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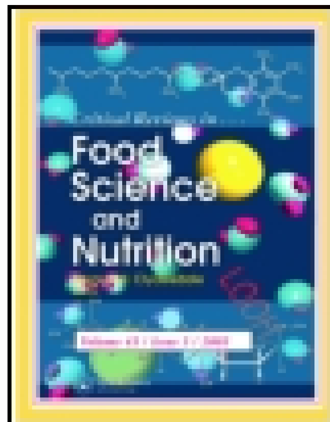

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Process-structure-function Relations of Pectin in Food

Stefanie Christiaens[a], Sandy Van Buggenhout[a], Ken Houben[a], Zahra Jamsazzadeh Kermani[a], Katlijn R.N. Moelants[a], Eugénie D. Ngouémazong[a], Ann Van Loey[a] & Marc E.G. Hendrickx[a]
[a] KU Leuven, Microbial and Molecular Systems, Heverlee, Belgium
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Process-structure-function relations of pectin in food

Dr. Stefanie Christiaens*, Dr. Sandy Van Buggenhout, Ken Houben, Zahra Jamsazzadeh Kermani, Katlijn R.N. Moelants, Dr. Eugénie D. Ngouémazong, Ann Van Loey, Marc E.G. Hendrickx PhD

KU Leuven, Microbial and Molecular Systems, Heverlee, Belgium

*Corresponding author: Dr Stefanie Christiaens KU Leuven, Microbial and Molecular Systems, Kasteelpark Arenberg 22, 3001 Heverlee, Belgium. E-mail: stefanie.christiaens@biw.kuleuven.be

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Abstract

Pectin, a complex polysaccharide rich in galacturonic acid, has been identified as a critical structural component of plant cell walls. The functionality of this intricate macromolecule in fruit- and vegetable-based derived products and ingredients is strongly determined by the nanostructure of its most abundant polymer, homogalacturonan. During food processing, pectic homogalacturonan is susceptible to various enzymatic as well as non-enzymatic conversion reactions modifying its structural and, hence, its functional properties. Consequently, a profound understanding of the various process-structure-function relations of pectin aids food scientists to tailor the functional properties of plant-based derived products and ingredients. This review describes the current knowledge on process-structure-function relations of pectin in foods with special focus on pectin's functionality with regard to textural attributes of solid plant-based foods and rheological properties of particulated fruit- and vegetable-derived products. In this context, both pectin research performed via traditional, *ex situ* physicochemical analyses of fractionated walls and isolated polymers and pectin investigation through *in situ* pectin localisation are considered.

Keywords: pectin, process-structure-function, texture, rheology, *ex situ*, *in situ*

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Introduction

The current inability of most consumers to achieve the recommended daily intake of fruits and vegetables (i.e. 600 g according to the World Health Organisation) triggers food research towards the generation of new and improved (particulated) fruit- and vegetable-based derived products and ingredients. Intelligent precision processing, which integrates structure-enabling and preservation techniques, can be applied to fully exploit the endogenous potential of raw materials in the creation of attractive, naturally structured food products that maximally retain bio-available, health-promoting components within the boundaries of consumer preference. An important structure-determining component in this context is the cell-wall polysaccharide pectin, which can undergo several structural modifications during food processing. A profound understanding of the various process-structure-function relations of pectin aids food scientists to tailor the functional properties of fruit- and vegetable-based derived products and ingredients. Current research towards this topic is performed at a molecular, microscopic and macroscopic level entailing both *ex situ* and *in situ* investigations. A schematic representation of the key concept discussed in this review (i.e. the process-structure-function relations of pectin) is displayed in **Figure 1**. Specifically, pectin structure, pectin conversions during food processing and process-structure-function relations of pectin will successively be discussed in this paper.

It is worth mentioning that during the past decade, several other pectin reviews have been published, each with a certain focus point. While Ridley et al. (2001) discussed pectin structure, biosynthesis and oligogalacturonide-related signaling, Willats et al. (2001a) placed the structural complexity of pectin in a cell biological and developmental context. A few years later, Willats et

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al. (2006) reviewed the tremendous progress in understanding the complex fine structure of pectin by the use of state-of-the-art techniques and discussed prospects for novel applications. Furthermore, a detailed overview on the structure, function and biosynthesis of pectic polysaccharides was provided by Mohnen (2008) and Caffall and Mohnen (2009). Voragen et al. (2009) on the other hand described the functional and structural characteristics of pectin with special emphasis on the structural elements making up the pectin molecule, their interconnections and present models of the macromolecule. In this context, Vincken et al. (2003) and Yapo (2011) published pectin reviews introducing new hypothetical models for the macromolecular structure of pectin. Finally, different aspects of pectin in processed fruits and vegetables were discussed in three complementary reviews by Duvetter et al. (2009), Sila et al. (2009) and Van Buggenhout et al. (2009).

Pectin structure

In order to be able to grasp the different process-structure-function relations of pectin, a detailed image on pectin structure is required. In this context, the methods currently available for analysing pectin structure are summarised at the end of this section.

Pectin structure and cross-linking

A family of complex polysaccharides rich in galacturonic acid (GalA) has generally been referred to as 'pectin'. This intricate macromolecule is composed out of as many as 17 different monosaccharides and contains over 20 types of different linkages (Vincken et al. 2003; Voragen et al. 2009). Three main pectin building blocks can be distinguished: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). In addition, other substituted galacturonans such as xylogalacturonan and apiogalacturonan have been described (Caffall and

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Mohnen 2009). HG is considered the most abundant pectic polysaccharide in plant cell walls, accounting for roughly 60% of the total pectin amount. This linear homopolymer consists of approximately 100 1,4-linked α-D-GalA residues in which some of the C-6 carboxyl groups are methyl-esterified. Depending on the plant source, GalA residues might also be O-acetylated at C-3 and/or C-2. HG in general and its methyl-esterification (degree and pattern) in particular strongly determine the functionality of pectin (Ridley et al. 2001; Willats et al. 2001a). RG-I on the other hand is a family of highly branched pectic polysaccharides that contain a backbone of the repeating disaccharide [→4)-α-D-GalA-(1→2)-α-L-Rha-(1→]. GalA residues in the RG-I backbone may be O-acetylated at C-3 and/or C-2 but are presumably not methyl-esterified or substituted with side chains. In contrast, 20-80% of the rhamnose residues are substituted at C-4 with neutral and acidic oligosaccharide side chains. These side chains consist mainly of galactosyl and/or arabinosyl residues and vary from a single glycosyl unit to polymeric linear or branched substitutions, such as (arabino)galactan and arabinan (Albersheim et al. 1996; Mohnen 2008). RG-II finally is, despite its name, not structurally related to RG-I. Instead, this highly conserved pectin structure consists of a HG backbone of around nine GalA residues which may be methyl-esterified at the C-6 position. The backbone is substituted by four hetero-oligomeric side chains with known and consistent composition and length, including very rare sugar residues such as apiose, aceric acid, deoxylyxoheptulopyranosylaric acid and ketodeoxymanno-octulopyranosylonic acid (Ishii et al. 1999; O'Neill et al. 1996).

Although the chemical composition and structure of the constituent polymers of pectin are well elucidated, it remains a matter of debate as to how the different structural elements are arranged into a macromolecular structure. The pectic polysaccharides are known to be covalently linked to

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each other, but the exact relative position of smooth (mainly HG) and hairy (RG-I, RG-II and side chains) regions is not yet revealed (Caffall and Mohnen 2009; Coenen et al. 2007). The conventional model postulates that HG, RG-I and RG-II form a continuous backbone in which smooth and hairy regions are alternating (Willats et al. 2006). In an alternative model proposed by Vincken et al. (2003) on the other hand, HG is considered a long side chain of RG-I next to arabinan and galactan. No conclusive evidence confirming or rejecting either of the models has been presented to date. Moreover, a new model in which both the traditional and the 'RG-I backbone' model are accounted for has very recently been presented by Yapo (2011). In this 'living thing-like' model, the backbone of the pectin-complex is alternately composed of two linear HG elements and one RG-I core.

Pectin macromolecules can further interact with each other through a number of covalent and non-covalent, intra- or intermolecular linkages resulting in the formation of a network. The predominant domain of pectin, HG, may self-associate depending on the pH and degree and pattern of methyl-esterification. Negatively charged, non-methyl-esterified GalA residues in the HG domain can ionically cross-link with divalent ions, particularly Ca^{2+}, according to the so-called 'egg-box' model (Braccini et al. 1999; Morris et al. 1982). Consequently, a low degree of methyl-esterification (DM) and a blockwise distribution of the non-methyl-esterified GalA residues enhance the Ca^{2+}-binding capacity of HG (Ngouémazong et al. 2012b; Ralet et al. 2001). The presence of acetyl groups on HG on the other hand, hinders the formation of Ca^{2+}-cross-links between HG chains (Ralet et al. 2005). Another type of cross-linking can be discerned in species of the family Amaranthaceae such as sugar beet and spinach. In these species, neutral sugars in the RG-I domain of pectin are substituted with ferulic acid. In

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particular, ferulic acid residues are mainly ester-linked to O-2 of Ara residues of the main core of arabinan chains and to O-6 of Gal residues of the main core of galactan chains (Micard and Thibault 1999; Morris et al. 2010; Ralet et al. 2005). These ferulic acid esters can undergo oxidative coupling reactions resulting in the formation of dehydrodimers, contributing to the creation of a pectic network (Fry 1986; Ishii 1997). Furthermore, RG-II exists in the primary cell wall as a dimer that is covalently cross-linked by a 1:2 borate-diol ester between the apiosyl residues of two RG-II monomers (Ishii et al. 1999; O'Neill et al. 1996). As RG-II is covalently linked to HG, dimerisation eventually contributes to the formation of a three-dimensional pectic network in the cell wall which is crucial for normal plant growth and development. Finally, hydrophobic interactions between methoxyl groups, hydrogen bonds between undissociated carboxyl and secondary alcohol groups and uronyl ester linkages between the carboxyl group of a GalA moiety in HG and a hydroxyl group of another glycosyl residue may also be involved in holding pectic polysaccharides within the plant cell wall (Marry et al. 2006; Vincken et al. 2003).

Another level of complexity is added considering pectin's presence in the plant cell wall. Besides pectin, two other major polysaccharides, cellulose and hemicellulose can be distinguished in the plant cell wall. Cellulose is a microfibrillar component consisting of linear chains of β-(1→4)-linked D-glucose, while the term hemicellulose refers to a heterogeneous group of polymers such as xyloglucans, xylans and mannans. Traditionally, the plant cell wall is perceived as a dynamic structure build up of cellulose microfibrils embedded in a matrix of pectins, hemicelluloses, proteins, phenolic compounds, lower molar mass solutes and water (Brett and Waldron 1996; Iiyama et al. 1994) (**Figure 2**). Simply put, cellulose microfibrils provide rigidity and resistance to tearing to the cell wall, while pectic polymers and hemicelluloses confer plasticity and the

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ability to stretch to this key structural entity. Ultrastructural models of the plant cell wall commonly rely on the presence of a pectin-pectin network (formed through different types of pectin cross-links) and a load-bearing xyloglucan-cellulose network (formed through hydrogen bonding) (Albersheim et al. 1996; Caffall and Mohnen 2009). Recently, there has been increasing evidence for a covalent, alkali-stable linkage between pectic RG-I and xyloglucan (Popper and Fry 2005) as well as hydrogen bonding between pectic side chains and cellulose (Zykwinska et al. 2005). Besides these polymer-polymer interactions based on specific bonds, physical entanglement might also contribute to the strength of the plant cell wall. In general, the polysaccharide configuration as well as the polysaccharide composition of the primary cell wall varies phylogenetically between plant species, temporally during plant development, spatially between plant tissues and even within a single cell (Jarvis et al. 2003; Knox 2002). The middle lamella on the other hand is considered to predominantly consist of pectin and to lack cellulose microfibrils.

Pectin structure determination methods

Large part of the knowledge on cell-wall polymers, including pectin, has been derived from the physicochemical analysis of fractionated walls and isolated polymers. In this context, cell-wall materials have frequently been isolated as alcohol-insoluble residue (AIR) (Selvendran and O'Neill 1987). This extraction procedure, involving alcohol and/or acetone, can be performed hot or cold and is suitable for tissues relatively poor in intracellular proteins, polyphenols and starch. Conversely, the isolation of cell-wall components of plant tissues has also been performed with water or buffer. Moreover, wet-ball milling and the application of sodium dodecylsulfate or sodium deoxycholate followed by extraction with phenol – acetic acid – water has been widely

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adopted for the isolation of cell-wall materials (Renard 2005). Due to differences in solubility between pectic polymers, isolated cell-wall material can be sequentially fractionated with different solvents such as water, aqueous solutions of chelating agents and aqueous solutions of alkali. Frequently, one or more of the following three main categories can be discerned: water-soluble pectin (WSP), containing pectic polymers that are loosely bound to the cell wall through non-covalent and non-ionic bonds, chelator-soluble pectin (CSP), consisting mainly of ionically cross-linked pectin, and Na_2CO_3-soluble pectin (NSP), which is predominantly linked to cell-wall polysaccharides through covalent ester bonds. Fractionated walls and isolated polymers have been characteristed towards their galacturonic acid content, degree of methyl-esterification (DM), pattern of methyl-esterification, distribution of DM among pectin chains, degree of acetylation, pattern of acetylation, degree of polymerisation, neutral sugar content, sugar linkages, linearity, degree of branching, molar mass distribution, size, ferulic acid cross-links and Ca^{2+} cross-links using many different methods (**Table 1**). A major limitation of these *ex situ* pectin characterisation methods however is the inherent loss of most spatial information on subtle variations and complexities occurring within individual cells and tissues. In order to elucidate pectin structure *in situ*, a series of specific probes were developed in the context of plant cell wall biology, providing insights into cell wall complexity, heterogeneity and dynamics (Knox et al. 1990; Liners et al. 1989; Verhertbruggen et al. 2009; Willats et al. 1999; Willats et al. 2001b). Among these probes, different monoclonal antibodies towards pectin are available, allowing the precise localisation of defined structural pectic domains in intact plant cell walls (see **Table 1**). Recently, the anti-HG antibodies were introduced in the field of food processing to investigate pectin (changes) *in situ* (Christiaens et al. 2011b).

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Besides the isolation of cell-wall components from plant matrices and the subsequent fractionation into different pectin fractions, which are carried out to follow pectic changes in the plant matrix itself during growth, ripening, storage and processing, pectin can also be extracted directly from the plant material to be employed as a functional food ingredient. In contrast to what is pursued using a combination of cell-wall material isolation and fractionation, a direct pectin extraction generally does not result in a reflection of the native state of pectin in the plant cell wall. On the other hand, depending on the extraction conditions used, the structure of pectin is modified in terms of molar mass, DM, etc. Conventionally, pectin is extracted in acidic conditions with reagents such as nitric acid and hydrochloric acid. The pH (1 – 3), temperature (50 – 90 °C) and extraction time (1 – 12 h) chosen will dictate the functional properties of the extracted pectin (Rolin 2002). In search for an improved extraction in terms of yield, extraction time and/or quality of the obtained products, different alternatives have been investigated including the application of ultrasound (Panchev et al. 1994), microwave heating (under pressure) (Fishman et al. 2000) and steam treatment (Fishman et al. 2003; Grohmann et al. 2012). Finally, the application of enzymes presents a more environmentally friendly way for the extraction of pectin. In this context, a distinction between the use of enzymes degrading the pectin backbone, i.e. endo-polygalacturonase combined with pectin methylesterase or pectin lyase (see further), releasing pectin fragments, and the application of proteases and cellulase, which allow the isolation of pectic polysaccharides by degrading the xyloglucan-cellulose network and protein networks, can be made (Panouillé et al. 2006; Zykwinska et al. 2008).

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Pectin conversions during food processing

Pectin is susceptible to various enzymatic as well as non-enzymatic conversion reactions during food processing modifying its structural and, hence, its functional properties. As HG is the major pectic domain governing the functionality of pectin, the overview on pectin conversions presented in this review will be mainly restricted to alterations in the linear HG chain. A schematic summary on HG conversion reactions is provided in **Figure 3** together with an indication on how these conversions impact on pectin's functionality in plant-derived food products and ingredients (see further).

Enzymatic conversions

A wide range of endogenous and exogenous enzymes can synergistically modify and degrade pectin. A concise overview of these pectinases and the reactions they catalyse is provided in **Table 2** in which the enzymes are classified according to the way they modify pectin structure. In the following paragraphs, pectin methylesterase, polygalacturonase, pectate/pectin lyase and some debranching enzymes will successively be discussed in detail.

Pectin methylesterase

Pectin methylesterase (PME, E.C. 3.1.1.11) catalyses the hydrolysis of methyl-esters at C-6 of GalA residues in HG, releasing methanol and protons while creating negatively-charged carboxyl groups on the pectin chain. This ubiquitous enzyme has been found in all higher plants examined and is also produced by phytopathogenic bacteria and fungi (Markovic et al. 2002; Pelloux et al. 2007). In many plants, the existence of multiple PME isoforms has been reported

(Pressey and Avants 1972). Most plant PMEs present a neutral to alkaline isoelectric pH (pI), which explains their tight association with the slightly acidic cell wall, while microbial PMEs often present more acidic pIs. Endogenous plant PMEs are involved in the metabolism of cell-wall pectin and, hence, take part in important physiological processes associated with vegetative and reproductive plant development (Pelloux et al. 2007; Wolf et al. 2009). In particular, pectin is synthesised in the Golgi apparatus, upon which it is secreted into the plant cell wall in a highly methyl-esterified form. Subsequently, pectin is demethoxylated by PME to obtain its (desired) functionality. Using a monoclonal antibody specifically recognising long blocks of non-methyl-esterified GalA residues (PAM1), it was demonstrated that in vivo pectin demethoxylation in tomato fruit is endogenously regulated by physical restriction of PME activity in the cell wall matrix (**Figure 4**). In this context, tissue disintegration was shown to abolish this limitation and, consequently, resulted in intensive demethoxylation of pectin by PME throughout the entire cell wall (Christiaens et al. 2012c).

PME's mode of action is of particular importance since it dictates the extent and nature of the blocks of unesterified GalA residues within the HG domain of pectin. It is generally believed that PMEs with an alkaline pI (mostly plant PMEs) hydrolyse methyl-esters in a blockwise fashion, creating long sequences of non-methoxylated GalA units on pectin. Plant PME-mediated demethoxylation has been suggested to occur via a single chain or multiple attack mechanism (Cameron et al. 2008; Denès et al. 2000; Kim et al. 2005). A single chain mechanism is in this context characterised by the formation of an enzyme-substrate complex in which the enzyme does not dissociate from the polymer until the end of the molecule or a blocking residue is reached, while in case of a multiple attack mechanism, a limited average number of subsequent

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residues is converted per enzymatic attack. Conversely, PMEs with an acidic pI (mostly microbial PMEs) demethoxylate more randomly, resulting in a rather random distribution of the unesterified GalA residues (Limberg et al. 2000; Ralet and Thibault 2002). A multiple chain mechanism, in which the enzyme-substrate complex dissociates after each enzymatic attack, has been put forward as the mode of action. However, the mode of action of PME may also be influenced by other factors, such as pH (Catoire et al. 1998; Denès et al. 2000). In addition, this intrinsic factor closely regulates the activity of PME.

PMEs, especially those of plants, appear to be very sensitive to their ionic environment. The activity of PME increases with salt concentration up to an optimal concentration above which activity usually decreases. This enzyme activation relies on the interaction of cations with the substrate rather than with the enzyme. It has been proposed that positive charges would interact with the free negatively charged carboxyl groups, formed upon pectin demethoxylation, which would otherwise trap the enzyme (since most plant PMEs are positively charged in the plant cell wall). Cations would then compete with the cationic proteins for fixation on pectin, thus allowing enzyme liberation (Nari et al. 1991). At high concentrations, especially divalent cations decrease the reaction rate of pectin demethoxylation due to ionic cross-link formation.

Each PME type is, depending on its biological origin and/or isoform, characterised by a certain thermal and pressure stability. In general, most (purified) PMEs are thermo-labile as they readily inactivate from 60 °C on (Crelier et al. 2001; Ly-Nguyen et al. 2002). In contrast to their temperature sensitivity, most PMEs are baro-tolerant. The pressure stability of the demethoxylating enzyme can nevertheless vary from moderately pressure-sensitive (> 600 MPa), like for carrot PME (Balogh et al. 2004; Ly-Nguyen et al. 2003), to extremely baro-tolerant (> 1

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GPa), like for tomato PME (Crelier et al. 2001; Rodrigo et al. 2006). Interestingly, temperature and pressure often exhibit an antagonistic effect on PME stability as thermal inactivation is often retarded by moderate pressure (Ly-Nguyen et al. 2003). PME in tissue-type systems is less vulnerable to thermal and high-pressure inactivation than the purified enzyme (Balogh et al. 2004; Sila et al. 2007). Besides processing inactivation, plant PME activity can also be inhibited by a proteinaceous PME inhibitor (PMEI), which has been abundantly found in kiwi fruit (Balestrieri et al. 1990) and recently been detected in tomato fruit (Reca et al. 2012). More detailed information on PMEI can be found in the reviews of Giovane et al. (2004) and Jolie et al. (2010).

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Pectin demethoxylation by PME is enhanced by increasing temperature until a point is reached where the rate of inactivation dominates the catalytic activity. Depending on the enzyme source and environment, thermal stimulation of the catalytic activity of PME is optimal between 45 and 55 °C. Due to thermal processing, cell membranes are more permeable to cations, hence allowing these positive charges to activate the cell wall-bound PME (Van Buren 1979). Besides the stimulating effect of temperature, pressure can also enhance the catalytic activity of PME. In fact, the substrate conversion rate increases within a certain pressure-temperature combination window for all PMEs. For example, the optimal conditions for substrate conversion for tomato PME in model systems are 55 °C and 300 MPa at pH 8.0 and 57 °C and 450 MPa at pH 4.4 (Verlent et al. 2004b), whereas for carrot PME in tissue-type systems 60 °C in combination with 200 to 400 MPa is optimal (Sila et al. 2007). The stimulating effect of pressure on PME activity can be explained by the principle of Le Châtelier: any phenomenon accompanied by a decrease in volume is enhanced by an increase in pressure. Solvation of the charged groups created by

pectin demethoxylation is accompanied by a volume reduction resulting from electrostriction, i.e. the compact alignment of water dipoles due to the coulombic field of the charged groups (Duvetter et al. 2006). In intact tissue systems, enzyme activation can also arise from pressure-induced decompartmentalisation (Hendrickx et al. 1998). Specifically, in intact tissues, enzymes and substrate are often separated by compartmentalisation which can be destroyed upon the application of elevated pressure, eventually resulting in enzyme-substrate contact. *In situ* pectin localisation showed that process-induced demethoxylation by endogenous PME mainly tends to take place at discrete regions of the inner face of the cell wall adjacent to the plasma membrane for carrot and in the tricellular junctions of adjacent cells for broccoli (Christiaens et al. 2011b; Christiaens et al. 2011c).

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Polygalacturonase

Polygalacturonase (PG) catalyses the hydrolytic cleavage of glycosidic α-(1,4) linkages in the HG domain of pectin. This enzyme can be either endo-acting (E.C. 3.2.1.15), resulting in a fast decrease in molar mass of pectin due to random substrate depolymerisation, or exo-acting, releasing monoGalA (E.C. 3.2.1.67) or diGalA (E.C. 3.2.1.82) from the non-reducing end of the HG chain. The exact substrate requirement for PG action is still subject to debate, but it is generally acknowledged that the enzyme will cleave only between non-methoxylated GalA residues. Consequently, PG shows increasing activity with decreasing DM, which gives occasion for a synergistic action between PME and PG in the degradation of pectin. PG has been found in fungi, bacteria, yeasts and several higher plants (Duvetter et al. 2009). Large amounts of the endo-acting form of this depolymerising enzyme have been detected in e.g. tomato and many other fruits, while vegetables such as carrot and broccoli only possess a negligible quantity of

exo-PG (Swoboda et al. 2004). PG plays an important role in the development of plants, e.g. during the ripening of fruits and is considered a key enzyme in the degradation of plant cell walls upon phytopathogenic attack. Most of the PGs of plant origin are endo-acting enzymes and are present in more than one isoform. Like PMEs, PGs are susceptible to their ionic environment. The optimal pH for PG activity, generally ranging between 4 and 6, is depending on both the biological origin and the PG isoform.

Each PG type is characterised by a certain thermal and pressure stability. In tomato fruit, PG is present in two isoforms, the thermo-labile PG2 and the thermo-stable PG1 (Pressey and Avants 1973). The latter, of which there is considerable controversy about its in vivo authenticity, is a dimer of PG2 and a heat-stable protein called the β-subunit (Fachin et al. 2004; Peeters et al. 2004). In contrast, PG1 and PG2 show the same pressure stability, both can be inactivated in the pressure range of 300-500 MPa at ambient temperatures (Fachin et al. 2004; Rodrigo et al. 2006). Whereas PME is a thermo-labile, pressure-stable enzyme, PG is thus a heat-resistant, pressure-labile enzyme. A high-pressure treatment at rationally-chosen conditions (e.g. 10 min, 550 MPa, 25 °C) hence enables the selective inactivation of PG, while PME activity is maintained (Crelier et al. 2001). The catalytic activity of PG is maximal at temperatures around 55-60 °C at atmospheric pressure (Verlent et al. 2004a).

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Pectate/pectin lyase

Pectate lyase (PL) catalyses the cleavage of glycosidic α-(1,4) linkages in the HG domain of pectin via a β-elimination reaction mechanism, resulting in the formation of a double bond between C-4 and C-5 of the newly formed non-reducing end. PLs can be endo- (E.C. 4.2.2.2) or exo-acting (E.C. 4.2.2.9) and require calcium ions for catalysis. Pectin lyase (PNL, E.C.

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4.2.2.10) performs the same pectin depolymerisation reaction as PL but requires the GalA residues adjacent to the scissile bond to be methyl-esterified. Consequently, PNL activity increases with increasing DM while PL activity is highest on pectins of moderate DM (20-50%). PLs have predominantly been isolated from bacteria and fungi, notably phytopathogenic micro-organisms, whereas putative PLs have been identified in plants. PNLs on the other hand have, so far, only been identified and isolated from micro-organisms (Yadav et al. 2009).

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Debranching enzymes

Next to the well-established HG-modifying enzymes, some pectin debranching enzymes, β-galactosidase and α-arabinofuranosidase in particular, are gaining more and more attention in the context of food processing. β-galactosidase (E.C. 3.2.1.23) hydrolyses terminal, non-reducing galactosyl residues from β-linked galactosides while α-arabinofuranosidase (E.C. 3.2.1.55) degrades branched arabinan to a linear chain by splitting off terminal residues from α-1,3-linked arabinofuranosyl sidechains and breaks α-1,5 links at the non-reducing end of linear arabinan chains (Prasanna et al. 2007). Both exo-acting enzymes play a role in cell wall modifications related with the development and softening of many fruits (Tateishi 2008). The thermal and pressure stability of β-galactosidase and α-arabinofuranosidase in tomato fruit have recently been determined by Houben et al. (2012). It was revealed that both enzymes were thermo-labile while being rather pressure-stable.

Non-enzymatic conversions

Pectin shows a higher sensitivity to non-enzymatic conversion reactions than cellulose and hemicellulose, the other cell wall polysaccharides. Around pH 3-4, the pK_a range of its carboxyl

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groups, pectin generally shows the highest stability. At specific conditions of pH, temperature and pressure however, HG demethoxylation and depolymerisation can occur.

Chemical demethoxylation

Methyl-esters at C-6 of GalA residues can be hydrolysed under alkaline or near-neutral conditions (pH $\geq$ 5). This chemical demethoxylation results, contrary to enzymatic de-esterification, in a completely random distribution of unesterified and methyl-esterified GalA residues on the HG chain (Denès et al. 2000; Limberg et al. 2000). Since saponification is initiated by hydroxyl ions, the reaction rate increases with increasing pH (Kravtchenko et al. 1992; Renard and Thibault 1996). The reaction rate constant of pectin demethoxylation decreases on the other hand with decreasing DM as less methyl-esters are available for demethoxylation, i.e. the reaction rate constant declines as the reaction proceeds (Fraeye et al. 2007). Furthermore, the saponification reaction is accelerated at high temperatures (De Roeck et al. 2009; Fraeye et al. 2007) and elevated pressures (De Roeck et al. 2009; Verlent et al. 2004b). The enhancing effect of high pressure on pectin demethoxylation can, as mentioned previously, be explained by the principle of Le Châtelier.

Beta-eliminative depolymerisation

When heating pectin at alkaline or near-neutral pH (T $\geq$ 80 °C; pH $\geq$ 5), splitting of the glycosidic linkages between GalA residues can occur through a β-eliminative depolymerisation reaction. β-elimination in uronic acid polymers such as pectin is known to proceed via an E1cB mechanism, i.e. a unimolecular elimination mechanism via the conjugate base (BeMiller and Kumari 1972). The hydrogen at C-5 of GalA is removed by a hydroxyl ion, leading to an

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unstable, intermediary anion. This anion is stabilised by eliminative cleavage of the glycosidic linkage in the β-position to the carbonyl function, resulting in the formation of a glycosyl anion and an unsaturated bond between C-4 and C-5 at the non-reducing end (Albersheim et al. 1960; Keijbets and Pilnik 1974; Kiss 1974). A prerequisite is the presence of a methyl-ester group at C-6, which renders H-5 sufficiently acidic to be removed by an alkali. Consequently, pectin with a high DM is more susceptible to β-elimination than pectin with a low DM (Albersheim et al. 1960; Fraeye et al. 2007; Sajjaanantakul et al. 1989). At elevated temperatures and near-neutral to alkaline pH, chemical demethoxylation can occur simultaneously with the β-eliminative depolymerisation of pectin, lowering the DM of pectin and, hence, reducing the β-elimination rate (De Roeck et al. 2009; Kravtchenko et al. 1992). Since hydroxyl ions initiate the β-elimination reaction, the reaction rate increases with increasing pH (Krall and McFeeters 1998; Kravtchenko et al. 1992; Sila et al. 2006b). β-eliminative breakdown of pectin is also stimulated in presence of cations and the enhancing effect of these ions generally increases with salt concentration and ion valency. It has been suggested that ions may associate with free carboxyl groups on pectin, resulting in a decrease of the overall negative charge, which may facilitate the approach of hydroxyl ions needed to initiate β-elimination (Keijbets and Pilnik 1974; Sajjaanantakul et al. 1993). At high concentrations however, calcium ions decrease the extent of β-elimination due to ionic cross-link formation (Sila et al. 2006b). β-eliminative pectin depolymerisation is also stimulated in presence of organic anions (Keijbets and Pilnik 1974; Vu et al. 2006). Moreover, this reaction is strongly temperature dependent and its rate increases with increasing temperature (De Roeck et al. 2009; Fraeye et al. 2007). More specifically, for every 10 °C increase in temperature, the reaction rate increases by a factor of 2 to 3.5 (Sila et al.

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2006b). In contrast, during high-pressure processing at room temperature, no β-elimination occurs (Kato et al. 1997). Combined high-temperature/high-pressure processing retards or even stops the β-elimination reaction. This phenomenon is assumed to be due to the extensive demethoxylation of pectin under these conditions, making pectin less susceptible to β-elimination (De Roeck et al. 2009).

Acid hydrolysis

A second mechanism resulting in pectin depolymerisation during heating is the acid-catalysed hydrolytic splitting of glycosidic bonds ($T \geq 60$ °C; $pH < 3$). This reaction proceeds through an initial protonation of the glycosidic oxygen to give the conjugated acid. Subsequently, the rate-limiting formation of a non-reducing end group and a cyclic oxocarbenium ion takes place via a unimolecular heterolysis of the conjugated acid. In a final step, water is added to the oxocarbenium ion leading to the formation of a reducing end group and a proton (Smidsrod et al. 1966). The rate of acid hydrolysis increases with decreasing pH, decreasing pectin DM and increasing temperature (Diaz et al. 2007; Fraeye et al. 2007; Krall and McFeeters 1998). During high-pressure processing at room temperature, no acid hydrolysis takes place (Kato et al. 1997).

Process-structure-function relations of pectin

Pectin's functionality to plant cell walls as well as to food is quite diverse. In the following paragraphs, special attention will be given to the functionality of this polysaccharide with regard to the textural attributes of solid plant-based foods and the rheological properties of particulated fruit- and vegetable-derived products. Particulated plant-derived products are in this respect defined as dispersions consisting of a certain combination of cell clusters, individual cells, cell

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fragments, cell organelles and insoluble polymers, immersed in a continuous serum phase containing the dissolved components. Furthermore, process-structure-function relations of pectin concerning the physical stability and health-related aspects of plant-based food products and the use of extracted pectin as an ingredient, will be discussed briefly at the end of this section.

Pectin in the plant cell wall

Pectin is a multifunctional component in plant cell walls (Mohnen 2008; Voragen et al. 2009; Willats et al. 2001a). First of all, pectin plays a key role in the adhesion between cells thereby contributing to tissue integrity and rigidity. In general, the strength of plant tissues is determined by the mechanical properties of the cell wall in conjunction with the internal turgor pressure of the cells and the intercellular adhesion. The turgor-generated forces that tend to separate adjacent cells are not evenly distributed over the cell surface but, instead, are concentrated at the tricellular junctions and, more specifically, at the corners of the intercellular spaces (Jarvis et al. 2003) (see **Figure 2**). Intercellular adhesion is therefore considered not to depend on the entire middle lamella but on the ‘reinforcing zones’ at the corners of the intercellular spaces which differ in polymer composition from both the primary cell wall and the middle lamella (Knox 2008; Waldron and Brett 2010). Via *in situ* immunolabeling experiments with anti-pectin antibodies, Ca^{2+}-cross-linked and low-esterified HG has been shown to be particularly abundant in these reinforcing zones in a wide range of dicotyledonous plant tissues (Christiaens et al. 2011b; Christiaens et al. 2011c; Manfield et al. 2005). Pectin is hence involved in complex physiological processes like cell growth and cell development. Secondly, pectin influences various cell wall properties such as porosity, surface charge, pH and ion balance and is consequently of importance to the ion transport in the cell wall, the permeability of the walls for

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enzymes and the water-holding capacity (Brett and Waldron 1996; Voragen et al. 2009). Finally, pectin oligosaccharides are known to act as signalling molecules, both as elicitors during wounding and pathogen attack and as hormone-like components during plant development (Caffall and Mohnen 2009).

Textural attributes of solid plant-based food systems

Texture of plant-based foods can mainly be attributed to the structural integrity of the cell wall and middle lamella, as well as to the turgor pressure generated within cells by osmosis (Jackman and Stanley 1995). In addition, the mode of tissue failure, cell breakage or cell separation, is central to the perception of texture of fruits, vegetables and derived products. Generally, cell breakage occurs when the adhesion between cells is stronger than the cell walls, whereas a weak intercellular adhesion favours cell separation through the middle lamella (Waldron et al. 1997b). During thermal or high-pressure processing of plant-based foods, several textural changes take place. These alterations can often be related to conversions of the critical cell-wall component, pectin.

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Thermal processing

Generally, extensive tissue softening occurs when fruits and vegetables are subjected to thermal processes like cooking, pasteurisation or sterilisation. Texture degradation can be ascribed to a combination of two factors. Initially, a rapid loss of hardness takes place due to membrane damage and the associated loss in turgor pressure (Greve et al. 1994). Furthermore, an important additional decrease in texture results from an increase in the ease of cell separation (Ng and Waldron 1997; Van Buren 1979). Thermally-induced cell separation is generally attributed to the β-eliminative depolymerisation and solubilisation of pectic polymers involved in cell-cell

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adhesion (Sila et al. 2006b; Vu et al. 2006; Waldron et al. 2003). Pectin depolymerisation through acid hydrolysis is in this context negligible since the pH of plant cell walls is generally between 4 and 6 (Brett and Waldron 1996; Van Buggenhout et al. 2009).

High-temperature processing of solid plant-based foods has been demonstrated to induce a shift from water-insoluble pectin to WSP in green beans (Recourt et al. 1996) and carrots (De Roeck et al. 2008; Ng and Waldron 1997; Sila et al. 2006a; Siliha et al. 1996). In addition, the applied thermal treatments altered the neutral-sugar content and composition of the different pectin fractions (De Roeck et al. 2008; Ng and Waldron 1997; Sila et al. 2006a; Siliha et al. 1996) and the induced pectin solubilisation and β-eliminative depolymerisation were apparent in the molar-mass distributions of the pectin fractions (De Roeck et al. 2008; Ng and Waldron 1997; Sila et al. 2006a). Furthermore, a decrease in the DM of pectin upon thermal processing has frequently been observed due to the occurrence of chemical pectin demethoxylation at high temperatures (De Roeck et al. 2009; Fraeye et al. 2007). Finally, anti-HG antibodies recognising highly methyl-esterified pectin (i.e. JIM7 and LM20) demonstrated the effect of thermal processing on pectin in broccoli tissue *in situ* (Christiaens et al. 2011c). As an illustration, representative pictures of immunofluorescence labeling of cortex parenchyma in raw and thermally processed broccoli with JIM7 are shown in **Figures 5 A** and **B** respectively. From the immunolabeling results it was evident that after thermal processing highly methyl-esterified, water-soluble pectic polymers are no longer located at the originally clearly defined spots in the cell wall but, instead, become detached and loosely bound to the cell wall.

In contrast to most fruits and vegetables, a number of edible, non-lignified parenchyma tissues, such as sugar beet, Chinese water chestnut, chufa, beans and lentils, fail to soften completely

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during thermal processing. These ‘hard-to-cook’ phenomena are related to a lack of cell separation during tissue failure. In sugar beet for example, resistance to thermal softening is ascribed to ferulic-acid cross-linking of pectin (Waldron et al. 1997a).

High-pressure processing

Consumer demand for minimally-processed, high-qualitative, additive-free yet microbiologically-safe and stable food products has stimulated the interest in a number of non-thermal approaches for food processing (Hendrickx et al. 1998; Ludikhuyze et al. 2001; Rastogi et al. 2007). High-pressure processing has been proven a valuable alternative to thermal processing as it only minimally affects organoleptic and nutritional quality attributes of foods while it can inactivate vegetative micro-organisms and spoilage enzymes. In addition, the application of high hydrostatic pressure on food products can introduce original functional properties. The effect of high pressure on the texture of fruits and vegetables is highly dependent on the plant type and the pressure-temperature-time combination applied. On the one hand, an initial loss of texture occurs ascribed to the instantaneous pulse of action of pressure (Basak and Ramaswamy 1998). This instantaneous pressure softening is, at low pressures (e.g. 100 MPa), caused by the compression of cellular structures without disruption and, at high pressures (> 200 MPa), due to the rupture of cell membranes leading to the loss of turgor pressure (Ludikhuyze et al. 2001). On the other hand, pressure holding time has, in most cases, a less detrimental effect on texture compared to the holding time during thermal processing. When the application of high pressure is not combined with elevated temperatures, further texture loss or gradual texture recovery occurs during the pressure holding phase (Araya et al. 2007; Basak and Ramaswamy 1998). When the use of high pressures on the other hand is combined with elevated temperatures,

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texture degradation of carrots is remarkably slowed down compared to thermal processing (De Roeck et al. 2008; De Roeck et al. 2010; Nguyen et al. 2007). Extensive demethoxylation of pectin during the high-pressure/high-temperature process is assumed to be responsible for retarded β-elimination, the main cause of thermal softening of carrots.

In situ pectin immunolabeling of high-pressure processed broccoli parenchyma lead to a surprising observation, i.e. the intercellular junctions between adjacent cells are often completely filled with label using any of the different anti-HG antibodies (**Figure 5 C**). It was hypothesised that at elevated pressures, intercellular spaces become very small due to the compression of air. As a result, a certain extent of entanglement of the pectic polysaccharides, previously present at different sides of the intercellular space, occurs that is not lost upon depressurisation (Christiaens et al. 2011c).

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Texture improvement through pectin engineering

Food scientists seek to counteract the textural damage of thermally-processed fruits, vegetables and derived food products, as consumers demand for products with an optimal textural quality. Texture improvement can be achieved through pectin engineering, i.e. *in situ* alteration of the pectin structure to change its functional properties in the cell wall and middle lamella. In this context, minimisation of β-eliminative pectin depolymerisation and enhancement of cell-cell adhesion are critical.

Low-temperature blanching, typically 15 to 45 min at 50 to 70 °C, has been successfully used to reduce the vulnerability of many vegetables such as carrot (Ng and Waldron 1997; Ni et al. 2005; Sila et al. 2006b; Sila et al. 2005; Smout et al. 2005; Stanley et al. 1995), cabbage (Ni et al. 2005), broccoli (Christiaens et al. 2011a; Ni et al. 2005; Wu and Chang 1990) and green beans

(Stanley et al. 1995) to softening during subsequent thermal processing. During blanching, the catalytic activity of PME is enhanced, resulting in the de-esterification of pectic polysaccharides. The increase in free pectic carboxyl groups provides a greater opportunity for pectic polymers to be cross-linked with divalent ions such as Ca^{2+}, leading to an increased intercellular adhesion. Moreover, the reduction in DM reduces the rate of β-eliminative pectin degradation during subsequent thermal processing. Alternatively, a high-pressure pretreatment has also been proven to be a highly effective technique to reduce texture degradation during subsequent thermal processing (Sila et al. 2004; Sila et al. 2005; Sila et al. 2006b; Sila et al. 2006a). As for low-temperature blanching, the positive effect of a high-pressure pretreatment is due to an enhanced catalytic activity of PME. Moreover, Sila et al. (2006b) showed the advantage of a high-pressure pretreatment over low-temperature blanching in the texture preservation of carrots. Both low-temperature blanching and high-pressure pretreating have been demonstrated to induce a decrease in WSP and a concomitant increase in CSP, consisting of Ca^{2+}-cross-linked pectin, and/or NSP (Canet et al. 2005; Recourt et al. 1996; Sila et al. 2006b; Sila et al. 2006a; Siliha et al. 1996; Wu and Chang 1990). During subsequent thermal processing, the conversion of water-insoluble pectin into WSP is significantly reduced (Ng and Waldron 1997; Sila et al. 2006a; Wu and Chang 1990) and shifts in the molar-mass distribution of the pectin fractions pointing at pectin solubilisation and β-eliminative depolymerisation are less pronounced (Ng and Waldron 1997). *In situ* pectin localisation with 2F4, an antibody specifically recognising Ca^{2+}-cross-linked HG dimers, revealed that both preprocessing techniques created pectin-Ca^{2+} cross-links in parts of the cell wall of broccoli tissue where these cross-links were originally absent (Christiaens et al. 2011c).

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The effectiveness of the PME-activating pretreatments is greatly influenced by the availability of divalent ions. Hence, thermal and high-pressure pretreaments are often combined with Ca^{2+} impregnation (Smout et al. 2005). Ca^{2+} ions as such have two opposite effects on texture. On the one hand, they can firm plant tissues through cross-linking of low-esterified pectic substances, while on the other hand they can increase tissue softening by enhancing the β-elimination reaction. The net result of Ca^{2+} addition, however, has been to enhance cell-cell adhesion and hence, firm the tissue (Van Buren 1979). When low-temperature blanching or high-pressure pretreating is combined with Ca^{2+} soaking, subsequent heat-induced softening is often further reduced (Sila et al. 2005; Sila et al. 2006a; Smout et al. 2005; Stanley et al. 1995). Ca^{2+} impregnation is most valuable when carried out after thermal or high-pressure pretreatments as tissue permeability is increased by these pretreatments (Sila et al. 2005).

The pretreatment strategy of stimulating endogenous PME activity is limited when PG or detrimental (off-flavour or oxidative) enzymes are present in the plant tissue or when only low amounts of endogenous PME are present. In porous tissues, like for example strawberries and eggplant, infusion of exogenous PME can be beneficial in this respect (Baker and Wicker 1996; Banjongsinsiri et al. 2004; Fraeye et al. 2009). Besides using thermal and high-pressure pretreatments, the DM of pectin can also be manipulated through genetic engineering.

Rheological properties of particulated plant-based food systems

In today's food industry, a global trend towards the manufacture of fruit- and vegetable-based products, such as soups, smoothies and sauces, is ongoing, as well as the incorporation of puréed vegetables into other food products (Blatt et al. 2011). The creation of this type of products involves the mechanical disruption of parenchyma-rich plant tissues. The resulting plant-food

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dispersions are a combination of a liquid phase (serum), containing pectic polysaccharides and other soluble substances, and a dispersed phase (pulp), containing the plant insoluble solids such as cell wall fragments, cells and cell clusters (Anthon et al. 2008; Bayod et al. 2005; Lopez-Sanchez et al. 2011a). The rheological properties of these plant-food dispersions are determined by both the particle properties of the dispersed phase and the properties of the serum phase (especially of the solubilised pectin) (Anthon et al. 2008; Den Ouden and Van Vliet 2002; Krokida et al. 2001; Tanglertpaibul and Rao 1987). Particle properties that affect the rheology of suspensions include concentration, size (distribution), shape, surface properties, deformability and interparticle forces (Day et al. 2010; Genovese et al. 2007; Moelants et al. 2012). Processing unit operations such as thermal, high-pressure and mechanical treatments are able to influence particle properties depending on the plant-type (Lopez-Sanchez et al. 2011a; Lopez-Sanchez et al. 2011b). Recently, the role of pectin, as influenced by different types of processing, on the rheological attributes of different types of particulated plant-based food systems was specifically investigated.

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Thermal and high-pressure (pre)processing

In the context of particulated plant-based food systems, a large number of studies have been performed on the rheological properties of tomato products like purées, pastes and juices due to their commercial importance. Two conventional thermal processes used in industrial tomato processing, termed ‘cold break’ and ‘hot break’, strongly affect the consistency of the resulting tomato-based products. These thermal processes impact on the pectin-modifying enzymes PME and PG, hence affecting the chemical composition of the particle and serum phase. In a ‘cold break’ process, tomatoes are heated at temperatures around 65 °C, whereby the activity of PME

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and PG is stimulated, leading to a loss in consistency. Conversely, a 'hot break' process, in which tomatoes are heated at temperatures around 85-90 °C, inactivates both PME and PG resulting in high-consistency products (Anthon et al. 2008; Goodman et al. 2002; Verlent et al. 2006). The required heat in a 'hot break' process however negatively influences other product characteristics like colour, flavour and nutritional value. High-pressure processing at rationally-chosen conditions (e.g. 10 min, 550 MPa, 25 °C) may present an interesting alternative for production of high-consistency tomato products as it selectively inactivates PG. In this way, the texture and viscosity of tomato-based products can be improved while the fresh product characteristics are better preserved.

In addition to research towards tomato-derived products, an increasing amount of studies have been performed on low-acid vegetables such as carrot and broccoli which, contrary to tomato, lack any appreciable amount of PG. Thermal processing of broccoli and carrot purée induces a shift in the solubility of pectin, i.e. from water-insoluble pectin to WSP, which is similar to the effect of thermal processing on pectin in solid plant-based foods (Christiaens et al. 2012b; Christiaens et al. 2012a). In addition, the induced pectin thermosolubilisation and β-eliminative depolymerisation were apparent in the molar-mass distribution of the pectin fractions. As an illustration, the influence of a thermal treatment on the solubility and molar-mass distribution of pectin in carrot purée is shown in **Figure 6 A-B**. Whereas the resulting weakening of intercellular adhesion could be related to texture softening in solid plant-based foods, a lowering in consistency and a decrease in the degree of syneresis (i.e. spontaneous separation of serum and pulp) is observed for particulated plant-based food systems. When carrots and broccoli were low-temperature blanched prior to purée preparation, a decrease in DM and increase in Ca^{2+}-

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cross-linked pectin at the expense of loosely bound WSP was observed. The resulting stronger intercellular adhesion was linked to a higher resistance of the plant tissue to physical disintegration, which in turn was reflected in a higher amount of large tissue particles. On a functional level, the increase in intercellular adhesion after low-temperature blanching was reflected in a purée with high consistency and a large degree of separation between serum and pulp (Christiaens et al. 2012b; Christiaens et al. 2012a).

Mechanical processing

High-pressure homogenisation, imposing high turbulence, shear and cavitation forces on a fluid passing the homogenisation valve, has quite recently been introduced in the area of processing of particulated plant-based food systems to further mechanically disrupt plant material compared to conventional blending (Betoret et al. 2009; Colle et al. 2010; Lopez-Sanchez et al. 2011b). High-pressure homogenisation has been demonstrated to result in a reduced viscosity for carrot and broccoli dispersions, which has been related to a reduced particle size, while high-pressure homogenised tomato dispersions showed an increased viscosity compared to conventionally-blended samples (Lopez-Sanchez et al. 2011a). Furthermore, this high-shear treatment has been shown to affect pectin solubility depending on the plant matrix. In case of carrot purées, high-pressure homogenisation led to an increase in loosely bound WSP at the expense of water-insoluble pectin (Christiaens et al. 2012b) (**Figure 6A**). The molar-mass distributions of the extracted water-soluble pectins revealed some pectin solubilisation and depolymerisation similar to the effect of a thermal treatment, although to a smaller extent (**Figure 6C**). Interestingly, the intriguing effect of high-pressure homogenisation on pectin solubility as noticed for carrot did not occur for broccoli nor tomato (Christiaens et al. 2012a; Christiaens et al. 2012c). Finally, in

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every fruit and vegetable matrix examined to date, high-pressure homogenisation had no distinctive effect on the DM of pectin.

Physical stability of plant-based foods

Besides its important role with regard to the textural attributes of solid plant-based foods and rheological properties of particulated fruit- and vegetable-derived products, pectin also affects the physical stability of some food products. Cloud loss, an important quality defect of juices, is generally attributed to the action of endogenous plant PME. By its action, blocks of free pectic carboxyl groups are formed, providing a greater opportunity for pectic polymers to be cross-linked with divalent ions such as Ca^{2+}. As a result, the apparent molar mass of aggregates is increased, reducing their solubility and leading to juice cloud precipitation (Baker and Cameron 1999; Croak and Corredig 2006). Cloud stabilisation is traditionally accomplished by heating the juice to inactivate PME. However, the discovery of PMEI by Balestrieri et al. in 1990 opened a new window for controlling PME activity with better flavour and nutrient retention compared to thermal processing (Giovane et al. 2004; Jolie et al. 2010). In particular, PMEI purified from kiwi fruit has been successfully exploited to control the PME activity in juice during storage (Castaldo et al. 1991). Nevertheless, the restricted occurrence of PMEI and its low extraction yield hampered the application of this protein in the juice industry. More recently, PMEI has been fruitfully expressed in Pichia pastoris and Escherichia coli which may allow large-scale industrial use (Hao et al. 2008; Mei et al. 2007).

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Health-related aspects of plant-based foods

Bio-accessibility of micronutrients

Food microstructure is strongly believed to govern the *in vitro* bio-accessibility of micronutrients, i.e. the fraction of the ingested micronutrients that is released from the food matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (Parada and Aguilera 2007). In this context, an inverse relation was observed between the structural quality of carrots, represented by the texture, and the β-carotene *in vitro* bio-accessibility by Lemmens et al. (2009). It was hypothesised that β-eliminative pectin degradation was the main cause of the increasing bio-accessibility of β-carotene upon thermal processing. Furthermore, upon high-pressure homogenisation of tomato pulp, Colle et al. (2010) observed that the applied homogenisation pressure was inversely related to the *in vitro* bio-accessibility of lycopene, the most important micronutrient in tomato. The decrease in lycopene bio-accessibility was attributed to the increased strength of the fiber network formed in tomato pulp after high-pressure homogenisation. Finally, ultrasound treatment of tomato pulp appeared to promote the formation of a new network due to hydrogen bonding and hydrophobic interactions among de-esterified pectin molecules, hence decreasing the lycopene *in vitro* bio-accessibility (Anese et al. 2013).

Satiety and satiation

While satiation is defined as the satisfaction of appetite that develops during feeding that marks the end of eating, satiety refers to the inhibition of hunger as a result of having eaten (Slavin and Green 2007). Short-term satiety and long-term intervention studies have shown that fruits and

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vegetables have high satiating efficiency per calorie (Ledoux et al. 2011; Rolls 2009). Moreover, dietary fibre is thought to impact on satiety and satiation due to its properties of producing viscosity (satiety) and adding bulk to the food (satiation). In this context, insoluble fibre has often been shown to have more impact on satiety than soluble fibre (Slavin and Green 2007). Pectin in particular can appear in both a soluble as well as an insoluble dietary form in food products. Pectin has been shown to significantly delay gastric emptying time, hence increasing satiety (Di Lorenzo et al. 1988). In addition, Tiwary et al. (1997) demonstrated that pectin in doses as small as 5 g added to 448 mL of orange juice increased satiety in US Army employees. Flood-Obbagy and Rolls (2009) in contrast found that adding naturally occurring levels of pectin to apple juice (4.8 g pectin/266 g apple juice) did not enhance satiety. Furthermore, low-esterified pectic polymers can affect fat digestion by their ability to bind calcium ions. As calcium increases the digestion rate of emulsified lipids by lipase, the presence of low-esterified pectin will decrease the lipid digestion rate (Hu et al. 2010; Isaksson et al. 1982; Kumar and Chauhan 2010). Finally, pectin has been demonstrated to lower cholesterol levels in the blood stream. By increasing the gut viscosity, pectin lowers the re-absorption of bile acids hence increasing the synthesis of bile acids from cholesterol and, consequently, reducing circulating blood cholesterol. Pectin source and type (DM and molar mass) affect the cholesterol lowering capacity (Brouns et al. 2012).

Bio-active role of pectin fragments

Recently, new health claims with regard to bio-active roles for modified pectin as an anti-cancer agent are emerging (Maxwell et al. 2012). Specifically, modified pectin, i.e. pectin that has been broken down into smaller fragments that in theory can be absorbed by the body, may bind to and

inhibit the various actions of the pro-metastatic protein galectin-3. This binding may block the interactions of galectin-3 with other proteins and peptides, inhibiting its ability to promote cell adhesion and migration, and to prevent apoptosis.

Pectin as food ingredient

Thanks to its gelling properties, extracted pectin (EU code E440) is widely used as a functional food ingredient in innumerable food products. In all its applications as a food ingredient, the nanostructure of pectin profoundly affects its functionality. The importance of this relationship is reflected in the fact that although most plant tissues contain pectin, commercial production is limited to a few sources that have the required properties, i.e. citrus peel and apple pomace (Endress et al. 2006; Thakur et al. 1997). Other potentially valuable sources (e.g. sugar beet) remain largely unused due to certain structural properties adversely affecting their gelling capacity.

Depending on the DM of pectin, two different mechanisms of pectin gelation can be distinguished. High-methoxylated pectins (DM > 50%) on the one hand can form gels in the presence of co-solutes (typically sucrose at a concentration > 55%) and under acidic conditions (pH < 3.5). The high concentration of the co-solute decreases the water activity of the system thereby promoting hydrophobic interactions between methyl groups whereas the low pH suppresses carboxylic dissociation, hence reducing the electrostatic repulsion of pectin chains and enhancing the hydrogen bonding between non-dissociated carboxyl and secondary alcohol groups (Endress et al. 2006; Thakur et al. 1997). Low-methoxylated pectins (DM < 50%) on the other hand form gels in the presence of divalent cations, particularly Ca^{2+}. Gelation is due to the formation of junction zones between HG regions of different pectin chains through calcium

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bridges between dissociated carboxyl groups (see pectin cross-linking). Several intrinsic and extrinsic factors determine the extent of pectin gelation (Fraeye et al. 2010). Intrinsic factors refer to pectin structural features such as degree and pattern of methyl-esterification, chain length, pectin side chains and acetylation while extrinsic parameters include gel composition and conditions of the environment such as calcium ion and pectin content, pH, sugar content and temperature. Furthermore, amidation increases the gel-forming ability of low-methoxylated pectins. In particular, amidated pectins require less Ca^{2+} ions to gel and form stronger gels, especially at low pH, by the presence of hydrogen bonds between amide groups (Lootens et al. 2003). Recently, Ngouémazong et al. (2012a) demonstrated that pectin debranching resulted in weaker Ca^{2+}-pectin gels through a reduction in polymer chain entanglements. In practice, high-methoxylated pectins are applied as gelling agent in sweetened fruit products such as jams, jellies and marmalades whereas low-methoxylated pectins are used in low-calorie surrogates as they do not require sugars for gelation. Furthermore, pectin is employed in the production of fruit juices, confectionary products and bakery filling and in the stabilisation of acidified milk drinks and yoghurts.

Next to its use as a gelling, thickening or stabilising agent, pectin can be applied as an emulsifying agent. In this respect, a lot of research has been performed towards the emulsification properties of sugar beet pectin. It’s emulsion-stabilising potential has been related to the hydrophobic character of acetyl groups, ferulic acid groups and the residual protein moieties present within the pectin (Funami et al. 2007; Kirby et al. 2006; Leroux et al. 2003; Williams et al. 2005). Recently, Funami et al. (2011) also identified the contribution of pectic neutral side chains to stabilise emulsions by the formation of a hydrated layer.

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Conclusions and outlook

Combining macroscopic, microscopic and molecular analyses has a high potential in elucidating the numerous process-structure-function relations of pectin in foods. A profound understanding of these relationships aids food scientists to tailor the functional properties of fruit- and vegetable-based derived products and ingredients. In particular, a sound basis to control the textural/rheological properties of plant-based foods and to identify targets for food-structure engineering can be acquired. The deliberate application of particular thermal, high-pressure and mechanical processes, impacting on pectin's nanostructure and hence its functional characteristics, makes it possible to design naturally structured/textured food products without the addition of texture-controlling agents such as starches, gums and stabilizers. In this context, split stream processing presents the opportunity to maximally exploit the individual endogenous potential of the different constituents of a multi-compound food product by subjecting them to separate treatment paths. As a result, high quality food products can be obtained meanwhile reducing the production of waste streams.

Until now, process-structure-function related pectin research has mainly focused on changes in the linear HG domain of pectin. Despite the great importance of HG modifications, contributions of pectin's branched domains should not be neglected and be subjected to further investigation. Specifically, tailoring the activity of endogenous plant enzymes such as β-galactosidase and α-arabinofuranosidase might present new routes for food-structure engineering. Furthermore,

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research towards the impact of processing on pectin has been predominantly restricted to unit operations such as thermal processing, high pressure processing and, more recently, high pressure homogenisation. It would however also be interesting to evaluate the effect of digestive processes in the mouth, stomach and small intestine on pectin structure, for example in relation to the bio-accessibility of health-promoting micronutrients. Finding the optimal balance between the (micro)structure and nutritional quality of fruit- and vegetable-based derived products and ingredients will be a major challenge for future food technologists.

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Table 1: Overview of methods for pectin characterisation (FID = flame ionisation detector, FT – IR = Fourier transform infrared spectroscopy, GC = gas chromatography; HPAEC = high-performance anion exchange chromatography, (RP)-HPLC = (reversed phase) high-performance liquid chromatography, HPSEC = high-performance size-exclusion chromatography, LALLS = low angle laser light scattering, MALLS = multi angle laser light scattering, MS = mass spectrometry, NMR = nuclear magnetic resonance, NIR = near infrared, PAD = pulsed amperometric detection, RI = refractive index, UV = ultra violet, Vis = visible).

Pectin characteristic	Determination methods	References
Galacturonic acid content	Acid-base titration	Food Chemicals Codex (1981)
	Acid/enzymatic hydrolysis + colorimetric reaction (spectrophotometric detection)	Dische and Rothschild (1967); Blumenkrantz and Asboe Hansen (1973); Ahmed and Labavitch (1977); Scott (1979); Anthon and Barrett (2008)
	Acid/enzymatic hydrolysis + derivatisation (trimethylsilylation or alditol acetates) + quantification with GC	Jones and Albersheim (1972); Ford (1982)
	Acid/enzymatic hydrolysis + quantification with HPAEC – PAD	Garleb et al. (1991); Garna et al. (2006)
Degree of methyl-esterification (DM)	Acid-base titration	Food Chemicals Codex (1981)
	Alkaline saponification + chemical/enzymatic oxidation to formaldehyde + colorimetric reaction (spectrophotometric detection)	Wood and Siddiqui (1971); Klavons and Bennett (1986)
	Alkaline saponification + quantification with GC – FID or MS	McFeeters and Armstrong (1984); Huisman et al. (2004); Savary and Nunez (2003); Nunes et al. (2006)
	Alkaline saponification + quantification with HPLC – RI	Voragen et al. (1986); Levigne et al. (2002)
	Direct measurement with NMR	Grasdalen et al. (1988)
	Direct measurement with capillary electrophoresis	Zhong et al. (1997)
	Direct measurement with FT – IR (and FT – Raman)	Gnanasambandam and Proctor (2000); Fellah et al. (2009); Synytsya et al. (2003b)
	Binding with monoclonal anti-HG antibodies (JIM5, JIM7, LM7, LM18, LM19, LM20, PAM1)	Knox et al. (1990); Willats et al. (1999); Willats et al. (2001b); Verhertbruggen et al. (2009)
Pattern of methyl-esterification	Ca^{2+} binding	Kohn et al. (1985)

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	Chemical/enzymatic fingerprinting via HPAEC – PAD combined with MS or NMR for structural characterisation	De Vries et al. (1986); Mort et al. (1993); Daas et al. (1999)
	Chemical/enzymatic fingerprinting via capillary electrophoresis	Guillotin et al. (2007)
	Direct measurement with NMR	Neiss et al. (1999)
	Binding with monoclonal anti-HG antibodies (JIM5, JIM7, LM7, LM18, LM19, LM20, PAM1)	Knox et al. (1990); Willats et al. (1999); Willats et al. (2001b); Verhertbruggen et al. (2009)
Distribution of DM among pectin chains	Direct measurement with capillary electrophoresis	Zhong et al. (1997)
Degree of acetylation	Hydroxamic acid method (spectrophotometric detection)	Downs and Pigman (1976)
	Alkaline saponification + quantification with HPLC – RI	Voragen et al. (1986); Levigne et al. (2002)
	Alkaline saponification + quantification with GC – FID or MS	Savary and Nunez (2003); Nunes et al. (2006)
	Direct measurement with FT – Raman and FT – IR	Synytsya et al. (2003b)
	Commercial enzymatic test kits	Buccholt et al. (2004); Ralet et al. (2008)
Pattern of acetylation	Enzymatic fingerprinting via HPAEC – PAD combined with MS or NMR for structural characterisation	Perrone et al. (2002); Ralet et al. (2005b); Ralet et al. (2008)
Degree of polymerisation	Spectrophotometric detection of reducing end groups with cyanoacetamide	Gross et al. (1982)
	Spectrophotometric detection of unsatured uronides	Sajjaanantakul et al. (1989)
Neutral sugar content	Orcinol - sulphuric acid method	Tollier and Robin (1979)
	Acid/enzymatic hydrolysis + quantification with HPAEC – PAD	Garleb et al. (1989); Matsuhashi et al. (1992); Garna et al. (2004)
	Acid/enzymatic hydrolysis + derivatisation (trimethylsilylation or alditol acetates) + quantification with GC – FID or MS	Englyst et al. (1982) ; Blakeney et al. (1983); Doco et al. (2001)
Sugar linkages	Acid/enzymatic hydrolysis + separation via HPAEC – PAD + MS or NMR for structural characterisation	Coenen et al. (2007)
	Acid/enzymatic hydrolysis + derivatisation (trimethylsilylation or alditol acetates) + structural characterisation with GC – FID or MS	Carpita and Shea (1989); van Casteren et al. (1998); Verhoef et al. (2009)
Linearity of pectic polymers	Visualisation of individual pectic polymers with atomic force	Round et al. (1997); Round et al. (2001); Yang

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Degree of branching	microscopy	et al. (2006)
	Determination via HPSEC – RI – MALLS	Corredig et al. (2000)
	Indirect estimation via calculation of sugar ratios	Houben et al. (2011)
Molar mass distribution	Low-speed sedimentation equilibrium determination via ultracentrifugation	Harding et al. (1991)
	Osmometry	Berth et al. (1990)
	Determination via HPSEC – RI	White et al. (1999)
	Determination via HPSEC – RI – LALLS	Berth et al. (1990)
	Determination via HPSEC – RI – MALLS	Fishman et al. (1997); Corredig et al. (2000)
	Determination via flow-field flow fractionation – RI – MALLS	Duval et al. (2001)
Size of pectin molecules	Dynamic light scattering with Zeta potential/particle sizer	Chen et al. (2012)
Ferulic acid (cross-links)	Alkaline saponification + quantification with RP-HPLC – UV	Waldron et al. (1996)
	Alkaline saponification + derivatisation + quantification with GC	Ralph et al. (1994)
	Spectrophotometric detection	Micard et al. (1994)
	Spectroscopic determination (UV – Vis, Vis – NIR, FT – IR, FT – Raman)	Synytsya et al. (2003a)
	Binding with a monoclonal antibody directed towards feruloylated galactan (LM9)	Clausen et al. (2004)
Ca^{2+} cross-links	Selective extraction with chelating agents	Fry (1986); Selvendran and O'Neill (1987)
	Binding with a monoclonal antibody directed towards Ca^{2+} cross-linked HG dimers (2F4)	Liners et al. (1989)
Location of specific pectin domains	Immunolabeling with anti-pectin antibodies (light microscopy equipped with epifluorescence illumination, confocal microscopy, transmission electron microscopy)	Liners et al. (1989); Knox et al. (1990); Willats et al. (1999); Willats et al. (2001b); Verhertbruggen et al. (2009)

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Table 2: Overview of ways to modify pectin structure enzymatically.

	Enzyme	**Reaction catalysed**
De-esterification	Pectin methylesterase	Hydrolysis of methyl-esters in HG
	Pectin acetylesterase	Hydrolysis of acetyl-esters in HG
	Rhamnogalacturonan acetylesterase	Hydrolysis of acetyl-esters in RG-I
	Feruloyl esterase	Hydrolysis of feruloyl-esters in arabinan/(arbino)galactan
Depolymerisation (pectin backbone)	Polygalacturonase	
	Endo-polygalacturonase	Hydrolysis of non-methyl-esterified glycosidic α-(1,4) linkages in HG
	Exo-polygalacturonase	Hydrolysis of monoGalA or diGalA from non-reducing end of HG
	Pectate lyase	
	Endo-pectate lyase	Transelimination of non-methyl-esterified glycosidic α-(1,4) linkages in HG
	Exo-pectate lyase	Transelimination of unsaturated diGalA from non-reducing end of HG
	Pectin lyase	Transelimination of methyl-esterified glycosidic α-(1,4) linkages in HG
	Rhamnogalacturonan hydrolase	Hydrolysis of α-D-GalA-(1→2)-α-L-Rha linkages in RG-I backbone
	Rhamnogalacturonan lyase	Transelimination of α-L-Rha-(1→4)-α-D-GalA linkages in RG-I backbone
	Rhamnogalacturonan rhamnohydrolase	Hydrolysis of Rha from non-reducing end of RG-I backbone
	Rhamnogalacturonan galacturonohydrolase	Hydrolysis of GalA from non-reducing end of RG-I backbone
Debranching (pectin side chains)	Arabinanase	
	Endo-arabinanase	Hydrolysis of α-arabinan linkages in arabinan/arabinogalactan

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Exo-arabinanase	Hydrolysis of Ara or short Ara oligosaccharides from non-reducing end of arabinan/arabinogalactan
α-arabinofuranosidase	Hydrolysis of Ara from non-reducing end of arabinan/arabinogalactan
Galactanase	
Endo-galactanase	Hydrolysis of β-galactan linkages in (arabino)galactan
Exo-galactanase	Hydrolysis of Gal or short Gal oligosaccharides from non-reducing end of (arabino)galactan
β-galactosidase	Hydrolysis of Gal from non-reducing end of (arabino)galactan

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Figure captions

Figure 1: Schematic overview of the basic concept of this review.

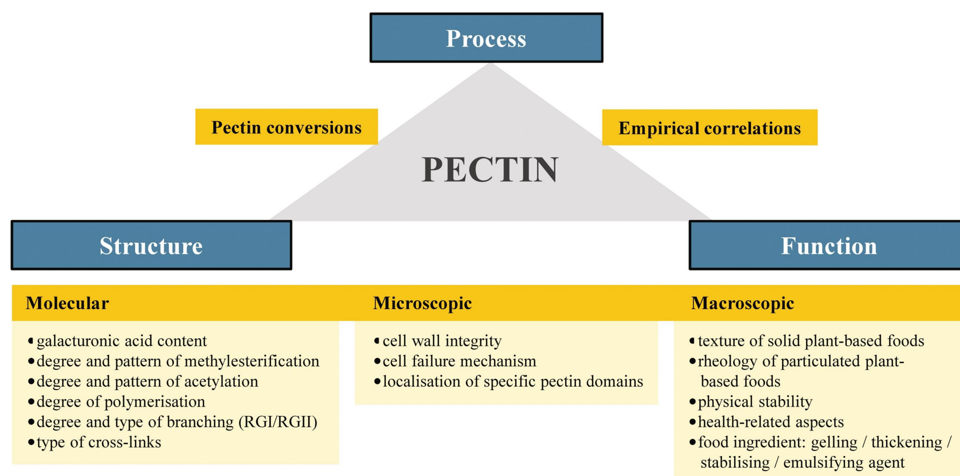

Figure 2: Schematic representation of the parenchyma cell wall in fruits and vegetables: (A) cross-section of a tricellular junction, (B) three-dimensional organisation of cell wall polymers (adapted from Davidson (2006)).

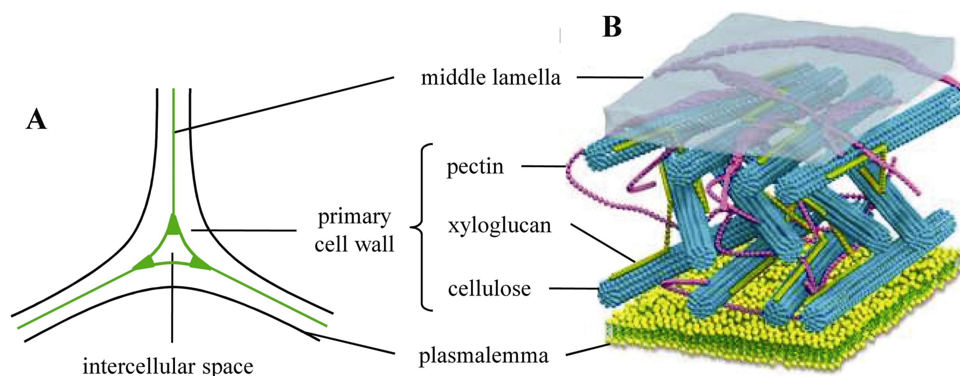

Figure 3: Schematic overview of process-structure-function relations related to the HG chain of pectin: PME = pectin methylesterase, PNL = pectin lyase, PL = pectate lyase, PG = polygalacturonase, T↑ = temperature increase.

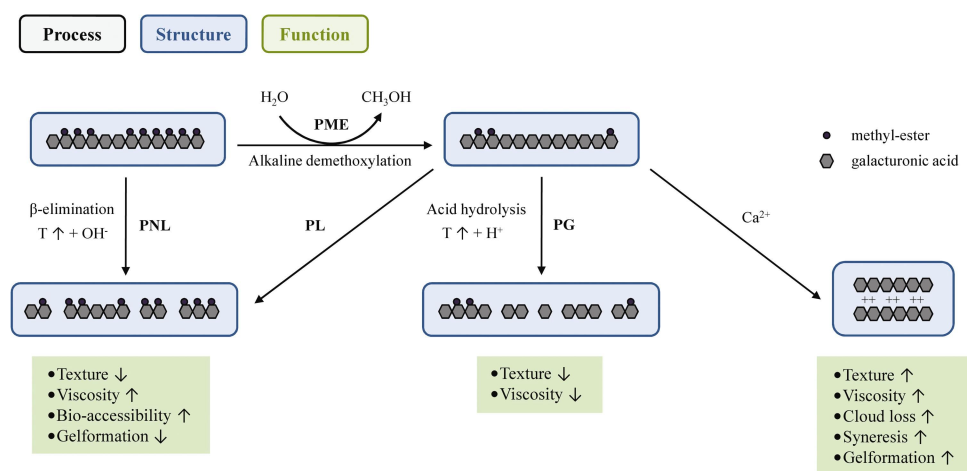

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Figure 4: Immunofluorescence labeling of tissue particles from high-temperature blanched tomatoes (A, B) and raw tomatoes (C) with PAM1, a monoclonal antibody specifically recognising long blocks of non-methyl-esterified GalA residues. In high-temperature blanched tomatoes PME was inactivated prior to tissue disruption, while in raw tomatoes PME was active during tissue disintegration. Observations with PAM1 demonstrate that in high-temperature blanched tomatoes long blocks of unesterified GalA residues are only present at discrete regions of the tomato cell wall, either at regions of the cell wall lining former intercellular spaces (A) or at random spots throughout the entire cell wall (B), pointing at a physical restriction of PME activity in intact red-ripe tomato fruit to these specific spots. In tissue particles originating from raw tomatoes, PAM1 epitopes were abundant at the entire surface of the tomato cells (C) indicating that the tight regulation of PME activity *in vivo* was lost and intensive demethoxylation of pectin by PME could occur throughout the entire cell wall. Scale bars = 200 µm. Pictures adapted from Christiaens et al. (2012c).

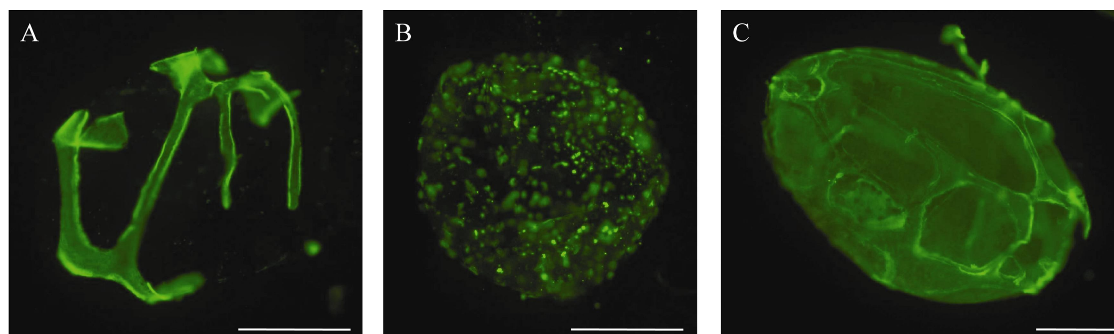

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Figure 5: Immunofluorescence labeling of broccoli cortex parenchyma: raw broccoli labeled with JIM7 (A), thermally processed (15 min at 90 °C) broccoli labeled with JIM7 (B) and high-pressure treated broccoli labeled with LM19 (C). Cryosections of ± 30 µm were used. Scale bars = 20 µm. Pictures adapted from Christiaens et al. (2011c).

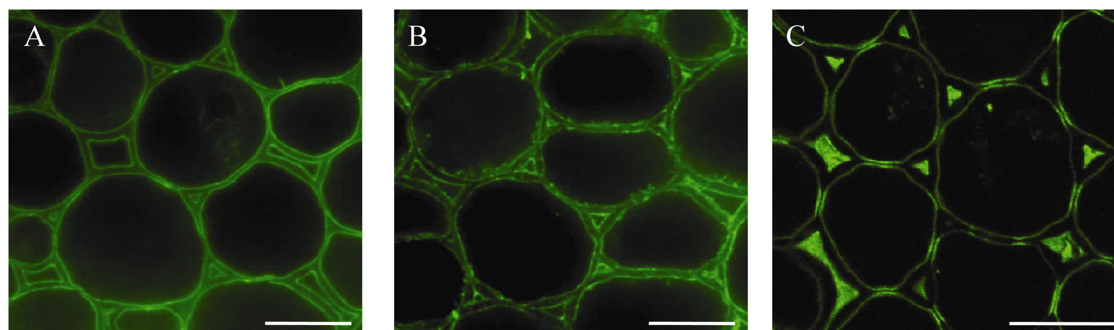

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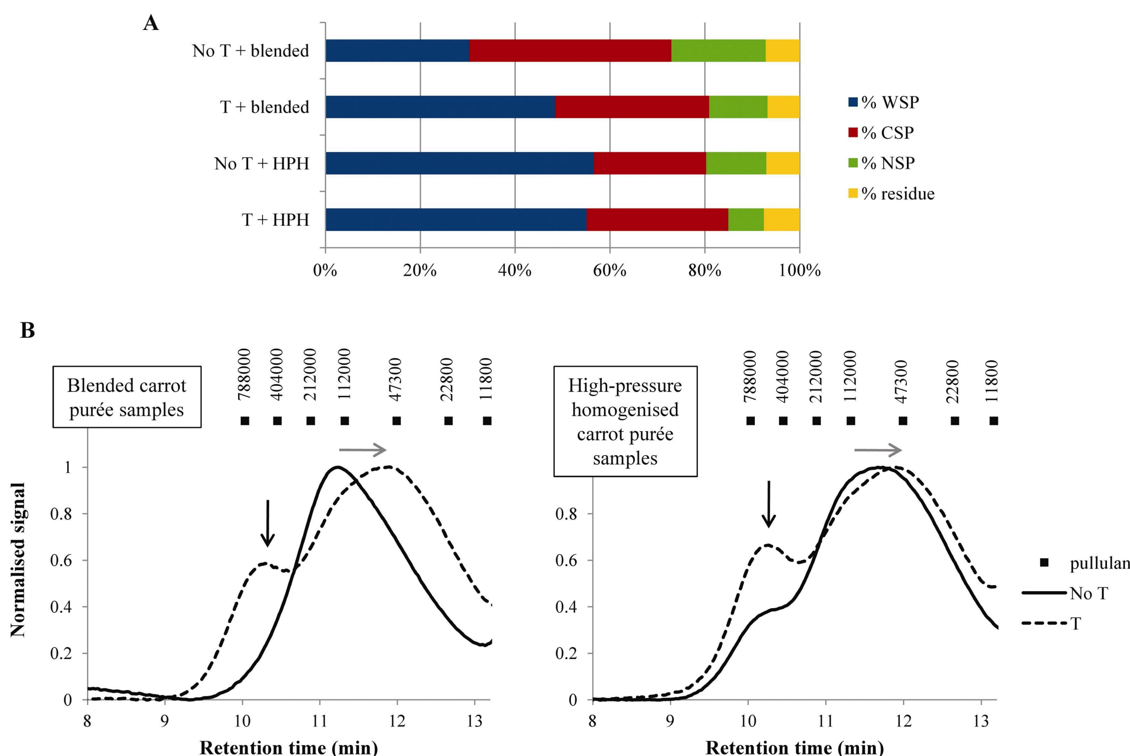
Figure 6: (A) Relative amount of pectin fractions (GalA in fraction relative to GalA in sum of fractions) in carrot purée samples. (B) Influence of different treatment conditions on the molar-mass distribution of WSP for blended and high-pressure homogenised carrot purée samples. Elution times of pullulan standards are indicated to allow for a rough estimation of the molar mass. Shifts are designated with arrows. No T = no thermal treatment, T = thermal treatment (5 min, 95 °C), HPH = high-pressure homogenisation (100 bar), WSP = water-soluble pectin, CSP = chelator-soluble pectin, NSP = Na_2CO_3-soluble pectin. Adapted from Christiaens et al. (2012b).



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