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Effect of nano-ZnO-packaging on chilling tolerance and pectin metabolism of peaches during cold storage

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ABSTRACT

The effects of nano-ZnO-based low-density polyethylene (NZLDPE)-packaging on chilling tolerance and pectin metabolism in postharvest "Hujingmilu" peaches were investigated. Peaches packed in low-density polyethylene (LDPE) and NZLDPE were stored at 2 °C for 40 d. Compared with the control, both packages alleviated the development of chilling injury, showed higher fruit firmness with lower browning index, electrolyte leakage, relative viscosity, and decay rate. NZLDPE-packaging performed better than LDPE-packaging because of the rapid formation of low O_2 and high CO_2. Moreover, NZLDPE-packaging inhibited pectin esterase and enhanced polygalacturonase and β-galactosidase, leading to the promotion of alkali soluble-pectin and water-soluble pectin, and the decrease of chelater-soluble pectin. These effects were of great benefit to the maintaining of cell wall structure and the degradation of calcium-pectate gel, which finally alleviated the chilling injury and therefore maintained good quality during chilling stress.

1. Introduction

Peach is a popular fruit for consumers because of its juiciness and attractive taste. Peaches ripen and decay quickly at room temperature after harvest, which leads to a loss of sales value. Cold storage is a widely used technology for postharvest fruit and vegetables to delay senescence and extend shelf-life (Wang, 1990). Nevertheless, as a climacteric fruit, peach suffers chilling injury at 2–5 °C. Several technologies, such as treatment with hot air (Yu et al., 2016), glycine betaine (Shan et al., 2016), and oxalic acid (Jin et al., 2014), have been reported to alleviate chilling injury in peaches during cold storage. However, reliable investigations on the practical techniques to inhibit or alleviate chilling injuries are still limited.

Chilling injury results in many macroscopic symptoms in peaches, including browning, wooliness, reddening and leatheriness, which are related to cell wall integrity and pectin metabolism (Lurie and Crisosto, 2005). Cao et al. (2009) indicated that chilling injury is partly due to biochemical modifications of cell wall polysaccharides. These processes involve hydrolytic enzymes such as pectin esterase (PE), polygalacturonase (PG) and β-galactosidase (β-Gal) (Fischer et al., 1991). Fruk et al. (2014) reported that the relatively higher PE and lower PG activities observed in peaches were response to chilling stress, which may lead to the formation of high-molecular-weight pectin with a low degree of esterification. This kind of pectin can combine with calcium to form calcium-pectate gel complexes, which then bind with free water and eventually lead to woolliness, a symptom of chilling injury (Brummell et al., 2004a).

In recent decades, nanomaterials used in food packaging have been actively explored (Chaudhry et al., 2008). Nanocomposites enhanced barrier and mechanical properties, and possessed antimicrobial effect along with spore germination inhibition ability in comparison to general polymer (Panea et al., 2014). Various nanocomposites including nano-ZnO-coated polyvinyl chloride (PVC) (Li et al., 2011), nano-Ag_2O-based low-density polyethylene (Zhou et al., 2011a), and nano-$CaCO_3$-based low-density polyethylene (Luo et al., 2014) applied in the preservation of fruit and vegetables have been reported. During cold storage, polyethylene with nano-Ag, nano-TiO_2 and montmorillonite has been proven to be efficient for inhibiting ethylene production, reducing degradation of nutritional components, extending organoleptic characteristics, preventing physiologic changes, and thus delaying the ripening and extending shelf-life of harvested kiwifruit (Hu et al., 2011). Li et al. (2011) stated that nano-ZnO-packaging film preserved fresh-cut 'Fuji' apple through reduction of fruit decay rate, depreciation of malondialdehyde (MDA) and ethylene accumulation, maintaining of total soluble solids (TSS) and titratable acid (TA) levels and inhibition of polyphenol oxidase (PPO) and pyrogallol peroxidase (POD) activities. However, to the best of our knowledge, the effect of nano-ZnO-packaging on chilling tolerance of peaches during cold storage has not been

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researched.

The objective of this study was to investigate the effect of nano-ZnO-based low-density polyethylene (NZLDPE)-packaging on chilling tolerance and pectin metabolism in postharvest "Hujingmilu" peaches during cold storage.

2. Materials and methods

2.1. Plant material and treatments

"Hujingmilu" peaches (*Prunus persica* L.) with 70–80% maturity (with a firmness of 16.1 ± 0.7 N, total soluble solids content of 11.2 ± 0.2%, and titratable acid content of 0.21 ± 0.02%) were harvested in Yuhang District of Hangzhou City and transferred to the laboratory in Zhejiang University immediately. The fruit were air cooled to 4 °C in 6 h. The first and second groups of peaches were packed into low-density polyethylene (LDPE)-packaging (6 bags) and NZLDPE-packaging (6 bags) with a size of 20 cm × 30 cm respectively, and then sealed and stored at 2 °C for up to 40 days. The third group of peaches were unpackaged and stored under the same condition as control. For each treatment, one bag of peaches was randomly taken every eight days during storage for subsequent analyses (totally 6 bags were used). And another three bags were used to observe the decay rate every eight days during the whole storage. Three biological replications were conducted.

2.2. Preparation of NZLDPE materials

A NZLDPE masterbatch containing 56% (w/w) of LDPE granule (softening point 95 °C, density 920 kg m^{-3}), 30% (w/w) of the commercial nano-ZnO (30 nm) and 14% (w/w) of cross-link reagent ester titanate was blended by a high-speed mixer for 1 h. After cooled down, the mixture was extruded to a NZLDPE masterbatch by a twin-screw extruder with a screw speed of 300 r min^{-1}. Then, 38.5 kg of LDPE granule and 1.5 kg of masterbatch were blended for 0.5 h. The above nanocompounds were made into films of 40 μm thickness by a plastic extruder, and the films were made into NZLDPE bags of 20 cm × 30 cm using a heat sealer after cooling. LDPE bags with the same thickness and size were made under the same condition.

2.3. Measurement of in-packaging atmospheric composition

Changes in CO_2 and O_2 concentrations were measured by injecting samples of headspace gases form the packages into a gas chromatograph with a thermal conductivity detector (GC-14A; Shimadzu, Suzhou, China). Each treatment was conducted for three biological replicates.

2.4. Measurement of browning index

For each replicate, eight peaches from one bag were used for evaluation of browning index. Peaches were cut along the fruit suture and the browning scale (B) was divided into four degrees according to the percentage of browning area of flesh; 0: no browning; 1: B ≤ 25%; 2: 25% < B ≤ 50%; 3: B > 50%. The browning index was calculated based on the following formula:

$$\text{Browing index}(\%) = \frac{\sum (\text{browning scale} \times \text{the amount of peaches at this scale})}{\text{total peach number} \times 3} \times 100$$

2.5. Measurement of electrolyte leakage

Discs of peach peel from one bag were excised randomly using a 6 mm diameter stainless steel borer. Ten grams of discs with 20 mL distilled water in a beaker were stirred slowly for 20 min and the electrolyte conductivity of leach liquor (C_1) was measured with a conductivity meter. Then the leach liquor with discs was boiled for 10 min and cooled down to the room temperature. Electrolyte conductivity (C_2) was measured. Each treatment was conducted for three biological replicates. The rate of electrolyte leakage was calculated as $(C_1/C_2) \times 100\%$.

2.6. Measurement of relative viscosity

Peach flesh from one bag was mashed and filtered using gauze. After centrifuged at 5000 × *g* for 15 min, 10 mL of supernatant was collected and the time supernatant (T_1) and distilled water (T_0) flowed through a capillary viscometer with 0.4 mm inner diameter at 25 °C were measured respectively. Each treatment was conducted for three biological replicates. The relative viscosity was presented as $(T_1\text{-}T_0)/T_0$.

2.7. Measurement of fruit firmness

A texture analyzer (TA-XT2i, Stable Micro Systems Ltd., Godalmin, UK) was used to determine the peach firmness. Three peaches form one bag were measured on opposite sides of the equator by a 5 mm diameter flat probe. The test speed was 1.5 mm s^{-1} and the distance was 8 mm. The maximum force (N_m) was recorded as peach firmness and the average values were calculated. Each treatment was conducted for three biological replicates.

2.8. Measurement of decay rate

For each treatment, another three bags were used to observe the decay rate every eight days during the whole storage. Any peach with visible mould growth was considered decay. The decay rate (%) was calculated as (the amounts of decayed peaches × 100)/(the total amounts of peaches). Each treatment was conducted for three biological replicates.

2.9. Isolation of cell wall material (CWM)

The CWM was prepared using the method described by Chen et al. (2015) with some modifications. One hundred grams frozen flesh pooled from six peaches was homogenized and kept boiling reflux with 500 mL 80% (v/v) ethanol for 0.5 h. After immediate filtration, the residue was collected and washed with 80% (v/v) ethanol for several times until no reducing sugar was detected in the filtrate. Then the residue was washed with acetone and incubated overnight with 90% (v/v) dimethylsulphoxide at 4 °C. The CWM was filtered, washed for several times with distilled water, dried in a vacuum oven and stored in a vacuum desiccator.

2.10. Extraction of CWM constituents

Extraction of CWM constituents was performed according to the methods described by Chen et al. (2015) with some modifications. The water-soluble pectin (WSP), chelater-soluble pectin (CSP) and alkali-soluble pectin (ASP) were extracted consecutively on the CWM. Three hundred milligrams of CWM was added to 100 mL 50 mM sodium acetate buffer (pH 6.5), oscillated and extracted at ambient temperature for 3 h. This process repeated for 3 times and the filtrate contained WSP was collected. Similarly, CSP and ASP was extracted by 50 mM sodium acetate buffer (pH 6.5) containing 50 mM ethylene diamine tetraacetic acid and 100 mM KOH respectively.

2.11. Measurement of CWM constituents

The CWM constituents were measured based on the method described by Bu et al. (2013). WSP, CSP and ASP extracts (5 mL) were