Pulsed electric field and mild thermal processing affect the cooking behaviour of carrot tissues (*Daucus carota*) and the degree of methylesterification of carrot pectin

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Abstract

For the first time, the effect of pulsed electric field (PEF) and mild thermal processing on the texture of cortex and vascular carrot tissue during subsequent thermal processing (i.e. cooking behaviour) was compared and the degree of methylesterification (DM) of pectin from the pretreated tissues was investigated. The PEF and mild thermal pretreatment slowed down the cooking behaviour of the carrot tissues, especially when the pretreatments were combined. The DM of pectin from vascular tissue was lowered after both types of pretreatments, the effect being most pronounced in the case of the combination of the PEF and mild thermal pretreatment. In contrast, the DM of cortex pectin only decreased after the mild thermal pretreatment and after the combination pretreatment. This study demonstrates that besides mild thermal pretreatments also PEF pretreatments can be considered in the context of texture preservation of thermally processed fruits and vegetables.

Keywords: pulsed electric fields, thermal processing, texture, pectin, carrot

1. Introduction

Pulsed electric field (PEF) is an emerging non-thermal technology used in food technology. PEF is able to improve diffusion processes in plant tissues, with effects on drying and extraction unit operations (Vorobiev & Lebovka, 2008). It can also be used to inactivate microorganisms in liquid food products (Pagan & Manas, 2006) without the detrimental effects of high temperatures on food quality attributes, such as sensorial and nutritional value (Janositz & Knorr, 2010). During the treatment, pulses of high voltage are applied for very short time periods (ms-µs) to biological cell material placed between two electrodes in a batch or continuous treatment chamber, filled with a conductive medium. The application of an external electric field results in an increased transmembrane potential of the cell membranes (plasma membrane and tonoplast), structural changes, and the reversible or irreversible formation of pores in the membranes. This phenomenon is called cell electroporation. The cell membrane permeabilisation is irreversible when the electric field is exceeding a critical electric field strength (Botero-Uribe et al., 2017; Pagan & Manas, 2006; Puértolas et al., 2012; Toepfl et al., 2006). The mechanism of cell electroporation is still under discussion. Although several theories exist, the electromechanical instability theory is the most accepted one (Kanduser & Miklavcic, 2009; Pagan & Manas, 2006; Toepfl et al., 2006). The efficiency of the PEF treatment depends on process parameters (electric field strength, treatment time, specific energy, pulse width and shape, frequency and temperature) as well as product (cell size and shape, orientation in the electric field, conductivity) and medium characteristics (conductivity, composition, pH) (Ben Ammar et al., 2011; Kanduser & Miklavcic, 2009; Puértolas et al., 2012; Vorobiev & Lebovka, 2008). The ranges of electric fields used

typically vary between 0.7-3 kV/cm for permeabilisation of plant cells and 10-15 kV/cm for inactivation of the smaller microbial cells (Toepfl et al., 2006). Although this technology is already in use, both for tissue and liquid treatments, its ability to disrupt the cell membrane may also be interesting for texture engineering purposes of food products consisting of fruit and vegetable tissue.

Texture is an important quality attribute of fruits and vegetables and is mainly determined by the turgor pressure in the cell, the strength of the cell wall and forces holding the cells together (Fincan & Dejmek, 2003; Gonzalez & Barrett, 2010; Jackman & Stanley, 1995; Sila et al., 2004). The main polysaccharides of the cell wall are pectin, hemicellulose and cellulose. While cellulose fibrils provide rigidity to the cell wall, hemicellulose and pectin provide plasticity (Christiaens et al., 2016). The middle lamella, the glue between adjacent cell walls, mainly consists of pectin. Therefore, the structure of this polysaccharide and its modifications during processing are important texture determining factors. Homogalacturonan (HG) is an important pectin building block and consists of galacturonic acid (GalA) residues that can be methylesterified and acetylated, depending on the plant source. HG can be demethylesterified enzymatically by plant pectinmethylesterase (PME), resulting in blocks of demethylesterified GalA residues, and by microbial PME, resulting in randomly distributed demethylesterified GalA residues. The degree (DM) and pattern of HG methylesterification are important characteristics determining the functionality of the polysaccharide. Blocks of negatively charged demethylesterified GalA residues can bind divalent cations such as Ca^{2+} and form intra- and intermolecular pectin crosslinks, referred to as the egg-box model. This results in improved cell adhesion and a firmer texture (Christiaens et al., 2016; Mohnen, 2008; Ridley et al., 2001; Willats et al., 2006). Thermal processing at temperatures higher than 80 °C induces the chemical depolymerisation of HG by beta-elimination, resulting in a softer texture of the plant tissue. The demethylesterification and ionic crosslinking of HG slows down this depolymerisation reaction, resulting in a slower texture degradation during thermal processing (i.e. cooking behaviour) (Christiaens et al., 2016). It is already known that a mild thermal pretreatment at 60 °C alters the cooking behaviour of carrots, especially in Ca^{2+} rich media (Lemmens et al., 2009; Sila et al., 2005; Smout et al., 2005). This temperature is optimal for PME activity and causes cell membrane disruption, facilitating enzymatic pectin demethylesterification and the formation of ionic crosslinks, and resulting in a slower texture degradation during subsequent thermal processing (Gonzalez & Barrett, 2010; Sila et al., 2004; Sila et al., 2005).

The effect of PEF on the texture of fresh plant tissues has already been studied. The electroporation of the cell membrane by an external electric field facilitates the mass transport of intracellular compounds, lowers the turgor pressure, softens the texture, and reduces the cutting force (Boussetta et al., 2013; Fincan & Dejmek, 2003; Leong et al., 2014). As for now, no clear effects of PEF on the cell wall structure in fresh plant tissues have been reported (Ben Ammar et al., 2011; El-Belghiti & Vorobiev, 2005; Fincan & Dejmek, 2002; Janositz & Knorr, 2010; Jemai & Vorobiev, 2002) and studies on the

structural changes of pectin after application of PEF are lacking. However, since PEF induces cell membrane permeabilisation, we hypothesised that this technology may also affect the cooking behaviour of plant tissues. The PEF treatment may facilitate the transport of intracellular compounds such as ions to the cell wall, the site of PME action. Moreover, the activity of PME, that is ionically bound to pectin, increases with cation concentration as cations compete with PME to bind the negatively charged demethylesterified carboxyl groups of pectin, releasing PME and allowing the enzyme to bind and demethylesterify additional GalA residues (Alonso et al., 2003; Christiaens et al., 2016; Nari et al., 1991). Consequently, the PEF treatment may facilitate PME activity, resulting in a lower DM, an increase of ionic pectin crosslinking and thus a slower cooking behaviour during subsequent thermal processing. The different tissues found in plant organs may have a different susceptibility to cell electroporation by PEF (Faridnia et al., 2015) and a different ionic composition. Therefore, it is interesting to know how these different tissues react at the combination of PEF and thermal processing. Studies considering the effect of PEF on the texture after thermal processing are scarce. In a study of Leong, Du, & Oey (2018) carrot cortex that underwent a mild thermal pretreatment at 60 °C and a PEF pretreatment prior to a 5 minutes blanching step at 100 °C had a similar texture. This suggests that not only mild thermal processing but also PEF may have an effect on the cooking behaviour of plant tissues.

Unlike previous studies on the effect of PEF on texture, this paper focuses on the effect of PEF on texture after thermal processing and on the pectin structure as texture determining factor. Therefore, this paper compares the effect of PEF and mild thermal processing on the cooking behaviour of carrot tissues and investigates the link with pectin structural changes, in particular its degree of methylesterification (DM). Since carrots contain relatively high amounts of pectin and show relatively high PME activity (Alonso et al., 2003; Houben et al., 2011), this matrix represents a good choice to study the effect of the different processing techniques on the relationship between textural changes and changes in the DM of pectin. A distinction was made between the two main types of carrot tissue: outer cortex and inner vascular tissue. Firstly, the parameters of the PEF treatment leading to effective cell electroporation of cells in both tissues were selected. Secondly, the cooking behaviour of the carrot tissues submitted to a PEF pretreatment, a mild thermal pretreatment and a combination of PEF and mild thermal pretreatment was determined and compared. The DM of pectin from the raw pretreated tissues was measured and linked to the cooking behaviour.

2. Materials and methods

2.1 Plant materials

Carrots (*Daucus carota*) of the variety Nerac were purchased from a local shop in Belgium and stored in a plastic bag at 4 °C for maximum one week before use (De Roeck et al., 2010). Different batches of

the same variety with similar texture (data not shown) were used. Carrots with cracks or bruises were excluded from the study.

2.2 Selection of pulsed electric field treatment parameters

2.2.1 Carrot sample preparation

The carrots were manually peeled and approximately 2-3 cm of both the crown and root end parts of the carrots were discarded to produce a carrot sample with 17 cm length. The carrots were washed in tap water and blotted dry with tissue paper (Leong et al., 2014).

2.2.2 Pulsed electric field equipment and treatment parameters

A batch pulsed electric field unit Cellcrack III of Elea-DIL (German Institute for Food Technologies, Quackenbrück, Germany) was used for the PEF treatments. The PEF unit was equipped with a treatment chamber consisting of two parallel stainless steel electrodes (24 x 22.5 x 0.5 cm, w x h x t) with an interelectrode distance of 29.7 cm. The insulator material had a thickness of 2 cm and the total volume of the treatment chamber was 12 l. Five carrots were positioned in the treatment chamber so that the carrot longitudinal axis (fibre direction) was perpendicular to the electrodes, ensuring a uniform distribution of the electric field through the five carrots (Leong et al., 2014). Standardised water (0.6156 g NaCl/l and 0.0923 g $CaCl_2.H_2O$/l) (Willemsen et al., 2017) with 1400 µS/cm conductivity at 25 °C (Ben Ammar et al., 2011; Liu et al., 2017) was added as treatment medium until the carrots were completely immersed. The total weight of the carrots and medium was 3.5 kg, with the weight ratio of carrot and medium being approximately 3:7.

The carrots were submitted to 0-60 exponential monopolar pulses with a pulse amplitude of 30 kV, resulting in an electric field strength of 1.01 kV/cm, which is the applied voltage divided by the interelectrode distance. The energy input per pulse of 450 J/pulse was defined as the energy provided by the capacitor with 1 µF capacitance and 30 kV voltage, and the specific energy input per pulse of 129 J/kg·pulse was calculated as the energy input per pulse divided by the total mass in the treatment chamber, being 3.5 kg. Therefore, the total specific energy input was calculated as the specific energy input per pulse multiplied by the amount of pulses. The frequency of the pulses was 2 Hz, the pulse width was 145 ± 9 µs (TBS 1102B-EDU digital oscilloscope, Tektronix, Köln, Germany) and the treatment temperature was approximately 20 °C. Within five minutes after the treatment, the carrots were vacuum packed and placed in a fridge at 4 °C, where they were stored for maximum 6 h before use.

2.2.3 *Carrot cylinder preparation*

Carrot cylinders (1 cm diameter, 1 cm height) were excised from the cortex and vascular tissue of untreated and PEF treated carrots, parallel with the fibre direction, using a stainless steel bore. For each total specific energy input tested, 24 cylinders were excised from the five carrots that were submitted to the same treatment. Three times eight carrot cylinders were encapsulated in stainless steel tubes (110 mm length, 13 mm internal diameter, and 1 mm thickness), that were filled with demineralised water (De Roeck et al., 2010) and equilibrated to 20 °C for 20 min.

2.2.4 *Texture measurement*

The texture measurements of the carrot cylinders were performed using the TA.XT2i Texture Analyzer (Stable Micro Systems, Godalming, United Kingdom) with a 25 kg load cell and heavy duty platform. The texture of the carrot cylinders was measured by means of a compression test, using an aluminum cylindrical probe with 25 mm diameter (P25, Stable Micro Systems, Godalming, United Kingdom) and test speed 1 mm/s. The force as a function of compression time was monitored by Texture expert exceed software (version 2.64, Stable Micro Systems, Godalming, United Kingdom). The hardness was defined as the peak force required to compress one cylinder to a 30% strain level (De Roeck et al., 2010).

2.3 Cooking behaviour of carrot tissues after pulsed electric field and mild thermal pretreatment

The cooking behaviour of carrot cortex and vascular tissue was evaluated after four types of pretreatment: no pretreatment (untreated), PEF pretreatment causing cell permeabilisation (PEF) (Pagan & Manas, 2006), mild thermal pretreatment at the optimal temperature (60 °C) for PME activity (T60) (Smout et al., 2005) and a combined PEF and mild thermal pretreatment (PEF-T60).

2.3.1 *Pulsed electric field treatment*

The carrots were prepared as indicated in 2.2.1, and submitted to 20 exponential monopolar pulses (total specific energy input of 2.571 kJ/kg) with the same characteristics as described in 2.2.2. Within five minutes after the treatment, the carrots were vacuum packed and placed in a fridge at 4 °C, where they were stored for maximum 16 h before use.

2.3.2 Thermal treatments

2.3.2.1 Carrot sample preparation

Carrot cylinders (1 cm diameter, 1 cm height) were excised from the cortex and vascular tissue of untreated and PEF treated carrots and encapsulated in stainless steel tubes (110 mm length, 13 mm internal diameter, and 1 mm thickness) filled with demineralised water, as explained in 2.2.3 (De Roeck et al., 2010).

2.3.2.2 Thermal treatments

The carrot cylinders, encapsulated in stainless steel tubes, were thermally pretreated in a temperature-controlled water bath at 60 °C for 20 min (including 3 min come up time) to promote PME activity (Smout et al., 2005).

Both the thermally pretreated and non-thermally pretreated carrot cylinders, encapsulated in stainless steel tubes, were transferred to a temperature-controlled water bath at 95 °C. The carrot cylinders were heated for 0-420 min holding time at 95 °C and at each holding time, three tubes containing eight carrot cylinders were withdrawn. The sample with holding time 0 min was withdrawn after a come up time of 5 min (De Roeck et al., 2010). After the treatment, the cylinders were immediately cooled for 5 min in an ice bath and conditioned in a water bath at 20 °C for 20 min before texture measurement.

2.3.2.3 Texture measurement

The texture measurements were performed as indicated in 2.2.4. The relative hardness was calculated as the ratio of the absolute hardness at a certain holding time and the absolute hardness of the raw untreated carrot tissue.

2.4 Degree of methylesterification of pectin

The degree of methylesterification (DM) of carrot pectin was determined analyzing thin sections of raw pretreated carrot tissue using Fourier-transformation infrared (FT-IR) spectroscopy (Shimadzu FTIR-8400S, Japan). The sections with thickness 50 µm (approximately 7 x 7 mm) were cut transversally from raw pretreated carrot cortex and vascular tissue using a cryomicrotome (Reichert, Austria) and were stored in 70% ethanol at 4 °C until analysis. Each type of pretreatment was performed on five different carrots and repeated twice. Hence, sections were cut from raw pretreated tissues derived from ten different carrots. Before analysis, the sections were washed, firstly in phosphate buffered saline solution (PBS) at pH 7.4 containing 40 mM NaCl, 2.7 mM KCl, 8.0 mM Na_2HPO_4 and 1.5 mM KH_2PO_4

(Christiaens et al., 2011), and secondly in demineralised water. The sections were dried on a parafilm after applying a drop of 70% ethanol and were analysed by the method of Kyomugasho, Christiaens, Shpigelman, Van Loey, & Hendrickx (2015). The ratio of the absorbance at 1740 cm^{-1} ($-COOCH_3$) and the sum of the absorbances at 1600 cm^{-1} ($-COO^-$) and 1740 cm^{-1} ($-COOCH_3$) was determined. The DM was calculated using the calibration curve of Kyomugasho et al. (2015) linking this ratio to the DM.

2.5 Characterisation of carrot tissues

Differences in cell size, conductivity, calcium content, dry matter content and PME activity of cortex and vascular tissue were investigated in the attempt to explain differences in texture and the DM of pectin after processing. Therefore, cortex and vascular tissue were excised from 13 fresh untreated carrots, cut in cubes, frozen and stored at -40 °C until analysis.

2.5.1 Determination of cell size in cortex and vascular tissue

The cryosections of cortex and vascular tissue excised from ten raw untreated carrots (2.4) were visualised under the microscope (Olympus BX-41, Optical Co. Ltd., Hamburg, Germany) under magnification 10x. The cell diameter of ten cells per cryosection was determined using the arbitrary line function in cellSens software (version 2.3, Olympus, Hamburg, Germany), resulting in an average cell size representative for 100 cells in the cortex and vascular tissue.

2.5.2 Conductivity of carrot puree

The conductivity of carrot puree from cortex and vascular tissue was measured for two reasons. Firstly, a difference in conductivity between cortex and vascular tissue may result in a different susceptibility to cell electroporation by the PEF treatment (Ben Ammar et al., 2011; Puértolas et al., 2012). Since we were not able to measure the conductivity of the intact tissues, and the conductivity of the tissue may change during the PEF treatment (Ben Ammar et al., 2011), the conductivity of puree consisting of one of the untreated tissues was measured to compare the conductivity between the tissues. Secondly, the conductivity of the purees was used as an indicator of the ionic cell content that can possibly migrate to the cell wall after cell electroporation and affect cell wall chemistry. Therefore, the frozen untreated tissues were homogenised (Grindomixer GM200, Retsch, Germany) and thawed in a 20 °C water bath. The conductivity at 20 °C was measured in triplicate using the Testo 240 conductivity meter (Ternat, Belgium).

2.5.3 Calcium content

The frozen untreated tissues were homogenised (Grindomixer GM200, Retsch, Germany) and freezedried for 22 h (Christ Alpha 2-4 LSC, Osterode, Germany). The dried puree was grinded using a mortar and vessel, obtaining a fine powder. Ten mg of the powder was ashed in triplicate in porcelain crucibles placed in a muffle furnace (Nabertherm Controller P330, Lilienthal, Germany) at 550 °C for 20 h. The cooled ashes were dissolved in 9.9 ml ultrapure water (organic free, 18 MΩ·cm resistance), acidified with 0.1 ml of 65% nitric acid and left overnight at 4 °C. Next, the solutions were filtered using a 0.45 µm membrane filter (Chromaphil® A-45/25, Macherey-Nagel, Düren, Germany) and analysed using inductively coupled plasma optical emission spectroscopy (iCAP 7400 ICP-OES Duo spectrometer, Thermo Scientific, USA). The calcium content was measured radially at 318 nm and calibration was performed using SPS-SW2 certified standard (Gwala et al., 2020). The calcium content was calculated in mg per g carrot powder.

2.5.4 Dry matter determination

The frozen untreated tissues were homogenised using the grindomixer (GM200, Retsch, Germany) and thawed in a 20 °C water bath. The dry matter content of the purees was determined in triplicate using a method slightly modified from Nguyen et al. (2016). The puree was dried in porcelain crucibles placed in a vacuum oven (UniEquip 1445-2, Planegg, Germany) at 70 °C under 0.8-0.2 bar pressure with pressure reduction steps of 0.2 bar every hour, followed by overnight drying at 40 °C and atmospheric pressure.

2.5.5 Pectin methylesterase extraction and activity measurement

PME was extracted in duplicate, based on the extraction procedure of Ly-Nguyen et al. (2003) with some adjustments. The frozen untreated tissues were homogenised (Grindomixer GM200, Retsch, Germany) and washed twice by mixing the puree with 0.05% cold sodium bisulfite (0.6 l per kg puree) and centrifuging the mixture 30 min at 10 000g at 4 °C (Beckman Coulter J2-HS, Fullerton, United States). The cell wall bound enzyme was extracted by adding 0.2M tris(hydroxymethyl)aminomethane buffer at pH 8.0 containing 1M NaCl to the washed pellet (1.3 l per kg puree) and overnight end-over-end rotation at 4 °C. The mixture was centrifuged 30 min at 18 000g at 4 °C and the pellet was removed. The supernatant was rotated end-over-end 30 min at 4 °C with a 30% ammonium sulfate concentration and centrifuged 1 h at 18 000g at 4 °C to precipitate large proteins. Ammonium sulfate was added to the supernatant to obtain a 80% concentration to precipitate PME. After 30 min end-over-end rotation at 4 °C, the mixture was centrifuged 1 h at 18 000g at 4 °C. The pellet was dissolved in 0.02M tris(hydroxymethyl)aminomethane buffer at pH 7.0 and stored at -40 °C until activity measurement.

The PME activity of each extract was measured in duplicate using an automatic pH stat titrator (718 STAT Titrino, Metrohm, Herisau, Switzerland) with cryostat (DC Haake) at 22 °C (Ly-Nguyen et al., 2003). The PME extract was mixed with 30 ml 0.35 % (w/v) apple pectin solution (DM 70.3%) containing 0.117 M NaCl at pH 6.5 that acted as substrate for PME. Demethoxylation of pectin by PME generates COO^- groups, lowering the pH. The pH of the mixture was kept at 7 for 1000 s by adding 0.01 M NaOH. The PME activity was calculated from the rate at which NaOH was added to maintain the neutral pH. The activity was expressed in units: one unit PME activity was defined as the amount of enzyme necessary to generate 1 µmol COO^- groups per minute at 22 °C and pH 7 (Ly-Nguyen et al., 2003). As cortex and vascular tissue may differ in dry matter content, the PME activity was expressed in U/g dry matter.

2.6 Statistical data analysis

The hardness of the carrots submitted to the PEF treatments with increasing total specific energy input was fitted to a fractional conversion model using the nonlinear regression procedure in SAS software (version 9.4, SAS Institute, Belgium). This model describes the exponential decrease in hardness when enhancing the total specific energy input, reaching a residual hardness: $H = H_r + (H_0 - H_r) \cdot exp(-k \cdot W_T)$ with H = hardness of carrot tissue submitted to total specific energy input W_T (N), H_0 = hardness of untreated tissue (N), H_r = residual hardness (N), k = exponential factor expressing the sensitivity of the carrot texture for tissue softening by the total specific energy input of the PEF treatment (kg/kJ) and W_T = total specific energy input (kJ/kg). The corrected correlation coefficient was calculated as $R^2_{corr} = 1 - (m-1)(1 - SSR/SST)/(m-j)$ with m = number of observations, j = number of model parameters, SSR = sum of squares due to regression and SST = total sum of squares (De Roeck et al., 2010). The model parameters of the fractional conversion model of both tissues were compared using their 95% confidence intervals.

The texture evolution during cooking (cooking behaviour) of the carrot tissues was modelled using the fractional conversion model, as was reported earlier (De Roeck et al., 2010; Smout et al., 2005): $H = H_r + (H_0 - H_r) \cdot exp(-k \cdot t)$ with H = relative hardness of carrot tissue at holding time t (% raw untreated), H_0 = initial hardness at t = 0 min (% raw untreated), H_r = residual hardness (% raw untreated), k = rate constant of texture degradation at 95 °C (min^{-1}) and t = holding time at 95 °C (min). The corrected correlation coefficient of each model was calculated and the model parameters were compared using their 95% confidence intervals. Differences in raw hardness (t = -5 min) of the pretreated tissues relative to the hardness of the raw untreated tissues were determined using the Tukey honest significant difference (HSD) test at significance level 5% using JMP Pro (version 14.2.0, SAS Institute, Belgium).

Significant differences in DM between both tissues and treatments were investigated using the Tukey HSD test with a significance level of 5%. A relationship between the rate constants of the fractional

conversions models describing the cooking behaviour of the pretreated carrot tissues, and the DM of the raw pretreated tissues was found using an exponential model: $k = k_0 \cdot exp(b \cdot DM)$ with k = rate constant of texture degradation at 95 °C of pretreated carrot tissue (min^{-1}), DM = degree of methylesterification of raw pretreated carrot tissue (%), k_0 = rate constant of texture degradation at 95°C if DM = 0%, b = exponential factor expressing the sensitivity of the rate constant of texture degradation of carrot tissue at 95 °C for the DM of carrot pectin. The data were modelled using the nonlinear regression procedure in SAS software and the corrected correlation coefficient was calculated.

Differences in cell size, conductivity, calcium content, dry matter content and PME activity between the two tissues were investigated using the Student's t-test in JMP Pro (version 14.2.0, SAS Institute, Belgium) with a significance level of 5%.

3. Results and discussion

3.1 Selection of pulsed electric field treatment parameters

The hardness of cortex and vascular carrot tissue was measured as a function of the total specific energy input of the PEF treatments (**Figure 1**). As expected, the PEF treatments reduced the hardness of both tissues. This may be due to cell electroporation and the loss of turgor pressure (Fincan & Dejmek, 2003). The decrease in hardness was exponential, reaching a constant residual hardness when further increasing the total specific energy input. Hence, the data were modelled using the fractional conversion model (**Figure 1**) and the model parameters were estimated (**Table 1**). Untreated cortex was harder than untreated vascular tissue. In contrast, vascular tissue remained harder than the cortex after the PEF treatments. This suggests that the cell walls and the forces holding the cells of vascular tissue together are stronger compared to the cortex. Based on the larger cell size and higher conductivity of vascular tissue (**Table 2**), one would expect the vascular tissue to be more susceptible to membrane electroporation (Ben Ammar et al., 2011; Faridnia et al., 2015; Kanduser & Miklavcic, 2009). Instead, the exponential factors describing the sensitivity of the tissue to softening by the PEF treatments were not significantly different for both tissues. Since there were only few datapoints in the exponential region to estimate the exponential factor of vascular tissue, the standard error of this estimate was quite large. Also the corrected correlation coefficient for vascular tissue was small (**Table 1**). As it was only possible to increase the total specific energy input with steps of 0.129 kJ/kg, the amount of datapoints that could be measured within the exponential phase of the curve was limited.

Considering these results, the total specific energy input of the PEF treatments used for the determination of the cooking behaviour after the PEF and combined PEF and thermal pretreatment was set at 2.571 kJ/kg. The plateau value of hardness reached at this intensity was assumed to represent effective and maximal cell electroporation in both tissues.

3.2 Cooking behaviour of carrot tissues after pulsed electric field and mild thermal processing

The PEF pretreatment, the mild thermal pretreatment and the combined PEF and mild thermal pretreatment reduced the raw hardness of both cortex and vascular tissue (**Figure 2**). This was expected as cell permeabilisation can occurr during these pretreatments: the application of electric pulses can result in structural changes in the cell membrane and the formation of electropores (Pagan & Manas, 2006), while mild preheating at 60 °C can result in thermal cell membrane destabilisation (Gonzalez et al., 2010; Gonzalez & Barrett, 2010; Sila et al., 2004). This will lead to loss of turgor pressure and a reduced hardness (Fincan & Dejmek, 2003; Gonzalez & Barrett, 2010). It seems that PEF reduces the hardness of raw tissue to a larger extent than the thermal pretreatment, for both tissues. The effects of the PEF and the thermal pretreatment on the texture of raw cortex tissue were not cumulative, suggesting both pretreatments may alter the same texture determining factor of raw tissue. In the case of vascular tissue, on the other hand, the combined pretreatment resulted in the lowest hardness.

The cooking behaviour of untreated and PEF and/or thermally pretreated carrot cortex and vascular tissue at 95 °C was modelled using the fractional conversion model and is shown in **Figure 3**. The parameter estimates describing the texture degradation kinetics are given in **Table 3**.

The initial hardness (holding time = 0 min) of the pretreated tissues was higher than the initial hardness of the untreated tissues (**Table 3**), although the hardness of the raw pretreated tissues was lower than the hardness of the raw untreated tissues (**Figure 2**). This suggests that the pretreated tissues are less susceptible to texture degradation during the come up phase, and possibly also during further heating at 95 °C.

No difference in rate constant between the untreated cortex and vascular tissue was observed (**Table 3**). The PEF treated cortex showed a similar cooking behaviour compared to the untreated tissue. The texture degradation of the PEF treated vascular tissue, on the other hand, was almost four times slower compared to the untreated tissue. The preheating at 60 °C slowed down the texture degradation at 95 °C for both cortex and vascular tissue. The effect of the PEF treatment on the cooking behaviour of vascular tissue was larger compared to the effect of the thermal pretreatment. The combined PEF and thermal pretreatment resulted in the slowest cooking behaviour for both types of tissue. Furthermore, the PEF pretreatment had a larger effect on the texture degradation kinetics of vascular tissue compared to cortex, as the texture degradation of vascular tissue that was submitted to the PEF pretreatment and to the combination pretreatment was slower compared to the texture degradation of cortex after the same pretreatments.

The pretreatments didn't affect the residual hardness of the cortex (**Table 3**). The texture of vascular tissue, on the other hand, may remain harder after the combination pretreatment and prolonged heating. However, it may also be that the real residual hardness is lower than the estimated residual hardness as there were only few datapoint to estimate this parameter.

3.3 PEF and mild thermal pretreatment facilitate pectin demethylesterification

In order to investigate the influence of pectin demethylesterification on the cooking behaviour of the untreated and pretreated tissues, the DM of pectin from raw untreated and pretreated cortex and vascular tissue was determined (**Figure 4**). The untreated cortex and vascular tissue contained pectin with a similar DM. Moreover, the DM of pectin from PEF treated cortex was similar to the DM of pectin from untreated cortex. This was consistent with the observed cooking behaviour of cortex that remained unchanged after the PEF pretreatment (**Table 3**). The DM of pectin from PEF treated vascular tissue, on the other hand, was lower than the original DM (**Figure 4**). This could explain the slower texture degradation after the PEF treatment of vascular tissue (**Table 3**). This difference in DM between PEF treated cortex and vascular tissue could not be explained by the difference in the activity of PME that was extracted from both tissues (**Table 2**), as the PME activity in the extract from vascular tissue was lower compared to its activity in the extract from cortex. On the other hand, the activity of the extracted enzyme may not be representative for the activity *in situ*. It seems that, even at suboptimal temperatures for PME activity, and in contrast with cortex, pectin from vascular tissue can be demethylesterified after cell electroporation. The disintegration of the membrane by PEF may improve the transport of intracellular ions (Puértolas et al., 2012; Toepfl et al., 2006) to the cell wall, the site of PME action. As PME activity increases with ionic strength (Alonso et al., 2003; Christiaens et al., 2016; Nari et al., 1991), the PEF pretreatment may facilitate pectin demethylesterification. The higher *in situ* PME activity in vascular tissue after cell electroporation compared to the activity in the cortex after the same pretreatment may be explained by a higher ionic strength at the cell wall in vascular tissue, indicated by the higher conductivity of the puree from vascular tissue (**Table 2**).

Pectin from both cortex and vascular tissue had a lower DM after the mild thermal pretreatment at 60 °C compared to the pectin from untreated tissues (**Figure 4**). Demethoxylation of pectin during such pretreatment was already observed by Lemmens et al. (2009), Sila et al. (2005) and Smout et al. (2005) among others, where the combination of cell permeabilisation, decompartimentalisation of the cell and an optimal temperature for PME activity could explain the decrease in DM. In contrast with the cooking behaviour after the PEF pretreatment, the slower cooking behaviour after the thermal pretreatment was similar for cortex and vascular tissue (**Table 3**). Surprisingly, the texture degradation of vascular tissue after the PEF treatment was slower than the texture degradation after the thermal pretreatment, although both pretreatments resulted in a similar DM of vascular pectin. It was already found that cell electroporation by PEF has a greater effect on the permeability of plant tissue than a mild thermal treatment (Jemai & Vorobiev, 2002). Possibly, more Ca^{2+} ions become available after cell electroporation compared to thermal destabilisation of the cell membrane, crosslinking pectin and slowing down texture degradation. Also, the slower cooking behaviour of vascular tissue may be linked to its higher calcium content compared to the cortex (**Table 2**). On the other hand, too high concentrations of cations, in particular divalent cations, may also counteract PME activity (Christiaens

et al., 2016). Therefore, a better understanding of the effect of PEF and mild thermal processing on ion availability at the cell wall, and the role of *in situ* ions on PME activity and Ca^{2+} crosslinking is necessary.

The use of a PEF pretreatment prior to a mild thermal pretreatment had a different effect on the DM of pectin from both tissues (**Figure 4**). Since the DM of cortex pectin after the PEF and mild thermal pretreatment was similar to the DM after the mild thermal pretreatment, it seems that the DM was only affected by the thermal pretreatment, even when this step was preceeded by PEF. This is in contrast with the slower texture degradation of cortex after the combination pretreatment compared to the texture degradation after the mild thermal pretreatment (**Table 3**). Hence, extra research is necessary to completely understand the differences in cooking behaviour after the PEF and mild thermal pretreatment, including the possibility of other enzymatic reactions that may alter the structure of pectin and strengthen the cell wall (e.g. phenolic crosslinks). In contrast, the DM of pectin from vascular tissue was lowest after the combination pretreatment (**Figure 4**). It seems that in vascular tissue the PEF treatment lowers the DM, additionally to the mild thermal pretreatment. Correspondingly, the texture degradation of vascular tissue was the slowest after the combination of the PEF and mild thermal pretreatment (**Table 3**).

An exponential relationship between the DM of pectin from the untreated and pretreated tissues and the rate constant of texture degradation of the corresponding tissues at 95 °C was found (**Figure 5**). It is clear that the rate constant decreases exponentially when lowering the DM. However, not all differences in cooking behaviour could be explained by differences in DM. Therefore, more research needs to be done on the effects of PEF and mild thermal processing on the structure and organisation of pectin.

4. Conclusion

In this paper the effect of PEF on the cooking behaviour of carrot tissues and on the DM of carrot pectin was investigated for the first time and compared to the effect of mild thermal processing on the texture and the DM. From this study it is clear that both PEF and mild thermal processing can slow down the cooking behaviour of carrot tissues, especially when the pretreatments are combined. Whereas the mild thermal pretreatment reduces the DM of carrot pectin from both cortex and vascular tissue, the PEF pretreatment only seems to affect the DM of pectin from vascular tissue. Further research is necessary to completely understand the mechanism behind the promotion of PME activity after cell electroporation and the tissue dependency of this phenomenon. The reduction of the DM leads to slower chemical depolymerisation of pectin during cooking. Moreover, an exponential relationship between the rate constant for texture degradation during thermal processing and the DM of pectin was found. However, not all differences in cooking behaviour could be explained by differences in DM. Possibly, a detailed study about the consequences of cell permeabilisation due to PEF and mild thermal processing

on PME activity, ionic pectin crosslinking, and other enzymatic reactions crosslinking pectin (e.g. phenolics) and strengthening the cell wall could clarify the observed differences in cooking behaviour between the carrot tissues after the different pretreatments.

Finally, the observation that a PEF pretreatment affects the texture of carrot tissue during subsequent thermal processing may be of great importance for the food processing industry. The PEF treatment not only leads to a softer texture of fresh fruit and vegetable tissues, reducing the cutting force (Leong et al., 2014), it is also slowing down texture degradation during cooking. Therefore, PEF may be considered as a technology for fruit and vegetable processing if a harder texture after thermal processing is desirable. Nevertheless, the use of PEF may also imply the loss of small molecules such as minerals, colorants, sucrose, phenolics and oils (Faridnia et al., 2015; Puértolas et al., 2012). Consequently, the advantages and disadvantages of using PEF as a technique for texture preservation during a specific production process should be taken into account.

Declaration of competing interest

The authors declare no conflict of interest.

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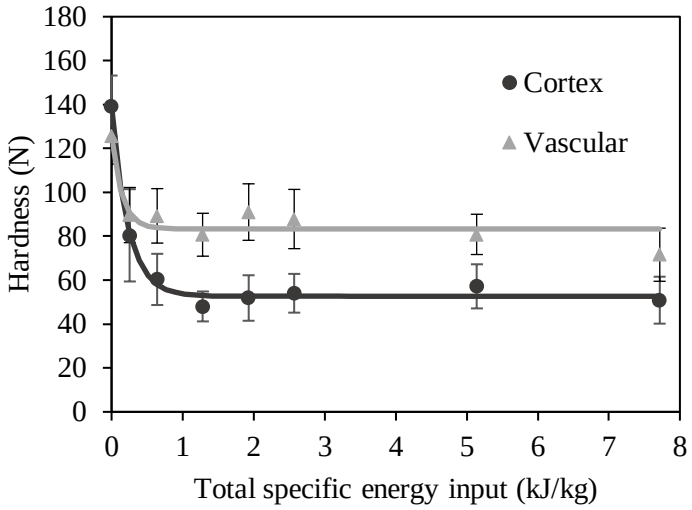


Figure 1: Hardness decay of carrot cortex and vascular tissue after PEF treatments with different total specific energy inputs at electric field strength 1.01 kV/cm, modelled using the fractional conversion model. Error bars represent the standard deviations of the texture measurements per treatment intensity.

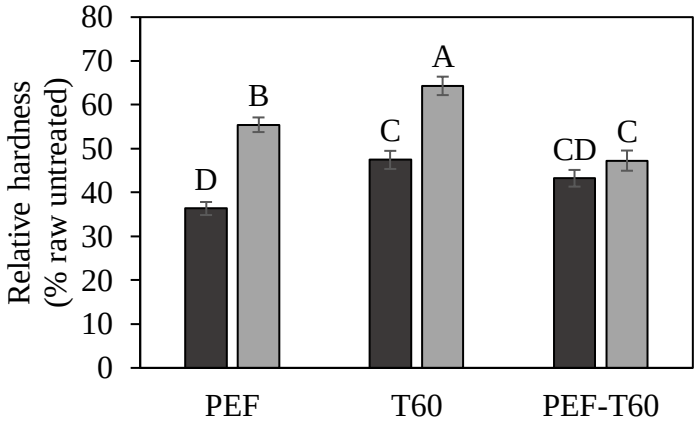
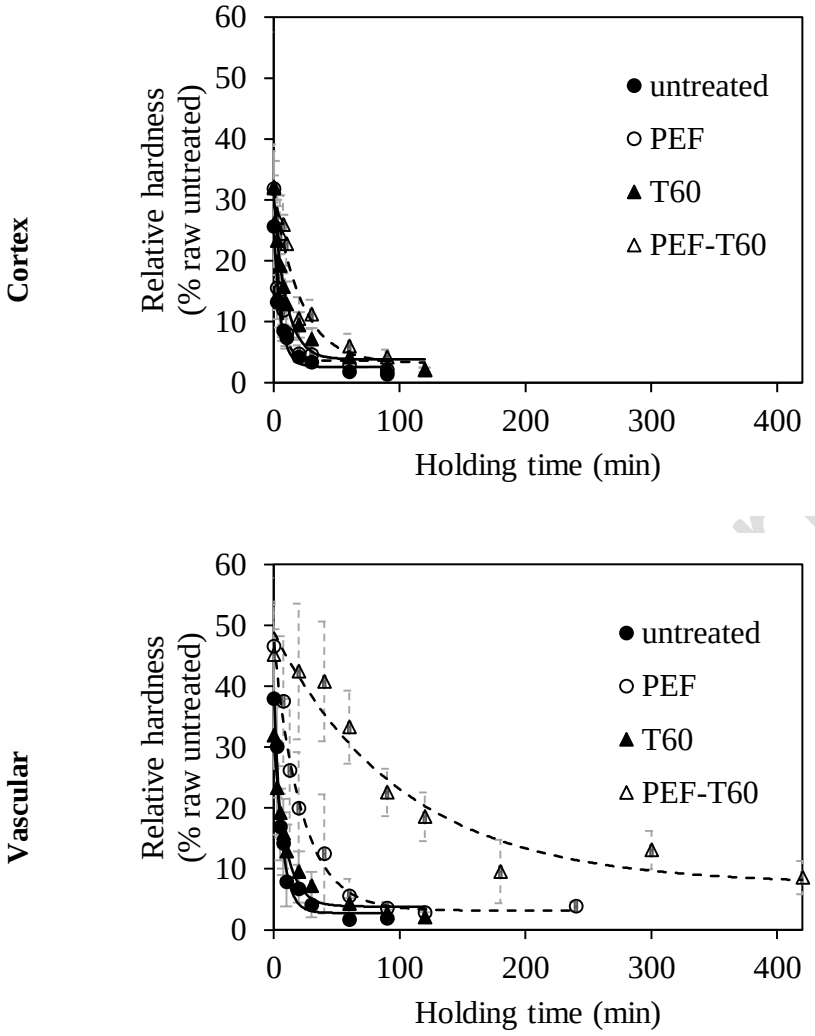


Figure 2: Hardness of raw carrot cortex (■) and vascular tissue (■) after PEF treatment (PEF), after mild thermal processing at 60 °C (T60) and after the combined PEF and thermal pretreatment (PEF-T60), relative to the hardness of the raw untreated tissue. Error bars represent standard errors on texture measurements. Different letters indicate significant differences between relative hardness based on the results from the Tukey HSD test with significance level 5%.



649 **Figure 3**: Cooking behaviour of carrot cortex and vascular tissue: relative hardness as a function of
650 holding time at 95 °C. PEF: pretreatment with pulsed electric fields, T60: mild thermal pretreatment at
651 60 °C, PEF-T60: combined PEF and thermal pretreatment. The data were modelled using the fractional
652 conversion model. Error bars represent standard deviations of the texture measurements.

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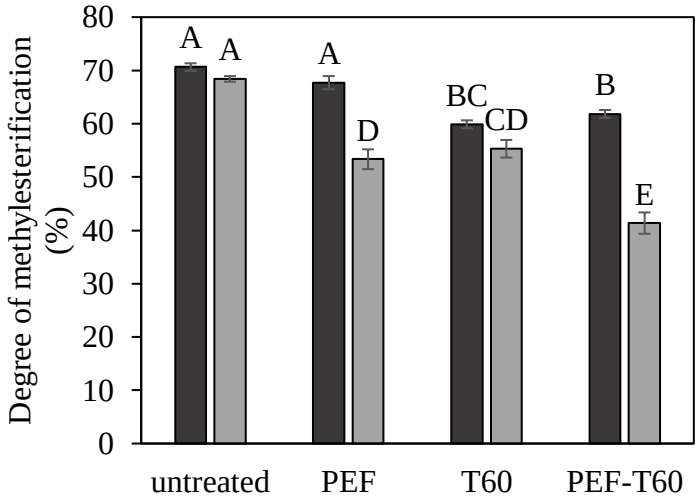
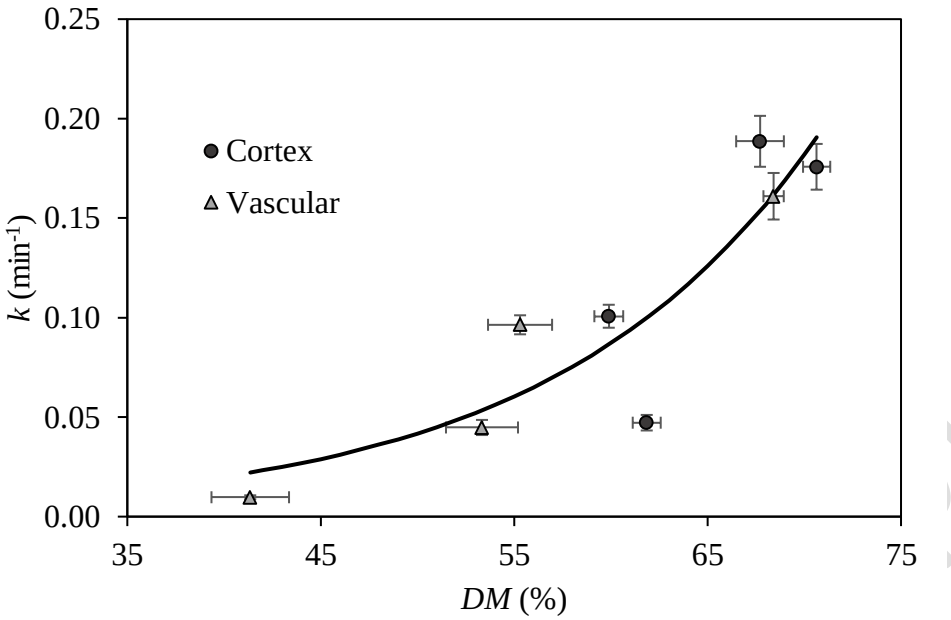


Figure 4: Degree of methylesterification (DM) of pectin from untreated and pretreated raw cortex (■) and vascular (■) carrot tissue. PEF: pretreatment with pulsed electric fields, T60: mild preheating at 60 °C, PEF-T60: combined PEF and thermal pretreatment. Error bars represent standard errors on the DM measurements. Letters indicate significant differences in DM, corresponding to the Tukey HSD test results with significance level 5%.



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678 **Figure 5**: Correlation between the rate constant *k* of texture degradation at 95 °C and the degree of
679 methylesterification (*DM*) of pectin from carrot tissues: $k = 0.001_{(\pm 0.001)} \cdot exp(0.074_{(\pm 0.020)} \cdot DM)$ with R^2_{corr}
680 = 0.941 and subscripts representing standard errors of regression. Error bars represent standard errors
681 on the estimated rate constant *k* and the measured *DM*.

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Tissue	H_0 (N)	k (kg/kJ)	H_r (N)	R^2_{corr}
Cortex	139.20 ± 2.52^{A}	4.311 ± 0.364^{A}	52.64 ± 1.11^{B}	0.842
Vascular	125.50 ± 3.53^{B}	6.843 ± 1.571^{A}	83.33 ± 1.14^{A}	0.418

Table 1: Kinetic parameter estimates with their standard error for the hardness decay of cortex and vascular tissue after PEF treatments with increasing total specific energy input, using the fractional conversion model. H_0: hardness of untreated tissue, k: exponential factor expressing the sensitivity of the tissue to softening by PEF treatments with different total specific energy input, H_r: residual hardness, R^2_{corr}: corrected correlation coefficient. Different letters indicate significant differences between parameters of cortex and vascular tissue, based on the overlap of their 95% confidence intervals.

	Cortex	Vascular
Cell diameter (µm)	89 ± 1^{B}	163 ± 3^{A}
Conductivity puree (mS/cm)	19.0 ± 0.4^{B}	23.2 ± 0.4^{A}
Calcium content (mg/g)	0.334 ± 0.210^{B}	1.933 ± 0.175^{A}
Dry matter content (%)	12.35 ± 0.09^{A}	12.01 ± 0.04^{B}
PME activity (U/g dry matter)	14.62 ± 1.26^{A}	6.32 ± 0.03^{B}

Table 2: Characterisation of carrot tissues: cell diameter, conductivity of puree, calcium content, dry matter content and PME activity, together with their standard error. Different letters indicate significant differences between cortex and vascular tissue, based on results of the Student's t-test with significance level 5%.

	Pretreatment	H_0 (% raw untreated)	k (min^{-1})	H_r (% raw untreated)	R^2_{corr}
Cortex	untreated	24.33 ± 0.60^{D}	0.1758 ± 0.0115^{A}	2.59 ± 0.34^{B}	0.838
	PEF	30.05 ± 0.76^{C}	0.1886 ± 0.0128^{A}	3.60 ± 0.43^{B}	0.827
	T60	30.43 ± 0.58^{C}	0.1007 ± 0.0058^{B}	3.83 ± 0.36^{B}	0.882
	PEF-T60	31.72 ± 0.67^{C}	0.0472 ± 0.0039^{C}	3.21 ± 0.65^{B}	0.832
Vascular	untreated	38.88 ± 1.09^{B}	0.1610 ± 0.0117^{A}	2.78 ± 0.64^{B}	0.810
	PEF	47.11 ± 1.37^{A}	0.0448 ± 0.0038^{C}	3.16 ± 0.88^{AB}	0.806
	T60	45.21 ± 0.76^{A}	0.0964 ± 0.0048^{B}	4.58 ± 0.49^{AB}	0.911
	PEF-T60	48.82 ± 1.24^{A}	0.0097 ± 0.0010^{D}	7.48 ± 1.38^{A}	0.766

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744 **Table 3**: Kinetic parameter estimates with their standard error describing the texture degradation
745 kinetics of untreated and pretreated carrot cortex and vascular tissue at 95 °C, using the the fractional
746 conversion model. H_0: initial hardness, relative to the hardness of raw untreated carrot tissue, k: rate
747 constant of texture degradation at 95 °C, H_r: residual relative hardness, R^2_{corr}: corrected correlation
748 coefficient. PEF: pretreatment with pulsed electric fields, T60: mild thermal pretreatment at 60 °C, PEF-
749 T60: combined PEF and thermal pretreatment. Significant differences between tissues and
750 pretreatments are indicated with different letters, based on the comparison of the 95% confidence
751 intervals of the estimated parameters.

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