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Pectin Fraction Interconversions: Insight into Understanding Texture Evolution of Thermally Processed Carrots

DANIEL N. SILA, EUGENIE DOUNGLA, CHANTAL SMOUT, ANN VAN LOEY, AND MARC HENDRICKX*

Center for Food and Microbial Technology, Laboratory of Food Technology, Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, B 3001 Heverlee, Belgium

In situ changes in pectin fractions for thermally processed carrots were related to textural changes. The texture of pretreated and subsequently thermally processed carrot disks was determined. Alcohol insoluble residue (AIR) was extracted from the pretreated and thermally processed tissues. The AIR was characterized in terms of the degree of methylation (DM) and changes in pectin fractions. Distinct differences in texture and DM were observed during thermal processing. Pretreatment conditions that induced a significant decrease in DM showed better textures. Demethoxylation caused interconversion of pectin fractions, water soluble pectin (WSP) changing into water insoluble pectin [chelator (CSP) and alkali (NSP) soluble pectin]. This process was reversed during cooking accompanied by remarkable alterations in molecular weight (MW) distribution patterns. The WSP depicted polydisperse MW distribution patterns, strongly dependent on the pretreatment condition. Confirmatory results of interconversions of pectin fractions (WSP, NSP) were demonstrated by the MW distribution patterns and neutral sugar profiles. All thermal related transformations of pectin structural parameters were decelerated by lowering the DM.

KEYWORDS: Degree of methylation; molecular weight distribution; neutral sugars

INTRODUCTION

Pectic polysaccharides, a group of complex structural polymers of the plant cell wall, are critical in many quality-related aspects of fruits/vegetables and other plant-based foods (*1*). Three major pectic polysaccharides (homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II) are thought to occur in all primary cell walls (*2*, *3*). Homogalacturonans (HG) are a family of unsubstituted galacturonic acid residues which can be methyl-esterified at C-6 and/or acetylated on O-2 and O-3 (*4*). Rhamnogalacturonan I (RGI) comprises a highly diverse population of spatially and developmentally regulated polymers, whereas rhamnogalacturonan II (RGII) represents a highly conserved and stable pectic domain (*5*). The RGI is structurally characterized by a sequence of alternating rhamnose and galacturonic acid residues (*6*, *7*). Acetylation is possible on the galacturonic acid residues in RGI; however, the presence of methyl esters is doubted (*8*). Some of the rhamnosyl residues may be decorated with arabinan and/or galactan side chains. Other minor structural elements such as xylogalacturonans are present (*9*). More recent structures of pectin indicate the HG as side chains of the RGI backbone (*3*, *4*).

Despite the fine structure of various pectic constituents being well-known, the interaction of the structural elements into macromolecular structures is still open for discussion. Specific biochemical and/or chemical transformations in relation to pectin occur during processing. During thermal processing, pectic polysaccharides exhibit marked changes in the degree of esterification, the degree of polymerization (molar mass distribution), neutral sugar content, and solubility characteristics. In the case of low-acid foods, part of these changes, which manifest as texture changes of plant-based foods, are directly related to the β-elimination reaction, one of the principal processes affecting pectin solubility and degradation (*10*−*12*). In this context, it has been shown that preprocessing techniques such as Ca^{2+} infusion, mild thermal pretreatments, high-pressure pretreatment, and exogenous pectinmethylesterase (PME) infusion prior to the actual thermal process can largely improve the texture of thermally processed plant-based foods (*12*−*18*). To improve the structural properties of processed plant-based foods, PME should be activated, leading to de-esterified homogalacturonan pectin chains that cross-link with divalent ions (Ca^{2+}), limiting the β-elimination reaction (*19*).

The complexity of plant tissues makes it difficult to identify with certainty in situ mechanisms determining thermal texture degradation (*20*). Consequently, model systems such as isolated pectin fractions or any other suitable cell wall material can be used reliably to illustrate structural and compositional changes during thermal processing. Understanding the mechanisms determining texture evolution during thermal processing requires fingerprinting the heterogeneity of matrix polymers, particularly pectin, during processing. A total mechanistic insight can be

* Author to whom correspondence should be addressed (telephone 32 16 321572; fax 32 16 321960; e-mail marc.hendrickx@biw.kuleuven.be).

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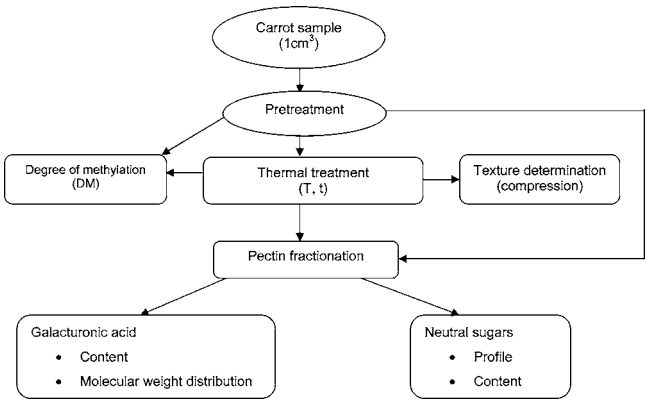

Figure 1. Schematic overview of the experimental setup and research strategy.

achieved by exploring the relationship between (pre-)processing conditions, the activity of texture-related enzymes, changes at the molecular level of matrix polysaccharides, microscopic changes at the tissue level, and the resulting macrostructural properties of the end products. In this study, using carrots (*Daucus carota*) as a food model, a mechanistic insight into the relationship between (pre-)processing conditions (pretreatment conditions and thermal processing), endogenous PME activity, in situ transformations in pectin (pectin fractions, degree of methylation, molecular weight distribution, neutral sugar), and the perceived structural properties (texture) of carrots is explored.

MATERIALS AND METHODS

The approach employed in this research involved a careful experimental design, maintaining variations in chemical characteristics minimally during analysis while using methods that would fingerprint differences at the molecular level that would clarify the link between composition, structure, and physical properties of pectin-containing food systems (**Figure 1**).

Vegetable Materials. Carrots (*D. carota* var. Nepal de Namur) were bought from a local shop in Belgium and stored at 4 °C before processing and analysis.

Pretreatments. The cores of carrots were chopped into disks of 10 mm height and 12 mm diameter before calcium, thermal, and/or high-pressure pretreatment. All pretreatment conditions were carried out as described by Sila et al. (*18*). The pretreatment conditions included low-temperature blanching (LTB; 60 °C, 40 min), LTB combined with 0.5% (w/v) calcium chloride soaking (LTB + Ca^{2+}), high-pressure pretreatment (HP; 400 MPa, 60 °C, 15 min), HP combined with 0.5% (w/v) calcium chloride soaking (HP + Ca^{2+}), and control (non-pretreated sample).

Thermal Processing. For texture studies, carrot disks (*10*) encapsulated in stainless steel tubes (110 mm long, 13 mm internal diameter, and 1 mm thickness) filled with brine (demineralized water or calcium chloride solution depending on the pretreatment) were subjected to thermal treatments (100 °C) in a temperature-controlled oil bath (synthetic oil: flash point = 227 °C, viscosity at 20 °C = 100 mPas, density at 20 °C = 0.86 kg/dm^3, and specific heat capacity = 1.96 kJ/kg·K). When galacturonic acid profiles in the brine solutions were determined, a lower sample size was used and the ratio of the sample to brine was maintained at 1:1.5 (w/v). 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 a stainless steel 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 prior to analysis. All of the other samples were further heat treated (100 °C) using various time intervals (withdrawing a sample after every 30 min during 2 h) and cooled in an ice bath, and subsequently the residual textures were determined.

Texture Measurements. Textural properties were measured using a TA-XT2 texture analyzer (Stable Micro Systems, Surrey, U.K.) equipped with a 25 kg force cell and a cylindrical flat head aluminum probe of 25 mm diameter. Texture was expressed as hardness, which is the peak force of the first 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, two independent pretreatments were carried out each with 10 carrot disks. The mean compression force value of the carrot cylinders was calculated. The variations in the 10 measurements were <10%.

Extraction of Alcohol Insoluble Residues. Alcohol insoluble residue (AIR) samples from carrot tissues after different pretreatments and thermal processing were prepared following the procedure described by McFeeters and Armstrong (*21*). Approximately 10.0 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 filter no. 413, ∅ 110 mm, made in the 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 stored in a desiccator until analysis. For each pretreatment condition, a stock of AIR sample was collected prior to subsequent characterization and fractionation experiments.

Determination of Degree of Methylation (DM). The DM of AIR was determined for all pretreated and thermally processed samples. First, anhydrous galacturonic acid was determined quantitatively by the colorimetric hydroxy phenyl phenol method (*22*) with an UV−vis spectrophotometer (Ultrospec 2100 pro from Amersham Biosciences, Uppsala, Sweden) at 520 nm.

The second step involved determining the amount of methanol spectrophotometrically (412 nm) according to the method of Klavons and Benett (*23*). Here, the pectin methyl esters in 20 mg of AIR were saponified using 2 M $NaOH_{(aq)}$ for 1 h at 20 °C followed by neutralization using an equal volume of 2 M $HCl_{(aq)}$ at 25 °C within 15 min. The resulting methanol was oxidized to formaldehyde using alcohol oxidase (1 unit per 1 mL of sample) and complexed to a colored compound at 58 °C for 15 min with 0.02 M pentandione in 2 M ammonium acetate and 0.05 M acetic acid. Finally, the DM 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.

Fractionation of Carrot Pectin. Water soluble pectin (WSP) was fractionated using a modified hot water extraction procedure according to the method described by Braga and co-workers (*24*). In this method, AIR samples from pretreated and thermally processed carrot tissues were weighed exactly (0.5 g). The samples were homogenized by stirring 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 & Schuell), keeping the filtrate for subsequent analysis. The residue was further fractionated in 100 mL of 0.05 M cyclohexane-*trans*-1,2-diamine tetraacetic acid (CDTA) in 0.1 M potassium acetate (pH 6.5) for 6 h at 28 °C (*25*). 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 (*25*). 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 of the pectin fractions (WSP, CSP, and NSP) were analyzed for galacturonic acid content prior to lyophilization followed by determination of molecular weight distribution patterns and neutral sugar content. Lyophilization was done using a freeze-dryer [Christ alpha (2-4)], and the dry powder was kept in a desiccator until subsequent analysis.

High-Performance Size Exclusion Chromatography (HPSEC). 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 L × 7.5 mm ∅, 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) in combination with a TSK guard column. A 25 μL injection loop was used. 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 = 188−788 000), which have structure and hydrodynamic characteristics similar to those of polygalacturonic acid, were used. Deionized water (organic free = 18 ΩM resistance) supplied by a Simplicity Millipore water purification system was used to prepare eluents and samples. Once the standard curve was established, monogalacturonic acid was used daily to validate the system.

All of the lyophilized samples (WSP and NSP) were dissolved in demineralized water (0.1% w/v) and extensively dialyzed in demineralized water. In the case of the CSP fractions, extensive dialysis in 0.1 M $NaCl_{(aq)}$ followed by dialysis in demineralized water was done. Brine solutions were also dialyzed in demineralized water. The samples were then adjusted to a concentration of 0.05 M $NaNO_3$ before analysis. Samples were injected in duplicate.

Neutral Sugar Composition. All of the fractionated samples were analyzed for neutral sugar content and composition. Approximately 0.01 g of lyophilized pectin fraction samples (WSP, CSP, and NSP) was weighed exactly and digested in 4 M trifluoroacetic acid (TFA) at 110 °C for 1.5 h. The samples were immediately cooled in an ice bath. The TFA was evaporated under vacuum from the cold samples using a rotavapor at 40 °C for 40 min. TFA-free samples were diluted in a 10.0 mL volumetric flask and analyzed for neutral sugars using high-performance anion exchange chromatography.

High-Performance Anion Exchange Chromatography (HPAEC). HPAEC was carried out to fingerprint the neutral sugars profiles in pectin. This 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). 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) deonized water, 18 ΩM (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; 46→60 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 L-xylose) were used for identification and quantification. The analysis was done in duplicate.

RESULTS

Influence of Pretreatment Conditions on Texture Evolution of Thermally Processed Carrots. Thermosoftening of carrots increased with increasing processing time for all of the pretreatment conditions studied (**Figure 2**). The impact of heat as a function of processing time was strongly dependent on the pretreatment condition applied. Inferior textural characteristics were observed for non-pretreated carrot samples as compared to pretreated ones. Combined pretreatment conditions demonstrated excellent thermal stability, the best case scenario being exhibited by high-pressure pretreatment condition combined with calcium soaking.

Modifications in the methyl ester content of carrot tissues were used to elucidate the observed differences in textural properties (**Table 1**). Pretreatment conditions that resulted in the most significant modifications in DM of AIR revealed superior textural properties (reduced thermosoftening). Combining pretreatment conditions with calcium soaking resulted in lower DM. In fact, the addition of salts, particularly low calcium concentrations, boosts PME activity endogenously, leading to increased demethoxylation, which in turn favors the formation of a pectin−calcium complex (*26*). In addition, because methyl esters are the main driving force of the β-elimination reactions, reductions in the DM of samples triggered decelerations in the β-elimination rate, consequently reducing thermal softening. These results complement our previous work (*12*, *18*). Thermal processing resulted in a further decline in the DM of AIR samples, most likely through β-elimination degradation of the methylated regions (*18*, *27*). However, other unknown but important cell wall related mechanisms might have played a role.

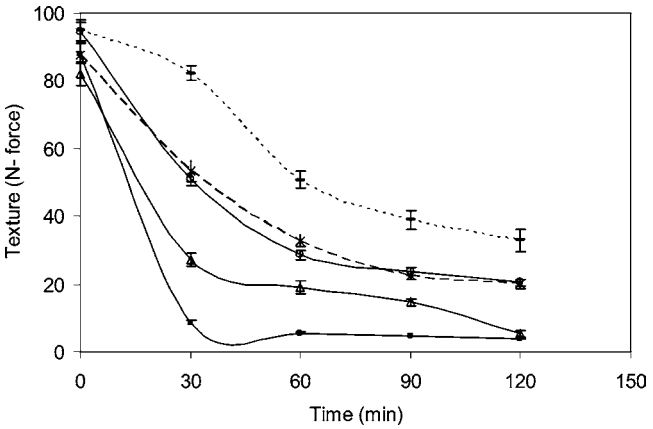

Figure 2. Changes in hardness of pretreated carrot disks after thermal processing (100 °C) at various times: control (■) = non-pretreated sample (time zero sample); LTB (△) = low-temperature blanching (preheating at 60 °C for 40 min); LTB + Ca^{2+} (∗) = low-temperature blanching followed by calcium 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.

Changes in Pectin Fractions during Preprocessing and Subsequent Thermal Processing. The turnover of matrix polysaccharides during preprocessing and subsequent thermal processing was related to the observed textural properties. The total galacturonic acid content obtained from summing all of the pectin fractions did not change considerably (0.6−1.0 mmol of galacturonic acid/g of AIR); however, a significant change in the proportions of pectin fractions was noted (**Table 2**). The recovery of galacturonic acid in the AIR was >90%. Non-pretreated carrots (control) contained predominantly water soluble pectin ($WSP_{control} \approx 55\%$), a substantial amount of sodium carbonate soluble pectin ($NSP_{control} \approx 32\%$), and a low amount of chelator soluble pectin ($CSP_{control} \approx 13\%$). Pretreatment conditions (100 °C, 0 min) resulted in the transformation of WSP into insoluble pectin (CSP and NSP). As in texture degradation, the extent of the modification was strongly related to the type of pretreatment condition applied and, consequently, the DM of AIR. A decrease in DM lowers the solubilization rate of pectin in water (*12*). Interestingly, during subsequent thermal processing, a thermoreversible mechanism resulted in significant changes in pectin fractions; the insoluble pectin fractions (CSP and NSP) were reconverted into water soluble pectin. This is clearly evident from the increasing proportions of the WSP fraction with increasing thermal severity, which is paralleled by declining proportions of insoluble pectin (NSP). This dynamic conversion of pectin was less obvious in the CSP fraction. Earlier on, we demonstrated the modification of the WSP into the NSP fraction (*12*). The phenomenon of increasing WSP content during thermal processing has been reported in

Table 1. Degree of Methylation of Carrot Disks during Pretreatment Conditions and Subsequent Thermal Processing (100 °C)[a]

	degree of methylation (%)				
pretreatment condition	0 min	30 min	60 min	90 min	120 min
non-pretreated sample (control)	69.8	64.8	64.7	62.5	62.1
low-temperature blanching (60 °C, 40 min)	57.0	53.2	52.2	52.2	51.1
low-temperature blanching + Ca^{2+} soaking	47.0	42.9	42.7	42.2	40.3
high pressure (400 MPa, 60 °C, 15 min)	55.3	53.6	46.7	46.5	45.6
high pressue + Ca^{2+} soaking	43.2	42.9	42.7	41.4	41.3

[a] The standard errors of all of the reported values were <10%. $n = 6$.

Table 2. Changes in Pectin Fractions during Pretreatment Conditions and Subsequent Thermal Processing (100 °C)[a]

	carrot pectin fraction (%)														
	0 min			30 min			60 min			90 min			120 min		
sample	WSP	CSP	NSP	WSP	CSP	NSP	WSP	CSP	NSP	WSP	CSP	NSP	WSP	CSP	NSP
control	54.9	12.7	32.4	72.1	12.7	15.2	75.6	14.2	10.2	75.2	13.0	11.8	77.0	11.7	11.3
low-temperature blanching	36.2	14.4	49.3	50.8	17.6	31.6	51.3	18.1	30.6	56.3	20.9	22.8	55.0	21.7	23.3
low-temperature blanching + Ca^{2+}	13.1	9.2	77.7	31.4	14.9	53.7	35.3	17.0	47.7	39.8	16.8	43.3	40.3	20.6	39.1
high pressure	26.7	16.4	56.9	45.6	16.8	37.6	47.8	18.0	34.2	55.6	16.9	27.5	56.5	16.6	26.9
high pressure + Ca^{2+}	11.3	9.1	79.6	15.4	12.0	72.6	20.2	16.2	63.6	23.0	17.1	59.9	28.4	18.9	52.3

[a] The standard errors of the reported values were <10%.

some plant-based foods (*10*, *28*−*30*). Non-pretreated samples portrayed the most pronounced changes in pectin fractions and textural attributes. Combining pretreatment condition with calcium soaking reduced the thermoconversion rate of pectin fractions, a characteristic that improved texture. Within the study domain, high-pressure pretreatment combined with calcium soaking exhibited the most thermotolerant characteristics. These results indicate that a substantial solubilization and degradation of matrix polysaccharides occurs during thermal processing. Nevertheless, it is unlikely that all of the observed changes in texture of plant-based foods can solely be explained by the changing solubility patterns of pectin fractions. Therefore, it was important to have an insight into in situ changes in molecular weight distribution patterns of pectin fractions with changing pretreatment condition.

Strong correlations ($r_{WSP} < -0.97$, $r_{NSP} > 0.98$) between the proportionate changes in pectin fractions and the resulting changes in residual textural properties were obtained (**Figure 3**). The complementary characteristics of the WSP and the NSP fraction were indicated by the counteracting pectin fraction contours for each pretreatment condition. This clearly indicates the importance of pectin solubility properties in relation to textural characteristics.

In Situ Changes in Molecular Weight (MW) Distribution of Matrix Polysaccharides. Further insight to understand the mechanism of texture degradation in plant-based foods was provided by the in situ changes in the degree of polymerization of matrix polysaccharides during thermal processing. High molecular weight polymers in native pectin fractions depicted homogeneous molecular weight distribution patterns (**Figure 4**). Each fraction was characterized by a distinct molecular weight average (WSP_{138kDa}, CSP_{289kDa}, and $NSP_{>788kDa}$), the WSP fraction depicting the lowest.

During thermal processing, pronounced changes in polymer concentrations and MW distribution patterns were apparent. The WSP fractions showed increasing concentrations of solubilized polymers characterized by a polydisperse MW range that covered the MW spectrum of all pectin fractions (WSP, CSP, and NSP). This was clearly depicted by two unresolved peaks of high molecular weight polymers (**Figure 5**), indicating

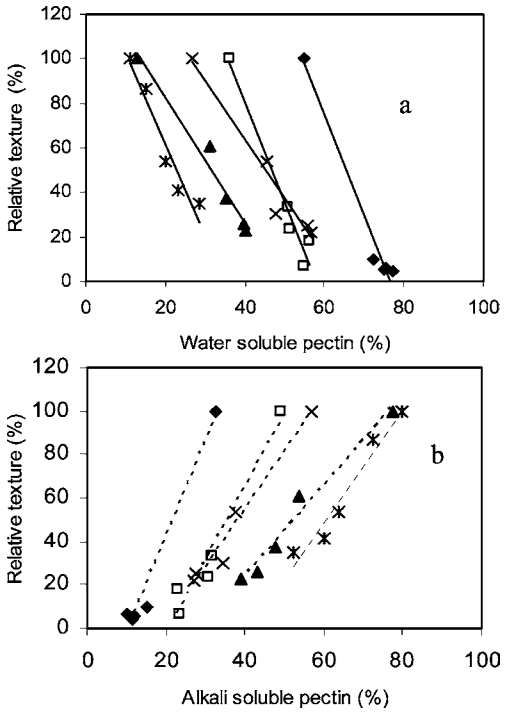

Figure 3. Correlation between the relative textural characteristics (hardness) and the proportion of (**a**) water soluble pectin (—) and (**b**) alkali soluble pectin (- - -) during thermal processing: control (◆) = non-pretreated sample; LTB (□) = low-temperature blanching (preheating at 60 °C for 40 min); LTB + Ca^{2+} (▲) = low-temperature blanching 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.

solubilization of both water soluble (WSP) and insoluble (CSP and NSP) pectin. The early eluting peaks represented the proportions of the CSP and NSP fractions, which were thermosolubilized together with the WSP fraction, whereas the later eluting peaks illustrated transformations in the WSP fractions (shifts toward low molecular weight fragments). This clearly

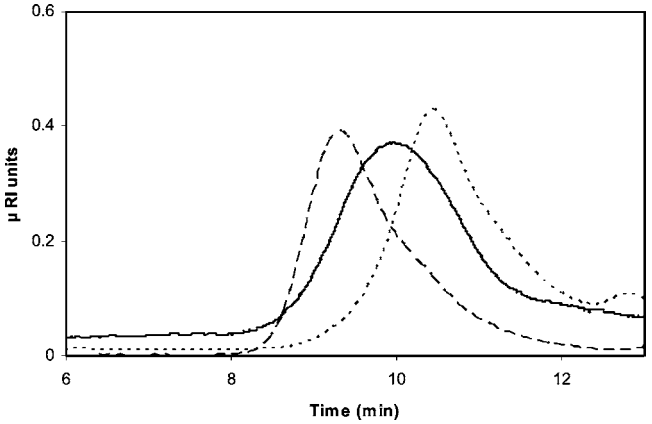

Figure 4. Differences in average molar mass of high molecular weight carrot pectins in non-pretreated samples before thermal processing: water soluble pectin (•••), chelator soluble pectin (—); sodium carbonate soluble pectin (- - -).

demonstrated a dynamic change in pectin fractions during thermal processing, a proof for the increasing concentrations of WSP. Thermosolubilization of pectin was greatly influenced by the type of pretreatment condition used. Non-pretreated carrot samples showed an extensive MW turnover (**Figure 5a**). The intensity of the transformations diminished with decreasing DM of AIR (**Figure 5b**,**c**).

A complementary trend in changes in molecular weight distribution was observed in the NSP fraction (**Figure 6**). The early-eluting peaks depicted the changes in high MW polymers, whereas the later-eluting peaks illustrated changes in low MW polymers. Unlike the WSP fraction, there were no clear shifts toward low molecular weight polymers. However, decreasing quantities of homogeneously distributed polymers were evident with increasing thermal severity. This counteracted the trend observed in the WSP fractions, thus verifying the thermal interconversion of pectin fractions (**Table 2**). It should be noted that pectin fractions exhibit different heat sensitivity, the most thermolabile being the WSP fraction (*12*).

Changes in Molecular Weight Distribution of Matrix Polysaccharides in Cooking Water. Analysis of the molecular weight distribution of matrix polymers in the cooking water provided more information on the degree of polymerization of thermal solubilization fragments. Diverse populations of thermal fragmentation products with discrete differences in MW distribution patterns were noticeable with changing pretreatment condition (**Figure 7**). Generally, the concentration of the extracts increased with increasing thermal processing time. In non-pretreated samples, a pronounced population of high molecular weight polymers with an average MW similar to that of the WSP fraction (intermediate peak) was observed (**Figure 7a**). As thermal processing progressed, increasing proportions of high MW polymers (early peak) comparable to the insoluble pectin fractions (CSP and NSP) were evident. In addition, minor fractions of undialyzed low molecular weight fragments (elution time ≈ 14 min) were detected. In the case of low-temperature blanching, the concentration of the solubilized polymers decreased when compared to that of non-pretreated samples. The extract contained both high and low molecular weight fragments (**Figure 7b**). Interestingly, high-pressure pretreatment combined with calcium soaking revealed minimal solubilization of high MW polymers; the bulk of the extracts were of low molecular weight (**Figure 7c**).

Pectin solubilization reduces the cementing ability of the polymer, prompting cell separation, a process that is closely

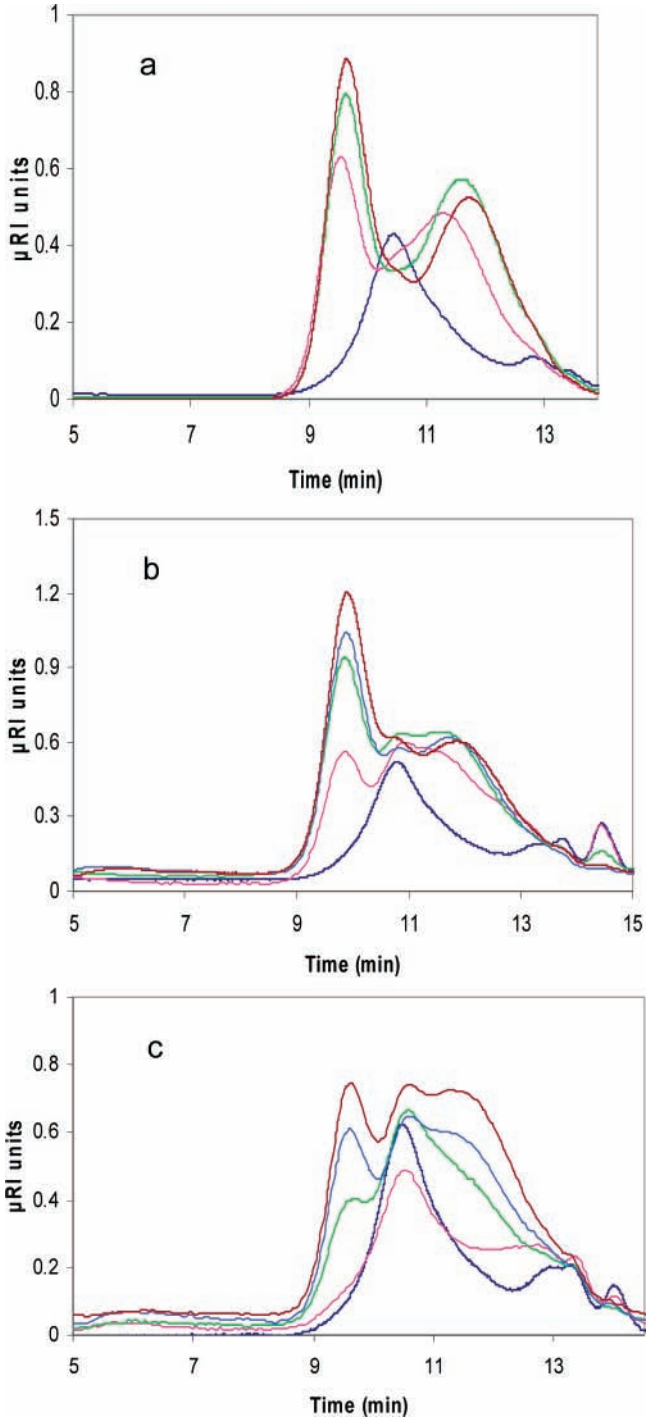

Figure 5. In situ changes in molecular weight distribution of water soluble pectin (0 min, black; 30 min, magenta; 60 min, green; 90 min, blue; and 120 min, red) in carrot samples that were (**a**) non-pretreated, (**b**) low-temperature-blanched, and (**c**) high-pressure-pretreated combined with calcium soaking.

linked to thermosoftening. These results strongly indicate the fundamental role of in situ modifications of matrix polymers prior to thermal processing in preserving the textural properties of plant-based foods.

Influence of Pretreatment Conditions and Thermal Processing on Neutral Sugar Content. Pectic polysaccharides contain a diverse neutral sugar composition that is associated with the "hairy region". Ion exchange chromatography combined with pulsed amperometric detection provided a reliable means of identifying and quantifying neutral sugars.

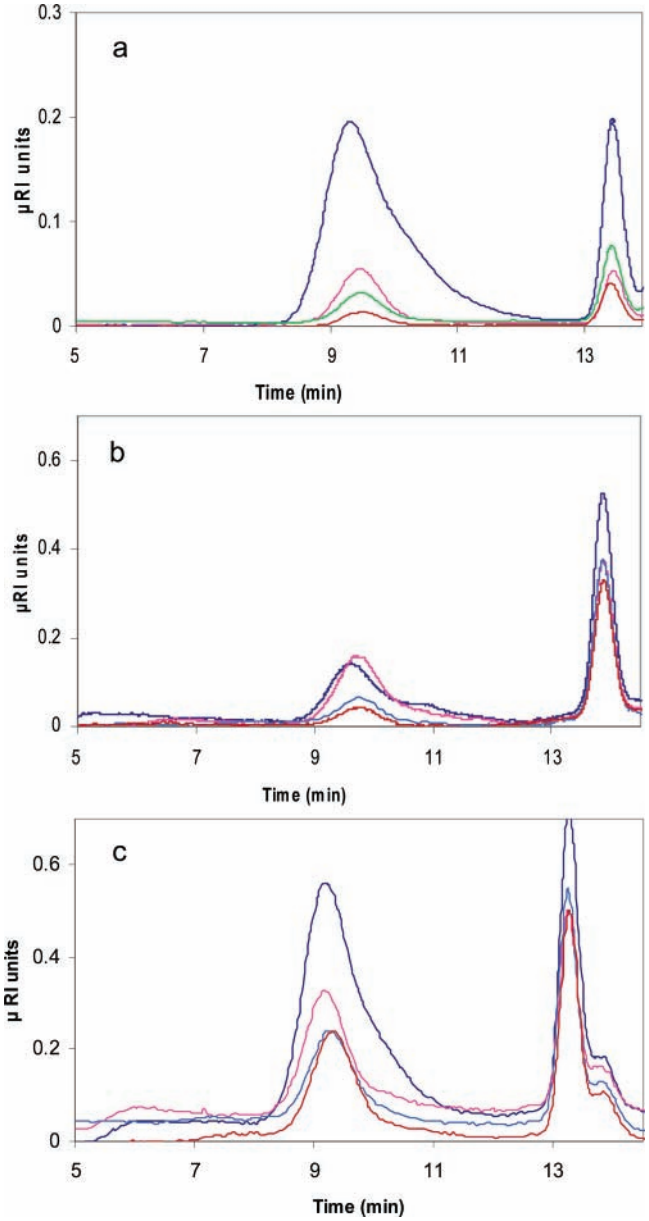

Figure 6. In situ changes in molecular weight distribution of sodium carbonate soluble pectin (0 min, black; 30 min, magenta; 60 min, green; 90 min, blue; and 120 min, red) in carrot samples that were (**a**) non-pretreated, (**b**) low-temperature-blanched, and (**c**) high-pressure-pretreated combined with calcium soaking.

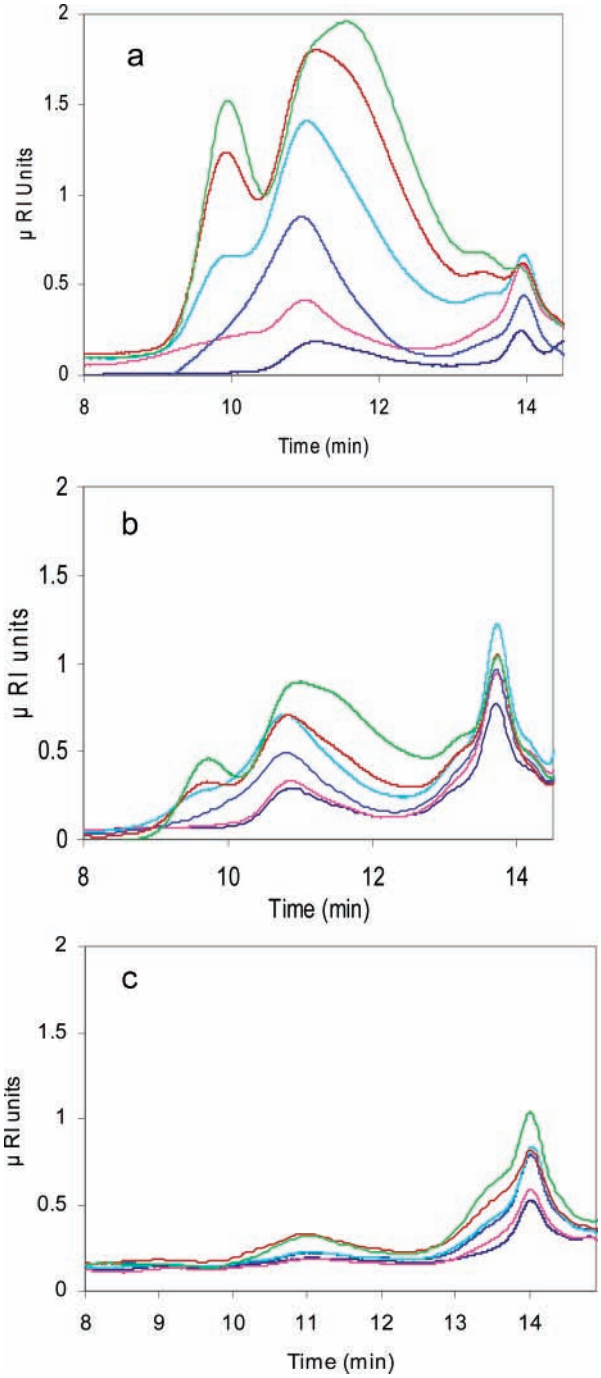

Figure 7. Changes in molecular weight distribution of solubilized matrix polymers in cooking water for carrot samples that were (**a**) non-pretreated (0 min, black; 10 min, magenta; 20 min, blue; 30 min, aqua; 40 min, red; and 60 min, green), (**b**) low-temperature-blanched (0 min, black; 10 min, magenta; 20 min, blue; 30 min, aqua; 40 min, red; and 60 min, green), and (**c**) high-pressure-pretreated combined with calcium soaking (0 min, black; 10 min, magenta; 20 min, blue; 30 min, aqua; 50 min, red; and 70 min, green).

Alterations in neutral sugar content as influenced by pretreatment conditions and thermal processing are summarized in **Tables 3**−**5**. In the WSP fractions, glucose, possibly a breakdown product of non-pectic polymers or a residue of soluble sugars that were not completely removed by ethanol in the AIR, was the most predominant before thermal processing. Pectin-related neutral sugars (rhamnose, arabinose, and galactose) occurred at high amounts except rhamnose (**Figure 8**). Xylose and an unidentified sugar (X; retention time ≈ 4.7 min) were also present. In the context of our study, changes in only pectin-related neutral sugars (galactose, arabinose, and rhamnose) are discussed. Clearly, all of the WSP fractions showed an increasing concentration of pectin-related neutral sugars (rhamnose, arabinose, and galactose) with increasing thermal processing time in accordance with pectin solubilization. The percentage of rhamnose in the total neutral sugars of the WSP fraction before thermal processing, in all cases, was approximately 2% but increased to about 5% during the entire cooking process (100 °C for 120 min). Arabinose and galactose occurred at relatively higher percentages (10−20%) before cooking, and a clear increasing trend in the absolute solubilized amounts with increasing thermal processing time was observed (**Figure 8**). In summary, these results strongly suggested a considerable thermal fragmentation of pectin hairy regions, an indication that part of the fractions solubilized with the WSP fraction contained high amounts of side chains.

Table 3. Changes in Neutral Sugar Composition of Non-pretreated Carrot Samples during Thermal Processing (Standard Error < 10%)[a]

	neutral sugar content of carrot pectin in lyophilized fractions (concentration, mg/g of solid)			
sugar	0 min	30 min	60 min	120 min
	Water Soluble Pectin			
rhamnose	7.4	26.2	27.5	33.5
arabinose	40.4	123.1	134.6	157.6
galactose	57.9	178.1	192.3	232.9
glucose	243.5	156.7	71.8	142.1
xylose	15.2	9.3	8.7	8.5
total	364.4	493.4	434.9	574.6
	Chelator Soluble Pectin			
rhamnose	1.27	1.21	1.03	0.84
arabinose	5.90	4.83	4.32	3.84
galactose	5.77	4.67	3.21	3.06
glucose	0.68	0.40	0.20	0.24
xylose	0.26	0.15	0.13	0.11
total	13.88	11.26	8.89	8.09
	Sodium Carbonate Soluble Pectin			
rhamnose	3.65	ND	3.47	0.50
arabinose	15.50	ND	14.57	2.92
galactose	25.03	ND	23.72	3.61
glucose	0.50	ND	0.00	1.70
xylose	0.55	ND	1.10	1.33
total	45.23	ND	42.86	10.06

[a] ND, not determined.

Table 4. Changes in Neutral Sugar Composition of Low-Temperature-Blanched Carrot Samples during Thermal Processing (Standard Error < 10%)[a]

	neutral sugar content of carrot pectin in lyophilized fractions (concentration, mg/g of solid)				
sugar	0 min	30 min	60 min	90 min	120 min
	Water Soluble Pectin				
rhamnose	5.09	16.57	23.73	25.53	27.85
arabinose	38.51	68.72	110.06	125.56	144.79
galactose	58.96	115.22	170.73	200.59	220.10
glucose	199.52	150.48	121.71	150.47	235.89
xylose	13.25	9.29	9.00	6.88	7.97
total	315.33	360.28	435.23	509.03	636.60
	Chelator Soluble Pectin				
rhamnose	0.51	0.69	0.92	1.25	0.98
arabinose	3.22	2.32	3.70	5.29	4.07
galactose	4.50	2.30	3.84	5.90	3.78
glucose	0.18	0.33	1.07	0.52	0.33
xylose	0.43	0.15	2.30	1.36	0.12
total	8.84	5.79	11.83	14.32	9.28
	Sodium Carbonate Soluble Pectin				
rhamnose	3.46	3.33	0.96	0.64	ND
arabinose	15.72	15.72	4.90	4.05	ND
galactose	22.67	22.40	6.82	3.40	ND
glucose	1.89	1.77	0.71	0.31	ND
xylose	1.73	1.99	0.68	0.46	ND
total	45.47	45.21	14.07	8.86	ND

[a] ND, not determined.

The CSP and NSP fractions were characterized by lower amounts of neutral sugars when compared to the WSP fraction. However, it should be noted that in the CSP fractions, CDTA salt was present in the lyophilized ground powder, thus influencing the absolute pectin amounts weighed per gram. Nonetheless, the fractions were characterized by different neutral sugar profiles and proportions when compared to the WSP fractions. The rhamnose content was higher ($CSP_{rhamnose} \approx 10\%$; $NSP_{rhamnose} \approx 7\%$) before cooking and remained almost constant

Table 5. Changes in Neutral Sugar Composition of High-Pressure-Pretreated and Calcium-Soaked Samples during Thermal Processing (Standard Error < 10%)[a]

	neutral sugar content of carrot pectin in lyophilized fractions (concentration, mg/g of solid)				
sugar	0 min	30 min	60 min	90 min	120 min
	Water Soluble Pectin				
rhamnose	4.40	ND	10.60	12.88	15.70
arabinose	38.94	ND	64.78	68.74	86.81
galactose	62.31	ND	103.81	113.58	139.50
glucose	125.10	ND	110.90	96.86	134.65
xylose	12.33	ND	7.74	0.00	0.00
total	243.08	ND	297.83	292.05	376.66
	Chelator Soluble Pectin				
rhamnose	0.90	1.04	1.50	1.80	1.85
arabinose	0.73	3.69	5.48	6.49	7.37
galactose	0.83	3.54	5.45	7.02	8.46
glucose	0.35	0.38	0.34	0.43	0.58
xylose	2.48	0.14	0.08	0.00	0.22
total	5.29	8.79	12.85	15.74	18.48
	Sodium Carbonate Soluble Pectin				
rhamnose	5.62	4.74	4.59	3.21	1.79
arabinose	22.58	20.46	18.80	14.69	6.43
galactose	29.32	27.45	25.94	20.69	8.26
glucose	0.00	0.90	0.93	1.00	0.15
xylose	0.70	1.24	0.00	0.00	4.31
total	58.22	54.79	50.26	39.59	20.94

[a] ND, not determined.

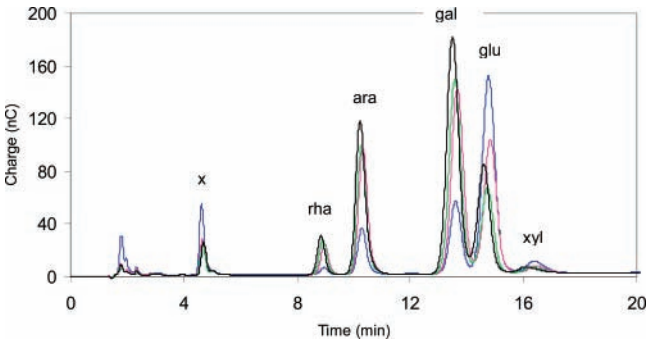

Figure 8. Neutral sugar profile of water soluble pectin (0 min, blue; 30 min, magenta; 60 min, green; and 120 min, black) from non-pretreated carrot samples during thermal processing (100 °C): x, unidentified sugar; rha, rhamnose; ara, arabinose; gal, galactose; glu, glucose; xyl, xylose.

during the cooking process. This suggested a high amount of side chains. In the CSP fractions, arabinose and galactose accounted for approximately 80% of the total neutral sugars. The NSP fractions contained approximately 35% arabinose and about 50% galactose. Interestingly, during thermal processing, a trend that was opposite and complementary to the one observed in the WSP fraction was conspicuous (**Figure 9**). The amounts of neutral sugars in the NSP fraction declined with increasing thermal processing time. Interestingly, the CSP fraction obtained from high-pressure pretreatment combined with calcium soaking showed an increasing trend of neutral sugars. Pretreatment conditions influenced the rate of thermo-solubilization; the absolute amounts of neutral sugars differed with decreasing DM of AIR, but the qualitative trends were similar. Nevertheless, thermal processing caused pronounced changes in pectin structure that were reflected in the profile of the thermal digest products and the corresponding changes in the MW distributions of the fragments. The counterbalancing trends in pectin fractions (content and composition) during

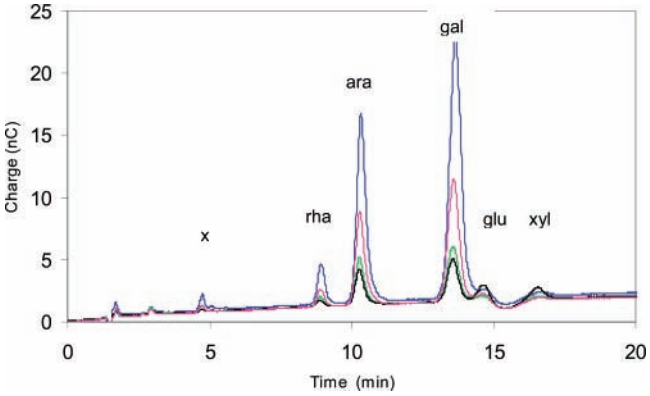

Figure 9. Neutral sugar profile of sodium carbonate soluble pectin (0 min, blue; 30 min, magenta; 60 min, green; and 120 min, black) from non-pretreated carrot samples during thermal processing (100 °C): x, unidentified sugar; rha, rhamnose; ara, arabinose; gal, galactose; glu, glucose; xyl, xylose.

thermal processing appeared to be key material characteristics that could be exploited in engineering better textured plant-based foods.

In conclusion, thermosoftening in carrots is strongly related to the solubility properties of pectin and the accompanying depolymerization mechanisms. In situ modification in the degree of methylation of pectin alters the thermal response of the embedding tissues. In fact, demethoxylation results in decreased thermal texture loss. Combining pretreatment conditions with calcium infusion confers enhanced thermostability of cooked plant-based foods. In carrots, texture degradation is strongly linked to pectin conversion. Pretreatment conditions induce a significant modification in matrix bonding, transforming the water soluble fraction into insoluble pectin. The extent of these changes, which are reversible during subsequent thermal processing, defines the textural properties of the end product. This phenomenon of pectin interconversion is an important material property, which can be tailored in situ and/or in vivo in an attempt to engineer novel textured plant-based foods. For the production of quality/value-added thermally processed foods, pectin structural manipulation and a fundamental insight into effect of the transformations are prerequisites.

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