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Towards a better understanding of the pectin structure–function relationship in broccoli during processing: Part I—macroscopic and molecular analyses

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ABSTRACT

To investigate the structure–function relationship of pectin during (pre)processing, broccoli samples (*Brassica oleracea* L. cultivar italica) were subjected to one of the following pretreatments: (i) low-temperature blanching (LTB), (ii) LTB in combination with Ca^{2+} infusion, (iii) high-pressure pretreatment (HP), (iv) HP in combination with Ca^{2+} infusion, or (v) no pretreatment (control sample), whether or not in combination with a thermal treatment of 15 min at 90 °C. The macroscopic attributes of broccoli were linked to the chemical structure of broccoli pectin. By enhancing the cross-linking of pectic polymers, both LTB and HP reduced the texture loss that occurred during thermal processing of broccoli. During these pretreatments, homogalacturonan was de-esterified by pectin methylesterase, which led to changes in pectin solubility. When LTB or HP was combined with Ca^{2+} infusion, changes in the structure of pectin occurred, however not always reflected at the macroscopic level. The degree of esterification of pectin in Ca^{2+}-soaked broccoli samples was lower compared to non-Ca^{2+}-soaked samples and, in addition, a higher amount of ionically cross-linked pectin was retrieved.

1. Introduction

Tailoring the textural properties of plant-based food products requires in-depth insight into the structure–function relationship of pectin. Pectin has been identified as a critical structure component of plant cell walls and is predominantly present in the middle lamella where it provides cell–cell adhesion and mechanical strength (Van Buren, 1979). The exact macromolecular structure of this complex and diverse polysaccharide is still under debate (Coenen, Bakx, Verhoef, Schols, & Voragen, 2007; Ridley, O'Neill, & Mohnen, 2001; Vincken et al., 2003). One of the most abundant polymers in pectin is homogalacturonan (HG), a linear chain of galacturonic acid (GalA) residues in which some of the C-6 carboxyl groups are methyl-esterified (Willats, McCartney, Mackie, & Knox, 2001). The degree of esterification (DE) of HG is an important factor for the pectin functionality in fruit and vegetable products. Besides HG, two branched domains, known as rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II), can also be distinguished in pectin.

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 the depolymerisation and solubilisation of pectic polymers involved in cell–cell adhesion (Van Buggenhout, Sila, Duvetter, Van Loey, & Hendrickx, 2009; Waldron, Parker, & Smith, 2003). In low-acid plant tissues, pectin depolymerisation occurs through a β-elimination reaction and is favoured by hydroxyl ions and methyl-esterified GalA residues (Sajjaanantakul, Van Buren, & Downing, 1989; Van Buren, 1979).

Food scientists seek to counteract the texture damage of heat-treated fruits, vegetables and derived food products by the use of different preprocessing techniques (Van Buggenhout et al., 2009; Waldron, 2004). Low-temperature blanching, typically 15 to 45 min at 50 to 60 °C, has been successfully used for firming many fruits and vegetables (Ng & Waldron, 1997; Ni, Lin, & Barrett, 2005; Sila, Smout, Vu, Van Loey, & Hendrickx, 2005). During blanching, the catalytic activity of cell-wall-bound pectin methylesterase (PME) is enhanced, resulting in the de-esterification of pectic polysaccharides. The increase in free pectic carboxyl groups provides a greater opportunity

Abbreviations: AIR, alcohol-insoluble residue; Ara, arabinose; CSP, chelator-soluble pectin; DE, degree of esterification; Gal, galactose; GalA, galacturonic acid; HG, homogalacturonan; HP, high-pressure pretreatment; HPAEC, high-performance anion exchange chromatography; HPSEC, high-performance size exclusion chromatography; LTB, low-temperature blanching; MM, molar mass; NSP, sodium-carbonate-soluble pectin; PME, pectin methylesterase; RG-I, rhamnogalacturonan-I; RG-II, rhamnogalacturonan-II; Rha, rhamnose; T, thermal treatment; U, unit; WSP, water-soluble pectin.

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for pectic polymers to be cross-linked with divalent ions such as Ca^{2+}, leading to an increased intercellular adhesion. The availability of divalent ions is critical, hence, blanching is often combined with Ca^{2+} infusion (Van Buggenhout et al., 2009). The reduction in DE has an additional positive effect: the rate of β-eliminative pectin degradation at high temperatures, occurring during the thermal processing of fruits and vegetables, is significantly reduced (Keijbets & Pilnik, 1974). Next to these more 'conventional' techniques, food technologists discovered the use of novel and less degradative technologies like high pressure processing to obtain plant-based foods with a high textural quality (De Roeck, Sila, Duvetter, Van Loey, & Hendrickx, 2008; Ludikhuyze, Van Loey, Indrawati, & Hendrickx, 2001; Sila, Smout, Vu, & Hendrickx, 2004).

To further increase our understanding of the perceived textural properties, a profound insight into the structure–function relationship of pectin is important. The intimate relation between pectin's fine structure and textural attributes has already been investigated for vegetables such as carrots and beans (Ng & Waldron, 1997; Sila, Smout, Elliot, Van Loey, & Hendrickx, 2006; Stanley, Bourne, Stone, & Wismer, 1995) and fruits like strawberries (Fraeye et al., 2009). However, for broccoli, a vegetable that is frequently studied in the context of health-related nutrients like vitamin C (Munyaka, Oey, Van Loey, & Hendrickx, 2009), folates (Munyaka, Oey, Verlinde, Van Loey, & Hendrickx, 2009) and glucosinolates (Shapiro, Fahey, Wade, Stephenson, & Talalay, 2001), research towards its textural quality as influenced by processing is lacking. To study this pectin structure–function relationship, a selective extraction of pectic fractions from cell wall material is commonly performed (Brett & Waldron, 1996; Selvendran & O'Neill, 1987). An enormous amount of information on the chemical structure of pectin can be obtained using this approach. However, a major restriction is that a certain number of bonds must be broken in order to extract the components from the cell wall, distorting the overall view of the cell wall architecture (Brett & Waldron, 1996).

The objective of the current study was to gain insight into the structure–function relationship of pectin. Therefore, a case study on broccoli (*Brassica oleracea* L. cultivar italica) was performed. The influence of a thermal or a high pressure pretreatment, whether or not in combination with Ca^{2+} infusion, on the textural quality of thermally processed broccoli was explored. In this part of the study, the macroscopic attributes of broccoli were linked to the chemical structure of broccoli pectin. In the second part of the study, this information is complemented by pectin analysis using anti-HG antibodies (see complementary publication by Christiaens et al.), entailing in situ (microscopy) and ex situ (immuno-dot assays) analyses. This approach has only recently been introduced to study process-induced changes in pectin's structure in fruits and vegetables (Christiaens et al., 2011) and is one of the few techniques available for analysis of the intact cell wall matrix. In combination with results from the current part of the study, it provides a detailed image of changes in the HG component of pectin during (pre)processing of plant tissues, which improves our understanding of the pectin structure–function relationship.

2. Materials and methods

A schematic overview of the experimental set-up is presented in Fig. 1.

2.1. Plant material

Broccoli was obtained from a local shop and stored at 4 °C for a maximum period of five days before use. Prior to processing, small broccoli stems were collected just underneath the florets and 20 cylinders (8 mm in diameter and 10 mm in height) were excised from the secondary stems (Fig. 2). For each sample, material was collected from at least four different broccoli tissues, and care was taken to obtain a maximal randomisation of centre and side stems. The small broccoli stems were used for chemical and microscopic analysis (see complementary publication by Christiaens et al.), whereas the cylinders were used in texture measurement.

2.2. Pretreatments

Broccoli stems and cylinders were subjected to five different pretreatment conditions selected on the basis of results obtained in previous studies (Ni et al., 2005; Sila et al., 2005) and some preliminary experiments (data not shown). These pretreatment conditions included low-temperature blanching (LTB = 30 min, 60 °C), LTB followed by 1% (w/v) calcium chloride soaking (LTB + Ca), high-pressure pretreatment (HP = 30 min, 60 °C, 400 MPa), HP followed by 1% (w/v) calcium chloride soaking (HP + Ca), and a control sample (non-pretreated sample).

2.2.1. Low-temperature blanching

Broccoli samples were vacuum-packed in a polyethylene bag followed by a thermal treatment of 30 min at 60 °C in a temperature-controlled

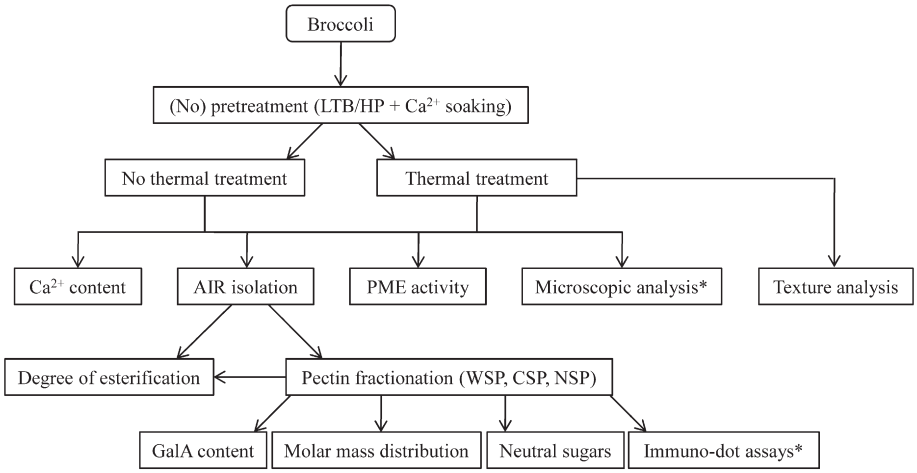

Fig. 1. Schematic overview of the experimental set-up (LTB = low-temperature blanching, HP = high-pressure pretreatment, AIR = alcohol-insoluble residue, PME = pectin methylesterase, WSP = water-soluble pectin, CSP = chelator-soluble pectin, NSP = sodium-carbonate-soluble pectin, GalA = galacturonic acid, * = experiments and results discussed in part II).