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 10000g 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$_2$CO$_3$ (Na$_2$CO$_3$, 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

molecular combing technique (exerting minimum force on the solution) using a glass cover slip was applied to comb the solutions, which pulls the pectin out without taking the chelating agent away (Yang, 2014). The solution on the mica was dried in air at room temperature and the tapping mode function for AFM imaging was performed using a Si_3N_4 cantilever with resolution of 0.1 nm in vertical and 1–2 nm in horizontal positions. The scan rate was about 0.5–2 Hz. AFM images were analysed offline using AFM software (Version 5.30r3sr3). Qualitative information was obtained through identification of pectin characteristics, such as branch chains, aggregates and others, and quantitative data was obtained using sectional analysis from the width of pectin chains.

2.4. Statistical analysis

Values of physicochemical properties, SSP contents and AFM results were recorded as means ± standard deviations. Analysis of variance (ANOVA) and Duncan's test were performed to examine the differences between groups by SAS software (SAS Institute Inc., Cary, NC, USA) with $P \leq 0.05$ was considered as significant.

3. Results and discussion

3.1. Effects of coating treatments on weight loss, decay rate and firmness of the Chinese cherry

Fig. 1 shows the weight loss, decay rate and firmness of Chinese cherries during a twenty days storage period using different coating treatments. The loss of fruit weight during postharvest storage can be approximately attributed to the water loss caused by transpiration and respiration processes (Petriccione et al., 2014). Fresh cherries lose significant water content from both the fruit and stem during postharvest, mainly due to their low skin diffusion resistance (Serrano et al., 2005) and relatively high surface/volume ratio (Conte et al., 2009). As shown in Fig. 1A, all groups exhibited increased weight loss during storage. Interestingly, the coated samples demonstrated slightly lower weight loss compared with the control group. More specifically, during the first five days, we observed no difference in weight loss between the samples coated with single chitosan and a composite of chitosan and nano-SiOx ($P > 0.05$); after five days, we observed notable differences between them. At the end of the storage period, the weight loss of the cherries coated with single chitosan and a composite of chitosan and nano-SiOx was only 14% and 9%, respectively, which was 29% and 51% lower than that of the control, respectively ($P \leq 0.05$). For Chinese cherry, the acceptable weight loss was about 10%. The values for control and single chitosan coated cherries were beyond 10% at 15 d while the number was less than 10% at 20 d for composite coated cherries. At least five extra days of storage life have been achieved with the composite coating, relative to the control and single chitosan coating. These data indicate that composite coating with chitosan and nano-SiOx on the fruit surface effectively prevents moisture loss of the Chinese cherry.

Chitosan coating acts as a semipermeable film that regulates gas exchange and coats the stomata. This leads to a decrease in transpiration and respiration rate, and therefore, limits the transference of water from the surface of the cherries to the surrounding environment (Bautista-Baños et al., 2006). The beneficial effect of chitosan coating has already been demonstrated in a wide range of fruits, including the sweet cherry (Yaman and Bayindirli, 2002), blueberry (Duan et al., 2011) and banana (Maqbool et al., 2010). In addition, the combination of chitosan and nano-SiOx has been previously demonstrated to have a superior protective effect on weight loss, potentially due to the additional barrier form nano-SiOx against diffusion through stomata (Shi et al., 2013). This result agrees well with a study on jujube (Yu et al., 2012) and longan (Shi et al., 2013).

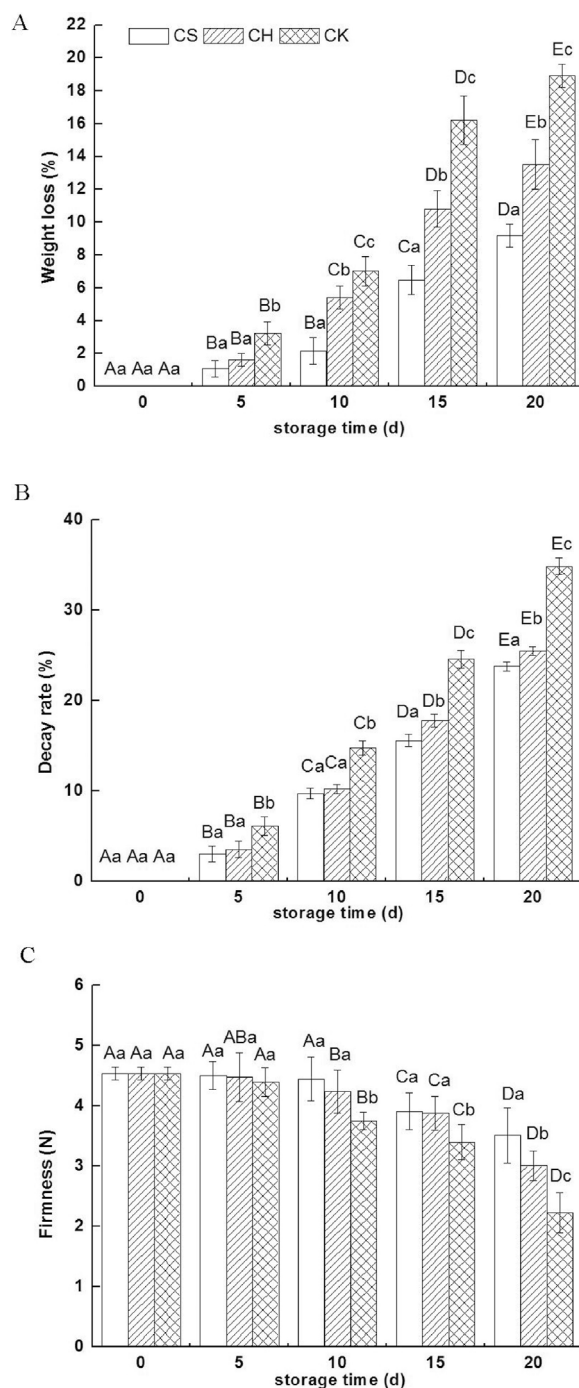In our study, the Chinese cherry decay rate increased with storage

Fig. 1. Different coating treatments affect weight loss, decay rate and firmness of the Chinese cherry. (A) Weight loss (n = 3); (B) Decay rate (n = 3); (C) Firmness (n = 20). Note: CK: control group; CH: Chinese cherry coated with chitosan; CS: Chinese cherry coated with chitosan combined with nano-SiOx.
Data are presented as means ± standard deviation. The different lowercase superscript letters in the same storage time indicate significant differences within different coating treatments ($P \leq 0.05$). The different capital superscript letters in the same coating treatment indicate significant differences within different storage times ($P \leq 0.05$)

time (Fig. 1B); after five days, all of the cherries began to rot. However, the rates of decay for the coated samples over the whole storage period were significant lower than that of the control group ($P \leq 0.05$). After ten days, the decay rates of the samples coated with a composite of chitosan and nano-SiOx were the lowest among all the groups ($P \leq 0.05$). The antifungal and antimicrobial actions of chitosan are believed to originate from its polycationic nature (Elsabee and Abdou, 2013). The antimicrobial activity of chitosan is due to the amino

protonation and subsequent cationic formation on its molecular side chain in acidic solution. The protonated amino groups (NH_2) have electrostatic forces with the anionic groups of microbial cell membranes, leading to the leakage of cell constituents and cell death (Qin et al., 2006; Li et al., 2016). Chitosan can also bind with microbial DNA to inhibit the synthesis of mRNA, which subsequently suppresses microbial growth (Sudarshan et al., 1992). According to Dhanasingh and Mallesha Hiriyannaiah, (2011), chitosan/silica films exhibit good antimicrobial activity against Gram-positive food-borne pathogens than chitosan alone. This is because nanoparticles are able to penetrate into the cells, causing a significant increase in membrane permeability, which ultimately leads to cell death (Kanmani and Rhim, 2014).

The storage of fruit is one of the most important factors affecting their firmness. Fig. 1C shows how firmness is affected in the different treatment groups and at different storage times. In general, cherry firmness decreased during the storage period. During the first five days, there was no significant change in firmness between the different groups ($P > 0.05$). Uncoated cherries began to soften at 10 d, while cherries coated with a plain and composite chitosan coating began to soften at 15 d. In general, during days ten and twenty of storage, the firmness of the coated samples was significant higher than the control group. The firmest samples were those treated with a composite of chitosan and nano-SiOx, of which the firmness was 3.51 N at 20 d, followed by the chitosan only treated samples (3.01 N) and the control group (2.23 N). Opposing firmness outcomes were obtained in different weight loss ranges. Regardless of the treatment and time in storage, Chinese cherry firming occurred consistently with low levels of weight loss whereas softening was simultaneously observed with higher weight loss (Fig. 1A). The results of this work are in agreement with the work of Paniagua et al. (2013) who reported that moisture loss is the major cause of firmness change during postharvest storage of blueberry. The firming of blueberries simultaneously with 0.22–1.34% weight loss but fruit softening when weight loss was 3.47–15.06%. Moisture loss has been previously suggested to induce postharvest softening in fruits due to reduced turgor (Heyes and Sealey, 1996; Allan-Wojtas et al., 2001). Alternatively, those changes also can be attributed to the loss of integrity of the cell wall structure, which leads to softening (Qi et al., 2011).

3.2. Effects of coatings on the SSC, TA and SSP content in the Chinese cherry

Fig. 2 shows the effects of chitosan coatings on the contents of SSC, TA and SSP content in the Chinese cherry. It is widely accepted that taste is the most important quality parameter in determining cherry consumer acceptability, which is mainly due to the ratio between SSC and TA. Fig. 2A shows the SSC for the samples during the twenty days storage period with different coating treatments. We observed a significant increase in SSC at the beginning of the storage period (Comabella and Lara, 2013; Petriccione et al., 2014). This increase could be attributed to the hydrolysis of starch and other polysaccharides to sugar (Comabella and Lara, 2013; Petriccione et al., 2014). After this initial increase, a decreasing trend of SSC in all sample groups was observed. At the end of the storage period, the samples treated with chitosan combined with nano-SiOx had the highest SSC (13.0%) compared with the chitosan-only coated samples (12.7%) and the control samples (12.3%). According to the results of weight loss (Fig. 1A), at the end of the storage, the weight loss (mainly water loss) of chitosan alone coated and uncoated cherries was more than 10%. Some increase of SSC value was possibly related to moisture loss (Abugoch et al., 2016). The more water lost the more SSC value increases. However, it did not affect the cherries coated with a composite of chitosan and nano-SiOx with the highest SSC at the end of storage. It has been well documented that chitosan combined with nano-SiOx formed an excellent semipermeable film around the cherry, modifying the internal atmosphere, leading to slower respiration and metabolic
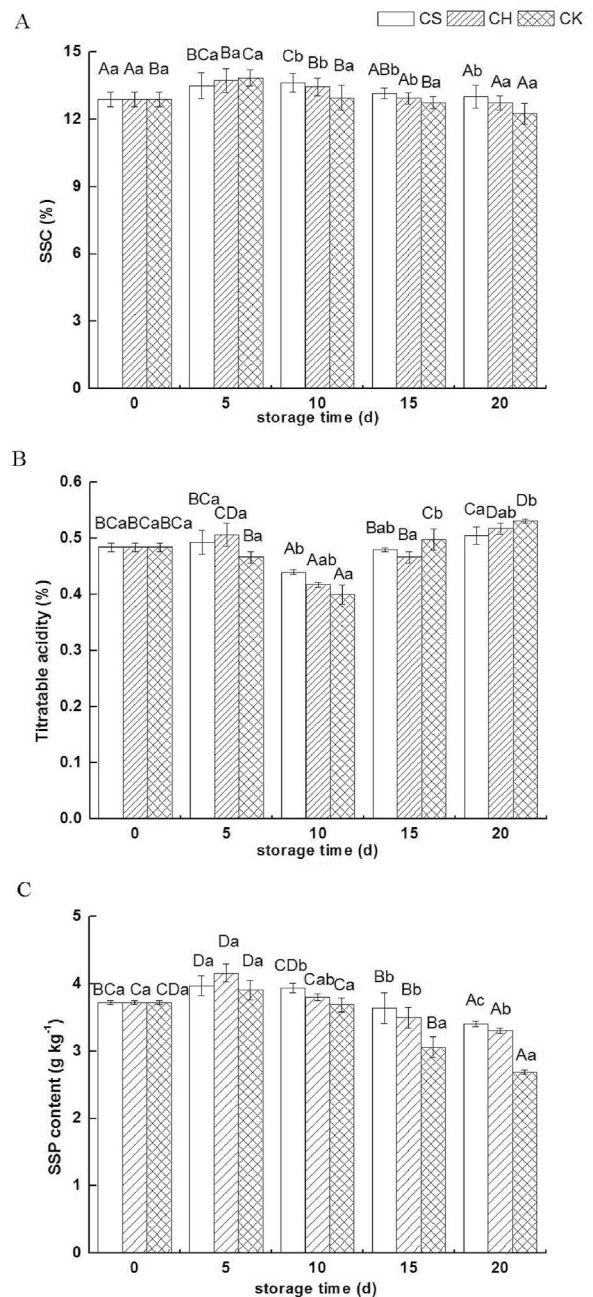

Fig. 2. Different coating treatments affect the content of SSC, TA and SSP of the Chinese cherry. (A) SSC (n = 10); (B) TA (n = 3); (C) SSP (n = 3).
Note: CK: control group; CH: Chinese cherry coated with chitosan; CS: Chinese cherry coated with chitosan combined with nano-SiOx.
Data are presented as means ± standard deviation. The different lowercase superscript letters in the same storage time indicate significant differences within different coating treatments ($P \leq 0.05$). The different capital superscript letters in the same coating treatment indicate significant differences within different storage times ($P \leq 0.05$)

activity, hence retarding the depletion of SSC (Shi et al., 2013). In addition, hydrogen bond interactions between chitosan and the hydroxyl on the surface of nano-SiOx adjusted the dissolving, diffusing and evaporation of gas in the coating, as such, the gas permeability of the coatings was affected by the dosage of nano-SiOx (Sun et al., 2016). During the storage period, a downward or rising trend of SSC was also reported in cherry treated with chitosan (Shi et al., 2013; Petriccione et al., 2014). However, other studies like honeydew melon coated with chitosan indicated that SSC didn't significant change in the untreated fruit (Chong et al., 2015). Many confounding factors may cause this discrepancy, e.g. the fruit varieties and species, its stage of ripeness, the
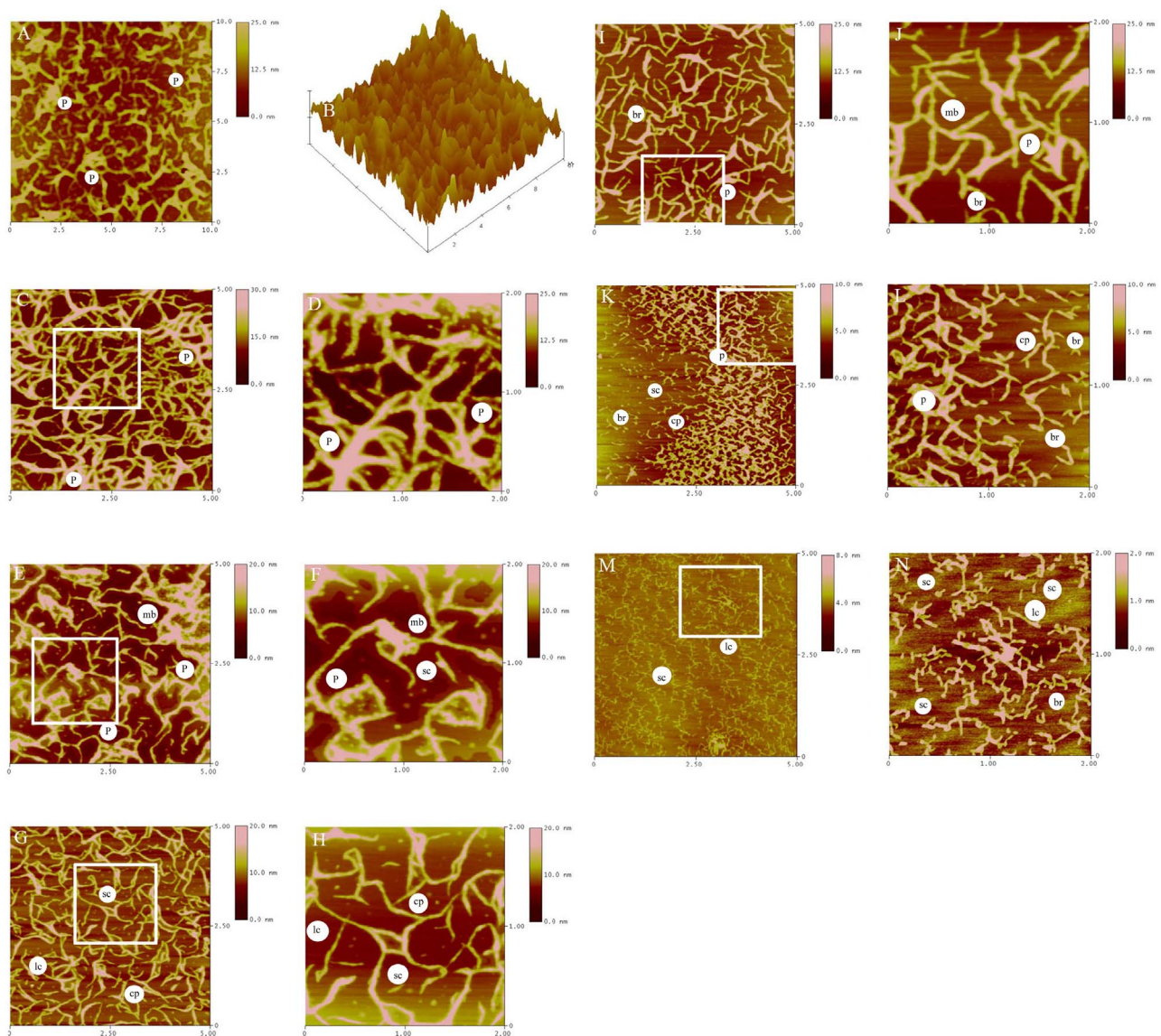
Fig. 3. AFM images of SSP chains from the Chinese cherry.

(A) Fresh cherry at 1 d; scan size: $10 \times 10\ \mu m^2$; (B) Its corresponding 3-dimensional image; scan size: $10 \times 10\ \mu m^2$; (C) Chitosan combined with nano-SiOx group at 10 d; scan size: $5 \times 5\ \mu m^2$; (D) Zoom plane image in the marked region of (C); scan size: $2 \times 2\ \mu m^2$; (E) Chitosan group at 10 d; scan size: $5 \times 5\ \mu m^2$; (F) Zoom plane image in the marked region of (E); scan size: $2 \times 2\ \mu m^2$; (G) Control group at 10 d; scan size: $5 \times 5\ \mu m^2$; (H) Zoom plane image in the marked region of (G); scan size: $2 \times 2\ \mu m^2$; (I) Chitosan combined with nano-SiOx group at 20 d; scan size: $5 \times 5\ \mu m^2$; (J) Zoom plane image in the marked region of (I); scan size: $2 \times 2\ \mu m^2$; (K) Chitosan group at 20 d; scan size: $5 \times 5\ \mu m^2$; (L) Zoom plane image in the marked region of (K); scan size: $2 \times 2\ \mu m^2$; (M) Control group at 20 d; scan size: $5 \times 5\ \mu m^2$; (N) Zoom plane image in the marked region of (M); scan size: $2 \times 2\ \mu m^2$.

Note: p: polymers; cp: cleavage point; lc: long chain; sc: short chain; br: branching; mb: multiple branched chain. Seventy parallel imaging tests for pectin were conducted by AFM to obtain reliable, representative results (Fig. 3).

storage conditions, the concentration and composition of coating solution, and so on.

Storage and distribution life of cherries ultimately affect the fruit's taste. This change in taste is caused by a decline in fruit acid content; therefore, reducing the rate of acidity loss is a critical objective for extending the potential marketing period (Mattheis et al., 1997). We noted a decrease in TA in both the coated and control cherries during the initial ten days of storage (Fig. 2B). This was because organic acids were substrates for respiratory processes (Díaz-Mula et al., 2011), which became depleted during storage. However, TA significantly increased after ten days of storage in all sample groups. The increment of TA at the last stage of storage was probably due to the accumulation of acids from D-glucose by glycolytic enzyme systems (Liu et al., 2009). During the different storage period, the acidity levels of cherry varied depending on what the main type of metabolic pathway in vivo was involved. Similar to SSC, at the end of storage, some increase of TA

value was possibly related to big water loss. But it did not affect the changes of TA during whole storage and the preservation effect of the composite coating. In the current study, chitosan combined with nano-SiOx coating treatments slowed down changes in TA of Chinese cherry and was thus effective in delaying fruit ripening. This was potentially due to the coating layer acting as a protection against metabolic changes. These results were similar to those reported by Shi et al. (2013) on the longan fruit, which showed that a combination of chitosan and nano-silica successfully inhibited TA changes during storage.

SSP content of the cherries under different treatment conditions are shown in Fig. 2C. WSP and CSP contents were all measured (Tables S1 and S2). However, Zhang et al. (2008) reported that Chinese cherry softening was closely related to the content and solubility of SSP. Therefore, only the changes of SSP were discussed. During storage, the SSP content decreased from 3.73 to 3.41 g kg^{-1} for the samples coated with chitosan combined with nano-SiOx; to 3.31 g kg^{-1} for the samples
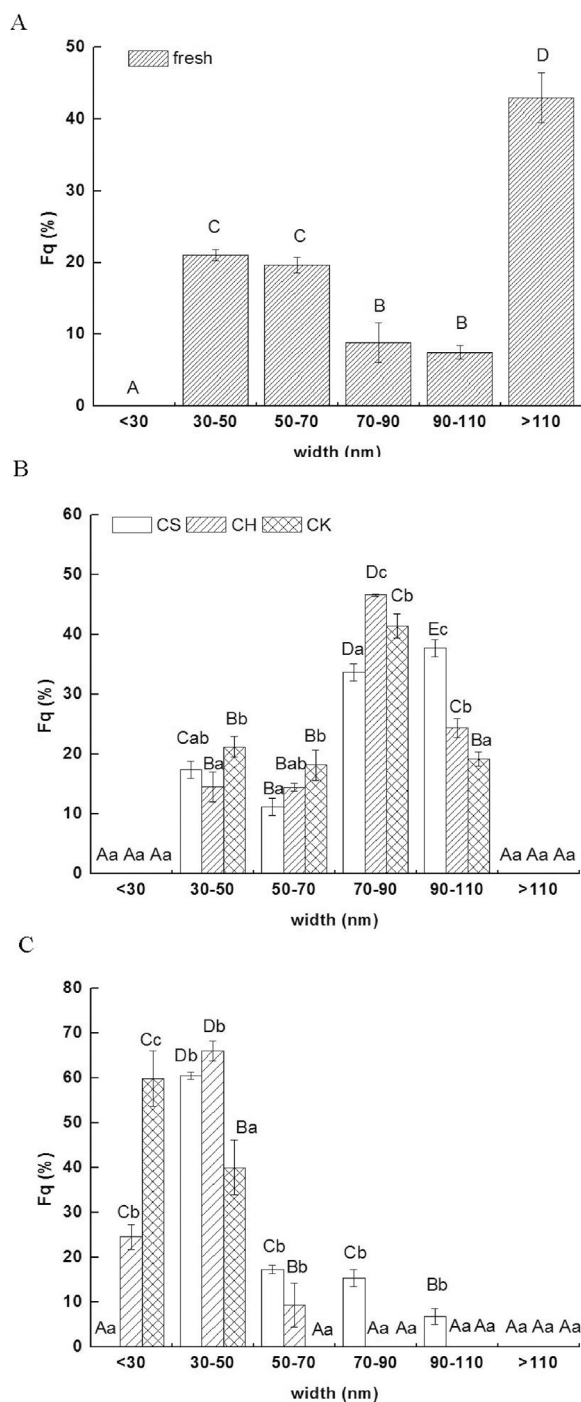
Fig. 4. Different coating treatments affect the width distribution of SSP chains of the Chinese cherry. Width at (A) 1 d; (B) 10 d; (C) 20 d.
Note: CK: control group; CH: Chinese cherry coated with chitosan; CS: Chinese cherry coated with chitosan combined with nano-SiOx.
Each result for different treatment group and storage time in Fig. 4 was from the quantitative information of thirty AFM images containing about fifty pectin chains. Data are presented as means ± standard deviation. Means within same ranges of width with different small case letters are significantly different ($P \leq 0.05$); means for same treatments on different ranges of width with different capital letters are significantly different ($P \leq 0.05$).

coated with chitosan only; and to 2.69 g kg^{-1} for the control group. At any given storage time, the SSP content of the coated samples was slightly higher than those of the control samples. Typically, SSP is solubilised and sequentially disassembled through increased depolymerisation of various pectinase classes (Cosgrove, 2005; Ortiz et al.,

2011a). Reports have shown that decreased content of SSP is significantly and closely related to fruit softening during ripening (Zhang et al., 2010; Chen et al., 2011; Ortiz et al., 2011b), which is consistent with our results (Fig. 1C). We noted reduced firmness, which corresponded with decreased SSP content during storage, especially our uncoated samples, which began to soften at 10 d. During days ten to twenty of storage, the firmness of the coated samples was significant higher than the control group, which corresponded with increased SSP content. At the end of storage, SSP content of the cherries coated with a composite of chitosan and nano-SiOx was significantly higher than the cherries coated with single chitosan. The results were consistent with the firmness (Fig. 1C).

3.3. Qualitative results of SSP nanostructures

Even though changes in SSP content are important for illustrating the texture and softening of fruits during storage, changes in the fruit cell wall better correlate with softening and are widely viewed as the nanostructural characteristics of polysaccharides and their interactions (Brummell 2006; Geitmann 2010; Wang et al., 2012). Previously, AFM has been successfully applied in describing pectin depolymerization and degradation during storage (Xin et al., 2011; Liu et al., 2017a,b; Yang et al., 2017; Zhang et al., 2017). Heterogeneous SSP nanostructures including polymers (p), cleavage point (cp), long chain (lc), short chain (sc), branching (br) and multiple branched chains (mb), have all been directly imaged by AFM (Zhang et al., 2008, 2010).

Fig. 3 shows the nanostructural morphologies of SSP chains including qualitative characteristics. The effects of coating and storage time on the SSP nanostructure are illustrated within the figure. The color bar legends beside the image are z-value scales, which represent the height of the scanned samples (Zhang et al., 2008). The AFM images of the SSP indicate that most of the SSPs form large aggregates (Fig. 3A, B). Compared with fresh cherries, it was observed that fewer polymers and more cleavage points, long chains, short chains, branching and multiple branched chains in cherries at 10 d (Fig. 3C–H) and 20 d (Fig. 3I–N). Besides, significantly different pectin morphologies was found between the control and coated groups. Compared with the control group, the coated cherries had more polymers that entangled together and multiple branched chains at 10 d (Fig. 3C–F). In contrast, in the control cherries, significantly more cleavage points, long chains and short chains were found (Fig. 3G, H). At the end of the storage period, SSPs from all the treatment groups exhibited branching, long chains and short chains. However, in the control cherries, there was a slight increase in the number of short chains, whereby most of the chains were randomly distributed instead of crosslinking with each other (Fig. 3M, N). In comparison, for the treated cherries, especially composite coating treated cherries many of the chains were still linked with other chains (Figs. 3I–L). In particular, fewer short chains in the cherries coated with chitosan combined with nano-SiOx. Most of the SSP chains exhibited a branched structure.

3.4. Coating effect on width distribution of SSP chains of the Chinese cherry

Qualitative information was obtained to elucidate the characteristics of SSP chains by applying the 'section analysis' function on the AFM software. Fig. 4 displays the effects of coating treatment and storage time on the width of SSP chains. The frequency of SSP chain width represents the number of times the specific width range is presented (Chong et al., 2015). As shown in Fig. 4, SSP chain width is heavily influenced by storage time; the frequency of small chains increased with storage time. For example, the width of SSP chains from cherries at the beginning of the storage period (0 d) was mostly greater than 110 nm (43.0%). The highest frequency of width decreased to the range of 70–90 nm during the middle stages of the storage period (10 d). At the end of the storage period (20 d), higher frequencies of widths in the range of 30–50 nm and shorter than 30 nm were found.

Cherries coated with chitosan combined with nano-SiOx contained fewer SSP short chains than the control and the chitosan-coated groups. The frequency of widths smaller than 30 nm was 60.0% for the control group and 24.5% for the chitosan-coated cherries, while frequency for cherries coated with chitosan combined with nano-SiOx was zero.

According to the AFM results, decreased chain width is associated with depolymerisation of SSP. Pectin chains depolymerise during fruit storage, which is caused by the effects of pectinesterase and poly-galacturonase (Yaman and Bayoindirli, 2002). This enzymatic action may contribute to cell wall disaggregation or loosening in-between cells, which leads to property changes in macroscopic firmness (Zdunek et al., 2014). In this study, chitosan combined with nano-SiOx coating treatment improved fruit firmness and inhibited the degradation of pectin chains. The reason may be that the composite coating inhibited the respiration and metabolism rate of cherry, thus reducing the activity of pectin degrading enzyme. Similar results were reported by Gol et al. (2013), who found that the carambola fruit coated with chitosan based coating maintained a high value of texture and low activity of PG, cellulase, PME and β-Gal. A combined treatment of chitosan and nano-SiOx was the most effective at maintaining firmness. These results suggest that a coating treatment of chitosan combined with nano-SiOx synergistically keeps the firmness of the Chinese cherry by maintaining the integrity of SSP.

4. Conclusions

Coating with 1% chitosan or a composite of 1% chitosan containing 0.2% nano-SiOx is effective in delaying the evolution of Chinese cherry postharvest ripening, including weight loss, decay rate, firmness, SSC and TA. In addition, combined chitosan and nano-SiOx treatment retarded the quality deterioration of the fruit, and maintained a higher content of SSP and inhibited the degradation of pectin chains. Qualitative and quantitative analyses on SSP suggest that the softening of Chinese cherry is due to the modifications of SSP, especially SSP polymers and branched chains. Altogether, these results indicate that coating cherries in chitosan combined with nano-SiOx poses an attractive alternative to preserve the quality of postharvest cherry fruit.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2017.06.010.

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