Food Bioprocess Technol (2017) 10:349–357
DOI 10.1007/s11947-016-1820-0

ORIGINAL PAPER

Influence of Rice Bran Wax Coating on the Physicochemical Properties and Pectin Nanostructure of Cherry Tomatoes

Lifen Zhang[1,2,3] · **Fusheng Chen**[1] · **Penglong Zhang**[1,2,3] · **Shaojuan Lai**[1,4] · **Hongshun Yang**[2,3]

Received: 6 August 2016 / Accepted: 30 October 2016 / Published online: 15 November 2016

Abstract The effects of rice bran wax coating on the physicochemical properties such as firmness, weight loss, titratable acidity (TA) and soluble solid content (SSC) of cherry tomatoes were studied during cold storage. The chemical and nanostructure properties of chelate-soluble pectin (CSP) were also investigated by high-performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FTIR) and atomic force microscopy (AFM). The results indicated that there was no significant difference of firmness between control (2.48 N) and waxed (2.87 N) fruits at the end of storage (20 days), while the weight loss of waxed fruits (13.54%) was lower than that of control fruits (16.02%). And the degree of esterification (DE) of both fruits decreased after cold storage by FTIR. The structural analysis by atomic force microscopy (AFM) indicated that rice bran wax coating inhibited the degradation of CSP. The CSP molecular widths ranged from 15 to 250 nm, and the vertical heights varied from 0.2 to 2.0 nm. Greater frequency (F_q) of large width and length CSP was found in waxed fruits than in control fruits. The results suggest that rice bran wax coating was an effective way to preserve fresh fruits.

Keywords Rice bran wax · Fruit coating · Chelate-soluble pectin · Cherry tomato · Edible coating · Atomic force microscopy · Nanostructure

✉ Fusheng Chen
fushengc@haut.edu.cn

✉ Hongshun Yang
chmynghs@nus.edu.sg

[1] College of Food Science and Technology, Henan University of Technology, Zhengzhou, Henan 450001, People's Republic of China

[2] Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

[3] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, People's Republic of China

[4] Guangzhou Pulu Medical Technology Co., Ltd, Guangzhou, Guangdong 510800, People's Republic of China

Introduction

Cherry tomato (*Lycopersicon esculentum*) is an abundant crop in China. It is rich in antioxidants (vitamin C, total phenols and carotenoids) and appreciated by consumers for its nutritional value, sweet taste and pleasant aroma (Raffo et al. 2002; Lenucci et al. 2006). In many countries, fresh cherry tomatoes play an important role in produce markets. However, considerable amounts of cherry tomatoes are spoiled during fruit growth and postharvest. These food losses are due to the diverse regional climates and undeveloped cold chain transportation, especially in some areas in China (Zhao et al. 2010).

Storage technologies have been developed to extend the shelf life of cherry tomatoes. For example, combined treatment of heat and modified atmosphere packaging delayed the colour development of cherry tomatoes (Ali et al. 2004). Controlled atmosphere storage and modified atmosphere packaging and edible coating improved the postharvest quality and shelf life of fruits (Baraiya et al. 2015; Daş et al. 2006). In addition, a complex coating (0.05% konjak powder, 0.05% sodium alginate and 0.02% ascorbic acid) reduced the decay rate of cherry tomatoes during cold storage (Gang et al. 2007). However, cassia oil, magnesium sulphate or their combination did not affect the quality of cherry tomatoes (Feng et al. 2008).

In recent years, wax coating has been widely used as a coating agent for preserving fruits and vegetables. It plays an important role in prolonging fruit quality. Candelilla wax

coating was shown to improve the shelf life and quality of avocado (Saucedo-Pompa et al. 2009). Carnauba wax or polyethylene coating supplemented with imazalil effectively preserved the fruit quality of sweet oranges (Njombolwana et al. 2013). Rice bran wax, a by-product from refining rice bran oil, composed of a mixture of esters of fatty alcohol and fatty acids, is becoming a popular food ingredient for health-conscious consumers. It could be used as a carrier of flavour and nutritional additives and a water-barrier coating for foods (Shih et al. 2011). Rice bran wax coating, a potential good source for processing organic foods, has the advantage of being cheap, stable and having good film-forming ability (Yu and Yang 2017; Zhang and Yang 2017; Zhao et al. 2017; Li et al. 2015). As a consequence, the use of rice bran wax to preserve fruits and vegetable has great future potential. In addition, better utilisation of cereal by-products including rice bran could enhance the food and water security globally (Valipour et al. 2015; Valipour 2015a, b; Yu et al. 2014).

To date, there are few reports on the rice bran wax coating of fruits. Most researchers focused on the influence of wax coating on the physicochemical properties and shelf life of fruits. But the mechanism underlying wax coating remains unclear. The aim of this current study was to elucidate the relationship between cell wall polysaccharides and texture of rice bran wax-coated fruits at the nanoscale. The effect of rice bran wax coating on certain physicochemical properties of cherry tomatoes was also studied. The relationship between the softening of fruit and chelate-soluble pectin was elucidated as well. The results provide a theoretical basis for the preservation of fruits and vegetables.

Materials and Methods

Materials

Cherry tomatoes (*Lycopersicon esculentum Mill*. cv. 'Mali') were picked by hand from a local farmland in Zhengzhou, Henan Province, China. The cherry tomatoes were transported to a laboratory set at 25 °C within 2 h after harvest and selected according to uniform colour (light-red stage), size, shape and the absence of visible physical injury (Lai et al. 2013; Wang et al. 2015). The selected samples were coated by rice bran wax and stored at 0 °C immediately.

Coating Treatment of Cherry Tomatoes

Rice bran wax coating was prepared as follows: 10 g rice bran wax (Beijing Jiade Biotechnology Co. Ltd., Beijing, China) and 10 g sucrose ester (Hangzhou Ruilin Chemical Co. Ltd., Hangzhou, Zhejiang, China) were mixed with 1000 mL distilled water. The solution was emulsified by high-shear homogeniser for 10 min at 10,000×*g* and diluted 100 times.

The selected cherry tomatoes were dipped in 0.01% coating solution for 3 min and kept at room temperature for 1 h. The control samples were dipped in the same solution except that the solution's rice bran wax was replaced by water. The treated and control samples were placed in fruit packing box and stored at 0 °C. Ten fruits of the treatment group and of the control group were randomly selected from storage and used for analyses every 5 days.

Cell Wall Material Preparation and Chelate-Soluble Pectin Extraction and Determination

Cell wall material preparation and chelate-soluble pectin (CSP) extraction were conducted according to Zhang and others (2008). Flesh samples (10 g) were placed in 200 mL ethanol (80%, *v/v*) for 20 min and cooled to room temperature. The samples were filtrated, and the residue was re-extracted two more times. Then, the residue was incubated overnight at 4 °C with 50 mL of dimethyl sulfoxide (DMSO) and water (9:1, *v/v*). After that, the residue was transferred to a 200 mL mixture of chloroform and ethanol (2:1, *v/v*) for 10 min. The final sample was washed with acetone until total whitening. The residue obtained was cell wall materials. The cell wall materials were added with sodium acetate buffer (10 mL, 50 mM, pH 6.5), agitated at 25 °C for 4 h, centrifuged at 10,000*g*, 4 °C for 10 min, and the supernatant was collected. The above process was repeated twice more. Then, the water-insoluble pellet was resuspended in 10 mL of 50 mM sodium acetate buffer (pH 6.5) containing 50 mM cyclohexane-trans-1,2-diamine tetra-acetate (CDTA). After the extraction process was repeated two more times, the supernatants were collected together and combined as CSP. The content of CSP was assayed by carbazole colorimetry method with galacturonic acid as standard. More detailedly, the CSP solution (2 mL) was mixed with sulphuric acid (12 mL, 98%, *w/w*) in a test tube and cooled immediately. Then, the mixture was boiled for 10 min and cooled using tap water. Carbazole ethanol solution (0.5 mL) was added into the solution and incubated at room temperature for 30 min. The absorbance at 530 nm was determined using a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd, Beijing, China). All experiments were conducted in triplicates.

Physicochemical Property Determination of Cherry Tomato

The firmness of cherry tomatoes was evaluated by using a texture analyser (TA-XT2i, Stable Micro System, UK). The samples were equilibrated to room temperature before analysis. A cylindrical probe with diameter 10 mm was used. The pre-test and test speed were 5.00 and 1.00 mm s^{-1}, respectively. The pressed distance was 2.5 mm. At least ten samples without destruction were determined individually for each

independent experiment, and an average hardness of ten samples was calculated (Chen et al. 2011).

Weight loss was also determined with ten fruits. The fruits were taken out from cold storage and placed at room temperature for 3 h before the test. Weight loss was calculated using the equation as follows: weight loss (%) = $(m_0 - m)\ m_0^{-1}$, where m and m_0 are the weight of each fruit at present and originally, respectively. Soluble solid content (SSC) was determined with fruit juice made from 20 samples by a portable digital refractometer (model WYT-J; Chengdu Xing Chen Optical Instrument Co., Ltd, Chengdu, Sichuan, China). Titratable acidity (TA) was assayed by a titration method according to Chen et al. (2011). Diluted juice (50 mL, diluted from 10 mL of pressed cherry tomato juice with distilled water) was titrated with 0.1 M NaOH until the solution colour changed into pink without fading in 30 s.

Monosaccharide Constituent Analysis of CSP

The monosaccharide constituent analysis of CSP was done by HPLC according to a previous method with slight modifications (Xin et al. 2010). CSP (2 mg) was dissolved in 2 M trifluoroacetic acid and hydrolyzed at 110 °C for 8 h. Each hydrolysed sample was dried and dissolved in 450 μL NaOH (0.3 M) and 450 μL 1-phenyl-3-5-pyrazolone (PMP). Then, lactose, as an internal standard, was added to each sample before derivatization. The mixture was reacted for 30 min at 70 °C in a thermostatic water bath (model DC-1006; Safe Corporation, Ningbo, Zhejiang, China), cooled to room temperature, neutralised with 450 μL HCl (0.3 M), and added with chloroform (1.0 mL). The above process was repeated for two more times. Finally, the solution was filtered through a 0.45-μm membrane before HPLC analysis.

Analysis of the PMP-labelled monosaccharides was carried out with a Waters 2695 HPLC system (Waters, Milford, MA, USA), with a PDA 2996 detector (Waters, Milford, MA, USA) and a Zorbax Aclips XDB-C18 column (4.6 mm × 250 mm, 5 μm; Agilent Technologies, Inc., Richardson, TX, USA). The mobile phase consisted of 0.05 M sodium phosphate (pH 6.9) with (A) 15% and (B) 40% acetonitrile. A linear gradient elution of 0–15% (0–10 min) then 15–25% buffer B (10–30 min) was used. The flow rate of mobile phase was 1 mL min^{-1}, and the wavelength of PDA detection was 250 nm.

FTIR Analysis of CSP

CSP (2 mg) was mixed with KBr (1:100, *w/w*) and pressed into KBr pellet for FTIR analysis. FTIR spectra Nicolet 5700 (Thermo Fisher Scientific, Boston, MA, USA) were collected in the frequency range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with absorbance mode. The results were analysed using Origin 8.0 software.

AFM Analysis of CSP

The nanostructural characterisation of CSP was conducted in air using a Multimode NanoScope IIIa AFM (Digital Instruments, Santa Barbara, CA, USA) in tapping mode (Chen et al. 2013). Each pectin solution (10 μg mL^{-1}) was mixed 3 min by a XW-80A Vortex mixer (Shanghai Jinke Co., Ltd., Shanghai, China). Then, 10 μL of the solution was pipetted onto a freshly cleaved mica sheet surface and dried in air at room temperature. The imaging was conducted with a Si_3N_4 tip. The resonance frequency of the tip was 330 kHz, and the scan rate was about 0.5–2 Hz.

The AFM images were analysed offline with section analysis (AFM software, version 5.30r3sr3) to obtain both qualitative and quantitative information of pectin. The quantitative parameters (width, length and height) of pectin molecules were recorded as W, L and V, respectively (Yang et al. 2006).

Statistical Analysis

All experiments were conducted independently in triplicates. The physicochemical properties and CSP structure of the cherry tomatoes were analysed using Excel 2010 and Origin 8.0 software. Dozens of parallel imaging tests for pectin were conducted by AFM to obtain reliable, representative and statistically valid results.

Results and Discussion

Effect of Rice Bran Wax Coating on the Physicochemical Properties of Cherry Tomatoes

The effect of rice bran wax coating on the physicochemical properties of cherry tomatoes is shown in Fig. 1. The firmness of cherry tomatoes decreased in both the control and waxed fruits during storage at 0 °C (Fig. 1a). However, the firmness of the control fruits decreased faster than the wax-coated group during the first 10 days of storage. On the fifth day, the firmness decreased about 50.6% for the control fruits while only 30.2% for the waxed fruits. Firmness is one of the most important textural components of fruits. This result indicated that rice bran wax coating could inhibit the rate of texture changes of cherry tomatoes during cold storage, although no significant differences of firmness were observed between the control (2.48 N) and waxed (2.87 N) fruits at the end of cold storage (20 days).

Significant increase of weight loss in both the control and wax-coated cherry tomatoes were found (Fig. 1b), although the weight loss of wax-coated fruits was significantly lower than that of the control (13.54 vs. 16.02%, at day 20). The difference might be caused by the enhanced resistance of water loss because of wax coating, as previous reports indicated

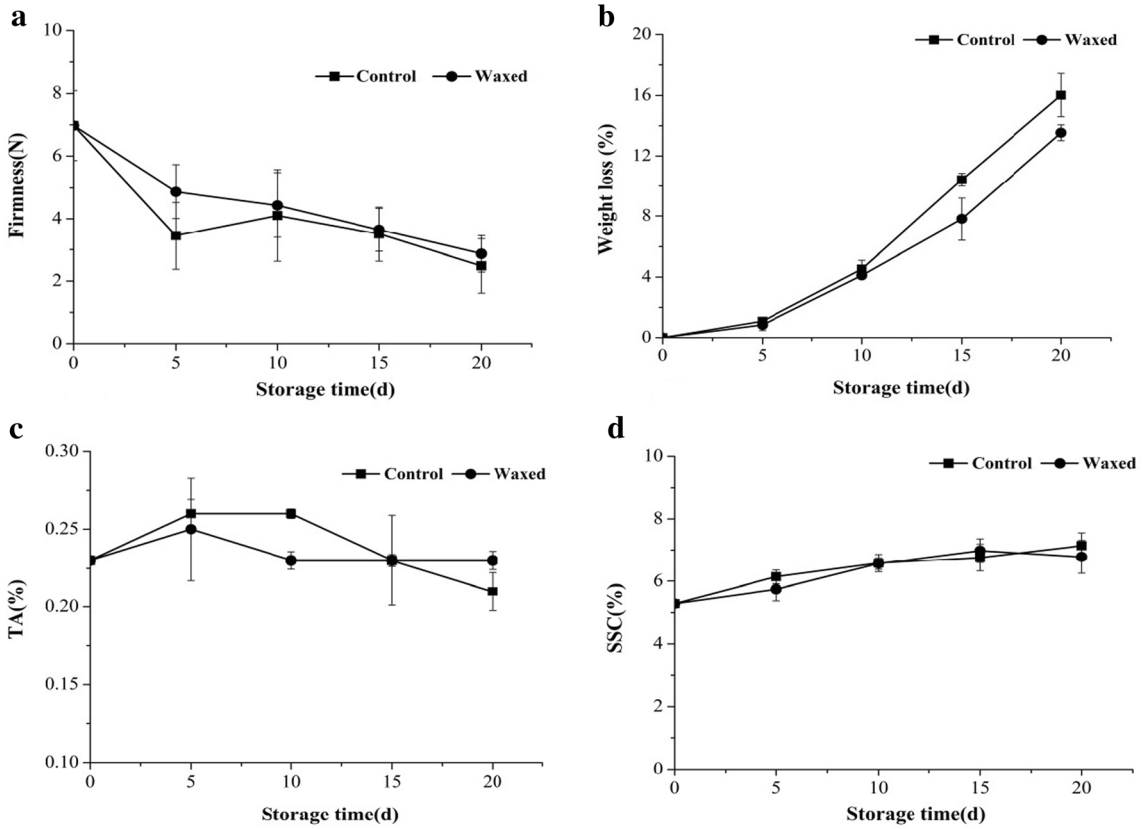

Fig. 1 Effect of rice bran wax coating on the firmness (**a**), weight loss (**b**), TA (**c**) and SSC (**d**) properties of cherry tomatoes. Note: *TA* titratable acidity, *SSC* soluble solid content

that lipid-based coatings such as beeswax and carnauba also had similar barrier property (Baldwin et al. 1995).

Citric acid, malic acid and oxalic acid are three main organic acids of cherry tomatoes (Raffo et al. 2002). In control fruits, the TA content was increased after 10 days of cold storage (Fig. 1c) (from 0.23 to 0.26%); however, from then, TA decreased until the end of storage (0.21%). As a comparison, the TA of wax-coated fruits remained unchanged during storage. Increasing TA could be caused by increased respiration rate and production of high level CO_2, affecting the glycolytic enzyme systems and resulting in accumulation of acids (Liu et al. 2009). Coating is generally regarded as inhibiting respiration rate of postharvest fruits (Chen and Nussinovitch 2000). Interestingly, the SSC between the control and treatment groups was not significantly different. For wax-coated fruits, SSC increased during the first 15 days but slightly decreased in the following storage period (Fig. 1d).

Table 1 shows the effect of rice bran wax coating on the CSP content of cherry tomatoes. The contents of CSP for the control and treatment groups were increased to the maximum level (31.24 vs. 28.58 mg 100 g^{-1}) at 10 days of storage ($P < 0.05$), compared to 15.66 mg 100 g^{-1} at harvest. The increased CSP during the first 10 days of storage might be due to the solubisation of other pectin components to CSP and then decreased due to the effect of polygalacturonase and pectinesterase (Liu et al. 2009). However, from then, the CSP content decreased to 21.18 and 18.15 mg 100 g^{-1}, respectively, for the control and treated groups at the end of storage period. The CSP was also significantly different between the control and coated groups when stored for 20 days ($P < 0.05$). However, there were no major modifications in CSP content of wax-coated fruits except at 10 days of storage (Table 1). These indicated that rice bran wax coating inhibited normal wall disassembly to some degree.

Effect of Rice Bran Wax Coating on the Monosaccharide Constitute of CSP

Table 2 shows the monosaccharide components of CSP of cherry tomatoes during cold storage. The monosaccharide of CSP was mainly composed of mannose (Man), galacturonic acid (GalUA), rhamnose (Rha), galactose (Gal) and arabinose (Ara). It can be observed that GalUA was the major sugar in CSP, while glucose (Glc) might be originated from non-pectic polysaccharides which were not completely removed during the extraction of the cell wall material. The content of all sugars increased after cold storage in both the control and wax-coated groups. However, the contents of GalUA and Rha of CSP in the wax-coated group increased less than those of the control group. The contents of GalUA and Rha of CSP

Table 1 Effect of rice bran wax coating on the CSP content (mg 100 g^{-1}) of cherry tomatoes

Sample	Storage time (days)				
	0	5	10	15	20
Control	$15.66 \pm 2.13^{c,A}$	$17.60 \pm 2.44^{bc,A}$	$31.24 \pm 1.85^{a,A}$	$16.44 \pm 1.53^{bc,A}$	$21.18 \pm 0.87^{b,A}$
Waxed	$15.66 \pm 2.13^{b,A}$	$12.85 \pm 4.23^{b,A}$	$28.58 \pm 4.54^{a,A}$	$14.46 \pm 0.95^{b,A}$	$18.15 \pm 1.05^{b,B}$

Different superscript lowercase letters in the same row and different superscript uppercase letters in the same column mean significant difference at $P < 0.05$. Waxed indicates rice bran wax-coated group

CSP chelate-soluble pectin

in the waxed fruits increased from 1.06 and 28.17 to 2.60 and 36.13 mg 100 g^{-1} fresh mass (FM), respectively. This result corresponded well to the result of CSP content.

The molar ratio of Rha to GalUA is a parameter indicating the existence of rhamnogalactoside-I (RG-I) segments within the pectin molecules. The molar ratio of Rha to GalUA is approximately 1:1 because the backbone is almost composed of alternating molecules of GalUA and Rha (Yapo 2011). To the pectin total population, the contribution of RG-I was 0.45 and 0.72 for the control and treated fruits, respectively, after storage for 20 days (Table 2). Therefore, treated fruits contained higher amount of RG-I regions than control fruits. The CSP of cherry tomatoes had two regions: smooth region and hairy region. Ara and Gal indicate the existence of arabinan side and galactan chains in the RG-I structure, while the molar ratio of (Ara + Gal) to Rha indicates the degree of branching of the RG-I segments. The molar ratio for control fruits was 3.46 while that for waxed fruits was 4.91 after 20 days of storage, suggesting the side chains of RG-I regions in the control fruits were shorter than those in the treated fruits. The side chains of RG-I regions might have a close relationship to the firmness of fruits (Yang 2014), explaining why the firmness of the wax-coated fruits was greater than that of the control fruits at the end of the storage period. The results of Rha/GalUA and (Gal + Ara)/Rha (Table 2) indicated that the rice bran wax coating inhibited the degradation of CSP during cold storage.

Effect of Rice Bran Wax Coating on the FTIR Properties of CSP

The infrared spectra of the control and wax-coated fruits are shown in Fig. 2. The broad and strong absorption between 3600 and 2500 cm^{-1} indicated O–H stretching absorption

Table 2 Effect of rice bran wax coating on the monosaccharide constitute of CSP

Monosaccharide (mg 100 g^{-1} FM)	Sample	Storage time (days)		
		0	10	20
Man	Control	1.11 ± 0.11^{c}	2.01 ± 0.04^{b}	2.36 ± 0.12^{a}
	Waxed	1.11 ± 0.11^{c}	1.85 ± 0.05^{b}	3.41 ± 0.05^{a}
Rha	Control	1.06 ± 0.06^{c}	1.41 ± 0.10^{b}	3.62 ± 0.24^{a}
	Waxed	1.06 ± 0.06^{c}	1.52 ± 0.03^{b}	2.60 ± 0.59^{a}
GalUA	Control	28.17 ± 1.21^{c}	38.10 ± 1.25^{b}	80.54 ± 4.83^{a}
	Waxed	28.17 ± 1.21^{b}	28.81 ± 2.92^{b}	36.13 ± 1.03^{a}
Glc	Control	5.17 ± 0.48^{ab}	7.22 ± 0.17^{a}	4.91 ± 1.39^{b}
	Waxed	5.17 ± 0.48^{b}	4.46 ± 0.21^{b}	33.44 ± 3.03^{a}
Gal	Control	2.49 ± 0.10^{c}	5.53 ± 0.42^{b}	6.81 ± 0.25^{a}
	Waxed	2.49 ± 0.10^{c}	4.75 ± 0.13^{b}	8.82 ± 0.20^{a}
Ara	Control	2.14 ± 0.13^{c}	4.12 ± 0.16^{b}	5.73 ± 0.20^{a}
	Waxed	2.14 ± 0.13^{c}	3.42 ± 0.28^{b}	3.95 ± 0.05^{a}
Rha/GalUA	Control	0.38	0.37	0.45
	Waxed	0.38	0.53	0.72
(Gal + Ara)/Rha	Control	4.37	6.84	3.46
	Waxed	4.37	5.38	4.91

Different superscript lowercase letters in the same row mean significant difference at $P < 0.05$. Waxed indicates rice bran wax-coated group

CSP chelate-soluble pectin, *Man* mannose, *Rha* rhamnose, *GalUA* galacturonic acid, *Glc* glucose, *Gal* galactose, *Ara* arabinose

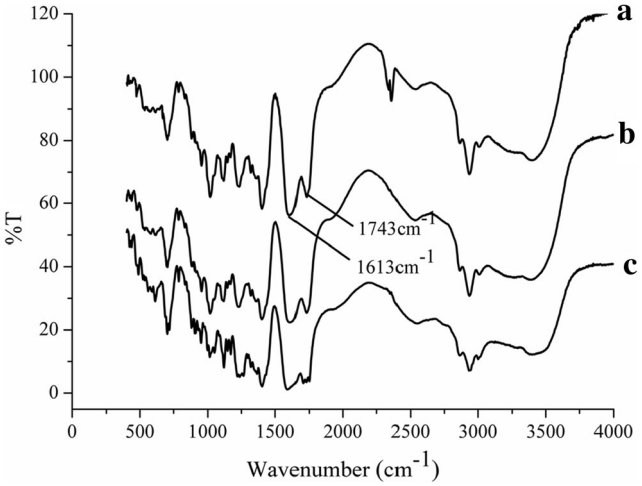

Fig. 2 Effect of rice bran wax coating on the FTIR properties of chelate-soluble pectin (CSP). *a* Fresh fruits; *b* control fruits; *c* waxed fruits

because of the inter- and intramolecular hydrogen bonds (Gnanasambandam and Proctor 2000). For CSP, the broad absorption in this region was attributed to the stretching of inter- and intramolecular hydrogen bonding of galacturonic acid polymer, while the absorption at about 2900 cm^{-1} referred to the C–H, CH_2 and CH_3 stretching and bending vibrations. In esterified pectin, an O–CH_3 stretching band was expected to be around 2900 cm^{-1} because of methyl esters of galacturonic acid. However, the O–CH_3 activity might be masked and thus not a reliable indicator of methoxylation (Gnanasambandam and Proctor 2000). The region between 1800 and 1500 cm^{-1} was of special interest with regards to the evaluation of the degree of esterification (DE). The averaged ratio of the peak intensity at 1743 cm^{-1} (COO–R) over the summed peak intensity of 1743 (COO–R) and 1613 cm^{-1} (COO–) was considered as the degree of methyl-esterification (Vriesmann and de Oliveira Petkowicz 2009). In the current study, the absorption at 1743 cm^{-1} decreased in the control and wax-coated fruits during storage, indicating a lower DE after storage. This result corresponded well to the changes of fruit firmness and to the previous study (Chatjigakis et al. 1998). The absorption between 800 and 1300 cm^{-1} was regarded as the fingerprint region of the pectin, while the absorption at 1380 cm^{-1} was C–H bending and 1000–

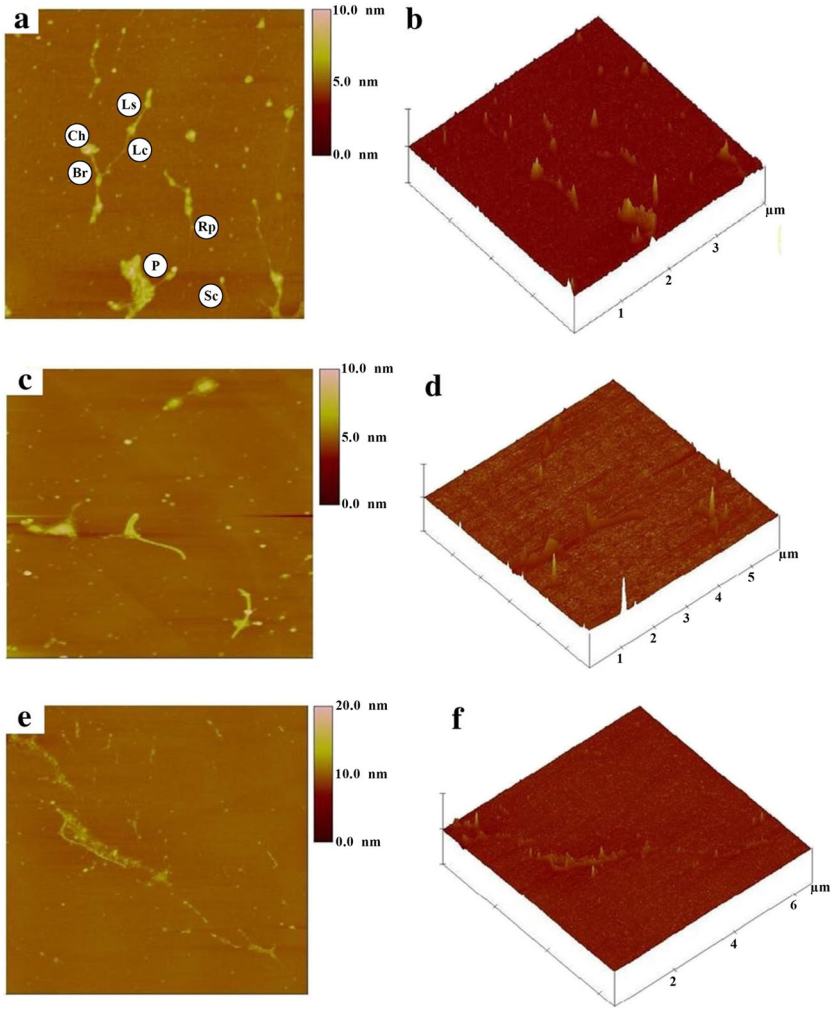

Fig. 3 AFM images of CSP of fresh- and cold-stored fruits. **a, b** Fresh fruits; scan area: 4.000 × 4.000 μm^2. **c, d** Control fruits; scan area: 5.766 × 5.766 μm^2. **e, f** Waxed fruits; scan area: 6.575 × 6.575 μm^2. Note: *Br* branch structure; *CSP* chelate-soluble pectin, *Ls* linear strands, *Lc* long chains, *Sc* short chains, *Ch* CDTA, *P* polymers, *Rp* stretched molecules from CDTA

1300 cm^{-1} was C–O stretching (Singthong et al. 2004). In general, the FTIR spectra were not significantly different between the control and rice bran wax-coated fruits.

Effect of Rice Bran Wax Coating on the Nanostructure of CSP

The AFM results of CSP of cherry tomatoes are shown in Fig. 3. The morphologies of CSP in fresh- and cold-stored samples were different. In the fresh sample, it mainly contained linear strands (Ls), branched (Br) and polymer structures (P) (Fig. 3a, b). After the cold storage, the branched and polymer structures of CSP reduced, meanwhile, shorter Ls of CSP increased in cold storage fruits. Compared to the control group, the Ls was longer in the wax-coated fruits (Fig. 3c–f), indicating that rice bran wax coating inhibited the degradation of pectin during cold storage.

AFM also provided quantitative dimensions of CSP (Fig. 4). The colour bar legends on the right of the images represented the scale of height of the samples scanned, and the other two ordinates at the below of the images were the scan areas (length × width) of the samples (Fig. 3); the width of pectin molecules was demonstrated as the peak width and the height expressed as peak height. F_q is referred to the times of the length, width and height of CSP chains observed. The quantitative values of pectin molecules were greater than the actual values due to the probe-broadening effects of AFM (Kirby et al. 1995; Chen et al. 2013).

Figure 4a shows the width distribution of CSP on day 20 of cold storage. The CSP molecular widths of fresh fruits ranged from 15 to 250 nm. The vertical heights of CSP molecules were 0.2 to 2.0 nm. However, the molecular width was mainly in the range of 51–200 nm for both the wax-coated and control fruits on day 20 of cold storage. Below 200 nm, the chain widths were not significantly different between the control and wax-coated fruits ($P > 0.05$). On the contrast, for chain width above 201 nm, F_q of the wax-coated fruits (12.83%) was greater than that of the control (5.01%) ($P < 0.05$), suggesting that the degradation of CSP molecules was inhibited by the wax coating during cold storage.

For many fruits, the length of CSP chains mostly fell within the range of 500–5000 nm, and longer than sodium carbonate-soluble pectin (SSP) (400–3600 nm for apricot and 100–700 nm for honeydew melon (Liu et al. 2009; Chong et al. 2015). After storage, the length of CSP chains of the control and treated fruits were mainly within 1000–5000 nm. Moreover, there was no significant difference in the F_q of CSP chain length between the control and wax-coated fruits ($P > 0.05$). However, the F_q of CSP chain length above 9000 nm in the wax-coated fruits (31.75%) was significantly greater than in the control (22.24%) ($P < 0.05$) (Fig. 4b), further supporting wax coating's protective effect on CSP chains. The quantitative dimensions (length and width) of CSP chains also correlated well with fruit firmness and the previous study (Chong et al. 2015).

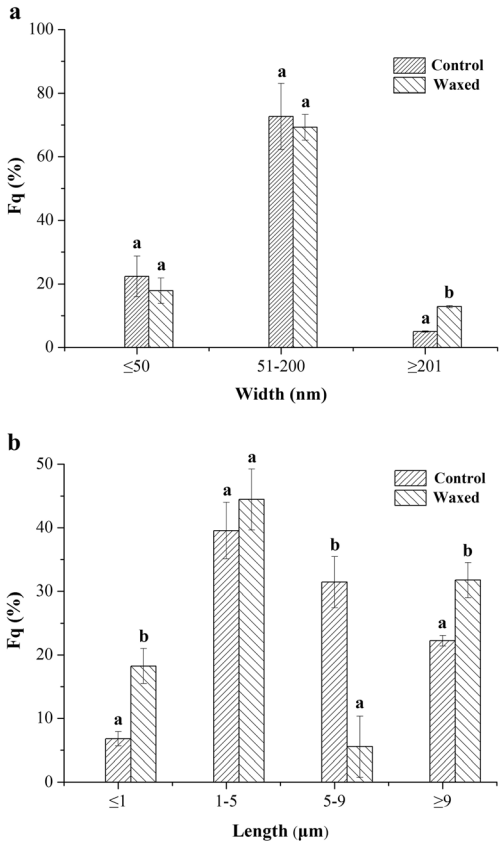

Fig. 4 The quantitative parameters of fruit CSP chains on day 20 of the cold storage. **a** The width distribution of CSP chains. **b** The length distribution of CSP chains. Note: 1–5 μm contains 5 μm but does not contain 1 μm; *different small letters* between the two groups mean significant difference at $P < 0.05$. Waxed indicates rice bran wax-coated group. *CSP* chelate-soluble pectin

The Relationship Between Physicochemical and Structured Properties

Compared to the control fruits, wax-coated fruits have greater firmness and less weight loss at the end of the cold storage (Fig. 1), indicating that rice bran wax coating inhibited the softening of fruits. In addition, the wax coating also inhibited the degradation of CSP chains according to the results of the monosaccharide, Rha/GalUA and (Gal + Ara)/Rha ratios, and the F_q of CSP chain width and length. These results suggested that the wax coating inhibited the softening of fruits at the nanoscale.

Fruit softening was accompanied by solubilisation and depolymerisation of the cell wall polysaccharides, resulting in disintegration of the cell wall (Billy et al. 2008). During fruit ripening, the arabinose and galactose side branches degraded (Kan et al. 2013). In the current study, rice bran wax coating inhibited the decrease of the (Gal + Ara)/Rha ratio, implying wax coating inhibited the cleavage of galactose and arabinose

side chains of uronide acid which prevented pectolytic enzymes from dissembling pectin and were accompanied by fruit softening (Ortiz et al. 2010). The softening of fruit was not only related to the chemical structure of pectin but also to the nanoproperties of the polysaccharides (Zdunek et al. 2014).

It should be noted that several limitations may affect the accuracy of the results. First, the fruits varied yearly and amongst different areas. Second, the AFM images may cause variations. AFM is good at examining the length of pectin molecules between 30 nm and 15 μm. Short-length chains might not be easily recognised from the images and might not be included in the final statistics (Yang 2014). A third possible error or limitation was that some other important components like hemicellulose might also be involved in the changes. In the future, to enhance the coating properties of rice bran wax, incorporating with proteins and polysaccharides from natural sources may be a good approach (Bu et al. 2015).

Conclusion

The effects of rice bran wax coating on the physicochemical properties and CSP structure of postharvest cherry tomatoes during cold storage were studied. There were no significant differences of firmness between the control (2.48 N) and wax-coated (2.87 N) fruits at the end of the storage (20 days), while the wax-coated (16.02%) samples had less weight loss than the control (13.54%). The structural analysis revealed that rice bran wax coating inhibited CSP degradation, which was further supported by the results of Rha/GalUA and (Gal + Ara)/Rha. After cold storage, DE decreased in both the control and coated fruits. AFM results revealed that the F_q of greater CSP chain width and length were higher in waxed fruits than in the corresponding control. Both the chemical and nanostructural morphologies of CSP played a role in the softening of cherry tomato fruits during cold storage. Overall, rice bran wax coating was an effective means to preserve cherry tomatoes.

Acknowledgments The authors acknowledge the financial support by the Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-583-112) and the National University of Singapore start-up grant (R-143-000-561-133). Projects 31371851 and 31071617 are supported by NSFC, Natural Science Foundation of Jiangsu Province (BK20141220), and the Applied Basic Research Project (Agricultural) Suzhou Science and Technology Planning Programme (SYN201522), and the Technology Innovation Talent Fund by Henan University of Technology (2014CXRC01) also contributed to this research.

References

Ali, M. S., Nakano, K., & Maezawa, S. (2004). Combined effect of heat treatment and modified atmosphere packaging on the color development of cherry tomato. *Postharvest Biology and Technology, 34*(1), 113–116.

Baldwin, E. A., Nisperos-Carriedo, M. O., & Baker, R. A. (1995). Edible coatings for lightly processed fruits and vegetables. *HortScience, 30*(1), 35–38.

Baraiya, N. S., Rao, T. V. R., & Thakkar, V. R. (2015). Improvement of postharvest quality and storability of jamun fruit (*Syzygium cumini* L. Var. Paras) by zein coating enriched with antioxidants. *Food and Bioprocess Technology, 8*(11), 2225–2234.

Billy, L., Mehinagic, E., Royer, G., Renard, C. M. G. C., Arvisenet, G., Prost, C., & Jourjon, F. (2008). Relationship between texture and pectin composition of two apple cultivars during storage. *Postharvest Biology and Technology, 47*(3), 315–324.

Bu, G., Zhu, T., Chen, F., Zhang, N., Liu, K., Zhang, L., & Yang, H. (2015). Effects of saccharide on the structure and antigenicity of β-conglycinin in soybean protein isolate by glycation. *European Food Research and Technology, 240*, 285–293.

Chatjigakis, A. K., Pappas, C., Proxenia, N., Kalantzi, O., Rodis, P., & Polissiou, M. (1998). FT-IR spectroscopic determination of the degree of esterification of cell wall pectins from stored peaches and correlation to textural changes. *Carbohydrate Polymers, 37*, 395–408.

Chen, S., & Nussinovitch, A. (2000). Galactomannans in disturbances of structured wax-hydrocolloid-based coatings of citrus fruit (easy-peelers). *Food Hydrocolloids, 14*(6), 561–568.

Chen, F., Liu, H., Yang, H., Lai, S., Cheng, X., Xin, Y., Yang, B., Hou, H., Yao, Y., Zhang, S., Bu, G., & Deng, Y. (2011). Quality attributes and cell wall properties of strawberry (*Fragaria annanassa* Duch.) under calcium chloride treatment. *Food Chemistry, 126*(2), 450–459.

Chen, Y., Chen, F., Lai, S., Yang, H., Liu, H., Liu, K., Bu, G., & Deng, Y. (2013). In vitro study of the interaction between pectinase and chelate-soluble pectin in postharvest apricot fruits. *European Food Research and Technology, 237*(6), 987–993.

Chong, J., Lai, S., & Yang, H. (2015). Chitosan combined with calcium chloride impacts fresh-cut honeydew melon by stabilising nanostructures of sodium-carbonate-soluble pectin. *Food Control, 53*, 195–205.

Daş, E., Gürakan, G. C., & Bayındırlı, A. (2006). Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella* enteritidis on cherry tomatoes. *Food Microbiology, 23*(5), 430–438.

Feng, W., Zheng, X., Chen, J., & Yang, Y. (2008). Combination of cassia oil with magnesium sulphate for control of postharvest storage rots of cherry tomatoes. *Crop Protection, 27*(1), 112–117.

Gang, D., Yang, H., & Zuo, Y. (2007). Study on coating preservation of plum and cherry tomato. *Chemistry & Bioengineering, 24*(3), 47–48.69.

Gnanasambandam, R., & Proctor, A. (2000). Determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy. *Food Chemistry, 68*, 327–332.

Kan, J., Liu, J., & Jin, C. (2013). Changes in cell walls during fruit ripening in Chinese 'honey' peach. *The Journal of Horticultural Science and Biotechnology, 88*(1), 37–46.

Kirby, A. R., Gunning, A. P., & Morris, V. J. (1995). Imaging xanthan gum by atomic force microscopy. *Carbohydrate Research, 267*, 161–166.

Lai, S., Chen, F., Zhang, L., Yang, H., Deng, Y., & Yang, B. (2013). Nanostructural difference of water-soluble pectin and chelate-soluble pectin among ripening stages and cultivars of Chinese cherry. *Natural Product Research, 27*, 379–385.

Lenucci, M. S., Cadinu, D., Taurino, M., Piro, G., & Dalessandro, G. (2006). Antioxidant composition in cherry and high pigment tomato cultivars. *Journal of Agricultural and Food Chemistry, 54*(7), 2606–2613.

Li, M., Chen, F., Yang, B., Lai, S., Yang, H., Liu, K., Bu, G., & Deng, Y. (2015). Preparation of organic tofu using organic compatible

magnesium chloride incorporated with polysaccharide coagulants. *Food Chemistry, 167*, 168–174.

Liu, H., Chen, F., Yang, H., Yao, Y., Gong, X., Xin, Y., & Ding, C. (2009). Effect of calcium treatment on nanostructure of chelate-soluble pectin and physicochemical and textural properties of apricot fruits. *Food Research International, 42*, 1131–1140.

Njombolwana, N. S., Erasmus, A., Van Zyl, J. G., du Plooy, W., Cronje, P. J. R., & Fourie, P. H. (2013). Effects of citrus wax coating and brush type on imazalil residue loading, green mould control and fruit quality retention of sweet oranges. *Postharvest Biology and Technology, 86*, 362–371.

Ortiz, A., Seymour, G. B., Tucker, G. A., & Lara, I. (2010). Cell wall disassembly during the melting phase of softening in 'snow queen' nectarines. *Postharvest Biology and Technology, 58*(2), 88–92.

Raffo, A., Leonardi, C., Fogliano, V., Ambrosino, P., Salucci, M., Gennaro, L., Bugianesi, R., Giuffrida, F., & Quaglia, G. (2002). Nutritional value of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1) harvested at different ripening stages. *Journal of Agricultural and Food Chemistry, 50*(22), 6550–6556.

Saucedo-Pompa, S., Rojas-Molina, R., Aguilera-Carbó, A. F., Saenz-Galindo, A., de la Garza, H., Jasso-Cantú, D., & Aguilar, C. N. (2009). Edible film based on candelilla wax to improve the shelf life and quality of avocado. *Food Research International, 42*(4), 511–515.

Shih, F. F., Daigle, K. W., & Champagne, E. T. (2011). Effect of rice wax on water vapour permeability and sorption properties of edible pullunlan films. *Food Chemsitry, 127*(1), 118–121.

Singthong, J., Cui, S., Ningsanond, S., & Goff, H. D. (2004). Structural characterization, degree of esterification and some gelling properties of Krueo Ma Noy (*Cissampelos pareira*) pectin. *Carbohydrate Polymers, 58*, 391–400.

Valipour, M. (2015a). Future of agricultural water management in Africa. *Archives of Agronomy and Soil Science, 61*(7), 907–927.

Valipour, M. (2015b). Land use policy and agricultural water management of the previous half of century in Africa. *Applied Water Science, 5*, 367–395.

Valipour, M., Ahmadi, M. Z., Raeini-Sarjaz, M., Sefidkouhi, M. A. G., Shahnazari, A., Fazlola, R., & Darzi-Naftchali, A. (2015). Agricultural water management in the world during past half century. *Archives of Agronomy and Soil Science, 61*(5), 657–678.

Vriesmann, L. C., & de Oliveira Petkowicz, C. L. (2009). Polysaccharides from the pulp of cupuassu (*Theobroma grandiflorum*): structural characterization of a pectic fraction. *Carbohydrate Polymers, 77*(1), 72–79.

Wang, W., Ma, X., Zou, M., Jiang, P., Hu, W., Li, J., Zhi, Z., Chen, J., Li, S., Ding, T., Ye, X., & Liu, D. (2015). Effects of ultrasound on spoilage microorganisms, quality, and antioxidant capacity of post-harvest cherry tomatoes. *Journal of Food Science, 80*(10), C2117–C2126.

Xin, Y., Chen, F., Yang, H., Zhang, P., Deng, Y., & Yang, B. (2010). Morphology, profile and role of chelate-soluble pectin on tomato properties during ripening. *Food Chemistry, 121*(2), 372–380.

Yang, H. (2014). *Atomic force microscopy (AFM): principles, modes of operation and limitations*. Hauppauge, NY: Nova Science Publishers, Inc.

Yang, H., Lai, S., An, H., & Li, Y. (2006). Atomic force microscopy study of the ultrastructural changes of chelate-soluble pectin in peaches under controlled atmosphere storage. *Postharvest Biology and Technology, 39*, 75–83.

Yapo, B. M. (2011). Rhamnogalacturonan-I: a structurally puzzling and functionally versatile polysaccharide from plant cell walls and mucilages. *Polymer Reviews, 51*(4), 391–413.

Yu, X., & Yang, H. (2017). Pyrethroid residue determination in organic and conventional vegetables using liquid-solid extraction coupled with magnetic solid phase extraction based on polystyrene-coated magnetic nanoparticles. *Food Chemistry, 217*, 303–310.

Yu, X., Yang, R., Gu, Z., Lai, S., & Yang, H. (2014). Anti-tumor and immunostimulatory functions of two feruloyl oligosaccharides produced from wheat bran and fermented by *Aureobasidium pullumans*. *BioResources, 9*, 6778–6790.

Zdunek, A., Koziol, A., Pieczywek, P. M., & Cybulska, J. (2014). Evaluation of the nanostructure of pectin, hemicellulose and cellulose in the cell walls of pears of different texture and firmness. *Food and Bioprocess Technology, 7*(12), 3525–3535.

Zhang, J., & Yang, H. (2017). Effects of potential organic compatible sanitisers on organic and conventional fresh-cut lettuce (*Lactuca sativa* Var. *crispa* L). *Food Control, 72*, 20–26.

Zhang, L., Chen, F., An, H., Yang, H., Sun, X., Gao, X., & Li, L. (2008). Physicochemical properties, firmness, and nanostructures of sodium carbonate-soluble pectin of 2 Chinese cherry cultivars at 2 ripening stages. *Journal of Food Science, 73*(6), N17–N22.

Zhao, Y., Tu, K., Tu, S. C., Liu, M., Su, J., & Hou, Y. (2010). A combination of heat treatment and *Pichia guilliermondii* prevents cherry tomato spoilage by fungi. *International Journal of Food Microbiology, 137*(1), 106–110.

Zhao, L., Zhang, Y., & Yang, H. (2017). Efficacy of low concentration neutralised electrolysed water and ultrasound combination for inactivating *Escherichia coli* ATCC 25922, *Pichia pastoris* GS115 and *Aureobasidium pullulans* 2012 on stainless steel coupons. *Food Control*. doi:10.1016/j.foodcont.2016.09.041.