Influence of Pretreatment Conditions on the Texture and Cell Wall Components of Carrots During Thermal Processing

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ABSTRACT: Diced carrots (*Daucus carota* var. Nerac) were subjected to different pretreatment conditions. The pretreated carrots were subsequently thermally processed in an oil bath (100 °C) and in a static retort (equivalent processes [$F_o \nabla$ 6 min] at 115 °C, 120 °C, and 125 °C). Changes in texture were analyzed as well as changes in the degree of methylation (DM) of pectin. From all the pretreatment conditions tested, high-pressure pretreated carrots (400 MPa, 60 °C for 15 min) exhibited the highest resistance to texture loss. The textural properties were significantly improved when calcium infusion was combined with low-temperature blanching condition (60 °C for 40 min). A significant reduction in the DM of carrot pectin was observed for all pretreatment conditions that resulted in a reduced texture loss after thermal processing. A strong negative correlation ($r \leq -0.90$) exists between the changes in the degree of methylation of carrot pectin and the observed changes in texture.

Keywords: pretreatment, texture loss, degree of methylation, calcium infusion, carrots

Introduction

The textural quality of fruits and vegetables is strongly dependent on cell wall polymers. This consists mainly of insoluble cellulose fibers meshed into a matrix of hemicellulose and pectin. Cellulose and hemicellulose exhibit a distinctive microfibril network via hydrogen bonds, which enhances cell wall rigidity and resistance to tearing. Pectin and hemicellulose confer plasticity and the ability to stretch. In the middle lamella, pectin plays a primary role in intercellular adhesion (van Buren 1979). The unique mixture of pectin, hemicellulose, and cellulose in cell walls, and the changes that occur during ripening and storage are highly implicated for the textural changes observed in plant foods (Abbott 1999).

Changes in the composition of cell wall polymers may result from the action of hydrolytic enzymes, mostly pectinases, and the activities of these enzymes often increase during ripening of fruits (Fischer and Bannett 1991). In carrots, research has been focused on pectinmethylesterase (PME) in relation to its influence on texture (Noriko and others 1997; Tijskens and others 1997; Roy and others 2001; Smout and others 2005). Endogenous polygalacturonase (PG), which depolymerizes PME demethylated pectin molecules, occurs in low amounts in carrots (Stratilova and others 1998). It, therefore, has a minor influence on the texture of carrots. In addition to pectinases, a number of cellulases (glycanases and glycosidases) have also been found to play a role in plant cell wall metabolism (Seymour and Gross 1996; Harker and others 1997). More recently, during cell growth, development and maturation in plants, the role of expansins in disruption of hydrogen bonds and the depolymerization of matrix glycans has been recognized in texture studies (Civello and others 1999; Cosgrove 2000; Brummell and Harpster 2001).

The most important changes in texture during thermal processing are related to the structure of both protopectin and pectin (Jarvis 1984; Van Buren 1986; Mc Cann and Roberts 1991; Carpita and Gibeaut 1993). Cell separation has been reported in cooking of carrots (Ng and Waldron 1997), which is related to solubilization of pectic components. The β-elimination reaction, which is dependent on the degree of methylation (DM) of pectic polymers, is associated with the heat induced softening of carrots (van Buren 1986; Sajjaanantakul and others 1992; Waldron and others 1997). Next to the influence of the pectin structure, the contribution of turgor pressure on the textural properties of carrots during thermal processing cannot be overlooked (Martens 1986; Greve and others 1994; Verlinden 1996).

In an attempt to meet consumer quality demands, the improvement of the texture of thermally processed carrots has been approached in various ways including low-temperature blanching (<70 °C) before sterilization (Lee and others 1979; Quintero-Ramos and others 1992; Roy 2001), calcium infusion before thermal processing (Siliha and others 1996; Gras and others 2003, Vu and others 2004), pH adjustment during processing (Van Buren and Pitifer 1992), and pressurization before thermal processing (Sila and others 2004). Irrespective of the available data on the effects of pretreatment conditions on carrot texture, information on the underlying cell wall chemistry is limited. To increase our understanding of the perceived textural attributes, correlations between measured textural properties and the changes in cell wall components are important. In this context, this study investigates heat-induced changes in the textural properties of carrots after exposure to different pretreatment conditions. Changes in cell wall components, in particular the methylation degree of pectins, will be related to the changes in textural properties of thermally processed carrot tissues.

Materials and Methods

Vegetable materials

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

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Table 1—A general overview of the main experimental set up

Sample pretreatment (Carrot discs: 1 cm^3)	Calcium soaking (0.5% $CaCl_2$)		
High-pressure pretreatment (HP): 400 MPa, 60 °C for 15 min	After HP Before HP	Thermal processing →	Compression Measurement
Low-temperature blanching (LTB): 60 °C for 40 min	After LTB Before LTB No calcium soak		Degree of methylation
High-temperature blanching (90 °C for 4 min)	No calcium soak		
Untreated carrots	Calcium soaked		
Control (untreated carrots)	No calcium soak		

Pretreatments

The cores of carrots were chopped into discs of 10-mm height and 12-mm dia before calcium, thermal, and/or high-pressure pretreatment. Eight different pretreatment conditions were selected on the basis of results obtained in previous studies (Lopez 1987; Sila and others 2004; Smout and others 2005). The treatment details are summarized in Table 1.

Calcium soaking. Calcium infusion was performed using a 0.5% (w/v) $CaCl_{2\ (aq)}$ solution at room temperature and pressure (25 °C). Rao and Lund (1986) proposed that the concentration of $CaCl_{2(aq)}$ in fruits and vegetables should be barely 1% of the weight of the final product. At this concentration, off-flavors are not detected. Calcium soaking was carried out by placing the carrot discs for 1 h in 0.5% (w/v) $CaCl_{2\ (aq)}$ solution. The infusion was done either before or after thermal/high-pressure pretreatment. The calcium solution was then drained and the samples taken to the subsequent step of the experiment.

High-pressure pretreatment. High-pressure pretreatments were carried out using a single-unit high-pressure apparatus (Engineered Pressure Systems Intl., Temse, Belgium, reactor volume ∇ 590 mL, temperature range ∇ –30 °C to 100 °C and maximum pressure level ∇ 600 MPa). The pressure transmitting medium was a mixture of propylene and glycol (60% Dowcal, Dow Chemical Co., Horgen, Switzerland). The pumping system uses an electrically driven high-pressure intensifier. Pressure was built up instantaneously (compression rate ∇ 16.7 MPa/s). A fluid flow heat exchanger allowed thermostating the system from outside of the vessel, which was done using 58% ethylene glycol solution (Cryostat Haake N8-KT 50W, Karlsruhe, Germany). The apparatus is fitted with thermocouples (type K), which allow recording of the temperature profile at different levels in the pressure vessel and 1 pressure sensor (data logger ∇ Cobra 7-10, Mess ≦ technik system GmbH, N.Y., U.S.A.). Before pressurization, the vessel was 1st thermally equilibrated at 60 °C. At least 10 carrot discs at room temperature and pressure were heat sealed under vacuum (until 11 mbar) in a double-film polyethylene bag and pressurized at 400 MPa, 60 °C for 15 min. At this pressure and temperature combination, the adiabatic rise in temperature was about 15 ± 2 °C. The temperature equilibrated at 60 °C after about 5 min. Decompression was done instantaneously (rate ≃ 10.0 MPa/s), and the final temperature dropped to about 47 ± 2 °C. Under these conditions, residual PME activity was detected (data not shown).

Thermal pretreatments. Thermal pretreatments were carried out in a temperature-controlled water bath for low-temperature blanching (60 °C for 40 min) and conventional high-temperature blanching (90 °C for 4 min) conditions. To ensure isothermal heating, 10 carrot discs were encapsulated in an aluminum tube (110 mm long, 13-mm internal dia, and 1-mm thickness). Demineralized water or calcium chloride solution (0.5% $CaCl_{2(aq)}$) was used as a heat-transfer medium (avoiding Ca^{2+} loss or uptake during the thermal pretreatment process). After the pretreatment, the samples were cooled immediately with cold water in a sink.

Thermal processing

Thermal treatment at laboratory scale. Thermal treatments were carried out 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) at 100 °C and 110 °C. Carrot discs (10) were encapsulated in aluminum tubes and filled with brine (demineralized water or calcium chloride solution depending on the pretreatment). There was no direct contact of the oil with the samples. To avoid the effects of heat lag during processing, a heat 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. In case of texture measurement, all the other samples were heat treated (100 °C) for an additional 20 min, cooled in an ice bath, and, subsequently, the residual texture was determined. To investigate the evolution of the DM of carrot pectin during processing, heat treated samples (110 °C) were withdrawn from the oil bath at preset time intervals and cooled in an ice bath. This was followed by the extraction of alcohol insoluble residues (AIR).

In-pack sterilization of carrots. Glass jars (370-mL volume, 99-mm height, and 80-mm dia) were used for thermal processing. Heat penetration data were collected for a maximum fill weight of 190 ± 0.5 g and a headspace of 5.0 mm. Consequently, equivalent processes (F_o ∇ 6min) were designed at 115 °C, 120 °C, and 125 °C using the Ball method (1923). Each glass jar contained 20 calibrated (1 cm^3) and pretreated carrot discs. Additional uncalibrated carrot filler material was added up to an approximate weight of about 190 ± 0.5 g. Depending on the pretreatment condition, either demineralized water or 0.5% $CaCl_{2(aq)}$ solution was used as a brine (avoiding Ca^{2+} loss or uptake during the sterilization process). Samples were sterilized using a static steriflow pilot retort (Barriquand, Roanne, France) at each of the respective temperatures (115 °C, 120 °C, and 125 °C) to obtain a process value (F_o) of 6 min.

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 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 sample, the mean value of the compression force of 10 carrot cylinders was calculated.

Determination of degree of methylation

The alcohol insoluble residue (AIR) was extracted from carrot tissue following the method of Mc Feeters and Armstrong (1984). Approximately 10.0 g of carrot sample (10 carrot discs) 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, ϕ 110 mm, Leuven, Belgium) and re-homogenized in 31.7 mL of 95% ethanol and filtered again. The residue was homogenized again in 31.6 mL acetone before a final filtration followed by drying overnight under vacuum at 40 °C. The AIR was then ground using a mortar and pestle and stored in a dessicator until analysis. Anhydrous galacturonic acid was determined quantitatively by the colorimetric hydroxyl-phenyl-phenol method (Blumenkrantz and Asboe-Hansen 1973) with an ultraviolet/visible light spectrophotometer (Ultrospec 2100 Pro, Amersham Biosciences, Uppsala, Sweden) at 520 nm in a 1-cm quartz cell.

The amount of methanol was determined spectrophotometrically (412 nm) according to the method of Klavons and Benett (1986) based on hydrolyzing pectinmethylesters to methanol and then complexing the methanol to a colored compound with pentandione. Finally, the DM was calculated as the ratio of the moles of methanol to the moles of anhydrous galacturonic acid content and expressed as a percentage.

Calcium determination

The dry ashing technique (calcination) as described by Vidal and others (2002) was used. Approximately 4.0 g of carrot (4 carrot discs) was weighed exactly into a porcelain crucible and dried in an oven for 2 h. The dry sample was calcinated in a muffle furnace at 600 °C for 2 h. The carbon free ash was then dissolved in 10.0 mL of 3 *M* $HCl_{(aq)}$ and diluted to 100.0 mL. Calcium content was determined using an atomic absorption spectrometer (Solaar 969, Cambridge, U.K.) at a wavelength of 422.7 nm.

Data analysis

For texture measurements, 2 independent pretreatments and 2 independent thermal treatments were carried out in parallel for each analysis, each based on an average of 10 determinations. The mean and standard deviation are reported. The DM was determined in triplicate for each of the 2 independent thermal treatments and the mean and standard deviation calculated. Calcium determination was done immediately after 3 independent pretreatments and the mean and standard deviation calculated. Correlations between compression forces (texture index) and DM were determined according to Moore and McCabe (1998). Statistical significance was examined using a 1-way analysis of variance (ANOVA table) (Moore and McCabe 1998).

Results and Discussion

Changes in texture of carrots as influenced by different pretreatments and thermal processing

Texture changes of carrots after different pretreatments. The hardness of carrot discs after the different pretreatment conditions is illustrated in Figure 1. Calcium soaking of raw samples increased the peak compression force of the samples. The increase in hardness for calcium soaked samples could be related to the protective role of calcium on membrane integrity (Marchiner 1986) and enhanced intramolecular and intermolecular cross-linking between pectic polymers and calcium ions. All the other pretreatment conditions resulted in texture degradation in comparison to non-pretreated samples (control). There was no significant difference ($P \geq 0.1$) in texture for the pretreatment conditions that resulted in texture degradation. Similarly, Verlinden and others (1997) observed that blanching of carrots at 55 °C and 65 °C resulted in comparable residual texture levels despite a short blanching time being used at 65 °C. The observed texture degradation might be ascribed to the loss of the integrity of the cell membranes, which subsequently might have led to turgor loss and increased sensitivity to compression force. This is consistent with the observations made by Knorr (1995) that the outer cell membrane is permeabilized at 250 to 350 MPa leading to loss in turgor pressure, which in turn results in a reduction of the final texture of a product. According to Basak and Ramaswamy (1996), texture degradation in carrots during pressurization is characterized by a sudden loss, followed by a more gradual loss with time as a result of pulse action of pressure (200 to 400 MPa).

Heat and pressure also affect the cell wall structure, leading to reduction in cell wall adhesion and cell separation. The heat-induced destruction of cell membranes is fast and starts at approximately 50 °C (Aguirela and Stanley 1990; De Belie and others 2002). In addition, microscopic studies on carrots reveal thickening of the cell wall coupled with disruption of the plasma membrane at about 60 °C (Ramana and others 1992), which results in tissue weakening. However, at these temperatures, cell wall pectins are not yet affected (Greve and others 1994). In conclusion, while calcium soaking increased the hardness value of raw carrots possibly because of cross-linking with pectic compounds, all other pretreatment conditions resulted to comparable declines in residual hardness levels, which might be related to tissue disruptions and turgor loss.

Texture changes of pretreated carrots after thermal processing at laboratory scale (100 °C). A simulation of a regular cooking process at 100 °C for 20 min resulted in marked differences in the residual hardness of pretreated carrots (Figure 2). Texture degradation of high-pressure pretreated samples in combination with calcium soaking was less than 10%. In contrast, conventional high-temperature blanched samples and non-pretreated samples re-

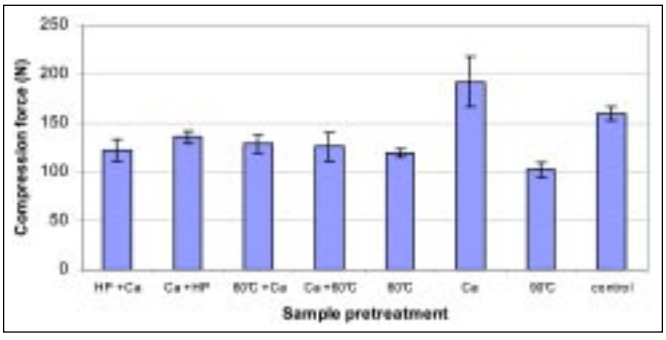

Figure 1—Hardness of carrot discs after different pretreatment conditions: HP + Ca = high-pressure pretreatment (400 MPa, 60 °C for 15 min) followed by calcium soaking; Ca + HP = calcium soaking before high-pressure pretreatment (400 MPa, 60 °C for 15 min); 60 °C = preheating at 60 °C for 40 min (low-temperature blanching); 60 °C + Ca = low-temperature blanching followed by calcium soaking; Ca + 60 °C = calcium soaking before low-temperature blanching; Ca = calcium soaking only; 90 °C = conventional high-temperature blanching (preheating at 90 °C for 4 min); control = non pretreated samples.

sulted in a texture loss of about 90%. Similar texture losses were observed with calcium soaking alone. On the other hand, low-temperature blanching alone resulted in a marked texture improvement compared with the control samples. A larger improvement in texture was observed after combining low-temperature blanching with calcium soaking. Irrespective of the pronounced enhancement in texture when combining calcium soaking with low-temperature blanching, there was no significant difference ($P \geq 0.1$) between applying the calcium pretreatment before or after the low-temperature blanching. These results underline the additional benefits obtained in texture preservation when combined pretreatment conditions are used. In fact, the best pretreatment is a combination of high-pressure pretreatment (400 MPa for 15 min), a low-temperature pretreatment (60 °C), and calcium pretreatment (either before or after the combined high-pressure, low-temperature pretreatment).

Texture changes of pretreated carrots after in-pack thermal processing in a pilot retort. When upscaling the laboratory-scale experiments to a pilot plant and applying equivalent processes in terms of safety ($F_0 \nabla 6$ min), the texture profiles showed a similar trend for all pretreatment conditions (Figure 3). Noticeable was the extreme softening of conventional high-temperature blanched carrots as opposed to the pronounced texture preservation of high-pressure pretreated samples. For each pretreatment, the results of texture measurements after different thermal processing temperatures were not significantly different ($P \geq 0.1$).

Cooking is often associated with the degradation of pectic polymers via β-elimination, which is usually related to the DM of pectins (Waldron and others 1997). Depolymerization through β-elimination reaction solubilizes esterified pectins at a pH above 4.5 and is enhanced by heating. Solubilization of pectic compounds in the middle lamella leads to intercellular weakening and cell separation causing texture degradation. The pH of carrot juice is approximately 6.3, which makes β-elimination reaction highly possible. In potatoes, the phenomenon of β-elimination was even found to be more pronounced at a pH of about 6.1 to 6.5 (Loh and Breene 1982). From this work, the texture index measured (compression force) seemed to be strongly influenced by the pretreatment method used and consequently by the chemical changes taking place at cellular level.

Influence of calcium soaking on the texture of carrots after thermal processing

The calcium content of carrots after different pretreatment conditions is presented in Table 2. The native concentration of calcium in fresh samples was estimated at 0.64 mg/g sample. A 13-fold increase in calcium content was obtained when calcium soaking was preceded by either a high-pressure pretreatment or low-temperature blanching. This is consistent with Buescher and Hudson (1986), who observed that post pretreatment infusion of calcium increases the amount of Ca^{+2} ions bound to pectin. In contrast, an insignificant increase in calcium content was observed in the case of calcium soaking before high-pressure pretreatment. These observations might be related to the state of tissues at the time of calcium soaking. In fact, pressures above 200 MPa have been reported to increase tissue permeability in plant cells (Knorr 1995; Prestamo and others 1998). Temperature, on the other hand, increases the rate of diffusion of ions in cells in addition to increasing cell membrane permeability. An 8-fold increase in calcium content was observed when calcium soaking preceded low-temperature blanching; such an increase was associated with increased calcium infiltration especially during the blanching operation because calcium solution was used as brine. The lowest levels of calcium were obtained in the conventionally high-temperature-blanched and low-temperature-blanched samples in the absence of calcium brine. The slight decrease compared with control samples was linked to leaching as explained by Vuksanovic and Juhas (1993).

A weak positive correlation (0.29 to 0.48) was found between the calcium content of pretreated carrot samples and the hardness values after thermal processing (115 °C, 120 °C, and 125 °C). Calcium soaking before and after high-pressure pretreatment resulted in a 9-fold difference in calcium content but did not lead to a significant difference ($P \geq 0.1$) in hardness after thermal treatment. Likewise, calcium soaking before and after low-temperature

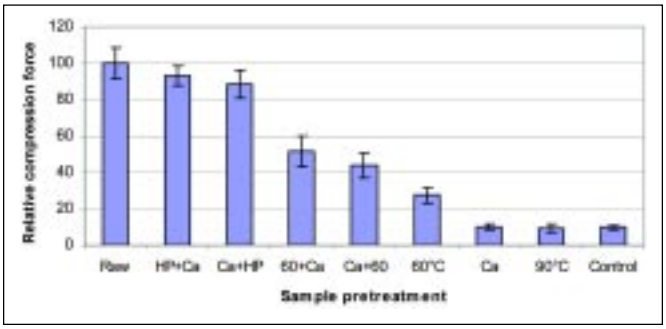

Figure 2—Relative hardness levels of pretreated carrot discs after thermal processing at 100 °C for 20 min under isothermal conditions: raw = fresh carrot sample after a heat equilibration time of 5 min (blank sample); HP + Ca = high-pressure pretreatment (400 MPa, 60 °C for 15 min) followed by calcium soaking; Ca + HP = calcium soaking before high-pressure pretreatment (400 MPa, 60 °C for 15 min); 60 °C = preheating at 60 °C for 40 min (low-temperature blanching); 60 °C + Ca = low-temperature blanching followed by calcium soaking; Ca + 60 °C = calcium soaking before low-temperature blanching; Ca = calcium soaking only; 90 °C = conventional high-temperature blanching (preheating at 90 °C for 4 min); control = non-pretreated samples.

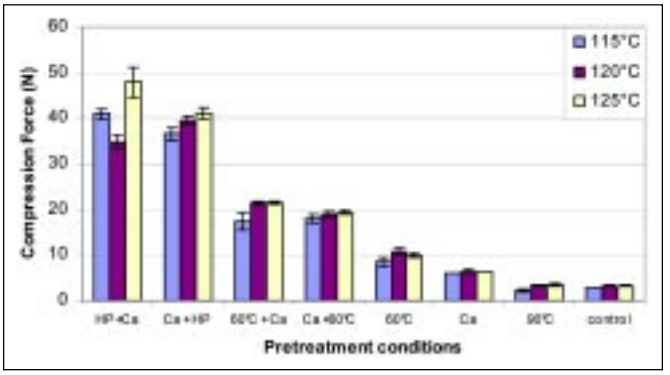

Figure 3—Effects of pretreatment on the texture of carrot discs after thermal processing at different temperatures for equivalent processes (F_o = 6 min): HP + Ca = high-pressure pretreatment (400 MPa, 60 °C for 15 min) followed by calcium soaking; Ca + HP = calcium soaking before high-pressure pretreatment (400 MPa, 60 °C for 15 min); 60 °C = preheating at 60 °C for 40 min (low-temperature blanching); 60 °C + Ca = low-temperature blanching followed by calcium soaking; Ca + 60 °C = calcium soaking before low-temperature blanching; Ca = calcium soaking only; 90 °C = conventional high-temperature blanching (preheating at 90 °C for 4min); control = non pretreated samples.

Table 2—Calcium content of carrots (*Daucus carota* var. Nerac) after pretreatment conditions[a,b]

Sample pretreatment	Calcium concentration (mg/g sample)
HP (60 °C for 15 min) + Ca^{2+} (0.5%)	8.40 ± 0.68
Ca^{2+} (0.5%) + HP (60 °C for 15 min)	0.93 ± 0.27
60 °C (40 min) + Ca^{2+} (0.5%)	8.43 ± 0.44
Ca^{2+} (0.5%) + 60 °C (40 min)	5.61 ± 1.28
60 °C for 40 min	0.56 ± 0.05
Ca^{+2} (0.5%)	1.41 ± 0.34
90 °C for 4 min	0.51 ± 0.07
Control	0.64 ± 0.16

[a]Values ± standard deviation.
[b]Ca^{+2} = calcium soaking; HP = high pressure.

Table 3—Degree of methylation of carrots pectin (*Daucus carota* var. Nerac) after different pretreatment conditions and subsequently after thermal processing at different temperatures

Sample pretreatment	DM after pretreatment	DM after thermal processing (F_o = 6 min)		
		115 °C	120 °C	125 °C
HP + Ca	39.46 ± 4.26	22.18 ± 0.07	27.78 ± 2.03	27.61 ± 0.07
Ca + HP	36.36 ± 3.23	23.78 ± 0.10	27.85 ± 1.66	30.11 ± 4.50
60 °C + Ca	45.99 ± 0.86	30.50 ± 0.62	34.05 ± 2.77	32.26 ± 0.53
Ca+ 60 °C	49.99 ± 0.70	31.95 ± 1.15	37.73 ± 3.36	34.73 ± 3.97
60 °C	53.52 ± 0.31	35.13 ± 1.41	37.83 ± 5.43	37.05 ± 3.05
Ca	58.90 ± 1.85	36.47 ± 0.36	32.40 ± 1.82	41.00 ± 3.08
90 °C, 4min	59.56 ± 0.08	41.38 ± 0.30	40.60 ± 3.52	42.77 ± 3.35
Control	60.12 ± 2.22	40.57 ± 3.55	41.83 ± 1.02	40.18 ± 0.53

[a]Values ± standard deviation.
[b]DM = degree of methylation.

blanching resulted in insignificant differences in hardness after thermal processing despite the distinct differences in calcium content after the pretreatment. On the other hand, low-temperature blanched samples reflected a better texture than control samples, which had a slightly higher calcium content. This implies that the endogenous level of calcium in carrots can lead to a significant texture improvement under mild heat conditions. However, as illustrated earlier for low-temperature blanching, a further improvement in texture is obtained when calcium soaking is combined with a pretreatment (thermal/pressure). Control samples, conventionally high-temperature-blanched samples and calcium-soaked samples indicated comparable textural properties irrespective of their clear differences in calcium contents. While the firming effect of calcium ions in combination with other pretreatment conditions was shown, it was demonstrated that texture retention is not solely dependent on the level of calcium in the tissues, but rather on other chemical reactions taking place in the cell wall. This prompted the conclusion that calcium plays a secondary role in texture improvement.

Influence of pretreatments and thermal processing on the DM of carrots

The DM and the extent of cross-linking between pectin molecules are important for the textural properties of plant tissues. Changes in the DM of carrot pectin after the respective pretreatments and immediately after the thermal process are summarized in Table 3.

The DM of raw carrot pectin (control) was estimated at about 60%. This is in close agreement with a DM of 60% to 69% reported for fresh carrots (Siliha and others 1996; Ng and Waldron 1997). Generally, the DM was strongly influenced by the pretreatment condition. The DM of control (raw), conventional high-temperature blanched and calcium-soaked samples was not statistically different ($P \geq 0.1$). The most significant reduction in DM was obtained in cases of high-pressure pretreatment. Low-temperature blanching showed an intermediate change in DM. Calcium soaking in combination with low-temperature blanching resulted in further reductions in DM, a lower DM being observed when blanching preceded calcium soaking. This is consistent with the observations of Hudson and Buescher (1985) that demethylation of pectin is increased when calcium soaking is delayed. Surprisingly, similar results were not observed for high-pressure pretreated samples. The DM was lower when high-pressure pretreatment was preceded by calcium soaking. The reason for this is not known, although it might be associated with some experimental errors, as indicated by the large standard deviation. In general, salts have been found to enhance PME activity (van Buren 1973), which might explain the observed decrease in DM. Indeed, a low concentration of calcium (5 to 25 m*M*) strongly enhances PME activity, whereas at higher concentrations, possibly because of the formation of pectate gels, it decreases the reaction rate (Rexova-Benkova and Markovic 1976).

Some pretreatments resulted in a transition from high methoxyl pectin to low methoxyl pectin in carrots. This phenomenon can be ascribed to stimulation of PME activity, which results in an increase in the demethylation of pectin polymers, eventually facilitating more cross-linking with divalent ions. Increased cross-linking of pectin often intensifies the resistance toward thermal degradation, as indirectly designated by compression forces. The optimal temperature for most plant PMEs has been found to be 50 °C to 80 °C (Van Buren 1973). Moreover, the optimal hardness of carrots has been observed after pretreatments at 50 °C to 77 °C (Lee and others 1979; Vu and others 2004), which also coincides with the optimal temperature for carrot PME activity (Stratilova and others 1998; Ly Nguyen and others 2002). Nevertheless, a complete inactivation of purified carrot PME is observed above 70 °C in a short time (Vora and others 1999; Ly Nguyen and others 2002). Balogh and coworkers (2004) reported that the D-values estimated for PME inactivation in carrot pieces ranged from $D_{66\,°C}$–648.5 min to $D_{74\,°C}$–5.2 min. Therefore, conventional blanching led to an almost instantaneous PME inactivation, hence little or no PME catalyzed demethylation. A reflection of this is expressed by the high DM and, correspondingly, the low texture measurements. Conversely, high-pressure pretreatment has been shown to enhance PME activity significantly (Cano and others 1997; Krebbers and others 2003). This is illustrated by the pronounced reduction in DM after a high-pressure pretreatment.

Thermal processing at each of the respective temperatures resulted in a further decrease in the DM (Table 3). The decrease was approximately 15% to 20% in all the cases after thermal processing. Ng and Waldron (1997) similarly reported a decrease in DM of fresh carrots from about 63% to about 52% after precooking and then to 41% after cooking. This behavior, which occurs simultaneously with β-elimination, is associated with chemical de-esterification of methylester groups as reported by Sajjaanantakal and others (1989) and Greve and others (1994).

The chemical demethylation of the pectin chain in carrots during thermal processing was observed to vary as a function of the pretreatment condition, time of application of calcium solution during the pretreatment stage and the exposure time during thermal treatment. Lower DM values were obtained after thermal processing when calcium soaking was preceded either by high-pressure

pretreatment or by a low-temperature pretreatment (Table 3). However, in spite of the clear trend, there was no significant difference ($P \geq 0.1$) in the DM of samples calcium soaked before or after the thermal/high-pressure pretreatment conditions. It is believed that the strong pectate network formed when calcium ions bind pectin polymers reduces the accessibility of methylester groups for the demethylation mechanism, hence the reason for high DM values in case of calcium soaking before other pretreatments and vice versa. To illustrate the evolution of the DM during thermal processing, the kinetics of demethylation of carrot pectin were studied (Figure 4). The demethylation profiles were dependent on the pretreatment condition used, and the trends were similar. As indicated earlier, the most pronounced decrease in DM was observed in high-pressure pretreated samples when compared with raw samples. In conclusion, pretreatment conditions clearly influenced the DM of the pectin polymer. These changes were considered very important for the observed textural changes during cooking.

Correlation between DM and texture of carrots

The relationship between the DM of carrot pectin and tissue hardness is demonstrated in Figure 5. The mechanism underlying this phenomenon has been only partially characterized. In this work, a strong negative correlation was observed between the DM and tissue hardness after thermal processing (Figure 5 and Table 4). The higher the methyl ester content, the lower the resistance toward texture degradation during cooking. Consequently, the level of pectin demethylation in carrots was pointed out to be the primary factor influencing the thermal softening mechanism. This obviates the marginal role of calcium infusion in texture improvement in comparison with the DM. From Figure 5, it is observed that at equivalent processes, a better texture of carrots is realized at higher temperatures as denoted by the shifts in the correlation curves.

This work complements similar correlations found between the methoxyl content of cooked vegetables and the softening process (Fuchigami and Okamoto 1984; Fuchigami 1987; Sajjaanantakal and others 1989). Overall, the DM was shown to play a very important role in the texture of carrots, whereby the residual hardness of cooked carrots increased with decrease in the methoxyl content. Below a DM of 40, a strong intermolecular interaction between calcium ions and low methoxy pectin has been experimentally illustrated (Garnier and others 1994), thus elucidating further the results of this work. In brief, information on the physicochemical changes taking place at the pectic level can be used to predict the textural properties of carrots after thermal processing. The DM, and to a lesser extent the amount of adsorbed calcium, contribute to the observed texture changes in carrots.

Table 4—Correlations between degree of methylation and texture of carrots after thermal processing

Temp. (°C)	Correlation coefficient (r)	Regression coefficient (R^2)
115	–0.97	0.95
120	–0.99	0.98
125	–0.95	0.92

Conclusions

The texture of carrots after thermal processing is strongly influenced by the type of pretreatment conditions performed. A high-pressure pretreatment (400 MPa at 60 °C for 15 min) combined with calcium soaking greatly reduces the level of thermal softening of carrots. Insignificant texture improvement is obtained by using conventional high-temperature blanching, whereas low-temperature blanching results in intermediate texture retention. Calcium soaking combined with low-temperature blanching leads to a pronounced improvement in texture, unlike calcium soaking alone. Pretreatments induce extensive modification of pectin in terms of their DM, which is extremely important for the texture of carrot products after thermal processing. While calcium infusion in carrots before thermal processing may be important, the DM of carrot tissues before thermal processing is more imperative. A strong negative correlation exists between the DM of carrot tissue and the residual hardness after thermal processing.

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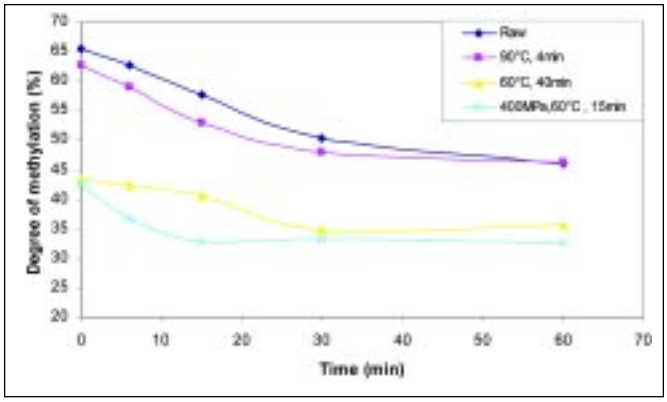

Figure 4—Demethylation of pretreated carrot tissues during thermal processing at 110 °C

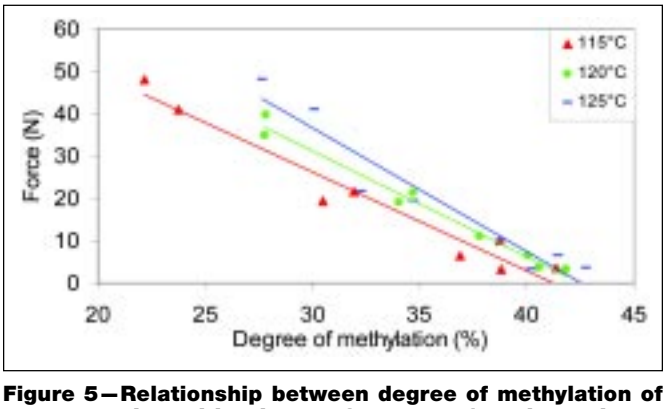

Figure 5—Relationship between degree of methylation of carrot pectin and hardness of carrots after thermal processing (Table 3)

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