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ORIGINAL PAPER

Effects of Vacuum Impregnation with Calcium Ascorbate and Disodium Stannous Citrate on Chinese Red Bayberry

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Abstract

This study aimed to improve the shelf life of Chinese red bayberries using vacuum impregnation. Vacuum pressure of 5 kPa for 15 min, atmospheric pressure for 10 min, an impregnation temperature of 20 °C, alone or in combination with isotonic sucrose solution, 1% food-grade disodium stannous citrate (DSC) and 2% food-grade calcium ascorbate were used for vacuum impregnation. Quality parameters, including firmness, weight loss, decay rate, microbial counts and polyphenol oxidase (PPO) and peroxidase (POD) activities, of red bayberries were studied at 2 °C for 10 days. The monosaccharide components, chemical structures and nanostructure properties of chelate-soluble pectin (CSP) were further studied using high-performance liquid chromatography, Fourier transform infrared spectroscopy and atomic force microscopy (AFM). The results indicated that vacuum impregnation with calcium ascorbate alone or calcium ascorbate combined with DSC showed significant effects on inhibiting the decrease of firmness (4–10 days), the increase of weight loss (2–10 days), decay rate (4–10 days) and microbial growth (2–10 days). In addition, vacuum impregnation with single calcium ascorbate or DSC or their combination significantly inhibited the increase of colour difference from day 6 to day 10 during storage, which was better than atmospheric impregnation. Furthermore, vacuum impregnation with DSC and calcium ascorbate had the best effect on sensory attributes. The nanostructure analysis by AFM showed CSP of large width and length when calcium ascorbate was impregnated, suggesting that vacuum impregnated with 2% calcium ascorbate inhibited the degradation and dissociation of CSP, although these fruits showed more branching of rhamnogalacturonan and a small change in chemical structure.

Keywords Nanostructure · Fruit · Atomic force microscopy · Polysaccharide · Monostructure

Introduction

Chinese red bayberry (*Myrica rubra Sieb. Et Zucc.*), which has good edible and medicinal values, is an economical crop native to Southeastern China (Ma et al. 2016). It is favoured by many consumers because of its unique flavour and rich nutritional components, including vitamins, soluble sugars, phenolics, minerals and organic acids (Cheng et al. 2016). Chinese bayberry can also be processed into a variety of forms, including jam, juice, wine, preserves and canned in syrup. However, it is an extremely perishable fruit and has a short shelf life because of moisture loss, mechanical damage, microbial growth, fast decay and other changes during storage (Wang et al. 2009). Therefore, there is an urgent need to find an effective preservation method to preserve its quality and prolong its shelf life.

Vacuum impregnation (VI) is used commonly as a preservation method to prolong the shelf life of production in food industry. VI can impregnate external solutions

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into the porous structure of fruit and vegetable tissues rapidly to improve the sensory, physicochemical and nutritional characteristics of the production (Radziejewska-Kubzdela et al. 2014; Yusof et al. 2017). The VI process of porous products mainly includes changes in internal gas and external solution. These changes are caused by hydrodynamic mechanism and deformation-relaxation phenomena (Fito 1994). VI with $CaCl_2$ could improve the textural properties and inhibited microbial growth of Zucchini (Occhinoa et al. 2011), pineapple (Lima et al. 2016) and melon (Tappi et al. 2016) to extend their shelf life. Furthermore, some studies also found that VI with isotonic trehalose solution or antibrowning agents could significantly inhibit browning of apple and pear during shelf life (Perez-Cabrera et al. 2011; Neri et al. 2016).

The main quality deteriorations of most fruit during storage include texture softening, colour change and microbial growth. Moreover, because calcium can strengthen the fruit cell wall by forming cross-links or bridges between uronic acid carboxyls, while protecting the functional and structural integrity of membranes (Tappi et al. 2016), calcium in fruit cell walls can prevent tissue softening during processing and storage. By contrast, the browning of fruit is closely related to the activity of polyphenol oxidase (PPO) and peroxidase (POD) in the fruit tissues. Disodium stannous citrate (DSC) can delay the adverse changes caused by food oxidation, thus maintaining the colour and flavour of food via its effects of antioxidation, anticorrosion and colour protection. Calcium ascorbate is a compound made from ascorbic acid and active calcium. It not only protects fruit colour and inhibits microbial growth, but also inhibits textural softening of fruits. Therefore, we selected DSC and calcium ascorbate as the auxiliary materials for vacuum impregnation.

Atomic force microscopy (AFM) is a powerful tool to examine the microstructure and molecules of food at the nanostructure level (Chong et al. 2015). Nanostructural changes of pectins during postharvest can be captured by AFM, which is able to characterise the qualitative and quantitative information of macromolecules (Yang et al. 2017; Zhang et al. 2012). Chelate-soluble pectin (CSP) is a type of pectin that is cross-linked with calcium in the mesoglea of fruit. Using AFM to capture the nanostructural changes of CSP can evaluate the effect of calcium on the quality of fruit during storage (Chong et al. 2015; Lara et al. 2004; Liu et al. 2017; Zhang et al. 2017).

The objective of this study was to analyse and evaluate the effects of VI with calcium ascorbate and DSC on the quality characteristics and polysaccharide morphology of Chinese red bayberry. This work evaluated the physicochemical and sensory properties, the physiological and biochemical metabolic processes and the nanostructures of CSP pectins under VI.

Materials and Methods

Raw Material

Red bayberries of the cultivar 'Lang dangzi' (*Myrica rubra Sieb. Et Zucc.*), selected on the basis of a similar size (2 cm in diameter), colour (deep red) and ripening degree (maturity 8~9), were transported at 4 °C to our laboratory within 10 h after harvesting by bayberry growers of Suzhou, Jiangsu, China. The °Brix of the juice produced by these berries was 9.54 ± 0.11. About 2580 (~ 23.6 kg) red bayberries were divided into six groups: untreated group (CK), impregnated under atmospheric pressure (Non VI), VI with isotonic sucrose solution (VI), VI with DSC (food-grade, CSPC Weisheng Pharmaceutical Co., Ltd. Shijiazhuang, Hebei, China) (No), VI with calcium ascorbate (food-grade, CSPC Weisheng Pharmaceutical Co., Ltd. Shijiazhuang, Hebei, China) (Ca) and VI with both DSC and calcium ascorbate (Ca + Na). Red bayberries were stored at 2 °C during the experiment.

VI Treatment and Storage Conditions

VI treatment was conducted in a vacuum chamber that included a vacuum pump (FY-1H-N, Zhejiang Feiyue electromechanical Inc., Zhejiang, China) and a thermostat water bath (DC-2006, Ningbo Xinzhi Biological Polytron Technologies Inc., Ningbo, Zhejiang, China). The VI device for red bayberries is shown in Fig. 1. The same levels of calcium ascorbate (2%) and distannous citrate (1%) were dissolved in an isotonic sucrose solution based on the average °Brix value (9.54 ± 0.11) of the red bayberries. In the experiment, a vacuum pressure of 5 kPa was applied for 15 min after red bayberries were immersed into the impregnation solution. The treatment temperature was 20 °C, and the mass ratio of fruit to solution was 1:3 (*w*/*w*).

After VI treatment, the atmospheric pressure was gradually restored within 10 s, and the bayberries continued to be immersed in the impregnation solution for 10 min. The ideal experimental conditions were obtained via preliminary experiments. The control group used in the experiment was fresh untreated bayberries. After VI treatment, each of 60 bayberries were packed in a polyethylene terephthalate box and stored at 2 °C.

Firmness, Weight Loss and Decay Rate Analysis

The firmness analysis of red bayberries was conducted using a TA-XT2i Texture Analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK). The operating parameters were as follows: an aluminium cylinder probe (35 mm diameter); pre-test speed, 2 mm/s; test speed, 1 mm/s; post-test speed, 3 mm/s; compression degree, 20%; trigger time, 5 s; and trigger

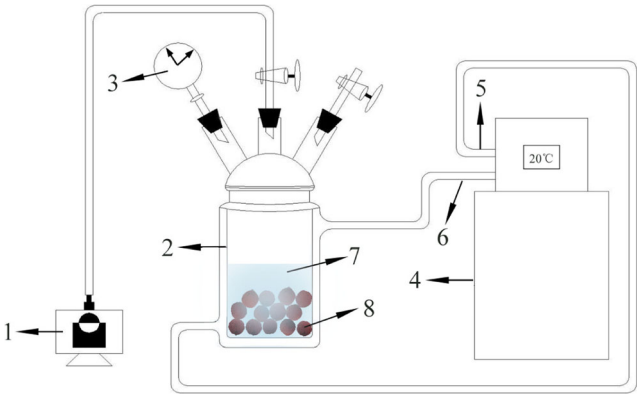


Fig. 1 Schematic diagram of the experimental facility used for vacuum impregnation of Chinese red bayberries. (1) Vacuum pump, (2) vacuum chamber, (3) vacuum pressure gauge, (4) thermostat water bath, (5) water outlet (used for conveying constant temperature circulating water to the interlayer of the vacuum chamber), (6) water inlet (used for transferring excess constant temperature circulating water to the thermostat water bath), (7) impregnation solution and (8) red bayberries

force, 5 g. Twenty bayberries were measured individually for each condition group.

The weight loss was determined according to the change between the initial and final weight of red bayberries in each box during the storage time. The percentage loss of the initial total weight was used to express the weight loss (Eq. (1)) (Wang et al. 2014). The decay rate was expressed as the percentage of the number of decayed bayberries and total bayberries in each box during storage time (Eq. (2)). There were 60 bayberries in each box.

$$Weight\ loss = \frac{m_0 - m_t}{m_0} \times 100\% \tag{1}$$

where m_0 is the initial total weight and m_t is the fruit's weight at time t (days) during storage.

$$\text{Decay } rate = \frac{N_t}{N_0} \times 100\% \tag{2}$$

where N_t is the number of decayed fruit at time t (days) and N_0 is the total number of fruits.

Colour Measurement

Surface colour measurement was carried out using a Minolta CR-400 chroma meter (Konica Minolta, Osaka, Japan). Before measurement, the instrument was calibrated using a white ceramic tile. The colour was measured by CIE $L^*a^*b^*$coordinates using a 10° standard observer and the illuminant D65 (Oms-Oliu et al. b). The colour of bayberries was expressed as L^* (lightness), a^* (+ red and − green) and colour difference (ΔE). Twelve bayberries were determined individually for each treatment group, and each fruit was measured three times at its equatorial position to obtain an average value. Then, the average of L^*, a^* and ΔE were calculated.

$$\Delta E = \sqrt{\left(L_t^* - L_0^*\right)^2 + \left(a_t^* - a_0^*\right)^2 + \left(b_t^* - b_0^*\right)^2} \tag{3}$$

where L_t^* is L^* at time t after processing; L_0^* is L^* of a fresh untreated bayberry; a_t^* is a^* at time t after processing; a_0^* is a^* of a fresh untreated bayberry; b_t^* is b^* at time t after processing; and b_0^* is b^* of a fresh untreated bayberry.

Determination of PPO and POD Activities

The extraction and determination of PPO and POD were carried out following the method of Soares et al. (2005) and Oms-Oliu et al. (2008a, b), with slight modifications. Each sample was measured in triplicate.

For PPO analysis, 20 g of fresh bayberry was homogenised for 3 min with 50 mL of phosphate buffer solution (50 mmol/L, pH 7.0) and centrifuged (Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 12,000×g for 10 min at 4 °C to extracted PPO. The supernatant was used to determine the PPO activity. Acetic acid sodium acetate buffer (4 mL 50 mmol/L, pH 5.5), 1 mL of 50 mmol/L catechol solution and 100 μL of enzyme extract were added to a test tube. Distilled water was used as reference, and the absorbance value was measured at 420 nm.

For POD analysis, 50 g of fresh bayberry was homogenised with 50 g 20 mmol/L sodium phosphate buffer in a mortar at 4 °C and centrifuged at 10,000×g for 15 min to extract the POD. The supernatant was used as the enzyme extract: 0.5 mL of the supernatant was mixed with 3 mL of 20 mmol/L guaiacol solution and 200 μL 0.5 mol/L H_2O_2 solution. The absorbance value was measured at 470 nm, with distilled water as the reference. Enzyme activity was expressed as unit min^{-1} mg^{-1} fresh weight (FW).

Microbial Analyses

The moulds, yeasts and total aerobic psychrophilic microbes in each group were evaluated during storage of red bayberries using standard methods (GB 478915-2010; Chong et al. 2015). For the enumeration of moulds and yeasts, a 25-g bayberry in 225 mL of sterilised distilled water was shaken in a conical flask and used to make 10-fold series dilutions. Meanwhile, a 25-g bayberry in 225 mL of normal saline was homogenised for 1 min for the total aerobic psychrophilic microorganisms count. One millilitre of each 10-fold serial dilution was spread on petri dishes containing 15 mL of culture medium of potato dextrose agar (PDA, poured at 46 °C) for moulds and yeasts and on 15 mL culture medium of plate count agar (PCA, poured at 46 °C) for aerobic psychrophilic count (APC). After agar freezing, the flat plate was inverted

and incubated at 28 °C for 5 days for moulds and yeasts and at 7 °C for 10 days for APC.

Microbial analyses were carried out in triplicate of at least two appropriate dilutions. Log colony forming units (CFU) per gramme was used to express the microbial counts.

Sensory Evaluation

Sensory evaluation of untreated and VI-treated bayberries was carried out at 2 days after processing by means of comparison tests, using ten trained panellists. Coded samples impregnated with and without vacuum pressure, sucrose solution, calcium ascorbate and DSC were compared in terms of the following sensory parameters: appearance, flavour, smell, colour and overall quality; each evaluation index was evaluated by a five-point scale (Cocci et al. 2014).

Extraction and Analysis of CSP

CSP Extraction

Cell wall and CSP were extracted according to the method described by Chen et al. (2009). About 10 g of red bayberry flesh was ground in a mortar in an ice bath and boiled in 80% (*v*/*v*) ethanol for 20 min. The mixture was then cooled to room temperature and filtered. This process was repeated three times. The flesh residue was then transferred to a solution of 50 mL dimethyl sulphoxide (DMSO)/H_2O (9:1, *v*/*v*) and kept overnight for 4 °C. After filtering, the residue was washed with deionised water and immersed in 200 mL of a chloroform/ethanol solution (2:1, *v*/*v*) for 20 min and then rinsed with acetone until totally white. The residue was the cell wall material (CWM).

The CWM of each group was shaken in 10 mL deionised water at 25 °C, 140 r/min for 4 h and then centrifuged at 4 °C, 10,000×*g* for 10 min (Shanghai Anting Scientific Instrument Factory, Shanghai, China). The above procedure was repeated two more times after centrifugation. The residue was further shaken and centrifuged three times in 10 mL 50 mM cyclohexane-trans-1,2-diamine tetra-acetate (CDTA) for CSP extraction. All the supernatants collected after centrifugation were used as the CSP.

FTIR Analysis of CSP

FTIR spectroscopy (WQF-510A, Thermo Scientific, Boston, MA, USA) was used to determine the structure and chemical characteristics of CSP. CSP (2 mg) was mixed with KBr (1:100, *w*/*w*) after vacuum drying (DZF-28, Beijing Yongguangming Medical Instrument, Co., Ltd. Beijing, China.) for FTIR analysis. The spectra of CSP were obtained from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. The results of FTIR were analysed using Origin 8.5 (OriginLab, Northampton, MA, USA).

Monosaccharide Constituent Determination of CSP

The monosaccharide constituents of CSP were analysed by HPLC referring to previously described methods (Xin et al. 2010; Zhang et al. 2017), with slight modifications. About 4 mg of freeze-dried CSP was dissolved in 2 mL of 2 mol/L trifluoroacetic acid solution and hydrolysed at 110 °C for 8 h after sealing. After hydrolysis, each CSP sample after was dried with N_2 using a MTN-2800D pressure blowing concentrator (Tianjin Autoscience Instrument Co., Ltd., Tianjin, China). Each sample was then dissolved in 450 μL of 0.3 mol/L NaOH and 900 μL of 0.5 mol/L 1-phenyl-3-5-pyrazolone (PMP). The mixture was reacted at 70 °C for 30 min in a DC-2006 thermostatic water bath (Ningbo Xinzhi Biological Polytron Technologies Inc., Ningbo, Zhejiang, China). After cooling to room temperature, 450 μL of 0.3 mol/L HCl was added for neutralisation and 1 mL chloroform was added for extraction. The above procedure was performed twice more until the chloroform was colourless. Finally, the solution was filtered through a 0.45-μm membrane.

Monosaccharide samples were analysed using a Waters 1525 HPLC system (Waters, Milford, MA, USA) equipped with a UV 2489 detector (Waters) and an Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm, Agilent Technologies, Inc., Richardson, TX, USA). The mobile phase (pH 6.9) consisted of 0.05 mol/L phosphate buffer with (A) 15% (*v*/*v*) and (B) 40% (*v*/*v*) acetonitrile. Test conditions for HPLC were as follows: detection wavelength, 250 nm; flow rate of the mobile phase, 1 mL/min; sample injection volume, 20 μL; column temperature, 25 °C; time gradient, 0–5–25–41 min; and concentration gradient of B phase, 0–15–25–0 (%).

AFM Analysis

The nanostructure of CSP was analysed according to methods described in previous reports (Chen et al. b; Yang 2014; Zhang et al. 2017) using a multimode NanoScope IIIa AFM (ZhuoLun MicroNano Equipment Co., Ltd. Shanghai, China). Each CSP solution (10 μg/mL) was mixed for 2 min with a QL-866 Vortex Mixer (Haimen Lindberg Instrument Manufacturing Co., Ltd. Haimen, Jiangsu, China). About 10 μL of pectin solution was then dropped onto a newly stripped mica sheet surface and dried naturally in air at room temperature. A Si_3N_4 probe was used for image scanning. The resonance frequency, the scanning frequency and the scanning vertical and horizontal distances of the probe were 330 kHz, 0.5–2 Hz, 0.1 nm and 1–2 nm, respectively. Each sample was scanned for at least 40 images.

The AFM images were analysed offline using AFM software (version 5.30r3sr3). The width (W), length (L) and height (V) of pectin molecules were obtained by section analysis (Chen et al. 2011; Yang 2014).

Statistical Analysis

Results were expressed as the mean ± standard deviation using analysis of variance (ANOVA). All the statistical analyses were performed using SPSS (20.0, SPSS Inc., Chicago, IL, USA) to determine the significant difference between the different treatment groups in storage time, and all graphs were plotted using Origin (8.5).

Results and Discussion

Effects of VI on the Firmness, Weight Loss and the Decay Rate of Red Bayberries

Chinese bayberries soften rapidly during storage because of their susceptibility to fungal contamination and thus have a short shelf life (Ma et al. 2016). Texture loss caused by moisture loss and metabolic changes is one of the most important changes of fruit during storage (Peretto et al. 2017). Figure 2a shows that the firmness of Chinese red bayberries decreased during storage. The firmness of the Ca and Na + Ca group was significantly higher than the control group during days 4 to day 10. The firmness of the Non VI group was significantly higher than the control group only at days 4 and 10. This could be explained by the formation of cross-links or bridges between uronic acid carboxyls caused by calcium, which was also reported in previous studies (Gras et al. 2003; Yang et al. 2017). This showed that calcium could limit the hardness loss of red bayberries during storage. This was also observed by Occhinoa et al. (2011) and Liu et al. (2009) for zucchini and apricot fruits, respectively. The Ca group had the greatest firmness, indicating that VI can effectively impregnate Ca^{2+} into fruit tissues when compared with atmospheric impregnation. Calcium significantly ($P < 0.05$) affected the firmness of red bayberries during storage.

Respiration and transpiration are the main causes of weight loss in stored fruit and vegetables (Zhu et al. 2008). Weight loss of Chinese red bayberries in all treatment and control groups increased continuously during storage (Fig. 2b). The Ca and Na + Ca group showed a significant delay the weight loss of Chinese red bayberries during 10 days of storage, while the Non VI group was significant better than the control only in the first 8 days ($P < 0.05$). Compared with other groups, the Ca group showed the lowest weight loss, except on the second day, during the storage period. This was likely caused by the effect of Ca^{2+} on maintaining pectin texture (Chen et al. 2011; Liu et al. 2017; Mao et al. 2017).

As shown in Fig. 2c, a gradual increase in the decay rate with storage time was observed in all treatment and control groups. This was consistent with previous studies on loquat fruit and Chinese bayberries (Chen et al. 2013a; Ma et al. 2016; Wang et al. 2016). The Non VI group significantly ($P < 0.05$) inhibited fruit decay compared to the control group from day 2 to day 8. Meanwhile, the Ca and Na + Ca groups significantly ($P < 0.05$) inhibited fruit decay, with the Ca group showing the lowest decay rate during 10 days of storage. At the end of storage, the decay rates of the Ca and Na + Ca groups were only 15.83 and 20%, respectively, while that of the control group was 31.67%. There was no significant difference ($P > 0.05$) in decay rate between the Na and control groups during the whole storage period, although Na group showed a lower decay rate. These results indicated that simultaneous impregnation with Ca^{2+} had the best effect on the preservation quality of Chinese red bayberries. This phenomenon was likely caused by the structural effects of calcium (Lara et al. 2004).

Effects of VI on Colour Development of Red Bayberries

Surface colour is an important index to evaluate the quality attributes of Chinese red bayberries because it is an important indicator to judge the degree of freshness and browning, even the level of contamination of fruit, and it affects the level of perception and acceptance by consumers (Chen et al. 2011; Pinheiro et al. 2016). The L^* value of the control group declined from 35.81 to 23.33 and the a^* value increased from 55.90 to 68.83, while VI treatment groups (Na, Ca and Na + Ca) showed less change (Fig. 3a, b). The results indicated that the surface colour of Chinese red bayberries gradually became dark during storage. This phenomenon was also found in figs (Reyes-Avalos et al. 2016). After treatment, the lightness of all VI groups decreased compared with the control group (Fig. 3a). This phenomenon was also reported in minimally processed pear (Perez-Cabrera et al. 2011). This could be explained by the increase of refractive index of the fruit tissue, which was caused by the substitution of the gas in the porous tissue of the fruit with the impregnation solution (Chiralt and Talens 2005; Yang et al. 2017).

As shown in Fig. 3b, there was a significant difference between the Na, Ca and Na + Ca groups and the control group for the a^* value from day 2 to day 10 during storage. The rise in the a^* value of the control group indicated that the surface colour of the red bayberries changed towards red during storage. Other researchers also observed this phenomenon in banana (Chaguri et al. 2017), mulberry fruit (Chen et al. 2015) and persimmon (Sanchis et al. 2016). The value of a^* (Fig. 3b) and colour difference (Fig. 3c) between Na, Ca and Na + Ca groups showed a similar change, indicating that there was no significant difference between these three treatments in

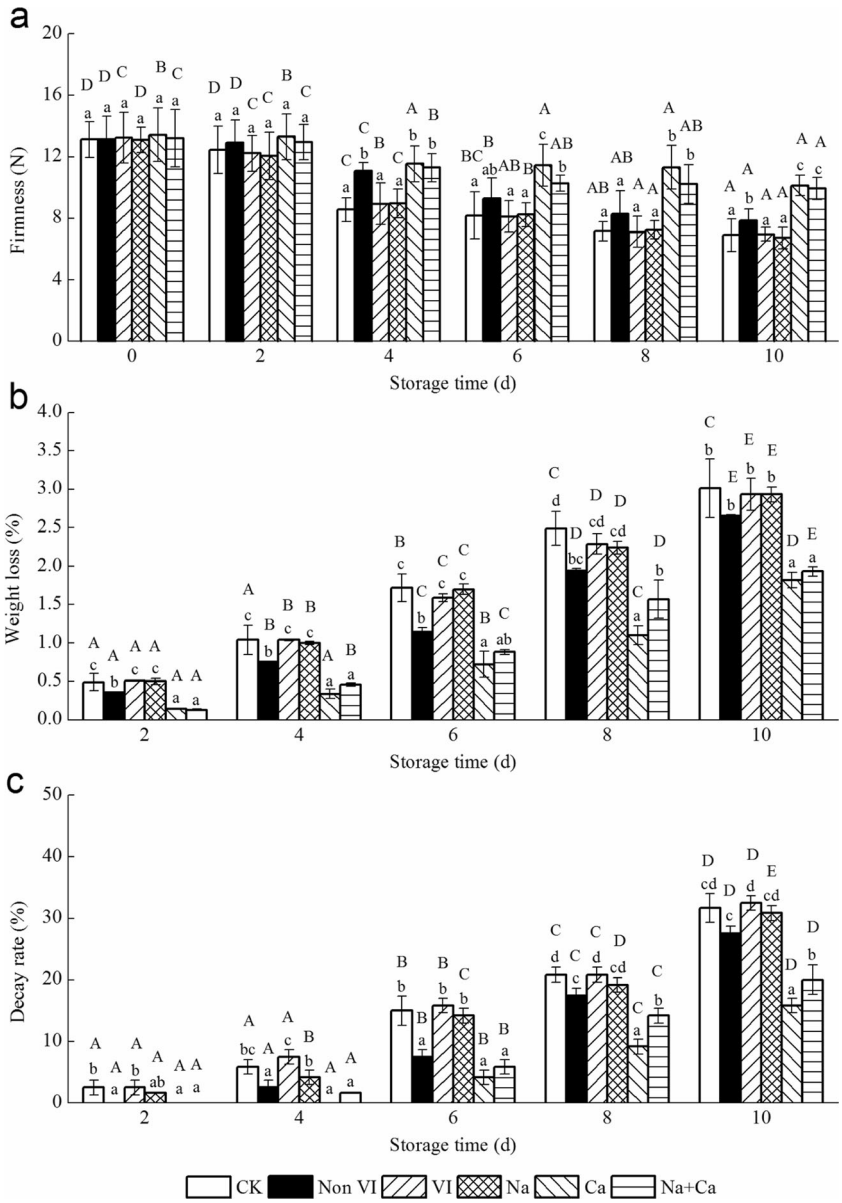


Fig. 2 Effects of vacuum impregnation on firmness (**a**), weight loss (**b**) and decay rate (**c**) of Chinese red bayberries during storage time at 2 °C. Note: CK untreated group, Non VI impregnated under atmospheric pressure, VI vacuum impregnation with isotonic sucrose solution, Na vacuum impregnation with disodium stannous citrate, Ca vacuum impregnation with calcium ascorbate, Na + Ca VI with both disodium stannous citrate and calcium ascorbate. Different capital letters (A–E) and lowercase letters (a–d) indicate a significant difference ($P < 0.05$) with different storage times and different treatments, respectively

limiting the colour change of red bayberries during storage. In addition, the colour difference of the Non VI group was similar to these three groups over the first 4 days and began to increase continuously after the sixth day. The results showed that vacuum impregnation with calcium ascorbate and DSC could effectively control the colour change of postharvest fruit. This was also reported for other fruits, such as honeydew melon (Chong et al. 2015).

Effects of VI on the PPO and POD Activities of Red Bayberries

PPO and POD catalyse the oxidation of phenolic compounds to form quinone compounds. Further polymerisation of the quinone compounds forms a darker substance. As shown in Fig. 4, the control and VI-treated fruits revealed higher enzyme activities compared with the other treated fruits for both PPO and POD. PPO and POD activities increased significantly ($P < 0.05$) in early storage and then decreased slowly after reaching a peak. A similar phenomenon was found for other fruit and vegetables, like eggplants (Fan et al. 2016a, b), blueberries (Martynenko and Chen 2016), pears (Fan et al. 2016a) and mulberry fruit (Chen et al. 2015). The maximum PPO and POD activities were obtained at day 4 (Fig. 4b). This was consistent with the previously noted colour change (Fig. 3).

The effects of DSC and calcium ascorbate with VI on PPO activities were similar. Both of them significantly ($P < 0.05$) inhibited the PPO activity of Chinese red bayberries during

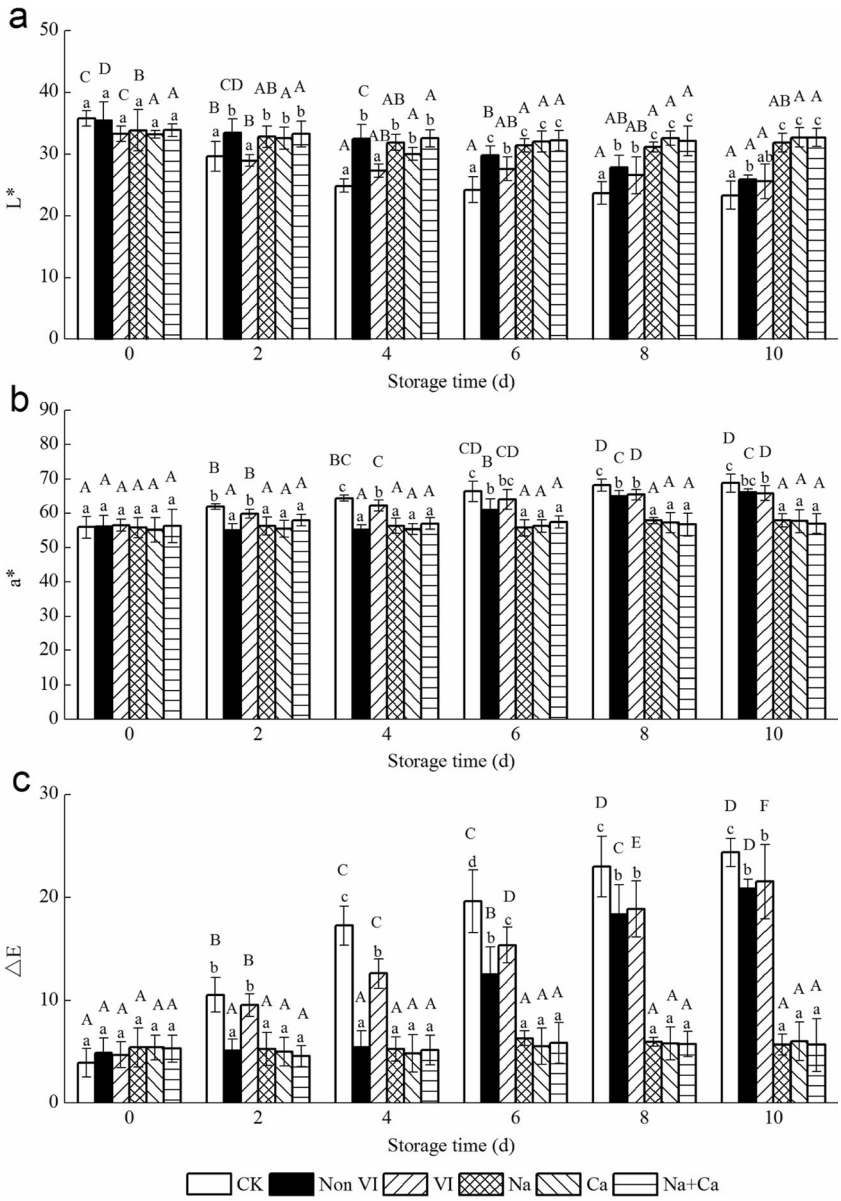


Fig. 3 Effects of vacuum impregnation on the colour of Chinese red bayberries during storage time at 2 °C. **a** Lightness, L^*. **b** Red and green value, a^*. **c** Colour difference, ΔE. Note: CK untreated group, Non VI impregnated under atmospheric pressure, VI vacuum impregnation with isotonic sucrose solution, Na vacuum impregnation with disodium stannous citrate, Ca vacuum impregnation with calcium ascorbate, Na + Ca VI with both disodium stannous citrate and calcium ascorbate. Different capital letters (A–F) and lowercase letters (a–c) indicate a significant difference ($P < 0.05$) with different storage time and different treatments, respectively

storage at 2 °C (Fig. 4a). Therefore, the colour changes of these groups were relatively stable (Fig. 3). This showed that DSC and calcium ascorbate effectively inhibited the oxidation of phenolic compounds by hydrogen peroxide to produce quinone compounds, thereby preventing the deterioration of red bayberry colour. However, there was no significant difference ($P > 0.05$) caused by DSC on the inhibition of POD activity. This result indicated that calcium ascorbate had a higher antioxidant effect on Chinese red bayberries than DSC.

Effects of VI on Microbial Growth of Red Bayberries

Red bayberries lack protection from the scarfskin; therefore, they are susceptible to microbial infection. Figure 5 shows the microorganisms present (moulds, yeasts and total aerobic psychrophilics) in each group during storage at 2 °C. The VI group had the highest growth of microorganisms, which could be attributed to the phenomenon of deformation relaxation caused by VI, which resulted in filling with isotonic sucrose solution, thereby improving the nutrient utilisation of microbial growth in intercellular space.

As shown in Fig. 5, fruit impregnated with calcium ascorbate only and with both DSC and calcium ascorbate showed significant ($P < 0.05$) inhibition of microbial growth during storage. The effectiveness of calcium ions to inhibit microbial growth was also observed for other fruits, including grapefruit (Moraga et al. 2009), pears (Perez-Cabrera et al. 2011) and honeydew melon (Chong et al. 2015). This was caused by calcium's ability to not only maintain the integrity of the fruit cell wall after harvest, resulting in resistance to microbial

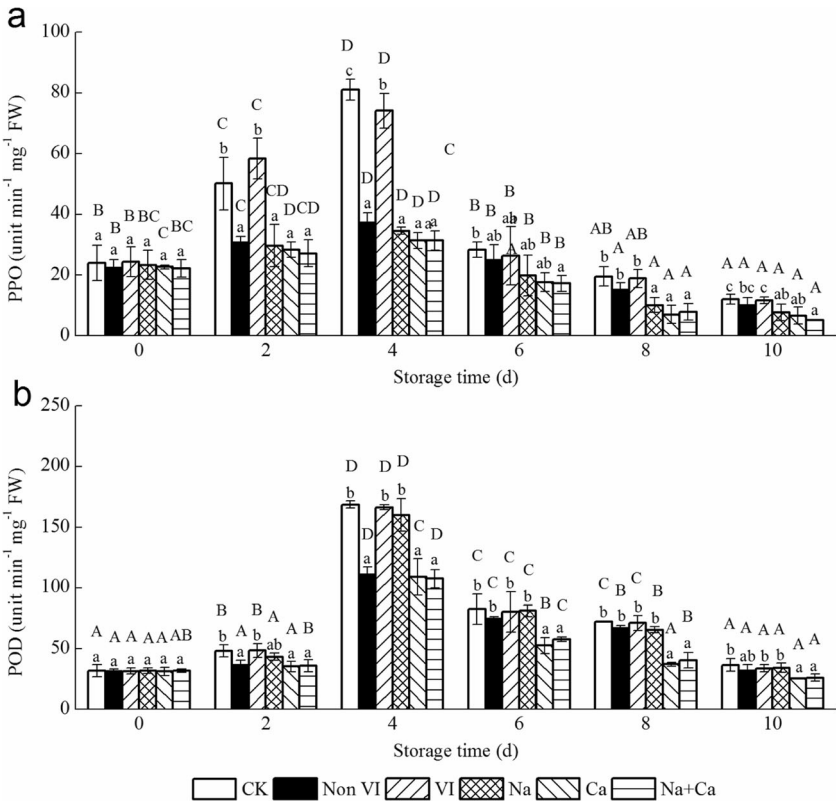


Fig. 4 Effects of vacuum impregnation on polyphenol oxidase (PPO) (**a**) and peroxidase (POD) (**b**) enzyme activities of Chinese red bayberries during storage time at 2 °C. Note: CK untreated group, Non VI impregnated under atmospheric pressure, VI vacuum impregn ation with isotonic sucrose solution, Na vacuum impregnation with disodium stannous citrate, Ca vacuum impregnation with calcium ascorbate, Na + Ca VI with both disodium stannous citrate and calcium ascorbate. Different capital letters (A–D) and lowercase letters (a–c) indicate a significant difference ($P < 0.05$) with different storage time and different treatments, respectively

infection (Chen et al. 2011), but also the reduction in the pH and water activity of the fruit, which inhibits microbial growth. Moreover, fruit impregnated with calcium ascorbate showed only minimal microbial growth.

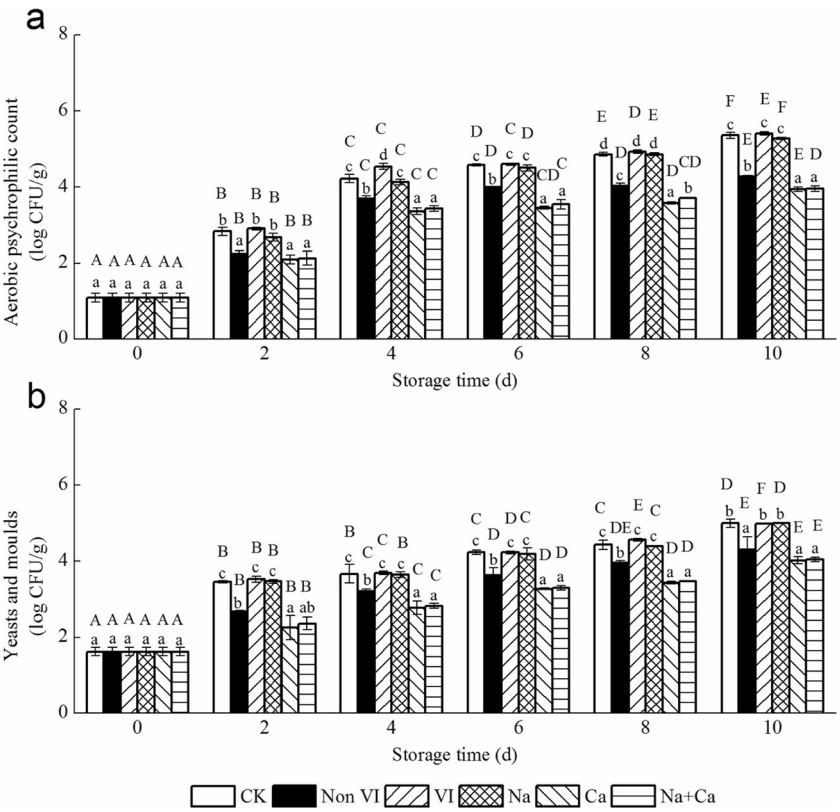


Fig. 5 Aerobic psychrotrophic microorganisms (**a**) and yeasts and moulds (**b**) growth in both control and treatment Chinese red bayberries. Note: CK untreated group, Non VI impregnated under atmospheric pressure, VI vacuum impregnation with isotonic sucrose solution, Na vacuum impregnation with disodium stannous citrate, Ca vacuum impregnation with calcium ascorbate, Na + Ca VI with both disodium stannous citrate and calcium ascorbate. Different capital letters (A–F) and lowercase letters (a–d) indicate a significant difference ($P < 0.05$) with different storage times and different treatments, respectively

Table 1 Sensory evaluation of Chinese red bayberries under different treatments. Different lowercase letters within each column indicate significant differences ($P < 0.05$) among different samples under the same evaluation index

Treatment	Evaluating indicator				
	Appearance	Flavour	Smell	Colour	Overall acceptability
CK	3.8 ± 0.8a	2.4 ± 1.1a	3.6 ± 0.9a	3.0 ± 1.2a	2.6 ± 1.5a
Non VI	3.8 ± 1.1a	3.4 ± 0.8ab	3.2 ± 0.4a	3.0 ± 1.4a	3.0 ± 1.0ab
VI	3.8 ± 0.8a	4.2 ± 0.8b	3.6 ± 0.5a	3.2 ± 1.1a	3.6 ± 0.9abc
Na	4.0 ± 0.7a	3.4 ± 0.5ab	3.6 ± 0.5a	4.2 ± 1.1a	3.8 ± 0.8abc
Ca	4.2 ± 0.8a	3.8 ± 0.4b	3.8 ± 0.8a	3.4 ± 0.5a	4.2 ± 0.8bc
Na + Ca	4.4 ± 0.6a	4.0 ± 0.7b	3.6 ± 0.5a	4.2 ± 0.8a	4.6 ± 0.5c

Sensory Evaluation

As seen from Table 1, there was no significant difference ($P > 0.05$) in the appearance, smell and colour of Chinese red bayberries between all groups after 2 days in cold storage. Impregnation with sucrose, calcium ascorbate or DSC had significant ($P < 0.05$) effects on the flavour of red bayberry fruit compared with the control group. Compared with the control group, the Ca and Na + Ca treatment groups showed a significant ($P < 0.05$) effect on the overall acceptability of red bayberries. Regarding the flavour and overall acceptability of bayberry fruit, those impregnated with sucrose and with both calcium ascorbate and DSC were preferred by the assessors.

Effects of VI on the Chemical Structure of CSP

As can be seen from the infrared spectra in Fig. 6, the control and treatment fruit had strong absorption peaks in two regions, 1200–1600 and 2200–3600 cm^{-1}. The strong absorption between 2200 and 3600 cm^{-1} corresponded to the stretching vibrations of O-H, which are induced by intermolecular and intramolecular hydrogen bonds. The strong and broad absorption peaks at 3421 cm^{-1} in the CSP indicated hydrogen bonds with galacturonic acid polymers after the stretching vibrations of O-H, while the absorption peaks at 2943 cm^{-1} were related to the stretching and bending vibrations of C-H, CH_2, and CH_3 (Zhang et al. 2017). The bending vibration of C-H caused the absorption at 1412 cm^{-1}. The strong absorption at 1597 cm^{-1} was attributed to the stretching vibration of C=O of -NH_2COCH_3 and the vibrations of cell wall lignin (Popescu et al. 2007).

Compared with fresh fruit, the control, VI and Na groups resulted in a large decrease at 2360 and 1421 cm^{-1} and a small decrease at 2943 and 3421 cm^{-1}. However, all characteristic absorption peaks of the Non VI group showed a small decrease, while the peak strengths of the Ca and Na + Ca groups were almost unchanged. Thus, the chemical analysis by FTIR indicated that calcium ascorbate treatment could maintain the chemical structures of pectin.

Effect of VI on the Monosaccharide Constitute of CSP

Pectin consists mainly of three basic structural units: homogalacturonan (HG), type I xylogalacturonan and rhamnogalacturonan (RG-I) and type II rhamnogalacturonan

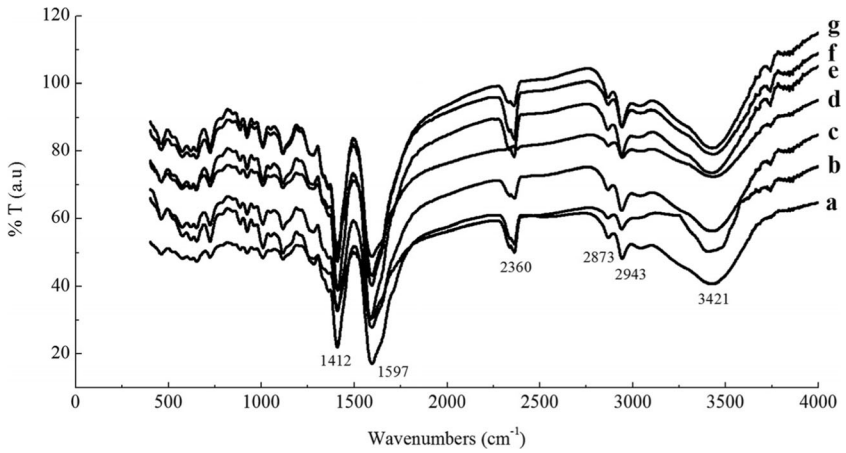


Fig. 6 Effect of vacuum impregnation on the chemical structure of chelate-soluble pectin. (a) fresh fruits, (b) vacuum impregnation with isotonic sucrose solution at 10 days, (c) vacuum impregnation with disodium stannous citrate at 10 days, (d) control fruits at 10 days, (e) vacuum impregnation with both disodium stannous citrate and calcium ascorbate at 10 days, (f) vacuum impregnation with calcium ascorbate at 10 days, (g) impregnated under atmospheric pressure at 10 days

Table 2 Effect of vacuum impregnation on the monosaccharide constitute of chelate-soluble pectin

Monosaccharide (mol%)	Day 0	Day 6						Day 10					
	CK	CK	Non VI	VI	Na	Ca	Na + Ca	CK	Non VI	VI	Na	Ca	Na + Ca
Man	2.6 ± 0.6	3.0 ± 0.2a	2.5 ± 0.2a	3.0 ± 1.0a	3.1 ± 0.2a	3.0 ± 0.4v	3.5 ± 0.7a	3.4 ± 0.1a	3.0 ± 0.1a	3.4 ± 0.4a	3.7 ± 0.6a	3.0 ± 0.3a	2.9 ± 0.1a
Rha	6.7 ± 0.2	7.8 ± 0.0a	7.3 ± 0.3a	7.2 ± 0.3a	7.7 ± 0.4a	7.7 ± 0.4a	7.3 ± 0.3a	15.9 ± 0.3b	14.3 ± 1.4b	15.4 ± 1.2b	15.6 ± 0.1b	10.5 ± 0.5a	10.8 ± 0.3a
GalUA	33.5 ± 0.1	35.4 ± 0.6a	35.4 ± 1.0a	34.9 ± 1.4a	36.2 ± 2.7a	37.8 ± 0.1a	34.6 ± 1.0a	39.1 ± 0.2ab	38.1 ± 0.1ab	39.8 ± 1.3b	39.0 ± 1.0ab	39.7 ± 0.4ab	37.9 ± 0.3a
Glc	31.1 ± 2.1	21.4 ± 0.2c	17.1 ± 1.0b	19.7 ± 2.3bc	19.6 ± 0.9bc	10.9 ± 0.0a	16.6 ± 0.6b	13.3 ± 1.5b	6.0 ± 1.3a	11.3 ± 0.4b	11.4 ± 1.3b	4.1 ± 0.1a	6.2 ± 0.1a
Gal	18.8 ± 2.0	22.3 ± 0.7a	28.4 ± 1.9b	24.3 ± 0.8a	23.9 ± 3.2a	30.7 ± 0.9b	28.6 ± 3.0b	25.1 ± 0.9a	30.9 ± 0.4c	26.4 ± 0.6b	26.1 ± 0.0ab	34.5 ± 0.1e	32.7 ± 0.5d
Xyl	1.6 ± 0.1	1.5 ± 0.5a	1.4 ± 0.0a	1.4 ± 0.1a	.4 ± 0.3a	1.2 ± 0.2a	1.2 ± 0.2a	1.2 ± 0.2a	.1 ± 0.0a	1.3 ± 0.3a	1.1 ± 0.1a	1.1 ± 0.1a	1.1 ± 0.1a
Ara	5.8 ± 0.1	7.8 ± 0.5ab	8.2 ± 0.1bc	7.5 ± 0.1a	7.8 ± 0.2ab	8.8 ± 0.1c	8.3 ± 0.3bc	2.2 ± 0.2a	6.8 ± 0.4c	2.9 ± 0.2b	3.2 ± 0.1b	7.5 ± 0.0d	8.5 ± 0.2e
GalUA/Rha	5.0	4.6a	4.9a	4.9a	4.7a	4.9a	4.8a	2.5a	2.6a	2.6a	2.5a	3.8b	3.5b
(Gal + Ara)/Rha	3.7	4.0a	5.0bc	4.4abc	4.1ab	5.2c	5.1bc	1.7a	2.7b	1.9a	1.9a	4.0c	3.8c

Different lowercase letters within each line indicate significant differences ($P < 0.05$) among different samples at the same storage time

CK untreated group, *Non VI* impregnated under atmospheric pressure, *VI* vacuum impregnation with isotonic sucrose solution, *Na* vacuum impregnation with disodium stannous citrate, *Ca* vacuum impregnation with calcium ascorbate, *Na + Ca* vacuum impregnation with both disodium stannous citrate and calcium ascorbate, *Man* mannose, *Rha* rhamnose, *GalUA* galacturonic acid, *Glc* glucose, *Gal* galactose, *Xyl* xylose, *Ara* arabinose

(RG-II) (Baum et al. 2017). Table 2 shows the changes in the monosaccharide components of CSP of Chinese red bayberries during cold storage at 2 °C. The CSP of red bayberries mainly contains galacturonic acid (GalUA), glucose (Glc), galactose (Gal), rhamnose (Rha) and arabinose (Ara). Among them, the content of GalUA was the highest. The molar ratios of Man (mannose), Rha, GalUA, Glc, Gal, Xyl (xylose) and Ara in the early storage stage were 2.6:6.7:33.5:31.1:18.8:1.6:5.8. Man, Rha, GalUA, Gal and Ara in both the treatment and control groups continued to increase during storage. This result showed that the CSP of red bayberries mainly comprised the HG backbone and RG-I side chain.

The main chain of the RG-I region comprises mainly GalUA and Rha, while Ara and Gal were the main components of its branched chain. Their dissociation and degradation could lead to a decrease in the ratio of GalUA to Rha and (Gal + Ara) to Rha. Previous studies have found that RG-I region was closely related to fruit texture and significantly affected its firmness (Yang 2014). As shown in Table 2, the molar ratios of GalUA/Rha and (Gal + Ara)/Rha in the control group decreased from 5.0 and 3.7 to 2.5 and 1.7, respectively. However, the molar ratios of GalUA/Rha and (Gal + Ara)/Rha in the Ca group after storage for 10 days were 3.8 and 4.0, while those for the Na + Ca group were 3.5 and 3.8, respectively. The molar ratio results indicated that vacuum impregnation with calcium ascorbate could maintain the firmness of red bayberries by inhibiting the degradation of CSP during storage at 2 °C.

Effects of VI on the Nanostructure of CSP of Red Bayberries

Qualitative Results of the Nanostructure of CSP

The changes to the cell wall structures, especially pectin components and nanostructures, are mostly correlated with the textural softening of fruit (Chen et al. 2011; Chong et al. 2015). AFM could display the complex structure of cell wall polysaccharides visually (Yang et al. 2017). Therefore, fresh fruit and all treated fruits at day 6 and day 10 during cold storage were subjected to AFM to characterise the changes in the CSP.

Figure 7a shows an AFM image of the CSP nanostructure of fresh red bayberries. In the early storage period, the pectin mainly consists of long chains (Lc), linear single fractions (Ls), short chains (Sc), branch structure (Br) and polymers (P). In contrast, AFM images of all treatment groups at day 6 show an increase in short pectin chains, branch structures and aggregates (Fig. 7c–h). This may be caused by the dissociation and dissolution of polymers and the backbone caused by PG and galactosidase. Furthermore, the AFM image of the

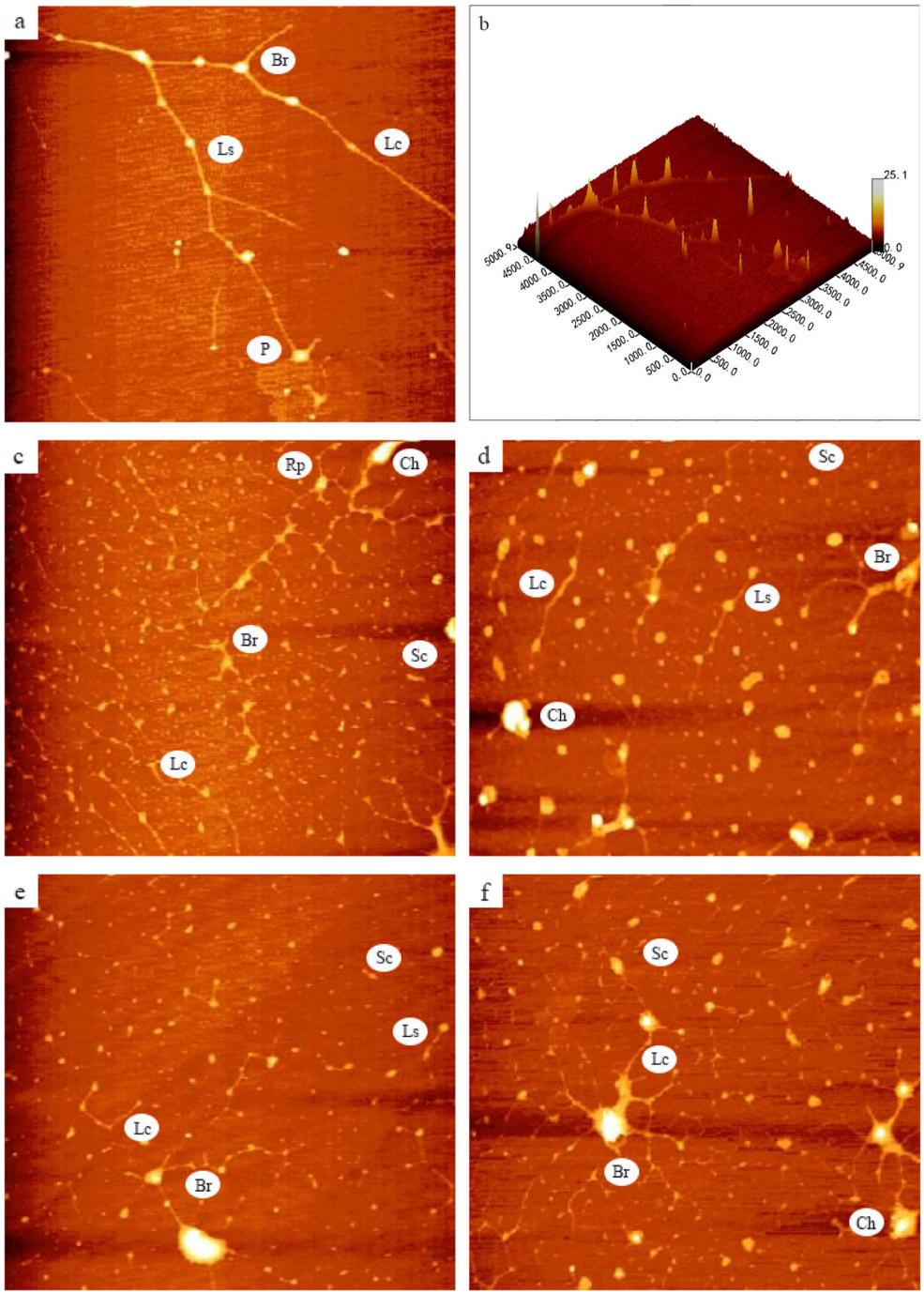
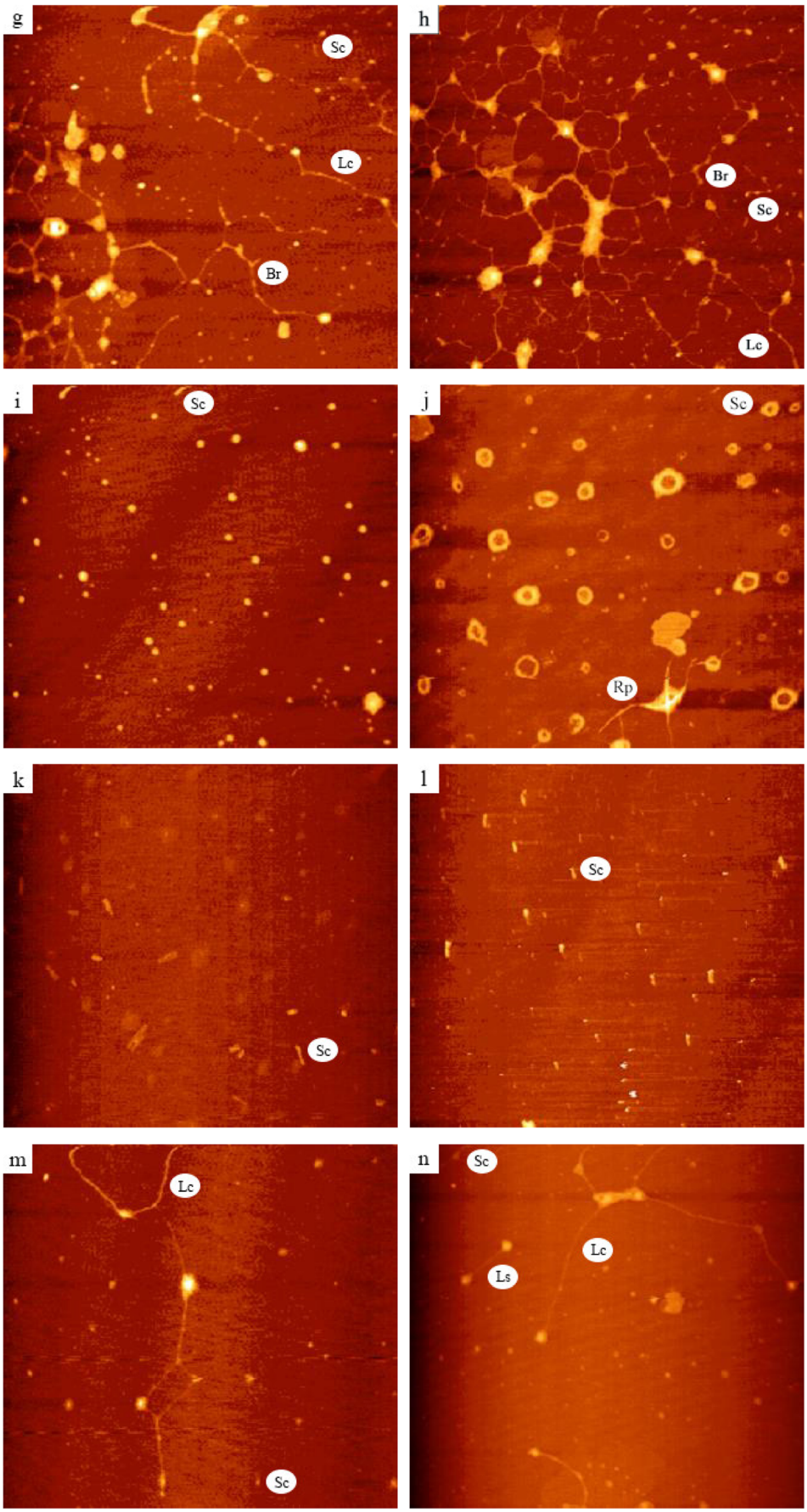


Fig. 7 Atomic force microscopy (AFM) images of chelate-soluble pectin of Chinese red bayberries. Note: scan size: 5 μm × 5 μm. **a**, **b** Fresh untreated. **c** Untreated group at day 6. **d** Impregnated under atmospheric pressure at day 6. **e** Vacuum impregnation with isotonic sucrose solution at day 6. **f** Vacuum impregnation with disodium stannous citrate at day 6. **g** Vacuum impregnation with calcium ascorbate at day 6. **h** Vacuum impregnation with both disodium stannous citrate and calcium ascorbate at day 6. **i** Untreated group at day 10. **j** Impregnated under atmospheric pressure at day 10, **k** Vacuum impregnation with isotonic sucrose solution at day 10. **l** Vacuum impregnation with disodium stannous citrate at day 10. **m** Vacuum impregnation with calcium ascorbate at day 10. **n** Vacuum impregnation with both disodium stannous citrate and calcium ascorbate at day 10. Lc long chains, Ls linear single fractions, Sc short chains, P polymers, Ch cyclohexane-trans-1, 2-diamine tetra-acetate, Br branch structure, Rp stretched chains from cyclohexane-trans-1,2-diamine tetra-acetate

Fig. 7 (continued)



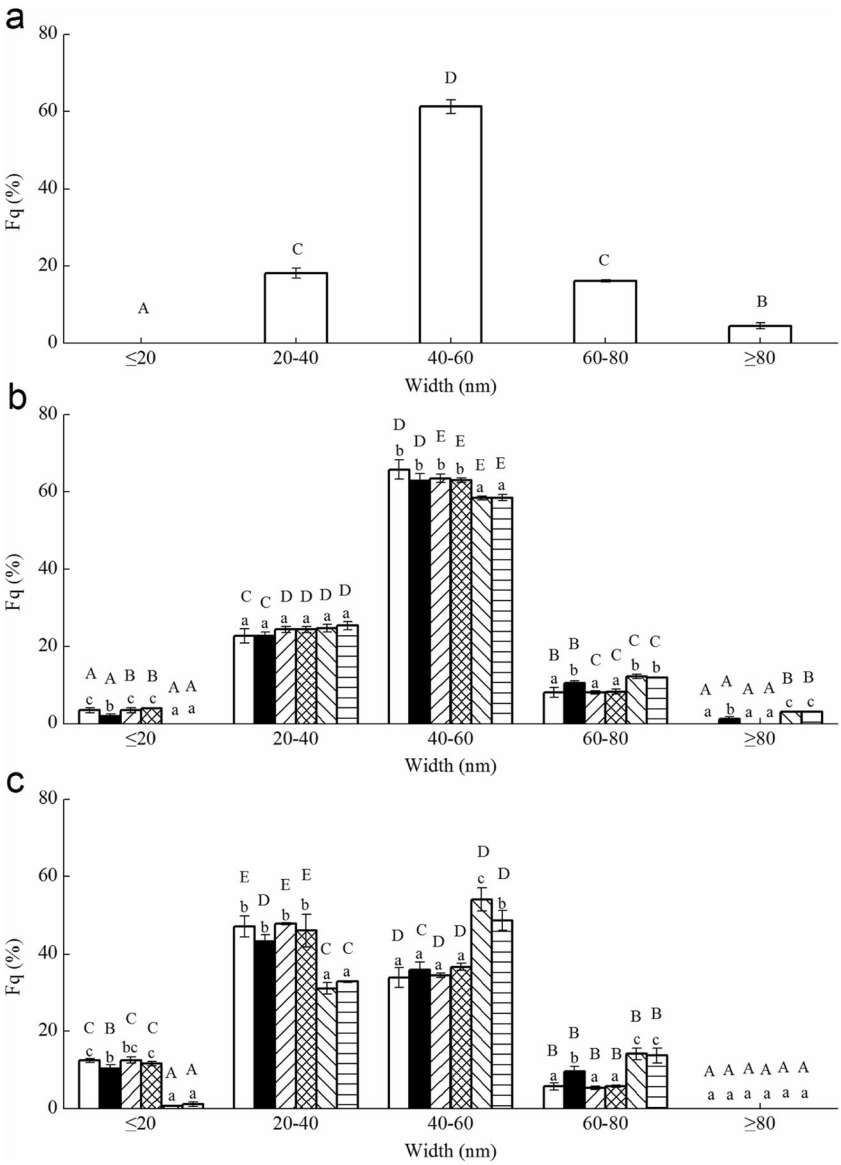


Fig. 8 Effect of different treatments on quantitative width and length distribution of chelate-soluble pectin chains of the Chinese red bayberry. **a** Width at day 0 (fresh fruit). **b** Width at day 6. **c** Width at day 10. **d** Length at day 0 (fresh fruit). **e** Length at day 6. **f** Length at day 10. Fq the frequency of the same range of width and length. Note: CK untreated group, Non VI impregnated under atmospheric pressure, VI vacuum impregnation with isotonic sucrose solution, Na vacuum impregnation with disodium stannous citrate, Ca vacuum impregnation with calcium ascorbate, Na + Ca VI with both disodium stannous citrate and calcium ascorbate. Different capital letters (A–E) and lowercase letters (a–d) indicate a significant difference ($P < 0.05$) with different ranges of length (width) and different treatments, respectively

Ca and Na + Ca group showed an obvious network structure (Fig. 7g–h). This indicated that calcium treatment increased the crosslinking between the pectin chains of the HG regions.

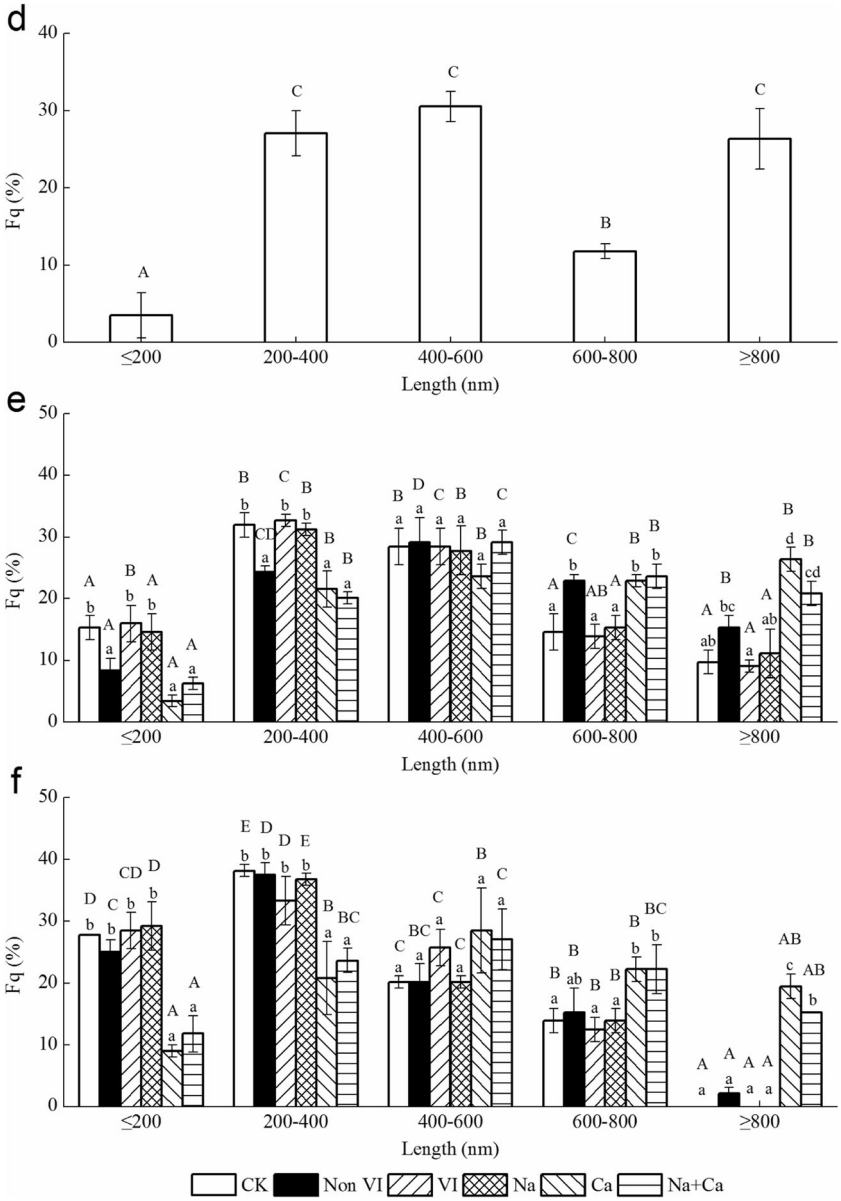
After cold storage for 10 days, compared with the other groups, the Ca and Na + Ca groups had longer pectin chains (Fig. 7i–n). The results showed that impregnation with calcium ascorbate could effectively retard the degradation and dissociation of CSP during cold storage.

Quantitative Results of the Nanostructure of CSP

Figure 8 shows the width and length distribution of CSP of red bayberries at days 0, 6 and 10. The width and length of the CSP chains of fresh red bayberries were mainly within 20–90 and 100–1200 nm, respectively. The height of the CSP chains of red bayberries ranged from 0.5 to 4.5 nm. The frequency of shorter and smaller pectin chains increased gradually during storage. Within 10 days after storage, the proportion of chains with a width less than 20 nm in the control group increased to 12.4% and the chain width greater than 80 nm decreased to zero. The VI and Na group showed a similar change to the control group.

After 10 days of storage, the proportion of chains with a width below 20 nm in the Ca (0.8%) and Na + Ca (1.2%) groups were significantly ($P < 0.05$) smaller than in the other groups (Fig. 8c). As shown in Fig. 8f, the Ca group had more long chains and fewer short chains than the control and other groups at the end of storage. The proportion of chains with a length shorter than 200 nm was 27.8% for the control group and 9.0% for the Ca group, while proportion of chains with a length longer than 800 nm was zero for the control group and 19.4% for the Ca group. This revealed that vacuum

Fig. 8 (continued)



impregnation with calcium could effectively inhibit pectin degradation of CSP during cold storage. This result was attributed to the addition of calcium ions, which greatly enhanced the cross-linking ability between the pectin chains (Mao et al... 2017; Yang et al. 2017). The higher percentage of wider and longer CSP chains after calcium treatment was also found in strawberries (Chen et al. 2011), papayas (Yang et al. 2017), apricots (Liu et al. 2017) and grapes (Mao et al. 2017).

Mechanism of Calcium Ascorbate and DSC

Diagrams of the interaction between Ca^{2+} and CSP are shown in Fig. 9a, b. These are consistent with the mechanism proposed by Yang et al. (2017). Ca^{2+} can combine with substances in the cell wall to form a solid calcium bridge after it enters the fruit. CSP mainly consists of the HG backbone and the RG-I side chain. After adding Ca^{2+}, the carboxyl group on the two galactose residues of the HG chain is linked with a Ca^{2+} to form a stable structure comprising interchain crosslinking. Thus, because of the higher degree of cross-linking, a stronger pectin structure is formed, and the mechanical strength of the cell wall increases. Therefore, the Ca and Na + Ca-impregnated fruit had a larger chain widths and lengths, which improved the firmness and structure of the red bayberries.

Figure 9c shows a diagram of the mechanism of the interaction between DSC and PPO. The PPO enzyme uses copper as the prosthetic group (Whitaker and Lee 1995). DSC can complex with the two copper groups of the oxygenated

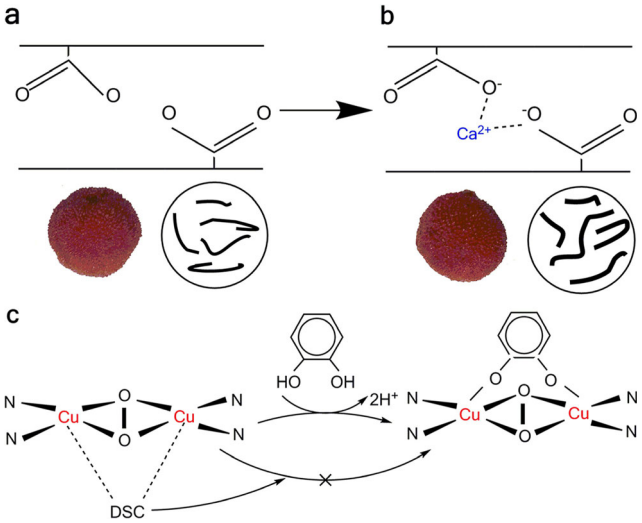


Fig. 9 Mechanism of calcium ascorbate and disodium stannous citrate. **a** Two homogalacturonan chains with free carboxyls of the fresh fruit, with shorter and smaller chain lengths. **b** The carboxyl group on galactose acid residues forms a cross-link with Ca^{2+} after the formation of a negative charge field; chain lengths and widths are longer and larger. **c** Two copper groups (red) of the oxygenated polyphenol oxidase (oxy-PPO) combined with the oxygen atoms of 2 hydroxyl groups of catechol to form O_2·catechol·PPO; the addition of disodium stannous citrate can complex with the two copper groups of the oxy-PPO to prevent the formation of O_2·catechol·PPO (colour figure online)

polyphenol oxidase (oxy-PPO) to prevent the formation of O_2·catechol·PPO. Adding DSC could inhibit PPO activity and prevent the formation of quinones compounds caused by the oxidation of phenols. Thus, the Na and Na + Ca-impregnated fruit showed a lower PPO activity and more stable colour.

Conclusion

Vacuum impregnation with 2% calcium ascorbate and 1% DSC effectively improved the quality of Chinese red bayberries during cold storage. In this study, vacuum impregnation with calcium ascorbate and DSC slowed down the rate of quality deterioration of red bayberries. There were significant differences of firmness between the fruits impregnated with calcium ascorbate (10.13 N) and control bayberries (6.91 N) at the end of storage, while the Ca bayberries maintained a lower moisture loss (1.82%) and decay rate (15.83%) than the control bayberries (3.01 and 31.67%). The higher value of GalUA/Rha (3.8) and (Gal + Ara)/Rha (4.0) ratios of Ca bayberries in structural analysis than control (2.5 and 1.7) indicated that calcium ascorbate inhibited CSP degradation. During cold storage, vacuum impregnation with calcium ascorbate maintained a lower PPO and POD activities, colour change and microbial growth than control bayberries while vacuum impregnation with DSC was only effective at inhibiting PPO activity and colour change. Sensory evaluation showed that fruit impregnated both with DSC and calcium ascorbate showed the best overall quality (4.6) while the control was 2.6. Vacuum impregnation with calcium ascorbate also maintained the morphological integrity of CSP during cold storage. By AFM, the Fq of greater chain width and length of CSP in the Ca and Na + Ca groups were higher than the control and other groups, indicating that vacuum impregnation with calcium delayed pectin degradation. Overall, vacuum impregnation with DSC and calcium ascorbate could prolong the shelf life of Chinese red bayberries during cold storage.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

Baum, A., Dominiak, M., Vidal-Melgosa, S., Willats, W. G. T., Sondergaard, K. M., Hansen, P. W., et al. (2017). Prediction of pectin yield and quality by FTIR and carbohydrate microarray analysis. *Food and Bioprocess Technology, 10*(1), 143–154.

Chaguri, L., Sanchez, M. S., Flammia, V. P., & Tadini, C. C. (2017). Green banana (*Musa cavendishii*) osmotic dehydration by non-caloric solutions: modeling, physical-chemical properties, color, and texture. *Food and Bioprocess Technology, 10*(4), 615–629.

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 strawberries (*Fragaria ananassa* Duch.) under calcium chloride treatment. *Food Chemistry, 126*(2), 450–459.

Chen, F., Zhang, L., An, H., Yang, H., Sun, X., Liu, H., Yao, Y., & Li, L. (2009). The nanostructure of hemicellulose of crisp and soft Chinese cherry (*Prunus pseudocerasus* L.) cultivars at different stages of ripeness. *LWT-Food Science and Technology, 42*(1), 125–130.

Chen, H., Gao, H., Fang, X., Ye, L., Zhou, Y., & Yang, H. (2015). Effects of allyl isothiocyanate treatment on postharvest quality and the activities of antioxidant enzymes of mulberry fruit. *Postharvest Biology and Technology, 108*, 61–67.

Chen, H., Yang, H., Gao, H., Long, J., Tan, F., Fang, X., & Jiang, Y. (2013a). Effect of hypobaric storage on quality, antioxidant enzyme and antioxidant capability of the Chinese bayberry fruits. *Chemistry Central Journal, 7*(1), 1–7.

Chen, Y., Chen, F., Lai, S., Yang, H., Liu, H., Liu, K., Bu, G., & Deng, Y. (2013b). 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.

Cheng, H., Chen, J., Chen, S., Xia, Q., Liu, D., & Ye, X. (2016). Sensory evaluation, physicochemical properties and aroma-active profiles in a diverse collection of Chinese bayberry (*Myrica rubra*) cultivars. *Food Chemistry, 212*, 374–385.

Chiralt, A., & Talens, P. (2005). Physical and chemical changes induced by osmotic dehydration in plant tissues. *Journal of Food Engineering, 67*(1), 167–177.

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.

Cocci, E., Sacchetti, G., Rocculi, P., & Dalla-Rosa, M. (2014). Response of Pink Lady® apples to postharvest application of 1-methylcyclopropene as a function of applied dose, maturity at harvest, storage time and controlled atmosphere storage. *Journal of the Science of Food and Agriculture, 94*(13), 2691–2698.

Fan, L., Shi, J., Zuo, J., Gao, L., Lv, J., & Wang, Q. (2016a). Methyl jasmonate delays postharvest ripening and senescence in the non-climacteric eggplant (*Solanum melongena* L.) fruit. *Postharvest Biology and Technology, 120*, 76–83.

Fan, M., Li, W., Hu, X., Sun, Y., Yu, G., & Zhang, X. (2016b). Effect of micro-vacuum storage on active oxygen metabolism, internal browning and related enzyme activities in Laiyang pear (*Pyrus bretschneideri Reld*). *LWT - Food Science and Technology, 72*, 467–474.

Fito, P. (1994). Modelling of vacuum osmotic dehydration of food. *Journal of Food Engineering, 22*(1-4), 313–328.

GB 478915-2010. *National food safety standard*, *Food microbiological examination: enumeration of moulds and yeasts*.

Gras, M. L., Vidal, D., Betoret, N., Chiralt, A., & Fito, P. (2003). Calcium fortification of vegetables by vacuum impregnation interactions with cellular matrix. *Journal of Food Engineering, 56*(2–3), 279–284.

Lara, I., Garcia, P., & Vendrell, M. (2004). Modifications in cell wall composition after cold storage of calcium-treated strawberry (*Fragaria × ananassa* Duch.) fruit. *Postharvest Biology and Technology, 34*(3), 331–339.

Lima, M., Tribuzi, G., Souza, J., Souza, I., Laurindo, J., & Carciofi, B. (2016). Vacuum impregnation and drying of calcium-fortified pineapple snacks. *LWT-Food Science and Technology, 72*, 501–509.

Liu, H., Chen, F., Lai, S., Tao, J., Yang, H., & Jiao, Z. (2017). Effects of calcium treatment and low temperature storage on cell wall polysaccharide nanostructures and quality of postharvest apricot (*Prunus armeniaca*). *Food Chemistry, 225*, 87–97.

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*(8), 1131–1140.

Ma, R., Yu, S., Tian, Y., Wang, K., Sun, C., Li, X., Zhang, J., Chen, K., & Fang, J. (2016). Effect of non-thermal plasma-activated water on fruit decay and quality in postharvest Chinese bayberries. *Food and Bioprocess Technology, 9*(11), 1825–1834.

Mao, J., Zhang, L., Chen, F., Lai, S., Yang, B., & Yang, H. (2017). Effect of vacuum impregnation combined with calcium lactate on the firmness and polysaccharide morphology of kyoho grapes (*Vitis vitis* x V. *labrusca*). *Food and Bioprocess Technology, 10*(4), 699–709.

Martynenko, A., & Chen, Y. (2016). Degradation kinetics of total anthocyanins and formation of polymeric color in blueberry hydrothermodynamic (HTD) processing. *Journal of Food Engineering, 171*(4), 44–51.

Moraga, M. J., Moraga, G., Fito, P. J., & Martinez-Navarrete, N. (2009). Effect of vacuum impregnation with calcium lactate on the osmotic dehydration kinetics and quality of osmodehydrated grape fruit. *Journal of Food Engineering, 90*(3), 372–379.

Neri, L., Biase, L. D., Sacchetti, G., Mattia, C. D., Santarelli, V., Mastrocola, D., & Pittia, P. (2016). Use of vacuum impregnation for the production of high quality freshlike apple products. *Journal of Food Engineering, 179*, 98–108.

Occhino, E., Hernando, I., Llorca, E., Neri, L., & Pittia, P. (2011). Effect of vacuum impregnation treatments to improve quality and texture of zucchini (*Cucurbita pepo* L). *Procedia Food Science, 1*(1), 829–835.

Oms-Oliu, G., Odriozola-Serrano, I., Soliva-Fortuny, R., & Martin-Belloso, O. (2008a). The role of peroxidase on the antioxidant potential of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres. *Food Chemistry, 106*(3), 1085–1092.

Oms-Oliu, G., Soliva-Fortuny, R., & Martin-Belloso, O. (2008b). Edible coatings with antibrowning agents to maintain sensory quality and antioxidant properties of fresh-cut pears. *Postharvest Biology and Technology, 50*(1), 87–94.

Peretto, G., Du, W., Avena-Bustillos, R. J., Berrios, J. D. J., Sambo, P., & Mchugh, T. H. (2017). Electrostatic and conventional spraying of alginate-based edible coating with natural antimicrobials for preserving fresh strawberry quality. *Food and Bioprocess Technology, 10*(1), 165–174.

Perez-Cabrera, L., Chafer, M., Chiralt, A., & Gonzalea-Martinez, C. (2011). Effectiveness of antibrowning agents applied by vacuum impregnation on minimally processed pear. *LWT - Food Science and Technology, 44*(10), 2273–2280.

Pinheiro, J. C., Alegria, C. S. M., Abreu, M. M. M. N., Goncalves, E. M., & Silva, C. L. M. (2016). Evaluation of alternative preservation treatments (water heat treatment, ultrasounds, thermosonication and UV-C radiation) to improve safety and quality of whole tomato. *Food and Bioprocess Technology, 9*(6), 924–935.

Popescu, C. M., Popescu, M. C., Singurel, G., Vasile, C., Argyropoulos, D. S., & Willfor, S. (2007). Spectral characterization of eucalyptus wood. *Applied Spectroscopy, 61*(11), 1186–1177.

Radziejewska-Kubzdela, E., Bieganska-Marecik, R., & Kidon, M. (2014). Applicability of vacuum impregnation to modify physicochemical, sensory and nutritive characteristics of plant origin products - a review. *International Journal of Molecular Sciences, 15*(9), 16577–16610.

Reyes-Avalos, M. C., Femenia, A., Minjares-Fuentes, R., Contreras-Esquivel, J. C., Aguilar-Gonzalez, C. N., Esparza-Rivera, J. R., & Meza-Velazquez, J. A. (2016). Improvement of the quality and the shelf life of figs (*Ficus carica*) using an alginate-chitosan edible film. *Food and Bioprocess Technology, 9*(12), 2114–2124.

Sanchis, E., González, S., Ghidelli, C., Sheth, C. C., Mateos, M., Palou, L., & Perez-Gago, M. B. (2016). Browning inhibition and microbial control in fresh-cut persimmon (*Diospyros kaki Thunb.* cv. Rojo Brillante) by apple pectin-based edible coatings. *Postharvest Biology and Technology, 112*, 186–193.

Soares, A. G., Trugo, L. C., Botrel, N., & Souza, L. (2005). Reduction of internal browning of pineapple fruit (*Ananas comusus* L.) by preharvest soil application of potassium. *Postharvest Biology and Technology, 35*(2), 201–207.

Tappi, S., Tylewicz, U., Romani, S., Siroli, L., Patrignani, F., Rosa, M., & Rocculi, P. (2016). Optimization of vacuum impregnation with calcium lactate of minimally processed melon and shelf-life study in real storage conditions. *Journal of Food Science, 81*(11), E2734–E2742.

Wang, K., Jin, P., Cao, S., Shang, H., Yang, Z., & Zheng, Y. (2009). Methyl jasmonate reduces decay and enhances antioxidant capacity in Chinese bayberries. *Journal of Agricultural and Food Chemistry, 57*(13), 5809–5815.

Wang, Z., Narciso, J., Biotteau, A., Plotto, A., Baldwin, E., & Bai, J. (2014). Improving storability of fresh strawberries with controlled release chlorine dioxide in perforated clamshell packaging. *Food and Bioprocess Technology, 7*(12), 3516–3524.

Wang, Y., Shan, Y., Chen, J., Feng, J., Huang, J., Jiang, F., et al. (2016). Comparison of practical methods for postharvest preservation of loquat fruit. *Postharvest Biology and Technology, 120*, 121–126.

Whitaker, J. R., & Lee, C. Y. (1995). *Recent in chemistry of enzymatic browning: an overview in enzymatic browning and its prevention*. Washington, DC: American Chemical Society.

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., Wu, Q., Ng, L. Y., & Wang, S. (2017). Effects of vacuum impregnation with calcium lactate and pectin methylesterase on quality attributes and chelate-soluble pectin morphology of fresh-cut papayas. *Food and Bioprocess Technology, 10*(5), 901–913.

Yusof, N. L., Wadsö, L., Rasmusson, A. G., & Galindo, F. G. (2017). Influence of vacuum impregnation with different substances on the metabolic heat production and sugar metabolism of spinach leaves. *Food and Bioprocess Technology., 10*(10), 1907–1917. https://doi.org/10.1007/s11947-017-1959-3.

Zhang, L., Chen, F., Yang, H., Ye, X., Sun, X., Liu, D., Yang, B., An, H., & Deng, Y. (2012). Effects of temperature and cultivar on nanostructural changes of water-soluble pectin and chelate-soluble pectin in peaches. *Carbohydrate Polymers, 87*(1), 816–821.

Zhang, L., Chen, F., Zhang, P., Lai, S., & Yang, H. (2017). Influence of rice bran wax coating on the physicochemical properties and pectin nanostructure of cherry tomatoes. *Food and Bioprocess Technology, 10*(2), 349–357.

Zhu, X., Wang, Q., Cao, J., & Jiang, W. (2008). Effects of chitosan coating on postharvest quality of mango (*Mangifera indica* L. cv. Tainong) fruits. *Journal of Food Processing and Preservation, 32*(5), 770–784.