Effect of pulsed electric field and mild thermal processing on texture-related pectin properties to better understand carrot (*Daucus carota*) texture changes during subsequent cooking

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Abstract

Pulsed electric field (PEF) and/or mild thermal (60 °C) processing distinctly affect the texture degradation of carrot tissues during subsequent cooking. The impact of PEF and/or mild thermal processing on texture-related pectin properties was studied in terms of pectin extractability, degree of methylesterification (DM) and Ca²⁺ crosslinking in carrot cortex and vascular tissue. In the case of cortex tissue, the treatments significantly ($\alpha=0.05$) reduced the extractability of pectin in water and alkali; the pectin DM and ionic crosslinking were not affected. In the case of vascular tissue, mainly the PEF and combination treatment reduced the extractability of pectin in water and alkali, reduced the pectin DM (approx. 60 to 50%), and improved its ionic crosslinking. Addition of Ca²⁺ ions may help preserve the texture of both tissues during subsequent cooking. Further research is necessary to investigate the effect of the treatments on covalent pectin crosslinking, especially in cortex tissue.

Keywords

pulsed electric field; thermal processing; texture; pectin; cell wall fractionation; calcium crosslinking

1. Introduction

Fruits and vegetables are an important part of the human diet. Their consumption is promoted because of their associated health benefits and their role in the prevention of chronic diseases (Gonzalez et al., 2010a; Southon & Faulks, 2002). Texture is one of the important sensorial quality attributes of fruit and vegetable products (Waldron et al., 2003) but may (deliberately or not) change during processing, e.g. soften during thermal processing used for product shelf life extension (Gonzalez et al., 2010a; Ramaswamy & Chen, 2002). Therefore, food scientists and the food industry are searching for technologies that help extend the product's shelf-life, while maintaining the desired quality (De Roeck et al., 2008). Understanding the relationship between food texture perception and food structure may facilitate the production of texturally attractive products (Sila et al., 2008; Wilkinson et al., 2000).

Texture of plant tissue based food products is mainly determined by the turgor pressure in the cell, and the cell wall and middle lamella polysaccharides (De Roeck et al., 2008; Fincan & Dejmek, 2003; Jackman & Stanley, 1995; Ranganathan et al., 2016). The turgor pressure is the hydrostatic pressure within the cell that results from an osmotic difference between the inside and outside of the cell and an intact semipermeable plasma membrane, and provides, together with the plant cell wall, rigidity and firmness to plant tissues (Gonzalez & Barrett, 2010b; Jackman & Stanley, 1995). However, the texture determining role of the turgor pressure of thermally processed fruit and vegetable tissue is considered

to be small, as the membrane integrity is quickly lost upon heating (Gonzalez et al., 2010a; Ranganathan et al., 2016).

The main polysaccharides of the primary plant cell wall are cellulose, hemicellulose, and pectin. Cellulose is composed of linear chains of D-glucose residues, that are associated into microfibrils. These microfibrils provide rigidity to the cell wall (Christiaens et al., 2016; Ranganathan et al., 2016).

Hemicelluloses are a heterogeneous group of complex cell wall polysaccharides that comprise xyloglucans, xylans, and mannans and provide plasticity to the cell wall (Christiaens et al., 2016; Houben et al., 2011; Ranganathan et al., 2016). Xyloglucans can bind cellulose through hydrogen bonding, providing a load-bearing xyloglucan-cellulose network (Albersheim et al., 1996; Caffall & Mohnen, 2009; Christiaens et al., 2016).

Pectin is the main polysaccharide of the middle lamella, that acts as an adhesive between cells (Christiaens et al., 2016; Ranganathan et al., 2016). Moreover, thermal processing mainly affects the structure of pectin, leaving the cellulose and hemicellulose structure almost unchanged (Houben et al., 2011; Sila et al., 2008; Williams & Besler, 1996). Therefore, the structure and organisation of pectin can be considered as one of the most important texture determining factors in thermally processed fruit and vegetable tissues.

Pectin comprises the linear subdomain homogalacturonan (HG), and the branched subdomains rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). HG is the most abundant pectic polymer (60% of pectin) and is composed of linked D-galacturonic acid (GalA) residues, that can be methylesterified and acetylated, depending on the plant source (Caffall & Mohnen, 2009; Christiaens et al., 2016; Ridley et al., 2001). HG can be demethylesterified under near-neutral and alkaline conditions ($\text{pH} \geq 5$) or by microbial pectin methylesterase (PME), resulting in randomly distributed demethylesterified GalA residues, or by plant PME, resulting in blocks of demethylesterified GalA residues. The pattern and degree of methylesterification (DM) largely determines the functionality of HG, as long regions of negatively charged demethylesterified GalA residues can bind divalent cations, such as Ca^{2+} , and crosslink HG intra- and intermolecularly. This improves cell adhesion and firms the texture (Christiaens et al., 2016; Houben et al., 2011; Sila et al., 2008; Willats et al., 2006). Acetyl groups, on the other hand, hinder the formation of ionic crosslinks (Christiaens et al., 2016; Ralet et al., 2005). Heating pectin at temperatures higher than 80 °C and at near-neutral or alkaline pH ($\text{pH} \geq 5$) promotes the β -eliminative depolymerisation of HG. Therefore, in general, thermal processing results in a softer texture. However, the demethylesterification and ionic crosslinking of pectin inhibits the β -elimination reaction (Christiaens et al., 2016).

The branched subdomain RG-I (20-35% of pectin) consists of a backbone of repeating disaccharides of GalA and rhamnose (Rha) residues, and of a variety of glycan side chains, mainly arabinan and galactan, that are attached to Rha and vary in size and degree of branching. The third subdomain RG-II (10% of

pectin) is structurally the most complex pectic polysaccharide and is largely conserved across plant species. It consists of a HG backbone with complex side chains (Caffall & Mohnen, 2009; Christiaens et al., 2016; Houben et al., 2011; Mohnen, 2008; Ridley et al., 2001; Willats et al., 2006).

The three pectin building blocks are covalently linked to each other, but the exact macromolecular structure is still under debate (Christiaens et al., 2016; Mohnen, 2008; Vincken et al., 2003; Willats et al., 2006; Yapo, 2011). Moreover, pectins are present as a pectin-pectin network through the presence of covalent and non-covalent crosslinks (Albersheim et al., 1996; Christiaens et al., 2016), such as ionic crosslinks (Celus et al., 2018), phenolic crosslinks (Ishii, 1997; Waldron et al., 1996), borate diol crosslinks (Mohnen, 2008; O'Neill et al., 2004), hydrophobic interactions, hydrogen bonds, and uronyl ester linkages. Next to the load-bearing hemicellulose-cellulose network and pectin-pectin network in the cell wall, also interactions between pectin and hemicellulose and between pectin and cellulose exist (Christiaens et al., 2016).

The structure and organisation of cell wall polymers affect the solubility, and thus extractability, of the polymers. Consequently, sequentially extracting the cell wall material (CWM) and structurally characterising the different fractions and residues may help to elucidate the structure and organisation of cell wall polymers. Loosely bound cell wall polymers can be extracted using water, while ionically crosslinked pectins can be extracted using a chelator. Cell wall polymers that are covalently bound to the cell wall can be extracted using alkaline solvents. Depending on the concentration of the alkali, pectins and hemicelluloses can be extracted (Christiaens et al., 2016; Selvendran & O'Neill, 1987).

Thermal processing of fruit and vegetable tissue is often required to extend the shelf stability through inactivation of micro-organisms and endogenous enzymes. The intensive heating softens the product texture, which may be both desired and undesired. However, there is an increasing demand for minimally processed fresh-like products with a sufficient shelf life (Ranganathan et al., 2016). Therefore, several technologies are available to preserve the texture of plant tissue based food products during thermal processing, such as mild thermal (pre)processing (15-60 min at 60-75 °C). This treatment promotes endogenous PME activity and thus the demethylesterification and ionic crosslinking of pectin. Consequently, the texture is better preserved during subsequent thermal processing, and this effect is even more pronounced if extra Ca^{2+} ions are added during the pretreatment (Lemmens et al., 2009; Sila et al., 2005; Smout et al., 2005). Moreover, these temperatures cause cell membrane disruption, which may further facilitate pectin changes by PME as more cations become available to activate PME (Ranganathan et al., 2016).

Recently, pulsed electric field (PEF) technology was also found to slow down the texture degradation of carrot tissue during cooking (i.e. cooking behaviour) (Moens et al., 2020). PEF technology refers to the application of short (μs -ms) intensive electric pulses to biological cell material that is surrounded by a conductive medium and placed between two electrodes. (Ir)reversible pores are formed in the cell

membrane, an event referred to as cell electroporation, with the electroporation being irreversible if the applied electric field strength exceeds the critical electric field strength (Pagan & Manas, 2006; Puértolas et al., 2012). Next to the electric field strength, other parameters can be varied, such as treatment time, specific energy, pulse width and shape, frequency and temperature. Also product characteristics (e.g. cell size) and medium characteristics (e.g. conductivity) determine the treatment efficiency (Ben Ammar et al., 2011; Kanduser & Miklavcic, 2009; Moens et al., 2020; Puértolas et al., 2012; Toepfl et al., 2006; Vorobiev & Lebovka, 2008). This technology is already in use to inactivate micro-organisms in liquid food products (10-15 kV/cm), and to promote diffusion processes in plant tissues (0.7-3 kV/cm), facilitating drying and extraction processes (Pagan & Manas, 2006; Toepfl et al., 2006; Vorobiev & Lebovka, 2008).

Additionally, the electroporation of the cell membranes affects the texture of fresh plant tissues. As the cell membranes are no longer intact, the turgor pressure is lost. This results in a softer texture and a lower cutting force of fresh tissue (Botero-Urbe et al., 2017; Leong et al., 2014; Moens et al., 2020). Moreover, Moens et al. (2020) recently showed that the slower cooking behaviour of PEF treated carrot tissue was correlated with the pectin DM. This indicates that PEF may affect the structure of pectin in the cell wall and middle lamella. Moens et al. (2020) hypothesised that the PEF pretreatment facilitates the enzymatic demethylesterification of pectin by PME: as the membranes are permeabilised by PEF, intracellular cations may migrate to the cell wall and middle lamella, promoting PME activity. However, the correlation between the texture degradation rate constant and pectin DM did not fully explain the observed differences in cooking behaviour after the PEF and/or mild thermal pretreatment and the different effects of processing on carrot cortex and vascular tissue. This indicates that the PEF pretreatment may also promote the formation of ionic and/or covalent crosslinks.

Therefore, the objective of this study is to further elucidate the effect of PEF and/or mild thermal processing on the texture-related structural properties of pectin in carrot tissues. The occurrence of ionic crosslinks, linked to changes in pectin DM, in both carrot cortex and vascular tissue is studied by sequentially extracting pectin and characterising the extracts and residues in terms of yield, pectin DM and Ca^{2+} content. As the effect of the different treatments on texture of fresh tissue and during subsequent thermal processing was already extensively studied by Moens et al. (2020), no texture measurements are included in this paper.

2. Materials and methods

2.1 Plant materials

Two batches of carrots (*Daucus carota*) of the cultivar Nerac were purchased from a local shop in Belgium in October 2019 and January 2020, respectively. They were stored in a plastic bag at 4 °C for maximum two weeks before use (De Roeck et al., 2010; Moens et al., 2020).

2.2 Treatments of carrot tissues

Carrot tissues were submitted to four types of treatment: no treatment (untreated), a pulsed electric field treatment (PEF), a mild thermal treatment at 60 °C (T60), and a pulsed electric field treatment followed by a mild thermal treatment at 60 °C (PEF-T60) (Moens et al., 2020). To avoid structural changes of cell wall components due to enzymatic activity during CWM extraction, fractionation and analysis, the (un)treated tissues were blanched. Finally, the cortex and vascular tissue were separated from each other. All treatments were performed in duplicate: one repetition for each batch of carrots.

2.2.1 Pulsed electric field treatment

The pulsed electric field (PEF) treatments were performed using the method described by Moens et al. (2020). Carrots were manually peeled, reduced to a length of 17 cm, rinsed in tap water, blotted dry with tissue paper (Leong et al., 2014) and treated with electric pulses using the batch PEF unit Cellcrack III of Elea-DIL (German Institute for Food Technologies, Quackenbrück, Germany). Five carrots were treated at a time in a treatment chamber with two parallel stainless steel plate electrodes (24 x 22.5 x 0.5 cm, w x h x t), an interelectrode distance of 29.7 cm, and 2 cm thick insulator material. The carrots (approximately 0.85 kg) were positioned with their longitudinal axis perpendicular to the electrodes and were immersed with standardised water (0.6156 g NaCl/l and 0.0923 g CaCl₂·2H₂O/l) with 1400 µS/cm conductivity at 25 °C (Ben Ammar et al., 2011; Liu et al., 2017; Willemsen et al., 2017) until a total weight of 3.5 kg was obtained. Twenty exponential monopolar pulses were applied at room temperature, with a pulse amplitude of 30 kV, an electric field strength of 1.01 kV/cm, an energy input per pulse of 450 J/pulse, a specific energy input per pulse of 129 J/kg·pulse, a total specific energy input of 2.571 kJ/kg, a frequency of 2 Hz, and a pulse width of 145 ± 9 µs (TBS 1102B-EDU digital oscilloscope, Tektronix, Köln, Germany). Within one minute after the treatment, the carrots were removed from the treatment chamber and medium, and within 5 min after the treatment they were vacuum packed and placed in a fridge at 4 °C, where they were stored for maximum 16 h before use.

2.2.2 Mild thermal treatment

Peeled untreated and PEF treated carrots were thermally treated at 60 °C. Therefore, carrots were cut transversally into discs of approximately 5 mm thickness, with the cutting being performed at 4 °C to limit enzymatic activity. The discs were vacuum packed as a monolayer and heated for 20 min in a temperature-controlled water bath (WNB29, Memmert, Schwabach, Germany) at 60 °C, which is the optimal temperature for PME activity (Smout et al., 2005).

2.2.3 Blanching step

To inactivate endogeneous enzymes (e.g. PME) that could alter the structure of pectin during extraction, fractionation and analysis, the (un)treated tissues were blanched. Discs of 5 mm thickness were cut transversally from peeled untreated and PEF treated carrots and vacuum packed at 4 °C. The vacuum

packs were heated for 8 min in a temperature-controlled water bath at 95 °C (WBU45, Memmert, Schwabach, Germany), after which they were immediately cooled in an ice bath. The carrot discs that already underwent a mild thermal treatment at 60 °C (Section 2.2.2) were directly transferred from the 60 °C water bath to the 95 °C water bath, blanched for 8 min, and cooled in an ice-bath.

2.2.4 Separation of cortex and vascular carrot tissue

The cortex and vascular tissue from the blanched untreated and PEF and/or mild thermally treated carrot discs were separated from each other. The separate tissues were vacuum packed and stored at -40 °C until CWM extraction.

2.3 Extraction and characterisation of cell wall material fractions

The different processing techniques may alter the structure and organisation of pectin, hereby affecting the extractability of the polymer. Therefore, the effect of PEF and mild thermal processing on the texture-related pectin properties was studied by extracting the CWM or alcohol insoluble residue (AIR) from the (un)treated carrot tissues, by fractionating the AIR into a water extractable fraction (WEF) and an alkaline extractable fraction (AEF), and by analysing the DM and ionic crosslinking of pectin in the obtained fractions and residues.

2.3.1 Extraction of alcohol insoluble residue

CWM from the blanched untreated and PEF and/or mild thermally treated carrot tissues was extracted as alcohol insoluble residue (AIR) using the method of McFeeters & Armstrong (1984). Firstly, the frozen tissue was homogenised (Grindomixer GM200, Retsch, Haan, Germany), and 60 g of the puree was suspended in 192 ml of 95% ethanol using a mixer (B-400, Buchi, Flawil, Switzerland). The suspension was filtered using a Büchner funnel with a paper filter (MN 615, Ø 90 mm, Macherey-Nagel, Düren, Germany). Secondly, the residue was resuspended in another 96 ml 95% ethanol and filtered analogously. Thirdly, the residue was resuspended in 96 ml acetone under magnetic stirring, and filtered. Finally, the residue was dried overnight at 40 °C after which it was ground using a mortar and pestle and stored in a desiccator (Willemsen et al., 2017). The residue was suspended in demineralised water (10 g/l), and the suspension was set at pH 7 and dialysed for 48 h at 4 °C against demineralised water while contained in a membrane (Spectra/Por® 3 dialysis membrane, MWCO 3.5 kD, Spectrum Laboratories Inc., CA, USA). The dialysed suspension was freeze-dried for 22 h (2-4 LSC, Christ-Alpha, Osterode, Germany), and the dry AIR was stored in a dessicator. For every combination of tissue and treatment type, two AIR samples were generated, corresponding to the two carrot batches.

2.3.2 Fractionation of alcohol insoluble residue

AIR was fractionated into a water extractable fraction (WEF), a water unextractable fraction (WUF), an alkaline extractable fraction (AEF), and an alkaline unextractable fraction (AUF) (**Figure 1**). The WEF is assumed to contain weakly bound pectin with a high DM (Houben et al., 2011; Selvendran & O'Neill, 1987). The WEF was extracted by stirring 1 g of dialysed and freeze-dried AIR in 200 ml demineralised water at pH 4.0 for 5 min while boiling. The suspension was immediately cooled in an icebath and filtered using a Büchner funnel and filter paper (MN 615, Ø 90 mm, Macherey-Nagel, Düren, Germany) (Houben et al., 2011). The filtrate (WEF) was frozen using liquid nitrogen, and stored at -40 °C. The residue after WEF extraction, the WUF, is assumed to contain pectin that is ionically and/or covalently bound. Therefore, an increase in calcium crosslinks should be noticed by a larger amount of WUF and/or a higher bound calcium content of WUF (Section 2.3.6). As this study was interested in the characteristics of pectin remaining in the WUF, the extraction of WEF from each AIR sample was performed twice: the resulting WUF was once stored at -40 °C for analysis, and once used for further fractionation (**Figure 1**).

Figure 1 Alcohol insoluble residue (AIR) was extracted from blanched untreated and PEF and/or mild thermally treated carrot tissues and used for analysis (bold) and for sequential pectin extraction. AIR was fractionated into a water extractable fraction (WEF) and a water unextractable fraction (WUF). This fractionation step was performed twice: the WEF and WUF from the first extraction (left) was used for analysis (bold), whereas the WUF from the second extraction (right) was used for further fractionation into an alkaline extractable fraction (AEF) and an alkaline unextractable fraction (AUF). The AEF and AUF were used for analysis (bold).

Although this study mainly focused on the formation of ionic pectin crosslinks, the occurrence of covalently bound pectin after the different processing techniques was also taken into consideration by further fractionating the WUF into an AEF and AUF (**Figure 1**). The increase of this fraction and residue may indicate the formation of covalently crosslinked pectin, e.g. by phenolic crosslinks, and the need for further investigation of this event. Therefore, the AEF was extracted by stirring the WUF in 200 ml of 1 M KOH with 20 mM NaBH₄ for 24 h at 20 °C under nitrogen atmosphere (Houben et al., 2011). The concentration of the alkaline extraction solvent was chosen in order to facilitate the extraction of the crosslinked pectin, including the hydrolysis of phenolic crosslinks (Parr et al., 1997). The suspension was filtered using a Büchner funnel and filter paper (MN 615, Ø 90 mm, Macherey-Nagel). The filtrate (AEF) and residue (AUF) were frozen using liquid nitrogen, and stored at -40 °C.

All fractions (WEF and AEF) were thawed, brought to pH 7, dialysed for 48 h at 4 °C against demineralised water while contained in a membrane (Spectra/Por® 3 dialysis membrane, MWCO 3.5 kD, Spectrum Laboratories Inc., CA, USA), and freeze-dried for 22 h (2-4 LSC, Christ-Alpha, Osterode, Germany). All residues (WUF and AUF) were thawed, suspended in 100 ml demineralised water, brought to pH 7, and dialysed and freeze-dried as mentioned above. The dialysed and freeze-dried fractions and residues were stored in a desiccator until analysis. For each combination of tissue and treatment type, two WEF, WUF, AEF and AUF samples were generated, corresponding to the two carrot batches.

2.3.3 Mass balance of fractions and residues

The mass of the dialysed and freeze-dried starting material for fractionation (AIR), and of the dialysed and freeze-dried fractions (WEF and AEF) and residues (WUF and AUF) was determined. The mass yield of each fraction and residue was calculated by dividing the mass of the fraction or residue by the mass of the starting AIR, and multiplying this ratio with 100%. The mass yield of each fraction and residue was calculated twice, corresponding to the two carrot batches.

2.3.4 Galacturonic acid content and yield

The galacturonic acid (GalA) content of AIR, WEF, WUF, AEF and AUF was measured spectrophotometrically (Ahmed & Labavitch, 1977) after hydrolysis of pectin using concentrated sulfuric acid (Blumenkrantz & Asboe-Hansen, 1973). The hydrolysis of pectin was performed in duplicate, and the GalA content of each hydrolysate was measured in triplicate. Consequently, six GalA measurements were performed for each cell wall fraction or residue corresponding to a specific tissue and treatment type per carrot batch.

The GalA content of AIR, WEF, AEF and AUF was used to calculate a GalA balance after fractionation. The GalA yield of WEF, AEF and AUF was calculated by dividing the GalA content of the fraction or residue multiplied by the mass of this fraction or residue (i.e. mass of GalA in total fraction or residue)

by the GalA content of AIR multiplied by the starting mass of AIR for fractionation (i.e. mass of GalA in AIR as starting material for fractionation), and by multiplying this ratio with 100%. Consequently, the GalA yield of each cell wall fraction or residue corresponding to a specific tissue and treatment type was calculated per carrot batch, and the standard deviation on this parameter was calculated by error propagation taking into account the repeated GalA measurements.

The GalA content of AIR and WUF was used to calculate the bound calcium content and the *R* value of AIR and WUF (Section 2.3.6).

2.3.5 Degree of methylesterification

The degree of methylesterification (DM) of pectin was measured in triplicate for each AIR, WEF and WUF sample using Fourier-transformation infrared (FT-IR) spectroscopy (FTIR-8400S, Shimadzu, Kyoto, Japan), according to the method of Kyomugasho et al. (2015). Consequently, an average DM and standard deviation, based on repeated measurements, was obtained for each AIR, WEF and WUF sample per batch.

2.3.6 Bound calcium content and *R* value

The calcium content of each AIR and WUF sample was determined in duplicate. Ten mg of the CWM was weighted in a porcelain crucible, hydrated with 1 ml ultrapure water (organic free, 18 MΩ·cm resistance), dried overnight at 100 °C, and ashed in a muffle furnace (Controller P330, Nabertherm, Lilienthal, Germany) at 550 °C for 20 h. The ashes were dissolved in 9.9 ml ultrapure water and acidified with 0.1 ml 65% nitric acid. The solutions were left overnight at 4 °C and filtered (0.45 µm membrane filter, Chromaphil® A-45/25, Machery-Nagel, Düren, Germany). Their calcium content was measured radially at 318 nm using inductively coupled plasma optical emission spectroscopy (iCAP 7400 ICP-OES Duo spectrometer, Thermo Scientific, MA, USA). An SPS-SW2 certified standard was used for calibration (Gwala et al., 2020; Moens et al., 2020).

The bound calcium content represents the proportion of GalA units that is bound to calcium and was calculated as the calcium content of AIR or WUF (mol/g) divided by the GalA content of AIR or WUF (mol/g). The bound calcium content of each AIR and WUF sample corresponding to a specific treatment and tissue type was calculated for every carrot batch, and the standard deviation on this parameter was calculated using error propagation taking into account the error on the repeated GalA and Ca²⁺ measurements.

The *R* value represents the proportion of demethylesterified GalA units that is bound to calcium, and was calculated as the calcium content of AIR or WUF (mol/g) divided by the content of demethylesterified GalA units of AIR or WUF (mol/g) (Celus et al., 2018; Fang et al., 2008). The content of demethylesterified GalA units was calculated using the DM and the GalA content of the particular cell wall fraction. The *R* value of each AIR and WUF sample corresponding with a specific

treatment and tissue type was determined per carrot batch, together with a corresponding standard deviation taking into account the error on the repeated Ca^{2+} , GalA, and DM measurements.

2.3.7 Statistical data analysis

Significant differences between the mass yields of different fractions and residues after the different types of treatment were investigated per type of tissue using the Tukey honest significant difference (HSD) test ($n=2$, $\alpha=0.05$).

The GalA yields of different fractions and residues after the different types of treatment were compared per type of tissue by means of their 95% confidence intervals. Also significant differences in DM, bound calcium content, and R values between treatments were investigated per fraction or residue and per type of tissue by comparing their 95% confidence intervals. The middle of the interval was equal to the average value of the parameter of both carrot batches, and the interval width was determined by the sample size ($n=2$), a significance level of 5%, and the standard deviation of the average parameter value of both batches, obtained using error propagation taking into account the standard deviation of the parameter value per batch. Samples with overlapping 95% confidence intervals were assumed to have a similar GalA yield, DM, bound calcium content, or R value.

3. Results and discussion

3.1 Mass balance fractionation

The PEF and mild thermal treatment may affect the structure and organisation of cell wall polymers, thereby affecting their extractability. Therefore, the alcohol insoluble residue (AIR) extracted from (un)treated carrot cortex and vascular tissue was fractionated into a water extractable fraction (WEF), an alkaline extractable fraction (AEF) and an alkaline unextractable fraction (AUF). The mass balance of the CWM from the (un)treated carrot tissues is illustrated in **Figure 2**. For both tissues, the total mass yield approaches 100%. This means only a small loss of CWM occurred during the fractionation protocol.

The mass yield of loosely bound CWM (WEF) from cortex decreased slightly but not significantly after the PEF and/or mild thermal treatment (**Figure 2 (a)**). Also a slight decrease of AEF mass yield was detected after the combination of the PEF and mild thermal treatment. The mass yield of AUF, on the other hand, slightly increased after the different treatments, with the increase being significant after the combination treatment.

Although no significant differences in the mass yield of WEF and AEF from vascular CWM were found between the different treatments, the mass yield of WEF and AEF seemed to decrease after the PEF and the combination treatment (**Figure 2 (b)**). The use of alternative statistical approaches (general linear models and mixed models, data not shown) confirmed this trend. Moreover, the mass yield of AUF was

significantly higher after these treatments. The mild thermal treatment affected the extractability of the vascular CWM only slightly.

Figure 2 Mass yield of water extractable fraction (■ WEF), alkaline extractable fraction (■ AEF), and alkaline unextractable fraction (■ AUF) from alcohol insoluble residue extracted from untreated, PEF treated (PEF), mild thermally treated (T60), and PEF and mild thermally treated (PEF-T60) carrot cortex (a) and vascular tissue (b). The error bars represent the standard error on the mass yield. The yields were compared between cell wall fractions and treatments per type of tissue using the Tukey HSD test with a significance level of 5%. Significant differences in extraction yields between cell wall fractions and treatments are indicated with different letters.

The change of the CWM extractability indicates that all treatments resulted in the formation of ionic and/or covalent crosslinks. However, a differentiation between the effect of the different treatments on the structure and organisation of the CWM can not be made based on the observed differences in mass yield. There might be different reasons for this, such as the loss of CWM during extraction and the uncomplete extraction of the crosslinked pectin under the alkaline conditions used.

GalA is an important building block of pectin. Therefore, determining the mass balance of GalA, instead of the overall mass balance, is a more specific approach to investigate the effect of the PEF and/or mild thermal treatment on the distribution of pectin over the different fractions and residues. For that reason, the GalA content of each fraction and residue was measured and the yield of GalA after each extraction step was determined (**Figure 3**). The range of the total GalA yield varied from 90 to 110%. As the total mass yield approached 100% (**Figure 2**), the range of the total GalA yield may be explained by the error on the GalA measurements.

The GalA yield of WEF extracted from cortex AIR was significantly lower after the PEF treatment, and lowest after the mild thermal and the combination treatment (**Figure 3 (a)**). The GalA yield of AEF

decreased in the order untreated, T60, PEF, and PEF-T60. Additionally, the GalA yield of AUF seemed lowest after the mild thermal treatment, and highest after the PEF and PEF-T60 treatment.

Figure 3 Galacturonic acid (GalA) yield of water extractable fraction (■ WEF), alkaline extractable fraction (■ AEF), and alkaline unextractable fraction (■ AUF) from alcohol insoluble residue extracted from untreated, PEF treated (PEF), mild thermally treated (T60), and PEF and mild thermally treated (PEF-T60) carrot cortex (a) and vascular tissue (b). The error bars represent the standard deviation on the GalA yield. The yields were compared between cell wall fractions and treatments per type of tissue by means of their 95% confidence intervals. Significant differences in extraction yields between cell wall fractions and treatments are indicated with different letters.

The GalA yield of WEF from vascular AIR decreased in the order untreated, T60, PEF, and PEF-T60, while the GalA yield of AEF was lower after the mild thermal treatment, and lowest after the PEF and the combination treatment (**Figure 3 (b)**). Finally, the GalA yield of AUF was lowest in case of the untreated vascular tissue, and largest after the PEF and the combination treatment.

In general, the results of the GalA yield (**Figure 3**) are in line with the results of the mass yield (**Figure 2**). In the cortex, the different treatments led to a decrease of loosely bound pectin, illustrated by a lower GalA yield of WEF. The decrease in GalA yield of AEF and the increase in GalA yield of AUF suggests that more pectin becomes difficult to extract by the used alkaline solution, especially after the PEF-T60 treatment. In vascular tissue, pectin becomes more difficult to extract using hot water, especially in the case of the PEF and the combination treatment. Moreover, a lower GalA yield of AEF and a larger GalA yield of AUF was found after these treatments, suggesting the formation of strong pectin crosslinks, impeding pectin extraction. To differentiate the effect of the different treatments on the texture-related properties of pectin, the DM (Section 3.2) and Ca^{2+} binding (Section 3.3) of pectin in the different fractions and residues was investigated.

3.2 Pectin demethylesterification

The PEF and mild thermal treatment may promote the action of PME, lowering the degree of methylesterification (DM) of pectin and facilitating the formation of ionic crosslinks. Therefore, the DM of pectin from untreated and PEF and/or mild thermally treated cortex and vascular tissue was determined (**Figure 4**). As the alkaline conditions during the AEF extraction promote demethylesterification of pectin, the DM of pectin in AEF and AUF is not representative for the DM of *in situ* pectin. Hence, only the DM of pectin in AIR, WEF and WUF was determined.

Figure 4 Degree of methylesterification (DM) of pectin in (a) alcohol insoluble residue (AIR), (b) water extractable fraction (WEF), and (c) water unextractable fraction (WUF) from untreated, PEF treated (PEF), mild thermally treated (T60), and PEF and mild thermally treated (PEF-T60) carrot cortex (■) and vascular tissue (□). The error bars represent the standard deviation on the DM values. The DM values were compared between treatments per type of tissue and per cell wall fraction by comparison of their 95% confidence intervals with significance level of 5%. Significant differences in DM between treatments are indicated with different letters.

Although a slight decrease in DM of cortex AIR can be observed after the mild thermal and the combination treatment in **Figure 4 (a)**, no significant difference could be found. Possibly, the mild thermal treatment was too short to have a significant effect on the DM. The DM of vascular AIR, on the other hand, was significantly higher after the mild thermal treatment. However, as an increase in DM is improbable, and a large variability between the two batches was observed for the DM of pectin in AIR from untreated vascular tissue, the DM of pectin in AIR from untreated vascular tissue may be higher than depicted in **Figure 4 (a)** and similar to the DM of pectin in AIR from mild thermally treated vascular tissue. In that case, the DM of pectin in vascular AIR may be reduced after the PEF and the combination treatment.

The results obtained for the different treatments do not differ to the same extent as those obtained by Moens et al. (2020), where the DM of pectin from cortex decreased significantly after the mild thermal treatment and the combination treatment, and the DM of pectin from vascular tissue decreased after all types of treatment, especially after the combination treatment. These differences may be explained by the sample preparation. Moens et al. (2020) determined the DM of pectin using cryosections cut from untreated and PEF and/or mild thermally treated carrot tissues, resulting in the DM of *in situ* pectin. The DM in **Figure 4 (a)** represents the DM of extracted pectin. Taking into account the possible loss of some pectins during the AIR extraction procedure, the DM values of AIR may be somewhat different from the DM values of cryosections. Moreover, Moens et al. (2020) analysed the DM of pectin from ten different carrots from a single batch, whereas the DM in **Figure 4** is representative for carrots from two different batches. This approach may lead to a more variable effect of the different treatments on the DM.

No clear effect of the different treatments on PME action could be deduced from the DM of pectin in cortex WEF (**Figure 4 (b)**). The DM of vascular WEF, on the other hand, was significantly lower after the mild thermal treatment and lowest after the PEF and the combination treatment. This is in line with the results obtained before (Moens et al., 2020).

No significant differences in DM of pectin in cortex WUF could be found after the different treatments. In contrast, the DM of pectin in vascular WUF was significantly lower after the PEF and the combination treatment.

In general, pectin from the vascular tissue seemed more prone to pectin demethylesterification in comparison with pectin from the cortex. Whereas the effect of the PEF and the mild thermal treatment on the DM of pectin from cortex was not very clear, the mild thermal treatment and especially the PEF treatment seemed to reduce the DM of pectin from vascular tissue. The differences in susceptibility to pectin demethylesterification between the carrot tissues were also observed by Moens et al. (2020). It was found that puree made from vascular carrot tissue has a higher conductivity than puree made from cortex, suggesting that in vascular tissue more cations can become available at the cell wall, the site of PME action, after cell permeabilisation.

3.3 Calcium binding

The demethylesterification of pectin may promote ionic crosslinking of pectin through pectin-Ca²⁺ crosslinks. An increase in the proportion of GalA units that is bound to Ca²⁺ ions may indicate the formation of such crosslinks. Therefore, the bound calcium content of AIR and WUF from untreated and PEF and/or mild thermally treated cortex and vascular carrot tissue was determined (**Figure 5**). Although the intrinsic Ca²⁺ content of vascular tissue is higher compared to the Ca²⁺ content of cortex tissue (Moens et al., 2020), the bound calcium content was not systematically higher in vascular CWM.

This might be explained by the higher GalA content of AIR from untreated vascular tissue (1.79 ± 0.04 mmol/g AIR) compared to the GalA content of AIR from untreated cortex (1.43 ± 0.02 mmol/g AIR).

Figure 5 Bound calcium content of (a) alcohol insoluble residue (AIR) and (b) water unextractable fraction (WUF) from untreated, PEF treated (PEF), mild thermally treated (T60), and PEF and mild thermally treated (PEF-T60) cortex (■) and vascular tissue (□). The error bars represent the standard deviation on the bound calcium content. The bound calcium content was compared between treatments per type of tissue and per cell wall fraction by comparing 95% confidence intervals. Significant differences in bound calcium content between treatments are indicated with different letters.

The bound calcium content of AIR extracted from the cortex is not affected by the different treatments (**Figure 5 (a)**). This corresponds with the observed DM of cortex AIR (**Figure 4 (a)**), which was also not significantly affected by the different treatments. It seems that in carrot cortex, the treatments have an insignificant effect on the DM and ionic crosslinking of pectin. Given the slower texture degradation of cortex tissue during thermal processing following the mild thermal and combination treatment, as observed by Moens et al. (2020), other phenomena might occur that tighten the pectin network and affect the cooking behaviour, such as the formation of phenolic crosslinks (Parr et al., 1997). Further research, beyond Ca²⁺ crosslinking, on the cell wall polymers after the different treatments seems necessary, e.g. on the formation of strong covalent crosslinks between pectin and hemicellulose and/or cellulose.

The proportion of GalA units bound to Ca²⁺ in vascular AIR, on the other hand, increased after the mild thermal treatment and was largest after the PEF and the combination treatment (**Figure 5 (a)**). The increased bound calcium content of the mild thermally treated tissue may be explained by a reduction of the DM. However, this was not the case in **Figure 4 (a)**, where the DM of AIR from mild thermally treated vascular tissue appeared to be high. However, large deviations between the carrot batches may explain the apparent low DM of AIR from the untreated vascular tissue and the apparent high DM of

AIR from mild thermally treated vascular tissue. The increased bound calcium content of vascular AIR after the PEF and combination treatment may be explained by a reduced DM (**Figure 4 (a)**). Additionally, the increased bound calcium content after the mild thermal treatment and especially after the PEF and the combination treatment is related to a decreased mass and GalA yield of WEF and AEF and an increased mass and GalA yield of AUF in **Figure 2 (b)** and **Figure 3 (b)**.

In general, the bound calcium content of WUF was higher than the bound calcium content of AIR (**Figure 5**). The removal of WEF may lead to an upconcentration of ionically crosslinked pectin, enhancing the bound calcium content of the residue (WUF).

The bound calcium content of WUF from the cortex was independent of the treatment used (**Figure 5 (b)**). Also no significant differences in DM were found that may explain a better calcium retention in WUF after certain treatments (**Figure 4 (c)**). Again, it seems that the explanatory role of pectin- Ca^{2+} crosslinking in the slower texture degradation during cooking of cortex is restricted. Texture measurements of the carrot tissues after the different treatments and subsequent thermal processing, on the other hand, were not performed during this study to confirm the texture changes observed by Moens et al. (2020) and to support a well-grounded elimination of pectin- Ca^{2+} crosslinking as an explanatory factor for the texture changes.

The proportion of GalA units bound to Ca^{2+} in vascular WUF increased to the same extent after all types of treatment (**Figure 5 (b)**). This is in contrast with the results obtained in **Figure 5 (a)**, where significant differences in bound calcium content were found between the treatments. This suggests that with the extraction of WEF, also ionically crosslinked pectin is removed, especially in the case of PEF and PEF and mild thermally treated vascular tissue. The similar bound calcium content of vascular WUF after the different treatments, on the other hand, does not correspond with the observed differences in DM of the same samples in **Figure 4 (c)**. Possibly, the intrinsic Ca^{2+} content of vascular tissue is not high enough to crosslink each additional demethylesterified GalA residue created as a result of the PEF and/or mild thermal treatment. Moreover, intrinsic Ca^{2+} ions may also leach from the tissue as a consequence of the thermal and/or electric cell permeabilisation. Although the role of covalent crosslinking as an explanatory factor for the texture changes in vascular tissue is not clear from this study, it can be concluded that the ionic crosslinking of pectin may, at least partially, explain the observed differences in texture of vascular tissue.

Based on the assumption that one Ca^{2+} ion crosslinks two demethylesterified GalA residues (Fraeye et al., 2010), the bound calcium content equals 0.5 if all GalA residues are demethylesterified and ionically crosslinked by Ca^{2+} . Given the presence of methylesterified GalA residues ($\text{DM} > 0\%$ in **Figure 4**), not all GalA residues can be ionically crosslinked. This is illustrated by the bound calcium content being smaller than 0.5 (**Figure 5**). To evaluate the proportion of demethoxylated GalA residues bound to

Ca²⁺, the *R* value was determined for AIR and WUF extracted from untreated and PEF and/or mild thermally treated cortex and vascular tissue (**Figure 6**).

Figure 6 *R* value of (a) alcohol insoluble residue (AIR) and (b) water unextractable fraction (WUF) from untreated, PEF treated (PEF), mild thermally treated (T60), and PEF and mild thermally treated (PEF-T60) cortex (■) and vascular tissue (□). The error bars represent the standard deviation on the *R* values. The *R* values were compared between treatments per type of tissue and per cell wall fraction by comparing their 95% confidence intervals. Significant differences in *R* value between treatments are indicated with different letters.

Both the *R* values of cortex AIR and WUF remain unchanged after the different treatments (**Figure 6**). This corresponds with the unchanged DM (**Figure 4**) and the unchanged bound calcium content (**Figure 5**): the proportion of demethylesterificated GalA residues that are bound to Ca²⁺ ions remains the same after the different treatments.

The *R* value of vascular AIR, on the other hand, increases significantly after each type of treatment (**Figure 6 (a)**), meaning a larger proportion of demethoxylated GalA units is bound to Ca²⁺ ions. The presence of more demethylesterified GalA residues after the different treatments (reduced DM in **Figure 4 (a)**) seems to be compensated by the increased bound calcium content, resulting in similar *R* values after the different treatments.

The *R* value of vascular WUF increased only significantly after the mild thermal treatment (**Figure 6 (b)**). The pectin in this sample has a relatively high DM in comparison with the DM of pectin in vascular WUF after the PEF and combination treatment (**Figure 4 (c)**). Moreover, the bound calcium content of vascular WUF is similar after all three treatments. Hence, in comparison with the PEF and the combination treatment, in the case of the mild thermal treatment less demethoxylated GalA residues are available to bind a similar amount of Ca²⁺ ions, resulting in a higher *R* value.

In **Figure 6**, all R values are lower than or slightly exceeding 0.25, the threshold value for ionic dimerisation of pectin chains (Celus et al., 2018). In the cell wall fractions with an R value smaller than 0.25 pectin- Ca^{2+} interactions mainly occur through monocomplexation and point-like crosslinks, resulting in a relatively weak gel. If the R value exceeds the threshold value of 0.25, dimerisation of pectin chains occurs, following the ordered egg-box model and resulting in a stronger gel (Celus et al., 2018; Fraeye et al., 2010). Consequently, the effect of the treatments on the Ca^{2+} binding and texture may be enhanced if more Ca^{2+} ions are available to crosslink the pectin chains. The demethylesterified GalA residues that are not bound to Ca^{2+} ions yet, may bind the extra ions and may further improve the texture. Therefore, the addition of Ca^{2+} ions to the treatment medium may be a strategy to enhance the texture preserving effect of the different treatments. Moreover, the infusion of Ca^{2+} ions may even be promoted if the cell membrane is damaged, by PEF (Leong et al., 2018) or by mild thermal processing (Sila et al., 2005).

4. Conclusion

To stimulate the consumption of health promoting fruit and vegetable tissue based food products, it is important to make these products texturally attractive for the consumer. To minimise the detrimental effect of thermal processing, used to extend the product's shelf life, on texture, PEF and mild thermal processing may be used. However, the exact mechanism explaining the texture preservation of PEF and/or mild thermally processed tissues during thermal processing is unclear. Especially the effect on the texture-related structural properties of pectin needs further understanding.

Therefore, in this study, the effect of these different processing techniques on texture-related structural properties of pectin was studied by sequentially extracting CWM from carrot cortex and vascular tissue, and by characterising these fractions structurally.

Although the extractability of cortex CWM changed after the different treatments, no clear relationship between the earlier reported texture changes (Moens et al., 2020), and the DM and ionic crosslinking of pectin was found. Further research is necessary to investigate the role of ionic and especially covalent crosslinking in the earlier observed texture preservation.

In contrast, the role of pectin demethylesterification and ionic crosslinking was clear in the case of the vascular CWM. It seems that both types of treatment reduce the DM and facilitate the ionic crosslinking of pectin, with the effect being largest in the case of the combination treatment. Moreover, the extractability of vascular CWM changed significantly, especially after the PEF and the combination treatment. A role for covalent crosslinking in the texture preservation during thermal processing can not be excluded.

In general, the effect of the different processing techniques on the studied structural properties of pectin was largest in the case of vascular tissue. However, regarding the relative small R values, the effect of the treatments on the texture may increase for both tissues if additional Ca^{2+} ions are added to the treatment medium.

Further research needs to be done to completely understand the effect of PEF and mild thermal processing on the texture-related structural properties of pectin, and to understand the different effects of the treatments on the CWM from cortex and vascular vegetable tissues. These insights might help to manage the texture of plant tissue based food products during processing.

Declaration of competing interest

The authors declare no conflict of interest.

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