Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 107 (2008) 1225–1235
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of high pressure/high temperature processing on cell wall pectic substances in relation to firmness of carrot tissue

Ans De Roeck, Daniel N. Sila, Thomas Duvetter, Ann Van Loey *, Marc Hendrickx

Laboratory of Food Technology, Centre for Food and Microbial Technology, Department of Microbial and Molecular Systems, Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, B-3001 Leuven, Belgium

Received 13 July 2007; received in revised form 28 August 2007; accepted 26 September 2007

Abstract

Thermal processing for food preservation results in undesired softening of fruits and vegetables. To explore the potential of high pressure sterilization in food processing, the effects of combined high pressure/high temperature (HP/HT) treatments on carrot pectic polysaccharides and the related textural properties were investigated and compared with that of samples thermally processed at atmospheric pressure. Disks of fresh carrot (*Daucus carota* var. Yukon) tissue were subjected to three different treatments (80 °C–0.1 MPa, 100 °C–0.1 MPa and 80 °C–600 MPa) for varying time intervals. Subsequently, the residual texture and microstructural changes of the carrots were evaluated. Alcohol-insoluble residues were prepared from the samples and sequentially fractionated with water, cyclohexane-trans-1,2-diamine tetra-acetic acid (CDTA) and Na_2CO_3 solutions. Thermal treatments at 0.1 MPa caused extensive tissue softening. This was marked by increased cell separation, an increase in water soluble pectin (WSP) paralleled by a decrease in chelator (CSP) and sodium carbonate (NSP) soluble pectin. HP/HT treated carrots showed minimal softening and negligible changes in intercellular adhesion. This was accompanied by a significant reduction in the degree of methyl esterification of pectin, low WSP in contrast to the high CSP and NSP fractions, minor changes in the different pectin fractions during treatment, and a substantial amount of pectin in the fractionation residue. There was a clear difference between HP/HT and thermally processed carrot pectin; HP/HT showing pronounced texture preservation.

Keywords: Carrot; Pectin; Texture; Beta-elimination; High pressure sterilization

1. Introduction

The texture of fresh, stored and processed fruits and vegetables is largely determined by the cell wall and middle lamella polysaccharides (Van Buren, 1979). Cell wall polysaccharides consist of pectin, hemicellulose and cellulose, while the middle lamella consists predominantly of pectic polysaccharides cross-linked with Ca^{2+} ions. Generally, pectin consists of a backbone, in which "smooth" (1→4)α-D-galacturonan regions, with varying degrees of methyl esterification, are interrupted by ramified rhamnogalacturonan regions substituted by side chains rich in neutral sugars, for example arabinose and galactose (Ridley, O'Neill, & Mohnen, 2001).

Specific biochemical and/or chemical transformations occur in pectin during processing, leading to texture changes of the resulting products. In vegetables and fruits, thermal processing causes a pronounced degradation of the pectic polysaccharides resulting in reduced intercellular adhesion and consequently in increased softening. At pH $\geq$ 4.5, softening is consistent with a depolymerization reaction that has the characteristics of a beta-elimination reaction catalyzed by hydroxyl ions and inhibited by demethoxylation of pectin (Sila, Smout, Elliot, Van Loey, & Hendrickx, 2006b). In most cases, a too extensive softening of fruits and vegetables is not desired by the consumer. Therefore, and to minimize other organoleptic and nutritional quality losses associated with thermal processing,

* Corresponding author. Tel.: +32 16 321567; fax: +32 16 321960.
E-mail address: Ann.Vanloey@biw.kuleuven.be (A. Van Loey).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.09.076

food scientists and the food industry are continuously searching for novel, less degradative technologies (Mertens & Knorr, 1992). One technology that has shown such potential is high pressure processing (Ludikhuyze, Van Loey, Indrawati, & Hendrickx, 2001). Currently, high pressure processing is successfully applied on a commercial scale for the pasteurization of a whole range of food products. Given the technical progress made in high pressure equipment, commercial high pressure sterilization is feasible in the near future. This process would involve both pressure and temperature contributing to sterilization by inactivating spores and enzymes (Meyer, Cooper, Knorr, & Lelieveld, 2000). Like in conventional thermal processing, the result is a shelf stable product, however a higher quality product is generally obtained (Matser, Krebbers, van den Berg, & Bartels, 2004). At present, the influence of combined high pressure/high temperature treatments on the texture of fruits and vegetables, and more in particular, on the molecular pectin changes which underpin the change in textural quality has not been studied in detail.

In this study, the influence of combined high pressure/high temperature treatments on the texture of carrots and the related cell wall chemistry was compared with that of high temperature treated samples. A detailed picture was obtained by abstracting information at the macro-, microscopic and molecular levels.

2. Materials and methods

Fig. 1 gives a schematic overview of the experimental setup.

2.1. Plant material

Carrots (*Daucus carota* var. Yukon) were obtained from a local shop in Belgium and stored at 4 °C for a maximum period of one week before use. Disks (10 mm height and 12 mm diameter) were excised from the core of the carrots before processing and analysis.

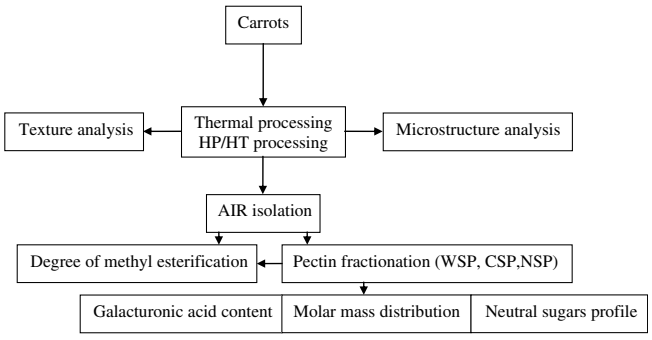

Fig. 1. Schematic overview of the experimental setup (HP/HT: high pressure/high temperature; AIR: alcohol-insoluble residue; WSP: water soluble pectin; CSP: chelator soluble pectin; NSP: sodium carbonate soluble pectin).

2.2. Thermal treatments

Carrot disks (10) encapsulated in stainless steel tubes (110 mm long, 13 mm internal diameter, and 1 mm thickness) filled with demineralized water as a brine (2.9 ml water/10 carrot disks) were subjected to thermal treatments (80 °C and 100 °C) in a temperature controlled oil bath. After a lag time of 5 min (experimentally determined by Smout, Sila, Vu, Van Loey, and Hendrickx (2005)) a first sample (time zero sample) was withdrawn. The rest of the samples were removed from the oil bath after preset time intervals. The samples were immediately cooled in an ice bath and the residual texture was determined. The brine solution was collected, frozen under liquid N_2 and stored at −40 °C for further analysis. Each sample consisted of three tubes and thus 30 carrot disks. 26 disks were used for texture measurement, the other four for microstructure analysis.

2.3. High pressure/high temperature treatments

Ten carrot disks were submerged in 2.9 ml of demineralized water in a double-film polyethylene bag. Isothermal–isobaric treatments were carried out in a laboratory scale multi-vessel high pressure equipment (Resato, Roden, The Netherlands). The temperature in the insulated vessels was first equilibrated at 80 °C using an external cryostat. Once the desired temperature was obtained, samples were put into the vessels and the vessels were closed within 2 min. Pressure was built up slowly (till 600 MPa) using a standard pressurization rate of 100 MPa/min to minimize the temperature rise due to adiabatic heating. After pressure build up, an equilibration period of 5 min was taken into account, to allow the samples to reach the desired temperature (Weemaes et al., 1997). After the equilibration time, the first sample was withdrawn (time zero sample). The other samples were removed after preset time intervals. The samples were immediately cooled in an ice bath. Subsequently, the residual texture was determined. The brine solution was frozen under liquid N_2 and stored at −40 °C for further analysis. Each sample consisted of three bags and thus 30 carrot disks.

2.4. Texture measurement

Texture is a multi-parameter attribute (Szczesniak, 2002). The parameter considered in this study is hardness. The carrot tissue hardness was evaluated by a compression test using a TA-XT2i Texture Analyzer (Stable Micro Systems, Surrey, UK). The following parameters were used: load cell = 25 kg, probe = 25 mm diameter aluminium cylinder, and test speed = 1 mm/s. The hardness of a carrot cylinder was defined as the maximum force needed to compress the carrot cylinder to 70% of its original thickness. The mean value of the compression forces of 26 carrot cylinders was considered as a single data point. After texture

measurement, the carrot cylinders were frozen under liquid N_2 and stored at −40 °C for further analysis.

2.5. Microstructure analysis

Treated carrot disks were cut axially in two pieces using a TA-XT2i Texture Analyzer equipped with a sharp knife blade which penetrated the disks at a constant speed of 1 mm/s. Subsequently, the pieces obtained were cut transversely in slices of 2–3 mm thick. These slices were fixed using glutaraldehyde followed by repeated washing and dehydration in a series of ethanol solutions (50%, 70% and 95%). The dehydrated carrot slices were infiltrated with a Historesin Embedding Kit (Leica, Bensheim, Germany) for five days, polymerized and cut into thin sections (5 μm) with a Microm HM355 microtome (Microm Laborgeräte GmbH, Walldorf, Germany). Sections were stained with 0.1% Toluidin Blue for 5 min followed by washing with water. Stained slices were examined with an Olympus BX-41 microscope (Olympus, Optical Co. Ltd, Tokyo, Japan). Micrographs were taken using image analysis software (analySIS pro 3.1 and analySIS 5, Soft Imaging System GmbH, Bensheim, Germany). To determine tissue failure characteristics, 10 micrographs (magnification × 40) were taken in series from the cut surface. Nine cross-sectional micrographs were also taken to examine the changes in cell wall area.

2.6. Preparation of alcohol-insoluble residue (AIR)

Cell wall material was isolated as AIR as described by McFeeters and Armstrong (1984). 30 carrot cylinders were completely homogenized in 192 ml of 95% ethanol using a mixer (Buchi mixer B-400, Flawil, Switzerland). The suspension was filtered (Machery-Nagel MN 615 Ø 90 mm) and the residue was re-homogenized in 96 ml of 95% ethanol and filtered again. The residue was homogenized in 96 ml of acetone before final filtration followed by drying overnight at 40 °C. The AIR was ground using a mortar and pestle and stored in a desiccator until analysis.

2.7. Determination of degree of methyl esterification (DM)

The DM of the pectin was estimated by taking the ratio of moles of methoxyl groups to the moles of anhydrous galacturonic acid in the AIR. To determine the latter, pectin was hydrolyzed with concentrated sulfuric acid according to the method of Ahmed and Labavitch (1977). Subsequently, the galacturonic acid content was determined colorimetrically as described by Blumenkrantz and Asboe-Hansen (1973). The methoxyl content was estimated by hydrolyzing the ester bonds of the pectin with NaOH (Ng & Waldron, 1997) and colorimetrical quantification of the released methanol as described by Klavons and Bennett (1986). In both procedures, the respective hydrolyses were performed in duplicate, and three colorimetrical analyses were carried out for each hydrolysate.

2.8. Pectin methylesterase (PME) extraction and activity assay

Thirty carrot cylinders were homogenized and mixed in 0.2 M Tris–HCl buffer containing 1 M NaCl (pH 8.0, 1:1.3 w/v) for 2 h at 4 °C to extract cell wall bound PME. The crude PME extract was obtained by filtration using a cheese cloth.

PME activity was determined by measuring the release of acid per time unit at pH 7.0 and 22 °C. The reaction mixture consisted of 2 ml crude extract and 30 ml of a 0.35% (w/v) apple pectin solution (DM 70–75%, Fluka) containing 0.117 M NaCl. During pectin hydrolysis, the pH was maintained at pH 7.0 (set-pH) by addition of 0.01 N NaOH using an automatic pH-stat titrator (Metrohm, Switzerland). One unit (U) of PME activity is defined as the amount of enzyme required to release 1 μmol of acid per minute, under the aforementioned assay conditions.

2.9. Fractionation of AIR

AIR was fractionated into various cell wall fractions. The AIR (0.25 g) was incubated, while stirring, in 45 ml of demineralized water for 5 min at 100 °C (Sila et al., 2006b). The suspension was cooled under running tap water, filtered using a filter paper (MN 615 Ø 90mm) and the filtrate (water soluble pectin, WSP) was collected. The volume was adjusted to 50 ml. The residue was re-suspended in 45 ml of 0.05 M cyclohexane-trans-1,2-diamine tetra-acetic acid (CDTA) in 0.1 M potassium acetate pH 6.5 for 6 h at 28 °C (Chin, Ali, & Lazan, 1999). Upon filtration, the volume of the filtrate was adjusted to 50 ml (chelator soluble pectin, CSP). The residue was re-incubated in 45 ml of 0.05 M Na_2CO_3 containing 0.02 M $NaBH_4$ for 16 h at 4 °C, and then for another 6 h at 28 °C (Chin et al., 1999). The mixture was filtered and the volume of the filtrate was adjusted to 50 ml (sodium carbonate soluble pectin, NSP). The fractionation was performed in duplicate.

All the pectin fractions (WSP, CSP, and NSP) were analyzed for galacturonic acid content prior to lyophilization, followed by determination of molar mass distribution patterns and neutral sugars contents. Lyophilization was done using a freeze-dryer (Christ alpha 2–4, Osterode, Germany), and the dry powder was kept in a desiccator until subsequent analysis. The degree of methyl esterification of the different lyophilized pectin fractions was determined as described previously.

2.10. High performance size exclusion chromatography (HPSEC)

Changes in molar mass distribution of carrot pectin during processing were studied using size exclusion chromatography. This was performed using an Äkta Purifier (Amersham Biosciences, Uppsala, Zweden) equipped with a mixed bed column of Bio-Gel TSK (dimensions = 300 mm L × 7.5 mm Ø, pore size = 100–1000 Å, particle size

= 13 μm, theoretical plates/column ⩾7000, pH range = 2–12, maximum pressure = 300 psi; Bio-Rad Labs, Richmond, CA, USA) in combination with a TSK guard column. A 20 μl injection loop was used. Elution was executed at 35 °C with 0.05 M $NaNO_3$ at a flow rate of 0.7 ml/min for 25 min. The eluent was monitored using a Shodex R101 refractive index detector (Showa Denko, K.K., Tokyo, Japan). Deionized water (organic free, 18 MΩ resistance) supplied by a Simplicity™ Millipore water purification system (Millipore, Billerica, USA) was used to prepare eluents and samples. Monogalacturonic acid was used daily to validate the system.

The lyophilized WSP and NSP samples dissolved in demineralized water (WSP: 0.25% w/v, NSP: 1.5% w/v) were extensively dialyzed against demineralized water. The CSP (5% w/v) sample was extensively dialyzed against 0.1 M NaCl, followed by dialysis against demineralized water. Brine solutions were also dialyzed against demineralized water. Samples were adjusted to a concentration of 0.05 M $NaNO_3$ before analysis.

2.11. High performance anion exchange chromatography (HPAEC)

HPAEC was carried out to fingerprint the neutral sugars profiles of the different pectin fractions. This was achieved using a Dionex system (DX 600) equipped with a GS 50 gradient pump, a CarboPac PA1 column, and a pulsed amperometric detector (Dionex, Sunnyvale, USA). The detector was equipped with a reference pH electrode (Ag/AgCl) and a gold electrode. Potentials $E_1 = 0.1$ V, $E_2 = -2.0$ V, $E_3 = 0.6$ V, and $E_4 = -0.1$ V were applied for duration times $t_1 = 400$ ms, $t_2 + t_3 = 40$ ms, and $t_4 = 60$ ms. The sample (25 μl) was injected and eluted (1 ml/min) in a gradient with 100 mM NaOH (A) and 1 M NaOAc (B) in deionized water (18 MΩ) (C) as follows: 0→23 min, 15% A and 85% C; 23→35 min, linear gradient of 15→30% A, 0→30% B, 85→40% C; 35→45 min, linear gradient of 30→15% A, 30→0% B, 40→85% C. The column temperature was set at 30 °C. Commercial neutral sugar standards (L-rhamnose, L-arabinose, D-galactose, D-glucose, and D-xylose) at varying concentrations (1–5 ppm) were used for identification and quantification.

Sample preparation was as follows: 0.01 g of lyophilized pectin fraction was digested in 4 M trifluoroacetic acid (TFA) at 110 °C for 1.5 h. After cooling, the TFA was evaporated under vacuum at 40 °C for 40 min. The TFA-free samples were diluted with demineralized water before analysis.

3. Results and discussion

3.1. Influence of processing method on the hardness of carrots

Fig. 2 illustrates the hardness of processed carrots relative to the hardness of raw carrots for the three different

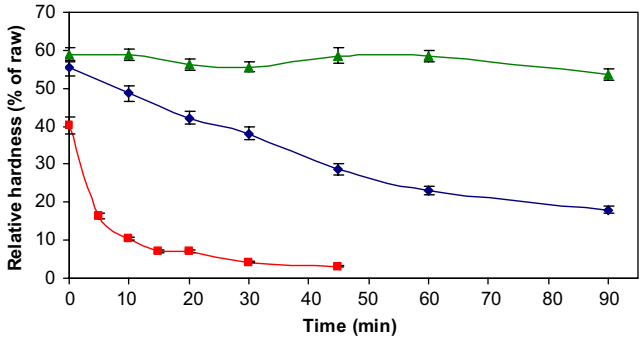

Fig. 2. Changes in carrot disks' hardness during processing: (♦) 80 °C–0.1 MPa, (■) 100 °C–0.1 MPa, and (▲) 80 °C–600 MPa. Hardness of treated carrots is expressed as a percentage of the hardness of raw carrots. Each point represents the mean value of 26 measurements (±standard error).

treatments. In all the cases, there was a rapid loss in the initial hardness due to membrane damage and turgor pressure loss (Basak & Ramaswamy, 1998; Greve, McArdle, Gohlke, & Labavitch, 1994). The hardness of the thermally treated carrots decreased further with increasing treatment time, probably due to beta-eliminative degradation of pectin (Greve et al., 1994; Sila et al., 2006b). In contrast, the high pressure/high temperature treated carrots did not undergo further softening, indicating that the beta-elimination reaction of pectin is inhibited at combined high pressure/high temperature treatments.

3.2. Influence of processing method on the microstructure of carrots

Raw carrot tissue showed well defined, conspicuously well stained cell walls (Fig. 3a). The intensity of the cell wall staining decreased progressively with increasing thermal impact accompanied by increased cell wall thickening (Fig. 3b and c). These observations are probably due to heat induced solubilization of the intercellular cementing pectin facilitating cell wall loosening (Waldron, 2004). In contrast, high pressure/high temperature treated tissue showed close resemblance to the raw tissue, indicating that pectin solubilization was limited (Fig. 3d).

To determine tissue failure characteristics, micrographs were taken from the cutting edge (results not shown). Tissue failure classically involves cell separation and/or cell breakage. If the forces holding the cells together are stronger than the cell walls, then failure will occur in the walls (cell breakage); if the forces holding the cells together are weaker than the cell walls, then cell separation will occur. Raw carrot tissue clearly showed cell breakage, indicating strong intercellular adhesion. In contrast, heat treated tissue showed cell separation. The results of the high pressure/high temperature treated tissues were intermediate, showing characteristics from both mechanisms.

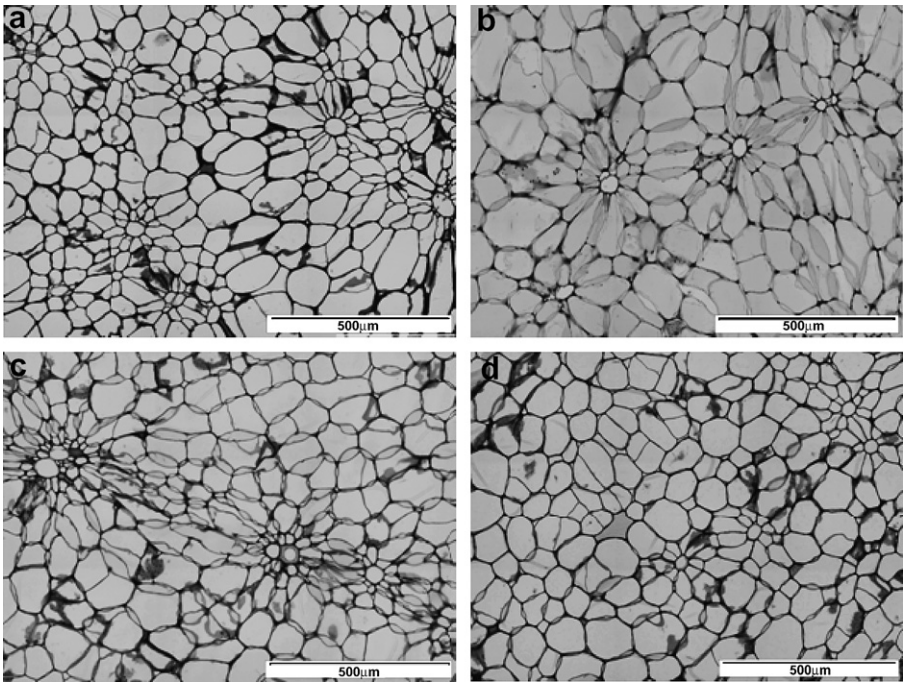

Fig. 3. Micrographs indicating changes in cell wall area: (a) raw carrots, (b) 100 °C–0.1 MPa–45 min, (c) 80 °C–0.1 MPa–90 min, and (d) 80 °C–600 MPa–90 min.

3.3. Influence of processing method on pectin structure

3.3.1. Degree of methyl esterification of pectin in AIR

The degree of methyl esterification (DM) of raw carrot pectin was estimated at 64%, which is in close agreement with the values reported for fresh carrots (DM = 60–69%) (Ng & Waldron, 1997; Sila, Doungla, Smout, Van Loey, & Hendrickx, 2006a; Siliha, Jahn, & Gierschner, 1996). For all the treatment conditions, the DM decreased. However, HP/HT treatment showed a more pronounced reduction (Table 1). The decline may be explained by the occurrence of chemical demethoxylation of pectin at high temperatures as reported by Sajjaanantakul, Van Buren, and Downing (1989). In case of the HP/HT treated samples, the high reduction may be explained by the combined effect of (a) chemical demethoxylation of pectin which is enhanced by pressurization (Verlent et al., 2004) and (b) enzymatic demethoxylation of pectin due to enhanced pectin methylesterase activity within the study domain. However, experiments on the stability and activity of the enzyme during HP/HT treatments are needed to verify this. Ly-Nguyen et al. (2003) investigated the stability of purified carrot PME under mild heat (⩽65 °C) and high pressure (⩽825 MPa). Carrot PME was rather thermolabile. Under isothermal–isobaric treatments, an antagonistic effect of temperature and pressure was observed at lower pressures (<300 MPa) and higher temperatures (>50 °C). Sila et al. (2007) noticed a pronounced stimulation of the catalytic activity of carrot PME in model systems as well as in food systems (shredded carrots) with increasing temperature (<55 °C) and pressure (⩽500 MPa). To verify whether the low DM of pectin of the pressure/temperature treated carrots could indeed be linked to PME activity at those conditions, carrots were checked for residual PME activity. Raw carrots showed a PME activity of around 2679 U/kg. After 10 min treatment at 80 °C/0.1 MPa, no residual PME activity could be detected. However, after 10 min at 80 °C/600 MPa, residual PME activity (~398 U/kg) was noticed, even after 60 min (~19 U/kg). This could be related to the stabilizing effect of pressure

Table 1
Pectin degree of methyl esterification of processed carrots

	Degree of methyl esterification (%)				
	0 min	5 min	20 min	45 min	90 min
80 °C–0.1 MPa	56.8 ± 2.1[a]	N.d.	52.0 ± 3.0	50.3 ± 2.3	52.7 ± 2.9
80 °C–600 MPa	42.6 ± 3.5	N.d.	37.4 ± 3.2	31.1 ± 2.0	34.2 ± 1.0
100 °C–0.1 MPa	57.7 ± 3.5	58.0 ± 2.5	55.0 ± 3.2	52.9 ± 1.9	N.d.
Raw	63.6 ± 5.3				

N.d.: Not determined.
[a] Standard deviation ($n = 6$).

on PME. Consequently, the reduction in DM of HP/HT samples could (partly) be due to PME activity. It was remarkable that after the equilibration time at 80 °C/600 MPa, the DM had already decreased from 64 to 43%. Sila et al. (2007) also noticed an extensive decrease in DM of pressure/temperature treated carrots during pressure build up and equilibration time (pressures up till 600 MPa in combination with temperatures up till 60 °C). It was suggested that the instantaneous release of PME from the cell wall and the enhanced contact with its substrate during pressurization were responsible for this phenomenon.

As methyl esters are one of the main driving forces of the beta-elimination reaction, it is likely that the high reduction in DM of the HP/HT treated carrots triggered inhibition of the beta-elimination reaction, consequently preventing softening.

3.3.2. Changes in pectin fractions during processing

Raw carrots contained predominantly water soluble pectin (~62%), a substantial amount of chelator soluble pectin (~26%), and a low amount of sodium carbonate soluble pectin (~12%). During processing, the pectin fraction ratios changed strikingly (Table 2). Thermally treated carrots were characterized by an increasing amount of water soluble pectin with increasing treatment time paralleled by a decreasing amount of chelator and sodium carbonate soluble pectin. These results are in accordance with the results found in literature (Ng & Waldron, 1997; Sila et al., 2006a; Sila et al., 2006b). This indicates that a substantial degradation and solubilization of pectin occurs during thermal processing. For the high pressure/high temperature treated carrots, at time zero, a low proportion of WSP (~15%) was noticed while the other fractions were proportionately higher (CSP ~ 45%, NSP ~ 40%). During HP/HT processing, the pectin fractions hardly changed, corresponding with the unaltered hardness of the carrots. The low amount of WSP and high amount of CSP of HP/HT treated carrots could be explained by increased ionic cross-linking as a result of the reduced DM. One possible explanation for the high amount of NSP could be that under HP/HT conditions particular (sodium carbonate soluble) ester bonds are formed. Another possible explanation could be that pectin with a very low DM, and consequently high degree of polymerization (because no or limited beta-elimination occurred), is very strongly ionically bound to the cell wall so that it could not be released with the used CDTA solution.

To evaluate the pectin fractionation, the amount of galacturonic acid found in AIR was compared to the amount of galacturonic acid found in WSP, CSP and NSP. The extraction yield of the pectin fractions for the carrots treated at 80 °C and 0.1 MPa for 20 min was 81% (Table 3). Interestingly, the extraction yields for the carrots treated at 80 °C and 600 MPa were clearly lower, ranging from 53% to 70%. To account for the difference, it is possible that there was loss of galacturonic acid or some pectin

Table 2
Changes in pectin fractions during processing

	Carrot pectin fraction (%)[a]											
	0 min			20 min			45 min			90 min		
	WSP	CSP	NSP	WSP	CSP	NSP	WSP	CSP	NSP	WSP	CSP	NSP
80 °C–0.1 MPa	50.6 ± 2.8[b]	36.7 ± 2.1	12.7 ± 0.6	56.5 ± 1.6	32.1 ± 2.3	11.4 ± 0.4	54.5 ± 3.0	32.8 ± 0.9	12.8 ± 0.5	57.1 ± 1.2	32.6 ± 0.8	10.3 ± 1.0
80 °C–600 MPa	14.7 ± 0.6	45.1 ± 1.3	40.1 ± 2.1	11.5 ± 0.6	45.9 ± 3.1	42.7 ± 1.4	12.2 ± 0.6	47.0 ± 1.2	40.8 ± 2.0	15.3*	45.6*	39.1*
100 °C–0.1 MPa	59.0 ± 1.8	29.5 ± 0.6	11.5 ± 0.5	67.1 ± 2.3	27.8 ± 0.7	5.1 ± 0.3	69.7 ± 2.2	26.7 ± 0.7	3.6 ± 0.1	N.d.	N.d.	N.d.
Raw	61.7 ± 2.6	26.3 ± 0.7	12.0 ± 0.4									

N.d.: Not determined.
[a] Galacturonic acid in fraction/galacturonic acid in (WSP + CSP + NSP)·100.
[b] Standard deviation ($n = 2$; *: $n = 1$).

Table 3
Galacturonic acid extraction yields in pectin fractions and fractionation residue

	AIR	WSP + CSP + NSP	Yield (%)[a]	Residue	Yield (%)[b]
80 °C–0.1 MPa–20 min	1.266[c]	1.027[c]	81	0.033[c]	84
80 °C–600 MPa–20 min	1.275	0.675	53	0.170	66
80 °C–600 MPa–45 min	1.358	0.946	70	0.207	85
80 °C–600 MPa–90 min	1.378	0.912	66	0.124	75

[a] Yield taking into account galacturonic acid found in WSP, CSP and NSP.
[b] Yield taking into account galacturonic acid found in WSP, CSP, NSP, and residue.
[c] mmol galacturonic acid/g AIR.

remained attached to the fractionation residue. Therefore, the galacturonic acid content of the fractionation residue was determined. From Table 3 it is obvious that a high amount of galacturonic acid remained associated to the residue of the high pressure/high temperature treated carrots, indicating that a part of the pectin is strongly bound to the other cell wall polymers, which cannot be solubilized with the procedure used. These differences in pectin solubility are a probable explanation for the differences in the textural characteristics of the samples.

Table 4
Degree of methyl esterification of water soluble pectin and chelator soluble pectin

	0 min	20 min	45 min	90 min
Degree of methyl esterification WSP (%)				
80 °C–0.1 MPa	77.8 ± 5.1[a]	70.3 ± 2.0	N.d.	69.0 ± 5.9
80 °C–600 MPa	53.7 ± 1.3	50.5 ± 4.2	50.0 ± 1.2	58.9 ± 1.4
100 °C–0.1 MPa	69.0 ± 3.7	63.4 ± 4.4	59.0 ± 2.2	N.d.
Raw	81.8 ± 2.4			
Degree of methyl esterification CSP (%)				
80 °C–0.1 MPa	37.7 ± 1.6	33.1 ± 2.7	34.2 ± 1.0	32.8 ± 0.8
80 °C-600 MPa	39.7 ± 1.0	32.1 ± 3.1	26.4 ± 0.8	36.6 ± 1.0
100 °C–0.1 MPa	30.7 ± 4.8	27.9 ± 1.0	21.5 ± 0.9	N.d.
Raw	36.3 ± 0.9			

N.d.: Not determined.
[a] Standard deviation ($n = 6$).

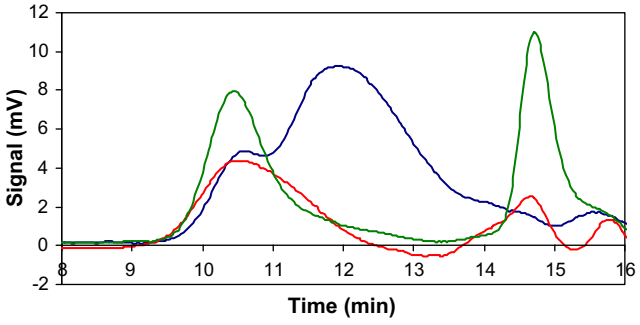

Fig. 4. Molar mass distribution of pectin in raw carrots: water soluble pectin (▬), chelator soluble pectin (▬), sodium carbonate soluble pectin (▬).

3.3.3. Degree of methyl esterification of WSP and CSP

In addition to changes in galacturonic acid concentrations, variations in the DM of the pectin fractions were observed (Table 4). WSP was highly methoxylated with a DM in the range 50–82%. In contrast, CSP was low methoxylated with a DM in the range 21–40%. The trend was similar to the one observed in the DM of the AIR: with increasing processing time generally the DM decreased. The DM of the NSP fraction could not be determined because the alkaline saponification procedure used in extraction renders it devoid of methyl esters.

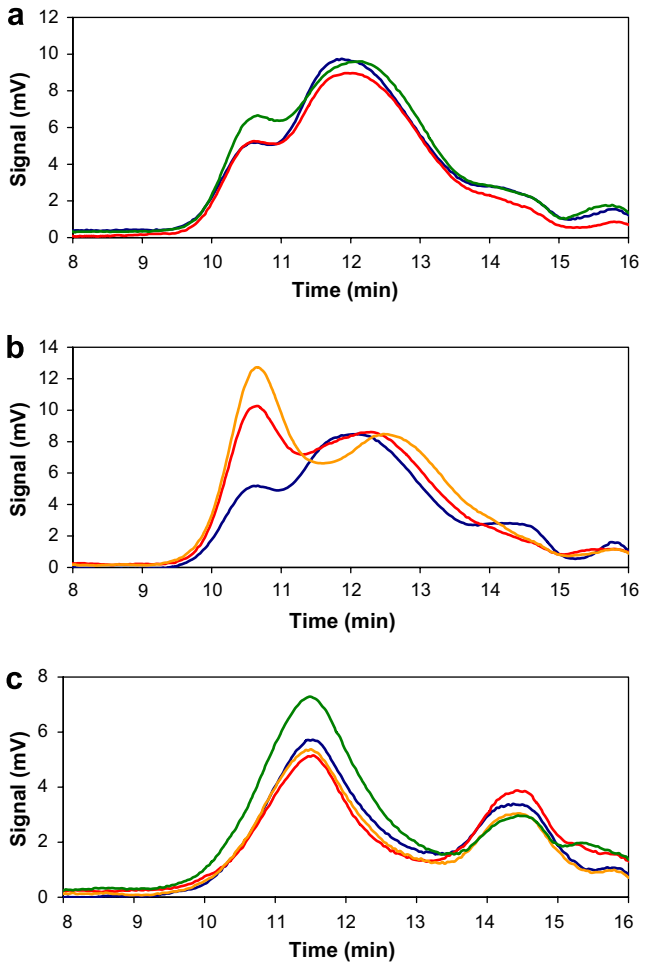

Fig. 5. Changes in molar mass distribution of water soluble pectin from carrots treated at (a) 80 °C – 0.1 MPa, (b) 100 °C – 0.1 MPa, and (c) 80 °C – 600 MPa. (0 min (▬), 20 min (▬), 45 min (▬), and 90 min (▬)).

3.3.4. Molar mass (MM) distribution of pectic polysaccharides

Molar mass distribution of various pectin fractions was monitored by HPSEC (Figs. 4–7). WSP of raw carrots comprised of two unresolved peaks, a small high MM peak (elution time ~ 10.5 min) coinciding with the elution profile of the CSP and NSP fractions and a larger peak (elution time ~ 12 min) having a slightly lower MM (Fig. 4). During thermal processing, distinct changes in polymer concentrations and MM distribution patterns were apparent; the most pronounced being at 100 °C. With thermal progression, two main observations could be deduced (Fig. 5a and b). First, the peak area of the early eluting peak increased with increasing processing time. This represents the proportions of the original CSP and NSP fractions which were thermosolubilized together with the WSP fraction. Secondly, there was a shift in the later eluting peak to lower MM, illustrating transformations in the WSP fractions due to depolymerization. This clearly demonstrated a dynamic change in pectin fractions during thermal processing, a proof for the increasing concentrations of WSP (Table 2). A complementary trend in changes in MM distribution was observed in the NSP fraction (Fig. 6a and b). Unlike the WSP fraction, there were no clear shifts toward low MM polymers. However, decreasing quantities of homogeneously distributed polymers were evident with increasing thermal severity. Contrary, high pressure/high temperature processing led to different MM patterns (Figs. 5 and 6c). The WSP fraction showed only one peak, which was neither clearly increasing in area nor shifting towards lower MM, indicating less thermosolubilization occurred.

MM distributions of the matrix polymers in the brine solution were also analyzed (Fig. 7). With increasing thermal processing time the polymer concentration of the brine increased. Furthermore, the polymers occupied a broader MM range, indicating extensive pectin solubilization.

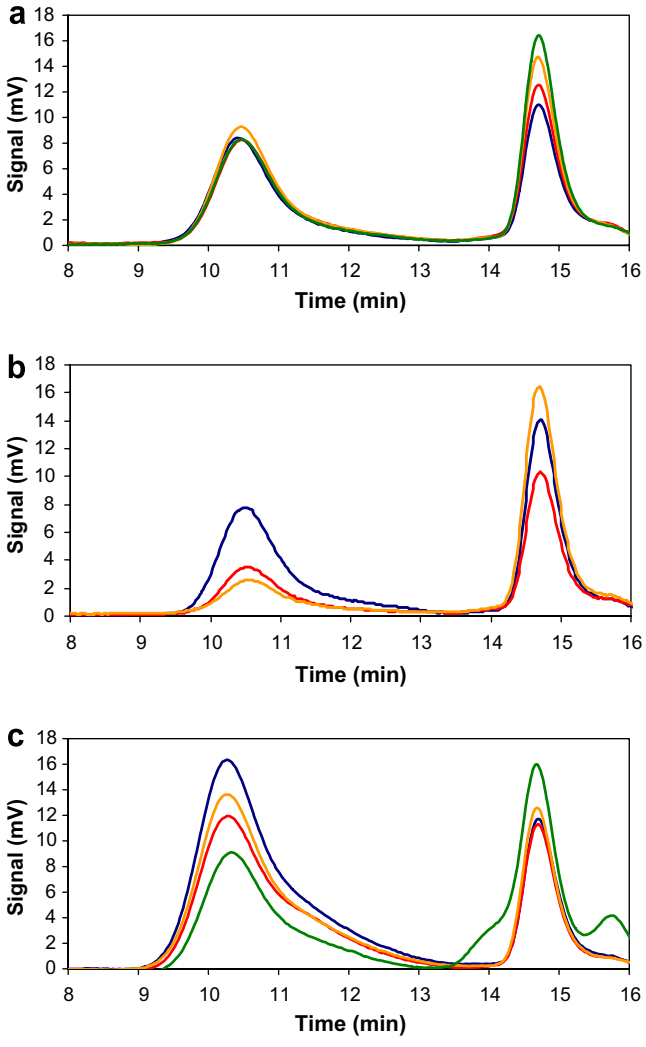

Fig. 6. Changes in molar mass distribution of sodium carbonate soluble pectin from carrots treated at (a) 80 °C – 0.1 MPa, (b) 100 °C – 0.1 MPa, and (c) 80 °C – 600 MPa. (0 min (▬), 20 min (▬), 45 min (▬), and 90 min (▬)).

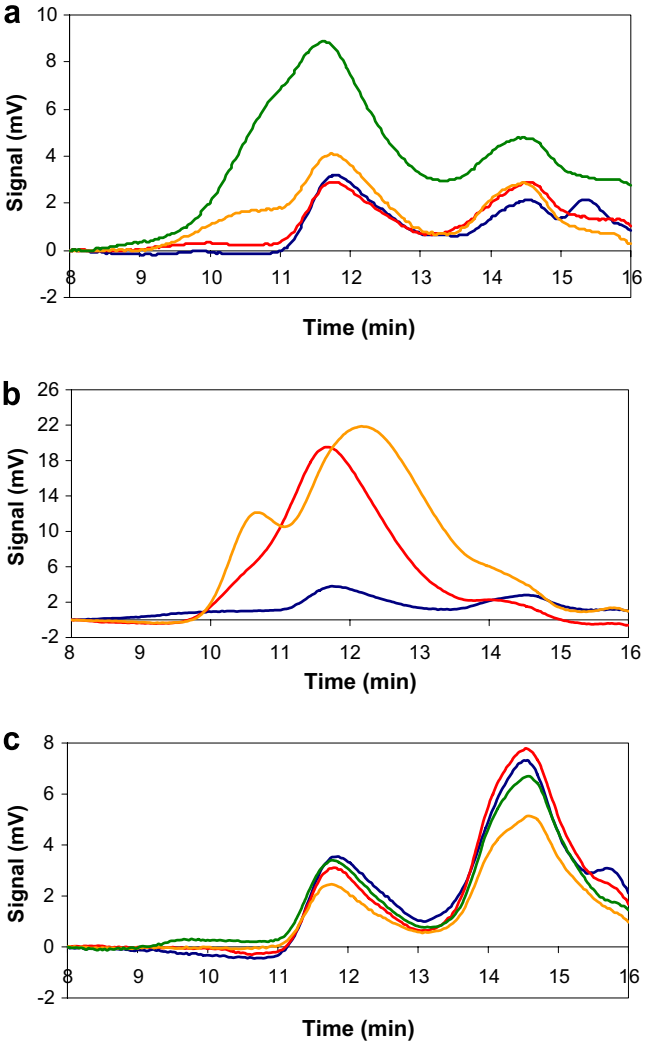

Fig. 7. Changes in molar mass distribution of solubilized matrix polymers in brine from carrots treated at (a) 80 °C – 0.1 MPa, (b) 100 °C – 0.1 MPa, and (c) 80 °C – 600 MPa. (0 min (▬), 20 min (▬), 45 min (▬), and 90 min (▬)).

Contrary, the brine solution associated with the high pressure/high temperature processed carrots contained almost no matrix polymers, indicating less solubilization.

3.3.5. Neutral sugars content of pectic polysaccharides

Pectic polysaccharides contain a diverse neutral sugar composition associated with the "hairy" region. Anion exchange chromatography combined with pulsed amperometric detection provided a reliable means of identifying and quantifying neutral sugars.

Alterations in neutral sugar content as influenced by processing are summarized in Tables 5–7. Besides the abundant pectin-associated neutral sugars, namely rhamnose, arabinose and galactose, glucose and xylose were also present in the different pectin fractions. Glucose was possibly a breakdown product of non-pectic polymers or a residue of soluble sugars that were not completely removed by ethanol during the AIR isolation. The CSP and NSP fractions were found to have lower amounts of neutral sugars when compared to the WSP fractions. It should be noted that the lyophilized CSP and NSP fractions contained salts, thus influencing the absolute pectin amounts weighed per gram. In the context of this study, only changes in rhamnose, arabinose and galactose are discussed.

Clearly, the WSP fractions showed an increasing concentration of pectin-related neutral sugars with increasing

Table 5
Changes in neutral sugar composition of carrots treated at 80 °C – 0.1 MPa

Sugar	Sugar content of carrot pectin in lyophilized fractions (mg/g solid)			
	0 min	20 min	45 min	90 min
Water soluble pectin				
Rhamnose	10.22	11.44	N.d.	12.45
Arabinose	37.26	40.77	N.d.	42.26
Galactose	67.53	74.67	N.d.	80.13
Glucose	69.28	46.28	N.d.	42.17
Xylose	6.78	5.23	N.d.	5.65
Galacturonic acid	278.67	300.70	N.d.	298.87
UA:NS[a]	2.42	2.37	N.d.	2.22
Chelator soluble pectin				
Rhamnose	0.52	0.51	0.35	0.52
Arabinose	1.64	1.81	1.15	1.67
Galactose	2.31	2.45	1.68	2.22
Glucose	0.20	0.28	0.26	0.23
Xylose	0.07	0.62	0.04	0.06
Galacturonic acid	9.96	11.85	10.46	10.39
UA:NS	2.23	2.48	3.29	2.35
Sodium carbonate soluble pectin				
Rhamnose	2.48	2.57	2.43	2.30
Arabinose	8.38	9.04	9.20	7.91
Galactose	17.09	18.50	18.03	16.06
Glucose	0.00	0.00	0.00	0.00
Xylose	0.49	0.57	0.59	0.70
Galacturonic acid	10.37	9.09	14.02	10.10
UA:NS	0.37	0.30	0.47	0.38

N.d.: Not determined.
[a] Uronic acid:neutral sugars (rhamnose + arabinose + galactose).

Table 6
Changes in neutral sugar composition of carrots treated at 100 °C – 0.1 MPa

Sugar	Sugar content of carrot pectin in lyophilized fractions (mg/g solid)		
	0 min	20 min	45 min
Water soluble pectin			
Rhamnose	10.30	15.68	17.59
Arabinose	38.24	56.78	66.13
Galactose	69.08	111.02	134.11
Glucose	42.87	39.80	22.82
Xylose	5.30	5.65	6.60
Galacturonic acid	302.47	299.84	279.24
UA:NS[a]	2.57	1.63	1.28
Chelator soluble pectin			
Rhamnose	0.39	0.29	0.36
Arabinose	1.19	1.18	1.30
Galactose	1.77	1.68	1.64
Glucose	0.19	0.10	0.09
Xylose	0.05	0.05	0.06
Galacturonic acid	9.50	8.26	7.69
UA:NS	2.84	2.63	2.33
Sodium carbonate soluble pectin			
Rhamnose	2.20	1.00	0.69
Arabinose	7.85	3.49	2.26
Galactose	15.85	7.32	4.39
Glucose	0.36	0.00	0.00
Xylose	0.37	0.46	0.49
Galacturonic acid	8.41	2.90	2.53
UA:NS	0.32	0.25	0.34

[a] Uronic acid:neutral sugars (rhamnose + arabinose + galactose).

Table 7
Changes in neutral sugar composition of carrots treated at 80 °C – 600 MPa

Sugar	Sugar content of carrot pectin in lyophilized fractions (mg/g solid)			
	0 min	20 min	45 min	90 min
Water soluble pectin				
Rhamnose	3.14	2.51	2.61	3.48
Arabinose	27.37	26.88	23.37	29.71
Galactose	49.99	46.93	39.44	49.76
Glucose	96.78	92.53	80.21	82.03
Xylose	9.83	10.92	8.86	7.81
Galacturonic acid	108.24	78.65	99.70	137.93
UA:NS[a]	1.34	1.03	1.52	1.66
Chelator soluble pectin				
Rhamnose	0.13	0.15	0.18	0.23
Arabinose	0.57	0.66	0.75	0.96
Galactose	0.58	0.62	0.75	1.13
Glucose	0.11	0.13	0.10	0.12
Xylose	0.05	0.10	0.10	0.09
Galacturonic acid	10.32	11.14	13.54	12.22
UA:NS	8.03	7.77	8.12	5.25
Sodium carbonate soluble pectin				
Rhamnose	4.17	2.78	3.76	3.48
Arabinose	14.70	15.27	11.79	11.74
Galactose	30.53	23.73	23.15	24.10
Glucose	0.00	0.00	0.00	0.59
Xylose	0.57	0.52	0.82	0.71
Galacturonic acid	37.54	24.34	24.34	19.59
UA:NS	0.76	0.58	0.63	0.50

[a] Uronic acid:neutral sugars (rhamnose + arabinose + galactose).

thermal processing time, in accordance with pectin solubilization (Tables 5 and 6). These results suggest a considerable thermal fragmentation of pectin hairy regions, an indication that part of the fractions solubilized contained high amounts of side chains. Interestingly, during thermal processing, a trend that was opposite and complementary to the one observed in the WSP fractions was conspicuous in the NSP fractions. These observations confirm our previous findings. In the pectin fractions of the high pressure/high temperature treated carrots (Table 7), the amounts of neutral sugars remained roughly constant; indicating the stability of these fractions.

To get an idea of the pectin composition of the different pectin fractions, the ratio of galacturonic acid to neutral sugars was calculated. It is obvious that the NSP fraction is mainly made up of neutral sugar-rich rhamnogalacturonan; in contrast to the WSP and CSP fractions which are relatively enriched with homogalacturonan. From the ratios, it can be deduced once more that pectin behaves differently under high temperature/high pressure conditions. As opposed to the thermally treated carrots, the WSP fractions of the high pressure/high temperature treated carrots contained more neutral sugars, the CSP and NSP fractions less.

4. Conclusion

The potential of high pressure sterilization, as alternative for heat sterilization, regarding texture preservation of processed fruits and vegetables was investigated.

Both high temperature and high temperature/high pressure treated carrots showed an initial loss of texture, probably due to loss of turgor pressure. In contrast to the thermally treated carrots which underwent further softening with treatment time, the high pressure treated carrots did not.

Texture loss of thermally treated carrots is mostly explained by the beta-eliminative depolymerization of pectin. Therefore, texture preservation of high pressure/high temperature treated carrots could be explained by inhibition of the beta-elimination reaction. Two possible explanations emerge. On the one hand, it is possible that the beta-elimination reaction does not occur under HP/HT conditions. On the other hand, it is possible that the low degree of esterification of the respective samples triggered inhibition of the beta-elimination. Moreover, the lowly methoxylated pectin could form fortifying networks with calcium ions present. However, other mechanisms may be playing a role. The HP/HT treated carrots contained a low WSP content as opposed to the CSP and NSP content, and a substantial amount of pectin remained in the fractionation residue. Conversion of WSP in CSP could be explained by the extensive demethoxylation. Some kind of newly formed ester cross-links could be responsible for the conversion of WSP in NSP; for instance diferulic bridges (Waldron, Smith, Parr, Ng, & Parker, 1997). What is more, it seems that under HP/HT conditions certain reactions occur by which pectin even becomes sodium carbonate insoluble.

In summary, regarding texture, combined high pressure/high temperature treatments offer an interesting alternative for thermal treatments. The exact mechanisms, responsible for the texture preservation, are the subject of further research.

Acknowledgement

This study has been carried out with financial support from the Commission of the European Communities, Framework 6, Priority 5 'Food Quality and Safety', Integrated Project NovelQ FP6-CT-2006-015710.

References

Ahmed, A. E. R., & Labavitch, J. M. (1977). A simplified method for accurate determination of cell wall uronide content. *Journal of Food Biochemistry, 1*, 361–365.

Basak, S., & Ramaswamy, H. S. (1998). Effect of high pressure processing on the texture of selected fruits and vegetables. *Journal of Texture Studies, 29*(5), 587–601.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*(2), 484–489.

Chin, L. H., Ali, Z. M., & Lazan, H. (1999). Cell wall modifications, degrading enzymes and softening of carambola fruit during ripening. *Journal of Experimental Botany, 50*(335), 767–775.

Greve, L. C., McArdle, R. N., Gohlke, J. R., & Labavitch, J. M. (1994). Impact of heating on carrot firmness: changes in cell wall components. *Journal of Agricultural and Food Chemistry, 42*, 2900–2906.

Greve, L. C., Shackel, K. A., Ahmadi, H., McArdle, R. N., Gohlke, J. R., & Labavitch, J. M. (1994). Impact of heating on carrot firmness: contribution of cellular turgor. *Journal of Agricultural and Food Chemistry, 42*, 2896–2899.

Klavons, J. A., & Bennett, R. D. (1986). Determination of methanol using alcohol oxidase and its application to methyl-ester content of pectins. *Journal of Agricultural and Food Chemistry, 34*(4), 597–599.

Ludikhuyze, L., Van Loey, A., Indrawati & Hendrickx, M. (2001). Combined high pressure thermal treatment of foods. In P. Richardson (Ed.), *Thermal technologies in food processing* (pp. 266–284). Cambridge: Woodhead Publishing Limited.

Ly-Nguyen, B., Van Loey, A. M., Smout, C., Ozcan, S. E., Fachin, D., Verlent, I., et al. (2003). Mild-heat and high-pressure inactivation of carrot pectin methylesterase: A kinetic study. *Journal of Food Science, 68*(4), 1377–1383.

Matser, A. A., Krebbers, B., van den Berg, R. W., & Bartels, P. V. (2004). Advantages of high pressure sterilisation on quality of food products. *Trends in Food Science & Technology, 15*(2), 79–85.

McFeeters, R. F., & Armstrong, S. A. (1984). Measurement of pectin methylation in plant cell walls. *Analytical Biochemistry, 139*, 212–217.

Mertens, B., & Knorr, D. (1992). Developments of nonthermal processes for food preservation. *Food Technology, 46*(5), 124–133.

Meyer, R. S., Cooper, K. L., Knorr, D., & Lelieveld, H. L. M. (2000). High-pressure sterilization of foods. *Food Technology, 54*(11), 67–72.

Ng, A., & Waldron, K. W. (1997). Effect of cooking and pre-cooking on cell-wall chemistry in relation to firmness of carrot tissues. *Journal of the Science of Food and Agriculture, 73*(4), 503–512.

Ridley, B. L., O'Neill, M. A., & Mohnen, D. A. (2001). Pectins: Structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry, 57*(6), 929–967.

Sajjaanantakul, T., Van Buren, J. P., & Downing, D. L. (1989). Effect of methyl-ester content on heat degradation of chelator-soluble carrot pectin. *Journal of Food Science, 54*(5), 1272–1277.

Sila, D. N., Doungla, E., Smout, C., Van Loey, A., & Hendrickx, M. (2006a). Pectin fraction interconversions: Insight into understanding texture evolution of thermally processed carrots. *Journal of Agricultural and Food Chemistry, 54*(22), 8471–8479.

Sila, D. N., Smout, C., Elliot, F., Van Loey, A., & Hendrickx, M. (2006b). Non-enzymatic depolymerization of carrot pectin: Toward a better understanding of carrot texture during thermal processing. *Journal of Food Science, 71*(1), E1–E9.

Sila, D. N., Smout, C., Satara, Y., Truong, V., Van Loey, A., & Hendrickx, M. (2007). Combined thermal and high pressure effect on carrot pectinmethylesterase stability and catalytic activity. *Journal of Food Engineering, 78*(3), 755–764.

Siliha, H., Jahn, W., & Gierschner, K. (1996). Effect of a new canning process on cell wall pectic substances, calcium retention and texture of canned carrots. In J. Visser & A. Voragen (Eds.), *Pectins and pectinases* (pp. 495–508). Amsterdam: Elsevier Science.

Smout, C., Sila, D. N., Vu, T. S., Van Loey, A. M. L., & Hendrickx, M. E. G. (2005). Effect of preheating and calcium pre-treatment on pectin structure and thermal texture degradation: A case study on carrots. *Journal of Food Engineering, 67*(4), 419–425.

Szczesniak, A. S. (2002). Texture is a sensory property. *Food Quality and Preference, 13*(4), 215–225.

Van Buren, J. P. (1979). The chemistry of texture in fruits and vegetables. *Journal of Texture Studies, 10*, 1–23.

Verlent, I., Van Loey, A., Smout, C., Duvetter, T., Nguyen, B. L., & Hendrickx, M. E. (2004). Changes in purified tomato pectinmethylesterase activity during thermal and high pressure treatment. *Journal of the Science of Food and Agriculture, 84*(14), 1839–1847.

Waldron, K. W., Smith, A. C., Parr, A. J., Ng, A., & Parker, M. L. (1997). New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. *Trends in Food Science & Technology, 8*, 213–221.

Waldron, K. W. (2004). Plant structure and fruit and vegetable texture. In D. Kilcast (Ed.), *Texture in food – volume 2: solid foods* (pp. 241–258). Cambridge: Woodhead Publishing Limited.

Weemaes, C. A., DeCordt, S. V., Ludikhuyze, L. R., VandenBroeck, I., Hendrickx, M. E., & Tobback, P. P. (1997). Influence of pH, benzoic acid, EDTA, and glutathione on the pressure and/or temperature inactivation kinetics of mushroom polyphenoloxidase. *Biotechnology Progress, 13*(1), 25–32.