Non-enzymatic Depolymerization of Carrot Pectin: Toward a Better Understanding of Carrot Texture During Thermal Processing

DANIEL N. SILA, CHANTAL SMOUT, FRANKLIN ELLIOT, ANN VAN LOEY, AND MARC HENDRICKX

ABSTRACT: Pretreated carrot discs were thermally processed (90 °C to 110 °C) in closed containers and the resulting textural characteristics were analyzed. The pretreatment conditions used include conventional high-temperature blanching (90 °C, 4 min), low-temperature blanching (LTB = 60 °C, 40 min), LTB combined with 0.5% calcium chloride soaking, LTB combined with 2% sodium chloride soaking, high pressure pretreatment (HP = 400 MPa, 60 °C, 15 min), HP combined with 0.5% calcium chloride soaking, and control (non-pretreated sample). Alcohol insoluble residues (AIR) from the pretreated carrot discs were characterized in terms of degree of methoxylation (DM). The AIR samples were further subjected to fractionation into water-soluble pectin (WSP), chelator-soluble pectin (CSP), and sodium carbonate–soluble pectin (NSP). Heat depolymerization patterns and β-elimination kinetics were investigated on the different pectin fractions. Thermal texture degradation was strongly influenced by the pretreatment condition used and the processing temperature during subsequent thermal treatment. Pretreatment conditions that showed a significant reduction in DM exhibited decreased WSP content, reduced β-elimination, and consequently superior textural characteristics. β-elimination was markedly pronounced in the highly methoxylated WSP fractions. CSP and NSP fractions were insensitive to β-elimination. A strong correlation ($r > 0.95$) between thermal texture loss of carrots and β-elimination kinetics exists. Overall, the benefits of controlled pectinmethylesterase activity in carrot processing were pointed out.

Keywords: carrot, pectin, pectinmethylesterase, degree of methoxylation, β-elimination

Introduction

Polysaccharides are the principal structural elements of plant cell walls. The cell wall polymers consist of cellulose, an interconnecting structure of hemicellulose and pectin within a matrix of globular and non-globular proteins. In view of structure and functional properties, pectin is probably one of the most interesting cell wall polymers because of its abundance, its solubility, its response to chemical reactions (Van Buren 1979), and its numerous industrial applications (Kashyap and others 2001). The ubiquitous presence of pectin in fruits and vegetables emphasizes its critical importance in determining the texture of plant derived processed products. Structurally, pectin represents a group of heterogeneous polysaccharides of substantial diversity depending on its botanical origin (Huisman and others 2001). It is mainly characterized by α- (1-4) linked D-galacturonic acid units esterified with methanol in different amounts (O'Neill 1990; Voragen and others 1995). Some of the hydroxyl groups can be acetylated on O-2 and /or O-3 (Rombouts and Thibault 1986). The poly-D-galacturonic acid backbone can be interspersed by the covalent insertions of (1-2)-linked L-rhamnosyl residues with neutral sugar residues (Voragen and others 1995). More recently, rhamnose insertions in the pectic backbone have been doubted (Zhan and others 1998; Vincken and others 2003).

During thermal processing, and in the context of texture degradation, solubilization of pectin and the accompanying β-elimination reactions are fundamental (Albersheim and others 1960; Kravtchenko and others 1992). The specific nature of these reactions is not very clear. However, β-elimination is quite prevalent above pH 4.5 (condition common in most vegetables) and at elevated temperature (Kravtchenko and others 1992). The reaction proceeds on uronic acids, which possess a glycosidic linkage on C_4 in the β-position of the carboxyl group at C_5. The reaction mechanism requires that the activated hydrogen atom on C_5 is removed by suitable proton-acceptors, which will result in the formation of unstable, intermediary anions that are stabilized by losing the C-O linkage in the β-position (Kiss 1974). Consequently, a double bond appears between C_4 and C_5 at the non-reducing end. Detection of the olefinic bond of the unsaturated ester by its UV absorption at 230 to 240 nm can be used to quantify β-elimination (Kravtchenko and others 1992; Ceci and Lazano 1998). The reaction is also affected by salts, malates, and phytates (Keijbets and Pilnik 1974).

Modifications in the structural characteristics of pectin may lead to alterations in the functional properties of the system and/or new applications. Because the β-elimination reaction is highly dependent on the methoxyl ester content of plant tissues (Sajjaanantakul and others 1989; Waldron and others 1997), targeted manipulation of the degree of methoxylation can lead to controlled β-elimination. This can be achieved in vivo through genetic engineering or by in situ activation of endogenous or exogenous pectinmethylesterase (PME) using pretreatment conditions. The beneficial effect of the latter has been shown for thermally processed carrots (Vu and others 2004; Sila and others 2005; Smout and others 2005). Very little has been published on the influence of pretreatment conditions on β-elimination (BeMiller and Kumari 1972; Keijbets and Pilnik 1974). In addition, quantitative data relating the kinetics of β-elimination

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to the perceived textural attributes is missing. In that perspective, carrot pectin from pretreated carrot discs was isolated and characterized with regard to thermal impact. The influence of targeted structural modifications of pectin and the extent of modification on β-elimination kinetics of carrot pectin was investigated. Finally, the relationship between β-elimination kinetics and the texture of thermally processed carrots was explored.

Materials and Methods

A general overview of the research strategy is summarized in Figure 1.

Vegetable materials

Carrots (*Daucus carota* var. Nerac) were bought from a local auction in Belgium and stored at 4 °C before processing and analysis.

Pretreatments

The cores of carrots were chopped into discs of 10-mm height and 12-mm dia before calcium/sodium, thermal, and/or high-pressure pretreatment. All pretreatment conditions, except for sodium infusion, were carried out as described by Sila and others (2005). The pretreatment conditions included conventional high-temperature blanching (HTB = 90 °C, 4 min), low-temperature blanching (LTB = 60 °C, 40 min), LTB combined with 0.5% calcium chloride soaking (LTB + Ca^{+2}), LTB combined with 2% sodium chloride soaking (LTB + Na^{+}), high-pressure pretreatment (HP = 400 MPa, 60 °C, 15 min), HP combined with 0.5% calcium chloride soaking (HP + Ca^{+2}), and control (non-pretreated sample). Table 1 gives a brief summary of the various pretreatment conditions used and the subsequent analysis performed.

Sodium soaking was performed at room temperature and pressure. The infusion was carried out by soaking carrot discs for 1 h in 2% (w/v) $NaCl_{(aq)}$ solution. This was done for LTB (60 °C for 40 min) samples only. The $NaCl_{(aq)}$ solution was then drained and the samples taken to the subsequent step in the experiment.

Thermal processing

Carrot discs (10) encapsulated in aluminum tubes filled with brine (demineralized water, sodium chloride, or calcium chloride solution depending on the pretreatment) were subjected to thermal treatments (90 °C to 110 °C) in a temperature controlled oil bath (synthetic oil: flash point = 227 °C, viscosity at 20 °C = 100 mPa, density at 20 °C = 0.86 kg/dm^3, and specific heat capacity = 1.96 kJ/kg.K). There was no direct contact of the oil with the samples. A heating lag study was carried out and a lag time of 5 min was determined using thermocouples placed at the center of the sample in an aluminum tube. Consequently, all samples were heat-treated in the oil bath and after 5 min, a blank sample (time zero sample) was withdrawn and immediately cooled in an ice bath before analysis. All the other samples were heat-treated (90 °C to 110 °C) for an additional 20 min, cooled in an ice bath, and subsequently the residual textures were determined.

Table 1—A summary of the pretreatment conditions used and the subsequent experiments carried out[a]

	Experiments carried out		
Pretreatment condition	Texture of processed carrot	Methyl ester content of AIR	β-elimination of pectin fractions
Control (non-pretreated)	√	√	√
90 °C, 4 min (HTB)	√	√	√
60 °C, 40 min (LTB)	√	√	√
LTB + $CaCl_2$ (0.5%)	√	√	√
LTB + NaCl (2%)	√	×	×
400 MPa, 60 °C, 15 min (HP)	√	×	×
HP + Ca^{+2}	√	√	√

[a]√ = analysis done; X = analysis not done; AIR = alcohol insoluble residue; LTB = low temperature blanching; HTB = high temperature blanching; HP = high pressure treatment.

Texture measurements

Textural properties were measured using a TA-XT2 texture analyzer (Stable Micro Systems, Surrey, England) equipped with a 25-kg force cell and a cylindrical flat-head aluminum probe of 25-mm dia. Texture was expressed as hardness, which is the peak force of the 1st compression of the sample. In this case, the peak force required to deliver a constant strain of 30% was measured at a compression rate of 1 mm/s. For a given thermal treatment condition, 2 independent pretreatments were carried out each with 10 carrot discs. The mean compression force value of the carrot cylinders was calculated.

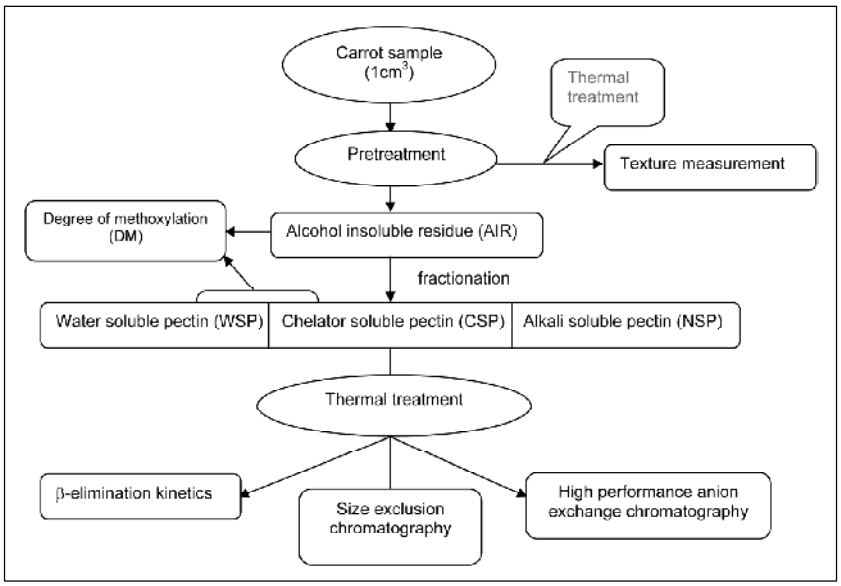

Figure 1—A schematic overview of the experimental set up and research strategy

Extraction of alcohol insoluble residues

Alcohol insoluble residue (AIR) samples from carrot tissues after different pretreatments were prepared following the procedure described by McFeeters and Armstrong (1984). Approximately 10 g of pretreated carrot sample was weighed exactly and completely homogenized in 63.3 mL of 95% ethanol using a mixer (Buchi mixer B-400, Flawil, Switzerland). The residue was filtered (Merck Eurolab filters nr 413, 110-mm dia, made in EU) and rehomogenized in 31.7 mL of 95% ethanol and filtered again. The residue was homogenized again in 31.6 mL of acetone before final filtration followed by drying overnight under vacuum at 40 °C. The AIR was ground using a mortar and pestle and put in a dessicator until analysis. For each pretreatment condition, a stock of AIR

sample was collected before subsequent fractionation and characterization experiments.

Fractionation of carrot pectin

Water-soluble pectins (WSP) were fractionated using a modified hot water extraction procedure according to Braga and others (1998). In this method, AIR samples from pretreated carrot tissues were weighed exactly (0.5 g). The samples were homogenized in 90 mL of hot water (100 °C) for 5 min. The resulting solution was cooled in a sink and the volume was adjusted to 100 mL after pH adjustment (pH 6.5). The mixture was filtered using a filter paper (Schleicher and Schuell, Belgium), keeping the filtrate for subsequent analysis. The residue was further fractionated in 100 mL of 0.05 *M* cyclohexane-trans-1,2-diamine tetra-acetic (CDTA) in 0.1 *M* potassium acetate (pH 6.5) for 6 h at 28 °C (Chin and others 1999). The resulting mixture was filtered and the residue taken to the next fractionation step. The filtrate was labeled chelator-soluble pectin (CSP). The residue was incubated again in 100 mL of 0.05 *M* Na_2CO_3 containing 0.02 *M* $NaBH_4$ for 16 h at 4 °C, with constant stirring, followed by re-incubation for another 6 h at 28 °C (Chin and others 1999). The resulting filtrate was designated sodium carbonate–soluble pectin (NSP). The NSP fraction was adjusted to pH 6.5 taking into account the dilution factor. The residue of the fractionation (cellulose and hemicellulose) was discarded.

All the pectin fractions (WSP, CSP, NSP) were analyzed for galacturonic acid content, degree of methoxylation, and β-elimination reaction.

Determination of degree of methylation

The degree of methoxylation (DM) of AIR samples and the consequent pectin fractions (WSP, CSP) was determined. First, anhydrous galacturonic acid was determined quantitatively by the colorimetric hydroxyl-phenyl-phenol method (Blumenkrantz and Asboe-Hansen 1973) with a UV/Vis spectrophotometer (Ultrospec 2100 pro from Amersham Biosciences, Sweden) at 520 nm.

The 2nd step involved determining the amount of methanol spectrophotometrically (412 nm) according to the method of Klavons and Benett (1986). Here, pectin methyl esters were hydrolyzed to methanol followed by complexing the methanol to a colored compound with pentandione. Finally, the degree of methoxylation was estimated by taking the ratio of moles of methanol to the moles of anhydrous galacturonic acid content, and expressed as a percentage. Two independent determinations were carried out each in triplicate.

β-elimination assay

Heat-induced β-elimination of carrot pectin was studied in a temperature-controlled oil bath from 90 °C to 110 °C. For each pectin fraction, exactly 4 mL of sample was pipetted into a screw capped pyrex tube and subjected to a thermal treatment at a given temperature. At specified time intervals (0 to 4 h), samples were withdrawn from the oil bath and cooled immediately in an ice bath.

To quantify the extent of β-elimination, the concentration of 4,5-unsaturated galacturonides in diluted samples was measured indirectly at 235 nm. An average molar extinction coefficient of 5412 M^{-1} cm^{-1} was used to estimate the amount of unsaturated galacturonides (Kravtchenko and others 1992). The concentration of unsaturated galacturonides was then expressed as m*M* unsaturated galacturonides per gram AIR.

The influence of pH on the β-elimination reaction of WSP fractions was studied. The samples were adjusted into 3 different pH regimes (pH 3.5, 6.5, and 9) using NaOH or HCl. β-elimination was investigated at 100 °C.

To investigate the effect of salts on β-elimination of WSP fractions, NaCl and $CaCl_2$ were used. The samples were adjusted to various salt concentrations (0 to 0.5 *M*). For each salt concentration, the amount of unsaturated galacturonides in 4 mL of sample was assayed in a blank (sample before heat treatment) and thermally processed sample (100 °C for 1 h). All analyses were done in duplicate.

High performance size exclusion chromatography

Changes in molecular weight distribution of carrot pectin during thermal processing were studied using size exclusion chromatography. This was performed using a Dionex system (DX 600) equipped with a mixed bed column of Bio-Gel TSK (dimensions = 300-mm length × 7.5-mm dia, pore size = 100 to 1000 Å, particle size = 13 μm, theoretical plates/column = >/= 7000, pH range = 2 to 12, maximum pressure = 300 psi, Bio-Rad Labs, Richmond, Calif., U.S.A.) in combination with a TSK guard column. Elution was executed at 35 °C with 0.05 *M* $NaNO_3$ buffer, pH 6.9 at a flow rate of 0.7 mL/min for 20 min. The eluent was monitored using a Shodex R101 refractive index detector (Showa Denko, K.K., Tokyo, Japan). Pullulan standards (MW range 188 to 788000), which have a similar structure and hydrodynamic characteristics with polygalacturonic acid, were used. Deionized water (organic free = 18 MΩ resistance) supplied by a Simplicity™ Millipore water purification system (Brussels, Belgium) was used to prepare eluents and samples. Once the standard curve was established, monogalacturonic acid was used daily to verify the system. The samples were injected in duplicate.

High-performance anion exchange chromatography

High-performance anion exchange chromatography of β-elimination products was achieved using a Dionex system (DX 600) equipped with a GP 50 gradient pump, a CarboPac PA1 column, and a pulsed amperometric detector (Dionex, Wommelgem, Belgium) as described by Verlent and others (2004). The sample (100 μL) was injected and eluted (1 mL/min) in a gradient with 100 m*M* NaOH (A) and 1 *M* NaOAc containing 100 m*M* NaOH (B) as follows: 0 → 3 min, 90% A and 10% B; 3 → 30 min, linear gradient of 10% → 100% B. Galacturonic acid monomer, dimmer, and trimer were used as standards. The analysis was done in duplicate.

Data analysis

β-elimination kinetics were determined using a 2-step approach. First, the rate constant at a given temperature (k-value) was determined using zero order reaction kinetics (Eq. 1 → 3) by plotting the concentration of unsaturated galacturonides as a function of time:

$$-\frac{\partial A}{\partial t} = k A^{\circ} \quad (1)$$

$$\int_{A_0}^{A_t} \partial A = -\int_{0}^{t} k \, \partial t \quad (2)$$

$$A_t = A_0 - k t \quad (3)$$

where A_o = concentration of unsaturated galacturonides at time t = 0 min, A_t = concentration at time t (min), and k is the β-elimination reaction rate constant (m*M*/min)/g AIR).

The 2nd step involved applying the Arrhenius model (Eq. 4) to determine the temperature dependence of k-values. The E_a values (kJ/mol) were estimated by plotting the natural logarithm of the k-value (m*M*/min)/g AIR) against the reciprocal of the respective absolute temperature (/K):

$$k_T = k_{ref} * \exp\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right) \quad (4)$$

where, E_a is the activation energy (kJ/mol), R is the universal gas constant (8.3143 J/K/mol), k_{ref} is the reaction rate constant (/min) at reference temperature T_{ref} (K), while k_T is the reaction rate constant (/min) at temperature T (K).

The goodness of fit of the linear curves was checked using regression coefficients (R^2).

Results and Discussion

Influence of pretreatment conditions on the texture of thermally processed carrots

The residual hardness of pretreated carrot discs after 20 min of thermal processing at different temperatures (90 °C to 110 °C) is shown in Figure 2.

Thermal texture degradation of carrots increased with increasing processing temperature for all the pretreatment conditions studied. The extent of texture degradation was strongly influenced by the pretreatment condition. The relative effect of pretreatment conditions was similar for all processing temperatures studied. Pretreatment conditions that resulted in a significant reduction in DM (Table 2) showed pronounced texture retention. This is in agreement with our previous results (Sila and others 2005).

High pressure-thermal pretreatment (400 MPa, 60 °C, 15 min) combined with calcium soaking (0.5% $CaCl_2$) resulted in the highest modification of carrot pectin and consequently the best texture preservation. Combining LTB with monovalent salt infusion ($NaCl_{aq}$) caused greater softening than when the carrots were blanched in distilled water. Contrary, when divalent salts ($CaCl_2$) were used, an enhancement of the texture stability of carrots was observed. Divalent cations facilitate formation of complexes between pectin molecules (Thibault and Rinaudo 2004) in addition to modifying the affinity of PME to pectin substrates (Rexova-Benkova and Markovic 1976). On the other hand, monovalent salts compete with divalent salts for interaction with the free carboxyl groups of the pectic acid (Waldron and others 2003) besides contributing largely to increased depolymerization during thermal processing (Sajjantanakul and others 1989). The advantages of a high-pressure pretreatment combined with calcium soaking over LTB combined with calcium soaking in texture preservation was more pronounced with increased processing temperature (110 °C). The observed variations in textural characteristics could be explained based on the amount of pectate formed and the inherent rate of β-elimination. Indirect but strong evidence points out that PME activity increases significantly during optimized pretreatment conditions (Sila and others 2005) resulting in increased pectate formation and inhibiting β-elimination. This decreases the heat susceptibility of pectin. More so, because texture preservation is strongly correlated to the degree of methoxylation (DM) of carrot pectin (Sila and others 2005), enhanced de-esterification is essential for improved texture. Overall, texture improvement was ascribed to enhanced PME activity during the pretreatment stage, making it interesting to investigate the effect of endogenous PME activity on the yield and composition of carrot pectin.

Table 2—Degree of methoxylation of alcohol insoluble residue (AIR) obtained from pretreated carrot discs

Pretreatment condition	Degree of methoxylation (%)
Non-pretreated sample (control)	62.68 ± 0.68[a]
HTB (90 °C, 4 min)	59.22 ± 0.78
LTB (60 °C, 40 min)	53.05 ± 0.05
LTB + Ca^{2+} Soaking	45.68 ± 0.68
HP + Ca^{2+} Soaking	36.71 ± 1.29

[a]Standard deviation; n = 6.

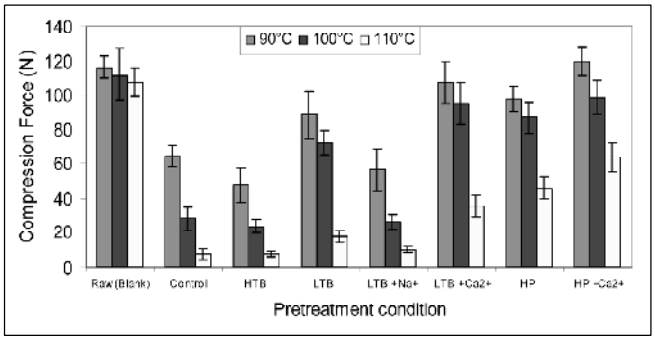

Figure 2—Residual hardness of pretreated carrot discs after thermal processing (90 °C to 110 °C) for 20 min: Raw = blank sample (processed for 5 min); Control = non-pretreated sample; HTB = conventional high-temperature blanching (preheating at 90 °C for 4 min); LTB = low-temperature blanching (preheating 60 °C for 40 min); LTB + Na^+ = LTB followed by sodium soaking; LTB + Ca^{2+} = LTB followed by calcium soaking; HP = high-pressure pretreatment (400 MPa, 60 °C for 15 min); HP + Ca^{2+} high-pressure pretreatment (400 MPa, 60 °C for 15 min) followed by calcium soaking.

Influence of pretreatment conditions on pectin fractionation

Alcohol insoluble residue (AIR) samples from carrots contains a large amount of polysaccharides, particularly pectin. Table 3 summarizes the changes in extraction yields and composition of carrot pectin (WSP, CSP, and NSP) with changing pretreatment conditions. The total extractable galacturonic acid yields were not affected ($P > 0.1$) by pretreatment conditions. However, distinct differences in the composition of pectin fractions were apparent with changing pretreatment conditions. The WSP fraction, which was the major pectin component in native carrots (48.7%), decreased with decreasing DM of AIR, whereas the NSP fraction increased correspondingly. Contrary, the proportion of the CSP fraction was low (8% to 15%) and not significantly influenced by the pretreatment condition. In comparison with non-pretreated sample, the proportion of WSP in high-pressure pretreated and calcium-soaked sample reduced to about 14% while the NSP fraction increased from 40% to approximately 78%. It is possible that a proportion of the WSP fraction was modified into the NSP fraction. Pectin solubilization was strongly influenced by the methoxyl ester content of AIR, and consequently the extent of pectin modification. Complete demethoxylation of pectin renders it insoluble in water (Hsu and others 1965). In addition, changes in methyl ester content influences matrix bonding (hydrogen bonds, ionic bonds, ester bonds), which subsequently affects pectin extractability. This observation is consistent with the fact that the water-insoluble pectin and chelator-insoluble pectin can be extracted from the cell wall in cold aqueous Na_2CO_3, a reagent that breaks many types of ester linkages (Selvedran and O'Neill 1987; Renard and others 1990).

In addition to changes in galacturonic acid concentrations, variations in the DM of pectin fractions were observed. The DM of WSP fraction was high and in the range 73% to 89%. Contrary, the DM of

Table 3—Effect of pretreatment conditions on the solubility and degree of methoxylation (DM) of carrot pectin fractions[a]

Sample pretreatment condition		Pectin characteristics			
		WSP	CSP	NSP	Total Gal
Non-pretreated	m*M* Gal/g AIR	0.25 (0.009)[b]	0.06 (0.002)	0.21 (0.008)	0.52
	DM (%)	89.39 (0.22)	14.19 (1.26)	ND	—
90 °C, 4 min (HTB)	m*M* Gal/g AIR	0.21 (0.026)	0.08 (0.004)	0.23 (0.003)	0.52
	DM (%)	83.01 (0.78)	11.16 (1.28)	ND	—
60 °C, 40 min (LTB)	m*M* Gal/g AIR	0.08 (0.008)	0.07 (0.001)	0.36 (0.007)	0.51
	DM (%)	80.12 (1.28)	13.57 (0.43)	ND	—
LTB + Ca^{+2} (0.5%)	m*M* Gal/g AIR	0.06 (0.003)	0.06 (0.005)	0.40 (0.004)	0.52
	DM (%)	88.67 (2.42)	14.84 (0.13)	ND	—
HP + Ca^{+2} (0.5%)	m*M* Gal/g AIR	0.07 (0.014)	0.04 (0.002)	0.40 (0.024)	0.51
	DM (%)	73.44 (1.92)	11.73 (0.18)	ND	—

[a]AIR = alcohol insoluble residue; CSP = chelator-soluble pectin; DM = degree of methoxylation; Gal = galacturonic acid; ND = not determined; NSP = sodium carbonate–soluble pectin; WSP = water-soluble pectin.
[b]Standard deviation.

the CSP fraction was low and almost constant (11% to 14%). Similar results (DM_{CSP} = 10%) have been reported for green bean pods (Stolle-Smits and others 1999). In contrast, Sajjantanakul and others (1989) estimated a DM_{CSP} of about 46% for carrot. This discrepancy with our results might be related to differences in extraction procedures used. On the other hand, the DM of the NSP fraction was not determined because the alkaline saponification procedure used in extraction renders it devoid of methyl esters (DM_{NSP} = 0).

Closely related to the observed changes in pectin fractions was the measured textural attributes. In fact, pectins are partially solubilized when plant tissues are softened by heating, mainly by β-elimination of internal cellular pectin (Chou and Kokini 1987; Hurtado and others 2002). Pretreatment conditions that resulted in a significant reduction in the solubilization of WSP showed increased texture retention. This implied decreased cell separation due to limited solubilization of pectin in the middle lamella. Our results concur with cell separation related changes observed during carrots cooking (Greve and others 1994). It is deduced that the endogenous modification of pectin has an effect on its solubilization consequently affecting the texture of the embedding matrix during thermal processing. Besides pectin solubilization, the heat depolymerization behavior of pectin was considered essential to obtain a better understanding on texture degradation.

Effect of heat on pectin depolymerization

Size exclusion chromatography (SEC) was carried out to qualitatively analyze the heat-induced depolymerization patterns and the molecular weight distribution of pectin fractions during thermal processing. Native pectin fractions were characterized by similar patterns of molecular weight distribution. Each fraction contained 2 polymeric populations of pectin (Figure 3). The 1st peak corresponded to high molecular weight (MW) pectin polymers, whereas the 2nd peak signified short chain oligogalacturonides. The molecular weight range (14.5 to > 788 kDa) of the high MW population was close to that of commercial apple pectin.

Heat degradation (110 °C) of the pectin fractions at varied times (0 to 4 h) resulted in clear differences in molecular weight distribution patterns. Figure 4 illustrates the heat response of WSP fraction. The amount of the high MW pectin decreased with increasing thermal processing time. Concurrently, a drift toward low MW pectin polymers was observed as the WSP fragmented to a non-homogeneous mixture of polymers. Therefore, a random depolymerization mechanism of the pectin chain was proposed.

In contrast, a more systematic and homogeneous depolymerization mechanism was observed for the CSP and NSP fractions. Thermal impact revealed a distinct decline in the concentration of the high MW polymers paralleled by a corresponding increase in the concentration of oligomers (Figure 5). The change in the concentration of oligomers is emphasized in Figure 6. No intermediate degradation products were observed unlike in the WSP fraction. Consequently, an end chain depolymerization mechanism was suggested.

β-elimination is quite dominant in highly methoxyl esterified samples, in this case the WSP fraction. The low degree of esterification of the CSP and NSP fractions possibly hampered thermal degradation via β-elimination. Despite the degree of esterification, the

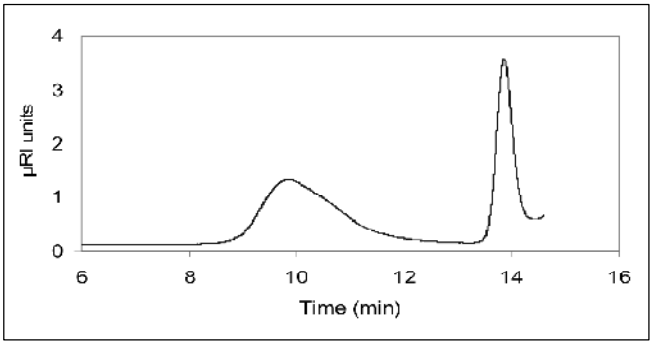

Figure 3—Molecular weight distribution of sodium carbonate–soluble pectin (NSP) before thermal processing

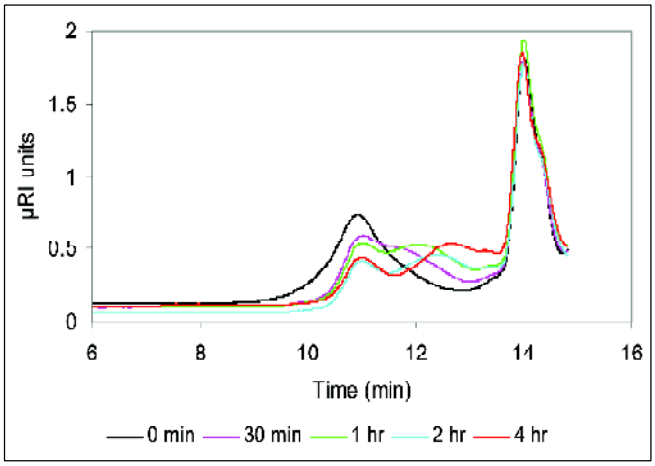

Figure 4—Thermal (110 °C) depolymerization pattern of water-soluble pectin (WSP)

distribution of the methoxyl esters over the entire pectin molecule is also important (Kravtchenko 1992).

A confirmation of the thermal digest products using high performance anion exchange chromatography combined with pulsed amperometric detection (HP-PAD) for the CSP and NSP fractions showed release of galacturonic acid monomers with increasing thermal processing time (Figure 7). The results were consistent with those obtained in SEC, a further prove for the proposed depolymerization mechanism. HP-PAD was also carried out for the WSP fractions, but the results were not clear due to the limitations of the method used (only applicable for oligomers with less than 12 galacturonic acid units). To gain a quantitative insight in the extent of depolymerization, β-elimination kinetics were investigated.

β-elimination kinetics of pectin fractions

In a preliminary experiment, the β-elimination reaction was investigated in each of the pectin fractions. Interestingly, a pronounced increase in the concentration of unsaturated galacturonides was observed in the WSP (Figure 8a). Conversely, β-elimination was limited in the CSP (Figure 8b) and NSP (Figure 8c) fractions. The susceptibility of pectin to β-eliminative depolymerization is determined by its methoxyl ester content (Jarvis and others 2003). Evidently, the highly methoxylated WSP fractions (DM = 73.4% to 88.7%) depolymerized extensively as opposed to the poorly/none esterified CSP and NSP fractions. As a result, further work on the kinetics of β-elimination was done only for the WSP fractions.

Table 4 shows β-elimination kinetics of the WSP fractions with changing pretreatment conditions. Zero order kinetics could adequately ($R^2 > 0.96$) describe β-elimination (Figure 9). The reaction rate increased with increasing temperature for all the pretreatment conditions studied. For every 10 °C increase in temperature, the reaction rate increased by a factor of 2 to 3.5. This indicated a strong temperature dependence of β-elimination. Similarly, at pH 6.9, Albersheim and others (1960) approximated a Q_{10} factor of 3.5 between 50 °C and 95 °C. An approximated Q_{10} factor of 3.3 for WSP in cucumber has also been reported (Krall and McFeeter 1998).

Like in texture degradation, β-elimination was strongly influenced by the pretreatment conditions applied and consequently the changes taking place in the pectin polymer. At each temperature, pretreatment conditions that showed enhanced pectin modification demonstrated reduced β-elimination. This emphasized the importance of methoxyl esters as the main driving force of β-elimination. Consequently, lowering the DM of WSP fractions generally led to retardations in the rate of depolymerization.

Temperature dependence of β-elimination reaction of WSP

The temperature sensitivity of β-elimination in the WSP fractions could be described adequately using the Arrhenius model (Figure 10). The estimated E_a values were in the range 82.9 to 129.3 kJ/mol (Table 4) and showed no specific trend with changing pretreatment condition. Closely matching this was the estimated activation enthalpies (112 to 127 kJ/mol) for WSP in cucumber (Krall and McFeeters 1998). More so, in trying to evaluate the effect of pretreatment conditions on texture degradation of thermally processed carrots, Sila and others (2004) predicted comparable E_a values (105.6 to 176.0 kJ/mol).

Influence of pH on β-elimination reaction kinetics

Softening of fruits/vegetables during heating is influenced by the pH of the environment. Table 5 summarizes the effect of pH (pH 3.5, 6.5. and 9) on β-elimination kinetics of WSP. Increasing the

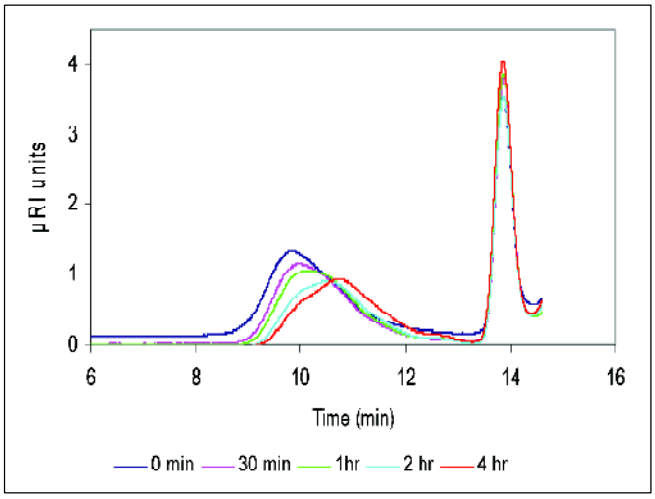

Figure 5—Thermal (110 °C) depolymerization pattern of sodium carbonate–soluble pectin (NSP)

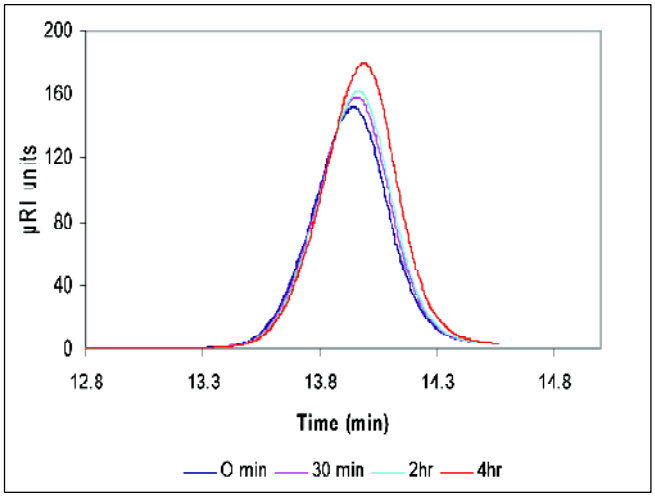

Figure 6—Thermal (110 °C) depolymerization pattern of chelaton-soluble pectin; only change in oligomers is shown.

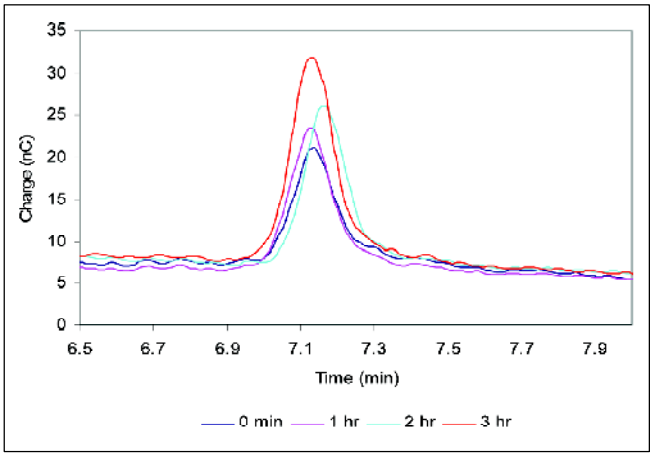

Figure 7—The influencing of processing time on the depolymerization of chelator soluble pectins using pulsed amperometry : processing temperature 110 °C

Table 4—Influence of pretreatment conditions on β-elimination kinetics of water-soluble pectin[a]

Sample pretreatment condition	β-elimination kinetics [unsaturated galacturonides (m*M*/min)/g AIR]			Ea value (kJ/mol)
	90 °C	100 °C	110 °C	
Non-pretreated	**0.024 (± 0.002)[b]**	**0.059 (± 0.001)**	**0.194 (± 0.016)**	**122.6 (± 11.0)**
90 °C, 4 min (HTB)	0.023 (± 0.001)	0.056 (± 0.003)	0.096 (± 0.008)	82.9 (± 10.6)
60 °C, 40 min (LTB)	0.013 (± 0.003)	0.026 (± 0.002)	0.090 (± 0.004)	111.7 (± 19.4)
LTB + Ca^{+2} (0.5%)	0.011 (± 0.001)	0.030 (± 0.003)	0.081 (± 0.010)	112.9 (± 2.4)
HP + Ca^{+2} (0.5%)	0.008 (± 0.001)	0.019 (± 0.001)	0.071 (± 0.005)	129.3 (± 14.4)

[a]AIR = alcohol insoluble residue; HP = 400 MPa, 60 °C for 15 min; HTB = high-temperature blanching; LTB = low-temperature blanching.
[b]Standard error of regression.

pH within the same pretreatment condition induced a pronounced acceleration of β-elimination (Figure 11 and Table 5). This could be attributed to the ability of hydroxyl ions or alkaline conditions to promote β-elimination reaction (Krall and McFeeters 1998).

Most fruits exhibit a pH > 4.5, making them very vulnerable to β-elimination. This makes the pH of the system a critical process parameter during thermal processing of pectin-containing foods.

Influence of monovalent and divalent salts on β-elimination reaction kinetics

The nature and quantity of ions and salts in plant tissues affect heat degradation of pectin consequently affecting the textural attributes of the tissues. An illustration of the effect of monovalent (NaCl) and divalent ($CaCl_2$) salts on β-elimination of WSP from carrots is given in Figure 12. A small concentration of NaCl provoked a sudden increase in the concentration of unsaturated galacturonides. Further increasing the concentration of NaCl did not significantly decrease the extent of β-elimination. Literature shows that heat degradation of CSP is favored by the addition of NaCl (Sajjanantakul and others 1993). Our results are consistent with the increased texture degradation of carrots observed when NaCl was used. Van Buren (1984) showed that 1 *M* Na^+ ions gave rise to about 30% reduction in tissue firmness.

On the other hand, low concentrations of divalent salts depicted

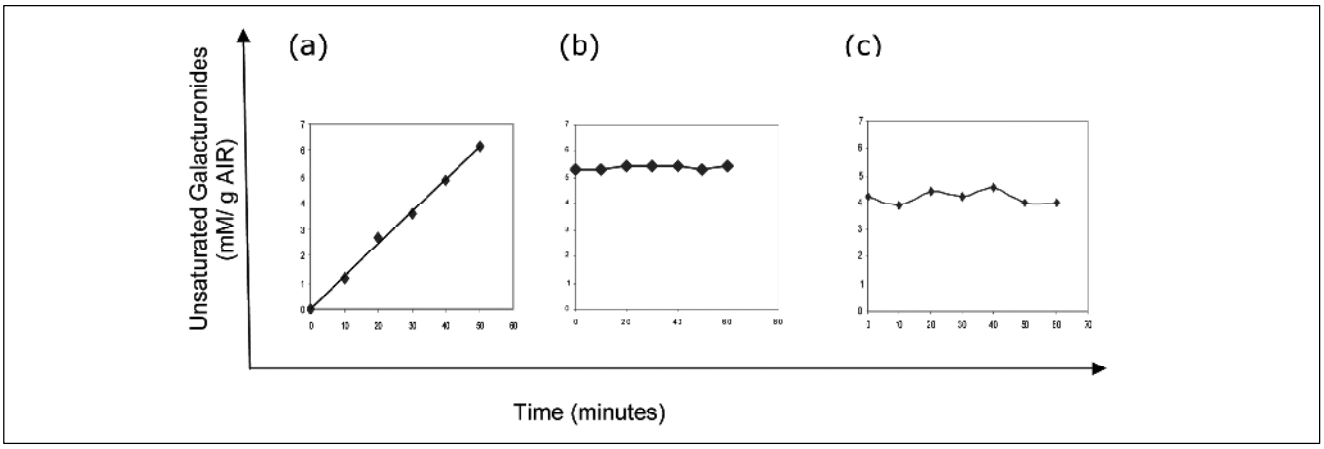

Figure 8—The effect of heat on β-elimination kinetics of (a) water-soluble pectin, (b) chelaton-soluble pectin, (c) sodium carbonate–soluble pectin obtained from non-pretreated carrot samples; processing temperature (110 °C).

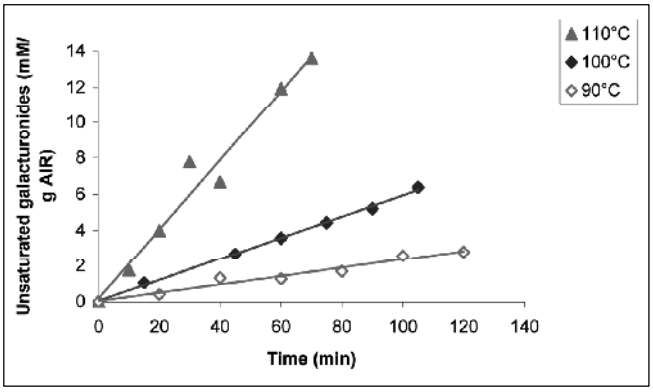

Figure 9—β-elimination kinetics of water-soluble pectin (WSP) obtained from non-pretreated carrot samples

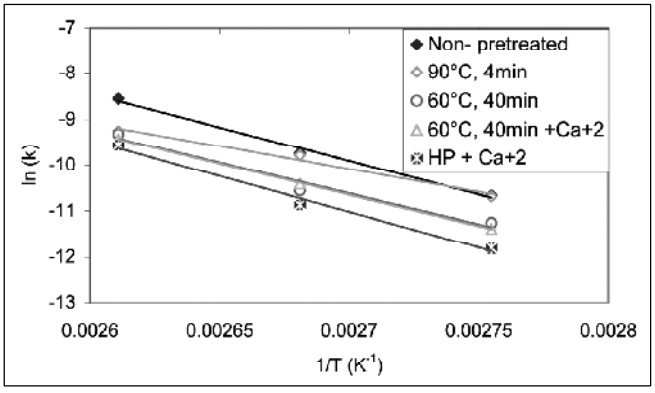

Figure 10—Temperature dependence of the k-values during β-elimination kinetics of carrot pectins

Table 5—Influence of pH on β-elimination kinetics of water-soluble pectin at a constant temperature (100 °C)[a]

	β-elimination kinetics (m*M*/min/g AIR)		
Sample pretreatment	**pH 3.5**	**pH 6**	**pH 9**
Non-pretreated sample	0.011 (± 0.002)	0.059 (± 0.001)	0.200 (± 0.016)
High pressure + Ca^{2+}	0.014 (±0.001)	0.019 (±0.001)	0.038 (±0.002)

[a]AIR = alcohol insoluble residue.

a similar effect; however, at higher concentrations (>0.05 *M*), a noticeable decline in β-elimination was observed. This could be attributed to the ability of divalent salts to form cross-links with the pectin chain. Differences in binding ability between cations and pectin carboxyl groups possibly contribute to the enhancement differences between monovalent and divalent cations. WSP from non-pretreated and high-pressure pretreated combined with calcium-soaked samples showed comparable results. In summary, there exists an important phenomenon in which salts and pectin interact influencing the texture of the product.

Correlations between the texture of thermally processed carrots and β-elimination kinetics of WSP

The relationship between the texture of thermally processed carrot and β-elimination kinetics of WSP is illustrated in Figure 13. There was a strong negative correlation ($r < -0.96$) between residual tissue hardness and the k-values of the β-eliminative degradation of the isolated WSP fraction. An increased β-elimination (increasing k-values) resulted in decreased texture preservation. The critical importance of process temperature in the texture/β-elimination relationship was emphasized by the shifts in the correlation curves. As a result, optimized process design and intricate structural engineering of pectin are the essential tools for new value-added fruits and vegetable products.

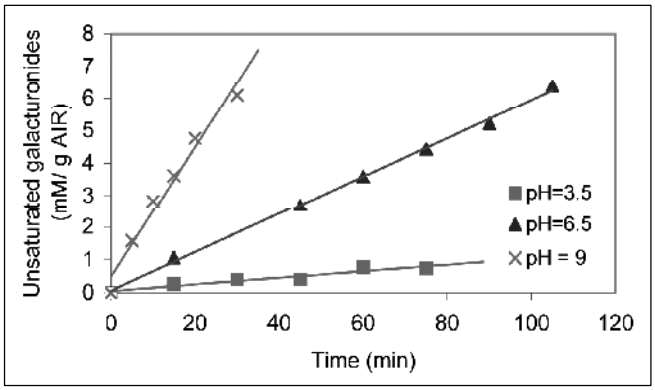

Figure 11—Effect of pH on β-elimination kinetics of water-soluble pectin (WSP) obtained from non-pretreated sample

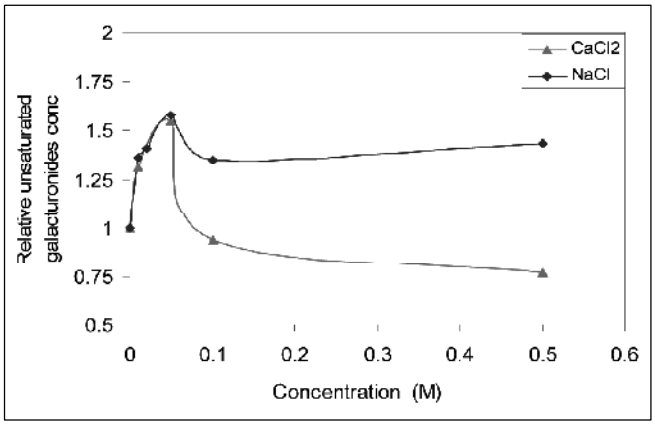

Figure 12—Effect of salt concentration on β-elimination kinetics of water-soluble pectin (WSP) obtained from non-pretreated sample (control)

Conclusions

Thermal texture degradation in carrots can partly be explained by pectin solubilization and the accompanying β-elimination reaction. Subjecting carrot tissues to optimized pretreatment conditions before thermal processing can lead to a significant reduction in texture loss. In situ modification of carrot pectin induces decreased solubilization of pectin besides decelerating β-elimination. Because pectin solubility and β-elimination are dependent on the degree of methoxylation, targeted control of the DM by enhancing PME activity is quite imperative. The depolymerization of the highly methoxylated WSP, which is markedly pronounced, is strongly correlated with thermal texture loss in carrots. This study indicates that pectin engineering is the key to understanding and optimizing the texture of thermal processed carrot. This knowledge could provide a valuable contribution to enhancing the quality of other plant based foods.

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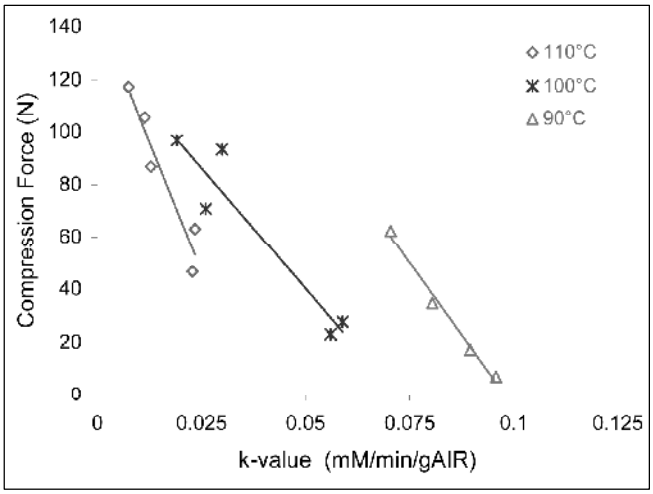

Figure 13—Relationship between residual texture of thermally processed carrots and the β-elimination kinetics (k-values) of water-soluble pectin (WSP); results are taken from the different pretreatment conditions studied (Figure 2 and Table 4).

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