Review

Pectin conversions under high pressure: Implications for the structure-related quality characteristics of plant-based foods

Ruben P. Jolie,
 Stefanie Christiaens,
 Ans De Roeck, Ilse Fraeye,
 Ken Houben,
 Sandy Van Buggenhout,
 Ann M. Van Loey and
 Marc E. Hendrickx*

Laboratory of Food Technology and Leuven Food Science and Nutrition Research Centre (LForCe), Department of Microbial and Molecular Systems (M2S), Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, PB 2457, BE-3001 Leuven, Belgium (Tel.: +32 16 321572; fax: +32 16 321960; e-mail: Marc.Hendrickx@biw.kuleuven.be)

Under conditions associated with fruit and vegetable processing, cell wall pectin can undergo numerous enzymatic and non-enzymatic conversion reactions, which are connected with and reflected in the structure-related quality characteristics of the final product (e.g., texture, viscosity, cloud stability). This paper reviews recent insights in the ways the

major pectin conversions (including depolymerisation and demethoxylation) are affected by application of high pressure (100–1000 MPa), a process parameter of increasing industrial relevance. Pressure-induced effects including (i) reaction acceleration and deceleration, (ii) pectic enzyme stimulation, inhibition, inactivation and stabilisation and (iii) enzyme-inhibitor and enzyme-subunit dissociation are discussed. Their food-technological implications and (potential) applications with regard to structure-related quality attributes of plant-based foods are illustrated, clearly showing the relevance of high-pressure processing of food systems for unique functional properties beyond preservation (pasteurisation and sterilisation).

Introduction

Subjecting biological systems to elevated pressure levels brings along changes at various organisational levels, ranging from macroscopic to molecular. Basically, two general scientific principles govern the behaviour of materials under high pressure (HP): the principles of Pascal and Le Châtelier. According to the Pascal or isostatic principle, pressure is transmitted uniformly and instantaneously throughout the entire sample. Products are not irreversibly deformed by pressure, except for those having a porous structure containing highly compressible air voids. The principle of Le Châtelier states that if a chemical system at equilibrium experiences a change in concentration, temperature, volume or partial pressure, the equilibrium shifts so as to counteract the imposed change and a new equilibrium is established. As a consequence, any phenomenon accompanied by a volume decrease is enhanced by an increase in pressure, and *vice versa*. Pressure-induced changes are hence volume-change-driven. Reaction equilibria will be directed according to reaction volume (i.e., volume difference of products and reactants), while reaction rates under pressure will depend on the activation volume of the reaction (i.e., volume difference between the transition state and the reactants). Based on this concept, chemical reactions (including enzyme-catalysed) can be accelerated or retarded and interactions between (bio)molecules can be promoted or broken. With regard to enzymes, the effect of HP may be twofold: stability (through protein denaturation) as well as catalytic activity can be affected, depending on the pressure level involved (Box 1). Over the years, several complementary reviews have been

* Corresponding author.

Box 1. General considerations on the effects of high pressure on enzymes

Pressurisation (at room temperature) can induce enzyme inactivation by perturbing the subtle balance of stabilising intramolecular and solvent–protein interactions, leading to changes in the global enzyme conformation or at/near the active site. According to Le Châtelier's principle, these changes are basically driven by volume reductions. HP inactivation at ambient temperature is considered to be initiated by forcing water into the interior of the protein matrix, leading to conformational transitions and resulting in unfolding. Loss of native tertiary structure is predominantly ascribed to disruption of hydrophobic and electrostatic interactions. The large hydration changes accompanying these structural modifications (i.e., ordering of solvent molecules around newly exposed polar and non-polar groups) are assumed to be the major source of volume decrease associated with HP denaturation. Changes in secondary structure, maintained by hydrogen bonds, occur only at very high pressure levels and mostly cause irreversible denaturation, whereas covalent bonds of the primary structure are almost unaffected (Balny, 2004; Knorr *et al.*, 2006; Ludikhuyze, Van Loey, Indrawati, Smout, & Hendrickx, 2003). Since an increase in pressure at constant temperature leads to an ordering of molecules, resulting in a decrease of entropy of the system (i.e., the principle of “microscopic ordering”), whereas a temperature rise induces disordering, the effects of pressure and temperature on proteins are often antagonistic. Although the molecular origin hereof is not well understood, an important role of (the physical properties of) water is beyond questioning (Balny, 2004; Boonyaratankornkit, Park, & Clark, 2002; Eisenmenger & Reyes-De-Corcuera, 2009; Knorr *et al.*, 2006; Meersman, Smeller, & Heremans, 2006).

HP-induced changes of enzyme action however exceed denaturation. Pressure may also exert an influence on the catalytic activity of the enzyme (at comparatively lower pressure levels). Both enhanced and reduced enzyme activity under HP have been observed. Either conformational changes of the protein structure, changes in the enzyme-substrate interaction or effects on the actual substrate-to-product conversion have been put forward as possible explanations. Again, the principle of Le Châtelier is involved, stating that pressure favours the reduction of a system's overall volume. For example, elevated pressure will hamper reactions that are accompanied by a volume increase upon substrate binding to the enzyme's active site and accelerate/retard reactions having a negative/positive activation volume (i.e. volume difference between the transition state and the ground state of the enzyme-substrate complex) (Aertsen *et al.*, 2009; Boonyaratankornkit *et al.*, 2002; Cheftel, 1992; Eisenmenger & Reyes-De-Corcuera, 2009). Moreover, in particular cases, increased enzyme activity can arise from HP-induced decompartmentalisation, with pressure damaging membranes and facilitating enzyme-substrate contact (Cheftel, 1992; Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998).

published dealing with these topics (Aertsen, Meersman, Hendrickx, Vogel, & Michiels, 2009; Balny, 2004; Cheftel, 1992; Knorr, Heinz, & Buckow, 2006).

These key principles of HP form the basis for its potential applications in the field of biological sciences in general and food science and technology in particular. Depending on the pressure-temperature combinations considered, applications such as pasteurisation, sterilisation, freezing, thawing and the creation of specific functional properties are within reach. The fact that the influence of pressure on different (bio-)chemical and physical changes is controlled by activation volumes while temperature effects are controlled by activation energies creates unique opportunities resulting from the new processing variable.

Over the past decades, HP processing (HPP) has increasingly being acknowledged as unit operation in food processing and preservation. One of its potentials is its use as an alternative technology to replace or complement traditional heating methods for food preservation, through effectively destroying undesired micro-organisms and enzymes while better retaining nutritional and sensory quality of the products. In current commercial applications, HPP is essentially a ‘non-thermal’ pasteurisation process, in which a food is subjected to pressures up to 600 MPa at initial temperatures below 45 °C for 1–20 min. Since the process involves minimal heating, many quality aspects can be remarkably similar to those of the unprocessed counterpart. Unfortunately, bacterial spores are very resistant at commercially achievable pressure levels (at room temperature). Consequently, HP-processed food products on today's market are chilled (e.g., ham, guacamole) and/or high-acid (e.g., fruit juices). In the quest of

obtaining ambient-stable low-acid foods, the combination of HP with elevated temperatures (“HP sterilisation” or “pressure-assisted thermal sterilisation”) is presently being investigated, achieving sterilisation conditions by starting the HP treatment at temperatures between 60 and 90 °C and using the compression heat to rapidly and uniformly reach temperatures of 90–130 °C (Bermudez-Aguirre & Barbosa-Canovas, 2011; Mujica-Paz, Valdez-Fragoso, Samson, Welti-Chanes, & Torres, 2011). In addition, in the high-pressure/low-temperature area (up to 400 MPa and down to –30 °C), several phase transitions are feasible including HP-shift freezing, HP-assisted freezing and HP thawing (Urrutia-Benet, Schlüter, & Knorr, 2004).

A product category for which HPP has proven useful is constituted by fruit- and vegetable-based foods. During processing of these products, HP affects both sensory properties like colour and flavour (Oey, Lille, Van Loey, & Hendrickx, 2008) and structure-related quality attributes like texture, viscosity, consistency, cloud stability etc. In many cases, processing-induced modifications in structure-related quality characteristics can be connected with transformations in the plant cell wall polysaccharides (Van Buggenhout, Sila, Duvetter, Van Loey, & Hendrickx, 2009; Waldron, Parker, & Smith, 2003). Cell wall pectin, specifically, is sensitive to numerous conversion reactions. A detailed insight in the influence of HP on pectin conversions allows exploring the unique prospects for HPP to specifically manage pectin-related functional properties of food systems next to its decontamination effects.

After a brief introduction on pectin structure and function, a concise overview of the major enzymatic and non-

enzymatic conversion reactions of pectin, supplemented with their food-technological relevance, is given. This is followed by the main focus point of the current review, namely recent insights in the ways these reactions are affected by application of HP (at ambient or elevated temperature). Food-technological implications and (potential) applications, with regard to structure-related quality attributes of plant-based foods, are discussed and illustrated.

Pectin structure, location and function

Pectin is a group of polysaccharides predominantly containing galacturonic acid (GalA). However, many other monosaccharides (such as Rha, Ara and Gal) occur as well. These monosaccharides are organised in a number of distinct polysaccharide building blocks, the three major ones being homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). HG is a linear chain of (up to approx. 200) (1 → 4)-linked α -D-GalA residues, part of which is methyl-esterified at the C-6 carboxyl group. The degree of this methoxylation (DM) is a major factor determining pectin functionality. Depending on the plant source, HG can also be O-acetylated on C-2 and/or C-3. RG-I, on the other hand, comprises a backbone of the repeating disaccharide [$\rightarrow$ 4)- α -D-GalA-(1 → 2)- α -L-Rha-(1 →)]. A large proportion (20–80%) of the Rha residues are substituted at C-4 with side chains, principally composed of Ara and Gal. These side chains may be branched and complex, and RG-I domains are often referred to as “hairy” regions when compared with the “smooth” regions of HG. RG-II has an HG backbone that is substituted by four hetero-oligomeric side chains of known and consistent composition. Besides these three main polysaccharides, other substituted galacturonans, such as xylogalacturonan (XGA) and apiogalacturonan (AGA), have been demonstrated in the cell walls of a restricted number of plants. The constituent pectin building blocks are covalently linked to each other, but the exact features still remain somewhat unclear.

In general, pectin is most abundant in the primary cell walls and the middle lamellae of plants. In the primary walls of dicotyledonous plants, it accounts for about one-third of the cell wall polymeric content and is often described as the cementing matrix in which the network of long, rigid cellulosic microfibrils is embedded. Grasses contain lower amounts of pectin (2–10%), while fruit cell walls are often pectin-enriched. The middle lamella, shared by two adjacent cells and considered to be an extension of the matrix material of the primary cell wall lacking the cellulose fibrils, predominantly contains pectin.

The functionality of pectin in the plant cell wall is diverse and not yet elucidated precisely. Basically, and most relevant to food technologists, it contributes to the mechanical strength of the wall and to the adhesion between cells. In this context, the ionic cross-linking of HG chains by the binding of calcium ions at blocks of negatively charged, non-methoxylated GalA units is considered crucial. Besides, pectin is involved in complex

physiological processes like cell growth and cell differentiation and influences various cell wall properties such as porosity, surface charge, pH and ion balance. For more details on structure and function of the pectic polysaccharides, the reader is referred to other, specific reviews (Caffall & Mohnen, 2009; Jarvis, 2011; Voragen, Coenen, Verhoef, & Schols, 2009; Waldron *et al.*, 2003).

Key pectin conversion reactions and their food-technological relevance

Under conditions associated with postharvest storage and processing of plant-based foods, pectin can undergo numerous enzymatic and non-enzymatic conversion reactions. Given the complex pectin composition and architecture, a particularly wide range of possible conversions exist. This review, however, will focus on the reactions taking place at the linear HG chain, including demethoxylation and depolymerisation. Through synergistic modification of pectin's fine structure, these reactions govern most of pectin's functional properties, both in plants and in foods derived thereof. A schematic overview is given in Fig. 1.

Enzymatic demethoxylation

Pectin methyl-esterases (PME, E.C. 3.1.1.11) catalyse the specific demethoxylation of HG at the O-6 of GalA, releasing methanol and protons and creating dissociable carboxyl groups on the pectin chain (Jolie, Duvetter, Van Loey, & Hendrickx, 2010; Pelloux, Rusterucci, & Mellerowicz, 2007). Free carboxyl groups in the vicinity of the active site appear to be required for PME action, explaining the preference of PME for partially demethoxylated pectins. PMEs have been identified in all higher plants examined, and are also produced by phytopathogenic bacteria and fungi. Different action patterns of PME on HG have been described, creating either long contiguous stretches (“blockwise” action, mainly for alkaline plant PMEs) or a more random distribution (mainly for acidic fungal PMEs) of de-esterified GalA residues (Jolie, Duvetter, Van Loey *et al.*, 2010; Limberg *et al.*, 2000; Ngouémazong *et al.*, 2010). In some plants (e.g., kiwi fruit), a proteinaceous PME inhibitor (PMEI) is present, capable of inhibiting plant PMEs through the formation of a reversible, non-covalent 1:1 complex with the enzyme (Giovane *et al.*, 2004; Jolie, Duvetter, Van Loey *et al.*, 2010).

Non-enzymatic demethoxylation

Methyl-esters at C-6 of GalA residues can be hydrolysed under alkaline or mild-acidic conditions (approx. pH $\geq$ 5) through saponification. This chemical demethoxylation is a random process, resulting in a statistical distribution of free and methoxylated GalA residues on the HG chain (Limberg *et al.*, 2000; Ngouémazong *et al.*, 2010; Van Buren, 1979). The reaction is accelerated at higher temperatures and, since saponification is initiated by hydroxyl ions, with increasing pH. Moreover, the rate is proportional

Fig. 1. Schematic overview of enzymatic and non-enzymatic conversion reactions occurring at the homogalacturonan chain of pectin. Names of enzymes and inhibitors are formatted *italic* (PME: pectin methyl-esterase; PMEI: PME inhibitor; PNL: pectin lyase; PL: pectate lyase; PG: polygalacturonase; R₁/R₂: initial/terminal fragment of the pectin polymer; ΔT: heating).

to the amount of esters remaining, so decreasing as the reaction proceeds (De Roeck *et al.*, 2009; Diaz, Anthon, & Barrett, 2007; Fraeye, De Roeck *et al.*, 2007; Renard & Thibault, 1996).

Additionally, demethoxylation can occur under strong-acidic conditions (approx. pH < 3) through a mechanism of acid hydrolysis. However, only few studies have focused hereon (Fraeye, De Roeck *et al.*, 2007; Krall & McFeeters, 1998).

Calcium-pectin cross-linking

Negatively charged, non-methoxylated GalA residues in the HG domain can ionically interact with divalent ions (such as Ca²⁺ and Mg²⁺) forming supramolecular networks and/or gels. Blocks of 7–20 free GalA residues have been reported to be required for building a stable linkage between chains (Thibault & Ralet, 2003; Voragen *et al.*, 2009).

Enzymatic depolymerisation

The pectin-depolymerising enzymes comprise hydrolases and lyases. Polygalacturonases (PG) cleave the α-(1 → 4)-D linkages between two adjacent GalA residues

in the HG domain of pectin by acid/base-assisted hydrolysis. Whereas endo-PGs (EC 3.2.1.15) hydrolyse the polymer substrate randomly (resulting in a fast decrease in molar mass of pectin), the exo-PGs are confined to cleave off GalA monomers (EC 3.2.1.67) or dimers (EC 3.2.1.82) from the non-reducing end of a chain (Benen & Visser, 2003b). The exact substrate requirement for PG action is still subject to debate, but it is generally acknowledged that the enzyme possesses increasing activity with decreasing DM, for example caused by action of PME (Tucker & Seymour, 2002). PG has been found in some fungi, bacteria, yeasts and many higher plants (Duvetter *et al.*, 2009; Tucker & Seymour, 2002). It is considered a key enzyme in the degradation of plant cell walls upon phytopathogenic attack, and many plants have evolved a resistance mechanism relying on PG-inhibiting proteins (PGIPs). These PGIPs are extracellular glycoproteins, found in virtually all tissues and organs of all plants investigated. They are active against microbial PGs, but do not interact with the plant-derived PGs studied (Juge, 2006; Protsenko, Buza, Krinitsyna, Bulantseva, & Korableva, 2008).

Pectate lyases (PL) catalyse the cleavage of α-(1 → 4)-D galacturonosidic linkages in HG via a β-elimination

mechanism, resulting in the formation of a double bond between C-4 and C-5 at the newly formed non-reducing end. They can be endo- (E.C. 4.2.2.2) or exo-acting (E.C. 4.2.2.9) and require calcium ions for catalysis (Benen & Visser, 2003a). For many PLs, highest specific activity was recorded using pectins of moderate DM (20–50%) instead of pectin with 0% DM. Initially, it was thought that PLs were secreted only by plant pathogens, their action resulting in the maceration of plant tissues. However, putative PLs have been identified in plants through sequencing of plant-tissue-specific cDNA libraries (Benen & Visser, 2003a; Marin-Rodriguez, Orchard, & Seymour, 2002; Vicente, Saladie, Rose, & Labavitch, 2007).

Pectin lyases (PNL, EC 4.2.2.10) perform the same reaction as PLs but this class of enzymes does not have a calcium requirement and prefers the GalA residues adjacent to the scissile bond to be methyl-esterified. Hence, the specific activity increases with increasing DM. Currently, merely endo-acting PNLs are known. PNLs have so far only been identified in and isolated from micro-organisms (Benen & Visser, 2003a; Yadav, Yadav, Yadav, & Yadav, 2009).

Non-enzymatic depolymerisation

When heating pectin at mild-acidic or alkaline pH (approx. $\text{pH} \geq 5$), splitting of glycosidic linkages between GalA residues can take place by a process of β -elimination. The hydrogen at C-5 of GalA is removed by a hydroxyl ion, followed by eliminative cleavage of the glycosidic linkage in the β -position, resulting in the formation of an unsaturated bond between C-4 and C-5 at the non-reducing end. A prerequisite is the presence of a methylester group at C-6, rendering H-5 sufficiently acidic to be removed by the alkali. Consequently, pectin with a high DM has an increased susceptibility towards β -eliminative depolymerisation (Albersheim, Neukom, & Deuel, 1960; Diaz *et al.*, 2007; Krall & McFeeters, 1998; Sajjaanantakul, Van Buren, & Downing, 1989). Chemical demethoxylation, proceeding under the same temperature and pH conditions and affecting the DM, will influence the β -elimination (De Roeck *et al.*, 2009; Fraeye, De Roeck *et al.*, 2007; Kravtchenko, Arnould, Voragen, & Pilnik, 1992). Since hydroxyl ions initiate the reaction, the rate increases with increasing pH, but β -elimination has been measured at pH values as low as 3.5 (Krall & McFeeters, 1998; Kravtchenko *et al.*, 1992; Sila, Smout, Elliot, Van Loey, & Hendrickx, 2006). β -Elimination is also stimulated in presence of cations (depending on concentration and valency) and organic anions (Keijbets & Pilnik, 1974), and accelerated with increasing temperature (De Roeck *et al.*, 2009; Diaz *et al.*, 2007; Fraeye, De Roeck *et al.*, 2007; Sila *et al.*, 2006).

A second mechanism leading to pectin depolymerisation during heating is the acid-catalysed hydrolysis of glycosidic bonds (approx. $\text{pH} < 3$). The reaction proceeds through an initial protonation of the glycosidic oxygen,

followed by a rate-limiting unimolecular heterolysis of the conjugated acid. Addition of water results in the formation of a reducing end group and a proton (Smidsrod, Haug, & Larsen, 1966; Van Buren, 1979). Pectin with a low DM hydrolyses faster and the rate of hydrolysis increases with decreasing pH, addition of NaCl and rising temperature (Diaz *et al.*, 2007; Fraeye, De Roeck *et al.*, 2007; Krall & McFeeters, 1998).

Food-technological relevance of the key pectin conversion reactions

The implications of non-enzymatic and enzyme-catalysed HG modifications on the structure-related quality of plant-based foods (texture, viscosity and cloud stability) can basically be explained in terms of either of two underlying phenomena: (i) fragmentation of cell wall pectins as a result of depolymerisation reactions and (ii) pectin cross-linking through salt bridges (mostly calcium). The role of demethoxylation reactions is rather indirect, the degree and pattern of HG methoxylation being determinant for pectin's susceptibility towards the aforementioned phenomena. Depending on the product at hand, specific conversion reactions can be considered desirable or detrimental, creating the need to tailor them in a certain direction.

Through depolymerisation of cell wall pectins, PGs induce loss of structural integrity of tissue systems (Van Buren, 1979), also thought to occur during fruit ripening (Waldron *et al.*, 2003). Moreover, this pectin fragmentation may cause a viscosity or consistency loss in, for instance, pastes and purées of plant sources with high levels of endogenous PG (e.g., tomato) (Crelrier, Robert, Claude, & Juillerat, 2001; Lopez, Sanchez, Vercet, & Burgos, 1997). In particular cases, PG-induced changes may deliberately be enabled or even enhanced (e.g., for reduced viscosity or for improved clarification/extraction of juices), but mostly inactivation of PG is aimed at, traditionally through thermal processing (e.g., “hot break” in tomato processing). Due to the occurrence of a thermostable PG fraction (“PG1”) in tomato-derived systems, intensive heating of these products is required for complete PG inactivation. This heat-resistant PG1 is presumably formed upon fruit handling and processing through tight association of the naturally occurring heat-labile catalytic PG subunit (“PG2”) with a heat-stable 38-kDa glycoprotein (“ β -subunit”) (Duvetter *et al.*, 2009). As opposed to the well-established role of PGs, little is known about the food-technological relevance of endogenous PLs and PNLs, if present at all. An appreciable contribution is anyhow suggested by their demonstrated role in fruit and vegetable textural changes during plant development and ripening (Vicente *et al.*, 2007).

Pectin fragmentation through β -elimination is considered the major driving force behind tissue softening upon thermal processing of fruits and vegetables (Sila *et al.*, 2006; Van Buren, 1979; Vu, Smout, Sila, Van Loey, &

Hendrickx, 2006; Waldron *et al.*, 2003), besides plasma-membrane disruption and associated loss of turgor (Greve *et al.*, 1994). Depolymerisation and solubilisation of pectic polysaccharides involved in cell–cell adhesion result in an increase in the ease of cell separation (Ng & Waldron, 1997) and changes in pectin solubility/extractability evolve (Sila *et al.*, 2009). Concomitantly, tissue failure characteristics shift from cell rupture to cell separation, pointing to failure of the tissue through the intercellular joints with minimal cell breakage and release of cell contents (Sila, Yue *et al.*, 2007; Van Buggenhout *et al.*, 2009; Waldron *et al.*, 2003). In case of acidic fruit tissues, softening upon heating may possibly be ascribed to acid hydrolysis of glycosidic bonds in cell wall polysaccharides, but information is scarce (Krall & McFeeters, 1998; Waldron *et al.*, 2003).

The second phenomenon, salt bridge formation between HG chains, owes its strong impact on the textural characteristics of plant-tissue-based foods to enhanced cell adhesion upon cross-linking of the middle-lamella pectins, bringing about cell wall reinforcement and firmness increase. In particular cases, the naturally present calcium can deliberately be supplemented by exogenously added ions, for example through soaking or infusion, to promote network formation (Baker & Wicker, 1996; Van Buggenhout *et al.*, 2009). Intermolecular networks/gels may also improve or preserve the viscosity of liquid plant-based products (Porretta, 1996). A similar cross-linking mechanism between demethoxylated pectin molecules and serum calcium ions in fruit and vegetable juices can give rise to precipitation of pectins, entraining pulp particles and leading to juice cloud/turbidity loss (Baker & Cameron, 1999; Jolie, Duvetter, Van Loey *et al.*, 2010).

Obviously, the sensitivity of pectins to cross-linking is related to the extent and distribution of non-methoxylated GalA units on the HG chains. Action of the de-esterifying enzyme PME, particularly the blockwise-acting types, hence promotes gel formation and cell wall reinforcement but also compromises juice cloud stability. Besides, PME-catalysed demethoxylation of HG chains also affects subsequent depolymerising reactions: demethoxylated pectin is more prone to enzymatic attack by PG or PL, inducing texture or consistency loss, whereas pectin demethoxylation reduces its susceptibility to non-enzymatic β -eliminative depolymerisation occurring during heating. Consequently, rigorous tailoring of PME-induced pectin conversions, through PME activity stimulation or inactivation, allows for the control of many pectin-related functional properties of plant-derived food products. Enhanced PME activity can be achieved by means of a mild-temperature pretreatment (since reactions are accelerated with temperature by increasing the fraction of collisions between molecules with sufficient energy) or enzyme infusion prior to the actual preservation process (Baker & Wicker, 1996; Ng & Waldron, 1997; Van Buggenhout *et al.*, 2009). In other cases, PME inactivation

is pursued (e.g., for cloud stabilisation), traditionally by thermal processing. Most PMEs are rather thermolabile, inactivating readily at temperatures below 70 °C (Duvetter *et al.*, 2009). However, thermoresistant isoforms exist (e.g., in the commercially important orange), needing more severe conditions for inactivation (Baker & Cameron, 1999). Besides PME inactivation, pre- or post-processing addition of PMEI (e.g., to a juice) has been put forward as an alternative strategy to prevent undesirable PME activity, but the current proofs-of-principle need further investigation (see Jolie, Duvetter, Van Loey *et al.*, 2010; Jolie *et al.* 2010 for a more detailed discussion).

Effects of high pressure on the key pectin conversions

The anticipated volume-change-driven effects of HP on reaction equilibria and reaction rates also apply to the key pectin conversions, both the enzyme-catalysed and the non-enzymatic. With regard to the former, stability as well as catalytic activity (see Box 1) of pectic enzymes can be affected. The information currently available in open literature, discussed below, is confined to the best-known pectinases, PME and PG. Compared to enzyme-catalysed pectin demethoxylation and depolymerisation, research on the non-enzymatic counterparts under HP has so far been limited, and restricted to saponification and β -elimination. Nevertheless, as will be illustrated in the next sections, the impact of pressure is substantial, particularly in the HT/HP (i.e., the emerging HP-sterilisation) domain.

PME stability

Since the pioneering study of Ogawa, Fukuhisa, Kubo, and Fukumoto (1990), numerous studies on the pressure stability of PME, including detailed kinetic studies, have been performed. Irreversible inactivation of a wide range of PMEs (e.g., citrus fruits, tomato, apple, carrot, strawberry, banana, plum and bell pepper) has been investigated, in model systems and in real food matrices (recently reviewed by Duvetter *et al.*, 2009). Generally speaking, PME can be regarded as a rather barotolerant enzyme (>600 MPa at room temperature). However, considerable variation in pressure stability exists between PMEs from various sources, ranging from moderately pressure-sensitive (e.g., orange and carrot PME) (Balogh, Smout, Ly Nguyen, Van Loey, & Hendrickx, 2004; Goodner, Braddock, & Parish, 1998; Nienaber & Shellhammer, 2001b; Polydera, Galanou, Stoforos, & Taoukis, 2004; Van den Broeck, Ludikhuyze, Van Loey, & Hendrickx, 2000b; Vervoort *et al.*, in press) to extremely pressure-stable (e.g., tomato PME, strawberry PME and fungal *Aspergillus aculeatus* PME) (Crelie *et al.*, 2001; Dirix, Duvetter, Van Loey, Hendrickx, & Heremans, 2005; Fachin *et al.*, 2002; Ly-Nguyen *et al.*, 2002). Also within one species, the different PME isoforms present can show significant differences in pressure stability (Castro,

Van Loey, Saraiva, Smout, & Hendrickx, 2006b; Plaza et al., 2007).

When HP is combined with mild heating (up to ca. 70 °C), different effects are generated, depending on the T,p region and the PME source. For many PMEs (e.g., orange, grapefruit, peach, carrot and tomato), a synergistic action of temperature and pressure in the low-temperature/high pressure domain (approx. <50 °C and >500 MPa) leads to an accelerated inactivation process compared to room temperature, whereas temperature and pressure often act antagonistically in the high-temperature/low-pressure region (approx. >50 °C and <400 MPa), moderate pressure levels retarding thermal inactivation of PME (Boulekou, Katsaros, & Taoukis, 2010; Guivarç'h, Segovia, Hendrickx, & Van Loey, 2005; Ly-Nguyen et al., 2003; Nienaber & Shellhammer, 2001b; Polydera et al., 2004; Van den Broeck et al., 2000b). This antagonistic effect of temperature and pressure is particularly striking for tomato PME (Crelie et al., 2001; Fachin et al., 2002; Stoforos, Crelie, Robert, & Taoukis, 2002; Van den Broeck, Ludikhuyze, Van Loey, & Hendrickx, 2000a). The antagonism is reflected by the elliptic shape of isorate contour plots in the T,p domain (i.e., plots representing T,p combinations resulting in the same inactivation rate constant) and has also been demonstrated at the conformational level through Fourier transform IR spectroscopy (Dirix et al., 2005). This pressure-induced “thermostabilisation” may indicate thermal unfolding to be associated with a positive volume change, which would then be counteracted by pressure. As a consequence, the optimal temperature for PME action can shift to higher temperatures at pressures beyond atmospheric pressure (cf. next section).

PME catalytic activity

For several PMEs, the rate of the enzyme-catalysed reaction (at a given temperature) has been shown to increase when an elevated pressure (below a certain critical pressure level) is applied. Examples are carrot, tomato, pepper and *A. aculeatus* PMEs, in purified form and/or *in situ* (Castro, Van Loey, Saraiva, Smout, & Hendrickx, 2006a; Duvetter et al., 2006; Fraeye, Duvetter et al., 2007; Jolie, Duvetter, Houben et al., 2009; Sila, Smout et al., 2007; Verlent, Van Loey, Smout, Duvetter, & Hendrickx, 2004; Verlent et al., 2004). This stimulating effect of pressure on enzyme activity has been attributed to the assumption that PME action is accompanied by a volume reduction as a result of so-called “electrostriction”: the compact alignment of water dipoles in the proximity of the charged free carboxyl groups created by pectin demethoxylation (Eisenmenger & Reyes-De-Corcuera, 2009; Verlent, Van Loey et al., 2004; Verlent et al., 2004). At pressures above the critical pressure level, PME action is however retarded. Reversible or irreversible PME inactivation is the most obvious explanation, but pressure-induced structural changes in the pectin, rendering the

substrate less susceptible for the enzyme, has been suggested as additional possibility (Jolie, Duvetter, Houben et al., 2009).

The PME-catalysed demethoxylation can be further accelerated (up to approx. 7-fold) by combining elevated pressures with moderate heating (Castro et al., 2006a; Duvetter et al., 2006; Fraeye, Duvetter et al., 2007; Sila, Smout et al., 2007; Verlent, Van Loey et al., 2004; Verlent et al., 2004), owing to the additional protective effect of pressure against thermal PME inactivation. In other words, the optimal temperature for PME activity can shift to higher values with increasing pressure (being function of the pressure level applied). Consequently, each PME possesses a characteristic T,p window for maximal turnover, to be determined experimentally (see, for example, Fig. 2 for carrot PME). Contrary to the reaction rate, the action pattern of enzymatic pectin de-esterification (i.e., by a fungal PME) seems not to be affected by temperature and/or pressure (Duvetter et al., 2006).

PME inhibition

The proteinaceous PME inhibitor found in kiwi fruit gradually loses its inhibitory capacity upon HP processing in the pasteurisation range (500–800 MPa at 25 °C), as it does upon heating (55–95 °C at 0.1 MPa). However, a processing-resistant PME inhibitor fraction, representing at least 25% of the total PME inhibitor (depending on the pressure level), retains its activity even after prolonged treatment times. PME inhibitor shows pH-dependent inactivation characteristics, readily inactivating at pH 6.5 but strikingly baroresistant at low pH values (no inactivation at ca. pH 4). These findings indicate that PME inhibitor (at least partly) survives an HP treatment and remains available for inhibition of residual, undesirable PME activity (Jolie, Duvetter, Houben et al., 2009; Jolie, Duvetter, Van Loey et al., 2010).

Fig. 2. *In situ* PME activity in shredded carrots (in µg MeOH/g carrot) as a function of temperature and pressure (Sila, Smout et al., 2007).

When subjecting a mixture of PME and PMEI to an HP treatment, dissociation of the enzyme-inhibitor complexes has been demonstrated to take place (i.e., carrot PME and kiwi PMEI) (Jolie, Duvetter, Houben *et al.*, 2009; Jolie, Duvetter, Verlinde *et al.*, 2009). Dissociation appears to be followed by gradual HP-induced inactivation of PME and PMEI separately (the latter in a pH-dependent fashion, as described above) and, upon decompression, reunion of the non-inactivated interaction partners. Consequently, in particular cases where a gradual loss of PME activity is combined with a (at least partial) retention of the PMEI (e.g., carrot PME and 800 MPa), HP treatment of an equimolar PME-PMEI mixture can result in an excess amount of PMEI (and no “free” PME) after processing (Jolie, Duvetter, Verlinde *et al.*, 2009). The same effect cannot be generated by thermal processing, since heating does not dissociate the PME-PMEI complex but rather denatures the complex as a single entity (Jolie, Duvetter, Houben *et al.*, 2009; Jolie, Duvetter, Verlinde *et al.*, 2009).

PG stability

Research on the HP stability of PG has so far been restricted to tomato fruits. The results reveal that PG, contrary to PME, is rather pressure-labile. Pressure levels around 500 MPa suffice for irreversible enzyme inactivation in tomato pieces, juice and purified PG solutions, even without the need for additional heating. Yet, when the HP treatment is combined with elevated temperatures, both factors act synergistically (Crelie *et al.*, 2001; Fachin *et al.*, 2003, 2004; Hsu, 2008; Peeters, Fachin, Smout, Van Loey, & Hendrickx, 2004; Rodrigo *et al.*, 2006; Shook, Shellhammer, & Schwartz, 2001; Tangwongchai, Ledward, & Ames, 2000). The large difference in processing stability between PG1 and PG2 isoforms, as noticed upon heating, does not exist upon pressurisation. Inactivation behaviours of PG1 and PG2 are equal, as illustrated in Fig. 3. Presumably, an HP-induced dissociation of PG1 into the catalytic PG polypeptide (i.e., PG2) and the β -subunit protein takes place, followed by separate action of HP on both interaction

partners. The β -subunit itself displays extreme processing stability (Fachin *et al.*, 2004; Peeters *et al.*, 2004).

Given the pronounced distinction in pressure stability of (tomato) PME and PG, suitable pressure-temperature conditions (e.g., 500 MPa, 25 °C, 15 min) exist where PG is inactivated without decrease in PME activity (the demethoxylation reaction may even be accelerated) (Fig. 3). This selective knockout of PG by processing is not achievable by thermal processing (Crelie *et al.*, 2001; Fachin *et al.*, 2003; Rodrigo *et al.*, 2006).

PG catalytic activity

Contrary to the HP-induced stimulation of PME, the catalytic activity of PG (purified from tomato) on both polygalacturonic acid and demethoxylated pectin decreases with increasing pressure (positive values for the activation volume) until the pressure level for irreversible inactivation is reached. This retarding effect is most pronounced at elevated temperatures (i.e., higher absolute values for the activation volume). The optimal temperature for PG-catalysed depolymerisation shifts to lower values at elevated pressures as compared to atmospheric pressure (Verlent, Smout, Duvetter, Hendrickx, & Van Loey, 2005; Verlent, Van Loey *et al.*, 2004).

In presence of PME, the picture is somewhat different. Since PG activity is enhanced with decreasing DM, PME activity indirectly affects the PG action through formation of a better substrate. Consequently, HP-induced activation of PME in the low-pressure range is accompanied by an increase in the PG reaction rate up to an optimal pressure level (Verlent, Hendrickx, Verbeyst, & Van Loey, 2007).

Non-enzymatic demethoxylation

Chemical demethoxylation of pectin through saponification is not only accelerated by temperature (according to the Arrhenius equation) but also by elevated pressure (Fig. 4A). As for the PME-catalysed demethoxylation, a possible explanation can be formulated based on Le Châtelier's principle, namely the volume reduction owing to the hydration of charged carboxylic acid groups upon hydrolysis of the methyl-esters. Obviously, a mild-acidic to alkaline pH environment (approx. pH $\geq$ 5) is imperative and the actual pH strongly determines the reaction rate (De Roeck *et al.*, 2009; Verlent, Van Loey *et al.*, 2004; Verlent *et al.*, 2004).

β -Elimination

At ambient temperatures, HP is not capable of inducing β -eliminative cleavage of pectin chains (Kato, Teramoto, & Fuchigami, 1997). However, when HP is combined with high temperatures, the pressure component does exert a marked influence on the β -elimination reaction. Contrary to its stimulatory effect on chemical demethoxylation, HP slows down or even stops β -elimination in treated pectin solutions (500–700 MPa, 90–115 °C) (Fig. 4B). The exact cause of the HP-induced inhibition of β -elimination, being a direct pressure effect, an indirect effect of the pressure treatment

Fig. 3. Pressure stability of purified tomato PG1 (■), PG2 (▲) and PME (◆) after 15 min at preset pressure levels and 25 °C (Rodrigo *et al.*, 2006).

Fig. 4. Demethoxylation and β -elimination under high pressure: (A) formation of methanol and (B) formation of unsaturated uronides as a function of treatment time when incubating apple pectin dissolved in 0.1 M MES pH 6.5 at 110 °C and 0.1 MPa (♦), 500 MPa (■), 600 MPa (▲) and 700 MPa (+) (De Roeck *et al.*, 2009).

(e.g., shift in the pectin's DM or the system's pH), or a combination of both, is not yet elucidated (De Roeck *et al.*, 2009).

Food-technological relevance of pectin conversions under high pressure

Considering the role of pectin conversions in determining the structure-related quality characteristics of many plant-based foods (texture, rheology, cloud stability) and the way these conversions are affected, to a certain extent and in a certain direction, by HP, it is obvious that the use of HPP in the production of fruit- and vegetable-derived food systems will automatically have implications for their structural quality. Besides, the additional pressure variable opens perspectives to specifically steer the conversions to create functionalities which are beyond reach of conventional thermal processing. In the various application domains where HPP is currently being used and/or investigated, namely HP as pre-processing step (moderate pressure and moderate temperature), HP for food pasteurisation purposes (high pressure and moderate temperature) and HP for food sterilisation purposes (high pressure and high temperature), data have been gathered and insight has been growing over the years.

High pressure as a pre-processing tool

In the moderate-pressure/moderate-temperature domain (limits around 500 MPa and 60 °C), the main pectin

conversion reaction that can be exploited is the enzymatic demethoxylation of HG by PME, which is accelerated under these conditions. Enhancing PME activity at selected T,p conditions leads to reduced DM values of pectin *in planta*. The concomitant, increased formation of ionically cross-linked pectin complexes then results in cell wall reinforcement. Since the availability of divalent ions is critical, combining PME stimulation with Ca^{2+} impregnation (through soaking or vacuum infusion; prior to, during or after the HP treatment) further enhances the positive texture effects. Moreover, pressure-induced permeation of the plant tissue promotes ion influx.

An HP pretreatment (with or without moderate heating) can be executed, as alternative to low-temperature blanching, to diminish thermo-softening of plant-based foods (e.g., carrot, bell pepper, broccoli) during subsequent thermal (HT) (Castro, Van Loey, Saraiva, Smout, & Hendrickx, 2007; Christiaens, Van Buggenhout, Houben *et al.*, 2011; De Roeck *et al.*, 2010; De Roeck, Mols, Duvetter, Van Loey, & Hendrickx, 2010; Kasai, Hatae, Shimada, & Iibuchi, 1995; Nguyen *et al.*, 2010; Sila, Yue *et al.*, 2007; Sila, Smout, Vu, & Hendrickx, 2004) or subsequent HT/HP processing (De Roeck, Mols, Duvetter *et al.*, 2010; Rastogi, Nguyen, & Balasubramaniam, 2008; Rastogi, Nguyen, Jiang, & Balasubramaniam, 2010), as illustrated for carrot pieces in Fig. 5A and Fig. 5B respectively. Besides salt bridge formation, an additional texture-preserving effect arises from the reduced rate and extent of β -eliminative pectin degradation and solubilisation during the heating step at lowered DM (De Roeck *et al.*, 2010).

Moreover, stimulating endogenous PME activity by means of an HP pretreatment (combined with a Ca^{2+} soak) has also shown potential in controlling the textural quality of frozen fruits and vegetables (case-studies on carrot and green pepper) (see, for example, Fig. 6), although the success depends on the freezing conditions. A controlled freezing operation that assures a high degree of supercooling and concomitant formation of many small ice crystals throughout the whole sample depth (e.g., HP-shift freezing) has proven to be a necessary factor (Castro *et al.*, 2007; Van Buggenhout *et al.*, 2006).

However, enhanced reduction of the pectin's DM by PME action can sometimes be disadvantageous. Juice extraction from HP-pretreated berries (15 min at 400 MPa and 35 °C) is hampered by the formation of a highly viscous pectin gel, reducing juice yield and phenolic compound extractability. Addition of commercial cell-wall-degrading enzyme mixtures, possibly stimulated by HPP, can overcome this problem (Hilz, Lille, Poutanen, Schols, & Voragen, 2006).

High-pressure processing at ambient temperatures (pasteurisation domain)

Of all studies about the effects of HP on pectin conversions, the highest number has been published in the context of juice cloud stabilisation, testing HP processing as

Fig. 5. Effect of selected pretreatments on the texture degradation of carrots during (A) a subsequent thermal (100 °C, 0.1 MPa) and (B) a subsequent HP/HT (100 °C, 600 MPa) treatment, modelled using a fractional-conversion model (De Roeck, Mols, Duvetter et al., 2010). The four different sequences pretreatment – treatment depicted are: (i) no pretreatment with the carrots processed in water (◆), (ii) no pretreatment with the carrots processed in a 1% (w/v) calcium chloride solution (■), (iii) HP pretreatment (15 min at 400 MPa and 60 °C) followed by treatment in H₂O (▲), and (iv) HP pretreatment with a subsequent calcium soak, followed by treatment in a calcium chloride solution (●). “Holding time t_2 ” refers to the time at isothermal-isobaric conditions.

an alternative to conventional thermal pasteurisation for elimination of PME. In summary, HPP at moderate temperatures can result in quick irreversible PME inactivation for certain PME sources (e.g., carrot, specific isoforms of

Fig. 6. Influence of pretreatments on the hardness (bars) and tissue damage (◆) of high-pressure shift frozen carrots: (1) no pretreatment, (2) calcium (0.5% CaCl₂) soaking and thermal pretreatment (30 min at 60 °C) and (3) calcium (0.5% CaCl₂) soaking and HP pretreatment (15 min at 300 MPa and 60 °C) (Van Buggenhout et al., 2006).

citrus), but fails as a useful alternative to thermal inactivation for some other (e.g., tomato) (Duvetter et al., 2009), except for conditions of HP sterilisation (Krebbes et al., 2003). Particularly important from a practical point of view is the observation that the thermostable orange PME isoform is also pressure-resistant, rendering a complete abolishing of PME activity within pasteurisation conditions impossible (Goodner et al., 1998; Van den Broeck et al., 2000b; Vervoort et al., in press). Nevertheless, cloud-stable orange juices could successfully be produced by HP treatment (Goodner et al., 1998; Nienaber & Shellhammer, 2001a). An additional dimension in the issue of juice cloud stabilisation with HPP is constituted by the remarkable pressure stability of the kiwi PMEI, especially at acidic pH, endorsing the principle of pre-HPP addition of PMEI to (high-acid) food systems for inhibition of residual undesired PME (e.g., fruit juices).

HPP at ambient temperatures also allows for PG inactivation, even at markedly lower pressure levels compared to PME inactivation (Fig. 3). Selective knockout of PG (eventually in a specific HP pretreatment step) offers valuable possibilities with regard to texture and viscosity engineering of tomato-based products. The remaining PME activity can indeed result in a strengthened pectin network through ionic cross-linking and, if relevant, reduced β -elimination, while inactivation of PG prevents enzymatic pectin fragmentation. An increased texture or viscosity can hence be obtained (Fig. 7), although some other rheological quality defects (e.g., syneresis, formation of a jelly-like translucent structure) may appear depending on the treatment temperature (Krebbes et al., 2003; Porretta, Birzi, Ghizzoni, & Vicini, 1995; Verlent, Hendrickx, Rovere, Moldenaers, & Van Loey, 2006).

Fig. 7. Comparison of the index of consistency, measured with the Bostwick consistometer, of tomato purée obtained from (A) untreated tomato pieces and (B) tomato pieces treated at 550 MPa and 25 °C for 10 min.

As a result of the inability of HP to induce β -elimination (at ambient T), HPP of fruits and vegetables is not accompanied by pronounced pectin depolymerisation and solubilisation, which are considered the main causes of thermal softening. The initial loss in firmness upon pressurisation, nonetheless observed for many fruits and vegetables (often referred to as “instantaneous pressure softening”), is mainly attributed to membrane rupture and concomitant turgidity loss, a direct result of the applied hydraulic pressure pulse (Araya *et al.*, 2007; Basak & Ramaswamy, 1998; Gonzalez, Jernstedt, Slaughter, & Barrett, 2010; Kato *et al.*, 1997; Knockaert *et al.*, 2011; Krebbers, Matser, Koets, & van den Berg, 2002). Generally, the outcome of HPP (at low T) with regard to texture strongly depends on the plant type and its morphological and structural features, ranging from nearly-fresh textures for plants with a compact, firm structure (e.g., cauliflower, carrot) (Araya *et al.*, 2007; Kato *et al.*, 1997; Prestamo & Arroyo, 1998) to structural collapse for highly porous fruits (e.g., strawberry) (Duvetter *et al.*, 2005).

In addition to the absence of β -elimination, activation (and incomplete inactivation) of PME during HPP at ambient temperatures, and the allied reduction of pectin's DM and increased susceptibility for network formation, have been pointed to as an explanation for firmness retention (or even firmness gain) of fruits and vegetables during pressurisation (Araya *et al.*, 2007; Basak & Ramaswamy, 1998; Gonzalez *et al.*, 2010; Kato *et al.*, 1997). Accelerated PME-

catalysed demethoxylation upon pressurisation can also explain the strong reduction in pectin's DM of strawberries when HP-processed after infusion of exogenous PME and Ca^{2+} (in comparison with non-infused samples or HT-treated strawberries). Hence, HP is able to magnify the effect of PME infusion. As a result, a firmness improvement of infused HP-treated strawberries is observed, unless the HP treatment is performed at elevated temperatures (Fig. 8) (Duvetter *et al.*, 2005; Fraeye *et al.*, 2010).

High-pressure processing at high temperatures (sterilisation domain)

The inhibitory effect of HP on the β -elimination reaction observed in pectin solutions at HT/HP conditions can be expected to have major implications for the textural quality of HT/HP-processed fruits and vegetables. Indeed, unlike thermally treated carrots, HT/HP-treated carrots (80 °C, 600 MPa) show minimal softening (except for a rapid initial hardness loss) and negligible changes in intercellular adhesion, attributable to limited occurrence of β -elimination in addition to increased cross-linking of the demethoxylated pectin polymers (De Roeck, Sila, Duvetter, Van Loey, & Hendrickx, 2008). Also at harsher processing conditions (95–121 °C, 500–700 MPa), HT/HP treatments result in a manifold slower, yet significant, texture degradation compared to thermal treatment (e.g., around 10-fold slower at 600 MPa) (De Roeck, Mols, Duvetter *et al.*, 2010; Nguyen, Rastogi, & Balasubramaniam, 2007) (Fig. 9).

Fig. 8. Effect of thermal (70 °C, 0.1 MPa), high pressure (25 °C, 550 MPa) and combined thermal/high pressure (70 °C, 550 MPa) processing on the firmness of non-infused (■) and Ca^{2+} /PME-infused (▒) strawberries and on the degree of methoxylation of the cell wall material (i.e., alcohol-insoluble residue) isolated from the strawberries (◆). Below, corresponding representative micrographs for the eight strawberry samples are depicted (adapted from Fraeye *et al.*, 2010).

Fig. 9. Texture degradation of carrots during thermal versus HP/HT treatments at various temperature and pressure levels, modelled using a fractional-conversion model (De Roeck, Mols, Duvetter et al., 2010). "Holding time t_2 " refers to the time at isothermal-isobaric conditions.

Since at all temperatures the reduced texture loss is accompanied by a markedly lower DM of the cell wall pectin, the explanation of a reduced DM retarding β -elimination under HP seems very likely, although it is not sure whether this is the sole mechanism (De Roeck, Mols, Duvetter et al., 2010). This reduction in DM is likely brought about by the combination of chemical and PME-catalysed demethoxylation during the HT/HP process. The respective contributions will however depend on the T, p and pH domains under consideration, the purely chemical component being mainly significant in the HT/HP and non-acidic (approx. pH > 6) region. For this type of processing, the "preprocess", passed through to reach the final HT/HP conditions, can be regarded as a DM-reducing HP pretreatment implicitly present. Hence, from a textural point of view, combined HT/HP processing (in an aqueous brine of dissolved Ca^{2+}) excludes the need for a separate pretreatment step (as is the case for regular thermal sterilisation) (see also Fig. 5B), allowing cost reduction and time savings (De Roeck, Mols, Duvetter et al., 2010).

Besides a probably decelerated β -elimination reaction, the shorter processing times in which thermal β -eliminative softening can occur in the case of HP sterilisation processes, compared to equivalent HT sterilisation, can obviously contribute to an enhanced texture of the final product (Knockaert et al., 2011; Krebbers et al., 2002; Leadley, Tucker, & Fryer, 2008).

Conclusions and outlook

HP exerts a marked effect on both enzymatic and non-enzymatic pectin conversions. According to fundamental scientific principles, chemical reactions can be accelerated or decelerated (e.g., demethoxylation and β -elimination), pectic enzymes can be stimulated, retarded, inactivated or stabilised (e.g., PME and PG), and enzyme-inhibitor and enzyme-subunit complexes can be dissociated (e.g., PME-

PMEI and PG2- β -subunit). The actual direction in which the pectin conversions are driven strongly depends on the T, p window applied. Since the HP-induced changes at the molecular level are directly or indirectly related to and reflected in the final structure-related quality of plant-based food products in terms of texture, viscosity, consistency and cloud stability, HPP offers unique possibilities (compared to HT processing) for structure engineering of these foods through deliberate tailoring of the allied pectin conversions. Although already substantial and still expanding, the current knowledge on pectin conversions under HP in model systems and real food matrices is yet incomplete. Some fields of potential progress can be identified.

To the present day, processing-related pectin research has mainly been focussing on the linear HG chains of pectin. Notwithstanding the belief that many structure-related quality attributes of plant-based foods are indeed governed to a great extent by HG modifications, important contributions of pectin's side-chain regions cannot be excluded and should be examined. Previously unknown routes for structure engineering may hence be uncovered.

Moreover, pectin characterisation has so far been restricted to *in vitro* analyses on isolated pectins. *In situ* analysis of processing-induced pectin structural changes and residual enzyme activity by means of antibodies against specific pectin epitopes or enzyme-directed probes, recently being explored (Christiaens, Van Buggenhout, Houben et al., 2011; Jolie et al., 2010; Vandevenne et al., 2011), will certainly broaden and deepen the current understanding of the structure-function relationship of pectin as affected by HP.

In addition, no studies have been performed on the effects of HP on pectin-directed enzymes other than PME and PG. To fully command pectin conversions by use of HPP, today's knowledge should be expanded to the stability and catalytic activity of many more pectinases, such as PL,

PNL and hairy-region-degrading enzymes (e.g., β -galactosidase, α -arabinofuranosidase), from a wide spectrum of plants. The use of enzyme inhibitors as processing aid for controlling residual enzymatic activity upon HPP should be further investigated.

The potential of HP technology as a texture-protecting pre-processing technique or as a less texture-degrading alternative for conventional thermal pasteurisation processes has extensively been documented. In recent years, research has been extended to the HP sterilisation domain, where the interplay of the two intervening factors HT and HP takes place, indicating retarded but significant pectin/texture degradation. In this area, however, there still is room for product-specific process optimisation.

Nevertheless, process optimisation in terms of structure-related quality is just one side of the picture, while nutritional quality is another (taking food safety for granted). As an important exponent of nutritional quality, bio-accessibility of micronutrients (such as carotenoids) for human uptake often requires a certain degree of disintegration of the plant matrix, which can be brought about by food processing. A recent study has indicated that textural differences between HT- and HP-treated products are accompanied by significant differences in micronutrient bio-accessibility, suggesting a possible conflict (Knockaert et al., 2011). Consequently, the quest towards optimal texture retention through HPP may possibly counteract the nutritional quality of fruit- and vegetable-based products. Equilibrating this balance constitutes a major future challenge for food technologists.

Acknowledgements

The authors gratefully acknowledge financial support from K.U. Leuven Research Fund (postdoctoral grant for RPJ) and Industrial Research Fund (KP/08/004), Research Foundation Flanders (FWO grants for SC, IF and SVB), the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT grant for KH) and the Commission of the European Communities (Framework 6, Priority 5 'Food Quality and Safety', Integrated Project NovelQ FP6-CT-2006-015710).

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