



# Impact of soybean protein isolate-chitosan edible coating on the softening of apricot fruit during storage

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## ABSTRACT

A soybean protein isolate (SPI)-chitosan edible coating was used to prolong the shelf life of apricots stored at 2 °C. Apricots were coated with two different coating formulations (SPI and SPI combined with chitosan). The changes to several parameters including weight loss, firmness, titratable acidity, soluble solids content, pectin contents, and the nanostructural properties of pectin were investigated to evaluate the effectiveness of the coatings. The coatings, especially the SPI-chitosan coating, significantly decreased the weight loss of apricots. Meanwhile, this treatment prevented the decrease in firmness and benefited the textural properties of the tissue. The atomic force microscopy (AFM) results showed a greater  $F_q$  (the percent of pectin chains of particular width or length among all the chains observed by AFM) for the width and length of pectin molecules in the SPI-chitosan coated samples (width  $\geq 61$  nm; length  $\geq 3 \mu\text{m}$ ), which indicated that the SPI-chitosan coating could inhibit pectin degradation. The results showed that the SPI-chitosan coating is an effective method to preserve the quality of apricots.

## 1. Introduction

Prolonging the shelf life of fresh-cut fruit and vegetables using edible coatings has attracted great interest, especially using environmentally friendly and biodegradable materials. Several researchers have reported the application of edible coatings to preserve fruit and vegetables, such as Chinese cherry (Xin, Chen, Lai, & Yang, 2017), fresh-cut apple (Ghidelli, Mateos, Rojas-Argudo, & Pérez-Gago, 2014; Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2017), grape berries (Oh et al., 2017) and eggplant (Singh et al., 2016).

Edible coatings, as biodegradable materials, can provide a semi-permeable barrier to gases and water vapour, reduce respiration, enzymatic browning, and water loss (Pérez-Gago, Serra, Alonso, Mateos, & del Río, 2005; Mannozi et al., 2017). The basic ingredients of edible coatings are proteins, polysaccharides, and lipids. The coatings' protective function can be enhanced by the addition of ingredients such as antioxidants. Weinbreck, Tromp, and de Kruif (2004b) reported that protein-polysaccharide interactions play significant roles in controlling the structure, texture, and stability of coating and packaging materials. Chitosan has the advantage of biodegradability, biocompatibility, non-toxicity, and antimicrobial activity; therefore, interest in its application

in edible coatings and films is increasing (Aider, 2010; Xin et al., 2017). However, chitosan films are highly permeable to water vapour. Many scientists have reported that chitosan combined with protein has a positive effect on the shelf life of fruit. The addition of quinoa protein and sunflower oil to chitosan resulted in low water vapour permeability and long shelf life of fresh blueberries (Abugoch et al., 2016). Simonaitiene reported that whey proteins-chitosan films with quince and cranberry juice inhibited the growth of *Penicillium expansum* on apples (Simonaitiene, Brink, Sipailiene, & Leskauskaitė, 2015).

The shelf life of fruit and vegetables is closely related to their texture. The firmness of fruit tissue is determined by the composition and morphology of the cell wall materials, which include pectin, cellulose, and hemicelluloses. Thus, changes in polysaccharides including pectin structure induce changes in the texture of fruit and vegetables (Chen et al., 2018; Liu, Jiang, Yang, & Yang, 2017; Liu, Tan, Yang, & Wang, 2017; Yang, 2014; van Buggenhout, Sila, Duvetter, Van Loey, & Hendrickx, 2009). It is believed that tissue softening of fruit is determined by pectin modifications and solubilisation. Chen reported that fruit firmness is associated with pectin polymers of chelate-soluble pectin (CSP) (Chen et al., 2013). Lara found that increasing the CSP content and lowering the levels of the water-soluble fraction reduced

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**Table 1**  
Effects of coating on the physicochemical properties of apricots during storage. <sup>a</sup>Different superscript uppercase letters in the same row and different superscript lowercase letters in the same column indicate a significant difference at  $P < 0.05$ ; CK indicates the control group; SPI and SPI-chitosan indicate soybean protein isolate coating and soybean protein isolate-chitosan coating group, respectively. SSC: soluble solids content; TA: titratable acidity; d: day.

Storage Time (d)	Weight Loss (%)			Firmness (N)			SSC (%)			TA (%)		
	CK	SPI	SPI-chitosan	CK	SPI	SPI-chitosan	CK	SPI	SPI-chitosan	CK	SPI	SPI-chitosan
0	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	11.07 ± 0.71 <sup>a</sup>	11.07 ± 0.71 <sup>a</sup>	11.07 ± 0.71 <sup>a</sup>	8.04 ± 0.18 <sup>a</sup>	8.04 ± 0.18 <sup>a</sup>	8.04 ± 0.18 <sup>a</sup>	1.00 ± 0.01 <sup>a</sup>	1.00 ± 0.01 <sup>a</sup>	1.00 ± 0.01 <sup>a</sup>
7	2.14 ± 0.02 <sup>b,c</sup>	1.84 ± 0.08 <sup>b,b</sup>	1.42 ± 0.04 <sup>b,a</sup>	10.80 ± 0.26 <sup>a,b</sup>	9.73 ± 0.78 <sup>b,a</sup>	10.68 ± 0.43 <sup>ab,b</sup>	8.32 ± 0.57 <sup>a</sup>	8.44 ± 0.27 <sup>a</sup>	8.60 ± 0.35 <sup>b,a</sup>	0.99 ± 0.01 <sup>a,b</sup>	1.01 ± 0.02 <sup>a,c</sup>	0.93 ± 0.00 <sup>c</sup>
14	7.13 ± 0.55 <sup>c,b</sup>	6.14 ± 0.14 <sup>c,a</sup>	5.73 ± 0.37 <sup>c,a</sup>	5.94 ± 0.29 <sup>c,a</sup>	9.21 ± 0.84 <sup>b,b</sup>	9.84 ± 0.67 <sup>ab,b</sup>	8.44 ± 0.44 <sup>ab</sup>	8.12 ± 0.34 <sup>a</sup>	8.80 ± 0.30 <sup>b,b</sup>	0.79 ± 0.00 <sup>c,a</sup>	0.86 ± 0.02 <sup>d,b</sup>	0.86 ± 0.03 <sup>b</sup>
21	9.44 ± 0.27 <sup>d,b</sup>	8.46 ± 0.60 <sup>d,a</sup>	8.32 ± 0.45 <sup>d,a</sup>	7.27 ± 0.43 <sup>b,a</sup>	8.06 ± 1.05 <sup>c,a</sup>	9.58 ± 0.91 <sup>c,b</sup>	9.36 ± 0.46 <sup>b,b</sup>	9.06 ± 0.25 <sup>ab</sup>	8.80 ± 0.16 <sup>b,a</sup>	0.76 ± 0.00 <sup>d,a</sup>	0.96 ± 0.01 <sup>b,b</sup>	0.97 ± 0.02 <sup>b,b</sup>
28	12.37 ± 0.48 <sup>e,a</sup>	10.78 ± 0.28 <sup>e,a</sup>	10.40 ± 1.58 <sup>e,a</sup>	5.18 ± 0.15 <sup>a</sup>	5.38 ± 0.32 <sup>a</sup>	5.84 ± 1.12 <sup>d,a</sup>	9.96 ± 0.27 <sup>c,a</sup>	9.76 ± 0.75 <sup>c,a</sup>	10.00 ± 0.36 <sup>c,a</sup>	0.85 ± 0.01 <sup>b,b</sup>	0.79 ± 0.01 <sup>f,a</sup>	0.83 ± 0.02 <sup>d,b</sup>
35	19.93 ± 1.03 <sup>f,c</sup>	18.10 ± 0.42 <sup>f,b</sup>	14.09 ± 0.35 <sup>f,a</sup>	4.40 ± 0.43 <sup>c,a</sup>	4.37 ± 0.29 <sup>c,a</sup>	5.39 ± 0.34 <sup>d,b</sup>	10.18 ± 0.25 <sup>c,a</sup>	10.98 ± 0.16 <sup>d,b</sup>	11.28 ± 0.36 <sup>d,b</sup>	0.81 ± 0.02 <sup>c,a</sup>	0.91 ± 0.01 <sup>c,b</sup>	0.79 ± 0.01 <sup>e,a</sup>
42	23.96 ± 1.78 <sup>g,b</sup>	22.13 ± 0.56 <sup>g,b</sup>	16.63 ± 0.85 <sup>g,a</sup>	2.69 ± 0.71 <sup>f,a</sup>	4.15 ± 0.09 <sup>c,b</sup>	4.26 ± 0.73 <sup>c,b</sup>	10.92 ± 0.37 <sup>d,a</sup>	12.94 ± 0.11 <sup>e,b</sup>	11.26 ± 0.43 <sup>d,a</sup>	0.76 ± 0.01 <sup>d,a</sup>	0.84 ± 0.00 <sup>c,b</sup>	0.97 ± 0.01 <sup>b,c</sup>

the degree of fruit dissolution and improved its texture (Lara, Garcia, & Vendrell, 2004).

Soybean protein materials are suitable for edible coatings because of their low permeability to oxygen and carbon dioxide, and reasonable cost (Kang, Kim, You, Lacroix, & Han, 2013). Soybean protein materials together with other materials have been used to extend the shelf life of fresh-cut eggplants and walnut kernels (Ghidelli et al., 2014; Kang et al., 2013). However, to the best of our knowledge, there has been little research focused on the effect of soy protein isolate (SPI) with chitosan coating on fruit. The aim of the current study was to investigate the physicochemical properties of apricots coated with an SPI-chitosan coating during storage. The softening mechanism of fruit was also studied from the aspect of the nanostructure of water-soluble and chelate-soluble pectin. The results will help to extend the use of SPI-chitosan coatings in postharvest fruit and vegetables.

## 2. Materials and methods

### 2.1. Materials

Apricot fruit (*Prunus armeniaca* L. 'Kaite') were harvested about one week before commercial maturity (their colour turned to light yellow) and were purchased from Zhengzhou, Henan, China. The fruit were transported to the laboratory within 2 h after harvest, and selected based on uniform colour, size, and absence of visible physical injury as the experiment materials. Food grade SPI (protein content 86.65%) was purchased from Shandong Wonderful Industrial Group Co. Ltd. (Dongying, Shandong, China). Food grade chitosan (viscosity < 100 mPa s and degree of deacetylation > 90%) was purchased from Zhejiang Aoxing Biotechnology Co. Ltd. (Hangzhou, Zhejiang, China). D-galacturonic acid (> 97.0%) was purchased from Sigma-Aldrich Co. Ltd. (St. Louis, MI, USA). All other reagents were of analytical grade.

### 2.2. Fruit coating

Fruit coating solutions were prepared according to previous reported methods with slight modifications (Huang, Sun, Xiao, & Yang, 2012; Xin et al., 2017). SPI (50 g) was dispersed in 1000 mL of distilled water, homogenised using ultrasound (power 80 W, time 5 min, and temperature 40 °C) (KQ-400E, Kunshan Shumei Ultrasonic Instrument Co. Ltd., Kunshan, Jiangsu, China), and used as the SPI coating solution (50 g SPI/1000 mL of water).

SPI solution (500 mL) was prepared by dispersing 50 g SPI in 500 mL distilled water, homogenised by ultrasound as the above. Chitosan solution (500 mL) was prepared by dissolving 1 g chitosan in 400 mL distilled water to which 32.5 mL glacial acetic acid was added. The solution was homogenised using ultrasound (power 80 W, time 5 min, and temperature 40 °C), adjusted to pH 5.6 with 0.1 M NaOH, and made up to 500 mL. The mixture of the SPI solution (500 mL) and chitosan solution (500 mL) formed the SPI-chitosan coating solution (50 g SPI/1000 mL of solution).

Two groups of 100 apricots were dipped into the SPI and SPI-chitosan coating solution, respectively, for 3 min, dried naturally, and defined as the SPI coated group and SPI-chitosan coated group. Another 100 apricots were dipped into distilled water for 3 min and defined as the control group (CK group). Fruit, harvested and delivered to the laboratory without any coating treatment, were defined as fresh fruit. Every 7 days, 10 fruit from each group were randomly selected from storage (temperature, 2 ± 1 °C; humidity, 75%) and analysed.

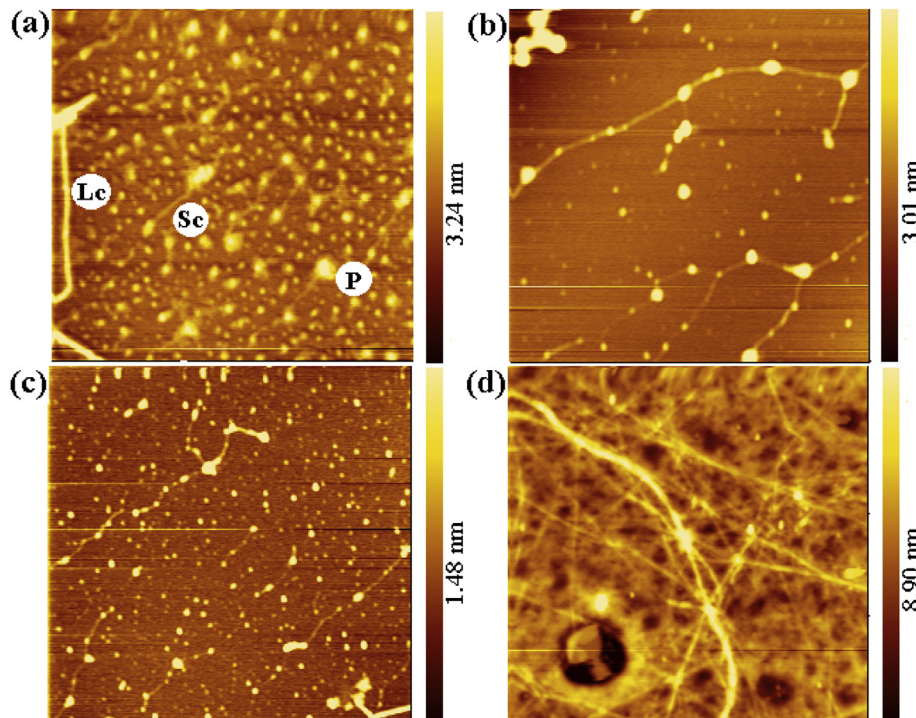
### 2.3. Firmness and weight loss determination

The firmness of the apricots was evaluated using a TA-XT2i texture analyser (Stable Micro System Ltd., Godalming, Surrey, UK). Based on a preliminary experiment (Liu et al., 2017), the operating settings were:

**Table 2**

Effects of coating on the pectin contents of apricots during storage. \*Different superscript uppercase letters in the same row and different superscript lowercase letters in the same column indicate a significant difference at  $P < 0.05$ ; CK indicates the control group; SPI and SPI-chitosan indicate soybean protein isolate coating and soybean protein isolate-chitosan coating group, respectively; WSP: water-soluble pectin; CSP: chelate-soluble pectin; d: day; FW: fresh weight.

Storage Time (d)	WSP (mg/100 g FW)			CSP (mg/100 g FW)		
	CK	SPI	SPI-chitosan	CK	SPI	SPI-chitosan
0	37.31 ± 0.59 <sup>g,A</sup>	37.31 ± 0.59 <sup>e,A</sup>	37.31 ± 0.59 <sup>f,A</sup>	4.21 ± 0.17 <sup>f,A</sup>	4.21 ± 0.17 <sup>d,A</sup>	4.21 ± 0.17 <sup>e,A</sup>
7	54.49 ± 1.14 <sup>f,A</sup>	51.61 ± 1.09 <sup>c,B</sup>	55.04 ± 0.33 <sup>c,A</sup>	7.50 ± 0.86 <sup>d,A</sup>	8.01 ± 0.82 <sup>c,A</sup>	7.81 ± 0.40 <sup>c,A</sup>
14	80.97 ± 1.25 <sup>a,A</sup>	64.60 ± 0.79 <sup>a,B</sup>	61.04 ± 0.51 <sup>a,C</sup>	6.18 ± 0.23 <sup>de,B</sup>	8.05 ± 0.94 <sup>c,A</sup>	6.23 ± 0.61 <sup>d,B</sup>
21	74.91 ± 0.77 <sup>b,A</sup>	52.13 ± 1.89 <sup>e,B</sup>	53.33 ± 0.41 <sup>d,B</sup>	12.52 ± 1.08 <sup>b,A</sup>	9.27 ± 0.70 <sup>b,B</sup>	7.95 ± 0.83 <sup>e,B</sup>
28	67.65 ± 0.86 <sup>c,A</sup>	65.76 ± 1.27 <sup>a,B</sup>	40.40 ± 0.76 <sup>e,C</sup>	4.99 ± 0.70 <sup>ef,C</sup>	8.39 ± 0.53 <sup>bc,A</sup>	7.11 ± 0.56 <sup>c,B</sup>
35	57.98 ± 0.27 <sup>e,A</sup>	45.47 ± 0.81 <sup>d,B</sup>	37.94 ± 0.33 <sup>f,C</sup>	10.93 ± 0.96 <sup>c,A</sup>	9.48 ± 0.48 <sup>b,B</sup>	8.92 ± 0.35 <sup>b,B</sup>
42	62.81 ± 1.04 <sup>d,A</sup>	55.29 ± 1.13 <sup>b,C</sup>	59.29 ± 1.13 <sup>b,B</sup>	16.16 ± 1.26 <sup>a,A</sup>	12.35 ± 0.31 <sup>a,B</sup>	10.58 ± 0.28 <sup>a,C</sup>



**Fig. 1.** The nanostructural morphologies of water-soluble pectin (WSP) determined by atomic force microscopy (AFM) (a) Fresh fruit; (b) Control fruit; (c) Soybean protein isolate (SPI) coated fruit; (d) SPI-chitosan coated fruit; scan area:  $3 \times 3 \mu\text{m}^2$  \*Lc long chains, Sc short chains, P polymers.

probe: 35 mm diameter aluminum cylinder; pre-test speed: 5 mm/s; test speed: 0.5 mm/s; post-tested speed: 0.5 mm/s; compression degree: 30%; time: 10 s; and trigger force: 3.0 g. Ten fruit from each group were measured individually.

The initial and final weights of ten apricots randomly selected from each group were recorded to monitor the change in weight with storage time. The equation used was as follows: Weight loss (%) =  $(m_0 - m) / m_0 \times 100$ , where  $m$  was the current weight of the fruit and  $m_0$  was the original weight.

#### 2.4. Titratable acidity, soluble solids, and pectin content determination

Titrate acidity (TA) and soluble solids content (SSC) were determined for 10 fruit from each group. TA was determined with 250 g well mixed juice titrated against NaOH (0.1 M). The results were expressed as percentage of malic acid (Zhang, Chen, Zhang, Lai, & Yang, 2017). SSC was determined using a portable digital refractometer (model 125 WYT-J; Chengdu Xing Chen Optical Instrument Co., Ltd, Chengdu, Sichuan, China).

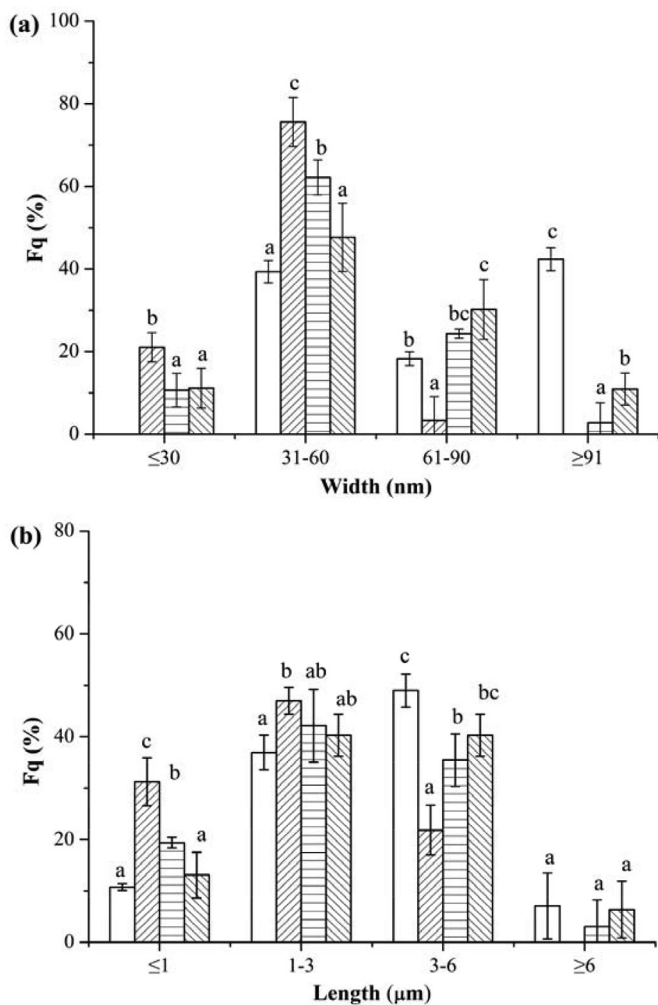
Cell wall material (CWM) was fractionated from fruit flesh according methods described in previous reports (Li, Zhang, Chen, Lai, &

Yang, 2018; Mao et al., 2017; Yang, Wu, Ng, & Wang, 2017). Water-soluble pectin (WSP) can be extracted from the CWM by suspending it in 10 mL ultra-purified water for 4 h at 25 °C. Thereafter, the sample was centrifuged at  $10,000 \times g$  for 10 min at 4 °C. The procedure was repeated two more times and the supernatants were collected as the WSP. Chelate-soluble pectin (CSP) was extracted using 0.05 M cyclohexane-trans-1, 2-diamine tetra-acetate (CDTA) using the above steps.

The pectin content analysis was determined by the carbazole colorimetry method with galacturonic acid as the standard. Pectin solution (2 mL) was hydrolysed by 12 mL sulfuric acid (98%, w/w) for 10 min in a boiling water bath, and then cooled using tap water. The cooled solution was mixed with 0.5 mL carbazole ethanol and incubated at room temperature for 30 min. The absorbance was then determined at 530 nm using a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd, Beijing, China). All experiments were conducted in triplicate.

#### 2.5. Pectin nanostructure analysis

The nanostructure of pectin was assessed by atomic force microscopy (AFM) using a multimode NanoScope IIIa instrument (Veeco



**Fig. 2.** The quantitative properties of water-soluble pectin (WSP) determined by atomic force microscopy (AFM) (a) The width distribution of WSP chains; (b) The length distribution of WSP chains. \*1–3 μm includes 3 μm but does not include 1 μm; 3–6 μm includes 6 μm but does not include 3 μm; different small letters between the two groups represent a significant difference at  $P < 0.05$ . Fresh fruit (□); CK (▨) indicates the control group; soybean protein isolate (SPI) (▤) and SPI-chitosan (▧) indicates SPI and SPI-chitosan coated fruit, respectively.

Metrology Group, Digital Instruments, Fremont, CA, USA) equipped with an E (J) scanner, according to the previous methods (Liu et al., 2017). About 5 μL of diluted pectin solution (10 μg/mL) was dropped onto the surface of freshly cleaved mica sheets to obtain optimal images. Imaging was conducted in tapping mode with an Si<sub>3</sub>N<sub>4</sub> tip in air. The resonant frequency of the tip was 330 kHz and the scan rate was approximately 0.5–2 Hz.

AFM images were analysed offline using AFM software (Version 5.30r3sr3). Section analysis was performed to determine the width (W) and length (L) of each sample. At least 30 measurements from 10 images were taken for each sample to provide representative results. The percent of pectin chains of a particular width or length among all the chains observed was recorded as the frequency (F<sub>q</sub>).

## 2.6. Statistical analysis

All experiments were conducted independently in triplicate, and the results were expressed as means ± standard deviation. The significance of the differences was determined using one-way analysis of variance (ANOVA), accompanied by Duncan's post hoc multiple

comparison test with a significance level of 0.05, using SPSS 16.0 software (International Business Machines Co. Armonk, NY, USA). The nanostructure analysis of pectin was conducted from dozens of parallel imaging tests by AFM. Principal component analysis (PCA) was used to reveal the relationships among the parameters measured in the experiment, and to check which variable was responsible for the quality of the apricots.

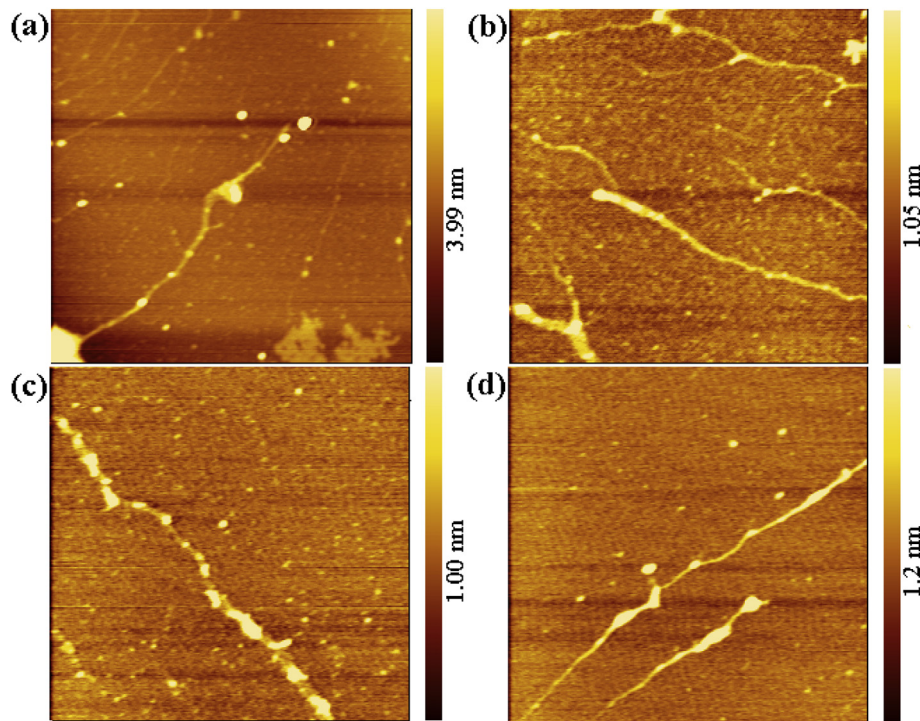
## 3. Results and discussion

### 3.1. Effects of SPI-chitosan coating on the physicochemical properties of apricots

Firmness is an important index of fruit texture and storability. A change in firmness is accompanied by fruit softening. The firmness of the apricots during storage is shown in Table 1. For the storage time from the 7th day to 14th day, there was a sudden drop in firmness in the CK group (Table 1). However, the firmness declined less drastically in SPI and SPI-chitosan coated groups compared with that observed in the CK group. By the end of storage, the firmness of the uncoated apricot fruit was 2.69 N compared with 4.15 and 4.26 N for samples treated with SPI and SPI-chitosan coating, respectively. The firmness of the CK group was significantly lower than that of the SPI and SPI-chitosan coated groups at the end of storage (42 day) ( $P < 0.05$ ). The results indicated that SPI and SPI-chitosan coating reduced the rate of softening of apricots significantly. This result agreed with previous studies that reported that the application of chitosan-based coatings inhibited the fruit softening process (Eshghi et al., 2014; Gardesh et al., 2016). In addition, Ghidelli reported that the application of an SPI edible coating significantly lowered the respiration rate of fresh-cut eggplant, resulting in high O<sub>2</sub> levels that could maintain the firmness of fruit and vegetables (Ghidelli et al., 2014).

Weight loss as a function of storage time in the CK, SPI, and SPI-chitosan coated apricots is shown in Table 1. Compared with the control, apricots with SPI-chitosan coating exhibited significantly ( $P < 0.05$ ) less weight loss (Table 1). On day 35, the weight loss of the control and SPI-coated fruit reached 19.93% and 18.10%, respectively. However, the weight loss of the apricots with the SPI-chitosan coating was only 14.09%. The results indicated that the SPI-chitosan coating prevented weight loss of apricots markedly. Loss of weight in fresh fruit and vegetables is mainly caused by the loss of water from transpiration and respiration (Zhu, Wang, Cao, & Jiang, 2007). Protein coatings are excellent barriers to carbon dioxide and oxygen but not to water, thus coatings with protein combined with other ingredients may better preserve fruit quality (Yousuf, Qadri, & Srivastava, 2018). As such, compared with the CK and SPI coated groups, the SPI-chitosan coating effects could be attributed to its better barrier properties against water evaporation.

The variations of TA and SSC for the control and coated apricots during storage are shown in Table 1. Compared with fresh fruit (0 day), the TA of all groups (CK, SPI, and SPI-chitosan coated groups) showed decreased during storage. However, compared with the CK and SPI-coated groups, the SPI-chitosan coated group showed a significant difference on the amount of TA at the end of storage (42 days) ( $P < 0.05$ ). TA decreased slightly more slowly in the coated fruit compared with that in the control fruit during storage. It was reported previously that organic acids are used as substrates for the respiration process; thus, the nanochitosan coating delayed TA losses significantly in apples after climacteric respiration (Gardesh et al., 2016). The SSC of the control and coated fruit increased significantly after 21 days of storage ( $P < 0.05$ ). The increase of SSC may be due to water loss, or due to synthesis of sugars from carbohydrates, except starch (Stanley, Prakash, Marshall, & Schröder, 2013). Compared with the SPI-coated group, the SPI-chitosan coating reduced moisture evaporation, which was mainly associated with weight loss, thus maintaining the SSC of apricots at the end of storage (42 days). However, the SSC of the SPI-



**Fig. 3.** The nanostructural morphologies of chelate-soluble pectin (CSP) determined by atomic force microscopy (AFM) (a) Fresh fruit; (b) Control fruit; (c) Soybean protein isolate (SPI) coated fruit; (d) SPI-chitosan coated fruit; scan area:  $3 \times 3 \mu\text{m}^2$ .

chitosan coated fruit showed no significant differences compared with the control fruit at the end of storage ( $P > 0.05$ ). Zhong and Xia also observed that fruit coating exhibited a best effect on maintaining the content of total soluble solids (Zhong & Xia, 2007).

Compared with the control and SPI coated groups, the SPI-chitosan coating could maintain the quality and prolong shelf life of apricots, as indicated by the firmness, weight loss, TA, and SSC. Previous research reported that soy protein biodegradable films released the inhibitor of ethylene action 1- methylcyclopropane (1-MCP) (Ortiz, Mauri, & Vicente, 2013). Thus, the application of the SPI-chitosan edible coating significantly lowered the respiration rate and maintained fruit quality (firmness, SSC, and TA) of apricots.

Pectin is a major component of cell wall materials. It is closely related to fruit tissue softening because of its solubilisation and depolymerisation during ripening and storage. As shown in Table 2, the WSP content of apricots increased from 37.31 mg/100 g FW (day 0) to 54.49, 51.61, and 55.04 mg/100 g FW for the CK group, SPI group, and SPI-chitosan group, respectively. This WSP content increased further from 54.49, 51.61, and 55.04 mg/100 g FW to 80.97, 64.60, and 61.04 mg/100 g FW, respectively, when the storage period was extended from 7 to 14 days. The WSP content of all groups decreased after storage at 14 days. Meanwhile, the WSP content of the control group was significantly higher than that of the other groups during the whole storage period ( $P < 0.05$ ). For the CSP, there was an increasing trend in all the coated fruit from day 0 to day 28 of the storage period. A significant difference in the CSP content between the control and coated fruit was observed at day 21 ( $P < 0.05$ ). The increasing WSP and CSP content of all samples at day 42 might be related to the loss of water during storage. Loss of water was mainly caused by transpiration and respiration, which were prevented by the SPI-chitosan coating. The increase in solubilisation or depolymerisation of the WSP fraction could be the reason for the loss of firmness during storage. The current result is consistent with that of Gwanpua et al. (2016), who reported that pectin depolymerisation and solubilisation was evident in the rapid softening of apples. Thus, the SPI-chitosan coating could inhibit the decrease in WSP and CSP.

### 3.2. Effect of SPI-chitosan coating on the nanostructure properties of WSP and CSP

WSP and CSP might contribute to cell wall loosening and dis-aggregation (Zhang et al., 2012). Pectin degradation is not only attributed to the modification of the pectin content, but also is caused by the alteration of pectin nanostructures (Chong, Lai, & Yang, 2015). Fig. 1 shows the nanostructural morphology of WSP molecular chains. The height-bar of the image represents differences in height, where white or light features are “high” and darker regions are “lower”. In the image of fresh fruit (Fig. 1a), linear strands and polymer structures were present. The consistent presence of these long chains (Lc), short chains (Sc), and polymers (P) reflected the heterogeneity and complexity of the pectin present in the cell wall materials (Fig. 1a). An increase in the number of cleavage points and smaller polymers was observed in the control and SPI-coated apricots at the end of storage (Fig. 1b–c). This indicated degradation of pectin in the apricots during storage. Micelle-like structures were present in SPI-chitosan coated fruit (Fig. 1d), which might be related to an inhibitory effect of the SPI-chitosan coating.

Using the section analysis software of AFM, the width and length of the WSP molecules were estimated (Fig. 2). The width of the WSP molecules was 11–187 nm. The  $F_q$  value for the higher width of all stored fruit was reduced during storage. At the end of storage, the  $F_q$  value for width ( $\geq 91$  nm) of the SPI-chitosan coated samples was significantly greater than that of the control and SPI-coated samples. The smallest  $F_q$  for width (31–60 nm) was observed in the SPI-chitosan coated samples, whereas the SPI-coated samples revealed a slightly higher  $F_q$  value for width between 31 and 60 nm, but not as high as the control fruit. The  $F_q$  for width ( $\leq 30$  nm) of the SPI and SPI-chitosan coated fruit was less than that of control. There was a significant ( $P < 0.05$ ) reduction in the  $F_q$  for molecules greater than  $6 \mu\text{m}$  in length. The greatest ( $\geq 6 \mu\text{m}$ ) and smallest ( $\leq 1 \mu\text{m}$ )  $F_q$  of length for WSP molecules was obtained in the SPI-chitosan coated apricots at the end of storage (Fig. 2b). These results indicated that SPI-chitosan coating could effectively inhibit the degradation of WSP molecules

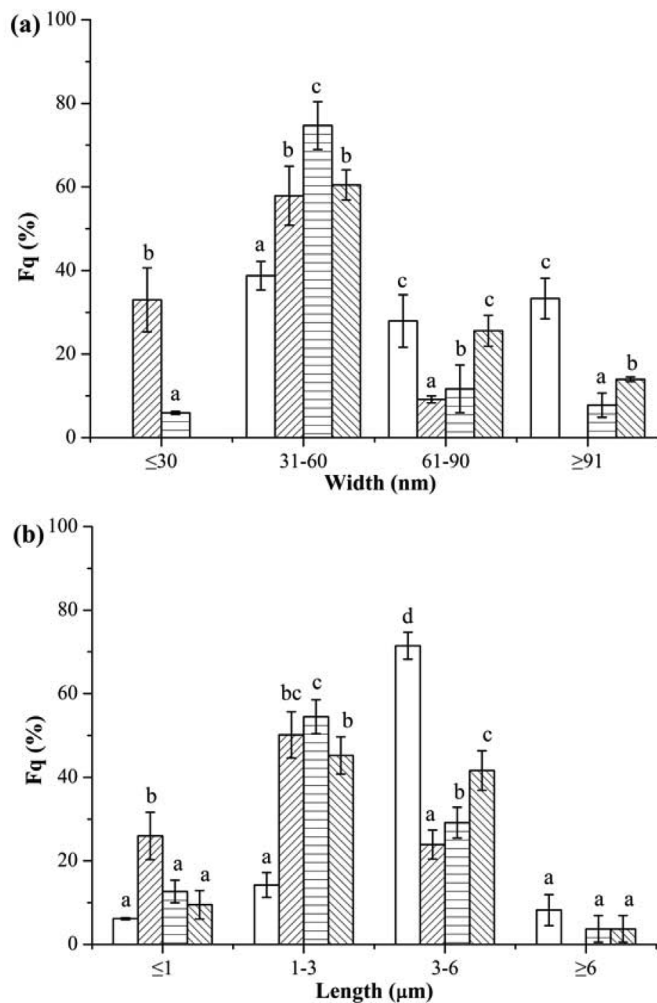


Fig. 4. The quantitative properties of chelate-soluble pectin (CSP) determined by atomic force microscopy (AFM) (a) The width distribution of CSP chains; (b) The length distribution of CSP chains. \*1–3  $\mu\text{m}$  includes 3  $\mu\text{m}$  but does not include 1  $\mu\text{m}$ ; 3–6  $\mu\text{m}$  includes 6  $\mu\text{m}$  but does not include 3  $\mu\text{m}$ ; different small letters between the two groups mean significant difference at  $P < 0.05$ . Fresh fruit ( $\square$ ); CK ( $\text{hatched}$ ) indicates the control group; soybean protein isolate (SPI) ( $\text{diagonal lines}$ ) and SPI-chitosan ( $\text{cross-hatched}$ ) indicates SPI and SPI-chitosan coated fruits, respectively.

during storage.

Fig. 3 presents examples of the AFM images of CSP of apricots during storage. Linear strands were present with a small proportion of them possessing branches (Fig. 3a–b). There was no obvious difference in the morphologies of CSP among the CK, SPI, and SPI-chitosan coated samples, except that no branch structures were observed in the SPI and SPI-chitosan coated samples (Fig. 3c–d). Cleavage points and smaller polymers were also found in the SPI-coated samples, similar to the WSP, at the end of storage (Fig. 3c).

The quantitative properties of CSP are shown in Fig. 4. The width of CSP of all the fruit was within the range of 11–187 nm, the same as for the WSP. A significant ( $P < 0.05$ ) shift to a lower  $F_q$  for width ( $\geq 91$  nm) of the CK, SPI, and SPI-chitosan coated samples was observed at the end of storage. In addition, there was a greater percentage of  $F_q$  in the SPI-chitosan coated fruit than in the CK and SPI-coated fruit for CSP width  $\geq 61$  nm. The lengths of the CSP chains from all the coated fruit were mostly within the range of 1–3  $\mu\text{m}$  (14%) followed by 3–6  $\mu\text{m}$  (71%), and above 6  $\mu\text{m}$  (8%). At the end of storage, the control group contained more CSP with short chains ( $\leq 1$   $\mu\text{m}$  (26%)) than did the coated groups. The  $F_q$  for the length of CSP in the SPI-chitosan

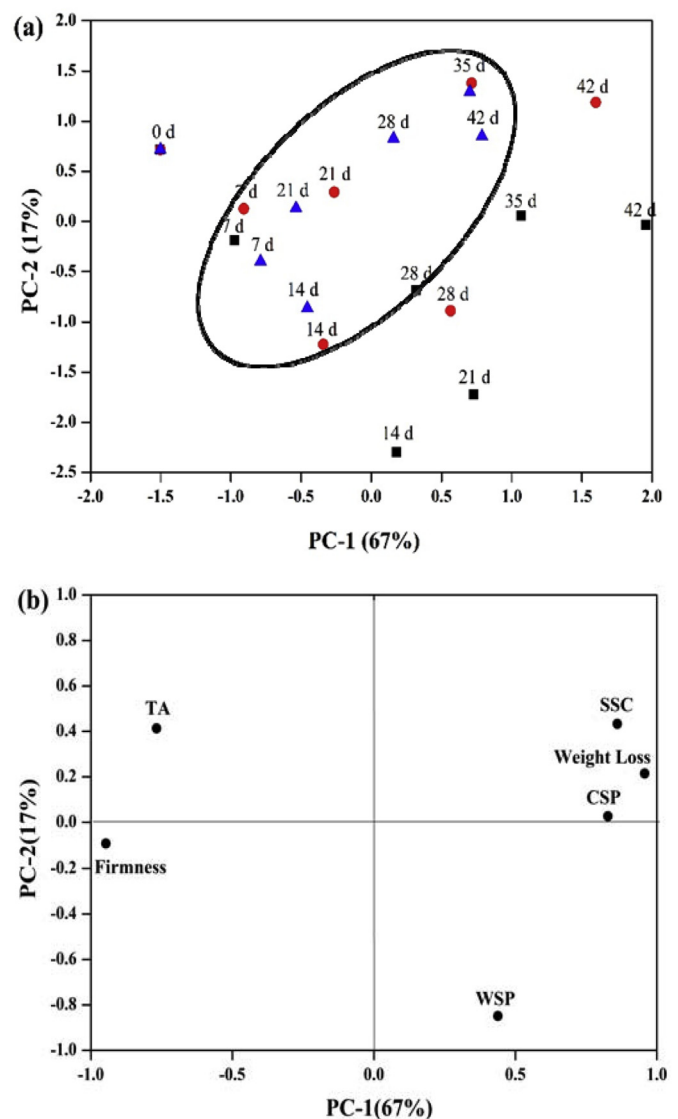


Fig. 5. Principal component analysis (along PC1 and PC2) of weight loss, firmness, soluble solids content (SSC), titratable acidity (TA), and pectin contents (water-soluble pectin (WSP) and chelate-soluble pectin (CSP)) for control, soybean protein isolate (SPI) and SPI-chitosan coated groups. (a) Scores plot; (b) loadings plot. CK ( $\blacksquare$ ), SPI ( $\bullet$ ), SPI-chitosan ( $\blacktriangle$ ).

coated group was:  $\leq 1$   $\mu\text{m}$  (9%), 1–3  $\mu\text{m}$  (45%), 3–6  $\mu\text{m}$  (42%), and greater than 6  $\mu\text{m}$  (4%), which indicated the preventive effects of the SPI-chitosan coating against shortening and degradation of CSP.

### 3.3. The relationship among the softening, pectin structure, and the physicochemical properties of apricots

To reveal the relationships among the parameters measured in the experiment, principal component analysis (PCA) on the mean values was performed. Fig. 5 shows the positions of treatment and variables in the PCA graph along the first two components: PC1 and PC2. The two components explained 84% of the groups' variability. Fig. 5a (scores plot) shows that the SPI-chitosan coated group had similar properties to the SPI-coated group except on day 28 and 42. However, the CK group had distinct properties from the other groups. Fig. 5b (loading graph) provides a comprehensive view of the relationships among the variables measured in this experiment. Weight loss, SSC, and CSP were highly positively correlated. Similarly, WSP was positively correlated with weight loss. Firmness and TA were negatively correlated with weight

loss. Weight loss and firmness were the major factors that affected the quality of apricots.

Softening of fruit is one of the main changes that occur during ripening and storage, and it is accompanied by changes in fruit firmness (Moggia, Graell, Lara, González, & Lobos, 2017; Zhou, Li, & Zhao, 2016). In the present study, the coated groups maintained better firmness than the control group (Table 1). Meanwhile, the WSP and CSP quantitative results suggested that the WSP and CSP chains are closely related to apricot firmness (Fig. 2 and Fig. 4). Higher values for the width and length of pectin molecule corresponded to lower weight loss and better firmness of the fruit.

Pectin, as one of the major components of cell wall materials, plays an important role in maintaining fruit texture (Moreira, Vázquez, Martín-Belloso, & Soliva-Fortuny, 2017). A high level of pectin depolymerisation was accompanied by softening of fruit during storage. Pectin methyl-esterase and polygalacturonase remove the methyl group of the galacturonic acid residues and depolymerise the demethylated pectin backbone, respectively (Gwanpua et al., 2016). Previous research reported that pectin-related enzyme activities were highly correlated with ethylene production (Gwanpua et al., 2014; Yulianti et al., 2015a). Edible coatings could reduce the respiration rate and inhibit the production of ethylene (Gao, Zhu, & Zhang, 2013; Gardesh et al., 2016). The higher quality of SPI-chitosan coated fruit probably resulted from lower levels of weight loss and degradation of pectin.

#### 4. Conclusions

The SPI-chitosan coating had a beneficial effect on weight loss, retention of firmness, TA, and SSC, as well as retention of the WSP and CSP contents of fruit. Weight loss, SSC, and CSP were highly positively correlated. PCA indicated that weight loss and firmness were the major factors affecting the quality of apricots. Compared with the control group, the SPI-chitosan coating showed a greater percentage of larger width and length pectin molecules (width  $\geq$  61 nm; length  $\geq$  3  $\mu$ m). This indicated that SPI-chitosan coating could inhibit the degradation of pectin molecules. The higher quality of SPI-chitosan coated fruit was probably caused by the lower levels of weight loss and degradation of pectin.

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