



Effect of edible coatings on enzymes, cell-membrane integrity, and cell-wall constituents in relation to brittleness and firmness of Huanghua pears (*Pyrus pyrifolia* Nakai, cv. Huanghua) during storage

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ARTICLE INFO

Article history:

Received 5 October 2009

Received in revised form 25 April 2010

Accepted 21 June 2010

Keywords:

Fruit

Edible film

Texture properties

Quality

ABSTRACT

To investigate the effects of edible coatings, such as shellac and Semperfresh™ (sucrose-polyester based coating) on the brittleness and firmness of Huanghua pears (*Pyrus pyrifolia* Nakai, cv. Huanghua), the changes in the cell-membrane permeability and cell-wall constituents, such as total pectin (TP), Na₂CO₃-soluble pectin (NSP), CDTA-soluble pectin (CSP), water-soluble pectin (WSP), hemicellulose and cellulose were periodically measured, for up to 60 days of cold storage (4 °C) after harvesting. The activities of peroxidase (POD), pectinesterase (PE), polygalacturonase (PG), and cellulase were also assayed. The data suggested that high POD activity and low activity of cell-wall-degrading enzymes, such as PE, PG, and cellulase in the coated pears were associated with a high integrity of the cell membrane and few changes in the cell-wall constituents, which contributed to high levels of brittleness and firmness in the pears during storage; further, the shellac coating provided a better effect than Semperfresh coating.

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1. Introduction

Huanghua pears (*Pyrus pyrifolia* Nakai, cv. Huanghua), which are well known for their thin peel, delicate flesh, rich juice, and good taste are one of the important fruits endemic to China (Zhou, Su, Yan, & Li, 2007). These are some of the main pear cultivars in Shanghai and are popularly known as Shanghaimi pears. However, these pears ripen at mid-summer; the rapid postharvest physiological changes account for a short postharvest ripening period, and pose a marketing challenge (Zhou et al., 2008). Softening changes, including a loss of brittleness and firmness, are the most important characteristics indicating the deterioration of Huanghua pears; these changes directly influence the quality of the fruits, as well as their storage life, transportability, and marketing.

Fruit softening is generally observed during ripening in various types of fruits (Murayama, Katsumata, Endou, Fukushima, & Sakurai, 2006). Loss of turgor pressure and degradation of the cell wall in climacteric fruits, such as Huanghua pears, contributes to the decrease in brittleness and firmness (Khin, Zhou, & Yeo, 2007; Zhou et al., 2008). The integrity of the cell membrane of fruits, which is involved in the maintenance of turgor pressure, can be destroyed by peroxides produced during storage. An efficient antioxidant sys-

tem can postpone the senescence process even though anti-oxidative activity in fruits decreases with ageing (Zheng, Tian, Meng, & Li, 2007). Peroxidase (POD), one of important antioxidant enzymes, is involved in the oxy-radical detoxification process in plant tissues (Yuan, Sun, Yuan, & Wang, 2010). The disassembly and degradation of cell wall structure and composition have been attributed to the action of enzymes (among other things), such as pectin methylesterase (PE) and polygalacturonase (PG) and cellulase on polysaccharides, such as pectin, hemicellulose, and cellulose present in the cell wall (Cheng et al., 2009; Fraeye et al., 2007; Lohani, Trivedi, & Nath, 2004; Zhou et al., 2007). However, the precise role of these enzymes in altering the cell membrane and cell wall, and the importance of related fruit-softening enzymes in Huanghua pears are little known.

Edible coatings, like modified atmosphere packaging, have been known to retard softening changes in fruits by inhibiting metabolic processes (Conforti & Zinck, 2002; Park, 1999). However, to our knowledge, there is no available scientific literature regarding the effect of edible coatings on retaining the texture of Huanghua pears during storage. Shellac wax and sucrose polyesters are widely used as edible coatings for fruit; these coatings have been successfully used in retaining the texture of fruits like apples (Bai, Baldwin, & Hagenmaier, 2002), cherries (Yaman & Bayoindirli, 2002), and quinces (Yurdugül, 2005). Thus, in the present study, we selected and designed two types of coatings, namely, shellac

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wax and Semperfresh™ (sucrose-polyester based coating), which had different coating characteristics for gas exchange, and examined the effects of edible coatings on maintaining the quality of Huanghua pears. The aim of this work was to (1) investigate the effects of coating treatments on POD activity and cell-membrane integrity of pears, (2) investigate changes in the hydrolase activity and cell-wall constituents during storage, and (3) clarify the contributions of changes caused by edible coatings to fruit softening and determine the most appropriate edible coating to preserve Huanghua pears.

2. Materials and methods

2.1. Fruit and coatings

Huanghua pears (*Pyrus pyrifolia* Nakai, cv. Huanghua) that had reached commercial maturity, which was determined on the basis of fruit skin colour and harvest date, were hand-harvested from an orchard in Fengxian, Shanghai, China. The fruits were selected on the basis of uniform colour and absence of bruises and disease. Then, the pears were transported to the laboratory within 2 h.

We used two types of experimental coatings. Shellac coating was prepared using refined, dewaxed, bleached, and food-grade shellac (14.3 g/100 ml; KFull-060715; Kunming Kfull Biotechnology Co. Ltd., Kunming, China), NH_3 (0.8 g/100 ml water), and food-grade polydimethylsiloxane antifoam (0.01 g/100 ml water; XP010; Runqi Food Technology Co. Ltd., Shanghai, China). The preparation steps of shellac coating were shown as follows: briefly, the shellac wax based on the ratio was homogenised with the heated ammonia water (65 °C), and filtered through 4 layers of cotton gauze (Shanghai Honglong Medical Supplies Equipment Co. Ltd., Shanghai, China). Food-grade polydimethylsiloxane antifoam (0.01 g/100 ml water) was homogenised with the filtrate. Then, the mixture was placed overnight and filtered again by following the aforementioned procedure. Semperfresh coating (Semperfresh™; AgriCoat Industries Co. Ltd., England; distributed by HongYuanXinDa Co. Ltd., Beijing, China) was acquired as a liquor concentrate (50 g/100 ml water), prepared from carboxymethyl cellulose and sucrose esters, and glycerides of fatty acids. The raw liquor was diluted with distilled water to obtain a concentration appropriate for pears (1.0 g/100 ml water), which is the recommended specification of the raw Semperfresh liquid. All other chemicals were of analytical grade.

The fruits were then coated by dipping in the shellac and Semperfresh solutions for 15 s, and then suspended by their stems and air dried using fans. The pears dipped in distilled water, following the same procedure, were used as controls. Then, all of pears were pre-cooled (7 °C, 12 h) after treatments. Finally, all pears were stored at 4 °C and approximately 95% relative humidity (RH).

2.2. Measurement of brittleness and firmness

The brittleness and firmness of the pears were determined on the basis of texture profile analysis (TPA) curves of the fruits. TPA was performed using a TA-XT2i texture analyser (Stable Micro Systems Ltd., UK) with a standard compression platen (SMS P/100). Five pears from each treatment group were used for the analysis. The fruits were peeled, and cylindrical plugs of 10-mm diameter were obtained from the equatorial region using a cork borer; these plugs were then cut into 10-mm thick slices. From the equatorial region of each fruit, four plugs were obtained from four opposite sides. Brittleness was measured as the distance (in mm) covered by the probe to reach the first peak of the TPA curve (Khin et al., 2007), and firmness was defined as the second peak in the TPA curve, expressed as N.

2.3. Membrane permeability of pear cells

The membrane permeability of pear cells was expressed in terms of relative electrical conductivity. The electrical conductivity of pears during storage was assayed using a digital conductometer (DDB-6200; Shanghai Leici Apparatus, Shanghai, China) with a DJS-1 type electrode. The assays were performed according to the procedure described by Feng, Yang, and Li (2005) with slight modifications. Five pears were randomly selected from each treatment group, rinsed with double-distilled deionized water, and then lightly wiped with filter paper.

We obtained 14.5-mm flesh samples from the equatorial region of pears using a cork borer, and then cut them into 2-mm thick wafers. Next, 5-g flesh samples were collected from each treatment group, rinsed three times with double-distilled deionized water, and immersed in 100 ml double-distilled deionized water for 1 h. The initial electrolyte leakage was measured. Subsequently, the samples were boiled for 5 min, and the total electrolyte leakage was assayed after the samples reached room temperature. The relative electrical conductivity was calculated as the percentage of the total electrolyte leakage. All the experiments were repeated three times.

2.4. Enzyme extraction and assays

The extraction method of POD was adapted from Dong, Cheng, Tan, Zheng, and Jiang (2004), with slight modification. The flesh of five pears was mixed and ground rapidly in an ice bath with a mortar and pestle. Fruit tissue (10 g) was obtained and homogenised with 40 ml of 0.05 M phosphate buffer (pH 6.8), and then filtered through two layers of middle-speed quantitative filter paper (Hangzhou Special Paper Industry Co. Ltd., Hangzhou, China). The filtrate was centrifuged at 10,000g for 20 min and the supernatant was used for further assays. POD activity was determined according to the method described by Jung et al., (2004). The reaction mixture comprised of 50 μl of 0.02 M guaiacol, 2.8 ml of 0.01 M phosphate buffer (pH 6.8), and 0.1 ml of enzyme supernatant. Next, we added 40 μl of 0.04 M H_2O_2 into the mixture, and immediately determined the reaction time. The changes in absorbance at 470 nm were recorded for 3 min. One unit of enzyme activity was defined as the amount of enzyme contributing to a change of 0.01 units in absorbance per minute.

The PE, PG, and cellulase activities were assayed according to the procedure described in previous studies (Zhou et al., 2007). Similarly, the flesh of 5 pears was mixed and ground in an ice bath. For PE extraction, 10 g fruit flesh was obtained and homogenised with 10 ml of 0.5 M Tris-HCl (pH 8.0) and centrifuged (8000g, 10 min) at 4 °C. The Tris-HCl solution contained 1 mM cyclohexane-trans-1, 2-diaminetetra-acetic acid (CDTA), 5% (w/v) polyvinylpyrrolidone, and 2 M NaCl. PE activity in the supernatant was assayed by an acid-base titration method described by Nagel and Patterson (1967). One unit of enzyme activity was the amount of the enzyme required to hydrolyse 1 μmol ester per minute, per gram of the original fresh flesh of pears.

For PG and cellulase extraction, fruit flesh (10 g) was homogenised with 10 ml of 0.1 M phosphate buffer (pH 7.0) and centrifuged (8000g, 10 min). The phosphate buffer contained 1 mM CDTA, 5% (w/v) polyvinylpyrrolidone, and 0.5 M NaCl. The PG activity in the supernatant was assayed by measuring the reducing groups released from citrus pectin (Sigma Chemical Co., St. Louis, Mo.), according to the method described by Zhou et al. (2007). One unit of enzyme activity was defined as 1 μmol of reducing groups released per hour, per gram of the original fresh flesh of pears. The cellulase activity was assayed by measuring the reducing groups released from carboxymethyl cellulose (Zhou et al., 2007). One unit of enzyme activity was the amount of the enzyme

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