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# Effects of high pressure nitrogen treatments on the quality of fresh-cut pears at cold storage

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ABSTRACT

When fresh-cut fruit and vegetables are subjected to HP nitrogen treatments, the nitrogen gas dissolves into water and forms clathrate hydrates to restricted water mobility and enzymatic reactions. In this study, effects of HP (120 MPa) nitrogen treatments on preserving fresh-cut pears were studied at 4 °C for 14 days. Water mobility in fresh-cut pears was significantly reduced by the HP (120 MPa) nitrogen treatment. The activities of catalase (CAT) and peroxidase (POD) were restricted by the HP (120 MPa) nitrogen treatment. Respiration rate and ethylene production of fresh-cut pears treated by the HP (120 MPa) nitrogen for 10 min were lower significantly in comparison with control and treated by flushing with nitrogen and the HP air for 10 min. The treatment also delayed proliferation of spoilage microorganisms of fresh-cut pears, because the populations of both mesophiles and psychrotrophs did not exceed 6.0 log cfu/g, and those for molds and yeasts did not exceed 3.0 log cfu/g after 14 day storage. HP nitrogen treatment did barely affect the content of titrated acidity and soluble solids, and significantly reduced the total phenolics decrease in fresh-cut pears ($p < 0.05$). Dipping into chemical solution, such as 0.3% ascorbic acid, and 0.5% calcium chloride for 3 min, could decrease the changes in the color and firmness of pear samples during HP (120 MPa) operation, and the combined HP nitrogen with dipping treatment maintained good sensory quality of fresh-cut pears during storage. These results suggested the fresh-cut pears treated by HP nitrogen at 120 MPa for 10 min could be kept in a fresh-like condition for 14 days at 4 °C.

*Industrial relevance:* Through this study, HP nitrogen (120 MPa) methods reduced the water mobility, antioxidative enzyme (CAT, POD) activities, respiration rate, and ethylene production of fresh-cut fruit and vegetables, and restrained the growth of microorganisms within 14 days at cold storage. This could be a promising approach to preserve fresh-cut pears. It was demonstrated that pressurized nitrogen is a suitable means of preserving fresh-cut pears. However, HP operation has a negative effect on the color and firmness to some degree. The combination treatment between HP nitrogen and dipping in solutions (0.3% AA, 0.5% CC) showed a better means of extending the shelf-life of fresh-cut fruit and vegetables.



## 1. Introduction

Nowadays, the demand for fresh-cut fruits and vegetables has significantly increased and consumers look out for safe and natural products. The fresh-cut process reinforces respiration rate and wound-induced $C_2H_4$ production and increases water mobility and enzymatic reactions. Thus, it is difficult to maintain the quality of fresh-cut fruits during storage (Soliva-Fortuny & Mnitrogentín-Belloso, 2003). Many methods have been proposed to extend the shelf life of fresh-cut fruits and vegetables, such as mild heat treatment, modified atmosphere package, dipping into chemical solutions, and ozone treatment (Albanese, Cinquanta, & Di Matteo, 2007; Soliva-Fortuny & Mnitrogentín-Belloso, 2003). However, mild heat treatment inevitably induces to loss of nutritional components and softening of fresh-cut produce (Abreu, Beiráo-da-Costa, Gonçalves, Beiráo-da-Costa, & Moldáo-Martins, 2003; Sgroppo & Pereyra, 2009; Alegria et al., 2012). UV irradiation may cause surface browning of fresh-cut fruits and vegetables (Gomez, Alzamora, Castro, & Salvatori, 2010; Pan & Zu, 2012). A major defect of ozone as a sanitizer is its bad stability in the presence of organic matter (Bermúdez-Aguirre & Barbosa-Cánovas, 2013).

Nitrogen gas as a major component of the atmosphere in modified atmosphere packaging has also been reported to decrease microbial growth and increase the quality retention of fresh produce (Day, 1998; Jamie & Saltveit, 2002). Zhang, Quantick, Grigor, Wiktorowicz, and Irven (2001) research shows that nitrogen effectively inhibits the activities of tyrosinase and malic dehydrogenase, which are specific key enzymes related to browning of fresh fruits and vegetables and respiratory metabolism (Zhang et al., 2001). Nitrogen is proved to be biochemically active, probably due to its enhanced solubility in water compared with Xe and possible disturb enzymatic oxygen receptor sites (Spencer, 1995).

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When certain gas such as nitrogen is dissolved in water under appropriate temperature and pressure condition, the ice-like crystal called clathrate hydrate or gas hydrate is produced (Chatti, Delahaye, Fournaison, & Petitet, 2005; Anderson, 2007; Disalvo et al., 2008; Ando et al., 2009; Ruffine, Donval, Charlou, Cremière, & Zehnder, 2010; Wu, Zhang, & Wang, 2012). The clathrate hydrate is stabilized relative to the structure of pure water ice and can exist well above 0 °C. The main conditions for gas hydrate formation are: presence of gas, water, high pressure and low temperature (Makogon, 2010). Oshita, Seo, and Kawagoe (2000) found that the formation of clathrate hydrate was effective in maintaining the quality of cut carnation and broccoli, due to inhibition of the activity of water and enzymatic reactions, resulting in inhabited vegetable metabolism. Fujii et al. (2002) found that addition of Ar increased the inactivation of *Bacillus cereus* spores under the pressure of 600 MPa due to nitrogen clathrate hydrates formation. Behnke (1969) discovered that high pressure (HP) nitrogen gases inhibit tyrosinase in non-fluid systems by decreasing oxygen availability. A study shown that mobility of water in fruits and vegetables can be significantly restricted due to the formation of clathrate hydrate (Meng et al., 2013). Encouragingly, nitrogen is chemically stable and they do not have an side effect in human (Ronald, 2001). It is desirable for using of low-cost nitrogen under high pressures to form clathrate hydrate to preserve fresh-cut pears (Zhan, 2005).

In this context, the goals of this research were to study the effect of HP nitrogen on physiological and physical aspects of fresh-cut pears during cold storage. The effects of pressurized nitrogen on the storage quality parameters such as activities of catalase (CAT) and peroxidase (POD), water mobility, respiration rate and ethylene production, color and firmness, titratable acidity and soluble solids, total phenolic compounds, sensory index and microbial quantity were measured.

## 2. Materials and methods

### 2.1. Material

Pears (Snow pear) at commercial maturity were picked from a local farm at PR China. The selected pears were of uniform size (about 70 mm diameter) and has no defects and placed in cold storage at 4 °C.

### 2.2. Pear wedge preparation and treatment

Whole pears were sanitized for 2 min in 0.01% (*V*/*V*) sodium hypochlorite solution, cleaned with deionized water and dried for about 10 min. Then they were cut into small wedges with thickness of 20 mm and finally placed into pressure vessels and subjected to pressurized nitrogen. Knife and cutting board were sanitized with 0.01% (*V*/*V*) sodium hypochlorite solution.

The fresh-cut pears were divided into six groups: (1) control; (2) flushing with nitrogen; (3) dip; (4) high pressure air processing; (5) high pressure nitrogen processing; and (6) dip plus high pressure nitrogen processing.

For treatment 2, samples were placed in polyethylene bags and flushed with nitrogen gases for 10 min. For treatment 3 and 6, samples were dipped into an chemical solution of 0.3% ascorbic acid (AA), and 0.5% calcium chloride (CC) for 3 min (Rocculi, Romani, & Dalla Rosa, 2004), then dried on a clean towel. High pressure processing was described as follows: samples were packed in pressure resistant polyethylene bags; using a multi-functional controlled atmosphere packing machine to flush with air or nitrogen. The air or nitrogen samples were pressurized at 120 MPa for 10 min using an ultra-high pressure machine and the pressure transmitting medium was water. This process condition was selected on the base of preliminary experiment (not described in the article). Time taken to reach the design pressure was about 30–60 s and depressurization only took 10 s. The pressure container temperature was maintained at 4 °C during the HP operation. Then samples were transferred from pressure-resistant polyethylene bags into low-density polyethylene bags and thermal-sealed. The permeability of films at 23 °C and 90% RH for water vapor, oxygen, and carbon dioxide was 94.96 g mm/$m^2$/22 h/atm, 0.026 mL/$m^2$/22 h/atm, and 0.104 mL/$m^2$/22 h/atm, respectively. All of these samples were stored at 4 °C for 14 days. Three replicates were used per treatment.

### 2.3. Relaxation time and water distribution

Relaxation time and water distribution were measured using a low field pulsed NMI 20-Analyst with 22.6 MHz as described by Han, Zhang, Fei, Xu, and Zhou (2008). $T_{2i}$ represents the water mobility in samples. $T_{21}$ refers to the transverse relaxation time of free water; $T_{22}$ and $T_{23}$ refer to the transverse relaxation times of bound water associated with some macromolecular substances such as proteins, starchs, etc. (Shao & Li, 2009). $M_{2i}$ represents the water distribution in samples. $M_{21}$ represents the corresponding water fraction to $T_{21}$; $M_{22}$ represents the corresponding water fraction to $T_{22}$; and $M_{23}$ represents the corresponding water fraction to $T_{23}$. The 2 g of sample was placed into a 15 mm glass tube and inserted in the nuclear magnetic resonance probe. The transverse relaxation time ($T_2$) and the water distribution ($M_2$) was determined using the Carr–Purcell–Meiboom–Gill pulse sequence. The 90–180° pulse spacing was set to 50 s. Relaxation curves were analyzed with Origin 8.0 software and each measurement was performed in triplicate.

### 2.4. Catalase (CAT) activity and peroxidase (POD) activity

CAT and POD activities were measured as described by Yuan, Sun, Yuan, and Wang (2010). Fresh-cut pears (1 g) were homogenised with 10 mL deionized water, 25 mM phosphate buffer (pH 7.8) including 0.8 g/L PVPP and 1 mM EDTA, then centrifuged at 15,000 $\times g$ for 25 min at 4 °C. For CAT, The 0.5 mL enzyme extract was added with 0.5 mL $H_2O_2$ (40 mM) and 2 mL sodium phosphate buffer (50 mM, pH 7.0). Then the sample absorbance was determined within 1 min at 25 °C. The CAT activity was measured using Eq. (1). CAT activity (U/g FW) $= 0.01 \times A_{240}$ nm/min (1). For POD, 0.5 mL enzyme extract was incubated in 2 mL buffered substrate for 5 min at 30 °C. The absorbance of samples was determined at 460 nm in every 30 s for 120 s after 1 mL of $H_2O_2$ (24 mM) was added. The POD activity was measured using Eq.(2). POD activity (U/g FW) $= 0.01 \times A_{470}$ nm/min (2)

### 2.5. Determination of the respiration rate and ethylene production

Respiration rate was measured as described by Ansorena, Marcovich, and Roura (2011). Pear samples (100 g each) were placed in 1000-mL airtight containers for 2 h at 4 °C. Then, a 5-mL gas sample was taken and injected into gas chromatographs. The gas chromatography settings for the detection of $CO_2$ were as follows: thermal conductivity detector (TDC), detector temperature of 80 °C, column temperature of 50 °C, and carrier gas (hydrogen) flow rate of 35 mL/min. Respiration rate was calculated as mg$CO_2$/(kg h).

Ethylene concentration was measured using the gas chromatograph equipped with a flame ionization detector and a capillary column. Gas flows for $N_2$, $H_2$ and air were 30, 47 and 400 mL/min and column temperature was isothermal at 60 °C. Ethylene production was calculated in mL $C_2H_4$/(kg h). Each measurement was made in triplicate.

### 2.6. Color and firmness measurement

The flesh color of pear samples was determined with a color measurement spectrophotometer (HunterLab ColorQuest XE, Hunter Associates Laboratory, Inc., Virginia, USA) in the reflectance mode. It provided L* (lightness), a* (green chromaticity), and b* (yellow chromaticity). Each measurement was made in triplicate.

Firmness was determined as the maximum force (N) required to puncture the flesh at a speed of 5 mm $s^{-1}$ to a depth of 10 mm (Mao,