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Influence of chitosan-based coatings on the physicochemical properties and pectin nanostructure of Chinese cherry

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ABSTRACT

The effects of chitosan-based coatings on the postharvest performance Chinese cherry during a twenty days storage period were investigated. The chitosan-based coatings effectively delayed postharvest ripening parameters including weight loss, decay rate, firmness, soluble solid content (SSC) and titratable acidity (TA). The most effective treatment was a combined chitosan and nano-SiOx coating, which led to 51% less weight loss, 32% less decay rate, 57% more firmness, and less SSC and TA content changes than the control group. This combined coating also maintained a higher content of sodium carbonate-soluble pectin (SSP), and inhibited pectin chain degradation. Qualitative and quantitative analysis revealed that the firmness of fruit was closely related to nanostructural morphologies and SSP chain width. In addition, modifications of SSP were correlated with fruit softening, especially SSP polymers and branched chains. These results demonstrate the effect of chitosan and nano-SiOx coating on extending the shelf life of Chinese cherries during postharvest storage.

1. Introduction

Chinese cherries (*Prunus pseudocerasus* L.) are a non-climacteric fruit with a high transpiration rate, and susceptibility to fungal rotting and physiological disorders (Alique et al., 2005). Therefore, they tend to have a short shelf life even under strict cold chain management (Wang and Long, 2014). The way cherries are stored affects their texture, which significantly impacts consumer appreciation. The rate of tissue softening is commonly considered to be largely due to depolymerisation and solubility of cell wall polysaccharides (Liu et al., 2009; Chen et al., 2013), driven by the cooperative action of numerous related proteins (Comabella and Lara, 2013). In particular, cherry softening is closely related to the content and solubility of pectin, especially sodium carbonate-soluble pectin (SSP) (Zhang et al., 2008).

Coating and cold storage can extend the shelf life and maintain the texture properties of postharvest fruit (Díaz-Mula et al., 2011). Chitosan, a deacetylated derivate of chitin, is a high molecular weight cationic polysaccharide. Chitosan-based coatings are widely used in the field of food preservation because of their excellent film forming ability (Chong et al., 2015), antimicrobial activity (Lei et al., 2014; Shankar et al., 2015) and safety for human consumption. These coatings have already been used to extend the shelf life of several fruits, including the sweet cherry (Petriccione et al., 2014; Pasquariello et al., 2015), melon (Carvalho et al., 2016), longan (Shi et al., 2013), pear (Kou et al., 2014) and carambola (Gol et al., 2013).

Conventional chitosan coatings, however, have poor mechanical properties and permeability (Sun et al., 2016). Current efforts are devoted to using nanotechnology to extend the shelf life of foods under storage and distribution conditions (Song et al., 2016; Youssef et al., 2016). Recently, nano-SiOx particles have been introduced to further improve the food preservation properties of chitosan coatings. Nano-SiOx particles have a small size effect and generate strong hydrogen bonds with chitosan molecules through surface hydroxyl, which improves the mechanical properties and water/oxygen permeability of the chitosan coating (Wang et al., 2008). Using transmission electron microscopy (TEM), Shi et al. (2013) observed the formation of Si-O−C and hydrogen bonds, and reported uniform dispersion of silica in the chitosan matrix. Intriguingly, while these nanoparticle coatings have been shown to improve chitosan film properties, it remains unclear how their application directly preserves postharvest fruit.

In this study, we applied chitosan-based coatings (chitosan alone or combined with nano-SiOx) in the storage of postharvest Chinese cherry to elucidate its preservation effects on inhibiting postharvest cherry deterioration. We evaluated the physical and chemical qualities of the

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postharvest cherry during a twenty days storage period and applied atomic force microscopy (AFM) to examine the SSP nanostructures of the cherry.

2. Materials and methods

2.1. Preparation of fruit materials and coating solutions

Chitosan coatings were prepared according to a method described by Petriccione et al. (2014) with slight modifications. Chitosan powder was purchased from Aoxing Biotechnology Co., Ltd (Taizhou, Zhejiang, China) and dissolved 5.0 g in 400 mL distilled water (containing 32.5 mL glacial acetic acid), which was then homogenised by ultrasound (40 kHz, 400 W, at 40 °C for 1 h). The pH was then adjusted to 5.6 with NaOH (1 mol L^{-1}) and the volume to 500 mL.

One gram Nano-SiOx powder (Zhoushan Nanomaterials Co., Ltd, Zhoushan, Zhejiang, China) and 5.0 g chitosan power were dissolved in 400 mL distilled water (containing 32.5 mL glacial acetic acid), which we then homogenised by ultrasound (40 kHz, 400 W, at 40 °C for 1 h). Then the pH was adjusted to 5.6 with NaOH (1 mol L^{-1}) and volume to 500 mL.

Fresh mature 'Wangzihong' Chinese cherries were harvested directly from an orchard in Zhengzhou (Henan, China). The cherries were harvested in the month of May early in the morning and immediately transported to the laboratory within 2 h. We selected Chinese cherries according to uniform size, colour, and absence of disease and other defects. The weight of each fruit was about 3 to 5 g. Then, we randomly divided the fruit into three lots for different coating treatments. We counted and weighed each lot before the following treatments: distilled water (control group), 1% chitosan and a composite of 1% chitosan containing 0.2% nano-SiOx, all modified to the same pH. We coated the Chinese cherries by dipping them in the coating solutions for 5 min before drying in air for 1 h. For each treatment, the cherries were loosely packed but not sealed in four commercial bags (double high-density polyethylene, 200 mm wide × 300 mm long × 40 μm thick, permeability of O_2: 7.64×10^{-9} mol μm^{-2} s^{-1} Pa^{-1}, permeability of CO_2: 8.09×10^{-9} mol μm^{-2} s^{-1} Pa^{-1}, Aodeju Co., Ltd, Dongguan, Guangdong, China) and stored in temperature controlled chambers at 2 ± 1 °C, RH 75% for up to twenty days. These conditions mimicked the storage conditions in supermarkets and represented adverse conditions for testing the effects of the coating (Abugoch et al., 2016). There were about 200 cherries in each bag.

2.2. Physiochemical analysis

At each sampling, the number of decayed fruits (development of mycelium on the fruit surface, brown spots and a softening of the injured zone) relative to the initial amount of fruits per each lot was counted as fruit decay rate (Chen et al., 2011). For each treatment, every five days, we randomly removed 40 fruit from each bag (total 160 fruit) for analysis (except for the decay rate analysis). We allowed the cherries to stand at 25 °C for about 2 h before we started the test.

Weight loss was determined with 20 fruit. Weight loss was calculated using the equation as follows: Weight loss (%) = $(m_0 - m)\, m_0^{-1}$, in which m and m_0 indicate the individual weight of fruit at present and initially, respectively (Chen et al., 2011). The experiment was conducted in triplicate. We assayed the titratable acidity (TA) with juice obtained from 20 fruit per lot. We assayed TA (expressed as percentage malic acid) by indicator titration of 50 mL diluted juice (we diluted 25 mL of pressed Chinese cherry juice to 250 mL using distilled water) with 0.1 mol L^{-1} NaOH (Wang et al., 2012). The experiment was conducted in triplicate. We determined SSC using a refractometer (WYT-J, Sichuan, China) at 25 °C (Mao et al., 2017). One fruit was used for SSC examination per replicate, and 10 randomly assigned fruit from each treatment were measured. We used a TA-XT2i Texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) to determine the firmness of the cherries. The test condition was set according to a previous report (Zhang et al., 2008) with the following modifications: probe diameter = 5 mm, test speed = 1 mm s^{-1}, pressed distance = 2.5 mm, trigger force = 5.0 g. Firmness was defined as the peak force, which was the maximum resistance force (N) obtained from the force-distance curves. We measured twenty cherries individually for each treatment lot.

2.3. SSP extraction and determination

2.3.1. Cell wall preparation and SSP extraction

Cell wall material was extracted from the Chinese cherry flesh using the method described by Liu et al. (2017a,b) with slight modifications. The peeled flesh of the Chinese cherry (10 g, random from 10 fruit pulp) and boiled it in 200 mL ethanol (80%, v/v) for 20 min. After cooling to room temperature, the samples were filtered using a vacuum pump. The residue was re-boiled twice with ethanol. Next, the residue was incubated overnight at 4 °C with 50 mL mixture of dimethysulphoxide (DMSO, Tianjin Resent Chemical Co., Ltd, China) and water (9:1, v/v) to remove the starch. Subsequently, the samples were transferred to a 200 mL mixture of chloroform and ethanol (2:1, v/v). After 10 min, the samples were filtered and washed using 200 mL acetone until they were totally whitened. This residue collected was the cell wall material.

The cell wall material was suspended in 10 mL double distilled water and shaken for 4 h at 25 °C, and centrifuged at 10000*g* at 4 °C for 10 min (Shanghai Anting Scientific Instrument Factory, Shanghai, China). The above procedure was repeated two more times and the residue was resuspended in 10 mL of 50 mmol L^{-1} cyclohexanetrans-1, 2-diamine tetra-acetate (CDTA, Tianjin Zinco Fine Chemical Institute, China), shaken for 4 h and centrifuged as described above. The residue was then re-extracted twice with CDTA. Finally, the residue was re-suspended in 10 mL of 50 mmol L^{-1} Na_2CO_3 (Na_2CO_3, Tianjin Zinco Fine Chemical Institute, China) containing 2 mmol L^{-1} CDTA, shaken for 4 h and centrifuged as described above. The supernatants were collected and the procedure was repeated two more times. All the three supernatants were collected together as the fraction of SSP. All experiments were conducted in triplicate.

2.3.2. Determination of SSP content

We assayed the extracted SSP content using the carbazole colorimetric method (Liu et al., 2017a,b), using Galacturonic acid (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA) as the standard. Sulfuric acid (12 mL, 98%, w/w) was added to the SSP solution (2 mL), which was then immediately cooled with ice water. Next, the mixture was boiled for 10 min before cooling with running tap water. Carbazole ethanol solution (0.5 mL) was added to the solution, mixed and incubated at room temperature for 30 min, before determining the absorbance at 530 nm using a UV-2000 spectrophotometer (Unico Instrument Co. Ltd., Shanghai, China) at room temperature. The extracted SSP content was expressed as g of galacturonic acid per kg of fresh weight. The SSP content was calculated using the equation as follows: SSP content (g kg^{-1}) = $c\ V\ m^{-1}\ 10^{-3}$, Where c is the concentration of galacturonic acid (μg mL^{-1}), V is the total volume of extracted SSP from fresh cherry (mL) and m is the weight of fresh cherry (g). All experiments were conducted in triplicate and results were expressed as g of galacturonic acid per kg of fresh weight.

2.3.3. Nanostructural characterisation of the SSP

Nanostructure analysis of the SSP was conducted using a Multimode NanoScope IIIa AFM (Digital Instruments, Santa Barbara, CA, USA) equipped with an E (J) scanner (Xin et al., 2010). AFM was performed with tapping mode in a glove box with 30–40% of relative humidity at about 25 °C. The relative humidity inside the glove box was adjusted and stabilised using silica gel before examining the samples. The samples was diluted to appropriate concentration (about 10 μg mL^{-1}) and pipetted 10 μL of samples on freshly cleaved mica sheets. A slight