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High hydrostatic pressure induced physiological changes and physical damages in asparagus spears

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ABSTRACT

High hydrostatic pressure (HHP) has been applied as a postharvest treatment in fresh fruits and vegetables for certain purpose, e.g. as a phytosanitary approach to control quarantine insect pests. However, detailed information on HHP-induced physiological changes of plant tissues in a broad range of pressure is limited. In this context, asparagus spears were treated by HHP at mild (10–200 MPa) and high pressure (400–600 MPa) for 0.5 to 30 min. At pressure lower than 200 MPa, the CO_2 production rate of asparagus spears increased with raising pressure and extending treatment times, while no sign of respiration was found at treatment pressures higher than 400 MPa. A shift from aerobic respiration to fermentation was noticed in the sample treated at mild pressure, which might be attributed to the HPP-induced hypoxia stress, enzyme activation, and cell damage. Generally, HHP treatment resulted in a decreasing firmness; however, slight but substantial recovery in firmness was observed in the treatments at pressure higher than 100 MPa for 20 min, which might be caused by fortification of intercellular adhesion through formation of new ionic linkages in cell wall pectic polysaccharides. Overall, it was suggested that 200 MPa could be a threshold for causing instant lethal metabolic disorder and severe physical damage in asparagus spears.

1. Introduction

High hydrostatic pressure (HHP) is generally regarded as a non-thermal technology for food processing, which based on applying high hydrostatic pressure to materials by compressing the surrounding water and transmitting pressure throughout the product uniformly and rapidly (Yaldagard et al., 2008). It is well known that HHP can better preserve many phytonutrients and delicate sensory properties of fruits and vegetables compared with traditional thermal processing, due to the fact that covalent bonds are almost unaffected under pressure treatment (Oey et al., 2008). Although the introduction of HHP in food preservation starting in early 1990s can be considered the beginning of industrial HHP technology, the field of HHP has spawned several other interesting applications over the past few years (Aertsen et al., 2009; Oey et al., 2008).

Among these applications, the past decade has seen an increasing interest in exploring the potential of HHP as an effective phytosanitary or postharvest treatment to control quarantine insect pests in plant materials, including Mexican fruit fly (*Anastrepha ludens* L.) (Candelario-Rodríguez et al., 2014), codling moth (*Cydia pomonella* L.), western cherry fruit fly (*Rhagoletis indifferens* C.) (Neven et al., 2007), and West Indian fruit fly (*Anastrepha obliqua*) (Ramírez et al., 2012), etc. According to previous studies, eggs exhibit a higher resistance than larvae to pressure, and pressures from 75 to 175 MPa are required for killing both eggs and larvae (Ramírez et al., 2012; Neven et al., 2007; Velazquez et al., 2010). Unlike thermal treatments, HHP kills the eggs and larvae of flies in host plants without significantly affecting the color, flavor, and some other organoleptic properties, which in most cases are heat-sensitive. Meanwhile, several researchers have studied the physiological changes after exposing plant materials to high hydrostatic pressure. Candelario-Rodríguez et al. (2014) found that high pressure treatment at 50–90 MPa significantly affected the respiration rate and ethylene production of mango, resulting in changes in other variables during storage, such as total

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soluble solid and titratable acidity. Vargas-Ortiz et al. (2013) also reported that when pressure of 100 MPa or higher were applied, CO_2 and ethylene production of mango fruits were reduced. Kato et al. (2002) found that the application of pressures lower than 220 MPa increased membrane permeability and caused membrane disorders, affecting ATPase activity as well as gel formation in cell membranes, and led to membrane destruction due to protein unfolding. Dörnenburg and Knorr (1997) found that a HHP treatment at 150 MPa caused irreversible permeabilization of cell membranes and loss of compartmentalization in the cells. A study on tomato showed that a HHP treatment at 90 MPa resulted in destruction and death of tomato cells, but a treatment at 50 MPa was shown to induce stress responses in the cells (Schreck et al., 1996). Besides, study on microorganisms showed that *Lactobacillus rhamnosus* treated at 100–400 MPa were sub-lethally injured and lost their ability to grown on agar, but the metabolic activity remained at a certain level (Ananta et al., 2004). There studies suggested that high hydrostatic pressures at relatively low pressure domain are generally assumed to be nonlethal but exert adverse effects on the physiology of plants that are adapted to atmospheric pressure. However, detailed knowledge regarding different pressure domains and treatment times on the HHP-induced physiological changes and cell damages is still limited. In addition, if cell walls and membranes can be damaged at low pressure, then a wound response is expected to occur. Moreover, it is of great interest to understand what range of pressure is the threshold or lethal dose for terminating respiratory metabolism.

The objective of this study was to study the influence of high hydrostatic pressure treatment at a broad range of pressures (10–600 MPa) and treatment time (0.5–30 min) on the physiology of asparagus spears, and to evaluate the extent and consequences of the physical damages caused by pressure treatment.

2. Materials and methods

2.1. Plant materials

Fresh green asparagus (*Asparagus officinalis* L.) spears were harvested from a local farm in Beijing. The spears were cut at ground level in the morning, placed in ice and transported to the laboratory within 3 h on the day of harvest. To obtain a stable respiration rate, all the asparagus spears were allowed to stay at 20 °C and 85% relative humidity for 24 h for recovering from wound-induced respiration (Brash et al., 1995). Straight, undamaged spears, 10–12 mm in diameter and 20 cm in length with closed bracts were used. The mass of each asparagus spear was 15 ± 2 g.

2.2. High hydrostatic pressure (HHP) treatment

HHP processing was carried out by using a laboratory high pressure processor (HHP L3-600, Huatai Senmiao Engineering and Technique Co., Ltd, Tianjin, China) with a processing chamber volume of 2 L, and a water jacket for temperature control. In order to minimize contact with the pressurizing fluid, twenty asparagus spears were placed in a zip-lock polyethylene bags. Distilled water (100 mL) was used as pressurizing fluid. Before sealing, the air inside the bag was removed manually. The samples were treated at 10, 20, 50, 100, 200, 300, 400, and 600 MPa for 0.5, 1, 2.5, 5, 10, 20, and 30 min, respectively. Although pressure around 200 MPa is viable for killing most eggs and larvae, the pressure range of commercial applications (<600 MPa) was also considered; therefore, with the aim of finding the threshold of lethal damage for plant tissues, a broader range of pressures (10–600 MPa) was selected. In addition, the processing time of HHP should be short to be profitable (Candelario et al., 2010). Therefore, processing times of 0.5–30 min were selected in this study. The initial temperature of the samples was 20 °C, and the highest temperature (38 °C) was recorded at pressure of 600 MPa due to the adiabatic heating. The target pressure was reached at pressurization rate of 4.2 MPa s^{-1}. The time for pressure releasing was always less than 10 s. After the treatment, the asparagus spears were took out from the bag, drained and equilibrated at room temperature (20 °C) for 2 h before analysis. HHP treatment was performed in triplicates.

2.3. Respiration rate, acetaldehyde and ethanol analysis

To determine the respiration rate, three replicates, each consisting of 6 asparagus spears, were weighted and introduced into 1020 mL sealed glass jars. The jar was placed at 20 °C for 30 min. Gas samples (1.0 mL) from the headspace were withdrawn by using a syringe though a rubber septum glued on the lid of the jar. Measurement of respiration rate was carried out as described by Jiang et al. (2004). The concentrations of CO_2 and O_2 were measured by a gas chromatography (Tianmei 102G, Tianmei Co., Ltd., Shanghai, China) equipped with a thermal conductivity detector, a molecular sieve, and a Porapak Q column (80–100 mesh) with helium as the stripping gas at a flow rate of 0.5 mL s^{-1}. The injector, column and detector temperatures were 120, 60 and 360 °C, respectively. N_2 was used as the carrier gas. The retention time for CO_2 and O_2 were 2 and 1 min, respectively. Calibrations were performed using three empty jars flushed with air. The headspace ethanol and acetaldehyde quantification were performed according to the method described by Lichter et al. (2002). Gas samples (1.0 mL) from the headspace was injected to a gas chromatograph (Varian-4000, Varian Inc., Palo Alto, USA) with flame ionization detector (GC-FID) at 180 °C, equipped with a stainless steel column, packed with 20% Carbowax 20 M, 80–100 mesh. Concentrations were calculated against standard aqueous solutions and by preparing the corresponding standard curve under the same conditions as those for the asparagus spear samples. All headspace analyses were conducted in triplicate.

2.4. Membrane permeability

Membrane permeability was determined according to the method of Li et al. (2008). Asparagus spears (2.0 g) were sliced into small discs (10–12 in diameter, 0.2 cm thick) and washed three times with deionized water. After draining with tissue paper, the discs were placed in a flask with 20 mL deionized water and shaken for 30 min at 25 °C. Electrical conductivity (L_t) of the solution was determined with a conductivity meter (DDS-llA, Shanghai Scientific Instruments Co., Ltd., Shanghai, China). The flask with solution was then heated at 96 °C for 15 min, quickly cooled and electrical conductivity of the solution was measured again (L_0). Membrane permeability was calculated and expressed by relative electrical conductivity (L_t/L_0).

2.5. Enzyme activities

Asparagus spears (0.4 g) which were treated by HHP at 10–600 MPa for 5 min were mixed with 4.0 mL of ice-cold extraction buffer (50 mmol L^{-1} phosphate buffer, pH 6.5 containing 10 mmol L^{-1} $MgCl_2$ and 2.0 mmol L^{-1} Na_2EDTA, 2.0 mmol L^{-1} dithiothreitol), and ground using mortar and pestle at 4 °C on an ice bath. The mixture was centrifuged at 9000*g* for 15 min and the supernatants were assayed for enzyme activities. Pyruvate decarboxylase (PDC) was determined according the method described by (Bouny and Saglio, 1996). Alcohol dehydrogenase (ADH) was measured according to (Kato-Noguchi, 1998). Pectinmethylesterase (PME) activity was determined by measuring the release of acid per unit time at pH 7.0 and 22 °C (Rodrigo et al.,