_{DBr} solutions. Therefore, it can be suggested that the very “weak” nature and the low elastic character of Ca^{2+} - DEP_{DBr} gels is mostly

attributed to the decrease in the length of RGI side chains of the DEP_{DBr} polymer. This may imply that the proposed side chain entanglements of the DEP solution contribute to the stability of Ca²⁺-pectin gel networks, and by this means promote the elastic character of the networks. These entanglements might also limit network chain mobility and improve the “true” gel behaviour of Ca²⁺-pectin gels.

3.5 Conclusion

The enzymatic modification of carrot pectins of low and high DM using a mixture of endo-arabinanase and α -L-arabinofuranosidase yielded partially debranched polymers with approximately 50% decrease in arabinose content and a slight decrease in degree of branching (DBr). In the presence of Ca²⁺, gels were formed from semi-dilute solutions of non-debranched and debranched pectins. Generally, the rheological characteristics of these gels varied with pectin structural features, particularly DBr. The net effects of pectin debranching on the rheological characteristics of Ca²⁺-pectin gels include lower gel strength as well as poor gel elastic character and “weaker” nature. This reduced gel elastic behaviour is mostly attributed to a reduction in polymer chain entanglements, emanating mainly from the proposed reduction in side chain entanglements in debranched pectin semi-dilute solutions.

Although the influence of partial depolymerisation of arabinose-containing RGI side chains on the rheological characteristics of pectin gels has been elaborated, the impact of the highly ramified galactose-containing AGII side chains on gel characteristics is still unknown. The experimental protocol and the results obtained from

590 this work can form the basis for further studies on the rheological characteristics of
591 Ca^{2+} -debranched pectin gels.

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References

- Berth**, G.; Anger, H.; Linow, F. (1977). Light scattering and viscometric studies for molecular weight determination of pectins in aqueous solutions. *De Nahrung*, 21, 936-946.
- Blumenkrantz**, N.; Asboe-Hansen, G. (1973). New methods for the determination of uronic acids. *Analytical Biochemistry*, 54 (2), 484-489.
- Boeriu**, C.; Stolle-Smits, T.; Van Dijk, C. (1998). Characterisation of cell wall pectins by Near Infrared Spectroscopy. *Journal of Near Infrared Spectroscopy*, 6, A299-A303.
- Budolfsen**, G.; Heldt-Hansen, H.P. (1997). Process for the improvement of gel formation or viscosity increase. *United States Patent* (Patent number: WO 97/10726).
- Cardoso**, S.M., Coimbra, M.A., Lopes Da Silva, J.A. (2003). Calcium-mediated gelation of an olive pomace pectic extract. *Carbohydrates Polymers*, 53, 125-133.
- Capel**, F.; Nicolai, T.; Durand, D.; Boulenguer, P. ; Langendorff, V. (2006). Calcium and acid induced gelation of (amidated) low methoxyl pectin. *Food Hydrocolloids*, 20, 901-907.
- De Roeck**, A.; Sila, D.; Duvetter, T.; Van Loey, A. ; Hendrickx, M. (2008). Effect of high pressure/high temperature processing on cell wall pectic substances in relation to firmness of carrot tissue. *Food Chemistry*, 107, 1225-1235.
- De Vries**, R.P.; Visser, J. (2003). Enzymes releasing L-arabinose and D-galactose from the side chains of pectin. In J.R. Whitaker, A.G.J. Voragen, D.W.S. Wong (Eds.). *Handbook of food enzymology* (pp 867-877). NY: Marcel Dekker.
- Doungla**, E.; Vandebril, S.; Duvetter, T.; Van Loey, A.; Moldenaers, P.; Hendrickx, M. (2009). Quick and reliable method of preparation of strong homogeneous calcium-pectin

616 gels. Proceedings of the 5th International Symposium on Food Rheology and Structure,
617 734-735.

618 **Fraeye**, I.; Duvetter, T.; Dounghla, E.; Van Loey, A.; Hendrickx, M. (2010). Fine-tuning
619 the properties of pectin-calcium gels by control of pectin fine structure, gel composition
620 and environmental conditions. *Trends in Food Science and Technology*, 21, 219-228.

621 **Graessley**, W.W. (1977). Effect of long branches on the flow properties of polymers.
622 *Accounts of Chemical Research*, 10, 332-339.

623 **Gross**, K. C. (1982). A rapid and sensitive spectrophotometric method for assaying
624 polygalacturonase using 2-cyanoacetamide. *HortScience*, 17(6), 933-934.

625 **Heukeshoven**, J.; Dernick, R. (1985). Simplified method for silver staining of proteins in
626 polyacrylamide gels and the mechanism of silver staining. *Electrophoresis*, 6, 103-112.

627 **Hwang**, J.; Kokini, J.L. (1992). Contribution of the side branches to the rheological
628 properties of pectins. *Carbohydrate Polymers*, 19 (1), 41-50.

629 **Hwang**, J.; Pyun, Y.R., Kokini, J.L. (1993). Side chains of pectins: some thoughts on
630 their role in plant cell walls and foods. *Food Hydrocolloids*, 7 (1), 39-53.

631 **Jolie**, R.; Duvetter, T.; Houben, K.; Clynen, E.; Sila, D.; Van Loey, A.; Hendrickx, M.
632 (2009). Carrot pectin methylesterase and its inhibitor from kiwi fruit: study of activity,
633 stability and inhibition. *Innovative Food Science and Emerging Technologies*, 10 (4),
634 601-609.

635 **Kaaber** L.; Kaack, K.; Kriznik, T.; Bråthen, E.; Knutsen, H. (2007). Structure of pectin in
636 relation to abnormal hardness after cooking in pre-peeled, cool-stored potatoes. *LWT-*
637 *Food Science and Technology*, 40 (5), 921-929.

638 **Klavons**, J.; Bennett, R. J. (1986). Determination of methanol using alcohol oxidase
639 and its application to methyl ester content of pectins. *Journal of Agriculture and Food*
640 *Chemistry*, 34(4), 597-599.

641 **Luzio**, G.A.; Cameron, R.G. (2008). Demethylation of a model homogalacturonan with
642 the salt-independent pectin methylesterase from citrus: Part II. Structure-function
643 analysis. *Carbohydrate Polymers*, 71(2), 300-309.

644 **Ly-Nguyen**, B.; Van Loey, A.; Fachin, D.; Verlent, I.; Indrawati, O.; Hendrickx, M. J.
645 (2002). Partial purification, characterisation and thermal and high-pressure inactivation
646 of pectin methylesterase from carrots (*Daucus carota* L.). *Journal of Agriculture and*
647 *Food Chemistry*, 50 (19), 5437-5444.

648 **MacDougall**, A.J.; Needs, P.W.; Rigby, N.M.; Ring, S.G. (1996). Calcium gelation of
649 pectic polysaccharides isolated from unripe tomato fruit. *Carbohydrate Research*,
650 923(2), 235-249.

651 **Macosko**, C.W. (1994). Rheology: Principles, measurements and applications (pp482-
652 486). Wiley-VCH: NY.

653 **Matthew**, J.A.; Howson, S.J.; Keenan, M.H.J.; Belton, P.S. (1990). Improvement of the
654 gelation of sugar beet pectin following treatment with an enzyme preparation derived
655 from *Aspergillus niger* - comparison with a chemical modification. *Carbohydrate*
656 *Polymers*, 12, 295-306.

657 **McFeeters**, R.F.; Armstrong, S.A. (1984). Measurement of pectin methylation in plant
658 cell walls. *Analytical Biochemistry*, 139 (1), 212-217.

659 **Mezger**, T. (2006). The rheology handbook: for users of rotational and oscillatory
660 rheometers (2nd ed). Hannover: Caotings Compedia.

661 **Ngouémazong**, D.E; Tengweh, F. F.; Duvetter, T.; Fraeye, I.; Van Loey, A.;
662 Moldenaers, P.; Hendrickx, M. (2010). Quantifying structural characteristics of partially
663 de-esterified pectins. *Food Hydrocolloids*, in Press, doi: 10.1016/j.foodhyd.2010.07.015.

664 **Oosterveld**, A.; Beldman, G.; Searle-van Leeuwen, M.J.F.; Voragen, A.G.J. (2000).
665 Effect of enzymatic deacetylation on gelation of sugar beet pectin in the presence of
666 calcium. *Carbohydrate Polymers*, 43, 249-256.

667 **Powell**, D. A.; Morris, E. R.; Gidley, M. J.; Rees, D. A. (1982). Conformations and
668 interactions of pectins: II. Influence of residue sequence on chain association in calcium
669 pectate gels. *Journal of Molecular Biology*, 155 (4), 517-531.

670 **Rao**, M.A. (1999). Measurement of flow and viscoelastic properties. In M.A. Rao (Ed),
671 *Rheology of fluids and semi-solid foods* (pp 25-139). New-York: An Aspen Publication.

672 **Ridley**, B.L.; O'Neil, M.A.; Mohnen, D. (2001). Pectins: structure, biosynthesis and
673 oligogalacturonide-related signalling. *Phytochemistry*, 57, 929-967.

674 **Schmelter**, T.; Wientjes, R.; Vreeker, R.; Klaffke, W. (2002). Enzymatic modifications of
675 pectins and impact on their rheological properties. *Carbohydrate Polymers*, 47, 99-108.

676 **Schols**, H.A.; Voragen, A.G.J. (1994). Occurrence of pectic hairy regions in various
677 plant cell wall materials and their degradability by rhamnogalacturonase. *Carbohydrate*
678 *Research*, 256 (1), 83-95.

679 **Schols**, H.A.; Voragen, A.G.J. (2003). Pectic polysaccharides. In J.R. Whitaker, A.G.J.
680 Voragen, D.W.S. Wong (Eds). *Handbook of food enzymology* (pp 829-841). NY: Marcel
681 Dekker.

Sila, D.; Dounsla N.; Smout, C.; VanLoey, A.; Hendrickx, M. (2006). Pectin fractions interconversions: Insight into understanding texture evolution of thermally processed carrots. *Journal of Agricultural and Food Chemistry*, 54 (22), 8471-8479.

Teroaka, I. (2002). Polymer solutions – An introduction to physical properties (pp.64-65). John Wiley and Sons: NY.

Verhoef, R.; Knox, J.P.; Voragen, A.G.J.; Schols, H.A. (2009). Fingerprinting complex pectins by chromatographic separation combined with ELISA detection. *Carbohydrate Research*, 344, 1808-1818.

Voragen, A.G.J.; Coenen, G.; Verhoef, R.P.; Schols, H.A. (2009). Pectin, a versatile polysaccharide present in plant cell walls. *Structural chemistry*, 20 (2), 263-275.

Waldron, K., Parker, M.; Smith, A. (2003). Plant cell walls and food quality. *Comprehensive review, Food Science and Food Safety*, 2, 101-119.

Willats, W. G. T.; Knox, J. P.; Mikkelsen, J. D. (2006). Pectin: New insights into an old polymer starting to gel. *Trends in Food Science and Technology*, 17 (3), 97-104.

Table

Table 1: Overview of structural characteristics of carrot WSP and modified carrot WSP

Sample Code	Pectin characteristics			
	<i>GalA (%)</i>	<i>Total composition^b (%)</i>	<i>DM (%)</i>	<i>DBr^c</i>
WSP	21.22 ±1.2 ^a	96.11 ±0.5	79.52 ±1.0	13.91 ±0.3
WSP_{DBr}	38.12 ±2.3	94.39 ±0.04	82.21 ±2.1	12.47 ±0.5
DEP	28.49 ±0.2	93.09 ±3.4	26.5 ±0.5	16.76 ±3.9
DEP_{DBr}	39.17 ±3.3	93.34 ±1.52	28.84 ±1.8	14.22 ±2.4

a: standard deviation; b: Total composition = GalA (%) + MeOH (%) + total NS (%); c: DBr = ([galactose] + [arabinose]) / [rhamnose]

Figure captions

Figure 1:

Enzyme activities in commercial enzyme preparations of ARASE (■) and AFASE (◆). (a): ARASE and AFASE activities in terms of reducing end groups produced in arabinan solution, (b): endo-PG activity in terms of reducing end groups produced in poly-D-GalA solution, (c): PME activity in terms of volume of NaOH added to apple pectin solution in order to titrate produced carboxyl groups.

Figure 2:

Effect of pectin structural modifications on individual RGI NS (rhamnose, galactose and arabinose) content and total NS content (sum of amounts of rhamnose, galactose, arabinose, fucose, glucose and xylose).

Figure 3:

Structure development of Ca^{2+} gels produced from non-debranched high methylesterified carrot pectin (WSP (■)) and debranched high methylesterified carrot pectin (WSP_{DBr} (●)). (a) and (b) represent low Ca^{2+} ($R=0.5$) and high Ca^{2+} ($R=2.0$) concentrations respectively. Filled and open symbols correspond, to G' and G'' , respectively. Curves without error bars are obtained from single measurements. Data are taken at an angular frequency of 1 rad/s.

Figure 4:

Effect of partial debranching of high-methylesterified pectin on Ca^{2+} -pectin gel strength (in terms of G') at different Ca^{2+} concentration (in terms of R-value). All samples have the same GalA content.

Figure 5:

Structure development of Ca^{2+} gels produced from non-debranched low-methylesterified carrot pectin (DEP (♦)) and debranched low-methylesterified carrot pectin (DEP_{DBr} (▲)). (a) and (b) represent low Ca^{2+} (R=0.5) and high Ca^{2+} (R=2.0) concentrations, respectively. Filled and open symbols correspond, to G' and G'' , respectively. Curves without standard errors (G' and G'' of Ca^{2+} -WSP_{DBr} gel at R=0.5) are obtained from single measurements. Data are taken at 1 rad/s.

Fig. 6:

Effect of partial debranching of low-methylesterified pectin on Ca^{2+} -pectin gel strength (in terms of G') at different Ca^{2+} concentration (in terms of R-value). All samples have the same GalA content.

Fig. 7:

Comparing the strength of Ca^{2+} gels produced from a non-debranched low-methylesterified carrot pectin solution (DEP) and a debranched low-methylesterified carrot pectin solutions (DEP_{DBr}) at the same polymer concentration but different GalA content (lower GalA content (DEP solution), higher GalA content (DEP_{DBr} solution)).

Gels were produced at low and high Ca^{2+} concentrations ($R=0.5$ and $R=2.0$, respectively).

Fig. 8:

Mechanical spectra of Ca^{2+} gels produced from non-debranched low-methylesterified carrot pectin (DEP (◆)) and debranched low-methylesterified carrot pectin (DEP_{DBr} (▲)), at low and high Ca^{2+} concentrations ($R=0.5$ and $R=2.0$, respectively). Filled and open symbols correspond, to G' and G'' respectively. (a) and (b) represent low Ca^{2+} ($R=0.5$) and high Ca^{2+} ($R=2.0$) concentrations, respectively. Curves without standard errors are obtained from single measurements.

Fig. 9:

Comparing the mechanical spectra of Ca^{2+} gels produced from non-debranched low-methylesterified carrot pectin (DEP) solutions at different polymer concentration (0.72% (◆) and 0.53% (—)). Gels were produced at low and high Ca^{2+} concentrations ($R=0.5$ and $R=2.0$, respectively). Filled and open symbols correspond, to G' and G'' , respectively. (a) and (b) represent low Ca^{2+} ($R=0.5$) and high Ca^{2+} ($R=2.0$) concentrations, respectively.

779 **Figures**

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781 **Ngouémazong et al. Figure 1**
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