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Effect of chitosan coatings on the evolution of sodium carbonate-soluble pectin during sweet cherry softening under non-isothermal conditions

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ABSTRACT

The inhibiting effect of chitosan coating (2%) on the softening and sodium carbonate-soluble pectin (SSP) evolution of sweet cherries during non-isothermal storage was investigated. Chitosan coating significantly extend the softening (6.4% greater than the control group), maintained the SSP content (6.6% greater than the control group), and reduced the degradation of SSP by inhibiting the expression of the *paPME1–5* genes, which regulating pectin methylesterase activity of sweet cherries under temperature variation. In addition, the results of methylation and monosaccharide composition indicated that the chitosan coating reduced demethylation of SSP and the loss of RG-I main and side chain neutral sugars. Atomic force microscopy images revealed that the coated sweet cherries contained more linked, branched, and long SSP chains and maintained the width of the pectin backbone (>140 nm). These results indicated that a chitosan coating is feasible to preserve postharvest fruit under non-isothermal conditions.

1. Introduction

The food cold chain is the special supply chain system that provides fruit and vegetables with a low temperature environment during their precooling, transportation, storage, distribution, and retail, until being sold to the consumers [1]. However, as fruit and vegetables travel through various points of the cold chain, they might be exposed to ambient temperatures ('broken cold chain'). In addition, temperature fluctuation can also be caused by temporary equipment failure and by incorrect response of temperature controlling devices during cooling or improper handling [2]. Compared with an isothermal cold chain, the quality of fruit and vegetables deteriorates faster under non-isothermal or higher temperature conditions [3–6].

Firmness reduction is one of the main phenomena of quality deterioration of fruit and vegetables under non-isothermal temperature conditions, and the texture characteristics of fruit body are primarily due to the physicochemical characteristics of the cell wall [7]. Previous studies showed that pectin as the skeleton of the cell wall has an important role in maintaining tissue firmness [8,9]. Pectin deterioration is significantly and closely related to the various pectinase classes and expression of related gene, such as pectin methyl esterase (PME), pectate lyase (PL), polygalacturonase (PG), alpha-arabinofuranosidase (α-AF), and beta-galactosidase (β-GAL) [10–12]. However, the temporal pattern and species of enzymes involved in softening vary greatly among fruit and vegetable species and cultivars. For example, during apple ripening, the expression levels of all *MdPMEs* were downregulated, while the expression levels of *MdPG1*, *Mdb-GAL1*, *2*, and *Mda-AF2* were upregulated [11]. In the process of grape softening, the activity of PG and β-GAL was high, but PME activity was low, and no PL activity was detected [13]. Wang reported that there has very high activity of PG and PME, and undergo extensive polyuronide solubilization and depolymerization during softening [14].

Chitosan as a cationic polysaccharide with high molecular weight is a deacetylated derivate of chitin. It has a selective permeability to gasses (CO_2 and O_2), an excellent film forming ability [15], and antimicrobial properties [16]. Therefore, chitosan-based coatings have received extensive attention and research for the preservation of fresh fruit and vegetable materials under storage and distribution conditions [15,17], including fresh-cut apple [18], apricot [19], mango [20] and Chinese cherry [8]. These coatings also have similar preservation effects on fruit and vegetables at room temperature, such as in mango [21],

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grape berry [22], and litchi [23]. Therefore, chitosan coating might effectively delay the decline in firmness of fruit and vegetables under non-isothermal conditions.

Sweet cherries (*Prunus avium* L.) have high economic value and rich in nutrition and contain certain health protection components [24]. However, sweet cherries are susceptible to adverse environmental factors, especially temperature variations. Base on the above, the purpose of this study was to elucidate the exact effect of temperature variations on sweet cherry texture quality. In addition, we applied chitosan coating in the non-isothermal storage of sweet cherries to clarify its inhibition on the softening of sweet cherry.

2. Materials and methods

2.1. Cherry materials and coating

Fresh mature sweet cherry (*Prunus avium* L. cv. 'Hongdeng') fruit samples were handpicked from the fruit trees with standard cultural on the farm (Zhengzhou, Henan, China) and delivered to the laboratory in less than 2 h. Sweet cherries with bright red colour and commercial maturity were harvested for research. Sweet cherries (per fruit weight >3 g, transverse diameter >9 mm,) with uniform shape, colour, and absence of blemishes or disease were selected. The chitosan coating solution (2%) was prepared according to the Xin's method [8]. A uniform chitosan film formed over the whole sweet cherry surface after the samples were immersed in the coating solution for 10 min. The coated fruit samples were then dried in air for 1 h.

2.2. Isothermal and non-isothermal storage conditions

Two kinds of storage regimes were considered. Regime I: isothermal storage, in which the fruit samples were stored at a constant temperature (temperature, 5 ± 1 °C). Regime II: non-isothermal storage, in which fruit samples were stored in sequence at 5 ± 1 °C (for 48 h), 25 ± 1 °C (for 6 h, simulation of a 'broken cold chain'), 5 ± 1 °C (for 42 h), 5–10 °C (for 9 h, simulation of temperature fluctuation), and 5 ± 1 °C (for 39 h), totalling 144 h.

The fruit samples were randomly divided into three groups with different coating treatments and storage regimes as mentioned below: distilled water + isothermal storage (IT), distilled water + non-isothermal storage (NIT), chitosan + non-isothermal storage (CNIT). All samples were then kept in commercial double high-density polyethylene bags (permeability of O_2: 7.64×10^{-9} mol μm m^{-2} s^{-1} Pa^{-1}, permeability of CO_2: 8.09×10^{-9} mol μm m^{-2} s^{-1} Pa^{-1}) and stored for up to 144 h.

2.3. Determination of firmness

Fruit firmness was determined using a TA-XT2i Texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK) with a 5-mm probe. The test speed was 1 mm s^{-1}, the pressed distance was 2.5 mm, and the trigger force was 5.0 g. Twenty fruit from each treatment were randomly selected for firmness measurement.

2.4. Pectin extraction and determination

The cell wall materials of sweet cherry flesh were separated using a previously described method [19,25]. SSP fraction was extracted using 50 mmol L^{-1} Na_2CO_3 containing 2 mmol L^{-1} CDTA. The extracted SSP content was determined using carbazole colourimetry and galacturonic acid was used as a standard [8].

2.5. Measurement of PME activities

The procedure of PME (EC 3.1.1.11) extraction was conducted using a previously described method with some modifications [26]. Pulp tissue (2 g) was from fifteen fruit and homogenised in 20 mL of 20 mmol L^{-1} Tris-HCl (pH 7.0), containing 20 mmol L^{-1} cysteine–HCl, 20 mmol L^{-1} EDTA, and 0.05% Triton X-100. The mixture was then centrifuged at 15,000 ×*g* for 30 min at 4 °C, and the supernatant was used to determine the enzyme activity.

The activity of PME was measured according to the Lohani's method with some modifications [26]. The reaction mixture contained 0.1 mL of enzyme extract, 1 mL of pectin solution (pH 7.5), 0.2 mL of NaCl (0.15 mol L^{-1}), 0.1 mL of bromothymol blue solution, and 0.2 mL of water. The reaction mixture was shaken and measured immediately at 620 nm. The PME activity was reflected by the change of absorbance values in 3 min.

2.6. Expression analysis of PME-related genes using qRT-PCR

Fifteen fruit were randomly selected for expression of *PME*-related genes test. An RNeasy plant mini kit column and a QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) were used to extract RNA from ground tissue and transcribed it into cDNA, respectively. The cDNA was synthesised using M-MLV reverse transcriptase. Primer pairs for the target genes (*paPME1–5*) (GenBank accession: XM_021964187, XM_021967675, XM_021964195, XM_021960415 and XM_021949424) were designed using Primer 5.0 software and their details were listed in Table S1. Quantitative RT-PCR was carried out using DNA binding dye SYBR Green I for detection of PCR products. The settings of the qRT-PCR cycling conditions included four stages: an initial denaturation step at 95 °C for 3 min, followed by 45 cycles of amplification at 95 °C for 10 s, annealing at 57 °C for 15 s and finally extension at 72 °C for 20 s.

2.7. FTIR analysis of pectin

Information on the pectin IR spectra and the degree of methylation (DE) were detected using FTIR spectroscopy (FTIR spectra Nicolet 5700, Thermo Fisher Scientific, Boston, MA, USA) in the frequency range of 4000–400 cm^{-1} [27].

2.8. Monosaccharide content of pectin

The monosaccharide content of SSP was analysed as described by Zhang [19]. SSP was hydrolysed with 2 mol L^{-1} trifluoroacetic acid. Then the hydrolysate was added 1-phenyl-3-methyl-5-pyrazolone (450 μL, 0.5 mol L^{-1} in methanol) and NaOH (450 μL, 0.3 mol L^{-1}) and incubated at 70 °C for 30 min. After that, the cooled mixture was mixed with HCl (450 μL, 0.3 mol L^{-1}) and chloroform (1.0 mL) for neutralization and extraction, respectively. Finally, the resulting aqueous phase was measured with a HPLC system (Waters2695, Milford, MA, USA). Elution was carried out with a mobile phase comprising acetonitrile (phase A: 15%, v/v; phase B: 40%, v/v) and sodium phosphate (0.05 mol L^{-1}, pH 6.9) at a flow rate of 1.0 mL min^{-1}. The gradient mode of 0–15% (over 0–25 min) then 15–25% buffer B (over 25–40 min) was used.

2.9. Pectin nanostructural characterisation and analysis

A Multimode NanoScope IIIa AFM (model Multimode VIII, Bruker Corp., Santa Barbara, CA) was used to observe and measure the pectin nanostructure. Diluted sample (about 10 μg mL^{-1}, 10 μL) was distributed on a mica sheet. The AFM measurement was taken with tapping mode at about 0.5–2 Hz. And the Nanoscope Analysis software (Bruker Corp., Santa Barbara, CA) was used for offline analysing of AFM images [28]. The reliable results were obtained from the analysis of at least 90 single chains for each sample.

2.10. Statistical analysis

The experiment was independently repeated in triplicate and all data was presented as the mean ± standard deviation. Statistical analysis was performed by analysis of variance (ANOVA, $P \leq 0.05$) and Duncan's test using the SAS software (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. The effect of chitosan coating on the firmness and SSP content of sweet cherries during non-isothermal storage

The firmness of sweet cherries in all groups decreased continuously during both isothermal and non-isothermal storage (Fig. 1A). In particular, under the action of temperature variations, the uncoated group (NIT) showed significantly accelerated softening. However, at the end of storage, the chitosan-coated cherries under non-isothermal storage (CNIT) maintained better firmness than the NIT group and isothermal group (IT) ($P < 0.05$). These data indicated that chitosan coating on the sweet cherry surface effectively prevented loss of firmness during non-isothermal storage.

The SSP content correlates positively with firmness [8]. As shown in Fig. 1B, the SSP content decreased during the whole storage period. The decrease in SSP could be attributed to the depolymerisation of the 'pectin-cellulose-hemicellulose' network and the effect of various pectinase classes [29]. After a 'broken cold chain' and 'temperature fluctuation', a significant decrease of SSP content was observed in the NIT group. However, over the whole study period, there were no statistically significant difference between the CNIT and IT groups ($P < 0.05$). This phenomenon suggested that chitosan coating inhibited normal SSP disassembly to some degree under non-isothermal condition. This trend was consistent with the firmness results (Fig. 1A).

The loss of firmness and the SSP content can be attributed to a variety of enzyme actions, which are differentially influenced by temperature [30]. The chitosan coating layer acts as a protection against increased endogenous enzyme activity and metabolic changes. The effect of a chitosan coating on delaying fruit softening has been reported in many fruits, including sweet cherry [31] and strawberry [16].

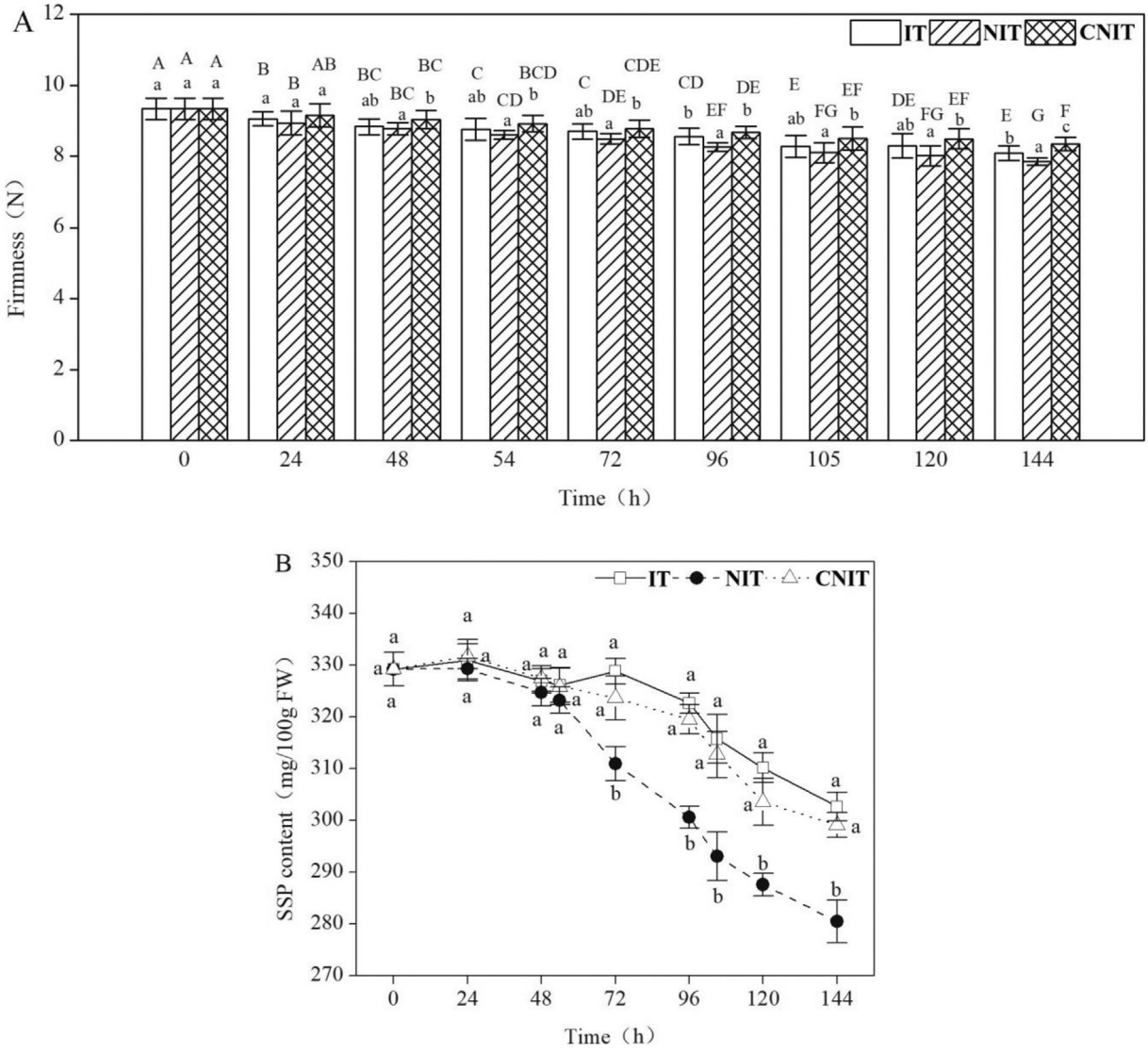


Fig. 1. Effect of chitosan coating on firmness and sodium carbonate-soluble pectin (SSP) content of sweet cherry during non-isothermal storage. (A) firmness; (B) SSP content. Note: IT means sweet cherry coated with distilled water under isothermal storage; NIT denotes sweet cherry coated with distilled water under non-isothermal storage; CNIT denotes sweet cherry coated with chitosan under non-isothermal storage. Data are presented as means ± standard deviation. The different lowercase superscript letters in the same storage time indicate significant differences within the different groups ($P < 0.05$). The different capital superscript letters in the same group indicate significant differences within the different storage times ($P < 0.05$).

3.2. The effect of chitosan coating on the DE of pectin, the PME activity, and PME-related gene expression of sweet cherry during non-isothermal storage

The FITR-derived conformation of SSP in fresh, isothermal, and non-isothermal stored samples is shown in Fig. 2A. The sweet cherries showed strong absorption peaks in three regions, 2500–3800, 1400–2000, and 800–1300 cm^{-1}. The absorption peaks at about 3400 cm^{-1} corresponds to the O—H stretching vibrations, which are attributed to the inter- and intra-molecular hydrogen bonding of the GalUA polymer [32]. The broad and strong absorption at about 2900 cm^{-1} refer to the bending and stretching vibrations of C—H (including C—H, C—H_2, and C—H_3) [33]. The obtained ratio between the intensity of the peak at 1743 cm^{-1} (COO–R) to the summed intensity of peak at 1743 and 1613 cm^{-1} (COO–) was considered as an evaluation of the degree of methylation (DE) [34]. In addition, the fingerprint region of pectin, including C—H bending and C—O stretching, caused absorption at 1380 cm^{-1} and 1000–1300 cm^{-1}, respectively [35]. After storage, the DE of SSP in the IT, NIT, and CNIT groups decreased. In addition, temperature variations accelerated the decline of DE. For example, the DE value of SSP in fresh sweet cherry was 0.45. At the end of storage, the DE value of the NIT group was only 0.38, but the DE value of the CNIT and IT groups remained at 0.40 and 0.41, respectively. This result indicated that the chitosan coating treatment has a negative effect on the demethylation of pectin during non-isothermal storage.

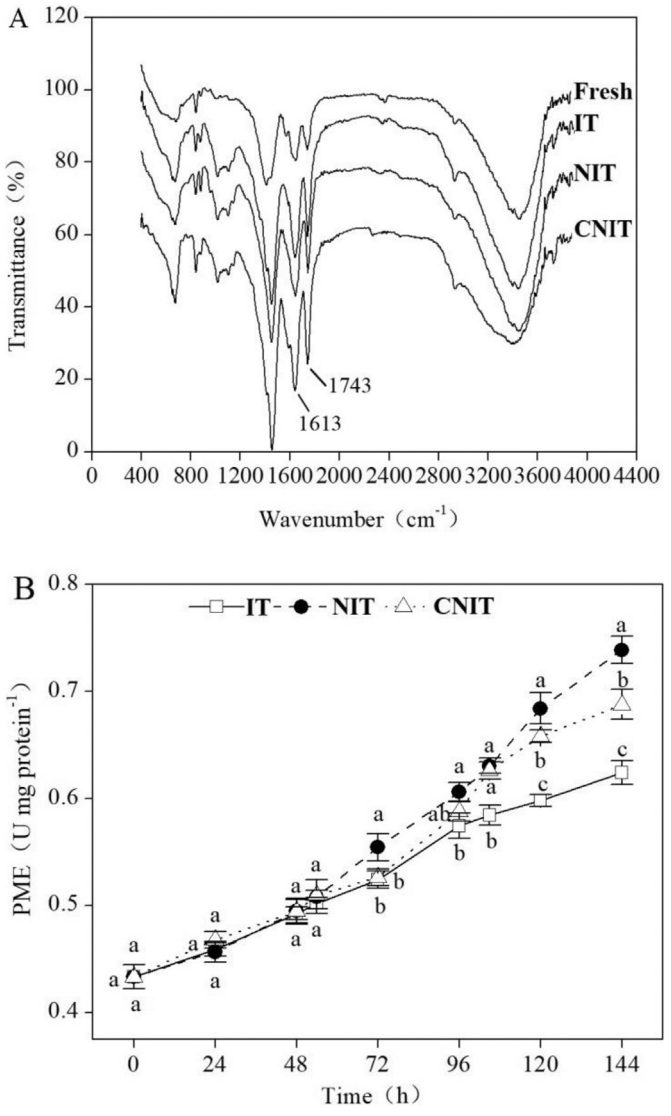


Fig. 2. Effect of chitosan coating on Fourier transform infrared (FTIR) spectroscopy properties of sodium carbonate-soluble pectin (SSP) and pectin methylesterase (PME) activity of sweet cherry during non-isothermal storage. (A) FTIR properties of SSP; (B) PME activity. Note: IT means sweet cherry coated with distilled water under isothermal storage; NIT denotes sweet cherry coated with distilled water under non-isothermal storage; CNIT denotes sweet cherry coated with chitosan under non-isothermal storage. Data are presented as means ± standard deviation. The different lowercase superscript letters in the same storage time indicate significant differences within the different groups ($P < 0.05$).

PME demethylate pectin polysaccharides and provide a substrate for pectin-metabolizing enzymes such as PG or PL. In addition, demethylation by PME also generates electrostatic repulsion between adjacent pectin molecules by increasing the number of negatively charged groups in pectin side chains, which might result in acceleration of the weakening of the cell wall [36]. The PME activity in sweet cherries increased gradually throughout the period of study (Fig. 2B). The observation of increased PME activity during the initial stage of postharvest fruit maturation agrees with previous reports [13,14]. Compared with that in the IT group, the PME activity started to increase sharply under the action of temperature variations. Either after 'broken cold chain' or after 'temperature fluctuation', the PME activity of the NIT group was significantly higher than that of the IT group. At the end of storage, PME activity had increased gradually in the chitosan-coated cherries under non-isothermal storage (CNIT), and was significant higher than that in the isothermal group (IT). Unlike the NIT group, the chitosan coating treatment delayed the increase in the enzymatic activity. The PME activity in the CNIT group was as same as the IT group at the first temperature change (at 54 and 72 h). In addition, after two temperature changes, the PME activity of the NIT group increased significantly faster than that of the CNIT group ($P < 0.05$). The data illustrated the inhibitory effect of chitosan coating on the increase in PME activity under non-isothermal conditions.

The expression levels of *paPME* genes were measured to further study the demethylation of pectin under non-isothermal conditions (Fig. 3). The results showed that temperature variations affected the expression levels of *paPME* genes compared with those in the isothermal storage group. In particular, at 54 h ('broken cold chain'), the expression levels of *paPME3* and *paPME5* in the NIT group were significantly higher than those in the IT group. At the end of storage, the expression levels of *paPME1–4* in the NIT group were still higher than those in the IT group. However, chitosan coating treatment could inhibit the adverse effect of temperature change on *paPME* gene expression. Under the temperature variations (at 54 h), the expression levels of *paPME1*, *paPME2*, *paPME3*, and *paPME5* in the CNIT group were significantly lower than those in the NIT group, and were even lower than those in the IT group. A similar pattern of *paPME1–4* expression was observed in CNIT group at 144 h. These gene transcription results indicated that upregulation of *paPME* gene expression was inhibited by the chitosan coating. Furthermore, coating treatment alleviated the increase of PME activity (Fig. 2B) and the demethylation of SSP (Fig. 2A).

3.3. The effect of chitosan coating on the monosaccharide constitute of SSP during non-isothermal storage

Pectins are complex plant cell wall polysaccharides structurally, which contain three basic classes of pectin: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II) [37]. As shown in Table 1, arabinose (Ara), galacturonic acid (GalUA), and rhamnose (Rha) were the major components of SSP (>80%), followed by galactose (Gal), and small amounts of xylose (Xyl), glucose (Glc), and mannose (Man), which may be possibly came from un-removed hemicelluloses in the pulp [38]. These results revealed that RG-I was the basic structural unit of SSP from sweet cherries. Previous studies found that a reduced ratio of GalUA:Rha and (Gal+Ara):Rha indicates the dissociation and degradation of the RG-I region, which correlated

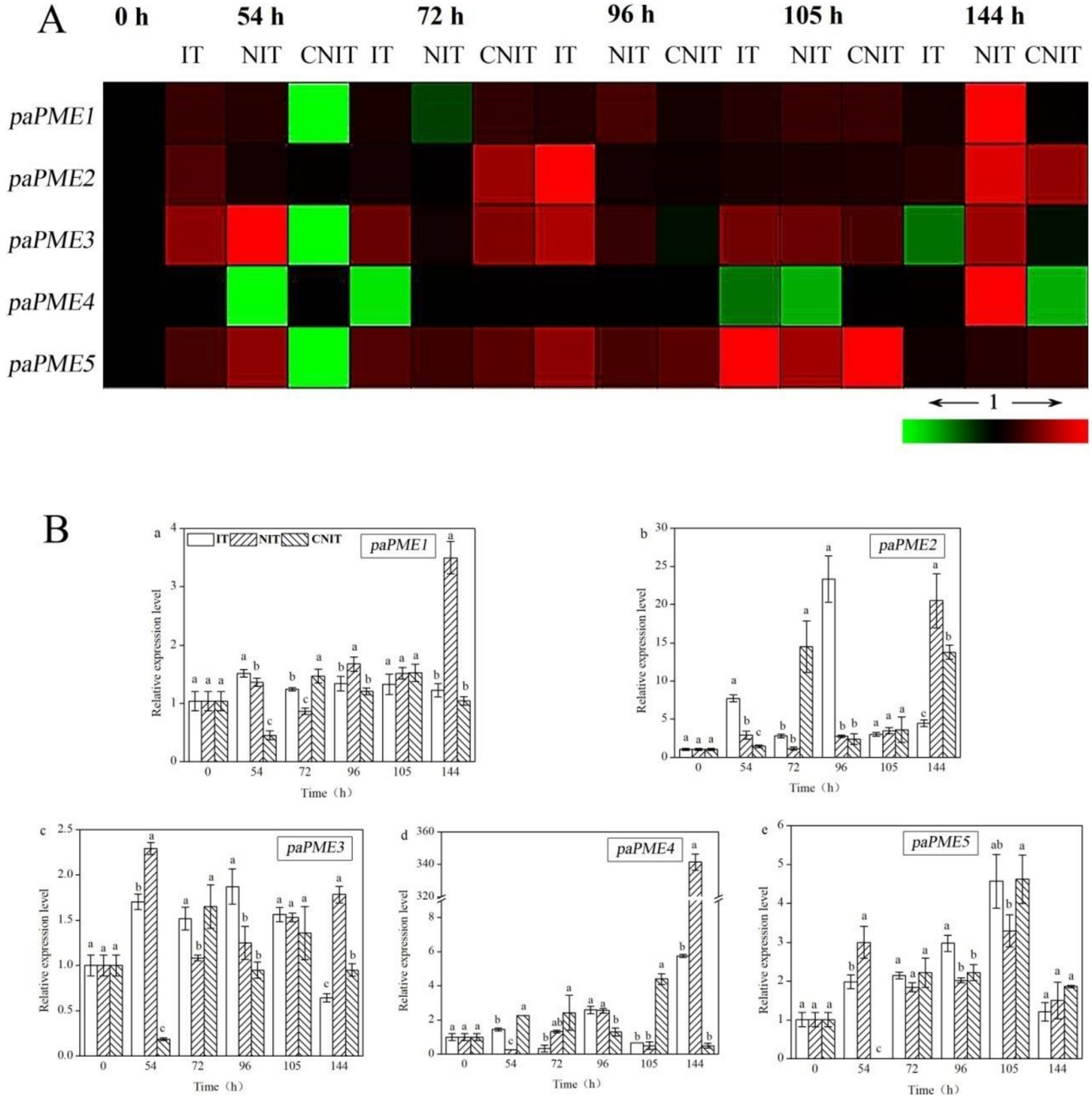


Fig. 3. Effect of chitosan coating on pectin methylesterase (PME)-related gene expression in sweet cherry during non-isothermal storage. (A) Heatmap of PME-related genes in sweet cherry. Levels of downregulated expression (green) or upregulated expression (red) are shown on a log2 scale for each gene; (B) relative expression of PME-related genes in sweet cherry. Note: IT means sweet cherry coated with distilled water under isothermal storage; NIT denotes sweet cherry coated with distilled water under non-isothermal storage; CNIT denotes sweet cherry coated with chitosan under non-isothermal storage. Data are presented as means ± standard deviation. The different lowercase superscript letters in the same storage time indicate significant differences within the different groups ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with the texture feature of fruit [32]. As shown in Table 1, the molar ratios of GalUA:Rha and (Gal+Ara):Rha in the IT group decreased to 1.03 and 2.48, while those in the NIT group were 0.89 and 2.40, respectively at the end of storage. These results indicated that the changes of RG-I main chains and side chains was affected by the temperature variations. And, more remarkable, the molar ratios of GalUA:Rha and (Gal+Ara):Rha in the CNIT group remained 1.03 and 2.53, respectively. Thus, during non-isothermal storage, the chitosan-coated samples contained the more main and side chains of RG-I segments than the untreated samples.

3.4. The effect of chitosan coating on the nanostructural characterisation of SSP during non-isothermal storage

The evolution of the SSP nanostructure plays a critical role in cell wall softening and disaggregation [39]. The nanostructural

Table 1
Effects of chitosan coating on the monosaccharide constitute of SSP during non-isothermal storage.

Monosaccharide (%)	0 h	144 h		
	IT	IT	NIT	CNIT
Man	0.84 ± 0.06^{c}	0.37 ± 0.08^{a}	0.61 ± 0.05^{b}	0.43 ± 0.08^{ab}
Rha	18.77 ± 0.34^{a}	20.98 ± 0.83^{b}	22.08 ± 0.41^{b}	20.57 ± 1.22^{b}
GalUA	22.95 ± 0.53^{c}	21.60 ± 0.78^{b}	17.77 ± 0.81^{a}	21.09 ± 1.03^{b}
Glc	1.36 ± 0.04^{a}	1.04 ± 0.06^{a}	2.18 ± 0.40^{b}	1.16 ± 0.25^{a}
Gal	8.65 ± 0.08^{a}	10.22 ± 0.21^{b}	13.89 ± 0.83^{c}	11.97 ± 0.76^{bc}
Xyl	3.48 ± 0.15^{a}	4.02 ± 0.04^{b}	4.31 ± 0.84^{b}	4.01 ± 0.81^{b}
Ara	43.96 ± 0.83^{c}	41.80 ± 0.45^{b}	39.18 ± 0.04^{a}	40.79 ± 1.06^{ab}
GalUA:Rha	1.22	1.03	0.89	1.03
(Gal+Ara):Rha	2.80	2.48	2.40	2.53

Note: IT means sweet cherry coated with distilled water under isothermal storage; NIT denotes sweet cherry coated with distilled water under non-isothermal storage; CNIT denotes sweet cherry coated with chitosan under non-isothermal storage.
Different superscript lowercase letters in the same row mean significant difference at $P < 0.05$.

morphologies of SSP are shown in Fig. 4. Heterogeneous and intricate nanostructures of SSP included linear strands and polymer structures. The nanostructures of SSP in fresh sweet cherries indicated that the interconnected SSPs form large polymer (Lp) [40] to maintain fruit firmness (Fig. 4Aa). After storage, the well-set structure was depolymerized to small polymer (Sp), loose structure (Ls), and long chain (Lc) [41] (Fig. 4Ac–e). Thereafter, the polymer structure was further dissolved and thick (Tk) and long chain degradation occurred. At the end of storage, multiple branched chain (Mb), branched chain (Br), and short straight chain (Ss) structures were found in the fruit (Fig. 4Af–h). Besides, there was a significant difference in pectin morphology between the IT and NIT groups. Under the action of temperature variations, the uncoated group (NIT) had more long chains and short straight chains that were randomly distributed compared with those in the IT group (Fig. 4Ad and g). By contrast, in the IT group, sweet cherries still maintained polymers, entangled thick chains and multiple branched chains cross linked with each other (Fig. 4Ac and f). This indicated accelerated degradation of SSP in the sweet cherries under non-isothermal storage. However, compared with NIT group, CNIT group has a slight increase of thick, long, and branched chains. During non-isothermal storage, the SSPs in the coated sweet cherries were still linked with other chains (Fig. 4Ae and h). This phenomenon indicated the preservation effect of chitosan coating [8].

The influence of chitosan coating treatment on the quantitative parameter of SSPs in sweet cherries during non-isothermal storage was shown in the Fig. 4B. The fresh sweet cherry showed the largest width, which mainly ranged from 120 to 140 nm (25%). The frequency of widths below 60 nm was approximately 5%. The frequency of wider pectin chains decreased gradually during storage. In particular, changes in the storage temperature markedly influenced the SSP chain width. For example, the width with the highest frequency decreased to the range of 100–120 nm (21.7%) and 60–80 nm (22.5%) after 'broken cold chain' (72 h) and 'temperature fluctuation' (144 h), respectively. In addition, compared with that in the IT group, the proportion of narrow chains (<60 nm) in the NIT group was significantly higher. Although stored under non-isothermal conditions, sweet cherries coated with chitosan (CNIT) maintained wider SSP chains than the uncoated cherries (NIT). At the end of storage, the content of wider SSP chains in the NIT group cherries was significantly lower than that in the CNIT and IT group cherries. The frequency of large widths (>140 nm) was 11.7% for CNIT group, but 2.5% in the NIT group.

3.5. Hypothetical schematic images for effect of chitosan coatings on the evolution of SSP during non-isothermal conditions

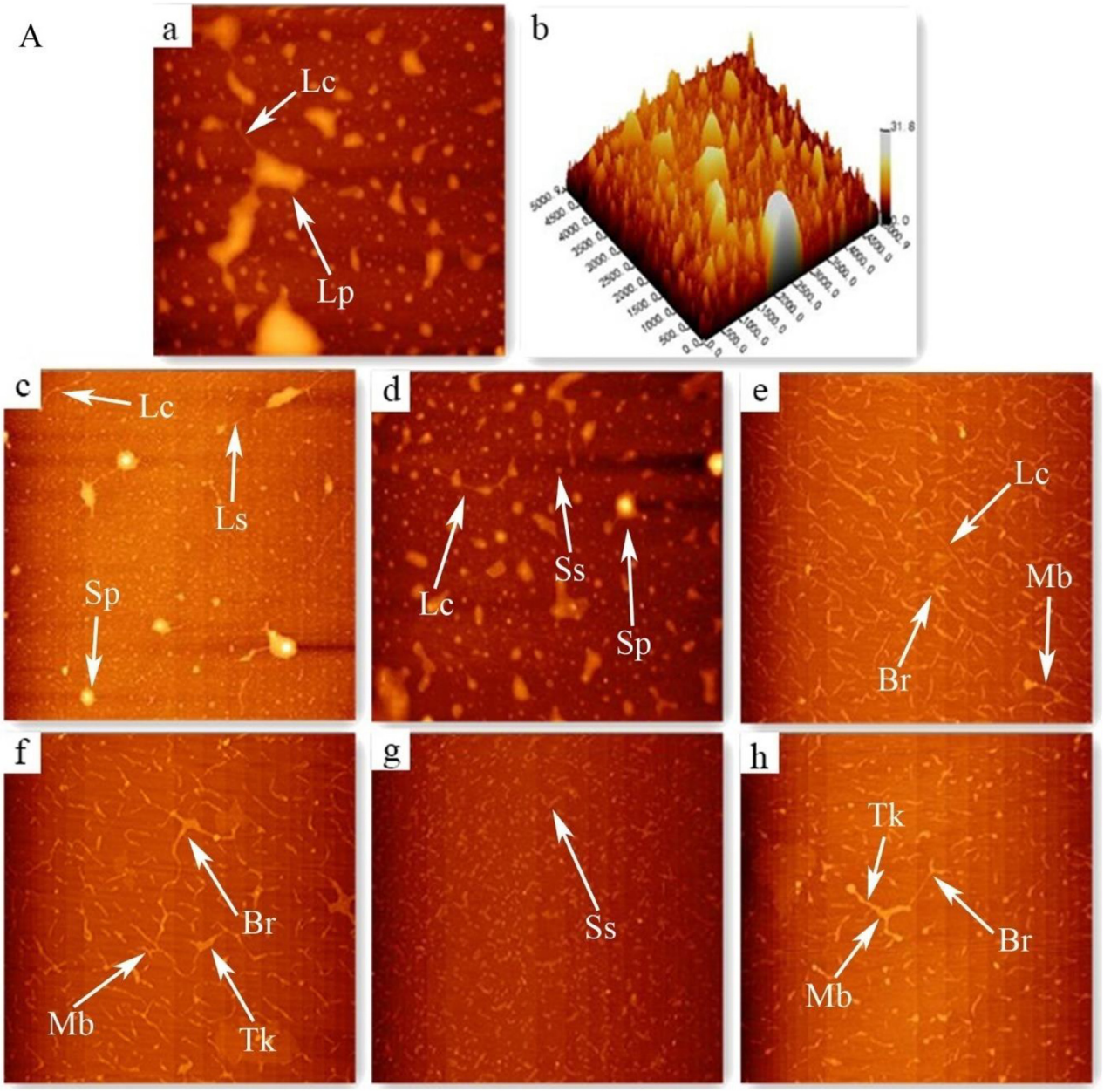
Fresh sweet cherries are rich in SSP, with a high level of methyl esters. SSP mainly contained HG backbone, RG-I main branch, and side chain. Those SSP chains were interconnected with each other and formed many large polymers to maintain fruit firmness (Fig. 5A). During cold storage, the expression levels of *paPME* genes and the activity of PME in sweet cherry increased gradually. PME demethylate pectin polysaccharides and provide a substrate for further degradation events. The SSP chains were demethylated, and then the number of negatively charged groups in pectin side chain was increased. The electrostatic repulsion between adjacent pectin molecules might result in acceleration of the weakening of the large polymers. The well-set structure was depolymerized to small polymers and long chains. Thereafter, SSP was further demethylated and degradation occurred. At the end of storage, pectin polymers and the cross-linking between pectin chains had decreased sharply. Branched and linear structures appeared (Fig. 5B).

Under the action of temperature variations, the expression levels of *paPME* genes were significantly upregulated, PME activity started to increase sharply, and the DE of pectin decreased. More pectin chains dissociated from the aggregates and distributed randomly in short and long chains. Under the action of temperature variations, the uncoated sweet cherries showed significantly accelerated softening (Fig. 5C). Chitosan coating could inhibit the upregulation of *paPME* gene expression, which are the key genes that regulate PME activity of sweet cherries under temperature variations. The coating treatment could alleviate the demethylation of SSP and hydrolysis of pectin main and side chains. Furthermore, the coated sweet cherries contained more linked branches and long SSP chains, and maintained the width of the pectin backbone. Finally, at the end of non-isothermal storage, the firmness of the sweet cherry was preserved due to the action of chitosan coating (Fig. 5D).

4. Conclusion

Unavoidable temperature variations often occur in the cold chain, accelerating loss of firmness of sweet cherries, a decline in SSP content, changes in DE, RG-I structural units, and the nanoscale morphology of SSP. This research demonstrated that chitosan coating could alleviate the fruit firmness decrease (6.4% higher than control group), loss of SSP content (6.6% higher than control group), and SSP degradation events through inhibiting the expression of *paPME1–5* genes, which regulate the PME activity in sweet cherries under temperature variation. The results of FTIR and HPLC indicated the inhibiting effect of chitosan coating on the demethylation of SSP, and the loss of RG-I main and side chain neutral sugars. Furthermore, the AFM images revealed that the coated sweet cherries contained more linked, branched, and long SSP chains and maintained the width of the pectin backbone

Fig. 4. Effect of chitosan coating on the nanostructural characterisation of sodium carbonate-soluble pectin (SSP) during non-isothermal storage. (A) Representative atomic force microscopy (AFM) morphology images of SSP chains in sweet cherries, scan size: 5.00 × 5.00 μm^2; (B) different coating treatments and storage regimes affect the width distribution of SSP chains of sweet cherry. Note: a, b: AFM images of fresh sweet cherry; c, d, e: AFM images at 72 h for IT (c), NIT (d) and CNIT (e); f, g, h: AFM images at 144 h for IT (f), NIT (g) and CNIT (h). IT means sweet cherry coated with distilled water under isothermal storage; NIT denotes sweet cherry coated with distilled water under non-isothermal storage; CNIT denotes sweet cherry coated with chitosan under non-isothermal storage. Lp: large polymer; Sp: small polymer; Ls: loose structure; Lc: long chain; Tk: thick chain; Br: branched chain; Mb: multiple branched chain; Ss: short straight chain Data are presented as means ± standard deviation. The different lowercase superscript letters in the same storage time indicate significant differences within the different groups ($P < 0.05$).


A
a
Lc
Lp
b
31.6
c
Lc
Ls
Sp
d
Lc
Ss
Sp
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Br
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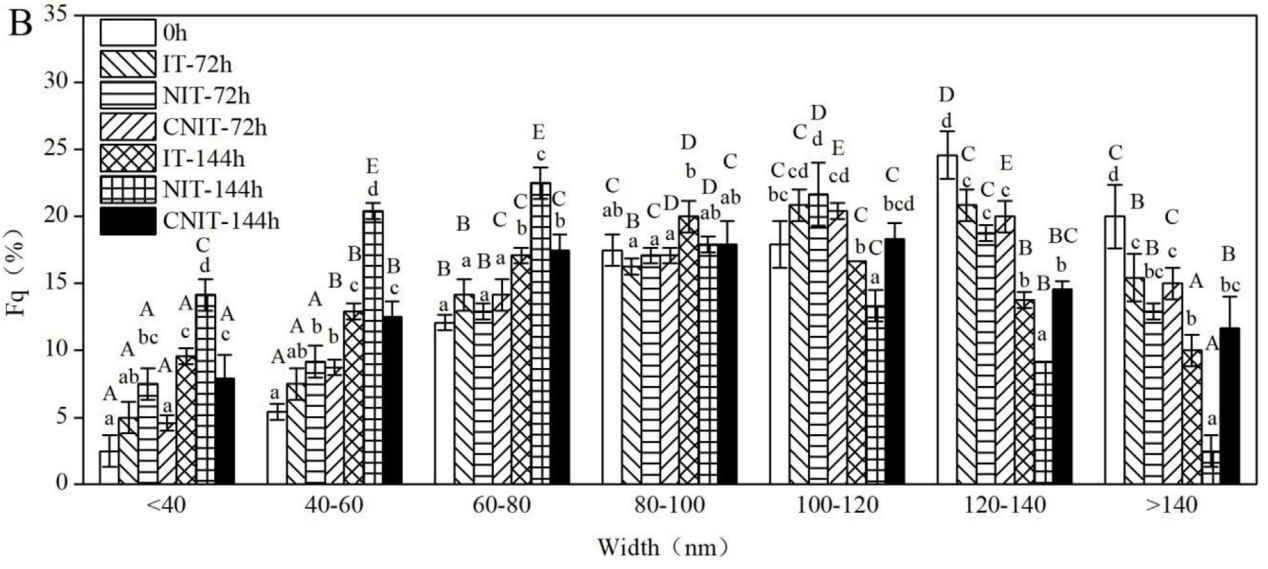
Br
Mb


B
0h
IT-72h
NIT-72h
CNIT-72h
IT-144h
NIT-144h
CNIT-144h
Fq（%）
0
5
10
15
20
25
30
35
<40
40-60
60-80
80-100
100-120
120-140
>140
Width（nm）

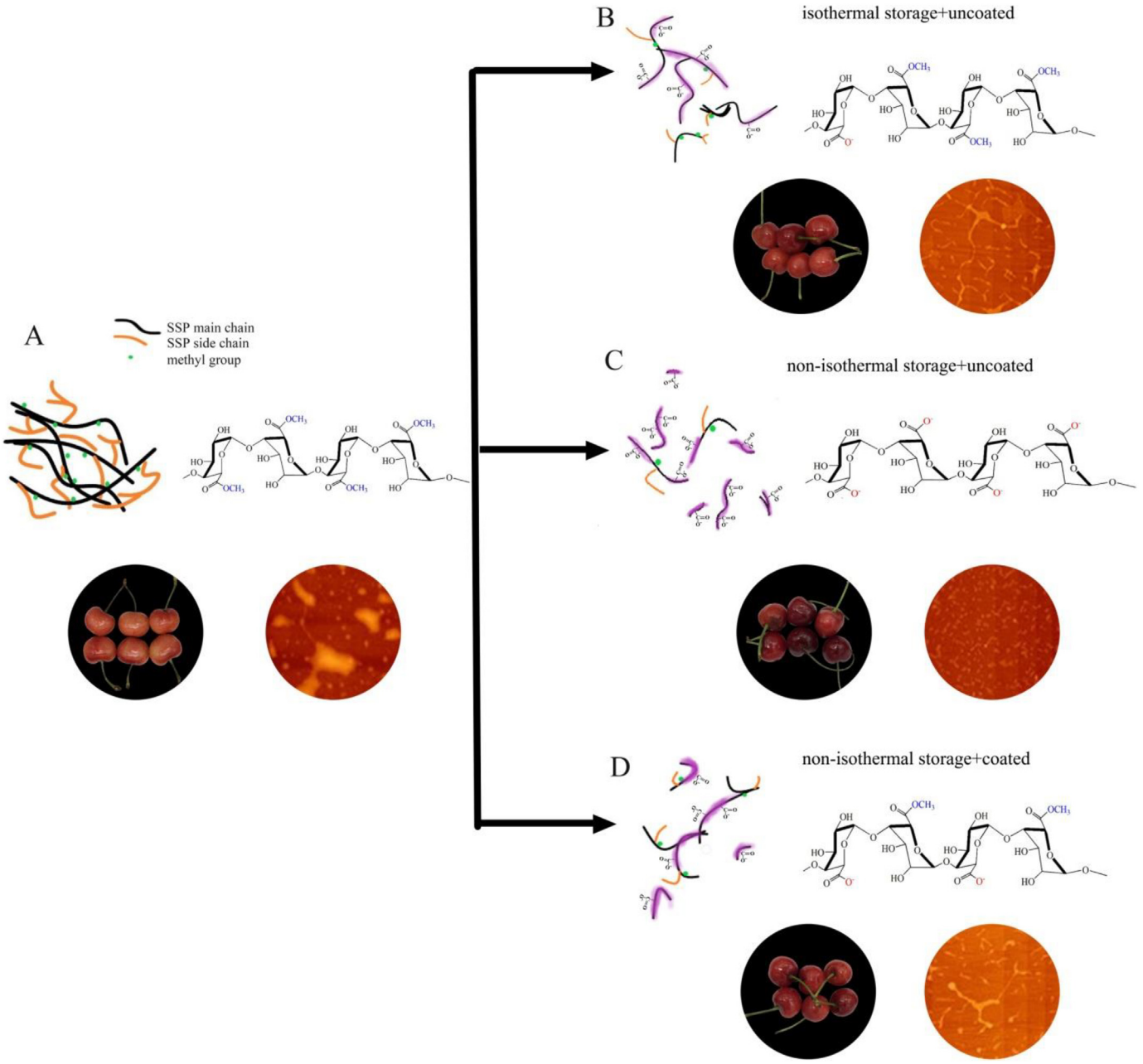


Fig. 5. Hypothetical schematic images for effect of chitosan coatings on the evolution of SSP during non-isothermal conditions.

(>140 nm). These results indicated that it is feasible to apply chitosan coating to preserve postharvest fruit under non-isothermal condition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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