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Microstructural and morphological behaviors of asparagus lettuce cells subject to high pressure processing

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ABSTRACT

To explore the mechanisms of texture change under high pressure (HP) processing, the effects of different pressures on cell microstructure and cell morphological behaviors of asparagus lettuce were investigated using different microscopy approaches. Pectin immunofluorescence labeling was adopted to analyze cell morphological change *in vivo* by *in situ* visualization of cell wall. Image analysis showed that cells in asparagus lettuce treated at lower pressures (100 MPa, 200 MPa and 300 MPa) exhibited rounder and smaller than the untreated samples. However, the shapes of cells in asparagus lettuce treated at 500 MPa were close to the control. The *in situ* analysis on pectin distribution by CLSM indicated that pectin was mainly restricted in the corners of tricellular junctions in the samples treated at 200 MPa and 300 MPa, whereas pectin uniformly distributed around the whole cell wall at 500 MPa. The results of cell activity staining were consistent with that of ultrastructure change of cell wall and membrane observed by TEM, which suggested that plasma membrane was ruptured at the pressure of 200 MPa, 300 MPa and 500 MPa. Meanwhile, the integrity of cell wall structure was retained under all HP conditions. Based on the above results, a 7-cell tissue model has been proposed to show the cell morphological behavior. On the one hand, moderate HP (100 MPa–300 MPa) caused an initial texture loss of asparagus lettuce tissue, probably due to the loss of turgor pressure and the loose skeleton of cell wall. On the other hand, HP (500 MPa) caused less apparent texture loss of asparagus lettuce due to the unchanged pectin distribution, which can maintain the rigid and flexible cell wall.

1. Introduction

Texture is one of the most important quality characteristics of fruits and vegetables. It is a major factor affecting sensory perception and consumer acceptance of foods (Oey, Lille, Van Loey, & Hendrickx, 2008; Van Buggenhout, Sila, Duvetter, Van Loey, & Hendrickx, 2009). Hardness or firmness is the most common quality indicator used in evaluating the texture of vegetables (stems, roots/tubers and leaves). Firmness is largely determined by the tissue structure, particularly by cell size, shape and packing, cell wall thickness and strength, and the extent of cell-to-cell adhesion, together with turgor status (Toivonen & Brummell, 2008). Moreover, it can be affected by the interaction of these factors. Processing could alter the initial mechanical properties of fruits and vegetables, leading to texture changes of the resulting products. For example, thermal processing causes a pronounced degradation of the pectic polysaccharides, resulting in the loss of intercellular adhesion and consequently, in softening which is not desired by the consumer (Bordoloi, Kaur, & Singh, 2012; De Roeck, Sila, Duvetter, Van Loey, & Hendrickx, 2008; Sila, Doungla, Smout, Van Loey, & Hendrickx, 2006). Therefore, to preserve a desirable texture and minimize other organoleptic and nutritional quality losses of fruit and vegetable products, there is a need to develop novel processing technologies.

High pressure (HP) processing has already become a commercially implemented technology which could preserve nutritional value and the sensory properties of fruits and vegetables (Basak & Ramaswamy, 1998; Oey et al., 2008; Sila et al., 2008; Van Buggenhout et al., 2009). However, some inevitable disadvantages after HP processing have been observed. Marigheto, Vial, Wright, and Hills (2004) found that rupture membranes of strawberry cells were shown at more than 100 MPa and cell wall damage was apparent at 300 MPa. Basak and Ramaswamy (1998) reported that HP showed a dual effect on the selected fruit and vegetable textures at 100 MPa to 400 MPa for 5 min to 60 min. First, initial loss in texture was ascribed to the expeditious effects of pressure, also called instantaneous pressure softening (IPS). The second and subsequent effect was described as gradual texture

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recovery with holding time. Tangwongchai, Ledward, and Ames (2000) found that the textural damage of whole cherry tomatoes increased with increasing pressures (0–400 MPa), the tomatoes appeared softer with some evidence of free water. However, the texture of tomatoes treated at 500 MPa and 600 MPa appeared more like the untreated samples.

This phenomenon could be attributed to the changes in the cell wall components, particularly pectins and divalent cations during HP treatment. In general, pectins can undergo enzymatic and non-enzymatic conversion reactions during processing (De Roeck, Mols, Duvetter, Van Loey, & Hendrickx, 2010; Sila, Smout, Elliot, Loey, & Hendrickx, 2006). HP processing facilitates the occurrence of conversions due to the increase of cell membrane permeability and cell disruption. Therefore, enzymes, substrates and cations are liberated from different compartments in the cells and interact with each other during HP treatment. HP treatment might be able to enhance the enzyme activity of PME or reduce the enzyme activity of PG (Krebbers et al., 2003; Oey et al., 2008). On the one hand, PME catalyzes the demethoxylation of pectin resulting in the formation of carboxylated pectin with release of methanol. Meanwhile, the de-esterified pectin (low-methoxy-pectin) is capable of forming a gel-network with divalent cations resulting in increased hardness (De Roeck et al., 2009; Jolie et al., 2012). On the other hand, PG is able to depolymerize pectin that has been demethylated by PME, leading to softening of fruits and vegetables. However, the exact mechanisms behind texture loss and or preservation under HP treatment are not known and further research is needed in this area.

The effect of pressure on texture changes is not only attributed to different (bio-)chemical conversions of pectin, but also to the role of mechanical compression of HP on plant tissues. Due to the isotropic homogeneity of mechanical stress, pressurization is accompanied by a simultaneous decrease in volume. In this situation, plant tissues suffer structural modifications favoring a more compact form. Therefore, morphological changes might occur initially in plant tissues subjected to HP. In the traditional theory, cell morphology, namely size and shape, cell wall thickness, as well as cell arrangement are controlled by turgor pressure and mechanical properties of cell walls (Guerriero, Hausman, & Cai, 2014). Therefore, morphological behavior of plant tissues and cells could be an interesting subject to be addressed.

Asparagus lettuce (*Lactuca sativa* var. *asparagine*) is a cultivar of lettuce grown primarily for its thick stem. Most people in China eat the fleshy stem by stir-frying and prefer the crispy texture (Mou, 2008; Wang et al., 2012). The aim of this study was to investigate HP-induced cell microstructural and morphological changes using different microscopy approaches and to explore the correlation between the mechanism of texture changes and the pressure gradient.

2. Materials and methods

2.1. Plant material

Asparagus lettuces (*L. sativa* var. *asparagine*), the cultivar of white bark, were harvested in December from a local vegetable greenhouse in Beijing and stored at 4 °C (for a maximum period of 2 days) until further use. Asparagus lettuce cylinders (5 cm diameter and 5 cm height) of cortex material were obtained by using a stainless steel borer (1 sample/treatment). The cylinder samples were divided into two groups, respectively (Fig. S1). The samples in the first group were vacuum packed in the polyethylene bags. The samples in the second group were cut into free-hand sections (1 cm diameter) which excised transversely from the pith of the lettuces stem. Then sections were suspended in phosphate-buffered saline (140 mM NaCl, 2.7 mM KCl, 8.0 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) and vacuum packed in the polyethylene bags. After that, both groups were treated by HP. The samples in the first group were used to investigate the ultrastructure of asparagus lettuce cells by transmission electron microscopy (TEM). The sections in the second group were used for neutral red (NR) and trypan blue staining, immunolabeling and cell morphologic analysis.

2.2. High pressure processing

Samples were treated at the designed pressure and time in a hydrostatic pressurization unit (Baotou Kefa High Pressure Technology Co., Ltd, Baotou, China), with a capacity of 7 L and a maximum pressure of 700 MPa. The pressure-transmitting fluid was distilled water. The rate of pressure increase was 200 MPa/min and the decompression was essentially instantaneous (<3 s). The HP-treatment time reported in this study did not include the pressure rise and release phases. Samples were treated at 100, 200, 300 and 500 MPa for 5 min, respectively. The initial temperature in the processing vessel was nearly 20 °C, when 500 MPa pressure was applied to the samples, the temperature reached 35 °C, which was estimated as 3 °C/100 MPa, since there was no temperature monitoring and recording system. Each HP treatment was performed in triplicate. After HP treatment, all samples were cooled in an ice bath and immediately analyzed.

2.3. Neutral red (NR) and trypan blue staining

Free-hand sections were stained with NR or trypan blue to assay cell viability by light microscope (Microscope-59XA with a Smart V2500 camera; Shanghai Optical Instrument Corporation, Shanghai, China). For NR staining, free-hand sections were dipped in 0.04% (w/v) NR solution (0.2 M Mannitol/0.01 M Hepes buffer, pH 7.8) for a period of 2 h, after which they were rinsed again for 30 min in the 0.2 M Mannitol/0.01 M Hepes buffer solution. Specimens were mounted on a microscope slide with a drop of deionized water, covered with a cover slip and observed immediately (Ersus & Barrett, 2010). Trypan blue staining was performed as described by Heese et al. (2007). Free-hand sections were stained with lactophenol–trypan blue (Sigma, USA) solution (10 mL lactic acid, 10 mL glycerol, 10 g phenol, and 10 mg trypan blue, dissolved in 10 mL distilled water). Whole tissue was boiled for approximately 1 min in the staining solution and then decolorized in 15.1 M chloral hydrate (Sigma, USA). Stained tissues were mounted on a microscope slide with 60% (v/v) glycerol.

2.4. Immunolabeling of pectic epitope with LM19 and JIM5

Free-hand sections without prior fixation were used for immunolabeling. Pectic epitope of cell wall in these sections was immunolabeled with the LM19 and JIM5 monoclonal antibody according to the method of Christiaens et al. (2012). It was started with an incubation with LM19 and JIM5 (PlantProbes, Leeds, United Kingdom) diluted in phosphate-buffered saline (PBS) (140 mM NaCl, 2.7 mM KCl, 8.0 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) containing 3% (v/v) milk powder (MPBS) for 90 min at room temperature. After primary labeling, sections were washed with PBS for 15 min. For the visualization of LM19 and JIM5, secondary labeling with an anti-rat Ig antibody coupled to fluorescein isothiocyanate (FITC) (Sigma, USA) was used. The secondary antibody was diluted 1/20 in 3% MPBS. After a final washing step with PBS, sections were mounted on a microscope slide with an anti-fade agent (Beyotime Institute of Biotechnology, China) and analyzed using the confocal laser scanning microscope Leica TCS SP5 II (Leica, Germany). The fluorescence labeling from FITC was captured at a wavelength of 488 nm, which was generated using an argon laser. Emission bands of 510–540 nm were used for FITC signal detection. Each image presented is composed of XY sections. Each section is the average of three scans performed at the resolution of 1024 × 1024 pixels. For each (pre)treatment condition, six different Asparagus lettuce stems were examined. Immunolabeling of these samples was carried out at least in duplicate.